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(54) **RECOMBINANT MULTIVALENT INFLUENZA VIRUSES**

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(57) **ABSTRACT**

The invention provides a composition useful to prepare influenza vaccine viruses, e.g., in the absence of helper virus, which includes internal viral segments from an influenza virus vaccine strain or isolate, e.g., one that is safe in humans, for instance, one that does not result in significant disease, and encodes a heterologous antigen.

Specification includes a Sequence Listing.

PR8(CAMBRIDGE)

PB2

AGCGAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAACTAAGAAATCTAATGTCGCAGTCTCGCACCCGCGAGATA
CTCACAAAAACCACCGTGGACCATATGGCCATAATCAAGAAGTACACATCAGGAAGACAGGAGAAGAACCCAGCACTTAGGATG
AAATGGATGATGGCAATGAAATATCCAATTACAGCAGACAAGAGGATAACGGAAATGATTCCTGAGAGAAATGAGCAAGGACAA
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CAGGATGTAATCATGGAAGTTGTTTTCCCTAACGAAGTGGGAGCCAGGATACTAACATCGGAATCGCAACTAACGATAACCAAA
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AGACTCACAGTTCTCGGAAAGGATGCTGGCACTTTAACCGAAGACCCAGATGAAGGCACAGCTGGAGTGGAGTCCGCTGTTCTG
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(SEQ ID NO:11)

Fig. 1A

PR8(CAMBRIDGE)

PB1

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TACTCAGAAAAGGGAAGATGGACAACAAACACCGAAACTGGAGCACCGCAACTCAACCCGATTGATGGGCCACTGCCAGAAGAC
AATGAACCAAGTGGTTATGCCCAAACAGATTGTGTATTGGAAGCAATGGCTTTCCTTGAGGAATCCCATCCTGGTATTTTTGAA
AACTCGTGTATTGAAACGATGGAGGTTGTTCAGCAAACACGAGTAGACAAGCTGACACAAGGCCGACAGACCTATGACTGGACT
TTAAATAGAAACCAGCCTGCTGCAACAGCATTGGCCAACACAATAGAAGTGTTCAGATCAAATGGCCTCACGGCCAATGAGTCA
GGAAGGCTCATAGACTTCCTTAAGGATGTAATGGAGTCAATGAAAAAAGAAGAAATGGGGATCACAACTCATTTTCAGAGAAAG
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TATCTAATTAGAGCATTGACCCTGAACACAATGACCAAAGATGCTGAGAGAGGGAAGCTAAAACGGAGAGCAATTGCAACCCCA
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ACCATCACTGGAGATAACACCAAATGGAACGAAAATCAGAATCCTCGGATGTTTTTGGCCATGATCACATATATGACCAGAAAT
CAGCCCGAATGGTTCAGAAATGTTCTAAGTATTGCTCCAATAATGTTCTCAAACAAAATGGCGAGACTGGGAAAAGGGTATATG
TTTGAGAGCAAGAGTATGAAACTTAGAACTCAAATACCTGCAGAAATGCTAGCAAGCATTGATTTGAAATATTTCAATGATTCA
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AATATGTTAAGCACTGTATTAGGCGTCTCCATCCTGAATCTTGGACAAAAGAGATACACCAAGACTACTTACTGGTGGGATGGT
CTTCAATCCTCTGACGATTTTGCTCTGATTGTGAATGCACCCAATCATGAAGGGATTCAAGCCGGAGTCGACAGGTTTTATCGA
ACCTGTAAGCTACTTGGAATCAATATGAGCAAGAAAAAGTCTTACATAAACAGAACAGGTACATTTGAATTCACAAGTTTTTTC
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GAGCAAACCCGTTCCAAAGCTGGACTGCTGGTCTCCGACGGAGGCCCAAATTTATACAACATTAGAAATCTCCACATTCCTGAA
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ACCATTGAAGAGCTCAGACGGCAAAAATAGTGAATTTAGCTTGTCCTTCATGAAAAAATGCCTTGTTTCTACT

(SEQ ID NO:10)

Fig. 1B

PR8(CAMBRIDGE)

PA

AGCGAAAGCAGGTACTGATTCAAAATGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGAGCTTGCGGAAAAAACA
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GAAAAGGCCAATAAAATTAAATCTGAGAAAACACACATCCACATTTTCTCGTTCACTGGGGAAGAAATGGCCACAAGGGCCGAC
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(SEQ ID NO:12)

Fig. 1C

PR8(CAMBRIDGE)

NP

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAATCATGGCGTCCCAAGGCACCAAACGGTCTTACGAACAGATG
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GGAGTCTTCGAGCTCTCGGACGAAAAGGCAGCGAGCCCGATCGTGCCTTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTC
GGAGACAATGCAGAGGAGTACGACAATTAAAGAAAAATACCCTTGTTTCTACT

(SEQ ID NO:13)

Fig. 1D

PR8(CAMBRIDGE)

M

AGCAAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCATCCCGTCAGGCCCCCT
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TAGACGCTTTGTCCAAAATGCCCTTAATGGGAACGGGGATCCAAATAACATGGACAAAGCAGTTAAACTGTATAGGAAGCTCAA
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CAACAGGATGGGGGCTGTGACCACTGAAGTGGCATTTGGCCTGGTATGTGCAACCTGTGAACAGATTGCTGACTCCCAGCATCG
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TTGATCGTCTTTTTTTCAAATGCATTTACCGTCGCTTTAAATACGGACTGAAAGGAGGGCCTTCTACGGAAGGAGTGCCAAAGT
CTATGAGGGAAGAATATCGAAAGGAACAGCAGAGTGCTGTGGATGCTGACGATGGTCATTTTGTCAGCATAGAGCTGGAGTAAA
AAACTACCTTGTTTCTACT

(SEQ ID NO:14)

NS

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(SEQ ID NO:15)

Fig. 1E

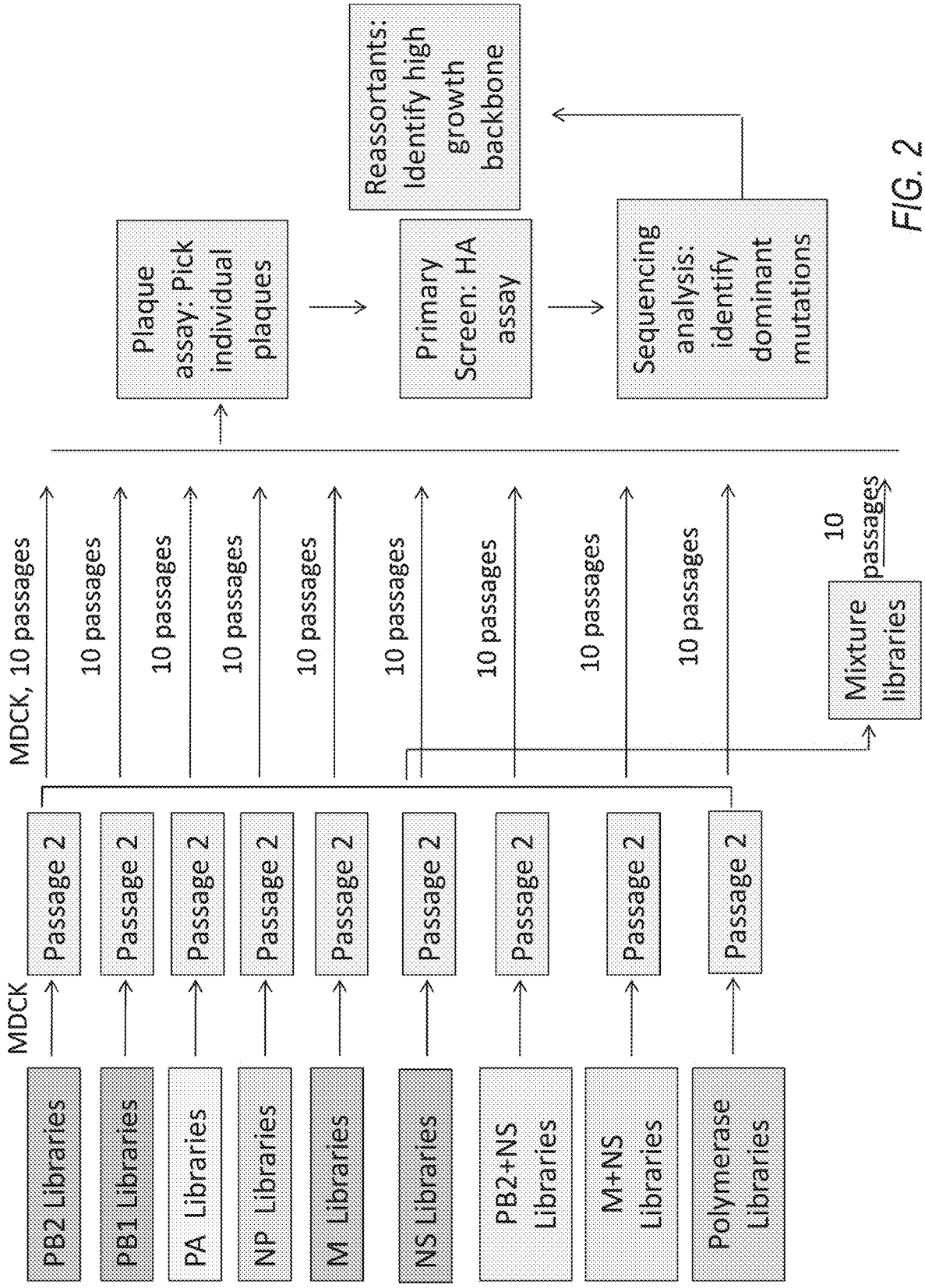

MDCK
Passage 2
MDCK, 10 passages
10 passages
10 passages
10 passages
10 passages
10 passages
10 passages
10 passages
10 passages
10 passages
Mixture libraries
PB2 Libraries
PB1 Libraries
PA Libraries
NP Libraries
M Libraries
NS Libraries
PB2+NS Libraries
M+NS Libraries
Polymerase Libraries
Plaque assay: Pick individual plaques
Primary Screen: HA assay
Sequencing analysis: identify dominant mutations
Reassortants: Identify high growth backbone

FIG. 2

Figure 3 Summary of HA assay of 1434 individual clones

Groups	Numbers of clone	Fold change	%
WT HA titer = 2^{7}	-	-	-
HA titer =$2^{>9\text{-}9.5}$	8	>4	0.6%
HA titer =$2^{>8.5\text{-}9}$	23	>2.8 - 4	1.6%
HA titer =$2^{7\text{-}8.5}$	748	1 - 2.8	52.2%
HA titer < 2^{7}	655	<1	45.6%
Total	1434	-	100%

Figure 4 Recombinant viruses generated from dominant mutations

Viruses	Gene backbone								Virus stock titer	
	HA	NA	PB2	PB1	PA	NP	M	NS	2^n	Pfu/ml
WT	Indo/NC/09 delHA	Indo/NC/09 NA	PR8-wt	PR8-wt	PR8-wt	PR8-wt	PR8-wt	PR8-wt	7	3.0E+07
1			M202L F323L	M507V V644A		I116L		K55E	9~9.5	2.0E+08
2			M202L F323L	Q247H	R401K			T49A	9	1.0E+08
3			I504V	M507V V644A	I550L	R74K N417D		K55E	8~8.5	5.7E+07
4			I505V	E112G	I550L	R74K		S161T	9	1.6E+08
5			M202L F323L	E112G				S161T	8.5	1.3E+08
6			M66R	M40I G180W		R74K		S161T	8~8.5	2.3E+07

Figure 5A Growth curve – PB2 mutants

(MOI=0.001)

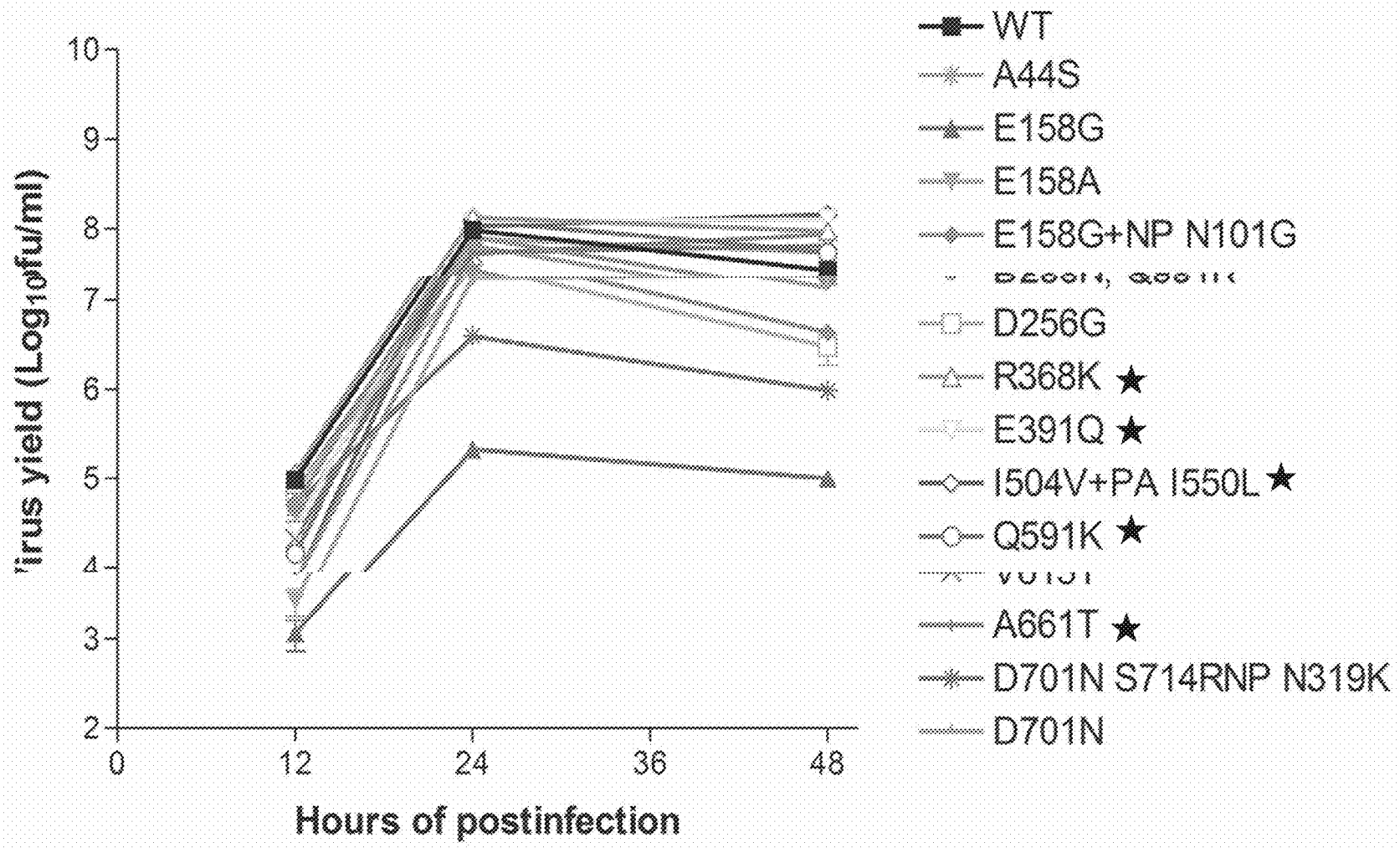


Figure 5B PB1 Mutants

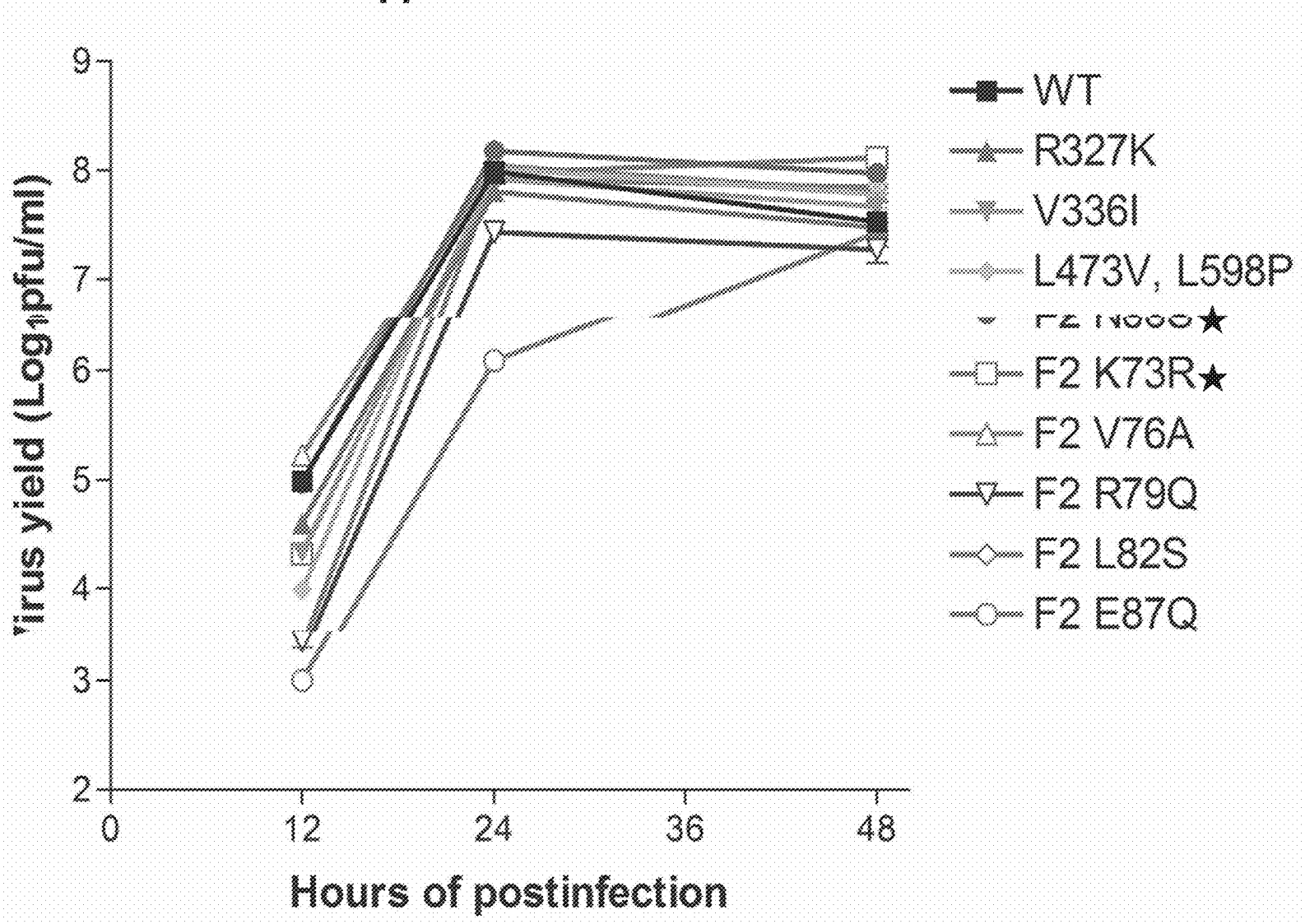


Figure 5C PA Mutants

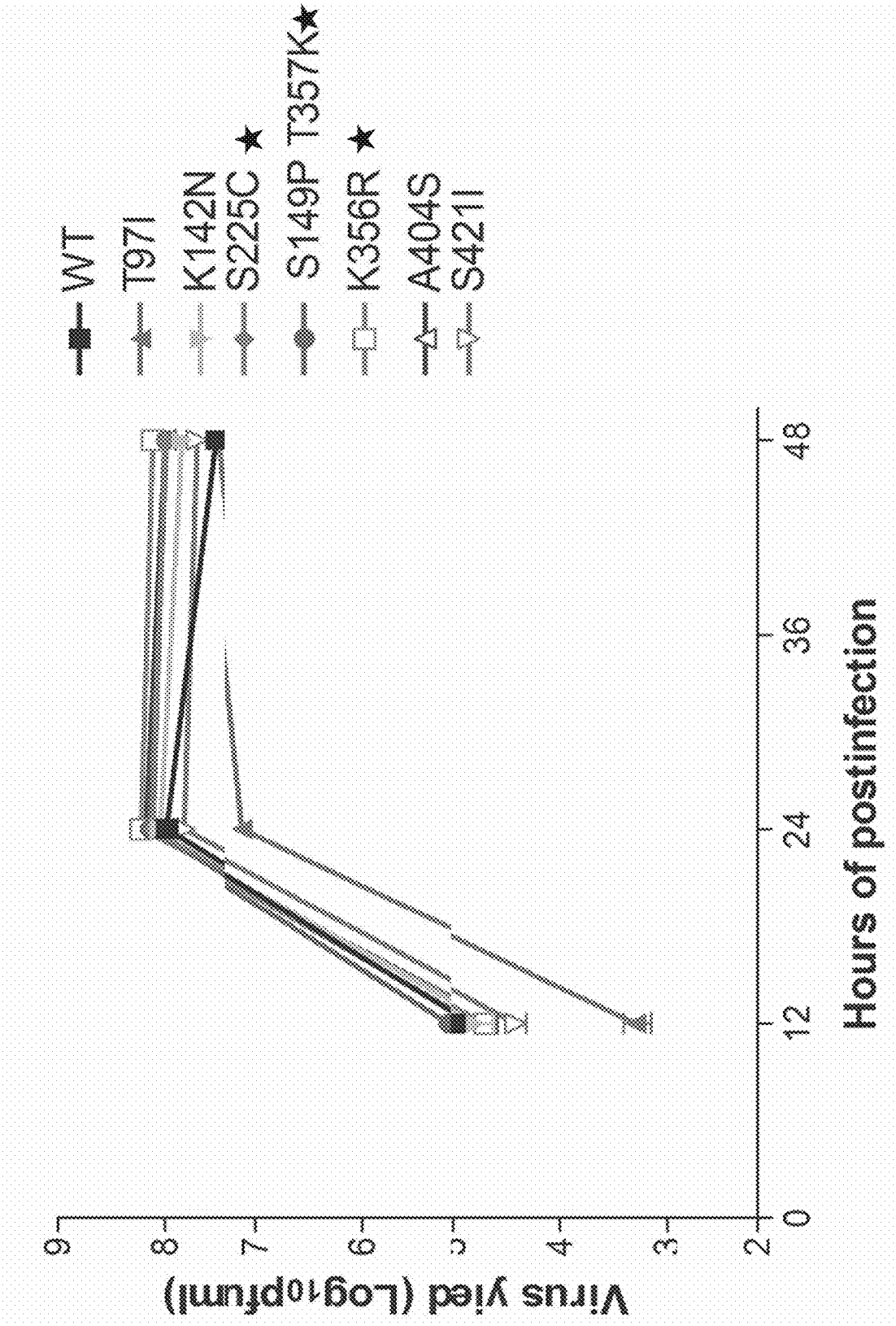


Figure 5D NP, M and NS1 mutants

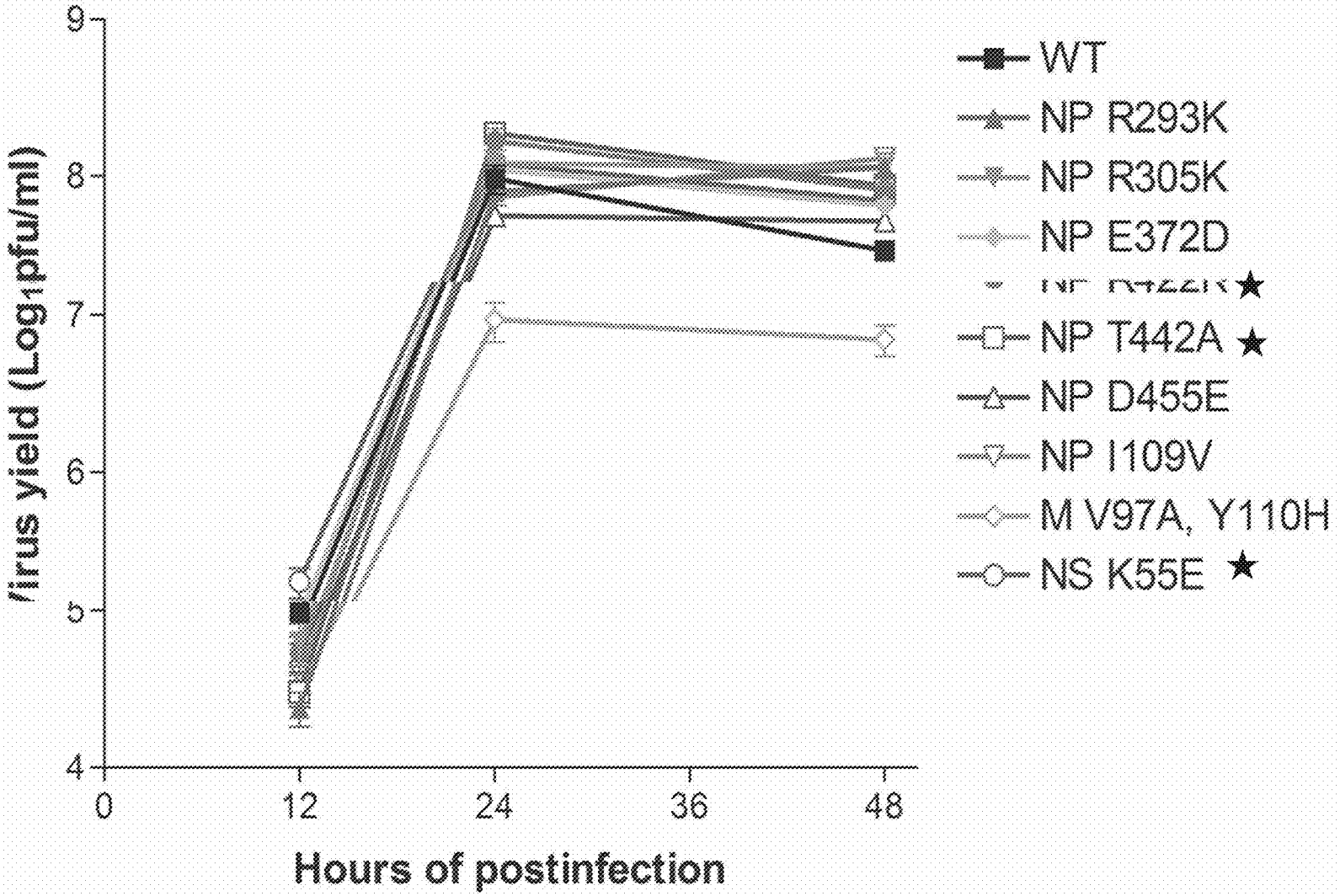


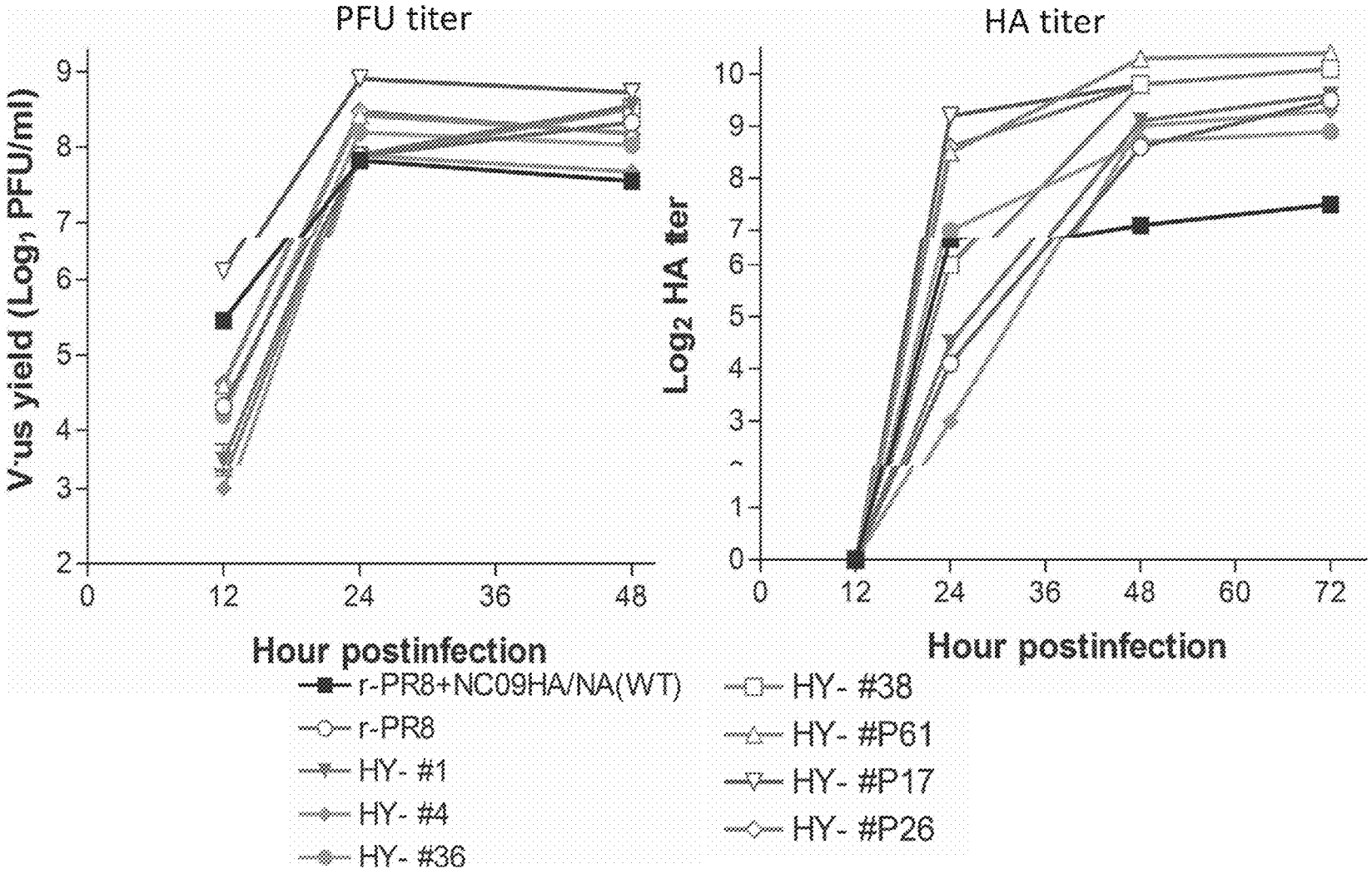
Figure 6 Confirmed high replicative mutations

Gene	Screened from viruses libraries	Described in literature
PB2	M202L F323L, I504V, M66R	A44S, E158G, E158A, D236N, D256G, R368K, E391Q, I504V, Q591K, V613T, A661T, D701N, D701N S714R
PB1	M507V V644A, V644A, R54I, Q247H, E112G, M40I G180W, I667T M714T	R327K, V336I, L473V L598P
PB1 F2	-	N66S, K73R, V76A, R79Q, L82S, E87Q
PA	F105C, R401K	T97I, K142N, S225C, S149PP T357K, K356R, A404S, S421I
NP	R293M, I116L, N224I, R74K, R74K N417D,	R293K, R305K, E372D, R422K, T442A, D455E, I109V, N101G, N319K
M	P90S	V97A, Y100H, V97A Y100H
NS	A30P, T49A, R140Q, S161T, A223E	K55E

Figure 7A Recombinant viruses generated by RGS

Virus #	Gene backbone								Virus stock titer	
	HA	NA	PB2	PB1	PA	NP	M	NS	2^n	Pfu/ml
wt	Inda/NC/09 delHA	Indo/NC/09 NA	wt	wt	wt	wt	wt	wt	7	3.0E+07
1			M202L F323L	M507V V644A		I116L		K55E	9~9.5	2.0E+08
4			M202L F323L	M507V V644A	K356R	T442A	V97A Y100H	K55E	10~10.5	1.6E+08
36			I504V	E112G	I550L	I112L	Y100H	R140Q	9.5	1.3E+08
38			M202L F323L	M507V V644A		I116L	Y100H	K55E	10~10.5	2.3E+08
HY-#17			I504V	E112G	S225C	R74K N417D	V97A Y100H	K55E	9.5~10	5.8E+08
HY-#61			M202L F323L	Q247H	K142N	R74K	V97A Y100H	K55E	10~10.5	2.0E+08
HY-#26			M202L F323L	M40L G180W	S225C	R422K	V97A Y100H	K55E	10	3.0E+08

Figure 7B Growth characteristics (MOI=0.001)



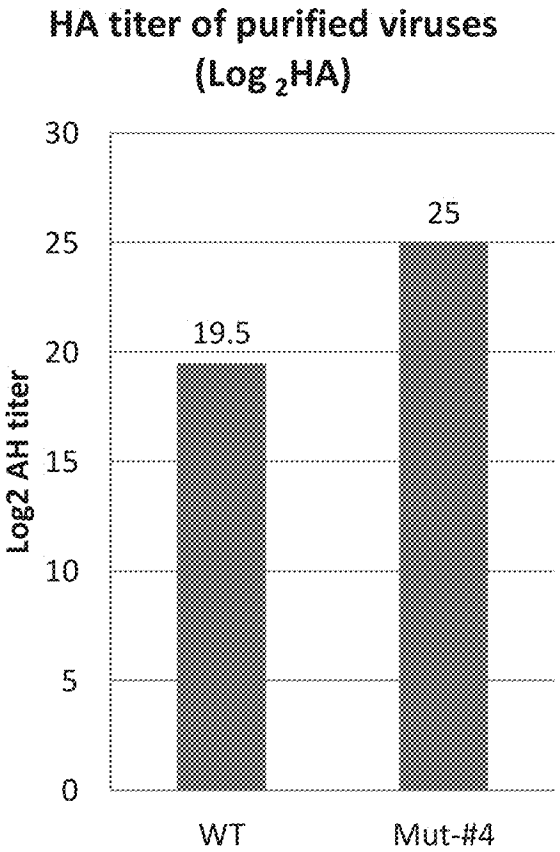


FIG. 8A

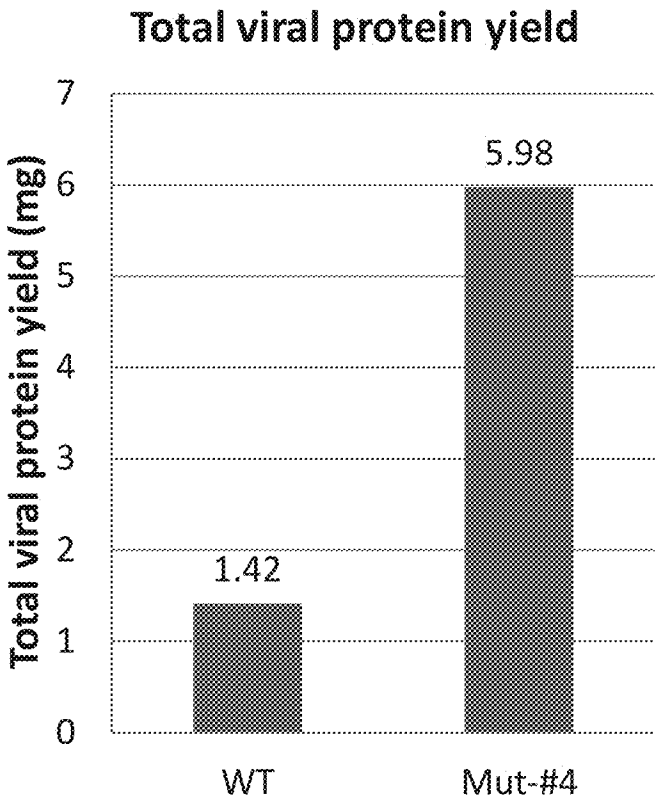
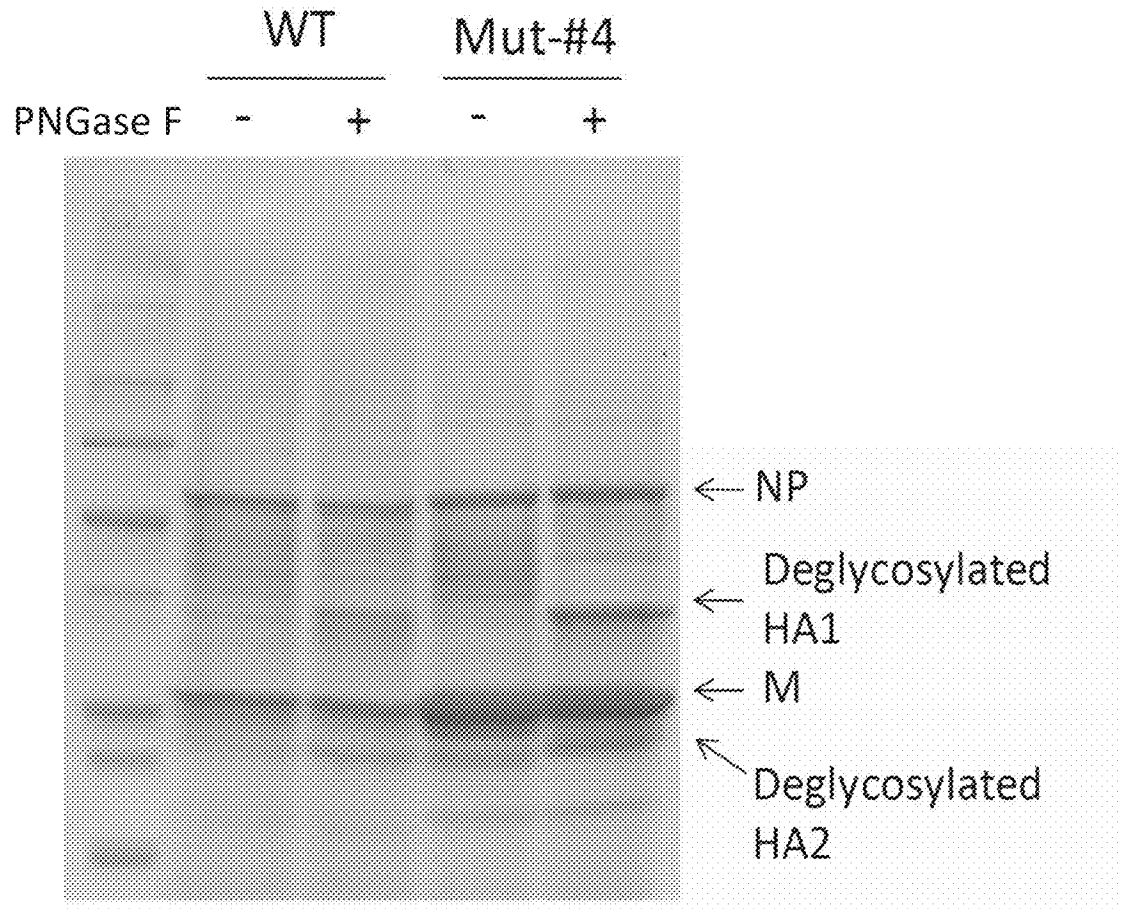


FIG. 8B

Total viral protein yield: 4.2 fold

Figure 8C SDS-PAGE analysis

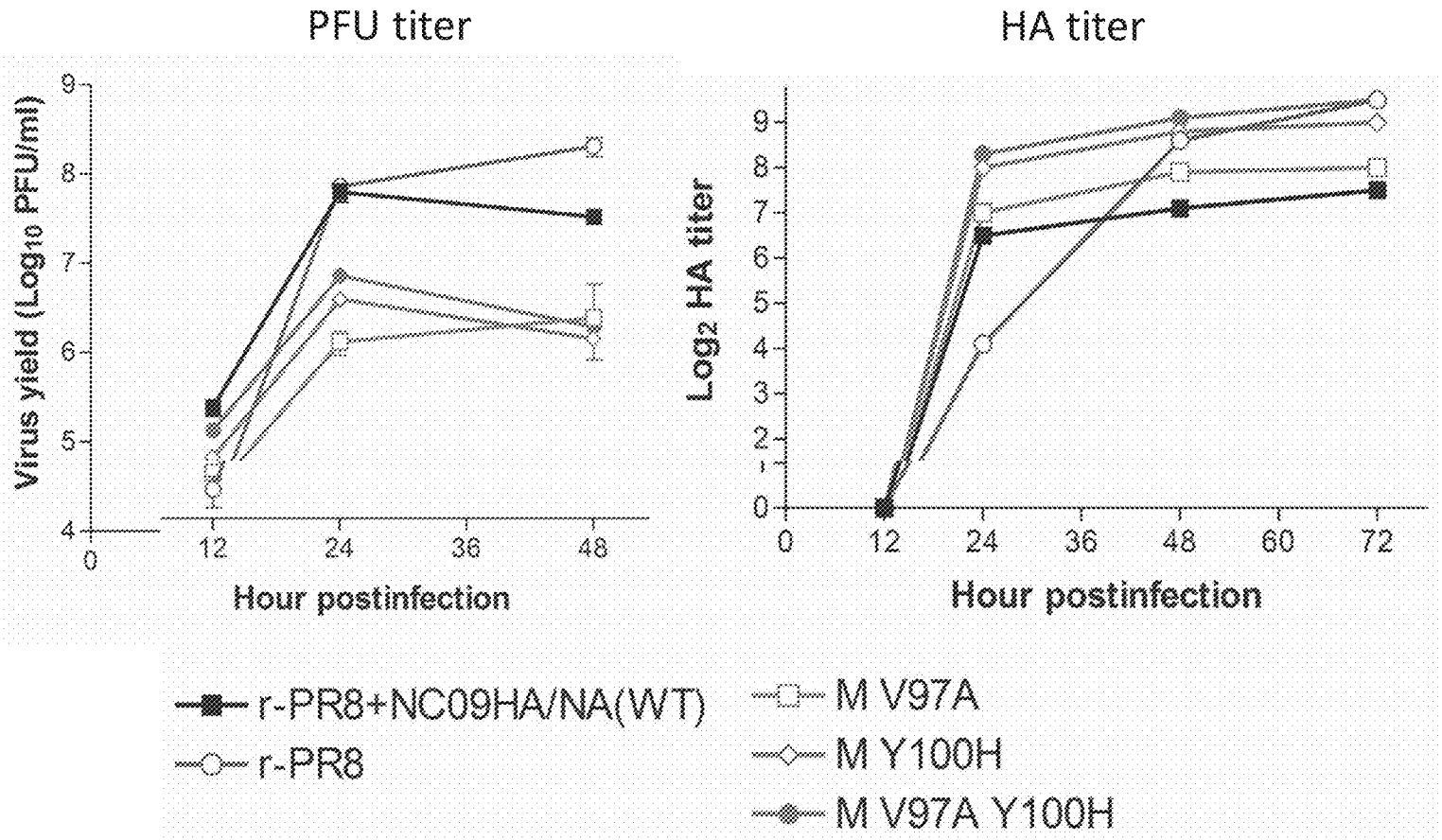


Mutant virus generates much more M1 and HA1 proteins than wild type.

