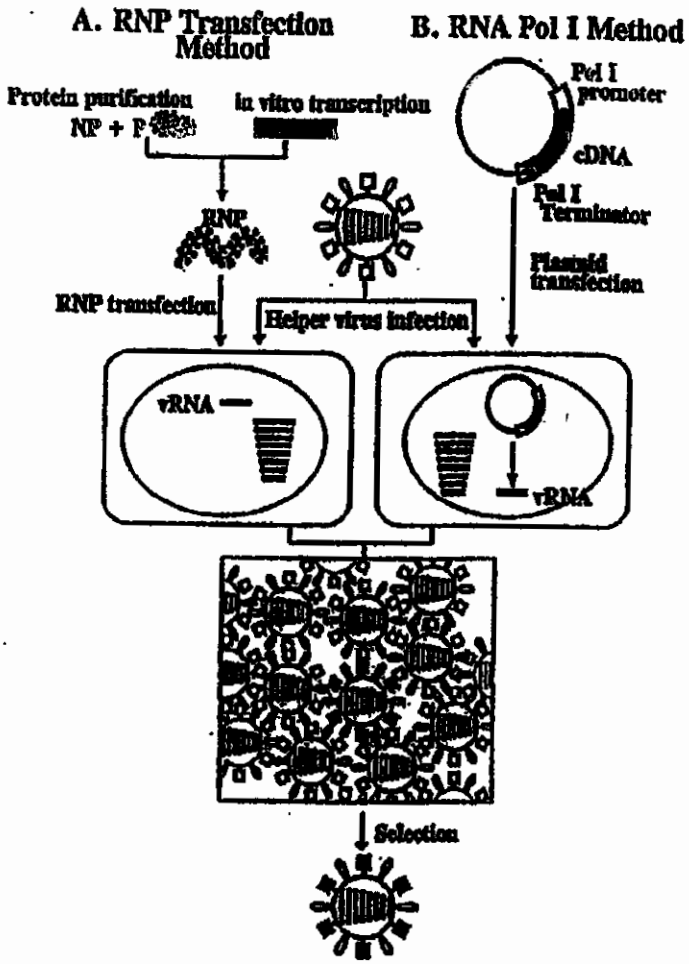

A. RNP Transfection Method
B. RNA Pol I Method
Protein purification
NP + P
In vitro transcription
Pol I promoter
cDNA
Pol I Terminator
Plasmid transfection
RNP
RNP transfection
Helper virus infection
vRNA
vRNA
Selection