Figure 9A Wild type VS. mutant

#	Gene backbone								Virus stock titer	
	HA	NA	PB2	PB1	PA	NP	M	NS	HA titer (2^n)	Pfu/ml
WT	Indo/NC/09 delHA	Indo/NC/09 NA	PR8-wt	PR8-wt	PR8-wt	PR8-wt	PR8-wt	PR8-wt	7	3.0E+07
4			M202L F323L	M507V V644A	K356R	T442A	V97A Y100H	K55E	10~10.5	1.6E+08

Figure 9B Growth kinetics (MOI=0.001)



CANIS FAMILIARIS [gbmam]: 1194 CDS's (559501 CODONS)

FIELDS: [TRIPLET] [AMINO ACID] [FRACTION] [FREQUENCY: PER THOUSAND] ([NUMBER])

UUU	F	0.41	17.1	(9540)	UCU	S	0.18	13.8	(7723)	UAU	Y	0.40	11.5	(6456)	UGU	C	0.42	10.1	(5665)
UUC	F	0.59	24.4	(13671)	UCC	S	0.24	18.4	(10299)	UAC	Y	0.60	17.5	(9786)	UGC	C	0.58	13.8	(7723)
UUA	L	0.06	5.8	(3270)	UCA	S	0.13	9.8	(5487)	UAA	*	0.27	0.6	(325)	UGA	*	0.53	1.1	(642)
UUG	L	0.12	11.8	(6627)	UCG	S	0.06	4.6	(2584)	UAG	*	0.21	0.5	(254)	UGG	W	1.00	13.8	(7704)
CUU	L	0.12	11.7	(6523)	CCU	P	0.27	15.6	(8713)	CAU	H	0.39	9.0	(5039)	CGU	R	0.07	3.9	(2163)
CUC	L	0.22	21.8	(12224)	CCC	P	0.35	20.4	(11422)	CAC	H	0.61	14.1	(7888)	CGC	R	0.20	10.6	(5943)
CUA	L	0.06	6.5	(3644)	CCA	P	0.25	14.6	(8157)	CAA	Q	0.25	11.0	(6149)	CGA	R	0.11	5.6	(3155)
CUG	L	0.43	42.8	(23966)	CCG	P	0.12	7.0	(3892)	CAG	Q	0.75	32.6	(18244)	CGG	R	0.21	11.0	(6132)
AUU	I	0.32	15.5	(8662)	ACU	T	0.22	12.3	(6886)	AAU	N	0.43	16.5	(9253)	AGU	S	0.14	10.8	(6029)
AUC	I	0.53	25.7	(14391)	ACC	T	0.39	21.4	(11979)	AAC	N	0.57	21.6	(12104)	AGC	S	0.25	18.9	(10595)
AUA	I	0.15	7.2	(4017)	ACA	T	0.26	14.2	(7972)	AAA	K	0.40	22.2	(12410)	AGA	R	0.20	10.5	(5847)
AUG	M	1.00	22.7	(12717)	ACG	T	0.13	7.2	(4005)	AAG	K	0.60	33.9	(18967)	AGG	R	0.21	11.1	(6228)
GUU	V	0.14	9.3	(5189)	GCU	A	0.25	17.2	(9609)	GAU	D	0.43	19.7	(11012)	GGU	G	0.16	11.3	(6298)
GUC	V	0.27	17.2	(9607)	GCC	A	0.44	30.3	(16927)	GAC	D	0.57	26.2	(14655)	GGC	G	0.35	24.2	(13513)
GUA	V	0.10	6.5	(3660)	GCA	A	0.20	13.7	(7651)	GAA	E	0.40	26.4	(14776)	GGA	G	0.24	16.9	(9465)
GUG	V	0.48	31.0	(17366)	GCG	A	0.11	7.9	(4431)	GAG	E	0.60	40.3	(22552)	GGG	G	0.25	17.4	(9718)

CODING GC 53.16% 1ST LETTER GC 55.35% 2ND LETTER GC 41.92% 3RD LETTER GC 62.22%

GENETIC CODE 1: STANDARD

Fig. 10A

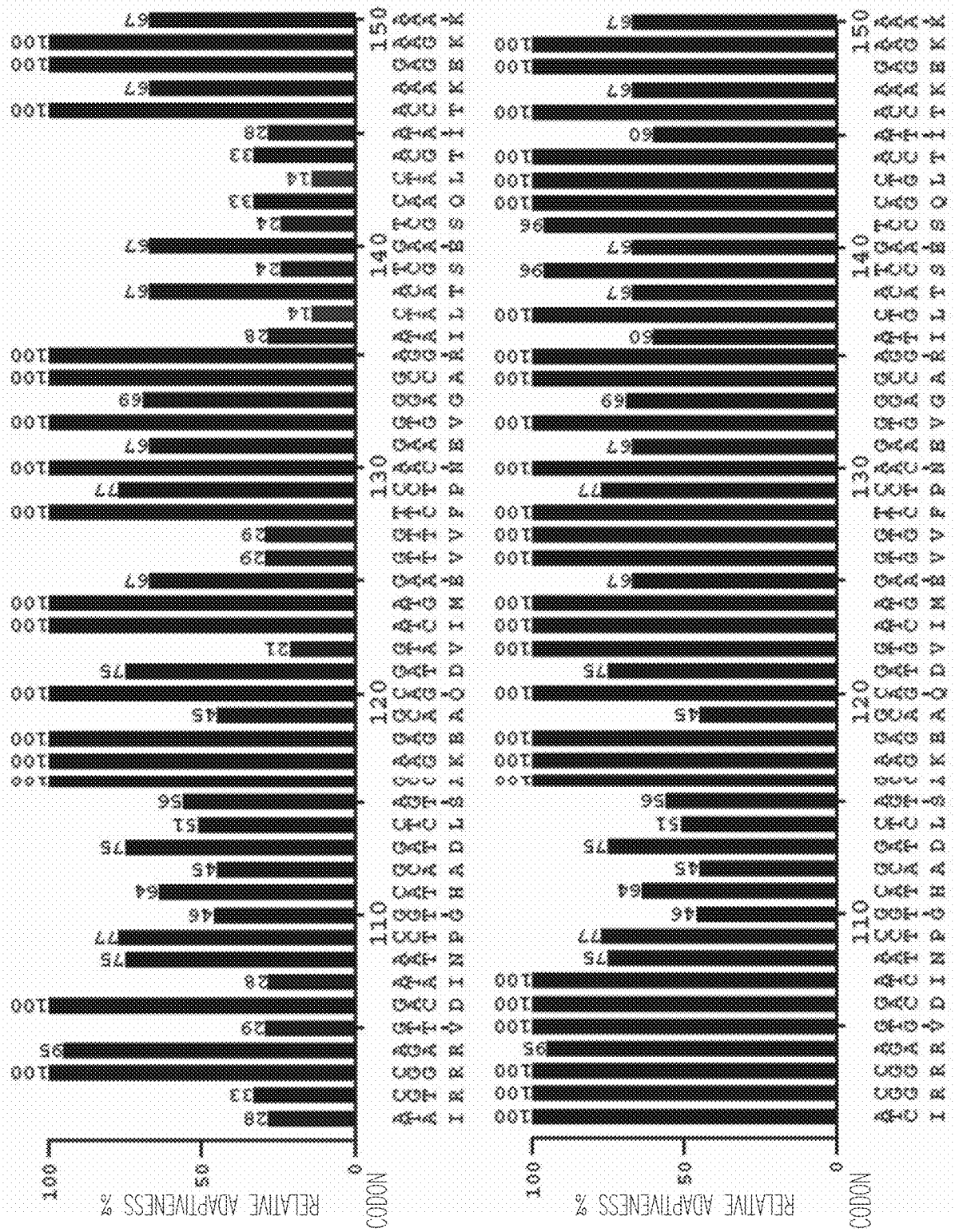

PR8-PB2wt:
BEFORE
OPTIMIZATION
RELATIVE ADAPTIVENESS %
CODON
TYPE 1:
ONLY MODIFY
RARE CODONS
RELATIVE ADAPTIVENESS %
CODON

Fig. 10B

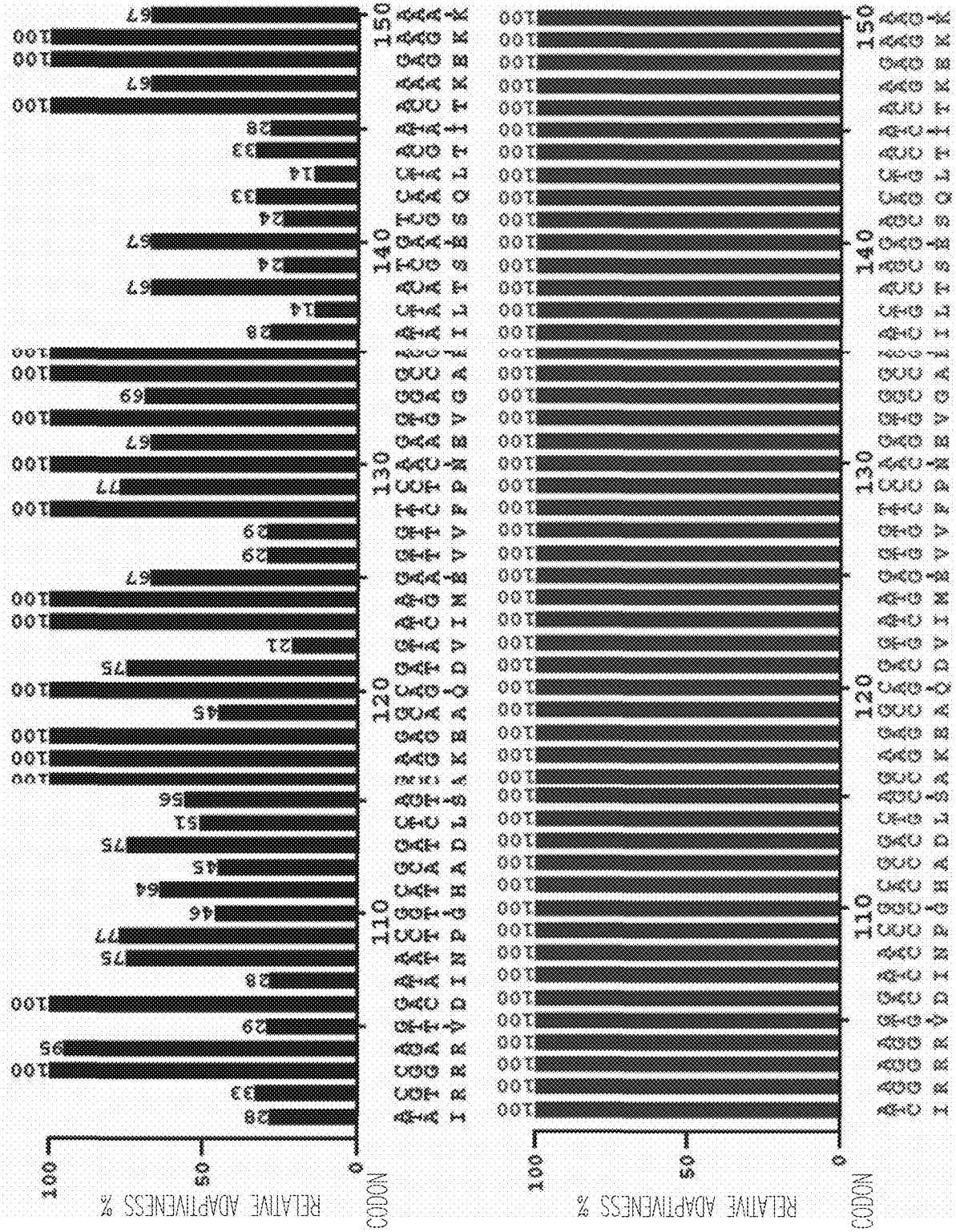
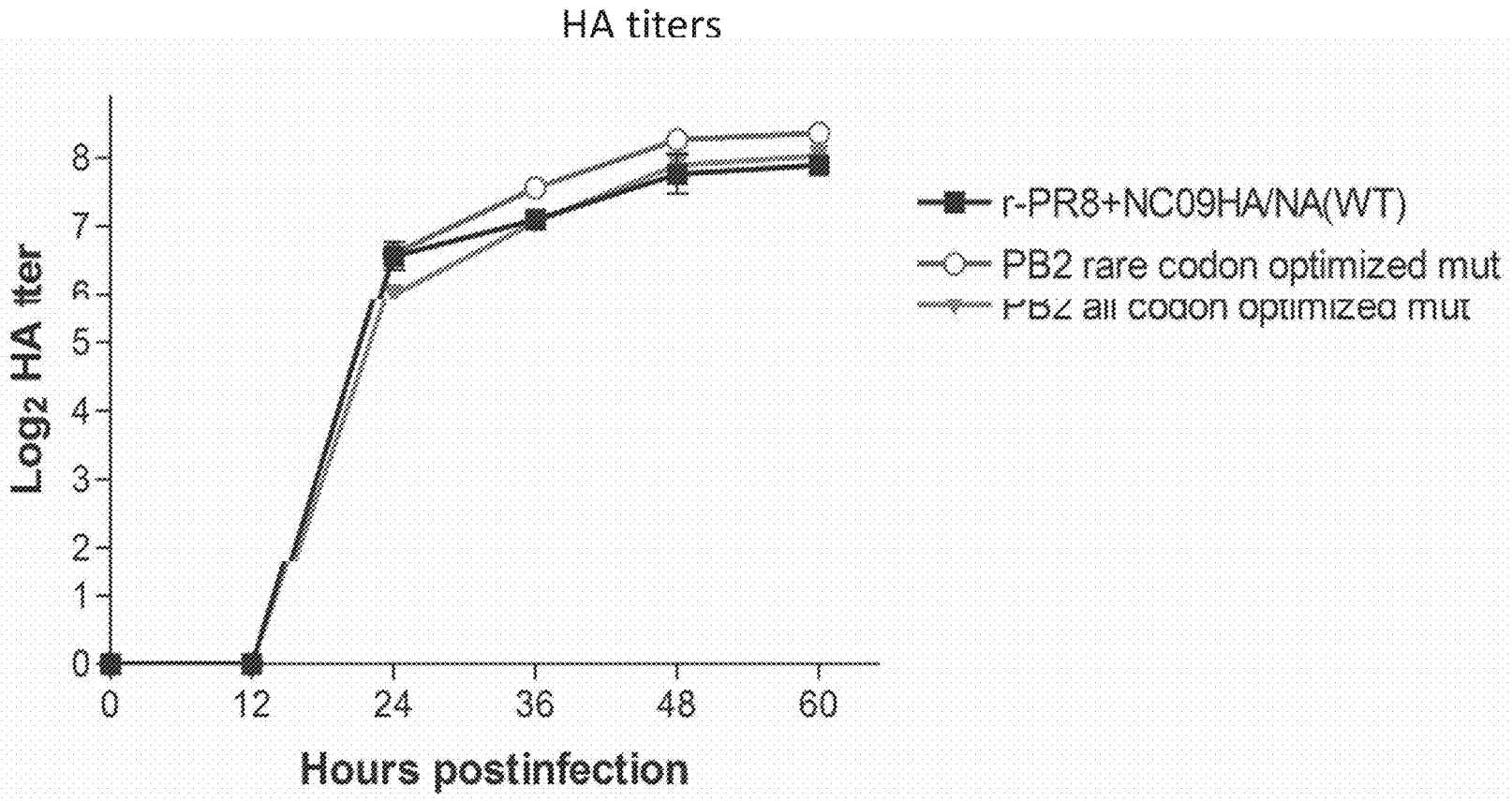

PR8-PB2wt
BEFORE
OPTIMIZATION
TYPE 2:
MODIFY ALL
CODONS
RELATIVE ADAPTIVENESS %
CODON

Fig. 10C

Figure 10D Growth kinetics in MDCK cells



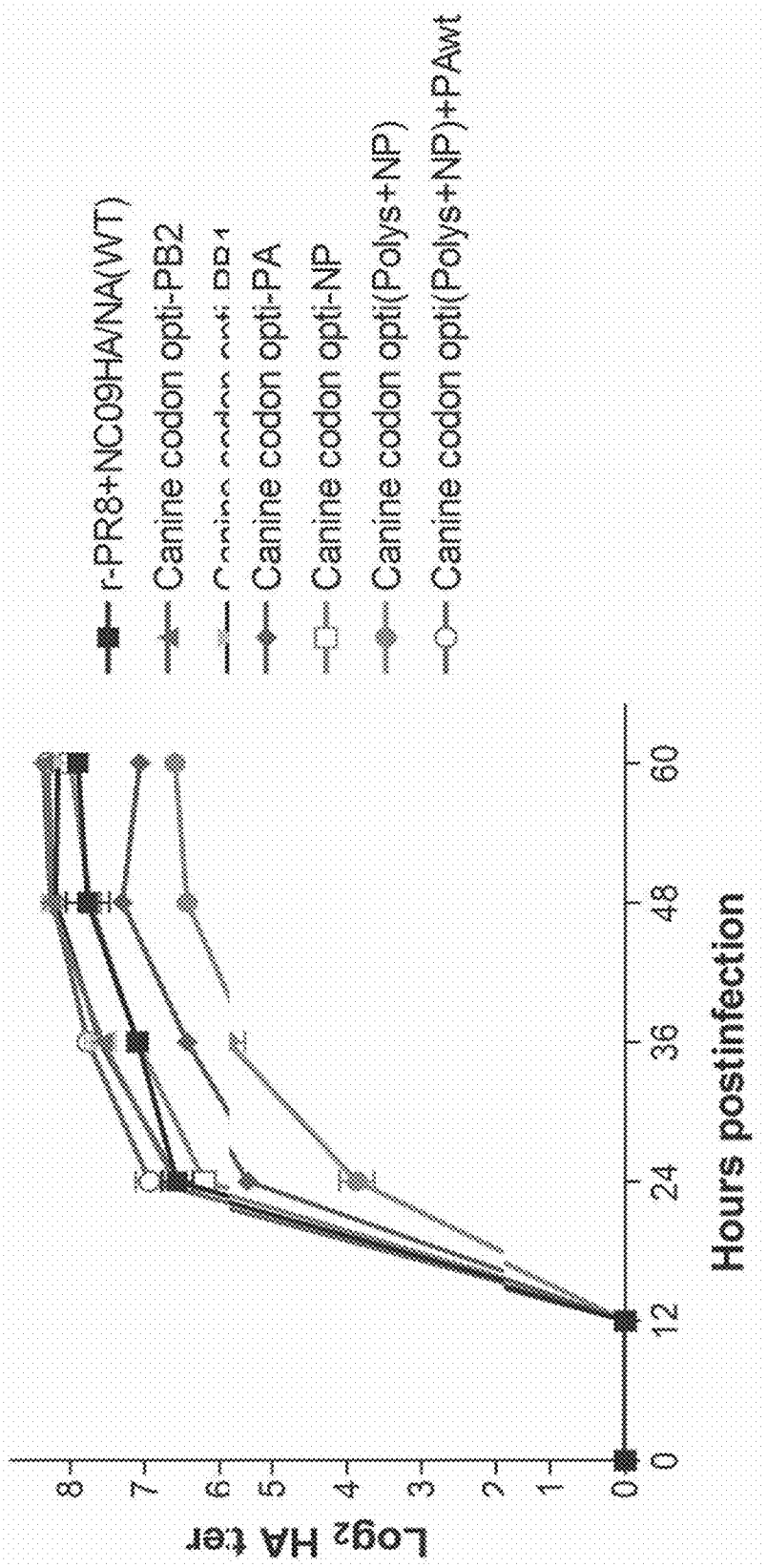

r-PR8+NC09HA/NA(WT)
Canine codon opti-PB2
Canine codon opti-PB1
Canine codon opti-PA
Canine codon opti-NP
Canine codon opti(Polys+NP)
Canine codon opti(Polys+NP)+PAwt
Log2 HA titer
0
1
2
3
4
5
6
7
8
0
12
24
36
48
60
Hours postinfection

Figure 10E

PR8-UW PB2:

AGCGAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAACTACGAAATCTAATGTCGCAGTCTCGCACCCGCGA

GATACTCACAAAAACCACCGTGGACCATATGGCCATAATCAAGAAGTACACATCAGGAAGACAGGAGAAGAACCCAGCAC

TTAGGATGAAATGGATGATGGCAATGAAATATCCAATTACAGCAGACAAGAGGATAACGGAAATGATTCCTGAGAGAAAT

GAGCAAGGACAAACTTTATGGAGTAAAATGAATGATGCCGGATCAGACCGAGTGATGGTATCACCTCTGGCTGTGACATG

GTGGAATAGGAATGGACCAATAACAAATACAGTTCATTATCCAAAAATCTACAAAACTTATTTTGAAAGAGTCGAAAGGC

TAAAGCATGGAACCTTTGGCCCTGTCCATTTTAGAAACCAAGTCAAAATACGTCGGAGAGTTGACATAAATCCTGGTCAT

GCAGATCTCAGTGCCAAGGAGGCACAGGATGTAATCATGGAAGTTGTTTTCCCTAACGAAGTGGGAGCCAGGATACTAAC

ATCGGAATCGCAACTAACGATAACCAAAGAGAAGAAAGAAGAACTCCAGGATTGCAAAATTTCTCCTTTGATGGTTGCAT

ACATGTTGGAGAGAGAACTGGTCCGCAAAACGAGATTCCTCCCAGTGGCTGGTGGAACAAGCAGTGTGTACATTGAAGTG

TTGCATTTGACTCAAGGAACATGCTGGGAACAGATGTATACTCCAGGAGGGGAAGTGAGGAATGATGATGTTGATCAAAG

CTTGATTATTGCTGCTAGGAACATAGTGAGAAGAGCTGCAGTATCAGCAGATCCACTAGCATCTTTATTGGAGATGTGCC

ACAGCACACAGATTGGTGGAATTAGGATGGTAGACATCCTTAGGCAGAACCCAACAGAAGAGCAAGCCGTGGATATATGC

AAGGCTGCAATGGGACTGAGAATTAGCTCATCCTTCAGTTTTGGTGGATTCACATTTAAGAGAACAAGCGGATCATCAGT

CAAGAGAGAGGAAGAGGTGCTTACGGGCAATCTTCAAACATTGAAGATAAGAGTGCATGAGGGATATGAAGAGTTCACAA

TGGTTGGGAGAAGAGCAACAGCCATACTCAGAAAAGCAACCAGGAGATTGATTCAGCTGATAGTGAGTGGGAGAGACGAA

CAGTCGATTGCCGAAGCAATAATTGTGGCCATGGTATTTTCACAAGAGGATTGTATGATAAAAGCAGTCAGAGGTGATCT

GAATTTCGTCAATAGGGCGAATCAACGATTGAATCCTATGCATCAACTTTTAAGACATTTTCAGAAGGATGCGAAAGTGC

TTTTTCAAAATTGGGGAGTTGAACCTATCGACAATGTGATGGGAATGATTGGGATATTGCCCGACATGACTCCAAGCATC

GAGATGTCAATGAGAGGAGTGAGAATCAGCAAAATGGGTGTAGATGAGTACTCCAGCACGGAGAGGGTAGTGGTGAGCAT

TGACCGTTTTTTTGAGAATCCGGGACCAACGAGGAAATGTACTACTGTCTCCCGAGGAGGTCAGTGAAACACAGGGAACAG

AGAAACTGACAATAACTTACTCATCGTCAATGATGTGGGAGATTAATGGTCCTGAATCAGTGTTGGTCAATACCTATCAA

TGGATCATCAGAAACTGGGAAACTGTTAAAATTCAGTGGTCCCAGAACCCTACAATGCTATACAATAAAATGGAATTTGA

ACCATTTCAGTCTTTAGTACCTAAGGCCATTAGAGGCCAATACAGTGGGTTTGTAAGAACTCTGTTCCAACAAATGAGGG

ATGTGCTTGGGACATTTGATACCGCACAGATAATAAAACTTCTTCCCTTCGCAGCCGCTCCACCAAAGCAAAGTAGAATG

CAGTTCTCCTCATTTACTGTGAATGTGAGGGGATCAGGAATGAGAATACTTGTAAGGGGCAATTCTCCTGTATTCAACTA

TAACAAGGCCACGAAGAGACTCACAGTTCTCGGAAAGGATGCTGGCACTTTAACTGAAGACCCAGATGAAGGCACAGCTG

GAGTGGAGTCCGCTGTTCTGAGGGGATTCCTCATTCTGGGCAAAGAAGACAAGAGATATGGGCCAGCACTAAGCATCAAT

GAACTGAGCAACCTTGCGAAAGGAGAGAAGGCTAATGTGCTAATTGGGCAAGGAGACGTGGTGTTGGTAATGAAACGGAA

ACGGGACTCTAGCATACTTACTGACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTCGAATAGTTT

AAAAACGACCTTGTTTCTACT (SEQ ID NO:3)

FIG. 10F

Canine codon optimized PR8-PB2:

AGCGAAAGCAGGTCAATTATATTCAATATGGAAAGAATAAAAGAACTACGAAATCTAATGTCGCAGTCTCGCACCCGCGA
GATACTCACAAAAACCACCGTGGACCATATGGCCATAATCAAGAAGTACACATCAGGAAGACAGGAGAAGAACCCAGCAC
TGAGGATGAAATGGATGATGGCAATGAAATATCCAATTACAGCAGACAAGAGGATCACCGAAATGATTCCTGAGAGAAAT
GAGCAGGGACAGACTCTGTGGAGTAAAATGAATGATGCCGGATCAGACCGAGTGATGGTGTCACCTCTGGCTGTGACATG
GTGGAATAGGAATGGACCAATCACAAATACAGTGCATTATCCAAAAATCTACAAAACTTATTTTGAAAGAGTCGAAAGGC
TGAAGCATGGAACCTTTGGCCCTGTCCATTTTAGAAACCAGGTCAAAATCCGGCGGAGAGTGGACATCAATCCTGGTCAT
GCAGATCTCAGTGCCAAGGAGGCACAGGATGTGATCATGGAAGTGGTGTTCCCTAACGAAGTGGGAGCCAGGATTCTGAC
ATCCGAATCCCAGCTGACCATTACCAAAGAGAAGAAAGAAGAACTCCAGGATTGCAAAATTTCTCCTCTGATGGTGGCAT
ACATGCTGGAGAGAGAACTGGTCCGCAAAACAAGATTCCTCCCAGTGGCTGGTGGAACAAGCAGTGTGTACATTGAAGTG
CTGCATCTGACTCAGGGAACATGCTGGGAACAGATGTATACTCCAGGAGGGGAAGTGAGGAATGATGATGTGGATCAGAG
CCTGATTATTGCTGCTAGGAACATTGTGAGAAGAGCTGCAGTGTCAGCAGATCCACTGGCATCTCTGCTGGAGATGTGCC
ACAGCACACAGATTGGTGGAATTAGGATGGTGGACATCCTGAGGCAGAACCCAACAGAAGAGCAGGCCGTGGATATTTGC
AAGGCTGCAATGGGACTGAGAATTAGCTCATCCTTCAGTTTTGGTGGATTCACATTTAAGAGAACAAGCGGATCATCAGT
CAAGAGAGAGGAAGAGGTGCTGACCGGCAATCTGCAGACACTGAAGATCAGAGTGCATGAGGGATATGAAGAGTTCACAA
TGGTGGGGAGAAGAGCAACAGCCATCCTCAGAAAAGCAACCAGGAGACTGATTCAGCTGATCGTGAGTGGGAGAGACGAA
CAGTCCATTGCCGAAGCAATTATTGTGGCCATGGTGTTTTCACAGGAGGATTGTATGATTAAAGCAGTCAGAGGTGATCT
GAATTTCGTCAATAGGGCCAATCAGCGACTGAATCCTATGCATCAGCTGCTGAGACATTTTCAGAAGGATGCCAAAGTGC
TGTTTCAGAATTGGGGAGTGGAACCTATCGACAATGTGATGGGAATGATTGGGATCCTGCCCGACATGACTCCAAGCATC
GAGATGTCAATGAGAGGAGTGAGAATCAGCAAAATGGGTGTGGATGAGTACTCCAGCACCGAGAGGGTCGTGGTGAGCAT
TGACAGATTTCTGAGAATCCGGGACCAGCGAGGAAATGTGCTCCTGTCTCCCGAGGAGGTCAGTGAAACACAGGGAACAG
AGAAACTGACAATTACTTACTCATCCTCAATGATGTGGGAGATTAATGGTCCTGAATCAGTGCTGGTCAATACCTATCAG
TGGATCATCAGAAACTGGGAAACTGTGAAAATTCAGTGGTCCCAGAACCCTACAATGCTGTACAATAAAATGGAATTTGA
ACCATTTCAGTCTCTGGTGCCTAAGGCCATTAGAGGCCAGTACAGTGGGTTTGTGAGAACTCTGTTCCAGCAGATGAGGG
ATGTGCTGGGGACATTTGATACCGCACAGATTATTAAACTGCTGCCCTTCGCAGCCGCTCCACCAAAGCAGAGTAGAATG
CAGTTCTCCTCATTTACTGTGAATGTGAGGGGATCAGGAATGAGAATCCTGGTGAGGGGCAATTCTCCTGTGTTCAACTA
TAACAAGGCCACCAAGAGACTCACAGTGCTCGGAAAGGATGCTGGCACTCTGACTGAAGACCCAGATGAAGGCACAGCTG
GAGTGGAGTCCGCTGTGCTGAGGGGATTCCTCATTCTGGGCAAAGAAGACAAGAGATATGGGCCAGCACTGAGCATCAAT
GAACTGAGCAACCTGGCCAAAGGAGAGAAGGCTAATGTGCTAATTGGGCAAGGAGACGTGGTGTTGGTAATGAAACGGAA
ACGGGACTCTAGCATACTTACTGACAGCCAGACAGCGACCAAAAGAATTCGGATGGCCATCAATTAGTGTCGAATAGTTT
AAAAACGACCTTGTTTCTACT (SEQ ID NO:16)

FIG. 10G

PR8-UW PB1:

AGCGAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACCTTACTTTTCTTAAAAGTGCCAGCACAAAATGCTATAAG
CACAACTTTCCCTTATACTGGAGACCCTCCTTACAGCCATGGGACAGGAACAGGATACACCATGGATACTGTCAACAGGA
CACATCAGTACTCAGAAAAGGGAAGATGGACAACAAACACCGAAACTGGAGCACCGCAACTCAACCCGATTGATGGGCCA
CTGCCAGAAGACAATGAACCAAGTGGTTATGCCCAAACAGATTGTGTATTGGAGGCGATGGCTTTCCTTGAGGAATCCCA
TCCTGGTATTTTTGAAAACTCGTGTATTGAAACGATGGAGGTTGTTCAGCAAACACGAGTAGACAAGCTGACACAAGGCC
GACAGACCTATGACTGGACTCTAAATAGAAACCAACCTGCTGCAACAGCATTGGCCAACACAATAGAAGTGTTCAGATCA
AATGGCCTCACGGCCAATGAGTCTGGAAGGCTCATAGACTTCCTTAAGGATGTAATGGAGTCAATGAACAAAGAAGAAAT
GGGGATCACAACTCATTTTCAGAGAAAGAGACGGGTGAGAGACAATATGACTAAGAAAATGATAACACAGAGAACAATGG
GTAAAAAGAAGCAGAGATTGAACAAAAGGAGTTATCTAATTAGAGCATTGACCCTGAACACAATGACCAAAGATGCTGAG
AGAGGGAAGCTAAAACGGAGAGCAATTGCAACCCCAGGGATGCAAATAAGGGGGTTTGTATACTTTGTTGAGACACTGGC
AAGGAGTATATGTGAGAAACTTGAACAATCAGGGTTGCCAGTTGGAGGCAATGAGAAGAAAGCAAAGTTGGCAAATGTTG
TAAGGAAGATGATGACCAATTCTCAGGACACCGAACTTTCTTTCACCATCACTGGAGATAACACCAAATGGAACGAAAAT
CAGAATCCTCGGATGTTTTTGGCCATGATCACATATATGACCAGAAATCAGCCCGAATGGTTCAGAAATGTTCTAAGTAT
TGCTCCAATAATGTTCTCAAACAAAATGGCGAGACTGGGAAAAGGGTATATGTTTGAGAGCAAGAGTATGAAACTTAGAA
CTCAAATACCTGCAGAAATGCTAGCAAGCATCGATTTGAAATATTTCAATGATTCAACAAGAAAGAAGATTGAAAAAATC
CGACCGCTCTTAATAGAGGGGACTGCATCATTGAGCCCTGGAATGATGATGGGCATGTTCAATATGTTAAGCACTGTATT
AGGCGTCTCCATCCTGAATCTTGGACAAAAGAGATACACCAAGACTACTTACTGGTGGGATGGTCTTCAATCCTCTGACG
ATTTTGCTCTGATTGTGAATGCACCCAATCATGAAGGGATTCAAGCCGGAGTCGACAGGTTTTATCGAACCTGTAAGCTA
CTTGGAATCAATATGAGCAAGAAAAAGTCTTACATAAACAGAACAGGTACATTTGAATTCACAAGTTTTTTCTATCGTTA
TGGGTTTGTTGCCAATTTCAGCATGGAGCTTCCCAGTTTTGGGGTGTCTGGGATCAACGAGTCAGCGGACATGAGTATTG
GAGTTACTGTCATCAAAAACAATATGATAAACAATGATCTTGGTCCAGCAACAGCTCAAATGGCCCTTCAGTTGTTCATC
AAAGATTACAGGTACACGTACCGATGCCATATAGGTGACACACAAATACAAACCCGAAGATCATTTGAAATAAAGAAACT
GTGGGAGCAAACCCGTTCCAAAGCTGGACTGCTGGTCTCCGACGGAGGCCCAAATTTATACAACATTAGAAATCTCCACA
TTCCTGAAGTCTGCCTAAAATGGGAATTGATGGATGAGGATTACCAGGGGCGTTTATGCAACCCACTGAACCCATTTGTC
AGCCATAAAGAAATTGAATCAATGAACAATGCAGTGATGATGCCAGCACATGGTCCAGCCAAAAACATGGAGTATGATGC
TGTTGCAACAACACACTCCTGGATCCCCAAAAGAAATCGATCCATCTTGAATACAAGTCAAAGAGGAGTACTTGAGGATG
AACAAATGTACCAAAGGTGCTGCAATTTATTTGAAAAATTCTTCCCCAGCAGTTCATACAGAAGACCAGTCGGGATATCC
AGTATGGTGGAGGCTATGGTTTCCAGAGCCCGAATTGATGCACGGATTGATTTCGAATCTGGAAGGATAAAGAAAGAAGA
GTTCACTGAGATCATGAAGATCTGTTCCACCATTGAAGAGCTCAGACGGCAAAAATAGTGAATTTAGCTTGTCCTTCATG
AAAAAATGCCTTGTTTCTACT (SEQ ID NO:2)

FIG. 10H

Canine codon optimized PR8 PB1:

AGCGAAAGCAGGCAAACCATTTGAATGGATGTCAATCCGACCTTACTTTTCTTAAAAGTGCCAGCACAAAATGCTATAAG
CACAACTTTCCCTTATACTGGAGACCCTCCTTACAGCCATGGGACAGGAACAGGATACACCATGGATACTGTCAACAGGA
CACATCAGTACTCAGAAAAGGGAAGATGGACAACAAACACCGAAACTGGAGCACCGCAACTCAACCCGATTGATGGGCCA
CTGCCAGAAGACAATGAACCAAGTGGTTATGCCCAAACAGATTGTGTATTGGAGGCGATGGCTTTCCTTGAGGAATCCCA
TCCTGGTATTTTTGAAAACTCGTGTATTGAAACGATGGAGGTTGTTCAGCAAACACGAGTGGACAAGCTGACACAGGGCC
GACAGACCTATGACTGGACTCTGAATAGAAACCAGCCTGCTGCAACAGCACTGGCCAACACAATCGAAGTGTTCAGATCA
AATGGCCTCACCGCCAATGAGTCTGGAAGGCTCATCGACTTCCTGAAGGATGTGATGGAGTCAATGAACAAAGAAGAAAT
GGGGATCACAACTCATTTTCAGAGAAAGAGACGGGTGAGAGACAATATGACTAAGAAAATGATTACACAGAGAACAATGG
GTAAAAAGAAGCAGAGACTGAACAAAAGGAGTTATCTGATTAGAGCACTGACCCTGAACACAATGACCAAAGATGCTGAG
AGAGGGAAGCTGAAACGGAGAGCAATTGCAACCCCAGGGATGCAGATTAGGGGGTTTGTGTACTTTGTGGAGACACTGGC
AAGGAGTATTTGTGAGAAACTGGAACAGTCAGGGCTGCCAGTGGGAGGCAATGAGAAGAAAGCAAAGCTGGCAAATGTGG
TGAGGAAGATGATGACCAATTCTCAGGACACCGAACTGTCTTTCACCATCACTGGAGATAACACCAAATGGAACGAAAAT
CAGAATCCTCGGATGTTTCTGGCCATGATCACATATATGACCAGAAATCAGCCCGAATGGTTCAGAAATGTGCTGAGTAT
TGCTCCAATTATGTTCTCAAACAAAATGGCCAGACTGGGAAAAGGGTATATGTTTGAGAGCAAGAGTATGAAACTGAGAA
CTCAGATTCCTGCAGAAATGCTGGCAAGCATCGATCTGAAATATTTCAATGATTCAACAAGAAAGAAGATTGAAAAAATC
CGACCCCTCCTGATTGAGGGGACTGCATCACTGAGCCCTGGAATGATGATGGGCATGTTCAATATGCTGAGCACTGTGCT
GGGCGTCTCCATCCTGAATCTGGGACAGAAGAGATACACCAAGACTACTTACTGGTGGGATGGTCTGCAGTCCTCTGACG
ATTTTGCTCTGATTGTGAATGCACCCAATCATGAAGGGATTCAGGCCGGAGTCGACAGGTTTTATCGAACCTGTAAGCTG
CTGGGAATCAATATGAGCAAGAAAAAGTCTTACATCAACAGAACAGGTACATTTGAATTCACAAGTTTTTTCTATCGCTA
TGGGTTTGTGGCCAATTTCAGCATGGAGCTGCCCAGTTTTGGGGTGTCTGGGATCAACGAGTCAGCCGACATGAGTATTG
GAGTGACTGTCATCAAAAACAATATGATCAACAATGATCTGGGTCCAGCAACAGCTCAGATGGCCCTGCAGCTGTTCATC
AAAGATTACAGGTACACCTACCGATGCCATATCGGTGACACACAGATTCAGACCCGAAGATCATTTGAAATCAAGAAACT
GTGGGAGCAGACCCGCTCCAAAGCTGGACTGCTGGTCTCCGACGGAGGCCCAAATCTGTACAACATTAGAAATCTCCACA
TTCCTGAAGTCTGCCTGAAATGGGAACTGATGGATGAGGATTACCAGGGGCGCCTGTGCAACCCACTGAACCCATTTGTC
AGCCATAAAGAAATTGAATCAATGAACAATGCAGTGATGATGCCAGCACATGGTCCAGCCAAAAACATGGAGTATGATGC
TGTGGCAACAACACACTCCTGGATCCCCAAAAGAAATCGATCCATCCTGAATACAAGTCAGAGAGGAGTGCTGGAGGATG
AACAGATGTACCAGAGGTGCTGCAATCTGTTTGAAAAATTCTTCCCCAGCAGTTCATACAGAAGACCAGTCGGGATCTCC
AGTATGGTGGAGGCTATGGTGTCCAGAGCCCGAATTGATGCACGGATTGATTTCGAATCTGGAAGGATCAAGAAAGAAGA
GTTCACTGAGATCATGAAGATCTGTTCCACCATTGAAGAGCTCAGACGGCAAAAATAGTGAATTTAGCTTGTCCTTCATG
AAAAAATGCCTTGTTTCTACT (SEQ ID NO:17)

FIG. 10I

PR8-UW PA:

AGCGAAAGCAGGTACTGATCCAAAATGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGAGCTTGCGGAAAA
AACAATGAAAGAGTATGGGGAGGACCTGAAAATCGAAACAAACAAATTTGCAGCAATATGCACTCACTTGGAAGTATGCT
TCATGTATTCAGATTTTCACTTCATCAATGAGCAAGGCGAGTCAATAATCGTAGAACTTGGTGATCCAAATGCACTTTTG
AAGCACAGATTTGAAATAATCGAGGGAAGAGATCGCACAATGGCCTGGACAGTAGTAAACAGTATTTGCAACACTACAGG
GGCTGAGAAACCAAAGTTTCTACCAGATTTGTATGATTACAAGGAGAATAGATTCATCGAAATTGGAGTAACAAGGAGAG
AAGTTCACATATACTATCTGGAAAAGGCCAATAAAATTAAATCTGAGAAAACACACATCCACATTTTCTCGTTCACTGGG
GAAGAAATGGCCACAAAGGCAGACTACACTCTCGATGAAGAAAGCAGGGCTAGGATCAAAACCAGACTATTCACCATAAG
ACAAGAAATGGCCAGCAGAGGCCTCTGGGATTCCTTTCGTCAGTCCGAGAGAGGAGAAGAGACAATTGAAGAAAGGTTTG
AAATCACAGGAACAATGCGCAAGCTTGCCGACCAAAGTCTCCCGCCGAACTTCTCCAGCCTTGAAAATTTTAGAGCCTAT
GTGGATGGATTCGAACCGAACGGCTACATTGAGGGCAAGCTGTCTCAAATGTCCAAAGAAGTAAATGCTAGAATTGAACC
TTTTTTGAAAACAACACCACGACCACTTAGACTTCCGAATGGGCCTCCCTGTTCTCAGCGGTCCAAATTCCTGCTGATGG
ATGCCTTAAAATTAAGCATTGAGGACCCAAGTCATGAAGGAGAGGGAATACCGCTATATGATGCAATCAAATGCATGAGA
ACATTCTTTGGATGGAAGGAACCCAATGTTGTTAAACCACACGAAAAGGGAATAAATCCAAATTATCTTCTGTCATGGAA
GCAAGTACTGGCAGAACTGCAGGACATTGAGAATGAGGAGAAAATTCCAAAGACTAAAAATATGAAGAAAACAAGTCAGC
TAAAGTGGGCACTTGGTGAGAACATGGCACCAGAAAAGGTAGACTTTGACGACTGTAAAGATGTAGGTGATTTGAAGCAA
TATGATAGTGATGAACCAGAATTGAGGTCGCTTGCAAGTTGGATTCAGAATGAGTTTAACAAGGCATGCGAACTGACAGA
TTCAAGCTGGATAGAGCTCGATGAGATTGGAGAAGATGTGGCTCCAATTGAACACATTGCAAGCATGAGAAGGAATTATT
TCACATCAGAGGTGTCTCACTGCAGAGCCACAGAATACATAATGAAGGGAGTGTACATCAATACTGCCTTGCTTAATGCA
TCTTGTGCAGCAATGGATGATTTCCAATTAATTCCAATGATAAGCAAGTGTAGAACTAAGGAGGGAAGGCGAAAGACCAA
CTTGTATGGTTTCATCATAAAAGGAAGATCCCACTTAAGGAATGACACCGACGTGGTAAACTTTGTGAGCATGGAGTTTT
CTCTCACTGACCCAAGACTTGAACCACATAAATGGGAGAAGTACTGTGTTCTTGAGATAGGAGATATGCTTATAAGAAGT
GCCATAGGCCAGGTTTCAAGGCCCATGTTCTTGTATGTGAGAACAAATGGAACCTCAAAAATTAAAATGAAATGGGGAAT
GGAGATGAGGCGTTGCCTCCTCCAGTCACTTCAACAAATTGAGAGTATGATTGAAGCTGAGTCCTCTGTCAAAGAGAAAG
ACATGACCAAAGAGTTCTTTGAGAACAAATCAGAAACATGGCCCATTGGAGAGTCCCCCAAAGGAGTGGAGGAAAGTTCC
ATTGGGAAGGTCTGCAGGACTTTATTAGCAAAGTCGGTATTCAACAGCTTGTATGCATCTCCACAACTAGAAGGATTTTC
AGCTGAATCAAGAAAACTGCTTCTTATCGTTCAGGCTCTTAGGGACAACCTGGAACCTGGGACCTTTGATCTTGGGGGGC
TATATGAAGCAATTGAGGAGTGCCTGATTAATGATCCCTGGGTTTTGCTTAATGCTTCTTGGTTCAACTCCTTCCTTACA
CATGCATTGAGTTAGTTGTGGCAGTGCTACTATTTGCTATCCATACTGTCCAAAAAAGTACCTTGTTTCTACT (SEQ ID NO:1)

FIG. 10J

Canine codon optimized PR8 PA:

AGCGAAAGCAGGTACTGATCCAAAATGGAAGATTTTGTGCGACAATGCTTCAATCCGATGATTGTCGAGCTTGCGGAAAA
AACAATGAAAGAGTATGGGGAGGACCTGAAAATCGAAACAAACAAATTTGCAGCAATATGCACTCACTTGGAAGTATGCT
TCATGTATTCAGATTTTCACTTCATCAATGAGCAAGGCGAGTCAATAATCGTAGAACTTGGTGATCCAAATGCACTTTTG
AAGCACAGATTTGAAATAATCGAGGGAAGAGATCGCACAATGGCCTGGACAGTAGTAAACAGTATTTGCAACACTACAGG
GGCTGAGAAACCAAAGTTTCTACCAGATTTGTATGATTACAAGGAGAATAGATTCATCGAAATTGGAGTAACAAGGAGAG
AAGTTCACATATACTATCTGGAAAAGGCCAATAAAATTAAATCTGAGAAAACACACATCCACATTTTCTCGTTCACTGGG
GAAGAAATGGCCACAAAGGCAGACTACACTCTCGATGAAGAAAGCAGGGCTAGGATCAAAACCAGACTATTCACCATAAG
ACAAGAAATGGCCAGCAGAGGCCTCTGGGATTCCTTTCGTCAGTCCGAGAGAGGAGAAGAGACAATTGAAGAAAGGTTTG
AAATCACAGGAACAATGCGCAAGCTTGCCGACCAAAGTCTCCCGCCGAACTTCTCCAGCCTTGAAAATTTTAGAGCCTAT
GTGGATGGATTCGAACCGAACGGCTACATTGAGGGCAAGCTGTCTCAAATGTCCAAAGAAGTAAATGCTAGAATTGAACC
TTTTCTGAAAACAACACCACGACCACTGAGACTGCCCAATGGGCCTCCCTGTTCTCAGCGGTCCAAATTCCTGCTGATGG
ATGCCCTGAAACTGAGCATTGAGGACCCAAGTCATGAAGGAGAGGGAATTCCCCTGTATGATGCAATCAAATGCATGAGA
ACATTCTTTGGATGGAAGGAACCCAATGTGGTGAAACCACACGAAAAGGGAATCAATCCAAATTATCTGCTGTCATGGAA
GCAGGTGCTGGCAGAACTGCAGGACATTGAGAATGAGGAGAAAATTCCAAAGACTAAAAATATGAAGAAAACAAGTCAGC
TGAAGTGGGCACTGGGTGAGAACATGGCACCAGAAAAGGTGGACTTTGACGACTGTAAAGATGTGGGTGATCTGAAGCAG
TATGATAGTGATGAACCAGAACTGAGGTCCCTGGCAAGTTGGATTCAGAATGAGTTTAACAAGGCATGCGAACTGACAGA
TTCAAGCTGGATTGAGCTCGATGAGATTGGAGAAGATGTGGCTCCAATTGAACACATTGCAAGCATGAGAAGGAATTATT
TCACATCAGAGGTGTCTCACTGCAGAGCCACAGAATACATCATGAAGGGAGTGTACATCAATACTGCCCTGCTGAATGCA
TCTTGTGCAGCAATGGATGATTTCCAGCTGATTCCAATGATCAGCAAGTGTAGAACTAAGGAGGGAAGGCGAAAGACCAA
CCTGTATGGTTTCATCATCAAAGGAAGATCCCACCTGAGGAATGACACCGACGTGGTGAACTTTGTGAGCATGGAGTTTT
CTCTCACTGACCCAAGACTGGAACCACATAAATGGGAGAAGTACTGTGTGCTGGAGATTGGAGATATGCTGATCAGAAGT
GCCATTGGCCAGGTGTCAAGGCCCATGTTCCTGTATGTGAGAACAAATGGAACCTCAAAAATTAAAATGAAATGGGGAAT
GGAGATGAGGCGCTGCCTCCTCCAGTCACTGCAGCAGATTGAGAGTATGATTGAAGCTGAGTCCTCTGTCAAAGAGAAAG
ACATGACCAAAGAGTTCTTTGAGAACAAATCAGAAACATGGCCCATTGGAGAGTCCCCCAAAGGAGTGGAGGAAAGTTCC
ATTGGGAAGGTCTGCAGGACTCTGCTGGCAAAGTCCGTGTTCAACAGCCTGTATGCATCTCCACAGCTGGAAGGATTTTC
AGCTGAATCAAGAAAACTGCTGCTGATCGTGCAGGCTCTGAGGGACAACCTGGAACCTGGGACCTTTGATCTGGGGGGGC
TGTATGAAGCAATTGAGGAGTGCCTGATTAATGATCCCTGGGTGCTGCTGAATGCTTCTTGGTTCAACTCCTTCCTTACA
CATGCATTGAGTTAGTTGTGGCAGTGCTACTATTTGCTATCCATACTGTCCAAAAAAGTACCTTGTTTCTACT (SEQ ID NO:18)

FIG. 10K

PR8-UW NP:

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAATCATGGCGTCTCAAGGCACCAAACGATCTTACGAACA
GATGGAGACTGATGGAGAACGCCAGAATGCCACTGAAATCAGAGCATCCGTCGGAAAAATGATTGGTGGAATTGGACGAT
TCTACATCCAAATGTGCACCGAACTCAAACTCAGTGATTATGAGGGACGGTTGATCCAAAACAGCTTAACAATAGAGAGA
ATGGTGCTCTCTGCTTTTGACGAAAGGAGAAATAAATACCTTGAAGAACATCCCAGTGCGGGGAAAGATCCTAAGAAAAC
TGGAGGACCTATATACAGGAGAGTAAACGGAAAGTGGATGAGAGAACTCATCCTTTATGACAAAGAAGAAATAAGGCGAA
TCTGGCGCCAAGCTAATAATGGTGACGATGCAACGGCTGGTCTGACTCACATGATGATCTGGCATTCCAATTTGAATGAT
GCAACTTATCAGAGGACAAGAGCTCTTGTTCGCACCGGAATGGATCCCAGGATGTGCTCTCTGATGCAAGGTTCAACTCT
CCCTAGGAGGTCTGGAGCCGCAGGTGCTGCAGTCAAAGGAGTTGGAACAATGGTGATGGAATTGGTCAGAATGATCAAAC
GTGGGATCAATGATCGGAACTTCTGGAGGGGTGAGAATGGACGAAAAACAAGAATTGCTTATGAAAGAATGTGCAACATT
CTCAAAGGGAAATTTCAAACTGCTGCACAAAAAGCAATGATGGATCAAGTGAGAGAGAGCCGGAACCCAGGGAATGCTGA
GTTCGAAGATCTCACTTTTCTAGCACGGTCTGCACTCATATTGAGAGGGTCGGTTGCTCACAAGTCCTGCCTGCCTGCCT
GTGTGTATGGACCTGCCGTAGCCAGTGGGTACGACTTTGAAAGGGAGGGATACTCTCTAGTCGGAATAGACCCTTTCAGA
CTGCTTCAAAACAGCCAAGTGTACAGCCTAATCAGACCAAATGAGAATCCAGCACACAAGAGTCAACTGGTGTGGATGGC
ATGCCATTCTGCCGCATTTGAAGATCTAAGAGTATTAAGCTTCATCAAAGGGACGAAGGTGCTCCCAAGAGGGAAGCTTT
CCACTAGAGGAGTTCAAATTGCTTCCAATGAAAATATGGAGACTATGGAATCAAGTACACTTGAACTGAGAAGCAGGTAC
TGGGCCATAAGGACCAGAAGTGGAGGAAACACCAATCAACAGAGGGCATCTGCGGGCCAAATCAGCATACAACCTACGTT
CTCAGTACAGAGAAATCTCCCTTTTGACAGAACAACCATTATGGCAGCATTCAATGGGAATACAGAGGGGAGAACATCTG
ACATGAGGACCGAAATCATAAGGATGATGGAAAGTGCAAGACCAGAAGATGTGTCTTTCCAGGGGCGGGGAGTCTTCGAG
CTCTCGGACGAAAAGGCAGCGAGCCCGATCGTGCCTTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAA
TGCAGAGGAGTACGACAATTAAAGAAAAATACCCTTGTTTCTACT (SEQ ID NO:4)

FIG. 10L

Canine codon optimized NP:

AGCAAAAGCAGGGTAGATAATCACTCACTGAGTGACATCAAAATCATGGCGTCTCAAGGCACCAAACGATCTTACGAACA
GATGGAGACTGATGGAGAACGCCAGAATGCCACTGAAATCAGAGCATCCGTCGGAAAAATGATTGGTGGAATTGGACGAT
TCTACATCCAGATGTGCACCGAACTCAAACTCAGTGATTATGAGGGACGGCTGATCCAGAACAGCCTGACAATCGAGAGA
ATGGTGCTCTCTGCTTTTGACGAAAGGAGAAATAAATACCTGGAAGAACATCCCAGTGCCGGGAAAGATCCTAAGAAAAC
TGGAGGACCTATCTACAGGAGAGTGAACGGAAAGTGGATGAGAGAACTCATCCTGTATGACAAAGAAGAAATCAGGCGAA
TCTGGCGCCAGGCTAATAATGGTGACGATGCAACCGCTGGTCTGACTCACATGATGATCTGGCATTCCAATCTGAATGAT
GCAACTTATCAGAGGACAAGAGCTCTGGTGCGCACCGGAATGGATCCCAGGATGTGCTCTCTGATGCAGGGTTCAACTCT
CCCTAGGAGGTCTGGAGCCGCAGGTGCTGCAGTCAAAGGAGTGGGAACAATGGTGATGGAACTGGTCAGAATGATCAAAA
GAGGGATCAATGATCGGAACTTCTGGAGGGGTGAGAATGGACGAAAAACAAGAATTGCTTATGAAAGAATGTGCAACATT
CTCAAAGGGAAATTTCAGACTGCTGCACAGAAAGCAATGATGGATCAGGTGAGAGAGAGCCGGAACCCAGGGAATGCTGA
GTTCGAAGATCTCACTTTTCTGGCACGGTCTGCACTCATCCTGAGAGGGTCCGTGGCTCACAAGTCCTGCCTGCCTGCCT
GTGTGTATGGACCTGCCGTGGCCAGTGGGTACGACTTTGAAAGGGAGGGATACTCTCTGGTCGGAATTGACCCTTTCAGA
CTGCTGCAGAACAGCCAGGTGTACAGCCTGATCAGACCAAATGAGAATCCAGCACACAAGAGTCAGCTGGTGTGGATGGC
ATGCCATTCTGCCGCATTTGAAGATCTGAGAGTGCTGAGCTTCATCAAAGGGACCAAGGTGCTCCCAAGAGGGAAGCTGT
CCACTAGAGGAGTGCAGATTGCTTCCAATGAAAATATGGAGACTATGGAATCAAGTACACTGGAACTGAGAAGCAGGTAC
TGGGCCATCAGGACCAGAAGTGGAGGAAACACCAATCAGCAGAGGGCATCTGCCGGCCAGATCAGCATTCAGCCTACCTT
CTCAGTGCAGAGAAATCTCCCTTTTGACAGAACAACCATTATGGCAGCATTCAATGGGAATACAGAGGGGAGAACATCTG
ACATGAGGACCGAAATCATCAGGATGATGGAAAGTGCAAGACCAGAAGATGTGTCTTTCCAGGGGCGGGGAGTCTTCGAG
CTCTCGGACGAAAAGGCAGCGAGCCCGATCGTGCCTTCCTTTGACATGAGTAATGAAGGATCTTATTTCTTCGGAGACAA
TGCAGAGGAGTACGACAATTAAAGAAAAATACCCTTGTTTCTACT (SEQ ID NO:19)

FIG. 10M

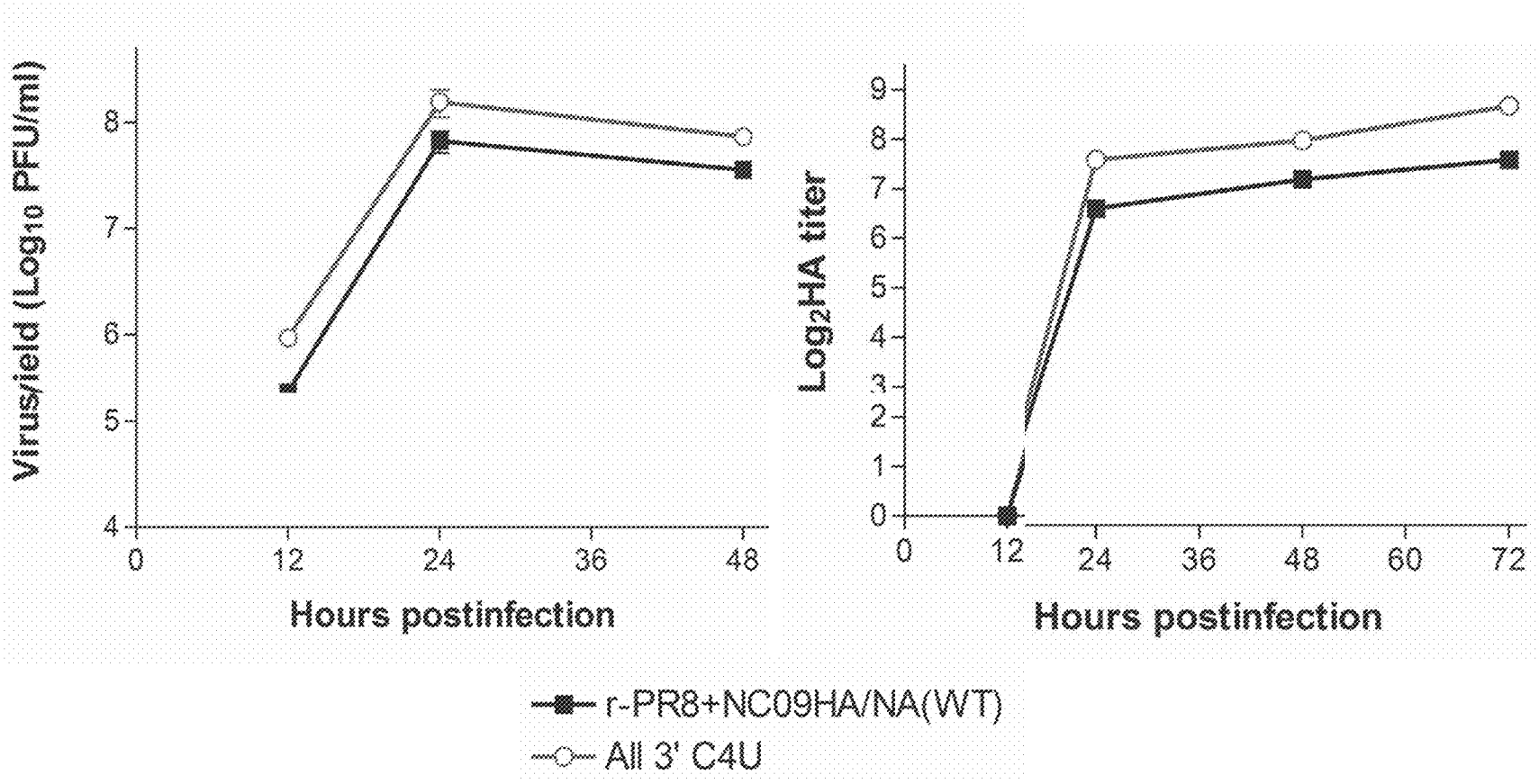
Figure 11 A Nucleotide mutation in position 4 of ea ch gene of PR8 and Indo/NC/09.

Genes	Position 4 of vRNA	
PR8 PB2	C	
PR8 PB1	C	
PR8 PA	C	
PR8 NP		U
PR8 M		U
PR8 NS		U
Inda/NC/09 HA		U
Inda/NC/09 NA		U

Figure 11B All 3'C4U mutant

Genes	Position 4 of vRNA	
PR8 PB2		U
PR8 PB1		U
PR8 PA		U
PR8 NP		U
PR8 M		U
PR8 NS		U
Inda/NC/09 HA		U
Inda/NC/09 NA		U

Figure 11C Growth kinetics of 3' C4U mutant



HA

atgaacactcaaatcctggtattcgctctgattgcgatcattccaacaaatgcagacaaaatctgcctcggacatcatgccgtgtcaaacggaaccaaagtaaa
cacattaactgaaagaggagtggaagtcgtcaatgcaactgaaacagtggaacgaacaaacatccccaggatctgctcaaaagggaaaaggacagttgacc
tcggtcaatgtggactcctggggacaatcactggaccacctcaatgtgaccaattcctagaattttcagccgatttaattattgagaggcgagaaggaagtgatg
tctgttatcctgggaaattcgtgaatgaagaagctctgaggcaaattctcagagaatcaggcggaattgacaaggaagcaatgggattcacatacagtggaat
aagaactaatggagcaaccagtgcatgtaggagatcaggatcttcattctatgcagaaatgaaatggctcctgtcaaacacagataatgctgcattcccgcaga
tgactaagtcatataaaaatacaagaaaaagcccagctctaatagtatgggggatccatcattccgtatcaactgcagagcaaaccaagctatatgggagtgg
aaacaaactggtgacagttgggagttctaattatcaacaatcttttgtaccgagtccaggagcgagaccacaagttaatggtctatctggaagaattgactttcat
tggctaatgctaaatcccaatgatacagtcactttcagtttcaatggggcttcatagctccagaccgtgcaagcttcctgagaggaaaatctatgggaatccag
agtggagtacaggttgatgccaattgtgaaggggactgctatcatagtggagggacaataataagtaacttgccatttcagaacatagatagcagggcagttg
gaaaatgtccgagatatgttaagcaaaggagtctgctgctagcaacagggatgaagaatgttcctgagattccaaagggaagaggcctatttggtgctatagc
gggtttcattgaaaatggatgggaaggcctaattgatggttggtatggtttcagacaccagaatgcacagggagagggaactgctgcagattacaaaagcaa
caatcggcaattgatcaaataacaggaaaattaaaccggcttatagaaaaaaccaaccaacaatttgagttgatagacaatgaattcaatgaggtagagaag
caaatcggtaatgtgataaattggaccagagattctataacagaagtgtggtcatacaatgctgaactcttggtagcaatggagaaccagcatacaattgatct
ggctgattcagaaatggacaaactgtacgaacgagtgaaaagacagctgaggagaatgctgaagaagatggcactggttgctttgaaatatttcacaagtgt
gatgatgactgtatggccagtattagaaataacacctatgatcacagcaaatacagggaagaggcaatgcaaaatagaatacagattgacccagtcaaacta
agcagcggctacaaagatgtgatactttggtttagcttcggggcatcatgtttcatacttctagccattgtaatgggccttgtcttcatatgtgtaaagaatggaaa
catgcggtgcactatttgtatataa (SEQ ID NO:20)

MNTQILVFAL IAIIPTNADK ICLGHHAVSN GTKVNTLTER GVEVVNATET VERTNIPRIC
SKGKRTVDLG QCGLLGTITG PPQCDQFLEF SADLIIERRE GSDVCYPGKF VNEEALRQIL
RESGGIDKEA MGFTYSGIRT NGATSACRRS GSSFYAEMKW LLSNTDNAAF PQMTKSYKNT
RKSPALIVWG IHHSVSTAEQ TKLYGSGNKL VTVGSSNYQQ SFVPSPGARP QVNGLSGRID
FHWLMLNPND TVTFSFNGAF IAPDRASFLR GKSMGIQSGV QVDANCEGDC YHSGGTIISN
LPFQNIDSRA VGKCPRYVKQ RSLLLATGMK NVPEIPKGRG LFGAIAGFIE NGWEGLIDGW
YGFRHQNAQG EGTAADYKST QSAIDQITGK LNRLIEKTNQ QFELIDNEFN EVEKQIGNVI
NWTRDSITEV WSYNAELLVA MENQHTIDLA DSEMDKLYER VKRQLRENAE EDGTGCFEIF
HKCDDDCMAS IRNNTYDHSK YREEAMQNRI QIDPVKLSSG YKDVILWFSF GASCFILLAI
VMGLVFICVK NGNMRCTICI (SEQ ID NO:21)

NA

atgaatccaaatcagaagattctatgcacttcagccactgctatcataataggcgcaatcgcagtactcattggaatagcaaacctaggattgaacataggact
gcatctaaaaccgggctgcaattgctcacactcacaacctgaaacaaccaacacaagccaaacaataataaacaactattataatgaaacaaacatcaccaa
catccaaatggaagagagaacaagcaggaatttcaataacttaactaaagggctctgtactatataattcatggcacatatatgggaaagacaatgcagtaaga
attggagagagctcggatgttttagtcacaagagaaccctatgtttcatgcgacccagatgaatgcaggttctatgctctcagccaaggaacaacaatcagagg
gaaacactcaaacggaacaatacacgataggtcccagtatcgcgccctgataagctggccactatcatcaccgcccacagtgtacaacagcagggtggaatg
cattgggtggtcaagtactagttgccatgatggcaaatccaggatgtcaatatgtatatcaggaccaaacaacaatgcatctgcagtagtatggtacaacagaa
ggcctgttgcagaaattaacacatgggcccgaaacatactaagaacacaggaatctgaatgtgtatgccacaacggcgtatgcccagtagtgttcaccgatgg
gtctgccactggacctgcagacacaagaatatactatttttaaagaggggaaaatattgaaatgggagtctctgactggaactgctaagcatattgaagaatgct
catgttacggggaacgaacaggaattacctgcacatgcagggacaattggcagggctcaaatagaccagtgattcagatagacccagtagcaatgacacaca
ctagtcaatatatatgcagtcctgttcttacagacaatccccgaccgaatgacccaaatataggtaagtgtaatgacccttatccaggtaataataacaatggag
tcaagggattctcatacctggatggggctaacacttggctagggaggacaataagcacagcctcgaggtctggatacgagatgttaaaagtgccaaatgcatt
gacagatgatagatcaaagcccattcaaggtcagacaattgtattaaacgctgactggagtggttacagtggatctttcatggactattgggctgaaggggact
gctatcgagcgtgttttatgtggagttgatacgtggaagacccaaggaagataaagtgtggtggaccagcaatagtatagtatcgatgtgttccagtacagaat
tcctgggacaatggaactggcctgatggggctaaaatagagtacttcctctaa (SEQ ID NO:22)

MNPNQKILCTSATAIIIGAIAVLIGIANLGLNIGLHLKPGCNCSHSQPETTNTSQTIINNYYNETNITNIQMEERTSRNFNNLTKGL
CTINSWHIYGKDNAVRIGESSDVLVTREPYVSCDPDECRFYALSQGTTIRGKHSNGTIHDRSQYRALISWPLSSPPTVYNSRVECI
GWSSTSCHDGKSRMSICISGPNNNASAVVWYNRRPVAEINTWARNILRTQESECVCHNGVCPVVFTDGSATGPADTRIYYFK

FIG. 12A

EGKILKWESLTGTAKHIEECSCYGERTGITCTCRDNWQGSNRPVIQIDPVAMTHTSQYICSPVLTDNPRPNDPNIGKCNDPYPG
NNNNGVKGFSYLDGANTWLGRTISTASRSGYEMLKVPNALTDDRSKPIQGQTIVLNADWSGYSGSFMDYWAEGDCYRACF
Y VELIRGRPKEDKVWWTSNSIVSMCSSTEFLGQWNWPDGAKIEYFL (SEQ ID NO:23)

HA

atgaacactcaaatcctggtattcgctctgattgcgatcattccaacaaatgcagacaaaatctgcctcggacatcatgctgtgtcaaacggaaccaaagtaaa
cacattaactgaaagaggagtggaagtcgtcaatgcaactgaaacagtggaacgaacaaacatccccaggatctgctcaaaagggaaaaggacagttgacc
tcggtcaatgtggactcctggggacaatcactggaccacctcaatgtgaccaattcctagaattttcagccgatttaattattgagaggcgagaaggaagtgatg
tctgttatcctgggaaattcgtgaatgaagaagctctgaggcaaatttctcagagaatcaggcggaattgacaaggaagcaatgggattcacatacagtggaat
aagaactaatggagcaaccagttcatgtaggagatcaggatcttcattctatgcagaaatgaaatggctcctgtcaaacacagataatgctgcattcccgcaga
tgactaagtcatataaaaatacaagaaaaaacccagctctaatagtatgggggatccatcattccggatcaactgcagagcaaaccaagctatatgggagtgg
aaacaaactggtgacagttgggagttctaattatcaacaatcttttgtaccgagtccgggagcgagaacacaagttaatggtcaatctggaagaattgacttca
ttggctaatgctaaatcccaatgatacagtcactttcagtttcaatggggctttcatagctccagaccgtgcaagcttcctgagaggaaaatctatgggaatccag
agtggagtacaggttgatgccgattgtgaaggggactgctattatagtggagggacaataataagtaacttgccatttcagaacatagatagcagggcagttgg
aaaatgtccgagatatgttaagcaaaggagtctgctgctagcaacagggatgaagaatgttcctgagattccaaagggaagaggcctatttggtgctatagcg
ggtttcattgaaaatggatgggaaggcctaattgatggttggtatggtttcagacaccagaatgcacagggagagggaactgctgcagattacaaaagcactc
aatcggcaattgatcaaataacaggaaaattaaaccggcttatagaaaaaaccaaccaacaatttgagttgatagacaatgaattcactgaggtagagaagc
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gctgattcagaaatggacaaactgtacgaacgagtgaaaagacagctgagagagaatgctgaagaagatggcactggttgctttgaaatatttcacaagtgtg
atgatgactgtatggccagcattagaaataacacctatgatcacagcaaatacagggaagaggcaatgcaaaatagaatacagattgacccagtcaaactaa
gcagcggctacaaagatgtgatactttggtttagcttcggggcatcatgtttcatacttctagccattgcaatgggccttgtcttcatatgtgtaaagaatggaaa
c atgcggtgcactatttgtatataa (SEQ ID NO:24)

MNTQILVFALIAIIPTNADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKGKRTVDLGQCGLLGTITGPPQCDQ
FLEFSADLIIERREGSDVCYPGKFVNEEALRQILRESGGIDKEAMGFTYSGIRTNGATSSCRRSGSSFYAEMKWLLSNTDNAAFP
QMTKSYKNTRKNPALIVWGIHHSGSTAEQTKLYGSGNKLVTVGSSNYQQSFVPSPGARTQVNGQSGRIDFHWLMLNPNDTV
TFSFNGAFIAPDRASFLRGKSMGIQSGVQVDADCEGDCYYSGGTIISNLPFQNIDSRAVGKCPRYVKQRSLLLATGMKNVPEIP
KGRGLFGAIAGFIENGWEGLIDGWYGFRHQNAQGEGTAADYKSTQSAIDQITGKLNRLIEKTNQQFELIDNEFTEVEKQIGNVI
NWTRDSITEVWSYNAELLVAMENQHTIDLADSEMDKLYERVKRQLRENAEEDGTGCFEIFHKCDDDCMASIRNNTYDHSKY
R EEAMQNRIQIDPVKLSSGYKDVILWFSFGASCFILLAIAMGLVFICVKNGNMRCTICI (SEQ ID NO:25)

NA

atgaatccaaatcagaagattctatgcacttcagccactgctatcataataggcgcaatcgcagtactcattggaatagcaaacctaggattgaacataggact
gcatctaaaaccgagctgcaattgctcacactcacaacctgaaacaaccaacacaagccaaacaataataaacaactattataatgaaacaaacatcaccaa
catccaaatggaagagagaacaagcaggaatttcaataacttaactaaagggctctgtactataaattcatggcacatatatgggaaagacaatgcggtaaga
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aaaacactcaaacggaacaatacacgataggtcccagtatcgcgccctgataagctggccactatcatcaccgcccacagtgtacaacagcagggtggaatg
cattgggtggtcaagtactagttgccatgatggcaaatccaggatgtcaatatgtatatcaggaccaaacaacaatgcatctgcagtagtatggtacaacagaa
ggcctgttgcagaaattaacacatgggcccgaaacatactaagaacacaggaatctgaatgtgtatgccacaacggcgtatgcccagtagtgttcaccgatgg
gtctgccactggacctgcagacacaagaatatactatttttaaagaggggaaaatattgaaatgggagtctctgactggaactgctaagcatattgaagaatgct
catgttacggggaacgaacaggaattacctgcacatgcaaggacaattggcagggctcaaatagaccagtgattcagatagatccagtagcaatgacacaca
ctagtcagtatatatgcagtcctgttcttacagacaatccccgaccgaatgacccaaatataggtaagtgtaatgacccttatccaggtaataataacaatggag
tcaagggattctcatacctggatggggctaacacttggctagggaggacaataagcacagcctcgaggtctggatacgagatgttaaaagtgccaaatgcatt
gacagatgatagatcaaagcccattcaaggtcagacaattgtattaaacgctgactggagtggttacagtggatctttcatggactattgggctgaggggact
gctatcgagcgtgtttttatgtggaattgatacgtggaagacccaaggaggataaagtgtggtggaccagcaatagtatagtatcgatgtgttccagtacagaat
tcctgggacaatggaactggcctgatggggctaaaatagagtacttcctctaa (SEQ ID NO:26)

MNPNQKILCTSATAIIIGAIAVLIGIANLGLNIGLHLKPSCNCSHSQPETTNTSQTIINNYYNETNITNIQMEERTSRNFNNLTKGL
CTINSWHIYGKDNAVRIGESSDVLVTREPYVSCDPDECRFYALSQGTTIRGKHSNGTIHDRSQYRALISWPLSSPPTVYNSRVECI
GWSSTSCHDGKSRMSICISGPNNNASAVVWYNRRPVAEINTWARNILRTQESECVCHNGVCPVVFTDGSATGPADTRIYYFK

FIG. 12B

EGKILKWESLTGTAKHIEECSCYGERTGITCTCKDNWQGSNRPVIQIDPVAMTHTSQYICSPVLTDNPRPNDPNIGKCNDPYPG
NNNNGVKGFSYLDGANTWLGRTISTASRSGYEMLKVPNALTDDRSKPIQGQTIVLNADWSGYSGSFMDYWAEGDCYRACF
Y VELIRGRPKEDKVWWTSNSIVSMCSSTEFLGQWNWPDGAKIEYFL (SEQ ID NO:27)

FIG. 12C

Figure 13A Construct chimeric HA &NA to increase virus replication

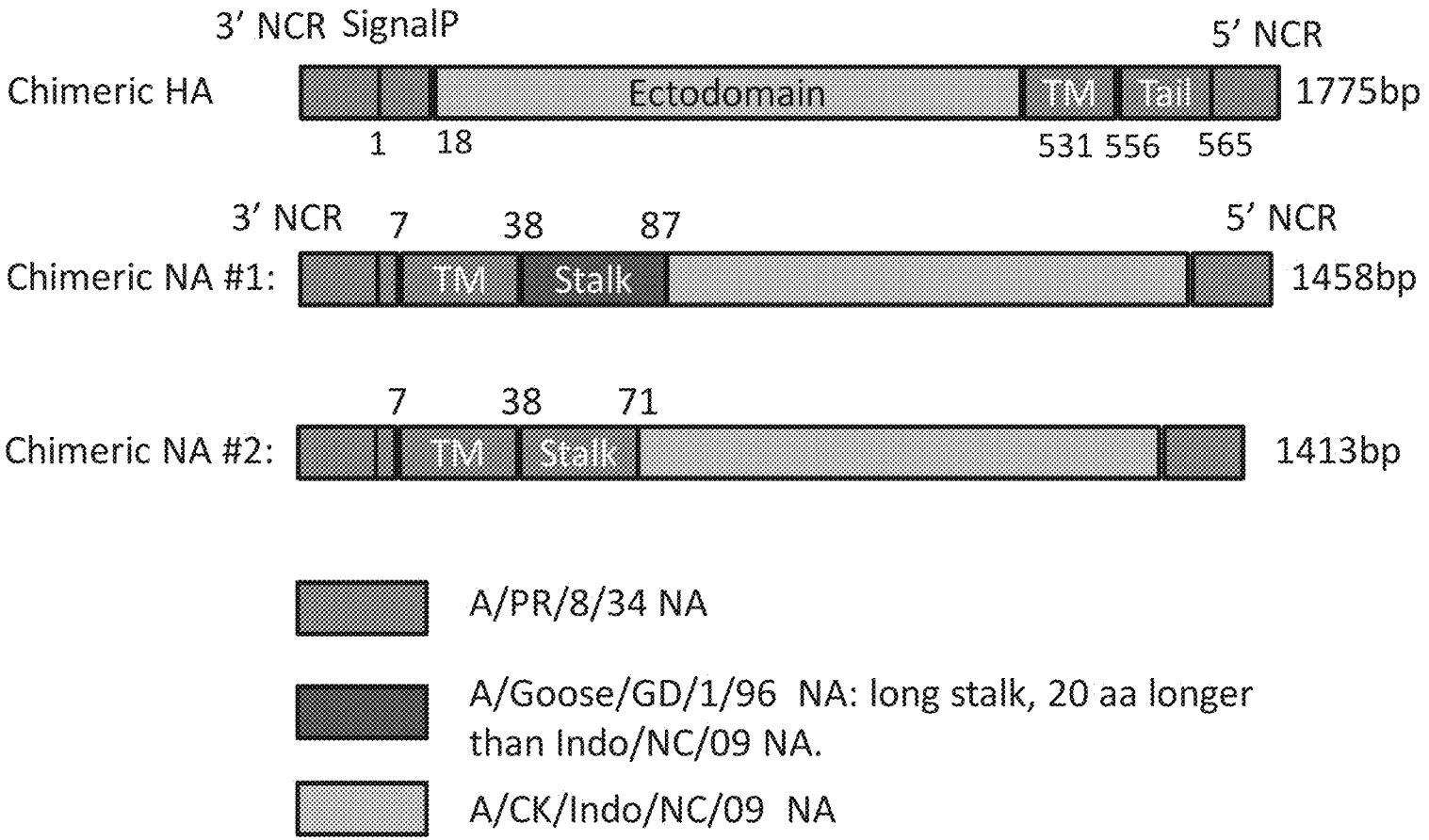


Figure 13B Growth kinetics in MDCK cells

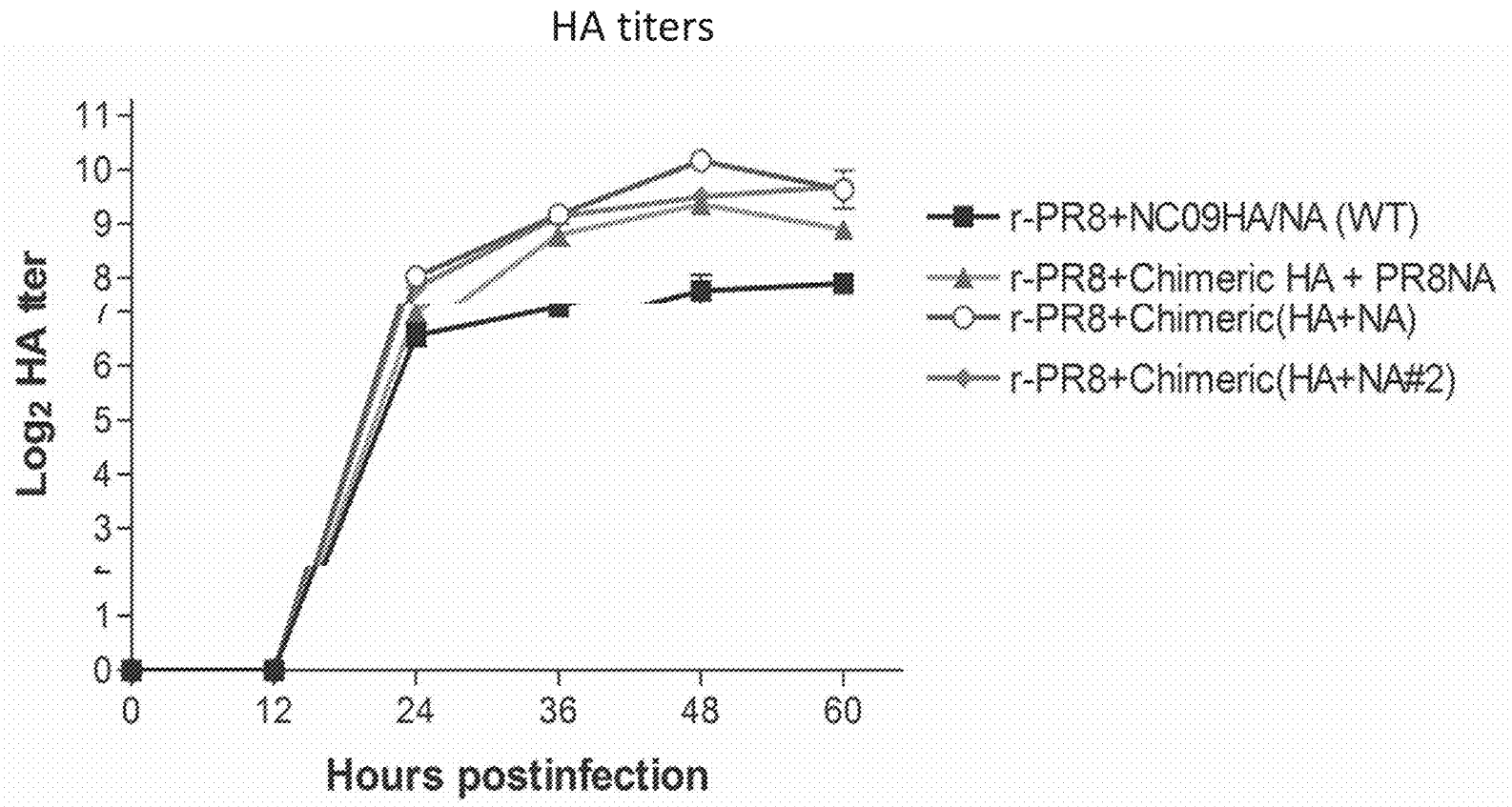
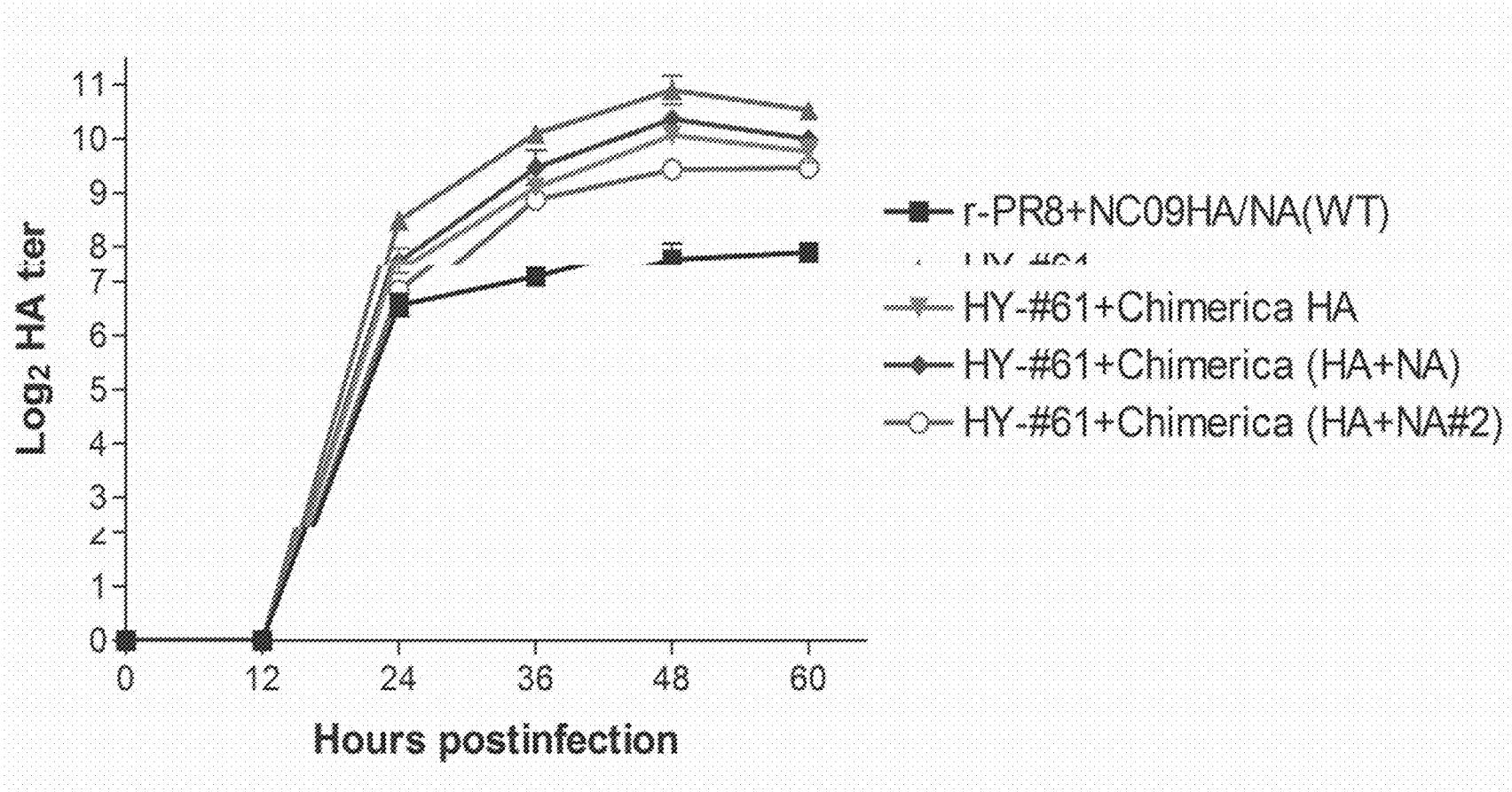
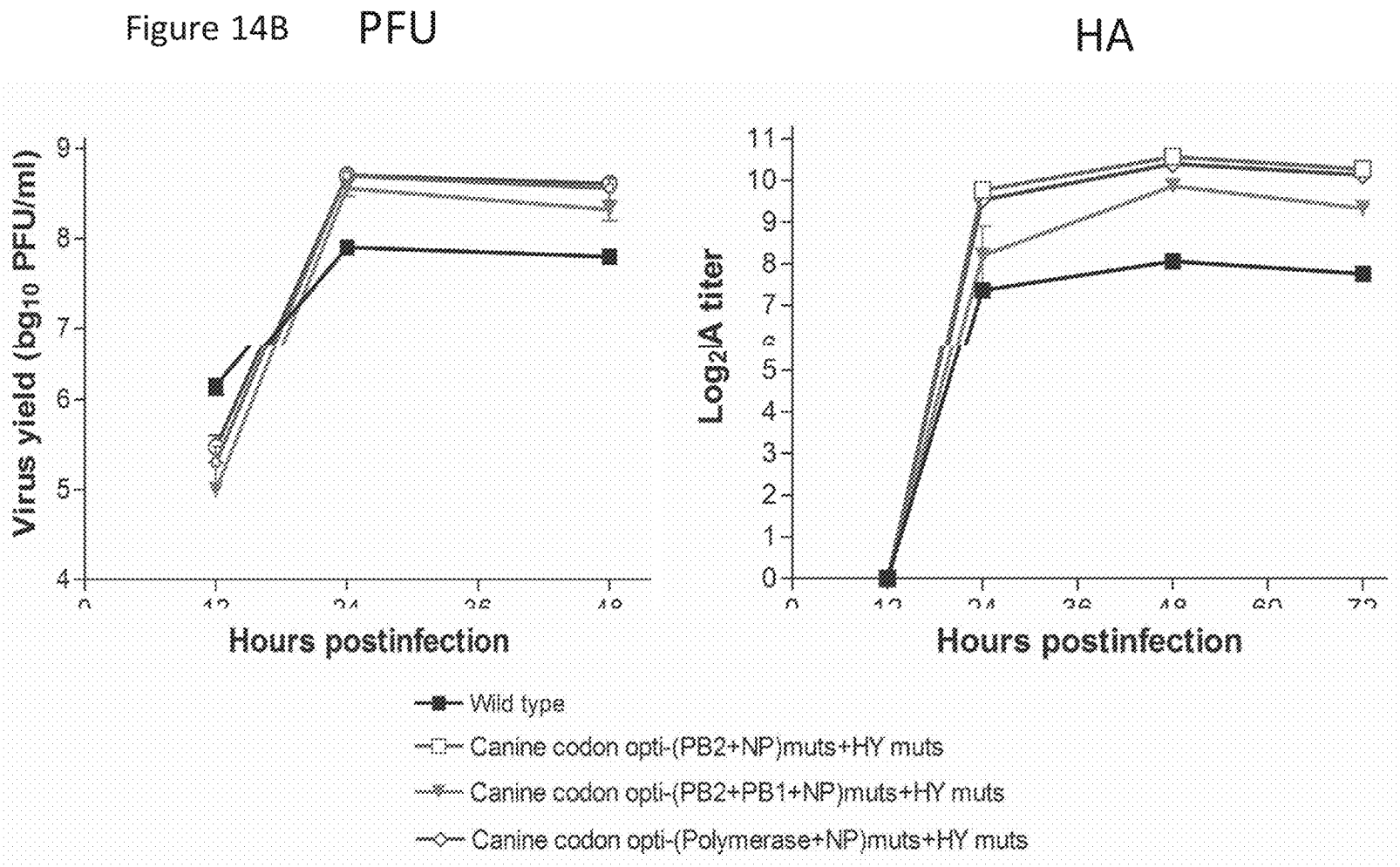


Figure 14A Growth kinetics in MDCK cells



HY-#61 includes PB2: M202L F323L, PB1 Q247H, PA K142N, NP R74K, M V97A Y100H and NS K55E mutations.