Fig. 1

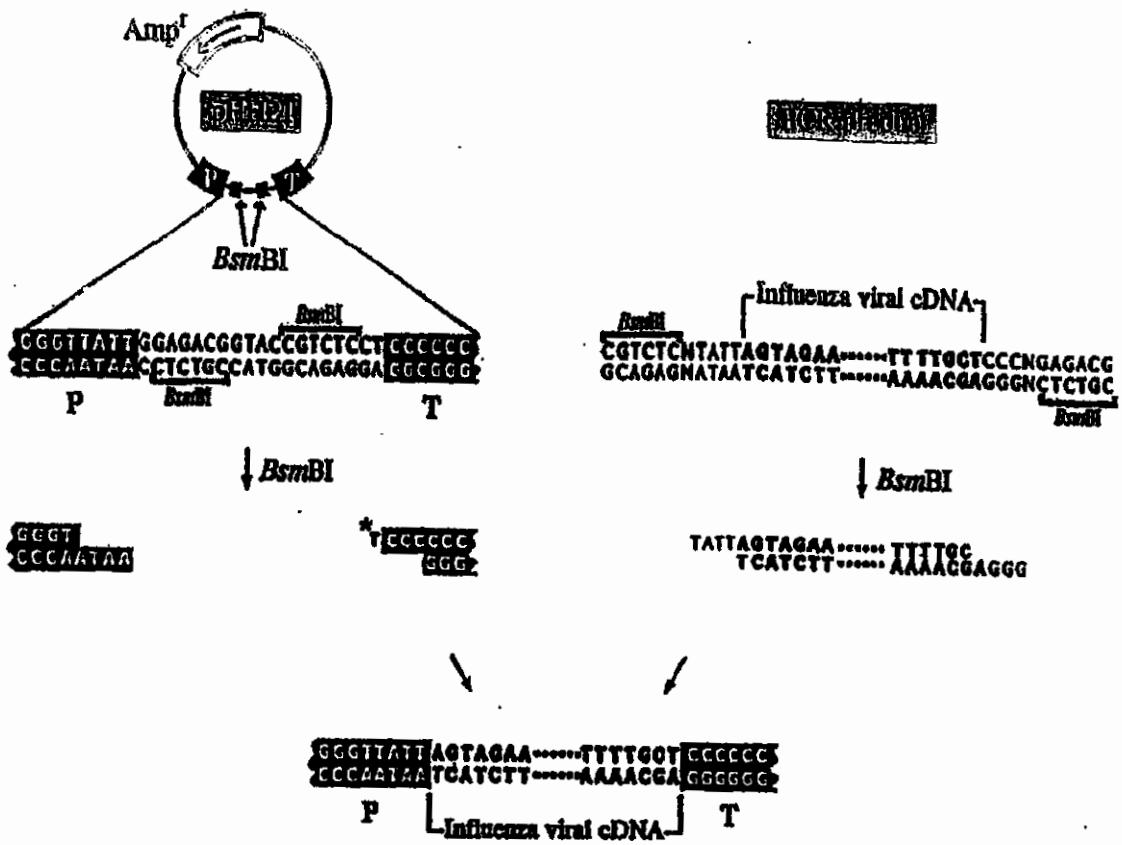

Amp^r
BsmBI
P
T
Influenza viral cDNA
BsmBI
P
T
Influenza viral cDNA

Fig. 2

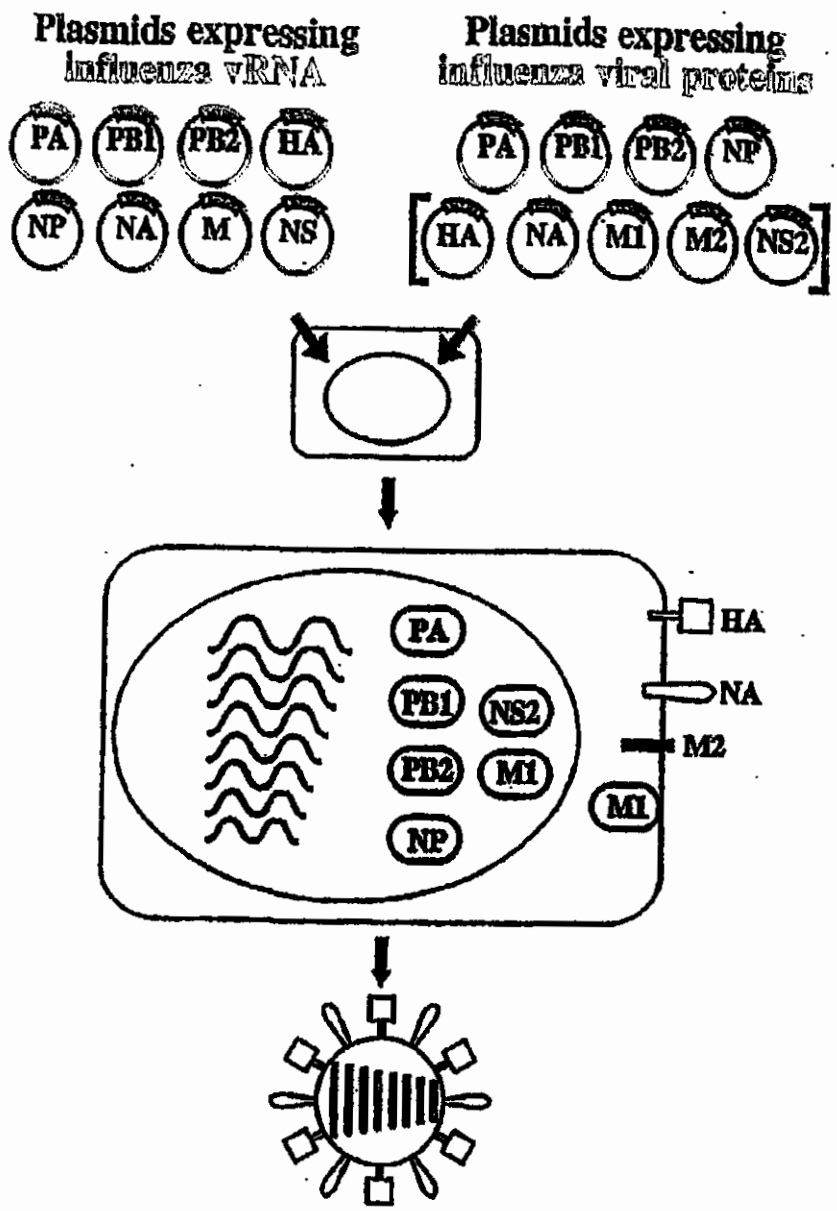

Plasmids expressing influenza vRNA
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PB1
PB2
HA
NP
NA
M
NS
Plasmids expressing influenza viral proteins
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PB2
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HA
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NS2
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PB1
PB2
NP
NS2
M1
HA
NA
M2
M1