Figure 14B



HY mutations include PB2: M202L F323L, PB1 Q247H, PA K142N, NP R74K, M V97A Y100H and NS K55E mutations.

Figure 15 Schematic diagram of screening high growth mutations in eggs.

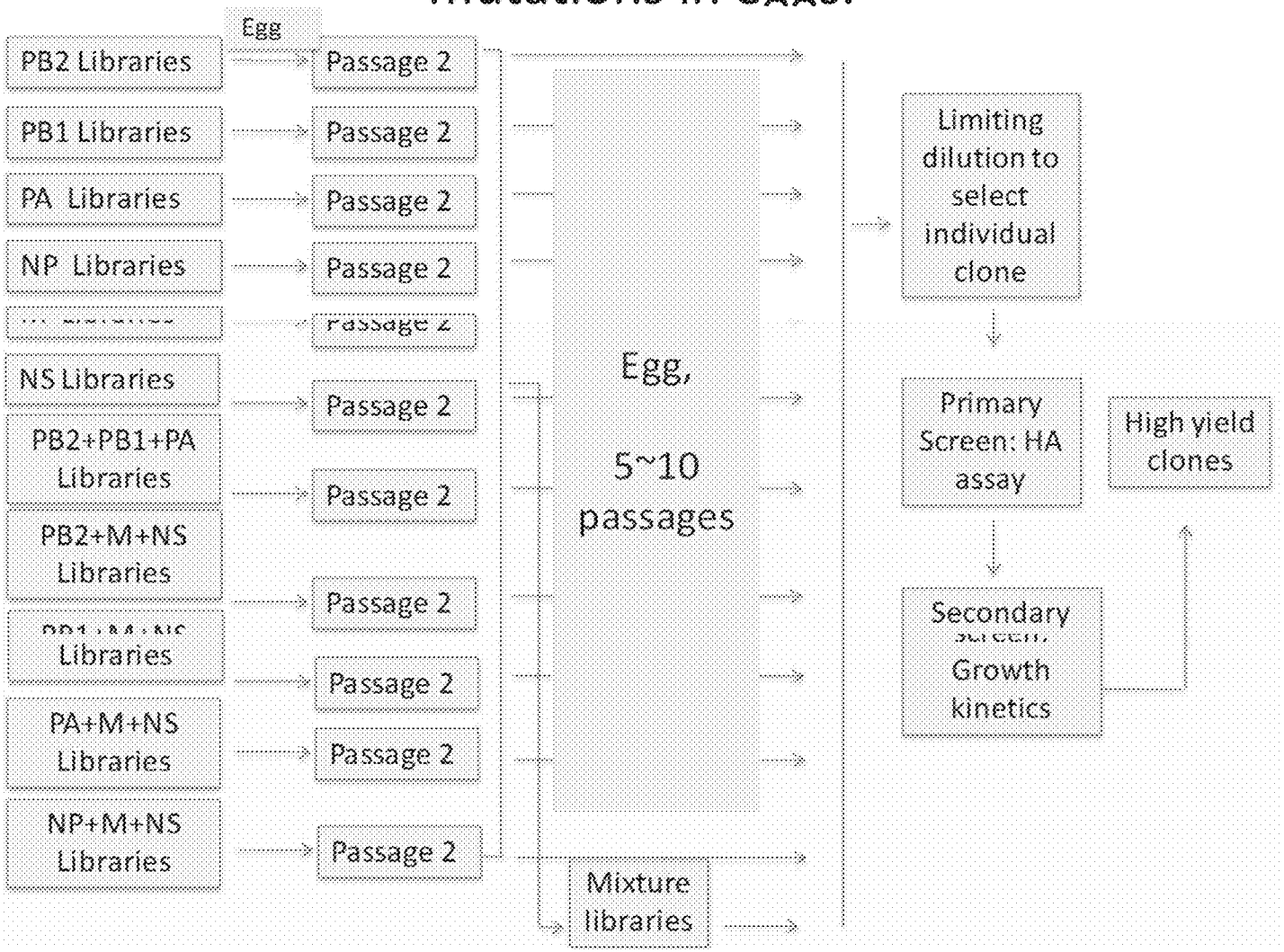


Figure 16 Summary of HA assay of individual clones purified from Vero cells

Groups	Numbers of clone	Fold change	%
WT HA titer = $2^{6.5}$	5	-	2.3%
HA titer =$2^{>9-9.5}$	16	>5.6	7.4%
HA titer =$2^{>8-9}$	91	>2.8 -5.6	42.2%
HA titer =$2^{6.5-8}$	80	1 - 2.8	37.0%
HA titer < $2^{6.5}$	24	<1	11.1%
Total	216	-	100%

Figure 17Recombinant viruses generated with different PR8 backbone mutants.

#	Del-HA & NA genes	PB2	PB1	PA	NP	M	NS
WT	CK/Indo/NC/09	WT	WT	WT	WT	WT	WT
HY-1		I504V	M40L G180W	R401K	I116L	WT	A30P R118K
HY-2		E391Q		I30T E31K K142N	R74K S377N	WT	S161T
HY-3		I504V		K142N	I116L	V97A Y100H	V136M S161T
HY-4		M202L F323L		K356R	I116L	V97A Y100H	K55E
HY-5				K356R	R422L	WT	K55E

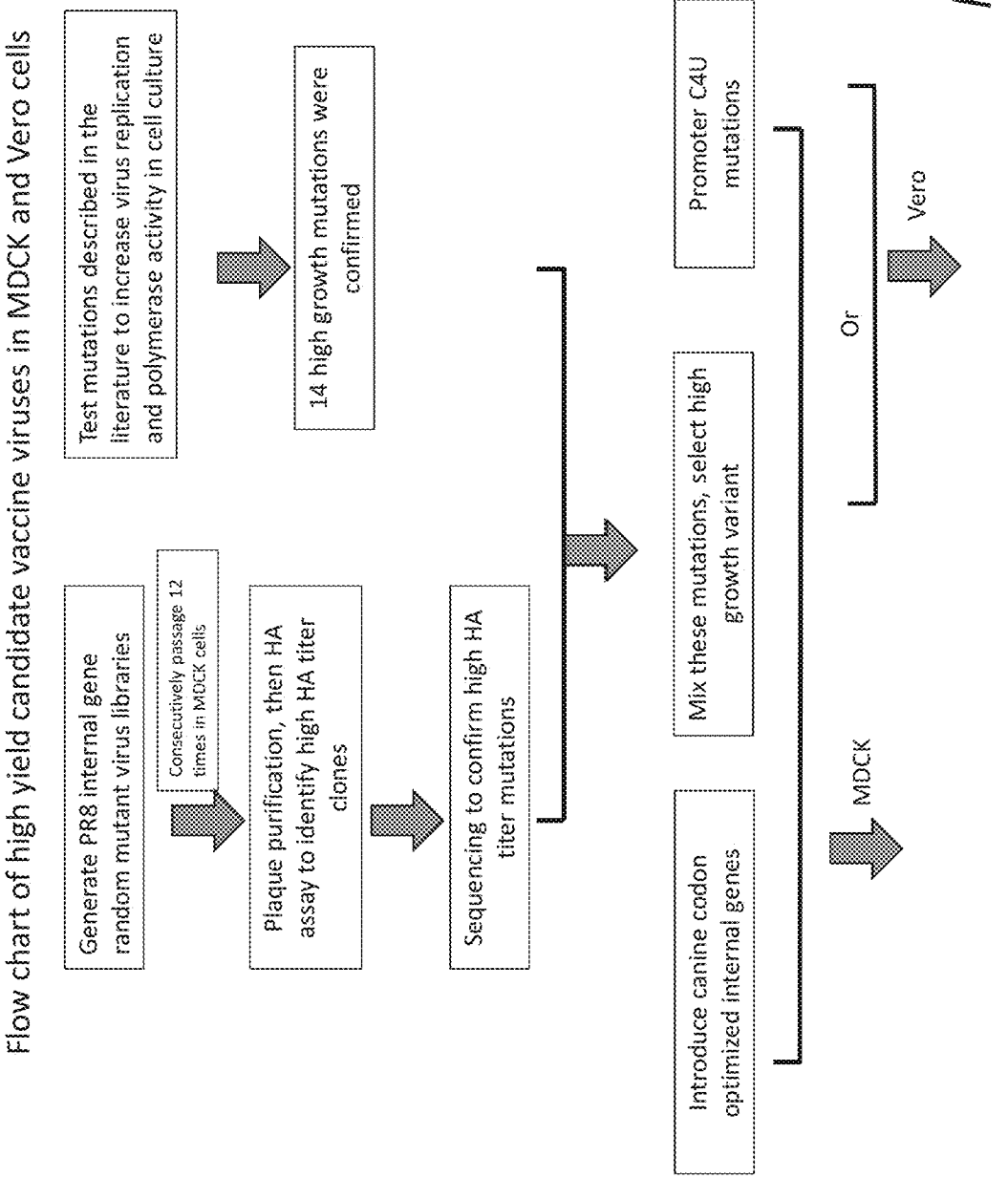

Flow chart of high yield candidate vaccine viruses in MDCK and Vero cells
Generate PR8 internal gene random mutant virus libraries
Consecutively passage 12 times in MDCK cells
Plaque purification, then HA assay to identify high HA titer clones
Sequencing to confirm high HA titer mutations
Test mutations described in the literature to increase virus replication and polymerase activity in cell culture
14 high growth mutations were confirmed
Introduce canine codon optimized internal genes
Mix these mutations, select high growth variant
Promoter C4U mutations
MDCK
Or
Vero

FIG. 18A

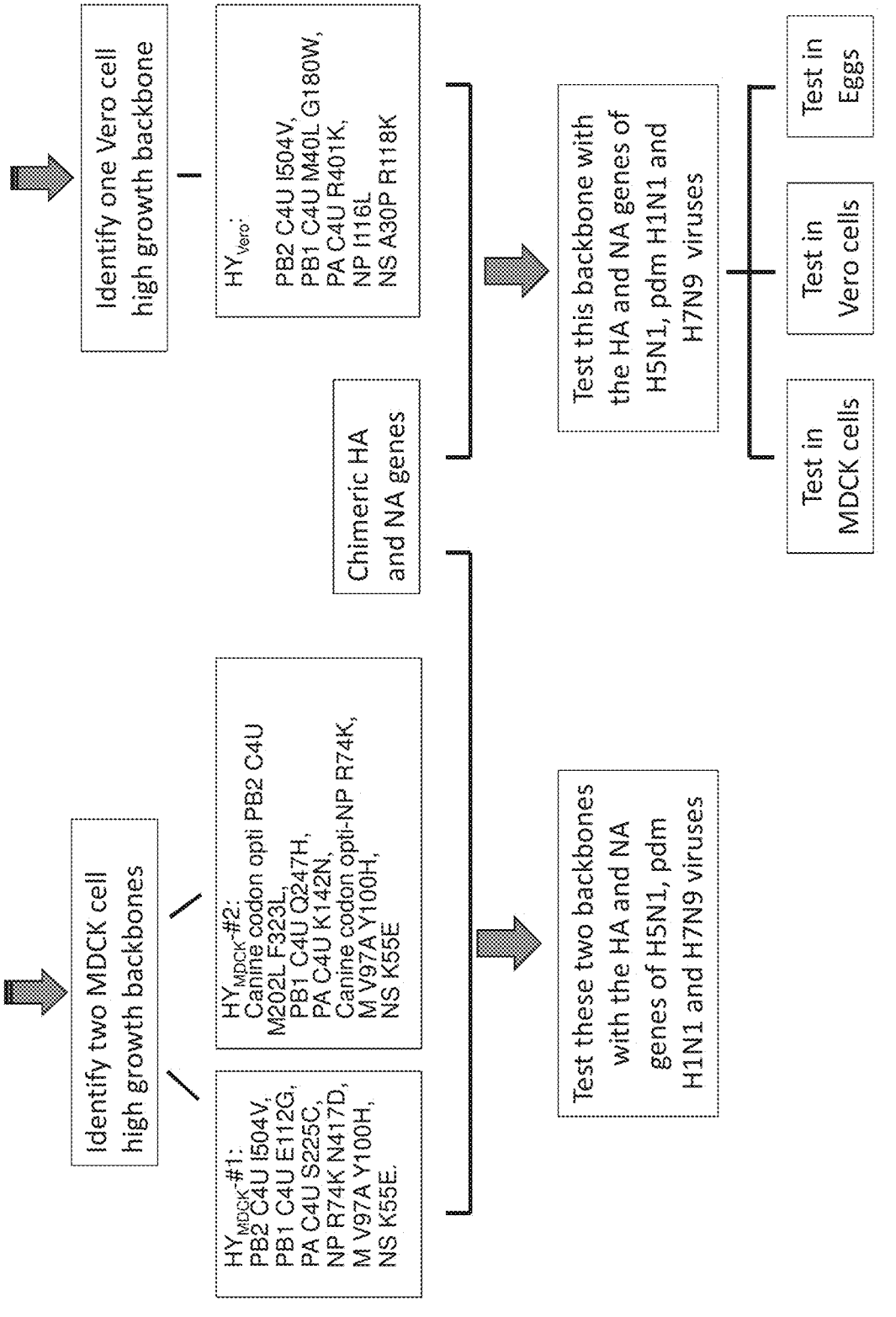

Identify two MDCK cell high growth backbones
HY MDCK-#1: PB2 C4U I504V, PB1 C4U E112G, PA C4U S225C, NP R74K N417D, M V97A Y100H, NS K55E.
HY MDCK-#2: Canine codon opti PB2 C4U M202L F323L, PB1 C4U Q247H, PA C4U K142N, Canine codon opti-NP R74K, M V97A Y100H, NS K55E
Test these two backbones with the HA and NA genes of H5N1, pdm H1N1 and H7N9 viruses
Identify one Vero cell high growth backbone
HY Vero: PB2 C4U I504V, PB1 C4U M40L G180W, PA C4U R401K, NP I116L NS A30P R118K
Chimeric HA and NA genes
Test this backbone with the HA and NA genes of H5N1, pdm H1N1 and H7N9 viruses
Test in MDCK cells
Test in Vero cells
Test in Eggs

FIG. 18B

Virus number	Surface genes		UW-PR8 internal gene						Growth substrate	Viral titer or HA titer	Fold change
	HA (H3 numbering)	NA	PB2	PB1	PA	NP	M1	NS1			
1	M476I		M202L F323L			R293M			MDCK	HA titer	2.8
2	F252I	L55S	M202L F323L			I116L			MDCK	HA titer	2.8
3			M202L F323L					A223E	MDCK	HA titer	2.8
4	L182V		M202L F323L						MDCK	HA titer	2.8~4
5				E112G (PB1-F2 R81G)[5]					MDCK	HA titer	2.8
6	E136D/ Q179L/ A194V			R54I					MDCK	HA titer	2.8~4
7	K162E			I667T/M714T					MDCK	HA titer	4
8	L182V					I116L		R140Q	MDCK	HA titer	2.8~4
9	L182V								MDCK	HA titer	2.8~4
10	L182V		M202L F323L						MDCK	HA titer	4
11	L182V		M66R						MDCK	HA titer	2.8~4
12	L182V		M202L F323L						MDCK	HA titer	4
13			M202L F323L	M507V/V644A					MDCK	HA titer	4
14	L182V		I504V			R74K/ N417D		A30P	MDCK	HA titer	2.8
15	K162E			E112G (PB1-F2-R61G)				S161T	MDCK	HA titer	2.8
16				I667T					MDCK	HA titer	2.8
17	K449E			M40L/G180W					MDCK	HA titer	2.8
18	K162E			E112G (PB1-F2-R81G)/ L624V				S161T	MDCK	HA titer	2.8~4

FIG. 19A

Virus number	Surface genes		UW-PR8 internal gene						Growth substrate	Viral titer or HA titer	Fold change
	HA (H3 numbering)	NA	PB2	PB1	PA	NP	M1	NS1			
19				E112G (PB1-F2-R81G)					MDCK	HA titer	2.8
20				M40L G180W				S161T	MDCK	HA titer	2.8~4
21			M202L F323L M243I	R54I					MDCK	HA titer	4~5.7
22	V184I		M202L F323L		F105C		P90S		MDCK	HA titer	2.8~4
23			M202L F323L	Q247H					MDCK	HA titer	2.8~4
24			M202L F323L			N224I			MDCK	HA titer	2.8~4
25	M476I		I504V			R74K/ N417D			MDCK	HA titer	2.8~4
26	M476I		M202L F323L	V644A	R401K			T49A	MDCK	HA titer	4~5.7
27	F252I		I504V	V644A		M371V			MDCK	HA titer	2.8
28	M476I	A265V	I504V	T59I G62A A63P V644A N694K L695T		R74K/ N417D			MDCK	HA titer	2.8
29	M476I		I504V						MDCK	HA titer	2.8~4
30	F252I		I504V	E75V D76G E78P P79V S80G V644A E697P F699L F700L P701H S702R Y705T		R74K			MDCK	HA titer	2.8~4
31	L182V		I504V			R74K			MDCK	HA titer	2.8~4
32	F252I		M202L F323L	V644A					MDCK	HA titer	4

FIG. 19B

Virus number	Surface genes		UW-PR8 internal gene						Growth substrate	Viral titer or HA titer	Fold change
	HA (H3 numbering)	NA	PB2	PB1	PA	NP	M1	NS1			
33			I57V T58G A59V K61Q E677D D678E P679M	E112G (PB1-F2-R81G) S713C					MDCK	HA titer	2.8
34				M40I G180W				S161T	MDCK	HA titer	2.8
35			R368K						MDCK	Viral titer	2.8
36			E391K						MDCK	Viral titer	3.8
37			I504V		I550L				MDCK	Viral titer	4.3
38				PB1 F2 N66S					MDCK	Viral titer	2.78
39				PB1 F2 K73R					MDCK	Viral titer	3.9
40					K142N				MDCK	Viral titer	2.1
41					K356R				MDCK	Viral titer	3.9
42						R293K			MDCK	Viral titer	3.36
43						R442K			MDCK	Viral titer	2.4
44						T442A			MDCK	Viral titer	2.6
46							V97A		MDCK	HA titer	8
47							Y100H		MDCK	HA titer	9
48							V97A Y100H		MDCK	HA titer	9.5
49								K55E	MDCK	Viral titer	2.1
50			M202L F323L	M507V V644A		I116L		K55E	MDCK	Viral titer	9.9
										HA titer	4.3
51			M202L F323L	M507V V644A	K356R	T442A	V97A Y100H	K55E	MDCK	Viral titer	1.3
										HA titer	3.7
52			I504V	E112G (PB1-F2-R81G)	I550L	I112L	Y100H	R140Q	MDCK	Viral titer	3
										HA titer	3
53			M202L	M507V		I116L	Y100H	K55E	MDCK	Viral titer	9

FIG. 19C

Virus number	Surface genes		UW-PR8 internal gene						Growth substrate	Viral titer or HA titer	Fold change
	HA (H3 numbering)	NA	PB2	PB1	PA	NP	M1	NS1			
[illegible]			F323L	V644A		[illegible]	[illegible]	[illegible]	[illegible]	HA titer	6.5
54			M202L F323L	Q247H	K142N	R74K	V97A Y100H	K55E	MDCK	Viral titer	4.3
										HA titer	9.2
55			I504V	E112G (PB1-F2-R81G)	S225C	R74K N417D	V97A Y100H	K55E	MDCK	Viral titer	14.9
										HA titer	6.5
56			M202L F323L	M40L G180W	S225C	R422K	V97A Y100H	K55E	MDCK	Viral titer	4.2
										HA titer	6.5
57			*C4U*	*C4U*	*C4U*				MDCK	Viral titer	3.3
										HA titer	2.1
58	X-181 HA &NA (Derived from A/California/ 07/2009 pdmH1N1)			G180E S261G S361R Q621R N654S					MDCK	Viral titer	3.5
										HA titer	2.1
59	A/Chicken/Indonesia/NC/2009, A/Vietnam/1203/2004, A/Hubei/1/2010, A/Egypt/N03072/2010, A/Indonesia/5/2005, A/Anhui/1/2013, X-181, X-223A		*C4U* I504V	*C4U* M40L G180W	*C4U* R401K	I116L		A30P R180K	Vero	Viral titer	5.1~269
										HA titer	2.2~134
									MDCK	Viral titer	1.8~29
										HA titer	1.3~5.5
									Eggs	Viral titer	4.6~172
										HA titer	1.3~17.7
60	A/Chicken/Indonesia/NC/2009		C4U I504V	C4U M40L G180W	C4U R401K	I116L	*G1012C, A1013U, U1014A*	A30P R180K	Vero	Viral titer	2.8
										HA titer	1.2
									Egg	Viral titer	1.5
										HA titer	1.6

Note: 1. The HA and NA genes of #1-58 come from A/Chicken/Indonesia/NC/2009 (H5N1) or mutant HA and NA of A/Chicken/Indonesia/NC/2009.
2:All the H5N1 HAs used in this study were mutated to remove multibasic cleavage sites to create lowly pathogenicity mutants.

FIG. 19D

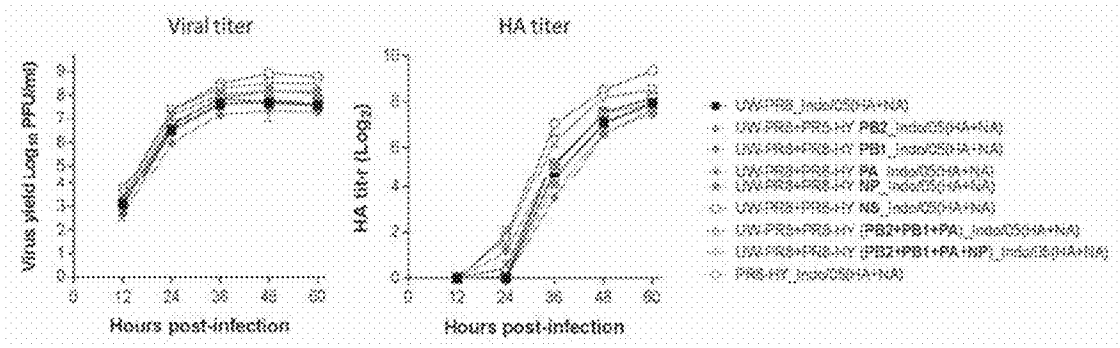
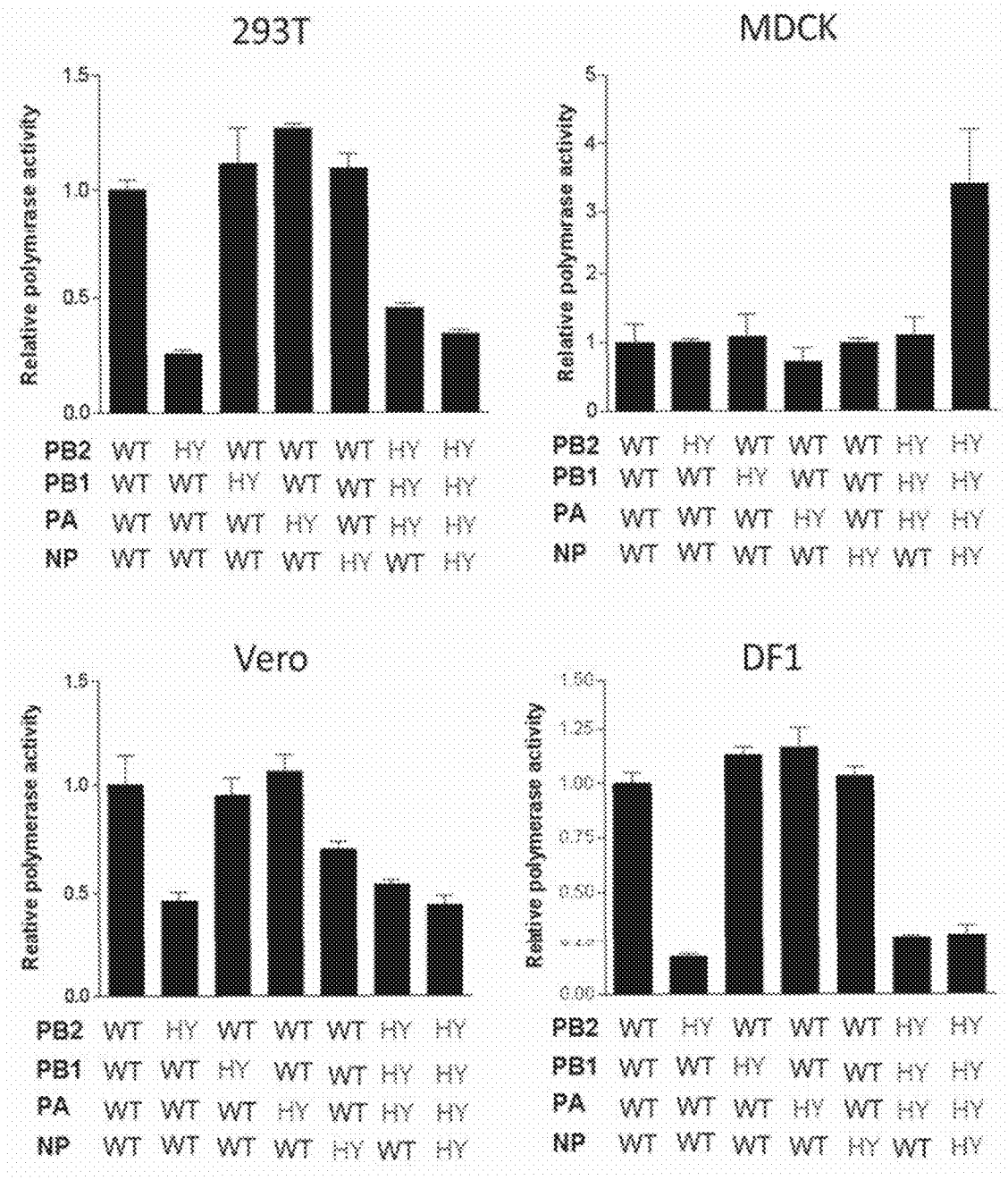

Viral titer
Virus yield (Log10 pfu/ml)
Hours post-infection
HA titer
HA titer (Log2)
Hours post-infection

Figure 20


293T
Relative polymerase activity
1.5
1.0
0.5
0.0
PB2 WT HY WT WT WT HY HY
PB1 WT WT HY WT WT HY HY
PA WT WT WT HY WT HY HY
NP WT WT WT WT HY WT HY
MDCK
Relative polymerase activity
5
4
3
2
1
0
PB2 WT HY WT WT WT HY HY
PB1 WT WT HY WT WT HY HY
PA WT WT WT HY WT HY HY
NP WT WT WT WT HY WT HY
Vero
Reative polymerase activity
1.5
1.0
0.5
0.0
PB2 WT HY WT WT WT HY HY
PB1 WT WT HY WT WT HY HY
PA WT WT WT HY WT HY HY
NP WT WT WT WT HY WT HY
DF1
Relative polymerase activity
1.50
1.25
1.00
0.75
0.50
0.00
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PB1 WT WT HY WT WT HY HY
PA WT WT WT HY WT HY HY
NP WT WT WT WT HY WT HY

Figure 21

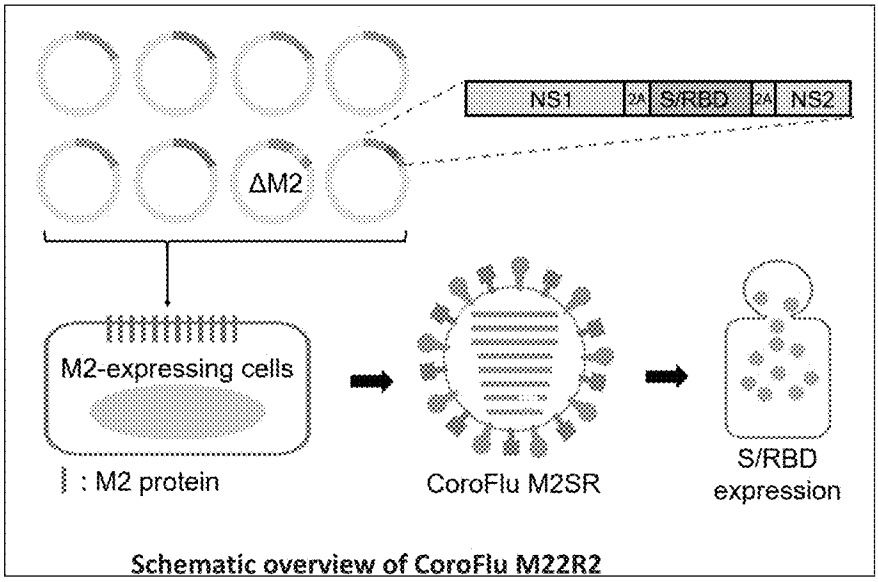
NS1
P2A
S/RBD
P2A
NS2
ΔM2
M2-expressing cells
: M2 protein
CoroFlu M2SR
S/RBD
expression
Schematic overview of CoroFlu M22R2

Fig. 22

QIA98554

```
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      241 llalhrsylt pgdsssgwta gaaayyvgyl qprtfllkyn engtitdavd caldplsetk
      301 ctlksftvek giyqtsnfrv qptesivrfp nitnlcpfge vfnatrfasv yawnrkrisn
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      661 ecdipigagi casyqtqtns prrarsvasq siiaytmslg aensvaysnn siaiptnfti
      721 svtteilpvs mtktsvdctm yicgdstecs nlllqygsfc tqlnraltgi aveqdkntqe
      781 vfaqvkqiyk tppikdfggf nfsqilpdps kpskrsfied llfnkvtlad agfikqygdc
      841 lgdiaardli caqkfngltv lpplltdemi aqytsallag titsgwtfga gaalqipfam
      901 qmayrfngig vtqnvlyenq klianqfnsa igkiqdslss tasalgklqd vvnqnaqaln
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     1021 sanlaatkms ecvlgqskrv dfcgkgyhlm sfpqsaphgv vflhvtyvpa qeknfttapa
     1081 ichdgkahfp regvfvsngt hwfvtqrnfy epqiittdnt fvsgncdvvi givnntvydp
     1141 lqpeldsfke eldkyfknht spdvdlgdis ginasvvniq keidrlneva knlneslidl
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BCA87361

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      181 gkqgnfknlr efvfknidgy fkiyskhtpi nlvrdlpqgf saleplvdlp iginitrfqt
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      661 ecdipigagi casyqtqtns prrarsvasq siiaytmslg aensvaysnn siaiptnfti
      721 svtteilpvs mtktsvdctm yicgdstecs nlllqygsfc tqlnraltgi aveqdkntqe
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QIK50427

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Figure 23 (Cont.)

```
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     1201 qelgkyeqyi kwpwyiwlgf iagliaivmv timlccmtsc csclkgccsc gscckfdedd
     1261 sepvlkgvkl hyt (SEQ ID NO:26)

QHU79173
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      421 ynyklpddft gcviawnsnn ldskvggnyn ylyrlfrksn lkpferdist eiyqagstpc
      481 ngvegfncyf plqsygfqpt ngvgyqpyrv vvlsfellha patvcgpkks tnlvknkcvn
      541 fnfngltgtg vltesnkkfl pfqqfgrdia dttdavrdpq tleilditpc sfggvsvitp
      601 gtntsnqvav lyqdvnctev pvaihadqlt ptwrvystgs nvfqtragcl igaehvnnsy
      661 ecdipigagi casyqtqtns prrarsvasq siiaytmslg aensvaysnn siaiptnfti
      721 svtteilpvs mtktsvdctm yicgdstecs nlllqygsfc tqlnraltgi aveqdkntqe
      781 vfaqvkqiyk tppikdfggf nfsqilpdps kpskrsfied llfnkvtlad agfikqygdc
      841 lgdiaardli caqkfngltv lpplltdemi aqytsallag titsgwtfga gaalqipfam
      901 qmayrfngig vtqnvlyenq kllanqfnsa igkiqdslss tasalgklqd vvnqnaqaln
      961 tlvkqlssnf gaissvlndi lsrldkveae vqidrlitgr lqslqtyvtq qliraaeira
     1021 sanlaatkms ecvlgqskrv dfcgkgyhlm sfpqsaphgv vflhvtyvpa qeknfttapa
     1081 ichdgkahfp regvfvsngt hwfvtqrnfy epqiittdnt fvsgncdvvi givnntvydp
     1141 lqpeldsfke eldkyfknht spdvdlgdis ginasvvniq keidrlneva knlneslidl
     1201 qelgkyeqyi kwpwyiwlgf iagliaivmv timlccmtsc csclkgccsc gscckfdedd
     1261 sepvlkgvkl hyt (SEQ ID NO:25)

YP_009724390
1 mfvflvllpl vssqcvnltt rtqlppaytn sftrgvyypd kvfrssvlhs tqdlflpffs
       61 nvtwfhaihv sgtngtkrfd npvlpfndgv yfasteksni irgwifgttl dsktqslliv
      121 nnatnvvikv cefqfcndpf lgvyyhknnk swmesefrvy ssannctfey vsqpflmdle
      181 gkqgnfknlr efvfknidgy fkiyskhtpi nlvrdlpqgf saleplvdlp iginitrfqt
      241 llalhrsylt pgdsssgwta gaaayyvgyl qprtfllkyn engtitdavd caldplsetk
      301 ctlksftvek giyqtsnfrv qptesivrfp nitnlcpfge vfnatrfasv yawnrkrisn
      361 cvadysvlyn sasfstfkcy gvsptklndl cftnvyadsf virgdevrqi apgqtgkiad
      421 ynyklpddft gcviawnsnn ldskvggnyn ylyrlfrksn lkpferdist eiyqagstpc
      481 ngvegfncyf plqsygfqpt ngvgyqpyrv vvlsfellha patvcgpkks tnlvknkcvn
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Figure 23 (Cont.)

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 541 fnfngltgtg vltesnkkfl pfqqfgrdia dttdavrdpq tleilditpc sfggvsvitp
 601 gtntsnqvav lyqdvnctev pvaihadqlt ptwrvystgs nvfqtragcl igaehvnnsy
 661 ecdipigagi casyqtqtns prrarsvasq siiaytmslg aensvaysnn siaiptnfti
 721 svtteilpvs mtktsvdctm yicgdstecs nlllqygsfc tqlnraltgi aveqdkntqe
 781 vfaqvkqiyk tppikdfggf nfsqilpdps kpskrsfied llfnkvtlad agfikqygdc
 841 lgdiaardli caqkfngltv lpplltdemi aqytsallag titsgwtfga gaalqipfam
 901 qmayrfngig vtqnvlyenq klianqfnsa igkiqdslss tasalgklqd vvnqnaqaln
 961 tlvkqlssnf gaissvlndi lsrldkveae vqidrlitgr lqslqtyvtq qliraaeira
1021 sanlaatkms ecvlgqskrv dfcgkgyhlm sfpqsaphgv vflhvtyvpa qeknfttapa
1081 ichdgkahfp regvfvsngt hwfvtqrnfy epqiittdnt fvsgncdvvi givnntvydp
1141 lqpeldsfke eldkyfknht spdvdlgdis ginasvvniq keidrlneva knlneslidl
1201 qelgkyeqyi kwpwyiwlgf iagliaivmv timlccmtsc csclkgccsc gscckfdedd
1261 sepvlkgvkl hyt (SEQ ID NO:50)
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QII57161

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1 mfvflvllpl vssqcvnltt rtqlppaytn sftrgvyypd kvfrssvlhs tqdlflpffs
  61 nvtwfhaihv sgtngtkrfd npvlpfndgv yfasteksni irgwifgttl dsktqslliv
 121 nnatnvvikv cefqfcndpf lgvyyhknnk swmesefrvy ssannctfey vsqpflmdle
 181 gkqgnfknlr efvfknidgy fkiyskhtpi nlvrdlpqgf saleplvdlp iginitrfqt
 241 llalhrsylt pgdsssgwta gaaayyvgyl qprtfllkyn engtitdavd caldplsetk
 301 ctlksftvek giyqtsnfrv qptesivrfp nitnlcpfge vfnatrfasv yawnrkrisn
 361 cvadysvlyn sasfstfkcy gvsptklndl cftnvyadsf virgdevrqi apgqtgkiad
 421 ynyklpddft gcviawnsnn ldskvggnyn ylyrlfrksn lkpferdist eiyqagstpc
 481 ngvegfncyf plqsygfqpt ngvgyqpyrv vvlsfellha patvcgpkks tnlvknkcvn
 541 fnfngltgtg vltesnkkfl pfqqfgrdia dttdavrdpq tleilditpc sfggvsvitp
 601 gtntsnqvav lyqdvnctev pvaihadqlt ptwrvystgs nvfqtragcl igaehvnnsy
 661 ecdipigagi casyqtqtns prrarsvasq siiaytmslg aensvaysnn siaiptnfti
 721 svtteilpvs mtktsvdctm yicgdstecs nlllqygsfc tqlnraltgi aveqdkntqe
 781 vfaqvkqiyk tppikdfggf nfsqilpdps kpskrsfied llfnkvtlad agfikqygdc
 841 lgdiaardli caqkfngltv lpplltdemi aqytsallag titsgwtfga gaalqipfam
 901 qmayrfngig vtqnvlyenq klianqfnsa igkiqdslss tasalgklqd vvnqnaqaln
 961 tlvkqlssnf gaissvlndi lsrldkveae vqidrlitgr lqslqtyvtq qliraaeira
1021 sanlaatkms ecvlgqskrv dfcgkgyhlm sfpqsaphgv vflhvtyvpa qeknfttapa
1081 ichdgkahfp regvfvsngt hwfvtqrnfy epqiittdnt fvsgncdvvi givnntvydp
1141 lqpeldsfke eldkyfknht spdvdlgdis ginasvvniq keidrlneva knlneslidl
1201 qelgkyeqyi kwpwyiwlgf iagliaivmv timlccmtsc csclkgccsc gscckfdedd
1261 sepvlkgvkl hyt (SEQ ID NO:51)
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QIJ96493

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1 mfvflvllpl vssqcvnltt rtqlppaytn sftrgvyypd kvfrssvlhs tqdlflpffs
  61 nvtwfhaihv sgtngtkrfd npvlpfndgv yfasteksni irgwifgttl dsktqslliv
 121 nnatnvvikv cefqfcndpf lgvyyhknnk swmesefrvy ssannctfey vsqpflmdle
 181 vkqgnfknlr efvfknidgy fkiyskhtpi nlvrdlpqgf saleplvdlp iginitrfqt
 241 llalhrsylt pgdsssgwta gaaayyvgyl qprtfllkyn engtitdavd caldplsetk
 301 ctlksftvek giyqtsnfrv qptesivrfp nitnlcpfge vfnatrfasv yawnrkrisn
 361 cvadysvlyn sasfstfkcy gvsptklndl cftnvyadsf virgdevrqi apgqtgkiad
 421 ynyklpddft gcviawnsnn ldskvggnyn ylyrlfrksn lkpferdist eiyqagstpc
 481 ngvegfncyf plqsygfqpt ngvgyqpyrv vvlsfellha patvcgpkks tnlvknkcvn
 541 fnfngltgtg vltesnkkfl pfqqfgrdia dttdavrdpq tleilditpc sfggvsvitp
 601 gtntsnqvav lyqdvnctev pvaihadqlt ptwrvystgs nvfqtragcl igaehvnnsy
 661 ecdipigagi casyqtqtns prrarsvasq siiaytmslg aensvaysnn siaiptnfti
 721 svtteilpvs mtktsvdctm yicgdstecs nlllqygsfc tqlnraltgi aveqdkntqe
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Figure 23 (Cont.)

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 781 vfaqvkqiyk tppikdfggf nfsqilpdps kpskrsfied llfnkvtlad agfikqygdc
 841 lgdiaardli caqkfngltv lpplltdemi aqytsallag titsgwtfga gaalqipfam
 901 qmayrfngig vtqnvlyenq klianqfnsa igkiqdslss tasalgklqd vvnqnaqaln
 961 tlvkqlssnf gaissvlndi lsrldkveae vqidrlitgr lqslqtyvtq qliraaeira
1021 sanlaatkms ecvlgqskrv dfcgkgyhlm sfpqsaphgv vflhvtyvpa qeknfttapa
1081 ichdgkahfp regvfvsngt hwfvtqrnfy epqiittdnt fvsgncdvvi givnntvydp
1141 lqpeldsfke eldkyfknht spdvdlgdis ginasvvniq keidrlneva knlneslidl
1201 qelgkyeqyi kwpwyiwlgf iagliaivmv timlccmtsc csclkgccsc gscckfdedd
1261 sepvlkgvkl hyt (SEQ ID NO:52)
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RECOMBINANT MULTIVALENT INFLUENZA VIRUSES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of U.S. application No. 62/994,738, filed on Mar. 25, 2020, the disclosure of which is incorporated by reference herein.

SUMMARY

[0002] In one embodiment, this disclosure provides a coronavirus/influenza virus vaccine. In one embodiment, the vaccine employs a single-replication (SR; a 'single cycle' that results in transcription of vRNAs but no progeny virus production in vivo; that is, a single cycle virus is replication incompetent as a result of not being capable of producing progeny) platform in which the genome of an influenza virus virion lacks at least a portion of a coding region for at least one influenza virus protein, which influenza virus protein is supplied in trans in vitro in cell lines but is restricted to a single round of replication in wild-type cells. In one embodiment, the vaccine employs a M2 Single-Replication (M2SR) platform in which an influenza virus lacking the M2 ion channel protein replicates to high titers in cell lines expressing M2 but is restricted to a single round of replication in wild-type cells. In one embodiment the open reading frame for M2 in M2SR has one or more stop codons, or one or more stop codons and a deletion in M2, rendering the protein non-functional, e.g., the transmembrane domain and/or cytoplasmic domain, or both are not expressed. As described herein, this platform was modified to generate a bivalent coronavirus/influenza virus vaccine (called CoroFlu M2SR) that expresses a secreted version of the spike (S) protein of coronavirus, e.g., SARS-CoV-2.

[0003] In one embodiment, a vaccine provides a humoral, mucosal, innate, and/or cell-mediated immune response. In one embodiment, this disclosure provides a replication competent coronavirus/influenza virus vaccine. In one embodiment, the vaccine is inactivated, including chemically inactivated, e.g., with formalin or β-propiolactone.

[0004] In one embodiment, a coronavirus/influenza virus expresses a full-length spike protein, e.g., but does not express HA and/or NA. In one embodiment, a bivalent coronavirus/influenza virus expresses a truncated version of the spike protein, e.g., including the S1 or receptor-binding (RBD) domain of S, in addition to at least one other antigenic molecule such as influenza HA and/or NA. In vaccinated individuals, the full-length protein or a portion thereof, e.g., a secreted S protein, elicits protective antibodies against SARS-CoV-2 after administration to a vertebrate, e.g., a mammal such as a human. In one embodiment, the vaccine is a bivalent vaccine where the virus also expresses influenza virus HA and/or NA proteins or other microbial proteins. In one embodiment, a bivalent coronavirus/influenza virus expresses a full-length spike protein. In one embodiment, a bivalent coronavirus/influenza virus expresses a truncated version of the spike protein, e.g., including the S1 or RBD domain of S, and influenza HA and NA. In vaccinated individuals, the full-length protein or a portion thereof, e.g., a secreted S protein, elicits protective antibodies against SARS-CoV-2, while the influenza viral HA protein will elicit protective antibodies against influenza virus, after administration to a vertebrate, e.g., a mammal such as a human. In contrast to the majority of influenza and experimental coronavirus vaccines, this vaccine mimics the natural infection process and stimulates mucosal, innate, humoral, and/or cell-mediated immune responses.

[0005] In one embodiment, an isolated, single cycle recombinant influenza virus having at least seven viral segments selected from PA, PB1, PB2, NP, NS, M, HA or NA (or HEF) viral segments, one of which segments comprises sequences for a heterologous antigen is provided. In one embodiment, the heterologous antigen comprises coronavirus protein sequences. In one embodiment, the coronavirus protein sequences comprise spike protein sequences or a soluble portion thereof. In one embodiment, the portion comprises S1. In one embodiment, the portion comprises the receptor binding domain. In one embodiment, the spike protein sequences or a portion thereof have at least 80% amino acid sequence identity to one of SEQ ID Nos. 25-28 and 50-52 and 50-52. In one embodiment, the spike protein has 1 to 7 proline residues (see Hsieh et al., which is incorporated by reference herein), which in turn stabilize the protein pre-fusion, e.g., proline at position 817, 892, 895, 899, 912, 942, or 946. In one embodiment, the virus comprises eight viral segments. In one embodiment, the virus comprises nine viral segments, where the ninth segment comprises the coronavirus sequences, e.g., on a PB2 or NS segment that does not express PB2 or NS1 or NS2, respectively. In one embodiment, the virus comprises nine viral segments, where the ninth segment comprises the coronavirus sequences, e.g., on a PA, PB1, NA, or M segment that does not express PA, PB1, NA, or M1 or M2, respectively. In one embodiment, the virus is an influenza A virus. In one embodiment, the virus is an influenza B virus. In one embodiment, the virus is an influenza C virus. In one embodiment, the virus is an influenza D virus. In one embodiment, the sequences for a heterologous antigen are inserted into or replace at least some of the coding sequences for one of PA, P81, PB2, NP, NS1, NS2, M1. M2, HA or NA (or HEF), e.g., at least a portion of the coding sequences for the influenza virus protein are deleted. In one embodiment, sequences for a heterologous antigen are inserted into or replace at least some of the coding sequences for one of PB1, PB2, NA, or M2. In one embodiment, sequences for a heterologous antigen are inserted into or replace at least some of the coding sequences for one of NS1, NS2, HA, or PA. Cell lines employed to prepare such viruses provide one or more influenza proteins in trans so as to complement any non-functional proteins resulting from the deletion. In one embodiment, the sequences for a heterologous antigen are inserted into coding sequences in the viral segment of one of PA, PB1, PB2, NP, NS, M, HA or NA (or HEF) viral segments. In one embodiment, sequences for a heterologous antigen are inserted, e.g., up to 3 to 4 kb into, e.g., expressed as a fusion polypeptide, coding sequences for one of PB1, PB2, NS1, NS2, or M2. Cell lines employed to prepare such viruses may provide one or more influenza proteins in trans so as to complement any non-functional proteins resulting from the insertion. In one embodiment, the virus is a single cycle bivalent virus. In one embodiment, the virus is a single cycle trivalent virus. Multivalent viruses within the scope of this disclosure may express at least two of the following: homologous influenza HA and/or NA, heterologous influenza HA and/or NA, heterologous viral gene products such as coronavirus gene products, or other viral gene products useful to elicit a protective immune response, rhabdovirus

GP protein, e.g., VSV-G, a filovirus protein, e.g., Ebolavirus GP, an alphavirus protein, a lentivirus protein, a retrovirus protein, a paramyxovirus protein, a rhinovirus protein, a bunyavirus protein an arenavirus protein a flavivirus protein, or a rhabdovirus protein, fungal gene products, or bacterial gene products. For example, a HA viral segment employed in the virus may replace the HA coding region with VSV-G coding sequences, or other host cell binding sequences, a NA viral segment employed in the virus may replace NA coding sequences with sequences from those from a paramyxovirus, e.g., type 3; heterologous antigenic coding sequences may be added to a viral coding region, e.g., added to the open reading frame for PB2 or NA, or may replace PB2 coding sequences. In one embodiment, the M viral segment is mutated so that upon viral replication the mutant M gene expresses a functional M1 protein and a mutant M2 protein with a deletion of the cytoplasmic tail and either lacking a transmembrane domain or having a mutated transmembrane domain, wherein the replication of the recombinant virus in vivo is limited to a single cycle (e.g., no progeny viruses are produced) relative to a corresponding influenza virus with a wild-type M viral segment. In one embodiment, the M2 coding region is modified to include one or more stop codons, e.g., at or near the splice site(s), and may also include a deletion of, e.g., downstream, coding sequences so as to result in a truncated M2 protein. In one embodiment, the mutant M2 protein comprises the M2 extracellular domain. In one embodiment, the M2 extracellular domain comprises less than 24 residues. In one embodiment, the M2 extracellular domain comprises at least 9 residues. In one embodiment, the mutation in the transmembrane domain comprises at least one amino acid substitution. In one embodiment, the mutation in the transmembrane domain comprises a deletion in the transmembrane domain. In one embodiment, the deletion in the transmembrane domain includes residues 29 to 31. In one embodiment, the deletion in the transmembrane domain comprises at least 10 residues. In one embodiment, the deletion in the M2 protein deletes the cytoplasmic tail which protein in turn when present in a virus, results in an attenuated virus. In one embodiment, one or more of the PA, PB1, PB2, NP, NS, and M viral segments have selected amino acid residues at positions 30, 31, 105, 142, 149, 225, 356, 357, 401, and/or 550 in PA: positions 40, 54, 59, 62, 63, 75, 76, 78, 79, 80, 112, 180, 247, 327, 507, 624, 644, 667, 694, 695, 697.699, 700, 701, 702, 705, 713, and/or 714 in PB1; positions 57, 58, 59, 61, 66, 202, 323, 368, 391, 504, 591, 677, 678, and/or 679, in PB2; positions 74, 112, 116, 224, 293, 371, 377, 417, 422 or 442 in NP; positions 90, 97 and/or 100 in M1; or positions 30, 49, 55, 118, 140, 161 and/or 223 in NS1. In one embodiment, the virus is bivalent. In one embodiment, the PB1 viral segment encodes a polypeptide having a residue other than glycine, serine, serine, glutamine or asparagine at position 62, 261, 361, 621, and/or 654 in PB1 or a residue other than arginine at position 81 in F2. In one embodiment, the virus is in a vaccine formulation. In one embodiment, the vaccine comprises influenza A HA, e.g., H1, H3, H5 or H7 HA. In one embodiment, the HA in the recombinant virus is modified at the HA cleavage site. In one embodiment, the vaccine further comprises a different influenza virus. In one embodiment, the vaccine further comprises two different influenza viruses.

[0006] For example, in one embodiment, to prepare recombinant virus, vectors for vRNA or cRNA are introduced to host cells expressing M2 in trans. One of vectors has sequences for the M segment and that segment is modified so that functional M2 is not expressed from that segment. In one embodiment, one or two stop codons are introduced, and optionally some M2 coding sequences are deleted. One of the vectors for vRNA or cRNA has the heterologous antigen sequences. For example, the heterologous antigen sequences may be inserted at the end of the coding region for NS1.

[0007] In another embodiment, to prepare recombinant virus, vectors for vRNA or cRNA are introduced to host cells expressing PB2 in trans. One of the vectors for vRNA or cRNA has the heterologous antigenic sequences. For example, the heterologous antigen sequences may be inserted at the end of the coding region for NS1 or into the coding sequences for PB2. One of vectors has sequences for the PB2 segment and that segment is modified so that functional PB2 is not expressed from that segment. In one embodiment, at least some PB2 coding sequences are deleted. In one embodiment, the modified PB2 viral gene segment includes 5' and/or 3' PB2 viral non-coding and coding incorporation sequences, optionally flanking a heterologous nucleotide sequence, and does not include contiguous sequences corresponding to sequences encoding a functional PB2. In one embodiment, the heterologous nucleotide sequence is about 30 to about 5,000, e.g., about 100 to about 4,500 or about 500 to about 4,000, nucleotides in length. In one embodiment, the deletion of PB2 coding sequences includes 1 or more contiguous or noncontiguous nucleotides of PB2 and may include a deletion of the entire coding region, e.g., a region encoding 759 amino acids. In one embodiment, the deletion includes at least 10%, 30%, 40%, 50%, 70%, 80%, 85%, 90%, 93%, 95% and up to 99%, or a percent numerical value that is any integer between 10 and 99, but not all, of the PB2 coding region. In one embodiment, the deletion of PB2 coding sequences does not include the deletion of 5' or 3' coding sequences that enhance incorporation of the resulting viral gene segment into virions, e.g., sequences that are contiguous to 3' or 5' non-coding PB2 sequences, relative to a recombinant viral gene segment with only non-coding PB2 incorporation sequences. For instance, if present in the PB2 segment, the heterologous nucleotide sequence may encode coronavirus sequences, and may be flanked by about 3 to about 400 nucleotides of the 5' and/or 3' PB2 coding region adjacent to non-coding sequence. In one embodiment, the 3' PB2 incorporation sequences correspond to nucleotides 3 to 400, nucleotides 3 to 300, nucleotides 3 to 100, nucleotides 3 to 50, or any integer between 3 and 400, of the N-terminal and/or C-terminal PB2 coding region. In one embodiment, the heterologous nucleotide sequence is flanked by about 3 to about 400 nucleotides of the 5' and/or 3' PB2 coding region adjacent to non-coding sequence. In one embodiment, the heterologous nucleotide sequence is flanked by about 100 to about 300, or 120 to about 150 nucleotides of the 5' and/or 3' PB2 terminal sequences which include coding and non-coding sequences. In one embodiment, heterologous sequences, e.g., antigenic sequences for a virus other than influenza virus or for an influenza virus protein from an isolate other than the strain employed to provide the internal viral segments or for the HA and NA viral segments, may be inserted at the end of the coding region for NS1.

[0008] In one embodiment, a method to immunize a vertebrate is provided. The method includes administering

to the vertebrate the vaccine. In one embodiment, the vertebrate is an avian. In one embodiment, the vertebrate is a mammal. In one embodiment, the vertebrate is a human. In one embodiment, the vaccine is intranasally administered. In one embodiment, the vaccine is intramuscularly administered.

[0009] In one embodiment, the internal viral segments (PA, PB1, PB2, NS, M, and NP viral segments) are from a vaccine strain, e.g., the PR8/UW, PR8HY or PR8/Cambridge strain. In one embodiment, the internal viral segments may be modified to enhance replication in host cell used to generate the vaccine. In one embodiment, in addition to the presence of certain amino acid residues in the coding regions of six internal viral segments, e.g., relative to PR8HY or the PR8/Cambridge strain, mutations in non-coding regions were observed to increase viral liters, 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. 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.

[0010] 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.

[0011] The invention provides isolated recombinant, e.g., reassortant, influenza viruses with, e.g., 7, 8 or 9 viral segments, one of which includes sequences for a microbial pathogen. e.g., sequences for a coronavirus spike protein, or a portion thereof. In one embodiment, the coronavirus sequences replace influenza virus sequences. e.g., replace coding sequences in one of PA, PB1, PB2, NP, M (encoding M1 and M2 proteins), NS (encoding NS1 and NS2 proteins), HA or NA (or HEF) viral segments. In one embodiment, the coronavirus sequences replace influenza virus sequences, e.g., replace coding sequences in one of PA, PB1, PB2, NP, M (encoding M1 and M2 proteins), or NS (encoding NS1 and NS2 proteins). In one embodiment, the coronavirus sequences are inserted into influenza virus sequences, e.g., into coding sequences in one of PA, PB1, PB2, NP, M (encoding M1 and M2 proteins), NS (encoding NS1 and NS2 proteins), HA or NA (or HEF) viral segments in influenza A viral segments. In one embodiment, the coronavirus sequences are inserted into, e.g., fused to, influenza virus coding sequences, e.g., into coding sequences for one of PA. PB1, PB2, NP, M (encoding M1 and M2 proteins), or NS (encoding NS1 and NS2 proteins). In one embodiment, the coronavirus sequences are expressed as a fusion with influenza virus protein sequences, e.g., a fusion with PA, PB1, PB2, NP, M1, M2, NS1, or NS2 proteins. In one embodiment, the coronavirus sequences are inserted into influenza virus coding sequences and the resulting fusion polypeptide is cleaved to release the coronavirus S protein sequences. For example, coronavirus coding sequences flanked by protease recognition sequences, e.g., self-cleaving sites such as those from foot and mouth disease or 2A sequences, for example, T2A (EGRGSLLTCGDVEENPGP; SEQ ID NO:53), P2A (ATNFSLLKQAGDVEENPGP; SEQ ID NO:54), E2A (QCTNYALLKLAGDVESNPGP; SEQ ID NO:55) or F2A (VKQTLNFDLLKAGDVESNPGP; SEQ ID NO:56) sequences, are inserted into the NS viral segment, e.g., between NS1 and NS2 coding sequences. In one embodiment, coronavirus sequences are introduced to a viral segment that, in the recombinant virus, is a ninth viral segment, where the other eight segments are the PA, PB1, PB2, NP, M, NS, HA and NA viral segments. In one embodiment, the M viral segment encodes a truncated M2 protein. In one embodiment, the coronavirus sequences replace coding sequences, e.g., PB1 or PB2 coding sequences. In one embodiment, the coronavirus sequences encode a protein having at least 80%, 82%, 84%, 85%, 87%, 90%, 92%, 94%, 95%, 97%, 98%, 99% or more amino acid sequence identity with one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the coronavirus sequences encode a S1 protein having at least 80%, 82%, 84%, 85%, 87%, 90%, 92%, 94%, 95%, 97%, 98%, 99% or more amino acid sequence identity with S1 in one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the coronavirus sequences encode a RBD having at least 80%, 82%, 84%, 85%, 87%, 90%, 92%, 94%, 95%, 97%, 98%, 99% or more amino acid sequence identity with the RBD in one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the coronavirus sequences encode a protein having at least 80%, 82%, 84%, 85%, 87%, 90%, 92%, 94%, 95%, 97%, 98%, 99% or more amino acid sequence identity with S1 in one of SEQ ID NOS. 25-28 and 50-52. In one embodiment, the coronavirus sequences encode a protein having at least 80%, 82%, 84%, 85%, 87%, 90%, 92%, 94%, 95%, 97%, 98%, 99% or more amino acid sequence identity with the RBD in one of SEQ ID Nos. 25-28 and 50-52.

[0012] In one embodiment, the influenza virus is a recombinant influenza virus 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.

[0013] In one embodiment, the influenza virus is a recombinant influenza virus 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. 1-8 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, R74 I112, I116, J442 or N417 in NP, V97 and/or Y100 in M1, and/or K55 or R140 in NS1.

[0014] In one embodiment, the influenza virus is a recombinant influenza virus 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. 1-8 or 10-15, e.g., a PB2 viral segment with a residue other than isoleucine and that is a conservative substitution for isoleucine at residue 504; a PB1 viral segment with a non-conservative substitution for E112; a PA viral segment with a substitution for S225; a NP viral segment with a conservative substitution for R74 and N417; a M viral segment with a conservative substitution for V97 and a non-conservative substitution for Y100; and a NS viral segment with a non-conservative substitution for K55.

[0015] In one embodiment, the influenza virus is a recombinant influenza virus 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 viral segment with a non-conservative substitution for M202 and F323; a PB1 viral segment with a non-conservative substitution for Q247; a PA viral segment with a non-conservative substitution for K142; a NP viral segment with a conservative substitution for R74; a M viral segment with a conservative substitution for V97 and a non-conservative substitution for Y100; and a NS viral segment with a conservative substitution for K55E.

[0016] In one embodiment, the influenza virus is a recombinant influenza virus 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 1504; 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 viral segment with a conservative substitution for A30 or R118.

[0017] In one embodiment, the influenza virus is a recombinant influenza virus 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 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 non-conservative substitution relative to K142 in PA, Q247 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.

[0018] Viral segments for PA, PB1, PB2, NP, M and/or NS may be combined with a viral segment for HA, e.g., H1. H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, or H18 and a viral 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. In one embodiment, the HA is H1, H5 or H7. In one embodiment the NA is N1 or N9. In one embodiment, the HA viral segment in the recombinant or reassortant virus is heterologous to the viral segments for PA, PB1, PB2, NP, M and NS. In one embodiment, the NA viral segment in the recombinant or reassortant virus is heterologous to the viral segments for PA, PB1, PB2, NP, M and NS. In one embodiment, the HA viral segment in the recombinant or reassortant virus has viral 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 viral 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 viral segments with sequences corresponding to SEQ ID Nos. 1-6 or 10-15. In one embodiment, the HA viral segment in the recombinant or reassortant virus is a chimeric HA viral 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 viral segment of the parent isolate or strain, or variant thereof. In one embodiment, the NA viral segment in the isolated recombinant virus is a chimeric NA viral segment e.g., a chimera of heterologous NA ectodomain sequences linked to NA transmembrane domain sequences from the NA viral 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 viral segment in the isolated recombinant virus is a chimeric NA viral segment e.g., a chimera of heterologous NA ectodomain sequences linked to NA transmembrane domain sequences from the NA viral 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 viral segment, a heterologous NA viral segment, a chimeric HA viral segment, a chimeric NA viral segment, or any combination thereof. The nucleic acid sequences employed to prepare vRNA or cRNA 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. Other reassortants with internal genes from other PR8 isolates or other vaccine viruses may be employed in recombinant reassortant viruses.

[0019] Vaccine viruses may be grown or passaged in cells in culture, e.g., MDCK or Vero cells or eggs. In one embodiment, the cells are canine or primate, e.g., human or monkey, cells.

[0020] The invention provides a plurality of influenza virus vectors, 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 is an influenza virus with 6 internal viral segments from a vaccine virus, a NA viral segment from a different (second) viral isolate, and a HA viral segment from a third isolate; a 6:2 reassortant within the scope of the present invention is an influenza virus with 6 internal viral segments from a vaccine virus, and a NA viral segment and a HA viral 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

viral segments and a NA viral segment from a vaccine virus, and a HA viral segment from a different viral source than the vaccine virus, or an influenza virus with 6 internal viral segments and a HA viral segment from the vaccine virus, and a NA viral segment is from a different viral source than the vaccine virus.

[0021] In one embodiment, the plurality of vectors includes vectors for vRNA or cRNA 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 or cRNA 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, e.g., humanized 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 or cRNA production of NA may be from any NA, e.g., any of N1-N11, and the DNA for vRNA or cRNA production of HA may be from any HA, e.g., H1-H18. In one embodiment, the DNAs for vRNA or cRNA production may be for an influenza B, C or D virus. The DNAs for vRNA or cRNA 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.

[0022] Viruses within the scope of the invention include viruses that have high titers in, for example, MDCK cells, e.g., titers of at least about 105 PFU/mL, e.g., at least 10^8 PFU/mL, 107 PFU/mL or 10^8 PFU/mL; high titers in embryonated eggs, e.g., titers of at least about 107 EID_{50}/mL, e.g., at least 10^8 EID_{50}/mL, 109 EID_{50}/mL or 10^{10} EID_{50}/mL; high titers in cells such as MDCK cells. e.g., titers of at least about 107 PFU/mL, e.g., at least 10^8 PFU/mL, or high titers in two of more of those host cells.

[0023] 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 viral 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.

[0024] 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 or cRNA, both native and recombinant vRNA or cRNA. The vectors comprise influenza cDNA, e.g., influenza A (e.g., any influenza A gene including any of the 18 HA or 11 NA subtypes), B, C or D DNA (see Fields *Virology* (Fields et al. (eds.), 7th edition, Wolter, Kluwer (2020), 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.

[0025] A composition or plurality of vectors comprises a heterologous gene or open reading frame of interest, e.g., a foreign gene encoding an immunogenic peptide or protein useful as a vaccine. 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 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 or cRNA corresponding to the heterologous sequences of the vector.

[0026] The promoter in a vector for vRNA or cRNA 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, tetrahymena 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.

[0027] The promoter or transcription termination sequence in a vRNA, cRNA 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 or cRNA 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.

[0028] In one embodiment, at least one vector for vRNA or cRNA 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 or cRNA 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 or cRNA vector may be the same or different as the RNA polymerase II promoter in any other vRNA or cRNA vector. Similarly, each ribozyme sequence in each vRNA or cRNA vector may be the same or different as the ribozyme sequences in any other vRNA or cRNA vector. In one embodiment, the ribozyme sequences in a single vector are not the same.

[0029] In one embodiment, the invention provides a plurality of influenza virus vectors for a recombinant or reassortant virus comprising a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PB1 DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PB2 DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus HA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NP DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus M DNA linked to a transcription termination sequence, and a vector for vRNA or cRNA 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 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.

[0030] 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 or cRNA production vector is on a separate plasmid. In one embodiment, each mRNA production vector is on a separate plasmid.

[0031] 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, cRNA or protein production vectors, prior to other vectors, either vRNA or protein production vectors. In one embodiment, the promoter for vRNA or cRNA 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 or cRNA vector employed in the method is on a separate plasmid. In one embodiment, the vRNA or cRNA 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.

[0032] 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 coronavirus, AIDS, influenza, hepatitis B, hepatitis C, rhinovirus, filoviruses, malaria, herpes, and foot and mouth disease).

[0033] The invention also provides isolated viral polypeptides, and methods of preparing and using a 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 live attenuated or single cycle influenza virus, and a mucosal adjuvant may induce virus-specific IgA and neutralizing antibody in the nasopharynx as well as serum IgG.

[0034] The influenza virus of the invention may employed with other anti-virals, e.g., protease inhibitors, for instance, remdesivir, anti-malarials, e.g., chloroquine, 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.

BRIEF DESCRIPTION OF THE FIGURES

[0035] FIGS. **1**A-**1**E. Nucleotide sequence for PR8(Cambridge) genes (SEQ ID NOs:10-15).

[0036] FIG. **2**: Overview of library passages and the identification of high-yield candidates.

[0037] FIG. **3**. Number of clones with random mutations having specified HA titers.

[0038] FIG. **4**. Titers of clones having selected mutations.

[0039] FIGS. **5**A-**5**D. Growth curves of UW-PR8 viruses possessing previously identified mutations in PB2 (A), PB1 (B), PA (C), and NP, M or NS1 (D).

[0040] FIG. **6**. Summary of mutations that confer high replicative property in MDCK cells.

[0041] FIGS. **7**A-**7**B. A) Virus stocks were tested for HA titers (in 2^n) and virus titers (in PFU/mL). B) Growth curves in MDCK cells.

[0042] FIGS. **8**A-**8**C. A) HA titer of wild type (UW-PR8) and clone #4. B) Viral protein for wild type (UW-PR8) and #4. C) SDS-PAGE analysis of viral proteins of wild type and #4.

[0043] FIGS. **9**A-**9**B. A) Comparison of titers of wild type virus (UW-PR8) and high replicative virus with mutations in M1. B) Growth kinetics of wild type virus (UW-PR8) and high replicative virus with mutations in M1.

[0044] FIGS. **10**A-**10**M. A) Codon usage table for canines. B) Relative adaptiveness of wild type (UW-PR8) and "rare" codon optimized PB2 viruses. C) Relative adaptiveness of wild type (UW-PR8) and 'all' codon optimized PB2 viruses. D) Growth kinetics of PB2 codon optimized viruses. E) Growth kinetics of viruses with codon optimized PB2, PB1, PA, or NP viral segment or combinations of segments. F) Sequence of PB2, PB1, PA and NP viral segments of UW-PR8 and sequence of canine codon-usage optimized PB2, PB1, PA and NP viral segments of UW-PR8 (SEQ ID NOs: 3, 13, 2, 12, 1, 11, 4).

[0045] FIGS. **11**A-**11**C. A) Nucleotide position 4 of each gene of PR8 and Indo/NC/09. B) All 3'C4U mutant. C) Growth kinetics of a recombinant UW-PR8 virus encoding 'C' at position 4 of the PB2, PB1, and PA genes (black), and a mutant encoding 'U' at position 4 of all eight segments (red).

[0046] FIG. **12**A-**12**C. Nucleotide and amino acid sequences for H7 and N9 which are exemplary sequences for use with the internal viral segment sequences disclosed herein useful to provide high titer influenza viruses for vaccines (SEQ ID NOs: 17-24).

[0047] FIGS. **13**A-**13**B. A) Schematic of chimeric HA and NA genes to increase virus titer. B) Growth kinetics of chimeric viruses.

[0048] FIGS. **14**A-**14**B. A) Growth kinetics of viruses with combinations of mutations. B) PFU and HA titers of viruses with combinations of mutations.

[0049] FIG. **15**. Screening in eggs.

[0050] FIG. **16**. HA titers of 216 clones isolated from Vero cells.

[0051] FIG. **17**. Recombinant viruses generated with different PR8 backbone mutations.

[0052] FIG. **18**A-**18**B. Overview of generation of viruses with enhanced growth in MDCK cells and Vero cells.

[0053] FIGS. **19**A-**19**D. Exemplary high yield substitutions (relative to PR8 (UW)).

[0054] FIG. **20**. Growth kinetics and HA titers of reassortant viruses possessing one or several vRNAs of PR8-HY virus.

[0055] FIG. **21**. Viral polymerase activity in mini-replicon assays in 293T, MDCK, Vero, and DF1 cells. The PB2, PB1, PA, and NP proteins were derived from UW-PR8 wild-type (WT) virus or from the high-yield PR8-HY (HY) variant.

[0056] FIG. **22**. Exemplary method to prepare bivalent viruses.

[0057] FIG. **23**. Exemplary SARS-CoV-2 spike (S) protein sequences (SEQ ID Nos. 25-28 and 50-52). The S1 portion of S is generally from residues 1 to 681 and the receptor binding domain in S1 is within residues 330 to 521 (see, e.g., Wrapp et al., *Science,* 67:1260 (2020), the disclosure of which is incorporated by reference herein).

DETAILED DESCRIPTION

Definitions

[0058] 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.

[0059] 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.

[0060] As used herein, "substantially free" means below the level of detection for a particular infectious agent using standard detection methods for that agent.

[0061] 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.

[0062] 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.

[0063] As used herein, a "heterologous" influenza virus gene or viral segment is from an influenza virus source that is different than a majority of the other influenza viral genes or viral segments in a recombinant, e.g., reassortant, influenza virus.

[0064] 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.

[0065] 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.

[0066] 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.

[0067] Computer implementations of 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.

[0068] 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 acid 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.

[0069] 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.nlm.nih.gov. Alignment may also be performed manually by inspection.

[0070] 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

[0071] 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.

[0072] 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 viral segment with both NA and NB sequences. Influenza C virus and Influenza D virus have only seven viral segments.

Cell Lines that can be Used in the Present Invention

[0073] 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, e.g., humanized MDCK cells (see U.S. application Ser. No. 16/785,449, filed on Feb. 7, 2020, which is incorporated herein by reference) or M2 expressing cell line (see Itwasuki et al., *J. Virol.,* 80:5233 (2006), the disclosure of which is incorporated by reference herein), 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.

[0074] 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).

[0075] 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

[0076] 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.

[0077] 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 or beta-propiolactone, for instance.

[0078] 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 (Laver & 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 a virus of the invention in a multivalent vaccine.

[0079] 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 a virus of the invention in a multivalent vaccine.

[0080] 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.

[0081] 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.

[0082] Live Attenuated Virus Vaccines. Live, attenuated influenza virus vaccines, can be used for preventing or treating influenza virus infection. In one embodiment, 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 may be derived from an attenuated parent. In this approach, genes that confer attenuation generally do not code for the HA and NA glycoproteins.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] The viruses in a multivalent vaccine can thus be attenuated, single cycle (live) 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, live single cycle 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 attenuated viruses.

Pharmaceutical Compositions

[0087] 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, live single cycle 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).

[0088] 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).

[0089] 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.

[0090] 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.

[0091] 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.

[0092] 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, foscamet, phosphonoacetic acid, acyclovir, dideoxynucleosides, a protease inhibitor, or ganciclovir.

[0093] 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

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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

[0099] 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 or single cycle 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.

[0100] 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).

[0101] 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.

[0102] A composition having at least one influenza virus of the present invention, including one which is single cycle, attenuated or inactivated 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.

[0103] 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.

[0104] 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.

[0105] 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.

[0106] 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^{18}, 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.

[0107] 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.

[0108] 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.

[0109] In one embodiment, the vaccine generally contains about 0.1 to 200 μg, e.g., 30 to 100 μg, 0.1 to 2 μg, 0.5 to 5 μg, 1 to 10 μg, 10 μg to 20 μg, 15 μg to 30 μg, or 10 to 30 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).

[0110] In one embodiment, the dosage of a live, attenuated or killed virus vaccine for an animal such as a mammalian adult organism may be from about 10^{20}-10^{20}, e.g., 10^3-10^{12}, 10^2-10^{10}, 10^5-10^{10}, 10^5-10^{15}, 10^2-10^{10}, or 10^{15}-10^{20} plaque forming units (PFU)/kg, or any range or value therein. The dose of one viral isolate vaccine, e.g., in an inactivated vaccine, may range from about 0.1 to 1000, e.g., 0.1 to 10 μg, 1 to 20 μg, 30 to 100 μg, 10 to 50 μg, 50 to 200 μg, or 150 to 300 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.

[0111] In one embodiment, the dosage of immunoreactive HA in each dose of replicated virus vaccine may be standardized to contain a suitable amount, e.g., 0.1 μg to 1 μg, 0.5 μg to 5 μg, 1 μg to 10 μg, 10 μg to 20 μg, 15 μg to 30 μg, or 30 μg 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.

[0112] 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 >3 years of age, and 7.5 μg per component for children <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 contain approximately 0.1 to 0.5 billion viral particles, 0.5 to 2 billion viral particles, 1 to 50 billion virus particles, 1 to 10 billion viral particles, 20 to 40 billion viral particles, 1 to 5 billion viral particles, or 40 to 80 billion viral particles.

Exemplary Embodiments for High Growth PR8 or Cambridge Variants

[0113] In one embodiment, the invention provides an isolated recombinant influenza virus having PA, PB1, PB2, NP, NS, and M viral segments from a first influenza vaccine virus isolate, a heterologous influenza virus NA viral segment, and a heterologous HA viral segment, wherein two or more of the PA, PB1, PB2, NP, NS, and M viral segments have selected amino acid residues at positions 30, 31, 105, 142, 149, 225, 356, 357, 401, and/or 550 in PA; positions 40, 54, 59, 62, 63, 75, 76, 78, 79, 80, 112, 180, 247, 327, 507, 624, 644, 667, 694, 695, 697, 699, 700, 701, 702, 705, 713, and/or 714 in PB1; positions 57, 58, 59, 61, 66, 202, 323, 368, 391, 504, 591, 677, 678, and/or 679, in PB2; positions 74, 112, 116, 224, 293, 371, 377, 417, 422 or 442 in NP; positions 90, 97 and/or 100 in M1; or positions 30, 49, 55, 118, 140, 161 and/or 223 in NS1. In one embodiment, the isolated virus has 142N, 225C, 358R, or 550L in PA; has one or more of 112G, 247H, 507V, or 644A in PB1; has one or more of 202L, 323L or 504V in PB2; has one or more of 74K, 112L, 116L, 417D, or 442A in NP; 97A and/or 100H in M1; and/or 55E and/or 140Q in NS1, or combinations thereof, e.g., has at least one of 202L and/or 323L in PB2, 247H in PB1 or 74K in NP and optionally at least one of 142N in PA1, 55K in NS1 or 97A and/or 100H in M1 or has at least one of 202L and/or 323L in PB2, 247H in PB1 or 74K in NP and at least one of 142N in PA1, 55K in NS1 or 97A and/or 100H in M1. In one embodiment, the virus has at least one of 202L and/or 323L in PB2, 247H in PB1 or 74K in NP and optionally at least one of 142N in PA1, 55K in NS1 or 97A and/or 100H in M1. In one embodiment, the virus has at least one of 202L and/or 323L in PB2, 247H in PB1 or 74K in NP and at least one of 142N in PA1, 55K in NS1 or 97A and/or 100H in M1. In one embodiment, the isolated virus has 202L and/or 323L in PB2, and optionally has 247H in PB1 and optionally 74K in NP. In one embodiment, the isolated virus has 247H in PB1 and optionally 74K in NP. In one embodiment, the isolated virus has 401, 40L,

112G, 180W, 247H, 507V, or 644A in PB1 and optionally has 202L and/or 323L in PB2, and optionally has 74K, 112L, 116L, 377N, 417D, or 422L in NP, and optionally has 30P, 118K, 161T or 140Q in NS1, and optionally has 142N, 225C, 356R, 401K, or 550L in PA. In one embodiment, the isolated virus has 401, 40L, 112G, 180W, 247H, 507V, or 644A in PB1. In one embodiment, the isolated virus has 202L and/or 323L in PB2. In one embodiment, the isolated virus has 74K, 112L, 116L, 377N, 417D, or 422L in NP. In one embodiment, the isolated virus has 30P, 118K, 161T or 140Q in NS1. In one embodiment, the isolated virus has 142N, 225C, 356R, 401K, or 550L in PA. In one embodiment, the selected amino acid residues at specified positions in the PA is/are at position(s) 97, 105, 142, 149, 225, 356, 357, 401, 404, and/or 421. In one embodiment, the selected amino acid residues at specified positions in the PB1 is/are at position(s) 12, 40, 54, 59, 62, 63, 66, 75, 76, 78, 79, 80, 180, 247, 507, 624, 644, 694, 695, 697, 699, 700, 701, 705, 713, 714, and/or 762. In one embodiment, the selected amino acid residues at specified positions in the PB2 is/are at position(s) 57, 58, 59, 61, 68, 202, 243, 323, 504, 677, 678, and/or 679. In one embodiment, the selected amino acid residues at specified positions in the NP is/are at position(s) 74, 112, 116, 224, 293, 417, and/or 442. In one embodiment, the selected amino acid residues at specified positions in the M1 is/are at position(s) 90, 97, and/or 100. In one embodiment, the selected amino acid residues at specified positions in the NS1 is/are at position(s) 49, 30, 55, 161, and/or 223. In one embodiment, the selected amino acid residues at specified positions in the PA is/are at position(s) 97, 105, 142, 149, 225, 356, 357, 401, 404, and/or 421; and optionally the selected amino acid residues at specified positions in the PB1 is/are at position(s) 12, 40, 54, 59, 62, 63, 66, 75, 76, 78, 79, 80, 180, 247, 507, 624, 644, 694, 695, 697, 699, 700, 701, 705, 713, 714, and/or 762, in any combination with the selected residues for PA; and optionally the selected amino acid residues at specified positions in the PB2 is/are at position(s) 57, 58, 59, 61, 66, 202, 243, 323, 504, 677, 678, and/or 679 in any combination with the selected residues for PA and/or PB1; and optionally the selected amino acid residues at specified positions in the NP is/are at position(s) 74, 112, 116, 224, 293, 417, and/or 442 any combination with the selected residues for PA, PB1 and/or PB2; and optionally the selected amino acid residues at specified positions in the M1 is/are at position(s) 90, 97, and/or 100 any combination with the selected residues for PA, PB1, PB2, and/or NP; and optionally the selected amino acid residues at specified positions in the NS1 is/are at position(s) 49, 30, 55, 161, and/or 223, or in any combination with the selected residues for PA, PB1, PB2, NP, and/or M1.

[0114] For any of the exemplary viruses disclosed above, in one embodiment, 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 ID 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 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.

[0115] For any of the exemplary viruses disclosed above, in one embodiment, at least one of the PA, PB1, PB2, NP, NS, and M viral segments has a C to U promoter mutation.

[0116] Any of the isolated viruses disclosed herein may be employed in a vaccine.

[0117] In one embodiment, the invention provides a plurality of influenza virus vectors for preparing a reassortant. In one embodiment, the plurality includes a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PB1 DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PB2 DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus HA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NP DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus M DNA linked to a transcription termination sequence, and a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the PB1, PB2, PA, NP, NS, and M DNAs in the vectors for vRNA or cRNA production are from one or more influenza vaccine virus isolates, wherein the NA DNA in the vector for vRNA production of NA has sequences for a heterologous NA, and wherein the HA DNA in the vector for vRNA or cRNA production of HA has sequences for a heterologous HA, 30, 31, 105, 142, 149, 225, 356, 357, 401, and/or 550 in PA; 40, 54, 59, 62, 63, 75, 76, 78, 79, 80, 112, 180, 247, 327, 507, 624, 644, 667, 694, 695, 697, 699, 700, 701, 702, 705, 713, or 714 and/or 247 in PB1; 57, 58, 59, 61, 66, 202, 323, 368,

391, 504, 591, 677, 678, or 679, 202 and/or 323 in PB2; 74, 112, 116, 224, 293, 371, 377, 417, 422 and/or 442 in NP; 90, 97 and/or 100 in M1; or 30, 49, 55, 118, 140, 161 and/or 223 in NS; 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, the PB1, PB2, PA, NP, NS, and M DNAs in the vectors for vRNA or cRNA production have a sequence corresponding to one that encodes a polypeptide having at least 95% amino acid sequence identity to a corresponding polypeptide encoded by SEQ ID NOs:1-6 or 10-15. In one embodiment, the promoter for vRNA or cRNA vectors is a RNA polymerase I promoter, a RNA polymerase II promoter, a RNA polymerase Ill promoter, a T3 promoter or a T7 promoter. In one embodiment, the NA is N9. In one embodiment, the HA is H7. In one embodiment, the PA, PB1, PB2, NP, NS, and/or M viral segments has/have a promoter C to a mutation.

[0118] In one embodiment, the invention provides a method to prepare influenza virus. The method includes contacting a cell with: a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PB1 DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus PB2 DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus HA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NP DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NA DNA linked to a transcription termination sequence, a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus M DNA linked to a transcription termination sequence, and a vector for vRNA or cRNA production comprising a promoter operably linked to an influenza virus NS DNA linked to a transcription termination sequence, wherein the PB1, PB2, PA, NP, NS, and M DNAs in the vectors for vRNA or cRNA production are from one or more influenza vaccine virus isolates, wherein the NA DNA in the vector for vRNA or cRNA production of NA has sequences for a heterologous NA, and wherein the HA DNA in the vector for vRNA or cRNA production of HA has sequences for a heterologous HA, 30, 31, 105, 142, 149, 225, 356, 357, 401, and/or 550 in PA; 40, 54, 59, 62, 63, 75, 76, 78, 79, 80, 112, 180, 247, 327, 507, 624, 644, 667, 694, 695, 697, 699, 700, 701, 702, 705, 713, and/or 714 and/or 247 in PB1; 57, 58, 59, 61, 66, 202, 323, 368, 391, 504, 591, 677, 678, and/or 679, 202 and/or 323 in PB2; 74, 112, 116, 224, 293, 371, 377, 417, 422 and/or 442 in NP; 90, 97 and/or 100 in M1; or 30, 49, 55, 118, 140, 161 or 223 in NS; 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 an amount effective to yield infectious influenza virus. In one embodiment, the cell is an avian cell or a mammalian cell, e.g., a Vero cell, a human cell or a MDCK cell. In one embodiment, the PB1, PB2. PA, NP. NS, and M DNAs in the vectors for vRNA productions have a sequence that corresponds to one that encodes a polypeptide having at least 95% amino acid sequence identity to a corresponding polypeptide encoded by SEQ ID NOs:1-6 or 10-15. In one embodiment, the method includes isolating the virus. In one embodiment, at least one of PA, PB1, or PB2 viral segments has a C to U promoter mutation.

[0119] Further provided is a vector for vRNA, cRNA or mRNA expression of influenza virus PA having at least 95% amino acid sequence identity to a polypeptide encoded by SEQ ID NO:1 and having a threonine at position 30, a lysine at position 31, cysteine at position 105 or a lysine at position 401; a vector for vRNA, cRNA or mRNA expression of influenza virus PB1 having at least 95% amino acid sequence identity to a polypeptide encoded by SEQ ID NO:2 and having a leucine at position 40, an alanine or isoleucine at position 54, glycine at position 112, histidine at position 247, valine at position 507, alanine at position 644, or cysteine at position 713; a vector for vRNA, cRNA or mRNA expression of PB2 having at least 95% amino acid sequence identity to a polypeptide encoded by SEQ ID NO:3 and a leucine at position 202 and/or 323; a vector for vRNA, cRNA or mRNA expression of influenza virus NP having at least 95% amino acid sequence identity to a polypeptide encoded by SEQ ID NO:4 and having a lysine at position 74, leucine at position 116, isoleucine at position 224, lysine at position 293, asparagine at position 377, or aspartic acid at position 417; a vector for vRNA, cRNA or mRNA expression of influenza virus NS1 having at least 95% amino acid sequence identity to a NS1 polypeptide encoded by SEQ ID NO:6 and having a proline at position 30, alanine at position 49, lysine at position 118, glutamine at position 140, threonine at position 161, or glutamic acid at position 223; and a

vector for vRNA, cRNA or mRNA expression of influenza virus M1 having at least 95% amino acid sequence identity to a M1 polypeptide encoded by SEQ ID NO:5 and having a serine at position 90.

Exemplary M Viral Segments

[0120] Wild-type influenza A virus M2 protein consists of three structural domains: a 24-amino-acid extracellular domain, a 19-amino-acid transmembrane domain, and a 54-amino-acid cytoplasmic tail domain. The M2 transmembrane domain has ion channel activity, which functions at an early stage of the viral life cycle between the steps of virus penetration and uncoating. The M2 cytoplasmic tail domain may also have an important role in viral assembly and morphogenesis. M1 protein and M2 protein share N-terminal sequences. The M2 protein is encoded by a spliced transcript and RNAs encoding the M1 protein and the M2 protein share 3' sequences, although the coding sequences for M1 and M2 in those 3' sequences are in different reading frames. The C-terminal residues of M1 and C-terminal portion of the extracellular domain of M2 are encoded by the overlapping 3' coding sequences.

[0121] A "functional" M1 protein provides for export of viral nucleic acid from the host cell nucleus, a viral coat, and/or virus assembly and budding. Thus, the M1 protein in the recombinant influenza viruses of the invention has substantially the same function (e.g., at least 10%, 20%, 50% or greater) as a wild-type M1 protein. Thus, any alteration in the M1 coding region in a mutant M viral segment in a recombinant influenza virus does not substantially alter the replication of that virus, e.g., in vitro, for instance, viral titers are not reduced more than about 1 to 2 logs in a host cell that supplies M2 in trans.

[0122] In one embodiment, an isolated recombinant influenza virus comprises a mutant M2 protein having a deletion of one or more residues of the cytoplasmic tail of M2, which virus replicates in vitro, e.g., producing titers that are substantially the same or at most 10, 100 or 1,000 fold less than a corresponding wild-type influenza virus, but wherein the replication of the recombinant virus in vivo is limited to a single cycle (e.g., no progeny viruses are produced). In one embodiment, the deletion includes 2 or more residues and up to 21 residues of the cytoplasmic tail of M2. In one embodiment, the M viral segment for the mutant M2 has one to two stop codons near the splice donor or splice acceptor site for the M2 transcript. In one embodiment, the coding region for the transmembrane and/or cytoplasmic domain of M2 is also deleted.

[0123] In one embodiment, the deletion of M2 includes 21 or more residues and up to 54 residues, i.e., the entire cytoplasmic tail, of the cytoplasmic tail of M2. In one embodiment, the mutant M2 protein may also comprise at least one amino acid substitution relative to a corresponding wild-type M2 protein. The substitution(s) in the M2 protein may be in the extracellular domain, the transmembrane (TM) domain, or the cytoplasmic domain, or any combination thereof. For example, substitutions in the TM domain may be at residues 25 to 43 of M2, e.g., positions 27, 30, 31, 34, 38, and/or 41 of the TM domain of M2. In another embodiment, the mutant M2 protein may also comprise a deletion in at least a portion of the extracellular domain and/or the TM domain, e.g., a deletion of residues 29 to 31, relative to a corresponding wild-type M2 protein. In yet another embodiment, the mutant M2 protein further comprises a heterologous protein, e.g., the cytoplasmic domain of a heterologous protein (a non-influenza viral protein), which may have a detectable phenotype, fused to the cytoplasmic tail or extracellular domain of M2, forming a chimeric protein. In one embodiment, a cytoplasmic domain of a heterologous protein is fused to the remaining residues of the cytoplasmic tail of the deleted M2 protein. In one embodiment, the presence of one or more substitutions, deletions, or insertions of heterologous sequences, or any combination thereof, does not substantially alter the properties of the recombinant influenza virus, e.g., the presence of one or more substitutions, deletions, or insertions of heterologous sequences does not result in virus titers in vitro that are more than about 1.5 to 2 logs lower, but allows for a single cycle of replication in vivo (e.g., no progeny viruses are produced) for a recombinant influenza virus comprising a mutant M2 protein having a deletion of one or more residues of the cytoplasmic tail of M2.

[0124] In one embodiment, the deletion in the cytoplasmic domain of M2 includes 2, 3, 4, 5 or more, e.g., 11, 12, 13, 14, or 15 residues, but less than 22 residues, of the C-terminus of the cytoplasmic tail of M2. In one embodiment, the deletion is 2 up to 10 residues, including any integer in between. In one embodiment, the deletion is from 1 up to less than 8 residues, including any integer in between. In one embodiment, the deletion is from 5 up to 21 residues, including any integer in between. In one embodiment, the deletion is from 5 up to less than 28 residues, including any integer in between. In one embodiment, the deletion is from 9 up to 15 residues, including any integer in between. In one embodiment, the deletion is from 9 up to 23 residues, including any integer in between.

[0125] In one embodiment, the deletion in the cytoplasmic domain of M2 includes 22, 23, 24, 25 or more. e.g., 41, 42, 43, 44, or 45 residues, but less than 54 residues, of the C-terminus of the cytoplasmic tail of M2.

[0126] In one embodiment, the deletion is from 22 up to 35 residues, including any integer in between. In one embodiment, the deletion is from 29 up to 35 residues, including any integer in between. In one embodiment, the deletion is from 35 up to 45 residues, including any integer in between. In one embodiment, the deletion is from 9 to less than 28 residues, including any integer in between.

[0127] In one embodiment, an isolated recombinant influenza virus is provided comprising a mutant M viral segment that is mutated so that upon viral replication, the mutant M gene expresses a functional M1 protein and a mutant M2 protein with a deletion of the cytoplasmic tail and a deletion of at least a portion of the transmembrane domain, e.g., internal or C-terminal deletions, and/or includes one or more substitutions in the transmembrane domain. In one embodiment, the mutant M2 protein has a deletion that includes the entire cytoplasmic tail and transmembrane domain of M2, and has one or more residues of the extracellular domain, e.g., has the first 9 to 15 residues of the extracellular domain. The replication of the recombinant virus is, in one embodiment, a single cycle in vivo relative to a corresponding virus without a mutant M viral segment. The recombinant influenza virus replicates in vitro in the presence of M2 supplied in trans, e.g., producing titers that are substantially the same or at most 10, 100 or 1,000 fold less than a corresponding wild-type influenza virus.

[0128] In one embodiment, a live single cycle or attenuated influenza virus elicits both systemic and mucosal

immunity at the primary portal of infection. In one embodiment, the live, single cycle or attenuated influenza virus has reduced replication in lung compared to wild-type influenza virus, e.g., the live, single cycle or attenuated influenza virus has titers in lung that are at least one to two logs less, and in one embodiment, replication in nasal turbinates is not detectable. The live, single cycle or attenuated virus may be employed in a vaccine or immunogenic composition, and so is useful to immunize a vertebrate, e.g., an avian or a mammal, or induce an immune response in a vertebrate, respectively.

[0129] In one embodiment, the mutations in the M2 gene result in a mutant M2 protein with a deletion of the entire cytoplasmic tail and deletion or substitution of one or more residues in the transmembrane (TM) domain of M2 and may also comprise at least one amino acid substitution in the extracellular domain, or a combination thereof, relative to a corresponding wild-type M2 protein encoded by a M viral segment. For example, substitutions in the TM domain may include those at residues 25 to 43 of M2, e.g., positions 27, 30, 31, 34, 38, and/or 41 of the TM domain of M2. Substitutions and/or deletions in the TM domain may result in a truncated M2 protein that is not embedded in the viral envelope. For example, a deletion of 10 residues at the C-terminus of the transmembrane domain may result in a truncated M2 protein that is not embedded in the viral envelope. In another embodiment, the mutant M2 protein may also comprise a deletion in at least a portion of the extracellular domain in addition to deletion of the cytoplasmic domain and a deletion in the TM domain. In one embodiment, the mutant M2 protein has a deletion of the entire cytoplasmic tail and the TM domain and at least one residue of the extracellular domain, e.g., 1 to 15 residues, or any integer in between, of the C-terminal portion of the extracellular domain. In yet another embodiment, the mutant M2 protein having at least a portion of the extracellular domain further comprises a heterologous protein, e.g., the cytoplasmic and/or TM domain of a heterologous protein (a non-influenza viral protein), which may have a detectable phenotype, that is fused to the C-terminus of at least the extracellular domain of M2, forming a chimeric protein. In one embodiment, the presence of one or more substitutions, deletions, or insertions of heterologous sequences, or any combination thereof, in the M2 gene does not substantially alter the properties of the recombinant influenza virus, e.g., the presence of one or more substitutions, deletions, or insertions of heterologous sequences does not result in virus titers in vitro that are more than about 1.5 to 2 logs lower, and/or but allows for a single cycle (e.g., no progeny viruses are produced) of replication in vivo for the recombinant influenza virus with a mutant M2 protein gene having a deletion of the cytoplasmic tail and TM domain of M2.

[0130] In one embodiment, the deletion in the TM domain of M2 includes 1, 2, 3, 4, 5 or more, e.g., 11, 12, 13, 14, or 15 residues, up to 19 residues. In one embodiment, the deletion is from 2 up to 9 residues, including any integer in between. In one embodiment, the deletion is from 15 up to 19 residues, including any integer in between. In one embodiment, the deletion is from 10 up to 19 residues, including any integer in between. In one embodiment, the deletion is the result of at least one substitution of a codon for an amino acid to a stop codon. In one embodiment, the deletion is the result of deletion of at least one codon for an amino acid. In one embodiment, the TM domain of M2 has one or more substitutions, e.g., includes 1, 2, 3, 4, 5 or more, e.g., 11, 12, 13, 14, or 15 substitutions, up to 19 residues of the TM domain. In one embodiment, the one or more amino acid deletions and/or substitutions in the TM domain in a mutant M2 protein that also lacks the cytoplasmic tail of M2, provides for a mutant M2 protein that lacks M2 activity and/or when expressed in a virus yields a live, single cycle virus.

[0131] In one embodiment, a deletion in the extracellular (ectodomain) domain of M2 may include 1, 2, 3, 4 or more, e.g., 5, 10, 15, or 20 residues, up to 24 residues of the extracellular domain. In one embodiment, the deletion in the extracellular domain is from 1 up to 15 residues, including any integer in between. In one embodiment, the deletion is the result of at least one substitution of a codon for an amino acid to a stop codon. In one embodiment, the deletion is the result of deletion of at least codon for an amino acid. In one embodiment, the extracellular domain of M2 may also include one or more substitutions. In one embodiment, the mutations in the M2 gene of a M viral segment that result in deletion(s) or substitution(s) in the extracellular domain of M2 do not substantially alter the function of the protein encoded by the M1 gene.

[0132] In one embodiment, fewer than 20%, e.g., 10% or 5%, of the residues in the TM domain or extracellular domain are substituted. In one embodiment, fewer than 60%, e.g., 50%, 40%, 30%, 20%, 10%, or 5% of the residues in the extracellular domain are deleted. In one embodiment, more than 20%, e.g., 30%, 40%, 50%, 80% or more, of the residues in the TM domain are deleted.

Exemplary PR8 Viral Segment Variants

Example A

Methods

Cells and Viruses

[0133] 293T human embryonic kidney cells are maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) with 10% fetal calf serum and antibiotics. Madin-Darby canine kidney (MDCK) cells are grown in MEM with 5% newbom calf serum and antibiotics. African green monkey Vero WCB cells, which had been established after biosafety tests for use in human vaccine production (Sugawara et al., 2002), are maintained in serum-free VP-SFM medium (GIBCO-BRL) with antibiotics. Cells are maintained at 37° C. in 5% CO_2. A WHO-recommended vaccine seed virus is NIBRG-14.

Construction of Plasmids and Reverse Genetics

[0134] To generate reassortants of influenza A viruses, a plasmid-based reverse genetics (Neumann et al., 1999) is used. The full-length cDNAs were cloned into a plasmid under control of the human polymerase I promoter and the mouse RNA polymerase I terminator (Poll plasmids).

[0135] A previously produced series of Poll constructs, derived from A/WSN/33 (H5N1; WSN) or PR8 strains is used, for reverse genetics (Horimoto et al., 2006; Neumann et al., 1999). The World Health Organization (WHO) recommends A/Puerto Rico/8/34 (H1N1; PR8) as a donor virus, because of its safety in humans (Wood & Robertson, 2004; Webby & Webster, 2003).

[0136] Plasmids expressing WSN or PR8 NP, PA, PB1, or PB2 under control of the chicken actin, e.g., beta-actin, promoter are used for all reverse genetics experiments (Horimoto et al., 2006; Neumann et al., 1999). Briefly, Poll plasmids and protein expression plasmids are mixed with a transfection reagent, Trans-IT 293T (Panvera), incubated at room temperature for 15 minutes, and then added to 293T cells. Transfected cells are incubated in Opti-MEM I (GIBCO-BRL) for 48 hours. For reverse genetics in Vero WCB cells, an electroporator (Amaxa) is used to transfect the plasmid mixtures according to the manufacturer's instructions. Sixteen hours after transfection, freshly prepared Vero WCB cells were added onto the transfected cells and TPCK-trypsin (1 μg/mL) is added to the culture 6 hours later. Transfected cells are incubated in serum-free VP-SFM for a total of 4 days. Supernatants containing infectious viruses are harvested, and may be biologically cloned by limiting dilution.

[0137] A recombinant virus having the HA and NA genes from A/Hong Kong/213/2003 (H5N1) and the remainder of the type A influenza virus genes from PR8(UW) was prepared. The titer of the recombinant virus was $10^{10.87}$ EID_{50}/mL, and the HA titer was 1:1600

TABLE 1

Virus possessing PR8 genes together with the following	HA titer (HAU/mL) in each dilition						
HA and NA genes	10-2	10-3	10-4	10-5	10-6	10-7	10-8
WSN-HA NA	160	40	40	320	40	640	<1
HK-HAavir NA	400	800	400	400	400	800	<1

[0138] The sequences of PR8 (UVV) genes are as follows: Exemplary viral sequences for a master vaccine strain (PR8UW)

HA

(SEQ ID NO: 22)

AGCAAAAGCAGGGGAAAATAAAAACAACCAAAATGAAGGCAAACCTACTG

GTCCTGTTATGTGCACTTGCAGCTGCAGATGCAGACACAATATGTATAGG

CTACCATGCGAACAATTCAACCGACACTGTTGACACAGTACTCGAGAAGA

ATGTGACAGTGACACACTCTGTTAACCTGCTCGAAGACAGCCACAACGGA

AAACTATGTAGATTAAAAGGAATAGCCCCACTACAATTGGGGAAATGTAA

CATCGCCGGATGGCTCTTGGGAAACCCAGAATGCGACCCACTGCTTCCAG

TGAGATCATGGTCCTACATTGTAGAAACACCAAACTCTGAGAATGGAATA

TGTTATCCAGGAGATTTCATCGACTATGAGGAGCTGAGGGAGCAATTGAG

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[0139] High-titer A/PR/8/34 (H1N1, PR8(UW)) virus grows 10 times better than other NAPR/8/34 PR8 strains 45 in eggs (10^{10} EID_{50}/mL; HA titer:1:8.000). Thus, replacement of the HA and NA genes of PR8(UW) with those of a currently circulating strain of influenza virus results in a vaccine strain that can be safely produced, and validates the use of PR8(UW) as a master vaccine strain.

[0140] Genes that contribute to different growth properties between PR8(UW) and PR8 (Cambridge), which provides the non-HA and -NA genes of the NIBRG-14 vaccine strain (FIG. **1**), were determined. Higher titers in eggs were obtained when the majority of internal genes were from PR8(UW). Highest titers were with the M viral segment of PR8(UW) and the NS gene of PR8 (Cambridge). The NS gene in PR8(UW) has a K (lysine) at residue 55 while the NS gene in PR8(Cam) has a E (glutamic acid). The polymerase subunit (PA, PB1, and PB2) and NP genes of PR8(UW) enhanced the growth of an H5N1 vaccine seed virus in chicken embryonated eggs, and the NS gene of PR8(Cambridge) enhanced the growth of an H5N1 vaccine seed virus in chicken embryonated eggs. A5tyrosine (Y) at position 360 in PB2 of PR8(UW) likely contributes to the high growth rate of that virus in MDCK cells.

Example B

[0141] To develop an high-yield A/PR/8/34 (H1N1; PR8) virus backbone for growth of vaccine virus in specific host cells, random mutagenesis of the internal genes of PR8(HG) (PRBUW) was conducted. Random mutations were introduced into the UW-PR8 (Example 1) internal genes by error-prone PCR after which plasmid libraries were prepared that possessed the random mutations in an individual UW-PR8 internal gene. Then virus libraries (PR8H5N) were generated that possessed random mutations in an individual UW-PR8 internal gene, along with the other wild type internal genes and the NA and 'detoxified' HA genes of A/chicken/IndonesiaNC/09 (H5N) virus (Table 1), to generate "6+2" recombinant viruses. Consecutive passages of the virus in MDCK cells were employed to select for variants with high-growth properties.

TABLE 1

Virus libraries generated

Number	Internal genes: Gene library	Internal genes: Other internal genes	HA + NA	Titer of virus library (pfu/ml)
Control	PR8 wild type		NC/09/H5N1	3×10^6
1	PB2	5 UW-PR8 genes	NC/09/H5N1	2.1×10^2
2	PB1	5 UW-PR8 genes	NC/09/H5N1	1.6×10^5
3	PA	5 UW-PR8 genes	NC/09/H5N1	7×10^3
4	NP	5 UW-PR8 genes	NC/09/H5N1	1.5×10^3
5	M	5 UW-PR8 genes	NC/09/H5N1	1×10^6
6	NS	5 UW-PR8 genes	NC/09/H5N1	1.8×10^6
7	PB2 + PB1 + PA	3UW-PR8 genes	NC/09/H5N1	75
8	PB2 + PB1 + PA + NP	2UW-PR8 genes	NC/09/H5N1	33
9	PB2 + NS	4UW-PR8 genes	NC/09/H5N1	2×10^2
10	M + NS	4UW-PR8 genes	NC/09/H5N1	5.7×10^5

Virus libraries were passaged 12 times in MDCK cells or, after 2 passages, the libraries were mixed and 10 more passages were carried out (FIG. **2**).

[0142] After 10 to about 12 consecutive passages in MDCK cells, plaque assays were performed and over 1,400 individual plaques were picked. FIG. **3** shows the numbers of clones with various HA titers. Growth enhancing mutations included: PB2: M202L, F323L, I504V, PB1: E1112G, V644A, NP: R74K, N417D, I116L, and NS: S161T. FIG. **4** provides the titers of recombinant viruses generated from selected mutations.

[0143] 38 viruses with the highest HA titers from the random mutagenesis libraries were sequenced (Table 2)

TABLE 2

Sequences of viruses with the highest HA titers

Clone #	Library	HA titer (2^n)	PB2	PB1	PA	HA (H3 numbering)	NP	NA	M	NS
WT		7								
329	Mix	9	M202L F323L			L182V				
154	Mix	8.5~9	M202L F323L			L182V				
347	Mix	9	M202L F323L			L182V				
94	Mix	8.5	M202L F323L			F252I	I116L	L55S		
1045	Mix	9	M202L F323L	V644A		F252I				
965	Mix	8.5~9	M202L F323L		F105C	V184I			P90S	
50	Mix	8.5	M202L F323L			M148I (HA2)	R293M			
1005	Mix	9~9.5	M202L F323L	V644A	R401K	M148I (HA2)				T49A
134	Mix	8.5	M202L F323L							A223E
387	Mix	9	M202L F323L	M507V V644A						
852	Mix	9~9.5	M202L F323L M243I	R54I						
981	Mix	8.5~9	M202L F323L	Q247H						
993	Mix	8.5~9	M202L F323L				N224I			

TABLE 2-continued

Sequences of viruses with the highest HA titers

Clone #	Library	HA titer (2^n)	PB2	PB1	PA	HA (H3 numbering)	NP	NA	M	NS
1043	Mix	8.5~9	I504V			L182V	R74K			
398	Mix	8.5	I504V			L182V	R74K, N417D			A30P
1007	Mix	8.5	I504V	V644A		F252I	M371V			
1042	Mix	8.5~9	I504V	E75V D76G E78P P79V S80G V644A E697P F699L F700L P701H S702R Y705T		F252I	R74K			
999	Mix	8.5~9	I504V			M148I (HA2)	R74K, N417D			
1014	Mix	8.5	I504V	T59I G62X A63P V644A N694K L695T		M148I (HA2)	R74K, N417D	A265V		
1016	Mix	8.5~9	I504V			M148I (HA2)				
540	PB1	8.5		E112G		K162E				S161T
548	PB1	8.5~9		E112G L624V		K162E				S161T
191	PB1	8~8.5		E112G						
571	PB1	9~9.5		E112G						
572	PB1	8.5		E112G						
573	PB1	8.5		E112G						
1404	PB1	8.5	I57V T58G A59V K61Q E677D D678E P679M	E112G S713C						
1408	PB1	8.5		M40I G180W						S161T
582	PB1	8.5~9		M40L, G180W						S161T
545	PB1	8.5		M40L, G180W		K121E (HA2)				
543	PB1	8.5		I667T						
219	PB1	9		I667T, M714T		K162E				
344	Mix	8.5~9	M66R			L182V				
312	Mix	8.5~9				L182V	I116L			R140Q
320	Mix	8.5				L182V				
209	PB1	8.5~9		R54I		E136D, Q179L, A194V				

[0144] In a second approach, potentially growth-enhancing mutations described in the literature were introduced into the background of UW-PR8 virus (see Table 3 for virus stock titers) and tested for replicative ability. FIGS. **5**A-D show growth curves for various viruses.

TABLE 3

UW-PR8 viruses possessing mutation(s) identified in the literature

Gene	Mutation(s)	Virus stock titer (Pfu/ml)
WT	—	2×10^7
PB2	A44S	4.5×10^7
	E158G	3.2×10^4
	E158G + NP N101G	7.5×10^4
	E158A	8.3×0^6
	D253N + Q591K	8.3×10^6
	D256G	2.8×10^7
	R368K	3.1×10^7
	E391Q	1.4×10^8
	I504V + PA I550L	1.1×10^8
	Q591K	4.4×10^7
	V613T	1.8×10^7
	A661T	2.2×10^7
	D701N + S714R + NP N319K	1×10^6
	D701N	2.1×10^7
PB1	R327K	1.3×10^7
	V336I	2.3×10^7
	L473V + L598P	3.9×10^6

TABLE 3-continued

UW-PR8 viruses possessing mutation(s) identified in the literature

Gene	Mutation(s)	Virus stock titer (Pfu/ml)
PB1F2	F2 N66S	1.6×10^8
	F2 K73R	1.1×10^8
	F2 V76A	4.4×10^7
	F2 R79Q	6.2×10^6
	F2 L82S	2.7×10^7
	F2 E87Q	1.5×10^6
PA	T97I	1.6×10^7
	K142N	3.3×10^7
	S225C	6.7×10^7
	S149P + T357K	3.4×10^8
	K356R	8.5×10^7
	A404S	5.2×10^7
	S421I	2.7×10^7
NP	R293K	4.7×10^7
	R305K	7.2×10^7
	E372D	2.2×10^7
	R422K	1.3×10^8
	T442A	5×10^7
	D455E	2.2×10^7
	I109V	3.9×10^7
M	V97A + Y100H	1.4×10^7
NS1	K55E	1.6×10^7

[0145] In a third approach, candidates from approaches 1 and 2 were combined and HA titers and PFU/mL determined (Table 4).

TABLE 4

High-growth candidates identified in approaches 1 and 2 were tested in various combinations.

	Gene origin								Virus stock titer	
#	HA	NA	PB2	PB1	PA	NP	M	NS	HA (2^n)	Pfu/ml
WT	Indo/NC/09 (detoxified)	Indo/NC/09	UW-PR8	UW-PR8	UW-PR8	UW-PR8	UW-PR8	UW-PR8	7	3.00E+07
1			M202L F323L	M507V V644A		I116L		K55E	9~9.5	2.00E+08
2			M202L F323L	R54I		N224I		K55E	5	1.00E+05
3			M202L F323L	Q247H	R401K			T49A	9	1.00E+08
4			M202L F323L	M507V V644A	K356R	T442A	V97A Y100H	K55E	10~10.5	1.60E+08
5			I504V	M507V V644A	I550L	R74K N417D		K55E	8~8.5	5.70E+07
6			I504V	M507V V644A	I550L	R74K N417D	V97A Y100H	K55E	9~9.5	4.40E+07
7			I505V	E112G	I550L	R74K		S161T	9	1.60E+08
8			M202L F323L	I667T M714T		I116L		R140Q	<1	<1E3
9			M202L F323L	E112G				S161T	8.5	1.30E+08

TABLE 4-continued

High-growth candidates identified in approaches 1 and 2 were tested in various combinations.

	Gene origin								Virus stock titer HA	
#	HA	NA	PB2	PB1	PA	NP	M	NS	(2^n)	Pfu/ml
10			M66R	M40I G180W		R74K		S161T	8~8.5	2.30E+07
12			R368K	PB1 F2 N66S	K356R	R422K		K55E	5.5	9.00E+02
13			E391Q	R327K	S149P T357K	R293K			3	1.60E+06
14			Q591K	PB1 F2 K73R	S225C	R422K		K55E	7.5	2.00E+07
23							V97A		8.5~9	1.50E+07
24							Y100H		9~9.5	2.90E+07
25	NCR 15-19 nt mut[1]	Indo/NC/09	M202L F323L	M507V V644A	K356R	R422K	V97A Y100H	K55E	9.5~10	7.50E+07
26	Indo/NC/09 (detoxified)	Indo/NC/09						A30P	6.5~7	1.00E+07
27								T49A	6.5~7	2.00E+07
28								R140Q	8	4.00E+07
29								S161T	7~7.5	1.40E+07
30								A223E	7.5	1.00E+07
31				I667T M714T					3.5	4.00E+05
32	NCR 15-19 nt mut	UW-PR8	M202L F323L	V644A	K356R	T442A	Y100H	K55E	7~7.5	4.30E+06
33	Indo/NC/09 (detoxified)	Indo/NC/09	M202L F323L	E112G	K356R	R74K	Y100H	K55E	9~9.5	7.00E+07
34	NCR 15-19 nt mut	UW-PR8	I504V	M507V V644A			V97A Y100H	K55E	7	2.00E+05
35	Indo/NC/09 (detoxified)	Indo/NC/09	M202L F323L	M507V V644A	R401K	T442A	Y100H	R140Q	9	3.20E+07
36			I504V	E112G	I550L	I112L	Y100H	R140Q	9.5	1.30E+08
37			M202L F323L	E112G	S149P T357K	T442A	Y100H	K55E	0	0.00E+00
38			M202L F323L	M507V V644A		I116L	Y100H	K55E	10.1	2.30E+08
39			M202L F323L	M507V V644A	K356R	T442A	Y100H	K55E	9.8	1.00E+08
40			I504V	M507V V644A	I550L	T442A	Y100H	K55E	9.2	6.00E+07
41			I504V	I112G	I550L	R74K	Y100H	K55E	9.2	7.50E+07
P17			I504V	E112G	S225C	R74K N417D	V97A Y100H	K55E	9.5~10	5.80E+08
P26			M202L F323L	M40L G180W	S225C	R422K	V97A Y100H	K55E	10	3.00E+08
P61		Indo/NC/09 NA P263T[2]	M202L F323L	Q247H	K142N	R74K	V97A Y100H	K55E	10~10.5	2.00E+08

[1]Mutation in the HA gene noncoding region;
[2]A P263T mutation was detected in the NA protein of this virus clone

As shown in Table 4, several recombinant viruses were identified that replicated better than wild type, such as #1, #4, #36, #38, P17, P16, and P61. To identify the growth characteristics of these viruses, growth kinetics in MDCK cells were determined (FIG. **7**). For one candidate, virus was purified on sucrose gradients and HA content and viral total protein evaluated. FIG. **8**A shows HA titer of wild type (UW-PR8) and #4, FIG. **8**B shows viral protein for wild type (UW-PR8) and #4, and FIG. **8**BC is a SDS-PAGE analysis of viral proteins of wild type (UW-PR8) and #4. Further analysis demonstrated that viruses possessing the V97ANY100H mutations in M1 yielded higher HA titers than the parental virus, although the virus titer was lower (see FIGS. **9**A-B). The V97A/Y100H mutations in M1 may result in particles with a larger surface into which more HA protein can be incorporated. Since inactivated influenza viruses are dosed based on their HA content, variants with high HA content are attractive vaccine candidates.

[0146] To identify mutations in the influenza promoter region that provide for enhanced replication, viruses possessing a 'U' at position 4 at the 3' end of all eight vRNA segments were prepared in the UW-PR8 PA, PB1 and PB2 internal genes (the UW-PR8 PB2, PB1, and PA segments possess a 'C' at position 4). The growth curves of the resulting viruses are shown in FIG. **11**C.

[0147] Viruses possessing combinations of promoter mutations and amino acid changes were prepared and titers determined (Table 5).

TABLE 5

Virus titers of high-growth candidates.

									Virus stock titer	
	Gene backbone								HA	
Viruses	HA	NA	PB2	PB1	PA	NP	M	NS	(2^n)	pfu/ml
Control	WT	WT	WT	WT	WT	WT	WT	WT	7	3.0E+07
1	WT	WT	3'C4U	3'C4U	3'C4U	R74K	V97A	K55E	10.5	2.2E+09
2	3' G3A U5C C8U & 5' U3C A8G		M202L F323L	Q247H	K142N		Y100H		8.5~9	5.6E+07
3	NCR 15-19 nt mut								9~9.5	1.4E+09
4	3' G3A U5C C8U & 5' U3C A8G & NCR 15-19 nt mut								7	7.0E+07

Codon usage optimization was also conducted. Alteration of codons may increase protein expression but could also alter RNA structure and stability. For example, codon usage optimization of the PB2 gene segment was performed to reflect the codon usage in canine cells (since MOCK cells are of canine origin) (FIG. **10**A), while leaving the packaging signals (located at the 5' and 3' ends of the vRNA) unaltered. In one approach, codon optimization was performed for all codons in the 'internal' region of the PB2 gene (FIG. **10**C) and in another approach, codon optimization was performed for so-called 'rare' codons (FIG. **10**B) (used at significantly lower frequency compared to the codon used most frequently for a given amino acid) (see SEQ ID NO:25 in FIG. **10**F). Analyses were carried out using the "Graphical Codon Usage Analyser" (www.gcua.de). The titers of those viruses are shown in Table 6 (see also FIGS. **10**B-C).

TABLE 6

Titers of viruses encoding codon-optimized PB2 genes.

									Virus stock titer	
	Gene backbone								HA	
Virus	HA	NA	PB2	PB1	PA	NP	M	NS	(2^n)	pfu/ml
Wild type	WT	WT	WT	WT	WT	WT	WT	WT	7~7.5	3.5E+07
PB2 codon optimization-1	WT	WT	Rare codon optimized PB2	WT	WT	WT	WT	WT	9	2.1E+08
PB2 codon optimization-2	WT	WT	All Codon optimized PB2	WT	WT	WT	WT	WT	3	9.0E+05

Optimization of rare codons in PB2 resulted in increased titers compared to wild type virus (UW-PR8) (see FIG. **10**D). Other gene segments were codon optimized and titers of viruses with those segments or combinations of optimized segments were determined (FIG. **10**E).

[0148] In another approach to increase virus titer in MDCK cells, chimeric HA and NA genes were prepared (FIG. **13**A) and titers of viruses having those genes were determined (FIG. **13**B).

[0149] Viruses with combinations of the above-mentioned mutations (high growth backbone mutations, promoter mutations, chimeric HA and NA genes and canine codon optimization) were prepared and growth kinetics, PFU and HA titers of those viruses were determined (see FIG. **14**).

[0150] An exemplary set of backbone mutations are canine codon opti-PB2+C4U+M202L, F323L; PB1: C4U+Q247H; PA: C4U+K142N; NP: Canine codon opti-NP+R74K; M: V97A, Y100H; and NS: K55E.

[0151] Any of the mutations described herein, or any combination thereof, may be combined with, for instance, seasonal H1N1 and H3N2, H3N2 Variant, PdmH1N1, H5N1, H7N9 or H9N2, or other clades or candidate vaccine strains. For example, HA and NA genes from A/Califoria/04/2009(pdm H1N1) were combined with the six internal genes of UW-PR/8 to generate "6+2" recombinant viruses. Eleven virus libraries were generated and passaged 10 times in eggs. Three rounds of limiting dilution were performed to screen for high growth mutants (FIG. **15**). In one embodiment, a variant with high growth properties in MDCK cells has a PB2 gene segment with a promoter mutation (C4U) and a mutation that results in I504V (relative to the parental virus); a PB1 gene segment with a promoter mutation (C4U) and a mutation that results in E112G; a PA gene segment with a promoter mutation (C4U) and a mutation that results in S225C; a NP gene segment with mutations that result in R74K and N417D; a M gene segment with mutations that result in V97A and Y100H; and a NS gene segment with a mutation that results in K55E, where optionally the sequence of one or more gene segments, e.g., the NP gene segment, is modified to include canine codon optimized

codons. In one embodiment, a variant with high growth properties in MDCK cells has a canine codon optimized PB2 gene segment with a promoter mutation (C4U) and mutations that result in M202L and F323L; a PB1 gene segment with a promoter mutation (C4U) and a mutation that results in Q247H; a PA gene segment with a promoter mutation (C4U) and a mutation that results in K142N; a canine codon optimized NP gene segment with a mutation that results in R74K; a M gene segment with mutations that result in V97A Y100H; and a NS gene segment with a mutation that results in K55E.

[0152] Similar experiments were conducted in Vero cells, e.g., after about 3 to 5 passages in Vero cells, using clones with high replicative properties in MDCK cells (see FIG. **16**). FIG. **17** shows 5 viruses likely to have high replicative properties in Vero cells. In one embodiment, a PR8(UW) variant with high-growth properties in Vero cells has the following mutations that may be used in various combinations to increase the replicative ability of PR8(UW) virus: PB2 segment: C4U (promoter mutation), I504V (amino acid change); PB1 segment: C4U (promoter mutation); M40L (amino acid change), G180W (amino acid change); PA segment: C4U (promoter mutation), R401K (amino acid change); NP segment: I116L (amino acid change); NS segment: A30P (amino acid change in NS1), or R118K (amino acid change in NS1).

[0153] In one embodiment, a PR8(UW) variant with high-growth properties has the following residues that may be used in various combinations with each other and/or other residues, e.g., those that enhance virus replication, to increase the replicative ability of reassortants having PR8 (UW) based viral segment(s): a HA segment with one or more of 136D, 162E, 179L, 182V, 184I, 252I, 449E, and/or 476I: a NA segment with 55S and/or 265V; a NS segment with NS1 having 118K; F2 with 81G; a PB1 segment with 62A, 261G, 361R, 621R, and/or 654S, and/or viral segment promoters with the growth-enhancing nucleotides described herein. e.g., having one or more of the nucleotide changes G1012C, A1013U, or U1014A in the M viral segment.

Example C

[0154] To assess the contribution of individual viral RNA (vRNA) segments to high-yield properties, a series of reassortant viruses was generated that possessed one or several vRNA segments of a high-yield PR8 (PR8-HY) variant in the background of the parental virus [UW-PR8_Indo/05 (HA+NA)]. Vero cells were infected in triplicate with the indicated viruses at a MOI of 0.005 and incubated at 37° C. in the presence of trypsin. At the indicated time points, virus titers and HA titers were determined by performing plaque or HA assays, respectively. The results are shown in FIG. **20**. These data indicated that several vRNA segments contribute to the properties of PR8-HY virus. In particular, the PB2+ PB1+PA+NP vRNAs of PR8-HY virus conferred an appreciable increase in virus and HA titers, evidencing the enhanced replicative ability of this virus.

[0155] To further assess which component of the viral replication complex that provides for high-yield properties, wild-type or high-yield PB2, PB1, PA, and NP proteins were tested in various combinations in minireplicon assays in human 293T, canine MDCK, African green monkey Vero, and avian DF1 cells. The results are shown in FIG. **21**. Interestingly, the PB2, PB1, PA, and NP proteins of PR8-HY virus attenuated the viral replicative ability in 293T, Vero, and DF1 cells; this effect was primarily conferred by the PB2 protein. In contrast, the combination of PB2+PB1+PA+ NP proteins derived from PR8-HY virus conferred a substantial increase in replicated ability in canine MDCK cells, which were used for the selection of PR8-HY virus. The findings suggested host-dependent mechanisms underlying the high yield of PR8-HY virus. For example, the combination of PB1+PA+NP proteins, or a subset thereof, derived from PR8-HY may confer enhanced viral replicative ability in 293T, Vero, and DF1 cells.

Exemplary Embodiments

[0156] An isolated, single cycle recombinant influenza virus is provided having at least seven viral segments selected from PA, PB1, PB2, NP, NS, M, HA or NA viral segments, or having at least six viral segments selected from PA, PB1, PB2, NP, NS, M, or HEF viral segments, one of which segments comprises coding sequences for an antigenic coronavirus protein or an antigenic portion thereof. In one embodiment, the antigenic coronavirus protein comprises coronavirus S (spike) sequences. In one embodiment, the antigenic coronavirus protein comprises S1 sequences. In one embodiment, the antigenic coronavirus protein comprises a soluble protein. In one embodiment, the antigenic portion comprises the receptor binding domain. In one embodiment, the antigenic coronavirus protein sequences or the portion thereof have at least 80% amino acid sequence identity to one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the virus comprises eight viral segments. In one embodiment, the virus comprises nine viral segments. In one embodiment, the virus is an influenza A or B virus. In one embodiment, the virus is an influenza C or D virus. In one embodiment, coding sequences for the antigenic coronavirus protein sequences or the portion thereof replace at least a portion of the coding sequences for one of PA, PB1, PB2, NP, NS1, NS2, M1, M2, HA, or NA. In one embodiment, coding sequences for the antigenic coronavirus protein sequences or the portion thereof are inserted into coding sequences in the viral segment of one of PA, PB1, PB2, NP, NS, M, HA or NA viral segments. In one embodiment, the virus is bivalent or trivalent. In one embodiment, the M viral segment is mutated so that upon viral replication the mutant M gene expresses a functional M1 protein and a mutant M2 protein with a deletion of the cytoplasmic tail and either lacking a transmembrane domain or having a mutated transmembrane domain. In one embodiment, the mutant M2 protein comprises the M2 extracellular domain. In one embodiment, the M2 extracellular domain comprises less than 24 residues. In one embodiment, the M2 extracellular domain comprises at least 9 residues. In one embodiment, the mutation in the transmembrane domain comprises at least one amino acid substitution. In one embodiment, the transmembrane domain is deleted. In one embodiment, the deletion in the transmembrane domain includes residues 29 to 31. In one embodiment, the deletion in the transmembrane domain comprises at least 10 residues. In one embodiment, two or more of the PA, PB1, PB2, NP, NS, and M viral segments have selected amino acid residues at positions 30, 31, 105, 142, 149, 225, 356, 357, 401, and/or 550 in PA; positions 40, 54, 59, 62, 63, 75, 76, 78, 79, 80, 112, 180, 247, 327, 507, 624, 644, 667, 694, 695, 697, 699, 700, 701, 702, 705, 713, and/or 714 in PB1; positions 57, 58, 59, 61, 66, 202, 323, 368, 391, 504, 591, 677, 678, and/or 679, in PB2; positions 74, 112, 116, 224, 293, 371, 377, 417, 422 or 442

in NP; positions 90, 97 and/or 100 in M1; or positions 30, 49, 55, 118, 140, 161 and/or 223 in NS1. In one embodiment, at least of the viral segments has a C to U promoter mutation. In one embodiment, at least one of PA, PB1, or PB2 viral segments has a C to U promoter mutation. In one embodiment, the PB2 segment has a C4U promoter mutation or 504V; the PB1 segment has one or more of C4U, 40L or 180W; the PA segment has C4U or 401K; the NP segment has 116L; or the NS segment has 30P in NS1 or 118K in NS1.

[0157] An isolated, single cycle recombinant influenza virus is provided having PA, PB1, PB2, NP, NS, M, HA or NA viral segments, or having PA, PB1, PB2, NP, NS, M, or HEF viral segments, wherein the NS or PB2 segment comprises coding sequences for an antigenic coronavirus protein or an antigenic portion thereof, and optionally the M viral segment is mutated so that upon viral replication the mutant M gene expresses a functional M1 protein and a mutant M2 protein with a deletion of the cytoplasmic tail and either lacking a transmembrane domain or having a mutated transmembrane domain. In one embodiment, the antigenic coronavirus protein comprises coronavirus S (spike) sequences. In one embodiment, the antigenic coronavirus protein comprises coronavirus S (spike) RBD sequences.

[0158] An isolated, single cycle recombinant influenza virus is provided having PA, PB1, PB2, NP, NS, M, HA or NA viral segments, or having PA, PB1, PB2, NP, NS, M, or HEF viral segments, wherein the NS segment comprises coding sequences for an antigenic coronavirus protein or an antigenic portion thereof, and optionally the M viral segment is mutated so that upon viral replication the mutant M gene expresses a functional M1 protein and a mutant M2 protein with a deletion of the cytoplasmic tail and either lacking a transmembrane domain or having a mutated transmembrane domain. In one embodiment, the antigenic coronavirus protein comprises coronavirus S (spike) sequences. In one embodiment, the antigenic coronavirus protein comprises coronavirus S (spike) RBD sequences.

[0159] Also provided is an isolated influenza virus having at least seven viral segments selected from PA, PB1, PB2, NP, NS, M, HA or NA viral segments, or having at least six viral segments selected from PA, PB1, PB2, NP, NS, M, or HEF viral segments, one of which segments comprises coding sequences for an antigenic coronavirus protein or an antigenic portion thereof. In one embodiment, the antigenic coronavirus protein comprises S1 sequences. In one embodiment, the antigenic portion comprises the receptor binding domain. In one embodiment, the antigenic coronavirus protein sequences or the portion thereof have at least 80% amino acid sequence identity to one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the virus comprises eight or nine viral segments. In one embodiment, the virus is an influenza A or B virus. In one embodiment, coding sequences for the antigenic coronavirus protein sequences or the portion thereof replace at least a portion of the coding sequences for one of PA, PB1, PB2, NP, NS1, NS2, M1, M2, HA, or NA. In one embodiment, coding sequences for the antigenic coronavirus protein sequences or the portion thereof are inserted into coding sequences in the viral segment of one of PA, PB1, PB2, NP, NS, M, HA or NA viral segments. In one embodiment, the virus is bivalent or trivalent. In one embodiment, the M viral segment is mutated so that upon viral replication the mutant M gene expresses a functional M1 protein and a mutant M2 protein with a deletion of the cytoplasmic tail and either lacking a transmembrane domain or having a mutated transmembrane domain, wherein the replication of the recombinant virus is abrogated or attenuated in vivo relative to a corresponding influenza virus with a wild-type M viral segment. In one embodiment, the mutant M2 protein comprises the M2 extracellular domain. In one embodiment, the M2 extracellular domain comprises at least 9 or 10 residues. In one embodiment, the mutation in the transmembrane domain comprises a deletion in the transmembrane domain. In one embodiment, two or more of the PA, PB1, PB2, NP, NS, and M viral segments have selected amino acid residues at positions 30, 31, 105, 142, 149, 225, 356, 357, 401, and/or 550 in PA; positions 40, 54, 59, 62, 63, 75, 76, 78, 79, 80, 112, 180, 247, 327, 507, 624, 644, 667, 694, 695, 697, 699, 700, 701, 702, 705, 713, and/or 714 in PB1; positions 57, 58, 59, 61, 66, 202, 323, 368, 391, 504, 591, 677, 678, and/or 679, in PB2; positions 74, 112, 116, 224, 293, 371, 377, 417, 422 or 442 in NP; positions 90, 97 and/or 100 in M1; or positions 30, 49, 55, 118, 140, 161 and/or 223 in NS1. In one embodiment, at least of the viral segments has a C to U promoter mutation. In one embodiment, at least one of PA, PB1, or PB2 viral segments has a C to U promoter mutation. In one embodiment, the PB2 segment has a C4U promoter mutation or 504V; the PB1 segment has one or more of C4U, 40L or 180W; the PA segment has C4U or 401K: the NP segment has 116L; or the NS segment has 30P in NS1 or 118K in NS1.

[0160] In one embodiment, a vaccine comprising an effective amount of the virus is provided. In one embodiment, the vaccine is formulated for intranasal delivery. In one embodiment, the virus is bivalent. In one embodiment, the recombinant virus comprises influenza A HA. In one embodiment, the virus comprises H1, H3, H5 or H7 HA. In one embodiment, the vaccine which further comprises a different influenza virus. In one embodiment, the vaccine further comprises at least two different influenza viruses. In one embodiment, the virus is inactivated.

[0161] Further provided is a method to immunize a vertebrate, comprising: administering to the vertebrate the vaccine disclosed herein. In one embodiment, the vertebrate is an avian. In one embodiment, the vertebrate is a mammal. In one embodiment, the vertebrate is a human. In one embodiment, the vaccine is intranasally administered. In one embodiment, the vaccine is intramuscularly administered. In one embodiment, more than one dose is administered.

[0162] The invention will be described by the following nonlimiting examples.

Example 1

[0163] In one embodiment, an eight segment single cycle recombinant influenza A virus is prepared. One of the viral RNA segments (for example, the NS segment) is modified to also express SARS-CoV-2 S (or portions thereof), e g., a fusion of NS1 and SARS-CoV-2 S protein or a portion thereof. For fusion protein between the flu and SARS proteins, proteases that autocatalytically cleave are employed to generate functional flu and SARS proteins. The addition of heterologous protein sequences does not result in the need for a helper cell to express a protein in trans. However, if influenza virus coding sequences on one or more the viral segments are deleted (either a portion thereof or in their entirety), the corresponding influenza virus pro-

tein(s) are supplied in trans. For example, the viral M segment is modified by inserting two stop codons into M2 (downstream of the splice acceptor site), and by deleting the coding region for the transmembrane domain of M2, referred to as M2SR, which undergoes only one round of replication and requires a helper cell line for propagation That is in contrast to live-attenuated viruses which undergo several rounds of slow replication). In one embodiment, one or more of the internal viral segments are from PR8HY. In one embodiment, the HA and NA viral segments are from a heterologous strain. The M2SR having coronavirus sequences (CoroFlu M2SR) is intranasally administrated. In other embodiments, inactivated coronavirus/influenza viruses may be intramuscularly administered.

[0164] In one embodiment, a nine-segment virus is generated with eight segments expressing the flu proteins (with M2 modified as described above), and a ninth viral segment in which (part of) the flu coding region is replaced with SARS-CoV-2 S (or portions thereof).

[0165] In one embodiment, an attenuated virus is generated, e.g., one having M2 mutations that result in attenuation, e.g., M2del29-31 or M2 cytoplasmic tail deletions (see, e.g., del11 or del 22 etc. in Iwatsuki-Horimoto et al. (2006) and Watanabe et al. (2008).

[0166] Other alterations in M2 include two stop codons to prevent expression of the transmembrane domain and cytoplasmic tails and two stop codons and deletion of the coding region of the transmembrane domain (see Watanabe et al. (2009) and Sarawar et al. (2016), which are incorporated by reference herein)

Example 2

[0167] An influenza vaccine that includes coronavirus sequences and is limited to a single round of replication in vaccinated individuals, but stimulates mucosal, innate, humoral, and/or cell-mediated immune responses, was prepared. Phase I and Phase IIa clinical studies with the vaccine virus (without coronavirus sequences) have demonstrated its safety (no serious adverse events; no virus shedding) and the ability to elicit neutralizing immune responses to homologous and antigenically mismatched influenza virus strains. Importantly, this vaccine mimics the natural infection process and stimulates mucosal, innate, humoral, and cell-mediated immune responses. Thus, this platform may be employed to generate a single-cycle bivalent influenza vaccine expressing a soluble portion of the spike protein (the major antigen) of a coronavirus, e.g., the new 2019 coronavirus. The immunogenicity and protective efficacy of this vaccine is likely to be superior to that of inactivated vaccines, which stimulate B cell responses, but fail to induce other immune responses.

Generate a Bivalent Coronavirus/Influenza Virus Vaccine Candidate and Test its Protective Efficacy in Animal Models

[0168] To generate the novel bivalent coronavirus/influenza virus vaccine based on the M2SR platform (called CoroFlu M2SR, FIG. **22**), cells ware transfected with plasmids for influenza virus generation. One plasmid possesses a deletion of the influenza viral M2 coding region. In another plasmid, the coding region for the receptor-binding domain (RBD) of the 2019-nCoV spike (S) protein is inserted between the influenza viral NS1 and NS2 coding regions, separated by foot-and-mouse virus protease 2A autoproteolytic cleavage sites (2A). In cells expressing the M2 protein, CoroFlu M2SR vaccine virus is generated.

[0169] In one embodiment, the coronavirus S amino acid sequence, or a portion thereof, has at least 80%, e.g., 90%, 92%, 95%, 97% or 99%, including any integer between 80 and 99, contiguous amino acid sequence identity to a polypeptide having one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the S polypeptide or a portion thereof 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 the residues, relative to a polypeptide having one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, a S polypeptide or a portion thereof 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 with one of the sequences disclosed herein. In one embodiment, the coronavirus sequence in the influenza virus has 1, 2, 3, 4 or 5 substitutions relative to one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the coronavirus S1 sequence in the influenza virus has 1, 2, 3, 4 or 5 substitutions relative to the S1 sequence in one of SEQ ID Nos. 25-28 and 50-52. In one embodiment, the coronavirus RBD sequence in the influenza virus has 1, 2, 3, 4 or 5 substitutions relative to the RBD sequence in one of SEQ ID Nos. 25-28 and 50-52.

[0170] For example, the amino acid(s) can be any amino acid within these positions such as any of the amino acids listed in the table below.

Original Residue	Exemplary Substitutions	Alternative Substitutions
Ala (A)	val; leu; ile	Val
Arg (R)	lys; gln; asn	Lys
Asn (N)	gln; his; lys; arg	Gln
Asp (D)	Glu, Asn	Glu, Asn
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	asn; gln; lys; arg; gln;	Arg; Gln
Ile (I)	leu; val; met; ala; phe norleucine	Leu
Leu (L)	norleucine; ile; val; met; ala; phe	Ile
Lys (K)	arg; gln; asn	Arg
Met (M)	leu; phe; ile	Leu
Phe (F)	leu; val; ile; ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser, Ala	Ser, Als
Trp (W)	Tyr	Tyr
Tyr (Y)	trp; phe; thr; ser	Phe
Val (V)	ile; leu; met; phe; ala; norleucine	Leu

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.

[0171] The basic characterization of CoroFlu M2SR includes assessment of virus titers in the Vero M2 production cell line; the virus is passaged 10 consecutive times (followed by sequence analysis) in Vero M2-expressing cells to assess the genomic stability of CoroFlu M2SR.

[0172] Animal studies are carried out in Syrian hamsters (in which SARS-CoV replicates efficiently), in ferrets (an animal model that has been used for SARS-CoV research) and in transgenic mice expressing the human angiotensin-converting enzyme-2 (ACE-2) receptor, the SARS-CoV receptor to which 2019-nCoV also binds. Animals are intranasally administered with different amounts of CoroFlu M2SR (e.g., 10^5 to 10^7 PFU). Control animals are administered with M2SR vaccine (expressing the same HA and NA genes as CoroFlu M2SR, but not expressing S/RDB). Another control group is mock-treated. On days 1, 3, 5, and 7 after vaccination, nasal swab samples are collected to confirm the lack of virus shedding. Three weeks post-vaccination, serum samples are collected and tested for antibodies to SARS-Cov2 S/RBD and influenza HA; if the titers are low, animals are boosted.

[0173] Animals are vaccinated and challenged with live SARS-Cov2 or influenza virus three weeks after the last immunization. Control groups are mock-vaccinated, followed by live virus challenge with SARS-Cov2 and influenza virus. Groups of animals are euthanized on days 3, 6, and 9 post-infection to titrate the amounts of virus in the nasal turbinates and lungs. Other groups of animals are observed for weight changes and clinical symptoms. Nasal swabs are collected every other day (starting on day 1 post-challenge) to determine the virus load in the challenged animals.

Assess Whether the Vaccine Candidates Cause Antibody-Dependent Enhancement (ADE) of Virulence

[0174] ADE (i.e., antibody-dependent enhancement of infectivity and disease severity) is a potential concern with the development of vaccines to a variety of viruses, including coronaviruses (Halsted, 2014; Huisman et al, 2009; Smatti et al., 2018; Wan et al., 2019; Wang et al., 2014; Yip et al., 2014; Takada et al., 2001; Takada et al., 2003; and Takada et al., 2007). Since ADE is most likely caused by non-neutralizing antibodies directed at sub-dominant epitopes, the use of S/RDB (instead of full-length S) may reduce the likelihood of ADE. To test this, animals are vaccinated with CoroFlu M2SR, M2SR, or mock-vaccinated, and sera will be collected three weeks later.

[0175] To assess ADE in vitro, the SARS-Cov2 is mixed with different dilutions of serum (obtained from vaccinated or control animals; see previous paragraph) and added to cells to determine virus titers. To assess ADE in vivo, two sets of experiments are carried out: In the first set of experiments, animals are administered different serum dilutions and subsequently infected with the SARS-Cov2. Control groups are treated with serum obtained from mock-vaccinated. In the other set of experiments, animals are vaccinated with CoroFlu M2SR, M2SR, or mock-vaccinated, and three weeks later infected with live SARS-Cov2. At different times post-infection, animals are euthanized to collect organs for virus titration and histopathological analysis, and sera are collected to determine antibody titers. The finding that sera obtained from CoroFlu M2SR-vaccinated animals and vaccination with CoroFlu M2SR do not increase virus titers, disease symptoms, or histopathology compared with the controls establishes the absence of ADE for CoroFlu M2SR vaccine.

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[0211] 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.

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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

gcaacttatc agaggacaag agctcttgtt cgcaccggaa tggatcccag gatgtgctct 540

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

ttgagagggt cggttgctca caagtcctgc ctgcctgcct gtgtgtatgg acctgccgta 900

gccagtgggt acgactttga aagggaggga tactctctag tcggaataga ccctttcaga 960

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agtcaactgg tgtggatggc atgccattct gccgcatttg aagatctaag agtattaagc 1080

ttcatcaaag ggacgaaggt gctcccaaga gggaagcttt ccactagagg agttcaaatt 1140

gcttccaatg aaaatatgga gactatggaa tcaagtacac ttgaactgag aagcaggtac 1200

tgggccataa ggaccagaag tggaggaaac accaatcaac agagggcatc tgcgggccaa 1260

atcagcatac aacctacgtt ctcagtacag agaaatctcc cttttgacag aacaaccatt 1320

atggcagcat tcaatgggaa 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

<210> SEQ ID NO 5

<400> SEQUENCE: 5

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<210> SEQ ID NO 6

<400> SEQUENCE: 6

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<210> SEQ ID NO 7

<400> SEQUENCE: 7

000

<210> SEQ ID NO 8

<400> SEQUENCE: 8

000

<210> SEQ ID NO 9

<400> SEQUENCE: 9

000

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

<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

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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

gctcagacgg caaaaatagt gaatttagct tgtccttcat gaaaaaatgc cttgtttcta 2340

ct 2342

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

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<400> SEQUENCE: 11

agcgaaagca ggtcaattat attcaatatg gaaagaataa aagaactaag 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 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 ttctcctttg 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

gctggcactt taaccgaaga cccagatgaa ggcacagctg gagtggagtc cgctgttctg 2100

aggggattcc tcattctggg caaagaagac aggagatatg ggccagcatt aagcatcaat 2160

gaactgagca accttgcgaa aggagagaag gctaatgtgc taattgggca aggagacgtg 2220

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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

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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

gagagtatga ttgaagctga gtcctctgtc aaagagaaag acatgaccaa agagttcttt 1860

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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

agcaaaagca gggtagataa tcactcactg agtgacatca aaatcatggc gtcccaaggc 60

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 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

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gaaataagat ggttgattga agaagtgaga cacaaactga aggtaacaga gaatagtttt 780

gagcaaataa catttatgca agccttacat ctattgcttg aagtggagca agagataaga 840

actttctcat ttcagcttat ttaataataa aaaacaccct tgtttctact 890

<210> SEQ ID NO 16
<211> LENGTH: 2341
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 16

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tcgcagtctc gcacccgcga gatactcaca aaaaccaccg tggaccatat ggccataatc 120

aagaagtaca catcaggaag acaggagaag aacccagcac tgaggatgaa atggatgatg 180

gcaatgaaat atccaattac agcagacaag aggatcaccg aaatgattcc tgagagaaat 240

gagcagggac agactctgtg gagtaaaatg aatgatgccg gatcagaccg agtgatggtg 300

tcacctctgg ctgtgacatg gtggaatagg aatggaccaa tcacaaatac agtgcattat 360

ccaaaaatct acaaaactta ttttgaaaga gtcgaaaggc tgaagcatgg aacctttggc 420

cctgtccatt ttagaaacca ggtcaaaatc cggcggagag tggacatcaa tcctggtcat 480

gcagatctca gtgccaagga ggcacaggat gtgatcatgg aagtggtgtt ccctaacgaa 540

gtgggagcca ggattctgac atccgaatcc cagctgacca ttaccaaaga gaagaaagaa 600

gaactccagg attgcaaaat ttctcctctg atggtggcat acatgctgga gagagaactg 660

gtccgcaaaa caagattcct cccagtggct ggtggaacaa gcagtgtgta cattgaagtg 720

ctgcatctga ctcagggaac atgctgggaa cagatgtata ctccaggagg ggaagtgagg 780

aatgatgatg tggatcagag cctgattatt gctgctagga acattgtgag aagagctgca 840

gtgtcagcag atccactggc atctctgctg gagatgtgcc acagcacaca gattggtgga 900

attaggatgg tggacatcct gaggcagaac ccaacagaag agcaggccgt ggatatttgc 960

aaggctgcaa tgggactgag aattagctca tccttcagtt ttggtggatt cacatttaag 1020

agaacaagcg gatcatcagt caagagagag gaagaggtgc tgaccggcaa tctgcagaca 1080

ctgaagatca gagtgcatga gggatatgaa gagttcacaa tggtggggag aagagcaaca 1140

gccatcctca gaaaagcaac caggagactg attcagctga tcgtgagtgg gagagacgaa 1200

cagtccattg ccgaagcaat tattgtggcc atggtgtttt cacaggagga ttgtatgatt 1260

aaagcagtca gaggtgatct gaatttcgtc aatagggcca atcagcgact gaatcctatg 1320

catcagctgc tgagacattt tcagaaggat gccaaagtgc tgtttcagaa ttggggagtg 1380

gaacctatcg acaatgtgat gggaatgatt gggatcctgc ccgacatgac tccaagcatc 1440

gagatgtcaa tgagaggagt gagaatcagc aaaatgggtg tggatgagta ctccagcacc 1500

gagagggtcg tggtgagcat tgacagattt ctgagaatcc gggaccagcg aggaaatgtg 1560

ctcctgtctc ccgaggaggt cagtgaaaca cagggaacag agaaactgac aattacttac 1620

tcatcctcaa tgatgtggga gattaatggt cctgaatcag tgctggtcaa tacctatcag 1680

tggatcatca gaaactggga aactgtgaaa attcagtggt cccagaaccc tacaatgctg 1740

tacaataaaa tggaatttga accatttcag tctctggtgc ctaaggccat tagaggccag 1800

tacagtgggt ttgtgagaac tctgttccag cagatgaggg atgtgctggg gacatttgat 1860

-continued

accgcacaga ttattaaact gctgcccttc gcagccgctc caccaaagca gagtagaatg 1920

cagttctcct catttactgt gaatgtgagg ggatcaggaa tgagaatcct ggtgaggggc 1980

aattctcctg tgttcaacta taacaaggcc accaagagac tcacagtgct cggaaaggat 2040

gctggcactc tgactgaaga cccagatgaa ggcacagctg gagtggagtc cgctgtgctg 2100

aggggattcc tcattctggg caaagaagac aagagatatg ggccagcact gagcatcaat 2160

gaactgagca acctggccaa 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 17
<211> LENGTH: 2341
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 17

agcgaaagca ggcaaaccat ttgaatggat gtcaatccga ccttactttt cttaaaagtg 60

ccagcacaaa atgctataag cacaactttc ccttatactg gagaccctcc ttacagccat 120

gggacaggaa caggatacac catggatact gtcaacagga cacatcagta ctcagaaaag 180

ggaagatgga caacaaacac cgaaactgga gcaccgcaac tcaacccgat tgatgggcca 240

ctgccagaag acaatgaacc aagtggttat gcccaaacag attgtgtatt ggaggcgatg 300

gctttccttg aggaatccca tcctggtatt tttgaaaact cgtgtattga aacgatggag 360

gttgttcagc aaacacgagt ggacaagctg acacagggcc gacagaccta tgactggact 420

ctgaatagaa accagcctgc tgcaacagca ctggccaaca caatcgaagt gttcagatca 480

aatggcctca ccgccaatga gtctggaagg ctcatcgact tcctgaagga tgtgatggag 540

tcaatgaaca aagaagaaat ggggatcaca actcattttc agagaaagag acgggtgaga 600

gacaatatga ctaagaaaat gattacacag agaacaatgg gtaaaaagaa gcagagactg 660

aacaaaagga gttatctgat tagagcactg accctgaaca caatgaccaa agatgctgag 720

agagggaagc tgaaacggag agcaattgca accccaggga tgcagattag ggggtttgtg 780

tactttgtgg agacactggc aaggagtatt tgtgagaaac tggaacagtc agggctgcca 840

gtgggaggca atgagaagaa agcaaagctg gcaaatgtgg tgaggaagat gatgaccaat 900

tctcaggaca ccgaactgtc tttcaccatc actggagata acaccaaatg gaacgaaaat 960

cagaatcctc ggatgtttct ggccatgatc acatatatga ccagaaatca gcccgaatgg 1020

ttcagaaatg tgctgagtat tgctccaatt atgttctcaa acaaaatggc cagactggga 1080

aaagggtata tgtttgagag caagagtatg aaactgagaa ctcagattcc tgcagaaatg 1140

ctggcaagca tcgatctgaa atatttcaat gattcaacaa gaaagaagat tgaaaaaatc 1200

cgacccctcc tgattgaggg gactgcatca ctgagccctg gaatgatgat gggcatgttc 1260

aatatgctga gcactgtgct gggcgtctcc atcctgaatc tgggacagaa gagatacacc 1320

aagactactt actggtggga tggtctgcag tcctctgacg attttgctct gattgtgaat 1380

gcacccaatc atgaagggat tcaggccgga gtcgacaggt tttatcgaac ctgtaagctg 1440

ctgggaatca atatgagcaa gaaaaagtct tacatcaaca gaacaggtac atttgaattc 1500

-continued

acaagttttt tctatcgcta tgggtttgtg gccaatttca gcatggagct gcccagtttt 1560

ggggtgtctg ggatcaacga gtcagccgac atgagtattg gagtgactgt catcaaaaac 1620

aatatgatca acaatgatct gggtccagca acagctcaga tggccctgca gctgttcatc 1680

aaagattaca ggtacaccta ccgatgccat atcggtgaca cacagattca gacccgaaga 1740

tcatttgaaa tcaagaaact gtgggagcag acccgctcca aagctggact gctggtctcc 1800

gacggaggcc caaatctgta caacattaga aatctccaca ttcctgaagt ctgcctgaaa 1860

tgggaactga tggatgagga ttaccagggg cgcctgtgca acccactgaa cccatttgtc 1920

agccataaag aaattgaatc aatgaacaat gcagtgatga tgccagcaca tggtccagcc 1980

aaaaacatgg agtatgatgc tgtggcaaca acacactcct ggatccccaa aagaaatcga 2040

tccatcctga atacaagtca gagaggagtg ctggaggatg aacagatgta ccagaggtgc 2100

tgcaatctgt ttgaaaaatt cttccccagc agttcataca gaagaccagt cgggatctcc 2160

agtatggtgg aggctatggt gtccagagcc cgaattgatg cacggattga tttcgaatct 2220

ggaaggatca agaaagaaga gttcactgag atcatgaaga tctgttccac cattgaagag 2280

ctcagacggc aaaaatagtg aatttagctt gtccttcatg aaaaaatgcc ttgtttctac 2340

t 2341

<210> SEQ ID NO 18
<211> LENGTH: 2233
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 18

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

cagtccgaga gaggagaaga gacaattgaa gaaaggtttg aaatcacagg aacaatgcgc 660

aagcttgccg accaaagtct cccgccgaac ttctccagcc ttgaaaattt tagagcctat 720

gtggatggat tcgaaccgaa cggctacatt gagggcaagc tgtctcaaat gtccaaagaa 780

gtaaatgcta gaattgaacc ttttctgaaa acaacaccac gaccactgag actgcccaat 840

gggcctccct gttctcagcg gtccaaattc ctgctgatgg atgccctgaa actgagcatt 900

gaggacccaa gtcatgaagg agagggaatt cccctgtatg atgcaatcaa atgcatgaga 960

acattctttg gatggaagga acccaatgtg gtgaaaccac acgaaaaggg aatcaatcca 1020

aattatctgc tgtcatggaa gcaggtgctg gcagaactgc aggacattga gaatgaggag 1080

aaaattccaa agactaaaaa tatgaagaaa acaagtcagc tgaagtgggc actgggtgag 1140

-continued

aacatggcac cagaaaaggt ggactttgac gactgtaaag atgtgggtga tctgaagcag 1200

tatgatagtg atgaaccaga actgaggtcc ctggcaagtt ggattcagaa tgagtttaac 1260

aaggcatgcg aactgacaga ttcaagctgg attgagctcg atgagattgg agaagatgtg 1320

gctccaattg aacacattgc aagcatgaga aggaattatt tcacatcaga ggtgtctcac 1380

tgcagagcca cagaatacat catgaaggga gtgtacatca atactgccct gctgaatgca 1440

tcttgtgcag caatggatga tttccagctg attccaatga tcagcaagtg tagaactaag 1500

gagggaaggc gaaagaccaa cctgtatggt ttcatcatca aaggaagatc ccacctgagg 1560

aatgacaccg acgtggtgaa ctttgtgagc atggagtttt ctctcactga cccaagactg 1620

gaaccacata aatgggagaa gtactgtgtg ctggagattg gagatatgct gatcagaagt 1680

gccattggcc aggtgtcaag gcccatgttc ctgtatgtga gaacaaatgg aacctcaaaa 1740

attaaaatga aatggggaat ggagatgagg cgctgcctcc tccagtcact gcagcagatt 1800

gagagtatga ttgaagctga gtcctctgtc aaagagaaag acatgaccaa agagttcttt 1860

gagaacaaat cagaaacatg gcccattgga gagtccccca aaggagtgga ggaaagttcc 1920

attgggaagg tctgcaggac tctgctggca aagtccgtgt tcaacagcct gtatgcatct 1980

ccacagctgg aaggattttc agctgaatca agaaaactgc tgctgatcgt gcaggctctg 2040

agggacaacc tggaacctgg gacctttgat ctgggggggc tgtatgaagc aattgaggag 2100

tgcctgatta atgatccctg ggtgctgctg aatgcttctt ggttcaactc cttccttaca 2160

catgcattga gttagttgtg gcagtgctac tatttgctat ccatactgtc caaaaaagta 2220

ccttgtttct act 2233

<210> SEQ ID NO 19
<211> LENGTH: 1565
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 19

agcaaaagca gggtagataa tcactcactg agtgacatca aaatcatggc gtctcaaggc 60

accaaacgat cttacgaaca gatggagact gatggagaac gccagaatgc cactgaaatc 120

agagcatccg tcggaaaaat gattggtgga attggacgat tctacatcca gatgtgcacc 180

gaactcaaac tcagtgatta tgagggacgg ctgatccaga acagcctgac aatcgagaga 240

atggtgctct ctgcttttga cgaaaggaga aataaatacc tggaagaaca tcccagtgcc 300

gggaaagatc ctaagaaaac tggaggacct atctacagga gagtgaacgg aaagtggatg 360

agagaactca tcctgtatga caaagaagaa atcaggcgaa tctggcgcca ggctaataat 420

ggtgacgatg caaccgctgg tctgactcac atgatgatct ggcattccaa tctgaatgat 480

gcaacttatc agaggacaag agctctggtg cgcaccggaa tggatcccag gatgtgctct 540

ctgatgcagg gttcaactct ccctaggagg tctggagccg caggtgctgc agtcaaagga 600

gtgggaacaa tggtgatgga actggtcaga atgatcaaaa gagggatcaa tgatcggaac 660

ttctggaggg gtgagaatgg acgaaaaaca agaattgctt atgaaagaat gtgcaacatt 720

ctcaaaggga aatttcagac tgctgcacag aaagcaatga tggatcaggt gagagagagc 780

cggaacccag ggaatgctga gttcgaagat ctcacttttc tggcacggtc tgcactcatc 840

ctgagagggt ccgtggctca caagtcctgc ctgcctgcct gtgtgtatgg acctgccgtg 900

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gccagtgggt acgactttga aagggaggga tactctctgg tcggaattga ccctttcaga 960

ctgctgcaga acagccaggt gtacagcctg atcagaccaa atgagaatcc agcacacaag 1020

agtcagctgg tgtggatggc atgccattct gccgcatttg aagatctgag agtgctgagc 1080

ttcatcaaag ggaccaaggt gctcccaaga gggaagctgt ccactagagg agtgcagatt 1140

gcttccaatg aaaatatgga gactatggaa tcaagtacac tggaactgag aagcaggtac 1200

tgggccatca ggaccagaag tggaggaaac accaatcagc agagggcatc tgccggccag 1260

atcagcattc agcctacctt ctcagtgcag agaaatctcc cttttgacag aacaaccatt 1320

atggcagcat tcaatgggaa tacagagggg agaacatctg acatgaggac cgaaatcatc 1380

aggatgatgg aaagtgcaag accagaagat gtgtctttcc aggggcgggg agtcttcgag 1440

ctctcggacg aaaaggcagc gagcccgatc gtgccttcct ttgacatgag taatgaagga 1500

tcttatttct tcggagacaa tgcagaggag tacgacaatt aaagaaaaat acccttgttt 1560

ctact 1565

<210> SEQ ID NO 20
<211> LENGTH: 1684
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 20

atgaacactc aaatcctggt attcgctctg attgcgatca ttccaacaaa tgcagacaaa 60

atctgcctcg gacatcatgc cgtgtcaaac ggaaccaaag taaacacatt aactgaaaga 120

ggagtggaag tcgtcaatgc aactgaaaca gtggaacgaa caaacatccc caggatctgc 180

tcaaaaggga aaaggacagt tgacctcggt caatgtggac tcctggggac aatcactgga 240

ccacctcaat gtgaccaatt cctagaattt tcagccgatt taattattga gaggcgagaa 300

ggaagtgatg tctgttatcc tgggaaattc gtgaatgaag aagctctgag gcaaattctc 360

agagaatcag gcggaattga caaggaagca atgggattca catacagtgg aataagaact 420

aatggagcaa ccagtgcatg taggagatca ggatcttcat tctatgcaga aatgaaatgg 480

ctcctgtcaa acacagataa tgctgcattc ccgccagatg actaagtcat ataaaaatac 540

aagaaaaagc ccagctctaa tagtatgggg gatccatcat tccgtatcaa ctgcagagca 600

aaccaagcta tatgggagtg gaaacaaact ggtgacagtt gggagttcta attatcaaca 660

atcttttgta ccgagtccag gagcgagacc acaagttaat ggtctatctg gaagaattga 720

ctttcattgg ctaatgctaa atcccaatga tacagtcact ttcagtttca atggggcttt 780

catagctcca gaccgtgcaa gcttcctgag aggaaaatct atgggaatcc agagtggagt 840

acaggttgat gccaattgtg aaggggactg ctatcatagt ggagggacaa taataagtaa 900

cttgccattt cagaacatag atagcagggc agttggaaaa tgtccgagat atgttaagca 960

aaggagtctg ctgctagcaa cagggatgaa gaatgttcct gagattccaa agggaagagg 1020

cctatttggt gctatagcgg gtttcattga aaatggatgg gaaggcctaa ttgatggttg 1080

gtatggtttc agacaccaga atgcacaggg agagggaact gctgcagatt acaaaagcac 1140

tcaatcggca attgatcaaa taacaggaaa attaaaccgg cttatagaaa aaaccaacca 1200

acaatttgag ttgatagaca atgaattcaa tgaggtagag aagcaaatcg gtaatgtgat 1260

aaattggacc agagattcta taacagaagt gtggtcatac aatgctgaac tcttggtagc 1320

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aatggagaac cagcatacaa ttgatctggc tgattcagaa atggacaaac tgtacgaacg   1380

agtgaaaaga cagctgagag agaatgctga agaagatggc actggttgct ttgaaatatt   1440

tcacaagtgt gatgatgact gtatggccag tattagaaat aacacctatg atcacagcaa   1500

atacagggaa gaggcaatgc aaaatagaat acagattgac ccagtcaaac taagcagcgg   1560

ctacaaagat gtgatacttt ggtttagctt cggggcatca tgtttcatac ttctagccat   1620

tgtaatgggc cttgtcttca tatgtgtaaa gaatggaaac atgcggtgca ctatttgtat   1680

ataa                                                               1684
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<210> SEQ ID NO 21
<211> LENGTH: 560
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 21

```
Met Asn Thr Gln Ile Leu Val Phe Ala Leu Ser Lys Gly Lys Arg Thr
1               5                  10                  15

Val Asp Leu Gly Ile Ala Ile Ile Pro Thr Asn Ala Asp Lys Gln Cys
            20                  25                  30

Gly Leu Leu Gly Thr Ile Thr Gly Ile Cys Leu Gly His His Ala Val
        35                  40                  45

Ser Asn Pro Pro Gln Cys Asp Gln Phe Leu Glu Phe Gly Thr Lys Val
    50                  55                  60

Asn Thr Leu Thr Glu Arg Ser Ala Asp Leu Ile Ile Glu Arg Arg Glu
65                  70                  75                  80

Gly Val Glu Val Val Asn Ala Thr Glu Thr Gly Ser Asp Val Cys Tyr
                85                  90                  95

Pro Gly Lys Phe Val Glu Arg Thr Asn Ile Pro Arg Ile Cys Val Asn
            100                 105                 110

Glu Glu Ala Leu Arg Gln Ile Leu Arg Glu Ser Gly Gly Ile Asp Lys
        115                 120                 125

Glu Ala Met Gly Phe Thr Tyr Ser Gly Ile Arg Thr Asn Gly Ala Thr
    130                 135                 140

Ser Ala Cys Arg Arg Ser Gly Ser Ser Phe Tyr Ala Glu Met Lys Trp
145                 150                 155                 160

Leu Leu Ser Asn Thr Asp Asn Ala Ala Phe Pro Gln Met Thr Lys Ser
                165                 170                 175

Tyr Lys Asn Thr Arg Lys Ser Pro Ala Leu Ile Val Trp Gly Ile His
            180                 185                 190

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

Lys Leu Val Thr Val Gly Ser Ser Asn Tyr Gln Gln Ser Phe Val Pro
    210                 215                 220

Ser Pro Gly Ala Arg Pro Gln Val Asn Gly Leu Ser Gly Arg Ile Asp
225                 230                 235                 240

Phe His Trp Leu Met Leu Asn Pro Asn Asp Thr Val Thr Phe Ser Phe
                245                 250                 255

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

Ser Met Gly Ile Gln Ser Gly Val Gln Val Asp Ala Asn Cys Glu Gly
        275                 280                 285
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Asp Cys Tyr His Ser Gly Gly Thr Ile Ile Ser Asn Leu Pro Phe Gln
    290                 295                 300

Asn Ile Asp Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val Lys Gln
305                 310                 315                 320

Arg Ser Leu Leu Leu Ala Thr Gly Met Lys Asn Val Pro Glu Ile Pro
                325                 330                 335

Lys Gly Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly
            340                 345                 350

Trp Glu Gly Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala
        355                 360                 365

Gln Gly Glu Gly Thr Ala Ala Asp Tyr Lys Ser Thr Gln Ser Ala Ile
    370                 375                 380

Asp Gln Ile Thr Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Gln
385                 390                 395                 400

Gln Phe Glu Leu Ile Asp Asn Glu Phe Asn Glu Val Glu Lys Gln Ile
                405                 410                 415

Gly Asn Val Ile Asn Trp Thr Arg Asp Ser Ile Thr Glu Val Trp Ser
            420                 425                 430

Tyr Asn Ala Glu Leu Leu Val Ala Met Glu Asn Gln His Thr Ile Asp
        435                 440                 445

Leu Ala Asp Ser Glu Met Asp Lys Leu Tyr Glu Arg Val Lys Arg Gln
    450                 455                 460

Leu Arg Glu Asn Ala Glu Glu Asp Gly Thr Gly Cys Phe Glu Ile Phe
465                 470                 475                 480

His Lys Cys Asp Asp Asp Cys Met Ala Ser Ile Arg Asn Asn Thr Tyr
                485                 490                 495

Asp His Ser Lys Tyr Arg Glu Glu Ala Met Gln Asn Arg Ile Gln Ile
            500                 505                 510

Asp Pro Val Lys Leu Ser Ser Gly Tyr Lys Asp Val Ile Leu Trp Phe
        515                 520                 525

Ser Phe Gly Ala Ser Cys Phe Ile Leu Leu Ala Ile Val Met Gly Leu
    530                 535                 540

Val Phe Ile Cys Val Lys Asn Gly Asn Met Arg Cys Thr Ile Cys Ile
545                 550                 555                 560


<210> SEQ ID NO 22
<211> LENGTH: 1398
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 22

atgaatccaa atcagaagat tctatgcact tcagccactg ctatcataat aggcgcaatc     60

gcagtactca ttggaatagc aaacctagga ttgaacatag gactgcatct aaaaccgggc    120

tgcaattgct cacactcaca acctgaaaca accaacacaa gccaaacaat aataaacaac    180

tattataatg aaacaaacat caccaacatc caaatggaag agagaacaag caggaatttc    240

aataacttaa ctaaagggct ctgtactata aattcatggc acatatatgg gaaagacaat    300

gcagtaagaa ttggagagag ctcggatgtt ttagtcacaa gagaacccta tgtttcatgc    360

gacccagatg aatgcaggtt ctatgctctc agccaaggaa caacaatcag agggaaacac    420

tcaaacggaa caatacacga taggtcccag tatcgcgccc tgataagctg gccactatca    480

tcaccgccca cagtgtacaa cagcagggtg gaatgcattg ggtggtcaag tactagttgc    540
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catgatggca aatccaggat gtcaatatgt atatcaggac caaacaacaa tgcatctgca    600

gtagtatggt acaacagaag gcctgttgca gaaattaaca catgggcccg aaacatacta    660

agaacacagg aatctgaatg tgtatgccac aacggcgtat gcccagtagt gttcaccgat    720

gggtctgcca ctggacctgc agacacaaga atatactatt ttaaagaggg gaaaatattg    780

aaatgggagt ctctgactgg aactgctaag catattgaag aatgctcatg ttacggggaa    840

cgaacaggaa ttacctgcac atgcagggac aattggcagg gctcaaatag accagtgatt    900

cagatagacc cagtagcaat gacacacact agtcaatata tatgcagtcc tgttcttaca    960

gacaatcccc gaccgaatga cccaaatata ggtaagtgta atgaccctta tccaggtaat   1020

aataacaatg gagtcaaggg attctcatac ctggatgggg ctaacacttg gctagggagg   1080

acaataagca cagcctcgag gtctggatac gagatgttaa aagtgccaaa tgcattgaca   1140

gatgatagat caaagcccat tcaaggtcag acaattgtat taaacgctga ctggagtggt   1200

tacagtggat ctttcatgga ctattgggct gaaggggact gctatcgagc gtgtttttat   1260

gtggagttga tacgtggaag acccaaggaa gataaagtgt ggtggaccag caatagtata   1320

gtatcgatgt gttccagtac agaattcctg ggacaatgga actggcctga tggggctaaa   1380

atagagtact tcctctaa                                                 1398
```

<210> SEQ ID NO 23
<211> LENGTH: 464
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 23

```
Met Asn Pro Asn Gln Lys Ile Leu Cys Thr Ser Ala Thr Ala Ile Ile
1               5                  10                  15

Ile Gly Ala Ile Ala Val Leu Ile Gly Ile Ala Asn Leu Gly Leu Asn
            20                  25                  30

Ile Gly Leu His Leu Lys Pro Gly Cys Asn Cys Ser His Ser Gln Pro
        35                  40                  45

Glu Thr Thr Asn Thr Ser Gln Thr Ile Ile Asn Asn Tyr Tyr Asn Glu
    50                  55                  60

Thr Asn Ile Thr Asn Ile Gln Met Glu Glu Arg Thr Ser Arg Asn Phe
65                  70                  75                  80

Asn Asn Leu Thr Lys Gly Leu Cys Thr Ile Asn Ser Trp His Ile Tyr
                85                  90                  95

Gly Lys Asp Asn Ala Val Arg Ile Gly Glu Ser Ser Asp Val Leu Val
            100                 105                 110

Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Asp Glu Cys Arg Phe Tyr
        115                 120                 125

Ala Leu Ser Gln Gly Thr Ile Ile Arg Gly Lys His Ser Asn Gly Thr
    130                 135                 140

Ile His Asp Arg Ser Gln Tyr Arg Ala Leu Ile Ser Trp Pro Leu Ser
145                 150                 155                 160

Ser Pro Pro Thr Val Tyr Asn Ser Arg Val Glu Cys Ile Gly Trp Ser
                165                 170                 175

Ser Thr Ser Cys His Asp Gly Lys Ser Arg Met Ser Ile Cys Ile Ser
            180                 185                 190

Gly Pro Asn Asn Asn Ala Ser Ala Val Val Trp Tyr Asn Arg Arg Pro
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        195                 200                 205

Val Ala Glu Ile Asn Thr Trp Ala Arg Asn Ile Leu Arg Thr Gln Glu
    210                 215                 220

Ser Glu Cys Val Cys His Asn Gly Val Cys Pro Val Val Phe Thr Asp
225                 230                 235                 240

Gly Ser Ala Thr Gly Pro Ala Asp Thr Arg Ile Tyr Phe Lys Glu Gly
                245                 250                 255

Lys Ile Leu Lys Trp Glu Ser Leu Thr Gly Thr Ala Lys His Ile Glu
            260                 265                 270

Glu Cys Ser Cys Tyr Gly Glu Arg Thr Gly Ile Thr Cys Thr Cys Arg
        275                 280                 285

Asp Asn Trp Gln Gly Ser Asn Arg Pro Val Ile Gln Ile Asp Pro Val
    290                 295                 300

Ala Met Thr His Thr Ser Gln Tyr Ile Cys Ser Pro Val Leu Thr Asp
305                 310                 315                 320

Asn Pro Arg Pro Asn Asp Pro Asn Ile Gly Lys Cys Asn Asp Pro Tyr
                325                 330                 335

Pro Gly Asn Asn Asn Asn Gly Val Lys Gly Phe Ser Tyr Leu Asp Gly
            340                 345                 350

Ala Asn Thr Trp Leu Gly Arg Thr Ile Ser Thr Ala Ser Arg Ser Gly
        355                 360                 365

Tyr Glu Met Leu Lys Val Pro Asn Ala Leu Thr Asp Asp Arg Ser Lys
    370                 375                 380

Pro Ile Gln Gly Gln Thr Ile Val Leu Asn Ala Asp Trp Ser Gly Tyr
385                 390                 395                 400

Ser Gly Ser Phe Met Asp Tyr Trp Ala Glu Gly Asp Cys Tyr Arg Ala
                405                 410                 415

Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Lys Glu Asp Lys Val
            420                 425                 430

Trp Trp Thr Ser Asn Ser Ile Val Ser Met Cys Ser Ser Thr Glu Phe
        435                 440                 445

Leu Gly Gln Trp Asn Trp Pro Asp Gly Ala Lys Ile Glu Tyr Phe Leu
    450                 455                 460


<210> SEQ ID NO 24
<211> LENGTH: 1683
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 24

atgaacactc aaatcctggt attcgctctg attgcgatca ttccaacaaa tgcagacaaa     60

atctgcctcg gacatcatgc tgtgtcaaac ggaaccaaag taaacacatt aactgaaaga    120

ggagtggaag tcgtcaatgc aactgaaaca gtggaacgaa caaacatccc caggatctgc    180

tcaaaaggga aaaggacagt tgacctcggt caatgtggac tcctggggac aatcactgga    240

ccacctcaat gtgaccaatt cctagaattt tcagccgatt taattattga gaggcgagaa    300

ggaagtgatg tctgttatcc tgggaaattc gtgaatgaag aagctctgag gcaaattctc    360

agagaatcag gcggaattga caaggaagca atgggattca catacagtgg aataagaact    420

aatggagcaa ccagttcatg taggagatca ggatcttcat tctatgcaga aatgaaatgg    480

ctcctgtcaa acacagataa tgctgcattc ccgcagatga ctaagtcata taaaaataca    540
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agaaaaaacc cagctctaat agtatggggg atccatcatt ccggatcaac tgcagagcaa 600

accaagctat atgggagtgg aaacaaactg gtgacagttg ggagttctaa ttatcaacaa 660

tcttttgtac cgagtccggg agcgagaaca caagttaatg gtcaatctgg aagaattgac 720

tttcattggc taatgctaaa tcccaatgat acagtcactt tcagtttcaa tggggctttc 780

atagctccag accgtgcaag cttcctgaga ggaaaatcta tgggaatcca gagtggagta 840

caggttgatg ccgattgtga aggggactgc tattatagtg gagggacaat aataagtaac 900

ttgccatttc agaacataga tagcagggca gttggaaaat gtccgagata tgttaagcaa 960

aggagtctgc tgctagcaac agggatgaag aatgttcctg agattccaaa gggaagaggc 1020

ctatttggtg ctatagcggg tttcattgaa aatggatggg aaggcctaat tgatggttgg 1080

tatggtttca gacaccagaa tgcacaggga gagggaactg ctgcagatta caaaagcact 1140

caatcggcaa ttgatcaaat aacaggaaaa ttaaaccggc ttatagaaaa aaccaaccaa 1200

caatttgagt tgatagacaa tgaattcact gaggtagaga agcaaatcgg taatgtgata 1260

aattggacca gagattctat aacagaagtg tggtcataca atgctgaact cttggtagca 1320

atggagaacc agcatacaat tgatctggct gattcagaaa tggacaaact gtacgaacga 1380

gtgaaaagac agctgagaga gaatgctgaa gaagatggca ctggttgctt tgaaatattt 1440

cacaagtgtg atgatgactg tatggccagc attagaaata acacctatga tcacagcaaa 1500

tacagggaag aggcaatgca aaatagaata cagattgacc cagtcaaact aagcagcggc 1560

tacaaagatg tgatactttg gtttagcttc ggggcatcat gtttcatact tctagccatt 1620

gcaatgggcc ttgtcttcat atgtgtaaag aatggaaaca tgcggtgcac tatttgtata 1680

taa 1683

<210> SEQ ID NO 25
<211> LENGTH: 1273
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 25

Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1 5 10 15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
20 25 30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
35 40 45

Tyr Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
50 55 60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65 70 75 80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
85 90 95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
100 105 110

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
115 120 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
130 135 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr

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145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
            180                 185                 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
        195                 200                 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
385                 390                 395                 400

Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
                405                 410                 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
            420                 425                 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
        435                 440                 445

Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
    450                 455                 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465                 470                 475                 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
                485                 490                 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
            500                 505                 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
        515                 520                 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
    530                 535                 540

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

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
           580                 585                 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
       595                 600                 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
   610                 615                 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625                 630                 635                 640

Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
               645                 650                 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
           660                 665                 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
       675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
   690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
               725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
           740                 745                 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
       755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
   770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
               805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
           820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
       835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
   850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
               885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
           900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
       915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
   930                 935                 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960
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Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990

Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
        995                 1000                1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu
    1010                1015                1020

Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val
1025                1030                1035                1040

Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ser Ala
                1045                1050                1055

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ala Gln Glu
            1060                1065                1070

Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His
        1075                1080                1085

Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val
    1090                1095                1100

Thr Gln Arg Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr
1105                1110                1115                1120

Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr
                1125                1130                1135

Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu
            1140                1145                1150

Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp
        1155                1160                1165

Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
    1170                1175                1180

Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
1185                1190                1195                1200

Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile
                1205                1210                1215

Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile
            1220                1225                1230

Met Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
        1235                1240                1245

Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val
    1250                1255                1260

Leu Lys Gly Val Lys Leu His Tyr Thr
1265                1270


<210> SEQ ID NO 26
<211> LENGTH: 1273
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 26

Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1               5                   10                  15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
            20                  25                  30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
        35                  40                  45
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His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
    50                  55                  60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65                  70                  75                  80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
                85                  90                  95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
            100                 105                 110

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
        115                 120                 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
    130                 135                 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
            180                 185                 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
        195                 200                 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
385                 390                 395                 400

Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
                405                 410                 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
            420                 425                 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
        435                 440                 445
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Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
    450                 455                 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465                 470                 475                 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
                485                 490                 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
            500                 505                 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
        515                 520                 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
    530                 535                 540

Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys Phe Leu
545                 550                 555                 560

Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala Val
                565                 570                 575

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
            580                 585                 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
        595                 600                 605

Ala Val Leu Tyr Gln Gly Val Asn Cys Thr Glu Val Pro Val Ala Ile
    610                 615                 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625                 630                 635                 640

Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
                645                 650                 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
            660                 665                 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
        675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
    690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
                725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
            740                 745                 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
        755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
    770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
                805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
            820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
        835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
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    850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
                885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
            900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
        915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
    930                 935                 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960

Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990

Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
        995                 1000                1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu
    1010                1015                1020

Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val
1025                1030                1035                1040

Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ser Ala
                1045                1050                1055

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ala Gln Glu
            1060                1065                1070

Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His
        1075                1080                1085

Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val
    1090                1095                1100

Thr Gln Arg Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr
1105                1110                1115                1120

Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr
                1125                1130                1135

Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu
            1140                1145                1150

Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp
        1155                1160                1165

Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
    1170                1175                1180

Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
1185                1190                1195                1200

Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile
                1205                1210                1215

Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile
            1220                1225                1230

Met Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
        1235                1240                1245

Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val
    1250                1255                1260
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Leu Lys Gly Val Lys Leu His Tyr Thr
1265                1270


<210> SEQ ID NO 27
<211> LENGTH: 1273
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 27

Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1               5                   10                  15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
            20                  25                  30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
        35                  40                  45

His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
    50                  55                  60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65                  70                  75                  80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
                85                  90                  95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
            100                 105                 110

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
        115                 120                 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
    130                 135                 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
            180                 185                 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
        195                 200                 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
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            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
385                 390                 395                 400

Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
                405                 410                 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
            420                 425                 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
        435                 440                 445

Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
    450                 455                 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465                 470                 475                 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
                485                 490                 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
            500                 505                 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
        515                 520                 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
    530                 535                 540

Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys Phe Leu
545                 550                 555                 560

Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala Val
                565                 570                 575

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
            580                 585                 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
        595                 600                 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
    610                 615                 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625                 630                 635                 640

Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
                645                 650                 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
            660                 665                 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
        675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
    690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
                725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
            740                 745                 750
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Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
        755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
    770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
                805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
            820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
        835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
    850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
                885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
            900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
        915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
    930                 935                 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960

Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990

Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
        995                 1000                1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu
    1010                1015                1020

Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val
1025                1030                1035                1040

Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ser Ala
                1045                1050                1055

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ala Gln Glu
            1060                1065                1070

Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His
        1075                1080                1085

Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val
    1090                1095                1100

Thr Gln Arg Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr
1105                1110                1115                1120

Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr
                1125                1130                1135

Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu
            1140                1145                1150
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Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp
        1155                1160                1165

Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
    1170                1175                1180

Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
1185                1190                1195                1200

Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile
                1205                1210                1215

Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile
            1220                1225                1230

Met Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
        1235                1240                1245

Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val
    1250                1255                1260

Leu Lys Gly Val Lys Leu His Tyr Thr
1265                1270


<210> SEQ ID NO 28
<211> LENGTH: 1273
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 28

Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1               5                   10                  15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
            20                  25                  30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
        35                  40                  45

His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
    50                  55                  60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65                  70                  75                  80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
                85                  90                  95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
            100                 105                 110

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
        115                 120                 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
    130                 135                 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
            180                 185                 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
        195                 200                 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240
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Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
385                 390                 395                 400

Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
                405                 410                 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
            420                 425                 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
        435                 440                 445

Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
    450                 455                 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465                 470                 475                 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
                485                 490                 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
            500                 505                 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
        515                 520                 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
    530                 535                 540

Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys Phe Leu
545                 550                 555                 560

Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala Val
                565                 570                 575

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
            580                 585                 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
        595                 600                 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
    610                 615                 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625                 630                 635                 640
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Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
                645                 650                 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
            660                 665                 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
        675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
    690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
                725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
            740                 745                 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
        755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
    770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
                805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
            820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
        835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
    850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
                885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
            900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
        915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
    930                 935                 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960

Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990

Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
        995                 1000                1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu
    1010                1015                1020

Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val
1025                1030                1035                1040

Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ser Ala
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               1045                1050                1055

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ala Gln Glu
           1060                1065                1070

Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His
       1075                1080                1085

Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val
   1090                1095                1100

Thr Gln Arg Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr
1105                1110                1115                1120

Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr
               1125                1130                1135

Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu
           1140                1145                1150

Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp
       1155                1160                1165

Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
   1170                1175                1180

Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
1185                1190                1195                1200

Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile
               1205                1210                1215

Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile
           1220                1225                1230

Met Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
       1235                1240                1245

Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val
   1250                1255                1260

Leu Lys Gly Val Lys Leu His Tyr Thr
1265                1270


<210> SEQ ID NO 29
<211> LENGTH: 560
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 29

Met Asn Thr Gln Ile Leu Val Phe Ala Leu Ile Ala Ile Ile Pro Thr
1               5                   10                  15

Asn Ala Asp Lys Ile Cys Leu Gly His His Ala Val Ser Asn Gly Thr
            20                  25                  30

Lys Val Asn Thr Leu Thr Glu Arg Gly Val Glu Val Val Asn Ala Thr
        35                  40                  45

Glu Thr Val Glu Arg Thr Asn Ile Pro Arg Ile Cys Ser Lys Gly Lys
    50                  55                  60

Arg Thr Val Asp Leu Gly Gln Cys Gly Leu Leu Gly Thr Ile Thr Gly
65                  70                  75                  80

Pro Pro Gln Cys Asp Gln Phe Leu Glu Phe Ser Ala Asp Leu Ile Ile
                85                  90                  95

Glu Arg Arg Glu Gly Ser Asp Val Cys Tyr Pro Gly Lys Phe Val Asn
            100                 105                 110

Glu Glu Ala Leu Arg Gln Ile Leu Arg Glu Ser Gly Gly Ile Asp Lys
        115                 120                 125
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Glu Ala Met Gly Phe Thr Tyr Ser Gly Ile Arg Thr Asn Gly Ala Thr
    130                 135                 140

Ser Ser Cys Arg Arg Ser Gly Ser Ser Phe Tyr Ala Glu Met Lys Trp
145                 150                 155                 160

Leu Leu Ser Asn Thr Asp Asn Ala Ala Phe Pro Gln Met Thr Lys Ser
                165                 170                 175

Tyr Lys Asn Thr Arg Lys Asn Pro Ala Leu Ile Val Trp Gly Ile His
            180                 185                 190

His Ser Gly Ser Thr Ala Glu Gln Thr Lys Leu Tyr Gly Ser Gly Asn
        195                 200                 205

Lys Leu Val Thr Val Gly Ser Ser Asn Tyr Gln Gln Ser Phe Val Pro
    210                 215                 220

Ser Pro Gly Ala Arg Thr Gln Val Asn Gly Gln Ser Gly Arg Ile Asp
225                 230                 235                 240

Phe His Trp Leu Met Leu Asn Pro Asn Asp Thr Val Thr Phe Ser Phe
                245                 250                 255

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

Ser Met Gly Ile Gln Ser Gly Val Gln Val Asp Ala Asp Cys Glu Gly
        275                 280                 285

Asp Cys Tyr Tyr Ser Gly Gly Thr Ile Ile Ser Asn Leu Pro Phe Gln
    290                 295                 300

Asn Ile Asp Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val Lys Gln
305                 310                 315                 320

Arg Ser Leu Leu Leu Ala Thr Gly Met Lys Asn Val Pro Glu Ile Pro
                325                 330                 335

Lys Gly Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly
            340                 345                 350

Trp Glu Gly Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala
        355                 360                 365

Gln Gly Glu Gly Thr Ala Ala Asp Val Lys Ser Thr Gln Ser Ala Ile
    370                 375                 380

Asp Gln Ile Thr Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Gln
385                 390                 395                 400

Gln Phe Glu Leu Ile Asp Asn Glu Phe Thr Glu Val Glu Lys Gln Ile
                405                 410                 415

Gly Asn Val Ile Asn Trp Thr Arg Asp Ser Ile Thr Glu Val Trp Ser
            420                 425                 430

Tyr Asn Ala Glu Leu Leu Val Ala Met Glu Asn Gln His Thr Ile Asp
        435                 440                 445

Leu Ala Asp Ser Glu Met Asp Lys Leu Tyr Glu Arg Val Lys Arg Gln
    450                 455                 460

Leu Arg Glu Asn Ala Glu Glu Asp Gly Thr Gly Cys Phe Glu Ile Phe
465                 470                 475                 480

His Lys Cys Asp Asp Asp Cys Met Ala Ser Ile Arg Asn Asn Thr Tyr
                485                 490                 495

Asp His Ser Lys Tyr Arg Glu Glu Ala Met Gln Asn Arg Ile Gln Ile
            500                 505                 510

Asp Pro Val Lys Leu Ser Ser Gly Tyr Lys Asp Val Ile Leu Trp Phe
        515                 520                 525

Ser Phe Gly Ala Ser Cys Phe Ile Leu Leu Ala Ile Ala Met Gly Leu
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    530                 535                 540

Val Phe Ile Cys Val Lys Asn Gly Asn Met Arg Cys Thr Ile Cys Ile
545                 550                 555                 560


<210> SEQ ID NO 30
<211> LENGTH: 1398
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 30

atgaatccaa atcagaagat tctatgcact tcagccactg ctatcataat aggcgcaatc     60

gcagtactca ttggaatagc aaacctagga ttgaacatag gactgcatct aaaaccgagc    120

tgcaattgct cacactcaca acctgaaaca accaacacaa gccaaacaat aataaacaac    180

tattataatg aaacaaacat caccaacatc caaatggaag agagaacaag caggaatttc    240

aataacttaa ctaaagggct ctgtactata aattcatggc acatatatgg gaaagacaat    300

gcggtaagaa ttggagagag ctcggatgtt ttagtcacaa gagaacccta tgtttcatgc    360

gacccagatg aatgcaggtt ctatgctctc agccaaggaa caacaatcag aggaaaacac    420

tcaaacggaa caatacacga taggtcccag tatcgcgccc tgataagctg gccactatca    480

tcaccgccca cagtgtacaa cagcagggtg gaatgcattg ggtggtcaag tactagttgc    540

catgatggca aatccaggat gtcaatatgt atatcaggac caaacaacaa tgcatctgca    600

gtagtatggt acaacagaag gcctgttgca gaaattaaca catgggcccg aaacatacta    660

agaacacagg aatctgaatg tgtatgccac aacggcgtat gcccagtagt gttcaccgat    720

gggtctgcca ctggacctgc agacacaaga atatactatt ttaaagaggg gaaaatattg    780

aaatgggagt ctctgactgg aactgctaag catattgaag aatgctcatg ttacggggaa    840

cgaacaggaa ttacctgcac atgcaaggac aattggcagg gctcaaatag accagtgatt    900

cagatagatc cagtagcaat gacacacact agtcagtata tatgcagtcc tgttcttaca    960

gacaatcccc gaccgaatga cccaaatata ggtaagtgta atgaccctta tccaggtaat   1020

aataacaatg gagtcaaggg attctcatac ctggatgggg ctaacacttg gctagggagg   1080

acaataagca cagcctcgag gtctggatac gagatgttaa aagtgccaaa tgcattgaca   1140

gatgatagat caaagcccat tcaaggtcag acaattgtat taaacgctga ctggagtggt   1200

tacagtggat ctttcatgga ctattgggct gaggggact gctatcgagc gtgtttttat   1260

gtggaattga tacgtggaag acccaaggag gataaagtgt ggtggaccag caatagtata   1320

gtatcgatgt gttccagtac agaattcctg ggacaatgga actggcctga tggggctaaa   1380

atagagtact tcctctaa                                                1398


<210> SEQ ID NO 31
<211> LENGTH: 462
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 31

Met Asn Pro Asn Gln Lys Ile Leu Cys Thr Ser Ala Thr Ala Ile Ile
1               5                   10                  15

Ile Gly Ala Ile Ala Val Leu Ile Gly Ile Ala Asn Leu Gly Leu Asn
            20                  25                  30

Ile Gly Leu His Leu Lys Pro Ser Cys Asn Cys Ser His Ser Gln Pro
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        35                  40                  45

Glu Thr Ile Asn Thr Ser Gln Thr Ile Ile Asn Asn Tyr Tyr Asn Glu
    50                  55                  60

Thr Asn Ile Thr Asn Ile Gln Met Glu Glu Arg Thr Ser Arg Asn Phe
65                  70                  75                  80

Asn Asn Leu Thr Lys Gly Leu Cys Thr Ile Asn Ser Trp His Ile Tyr
                85                  90                  95

Gly Lys Asp Asn Ala Val Arg Ile Gly Glu Ser Ser Asp Val Leu Val
            100                 105                 110

Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Asp Glu Cys Arg Phe Tyr
        115                 120                 125

Ala Leu Ser Gln Gly Thr Ile Ile Arg Gly Lys His Ser Asn Gly Thr
    130                 135                 140

Ile His Asp Arg Ser Gln Tyr Arg Ala Leu Ile Ser Trp Pro Leu Ser
145                 150                 155                 160

Ser Pro Pro Thr Val Tyr Asn Ser Arg Val Glu Cys Ile Gly Trp Ser
                165                 170                 175

Ser Thr Ser Cys His Asp Gly Lys Ser Arg Met Ser Ile Cys Ile Ser
            180                 185                 190

Gly Pro Asn Asn Asn Ala Ser Ala Trp Trp Tyr Asn Arg Arg Pro Val
        195                 200                 205

Ala Glu Ile Asn Thr Trp Ala Arg Asn Ile Leu Arg Thr Gln Glu Ser
    210                 215                 220

Glu Cys Val Cys His Asn Gly Val Cys Pro Trp Phe Thr Asp Gly Ser
225                 230                 235                 240

Ala Thr Gly Pro Ala Asp Thr Arg Ile Tyr Tyr Phe Lys Glu Gly Lys
                245                 250                 255

Leu Lys Trp Glu Ser Leu Thr Gly Thr Ala Lys His Ile Glu Glu Cys
            260                 265                 270

Ser Cys Tyr Gly Glu Arg Thr Gly Ile Thr Cys Thr Cys Lys Asp Asn
        275                 280                 285

Trp Gln Gly Ser Asn Arg Pro Val Ile Gln Ile Asp Pro Val Ala Met
    290                 295                 300

Thr His Thr Ser Gln Tyr Ile Cys Ser Pro Val Leu Thr Asp Asn Pro
305                 310                 315                 320

Arg Pro Asn Asp Pro Asn Ile Gly Lys Cys Asn Asp Pro Tyr Pro Gly
                325                 330                 335

Asn Asn Asn Asn Gly Val Lys Gly Phe Ser Tyr Leu Asp Gly Ala Asn
            340                 345                 350

Thr Trp Leu Gly Arg Thr Ile Ser Thr Ala Ser Arg Ser Gly Tyr Glu
        355                 360                 365

Met Leu Lys Val Pro Asn Ala Leu Thr Asp Asp Arg Ser Lys Pro Ile
    370                 375                 380

Gln Gly Gln Thr Ile Val Leu Asn Ala Asp Trp Ser Gly Tyr Ser Gly
385                 390                 395                 400

Ser Phe Met Asp Tyr Trp Ala Glu Gly Asp Cys Tyr Arg Ala Cys Phe
                405                 410                 415

Tyr Val Glu Leu Ile Arg Gly Arg Pro Lys Glu Asp Lys Val Trp Trp
            420                 425                 430

Thr Ser Asn Ser Ile Val Ser Met Cys Ser Ser Thr Glu Phe Leu Gly
        435                 440                 445
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Gln Trp Asn Trp Pro Asp Gly Ala Lys Ile Glu Tyr Phe Leu
450 455 460

<210> SEQ ID NO 32
<211> LENGTH: 1775
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 32

agcaaaagca ggggaaaata aaaacaacca aaatgaaggc aaacctactg gtcctgttat 60

gtgcacttgc agctgcagat gcagacacaa tatgtatagg ctaccatgcg aacaattcaa 120

ccgacactgt tgacacagta ctcgagaaga atgtgacagt gacacactct gttaacctgc 180

tcgaagacag ccacaacgga aaactatgta gattaaaagg aatagcccca ctacaattgg 240

ggaaatgtaa catcgccgga tggctcttgg gaaacccaga atgcgaccca ctgcttccag 300

tgagatcatg gtcctacatt gtagaaacac caaactctga gaatggaata tgttatccag 360

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

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 33
<211> LENGTH: 1413
<212> TYPE: DNA

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<213> ORGANISM: Influenza virus

<400> SEQUENCE: 33

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 34
<211> LENGTH: 2233
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 34

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

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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

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 35
<211> LENGTH: 2341
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 35

agcgaaagca ggcaaaccat ttgaatggat gtcaatccga ccttactttt cttaaaagtg 60

ccagcacaaa atgctataag cacaactttc ccttatactg gagaccctcc ttacagccat 120

gggacaggaa caggatacac catggatact gtcaacagga cacatcagta ctcagaaaag 180

ggaagatgga caacaaacac cgaaactgga gcaccgcaac tcaacccgat tgatgggcca 240

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ctgccagaag acaatgaacc aagtggttat gcccaaacag attgtgtatt ggaggcgatg 300

gctttccttg aggaatccca tcctggtatt tttgaaaact cgtgtattga aacgatggag 360

gttgttcagc aaacacgagt agacaagctg acacaaggcc gacagaccta tgactggact 420

ctaaatagaa accaacctgc tgcaacagca ttggccaaca caatagaagt gttcagatca 480

aatggcctca cggccaatga gtctggaagg ctcatagact tccttaagga tgtaatggag 540

tcaatgaaca aagaagaaat ggggatcaca actcattttc agagaaagag acgggtgaga 600

gacaatatga ctaagaaaat gataacacag agaacaatgg gtaaaaagaa gcagagattg 660

aacaaaagga gttatctaat tagagcattg accctgaaca caatgaccaa agatgctgag 720

agagggaagc taaaacggag agcaattgca accccaggga tgcaaataag ggggtttgta 780

tactttgttg agacactggc aaggagtata tgtgagaaac ttgaacaatc agggttgcca 840

gttggaggca atgagaagaa agcaaagttg gcaaatgttg taaggaagat gatgaccaat 900

tctcaggaca ccgaactttc tttcaccatc actggagata acaccaaatg gaacgaaaat 960

cagaatcctc ggatgttttt ggccatgatc acatatatga ccagaaatca gcccgaatgg 1020

ttcagaaatg ttctaagtat tgctccaata atgttctcaa acaaaatggc gagactggga 1080

aaagggtata tgtttgagag caagagtatg aaacttagaa ctcaaatacc tgcagaaatg 1140

ctagcaagca tcgatttgaa atatttcaat gattcaacaa gaaagaagat tgaaaaaatc 1200

cgaccgctct taatagaggg gactgcatca ttgagccctg gaatgatgat gggcatgttc 1260

aatatgttaa gcactgtatt aggcgtctcc atcctgaatc ttggacaaaa gagatacacc 1320

aagactactt actggtggga tggtcttcaa tcctctgacg attttgctct gattgtgaat 1380

gcacccaatc atgaagggat tcaagccgga gtcgacaggt tttatcgaac ctgtaagcta 1440

cttggaatca atatgagcaa gaaaaagtct tacataaaca gaacaggtac atttgaattc 1500

acaagttttt tctatcgtta tgggtttgtt gccaatttca gcatggagct tcccagtttt 1560

ggggtgtctg ggatcaacga gtcagcggac atgagtattg gagttactgt catcaaaaac 1620

aatatgataa acaatgatct tggtccagca acagctcaaa tggcccttca gttgttcatc 1680

aaagattaca ggtacacgta ccgatgccat ataggtgaca cacaaataca aacccgaaga 1740

tcatttgaaa taaagaaact gtgggagcaa acccgttcca aagctggact gctggtctcc 1800

gacggaggcc caaatttata caacattaga aatctccaca ttcctgaagt ctgcctaaaa 1860

tgggaattga tggatgagga ttaccagggg cgtttatgca acccactgaa cccatttgtc 1920

agccataaag aaattgaatc aatgaacaat gcagtgatga tgccagcaca tggtccagcc 1980

aaaaacatgg agtatgatgc tgttgcaaca acacactcct ggatccccaa aagaaatcga 2040

tccatcttga atacaagtca aagaggagta cttgaggatg aacaaatgta ccaaaggtgc 2100

tgcaatttat ttgaaaaatt cttccccagc agttcataca gaagaccagt cgggatatcc 2160

agtatggtgg aggctatggt ttccagagcc cgaattgatg cacggattga tttcgaatct 2220

ggaaggataa agaaagaaga gttcactgag atcatgaaga tctgttccac cattgaagag 2280

ctcagacggc aaaaatagtg aatttagctt gtccttcatg aaaaaatgcc ttgtttctac 2340

t 2341

<210> SEQ ID NO 36
<211> LENGTH: 2341
<212> TYPE: DNA

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<213> ORGANISM: Influenza virus

<400> SEQUENCE: 36

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

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

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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 37
<211> LENGTH: 1565
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 37

agcaaaagca gggtagataa tcactcactg agtgacatca aaatcatggc gtctcaaggc 60

accaaacgat cttacgaaca gatggagact gatggagaac gccagaatgc cactgaaatc 120

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

gcaacttatc agaggacaag agctcttgtt cgcaccggaa tggatcccag gatgtgctct 540

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

ttgagagggt cggttgctca caagtcctgc ctgcctgcct gtgtgtatgg acctgccgta 900

gccagtgggt acgactttga aagggaggga tactctctag tcggaataga ccctttcaga 960

ctgcttcaaa acagccaagt gtacagccta atcagaccaa atgagaatcc agcacacaag 1020

agtcaactgg tgtggatggc atgccattct gccgcatttg aagatctaag agtattaagc 1080

ttcatcaaag ggacgaaggt gctcccaaga gggaagcttt ccactagagg agttcaaatt 1140

gcttccaatg aaaatatgga gactatggaa tcaagtacac ttgaactgag aagcaggtac 1200

tgggccataa ggaccagaag tggaggaaac accaatcaac agagggcatc tgcgggccaa 1260

atcagcatac aacctacgtt ctcagtacag agaaatctcc cttttgacag aacaaccatt 1320

atggcagcat tcaatgggaa 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

<210> SEQ ID NO 38
<211> LENGTH: 1027
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

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<400> SEQUENCE: 38

agcaaaagca ggtagatatt gaaagatgag tcttctaacc gaggtcgaaa cgtacgtact 60

ctctatcatc ccgtcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtctt 120

tgcagggaag aacaccgatc ttgaggttct catggaatgg ctaaagacaa gaccaatcct 180

gtcacctctg actaaggggа 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 39
<211> LENGTH: 890
<212> TYPE: DNA
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 39

agcaaaagca gggtgacaaa aacataatgg atccaaacac tgtgtcaagc tttcaggtag 60

attgctttct ttggcatgtc cgcaaacgag ttgcagacca agaactaggc gatgccccat 120

tccttgatcg gcttcgccga gatcagaaat ccctaagagg aaggggcagt actctcggtc 180

tggacatcaa gacagccaca cgtgctggaa agcagatagt ggagcggatt ctgaaagaag 240

aatccgatga ggcacttaaa atgaccatgg cctctgtacc tgcgtcgcgt tacctaactg 300

acatgactct tgaggaaatg tcaagggact ggtccatgct catacccaag cagaaagtgg 360

caggccctct ttgtatcaga atggaccagg cgatcatgga taagaacatc atactgaaag 420

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

actttctcgt ttcagcttat ttagtactaa aaaacaccct tgtttctact 890

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<210> SEQ ID NO 40

<400> SEQUENCE: 40

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<210> SEQ ID NO 41

<400> SEQUENCE: 41

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<210> SEQ ID NO 42

<400> SEQUENCE: 42

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<210> SEQ ID NO 43

<400> SEQUENCE: 43

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<210> SEQ ID NO 44

<400> SEQUENCE: 44

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<210> SEQ ID NO 45

<400> SEQUENCE: 45

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<210> SEQ ID NO 46

<400> SEQUENCE: 46

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<210> SEQ ID NO 47

<400> SEQUENCE: 47

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<210> SEQ ID NO 48

<400> SEQUENCE: 48

000

<210> SEQ ID NO 49

<400> SEQUENCE: 49

000

<210> SEQ ID NO 50
<211> LENGTH: 1273
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

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<400> SEQUENCE: 50

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Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1               5                  10                  15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
            20                  25                  30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
        35                  40                  45

His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
    50                  55                  60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65                  70                  75                  80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
                85                  90                  95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
            100                 105                 110

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
        115                 120                 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
    130                 135                 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
            180                 185                 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
        195                 200                 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
385                 390                 395                 400
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Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
                405                 410                 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
            420                 425                 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
        435                 440                 445

Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
    450                 455                 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465                 470                 475                 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
                485                 490                 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
            500                 505                 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
        515                 520                 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
    530                 535                 540

Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys Phe Leu
545                 550                 555                 560

Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala Val
                565                 570                 575

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
            580                 585                 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
        595                 600                 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
    610                 615                 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625                 630                 635                 640

Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
                645                 650                 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
            660                 665                 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
        675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
    690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
                725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
            740                 745                 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
        755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
    770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800
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```
Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
                805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
            820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
        835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
    850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
                885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
            900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
        915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
    930                 935                 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960

Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990

Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
        995                 1000                1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu
    1010                1015                1020

Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val
1025                1030                1035                1040

Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ser Ala
                1045                1050                1055

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ala Gln Glu
            1060                1065                1070

Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His
        1075                1080                1085

Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val
    1090                1095                1100

Thr Gln Arg Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr
1105                1110                1115                1120

Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr
                1125                1130                1135

Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu
            1140                1145                1150

Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp
        1155                1160                1165

Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
    1170                1175                1180

Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
1185                1190                1195                1200

Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile
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               1205                1210                1215

Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile
            1220                1225                1230

Met Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
        1235                1240                1245

Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val
    1250                1255                1260

Leu Lys Gly Val Lys Leu His Tyr Thr
1265                1270


<210> SEQ ID NO 51
<211> LENGTH: 1273
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 51

Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1               5                   10                  15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
            20                  25                  30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
        35                  40                  45

His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
    50                  55                  60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65                  70                  75                  80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
                85                  90                  95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
            100                 105                 110

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
        115                 120                 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
    130                 135                 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
            180                 185                 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
        195                 200                 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285
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Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
385                 390                 395                 400

Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
                405                 410                 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
            420                 425                 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
        435                 440                 445

Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
    450                 455                 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465                 470                 475                 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
                485                 490                 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
            500                 505                 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
        515                 520                 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
    530                 535                 540

Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys Phe Leu
545                 550                 555                 560

Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala Val
                565                 570                 575

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
            580                 585                 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
        595                 600                 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
    610                 615                 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625                 630                 635                 640

Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
                645                 650                 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
            660                 665                 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
        675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
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    690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
                725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
            740                 745                 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
        755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
    770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
                805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
            820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
        835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
    850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
                885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
            900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
        915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
    930                 935                 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960

Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990

Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
        995                 1000                1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu
    1010                1015                1020

Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val
1025                1030                1035                1040

Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ser Ala
                1045                1050                1055

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ala Gln Glu
            1060                1065                1070

Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His
        1075                1080                1085

Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val
    1090                1095                1100
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Thr Gln Arg Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr
1105                1110                1115                1120

Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr
                1125                1130                1135

Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu
            1140                1145                1150

Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp
        1155                1160                1165

Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
    1170                1175                1180

Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
1185                1190                1195                1200

Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile
                1205                1210                1215

Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile
            1220                1225                1230

Met Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
        1235                1240                1245

Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val
    1250                1255                1260

Leu Lys Gly Val Lys Leu His Tyr Thr
1265                1270


<210> SEQ ID NO 52
<211> LENGTH: 1273
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

<400> SEQUENCE: 52

Met Phe Val Phe Leu Val Leu Leu Pro Leu Val Ser Ser Gln Cys Val
1               5                   10                  15

Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser Phe
            20                  25                  30

Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val Leu
        35                  40                  45

His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr Trp
    50                  55                  60

Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
65                  70                  75                  80

Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr Glu
                85                  90                  95

Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
            100                 105                 110

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
        115                 120                 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
    130                 135                 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145                 150                 155                 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
                165                 170                 175

Met Asp Leu Glu Val Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
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            180                 185                 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
        195                 200                 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
    210                 215                 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225                 230                 235                 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
                245                 250                 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
            260                 265                 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
        275                 280                 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
    290                 295                 300

Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
305                 310                 315                 320

Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys
                325                 330                 335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
            340                 345                 350

Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
        355                 360                 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
    370                 375                 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
385                 390                 395                 400

Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
                405                 410                 415

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
            420                 425                 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
        435                 440                 445

Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
    450                 455                 460

Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
465                 470                 475                 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
                485                 490                 495

Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
            500                 505                 510

Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
        515                 520                 525

Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
    530                 535                 540

Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys Phe Leu
545                 550                 555                 560

Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala Val
                565                 570                 575

Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys Ser Phe
            580                 585                 590
```

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```
Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
        595                 600                 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
    610                 615                 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
625                 630                 635                 640

Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
                645                 650                 655

Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
            660                 665                 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
        675                 680                 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
    690                 695                 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
705                 710                 715                 720

Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
                725                 730                 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
            740                 745                 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr
        755                 760                 765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
    770                 775                 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
785                 790                 795                 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
                805                 810                 815

Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
            820                 825                 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
        835                 840                 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
    850                 855                 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
865                 870                 875                 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
                885                 890                 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
            900                 905                 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
        915                 920                 925

Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
    930                 935                 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
945                 950                 955                 960

Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
                965                 970                 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
            980                 985                 990
```

-continued

```
Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
        995                 1000                1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu
    1010                1015                1020

Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys Arg Val
1025                1030                1035                1040

Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro Gln Ser Ala
                1045                1050                1055

Pro His Gly Val Val Phe Leu His Val Thr Tyr Val Pro Ala Gln Glu
            1060                1065                1070

Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His
        1075                1080                1085

Phe Pro Arg Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val
    1090                1095                1100

Thr Gln Arg Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr
1105                1110                1115                1120

Phe Val Ser Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr
                1125                1130                1135

Val Tyr Asp Pro Leu Gln Pro Glu Leu Asp Ser Phe Lys Glu Glu Leu
            1140                1145                1150

Asp Lys Tyr Phe Lys Asn His Thr Ser Pro Asp Val Asp Leu Gly Asp
        1155                1160                1165

Ile Ser Gly Ile Asn Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp
    1170                1175                1180

Arg Leu Asn Glu Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu
1185                1190                1195                1200

Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile
                1205                1210                1215

Trp Leu Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile
            1220                1225                1230

Met Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
        1235                1240                1245

Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val
    1250                1255                1260

Leu Lys Gly Val Lys Leu His Tyr Thr
1265                1270


<210> SEQ ID NO 53
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A synthetic sequence

<400> SEQUENCE: 53

Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro
1               5                   10                  15

Gly Pro


<210> SEQ ID NO 54
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A synthetic sequence
```

-continued

<400> SEQUENCE: 54

Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val Glu Glu Asn
1 5 10 15

Pro Gly Pro

<210> SEQ ID NO 55
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A synthetic sequence

<400> SEQUENCE: 55

Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp Val Glu Ser
1 5 10 15

Asn Pro Gly Pro
20

<210> SEQ ID NO 56
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A synthetic sequence

<400> SEQUENCE: 56

Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Ala Gly Asp Val Glu
1 5 10 15

Ser Asn Pro Gly Pro
20

What is claimed is:

1. An isolated recombinant influenza virus having at least seven viral segments selected from influenza virus PA, PB1, PB2, NP, NS, M, HA or NA viral segments, or having at least six viral segments selected from PA, PB1, PB2, NP, NS, M, or HEF viral segments, one of which segments comprises coding sequences for an antigenic coronavirus protein or an antigenic portion thereof.

2. The virus of claim **1** which is a single cycle virus.

3. The virus of claim **1** wherein the antigenic coronavirus protein comprises coronavirus S (spike) sequences.

4. The virus of claim **1** wherein the antigenic coronavirus protein comprises S1 sequences.

5. The virus of claim **1** wherein antigenic coronavirus protein comprises a soluble protein.

6. The virus of claim **1** wherein the antigenic portion comprises the receptor binding domain.

7. The virus of claim **1** wherein the antigenic coronavirus protein sequences or the portion thereof have at least 80% amino acid sequence identity to one of SEQ ID Nos. 25-28 and 50-52.

8. The virus of claim **1** which comprises eight or nine viral segments.

9. The virus of claim **1** which is an influenza A or B virus.

10. The virus of claim **1** wherein coding sequences for the antigenic coronavirus protein sequences or the portion thereof replace at least a portion of the coding sequences for one of PA, PB1, PB2, NP, NS1, NS2, M1, M2, HA, or NA.

11. The virus of claim **1** wherein coding sequences for the antigenic coronavirus protein sequences or the portion thereof are inserted into coding sequences in the viral segment of one of PA, PB1, PB2, NP, NS, M, HA or NA viral segments.

12. The virus of claim **1** wherein the virus is bivalent or trivalent.

13. The virus of claim **1** wherein the M viral segment is mutated so that upon viral replication the mutant M gene expresses a functional M1 protein and a mutant M2 protein with a deletion of the cytoplasmic tail and either lacking a transmembrane domain or having a mutated transmembrane domain.

14. The virus of claim **13** wherein the M2 lacks the transmembrane domain.

15. The virus of claim **1** wherein at least one of PA, PB1, or PB2 viral segments has a C to U promoter mutation.

16. The virus of claim **1** wherein the PB2 segment has one or more of a C4U promoter mutation, 202L/323L or 504V; the PB1 segment has one or more of C4U, 40L, 112G, 180W or 247H; the PA segment has one or more of C4U, 142N, 225C or 401K; the NP segment has 74K or 116L; or the NS segment has 30P in NS1 or 118K in NS1.

17. A vaccine comprising an effective amount of the virus of claim **1**.

18. The vaccine of claim **17** wherein the recombinant virus comprises influenza A HA.

19. A method to immunize a vertebrate, comprising: administering to the vertebrate the vaccine of claim **17**.

20. The method of claim **19** wherein the vertebrate is a human.

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