Fig. 3

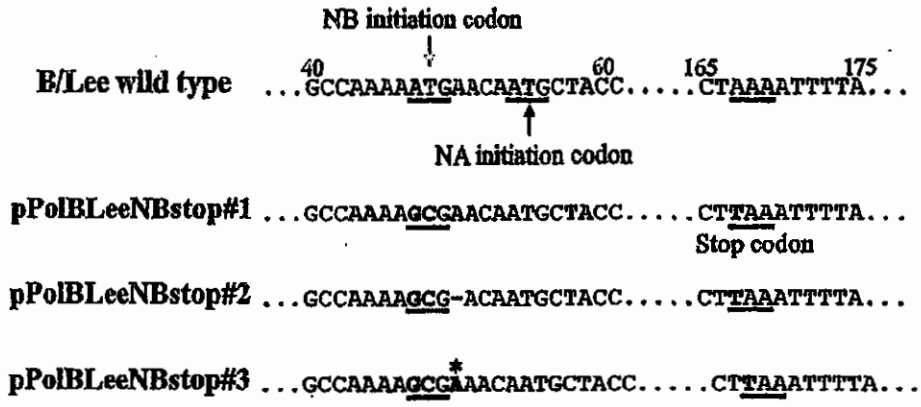

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B/Lee wild type
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Stop codon
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Fig 4

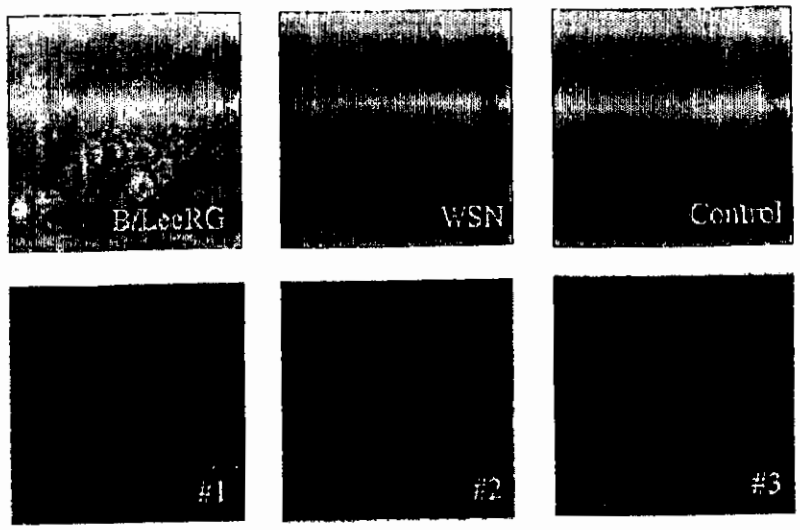


Fig 5A

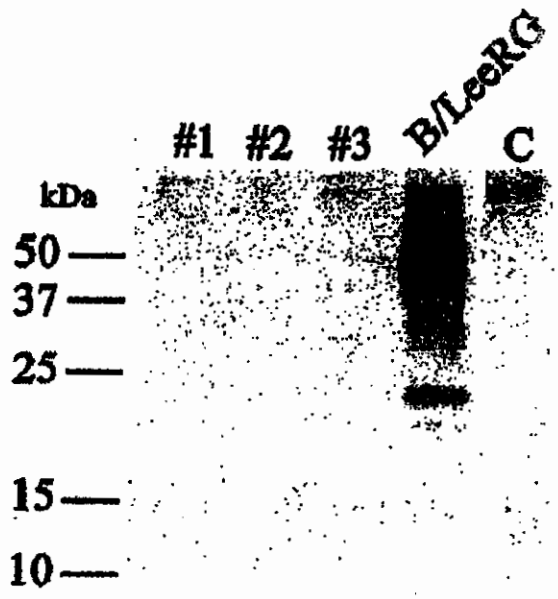


Fig 5B

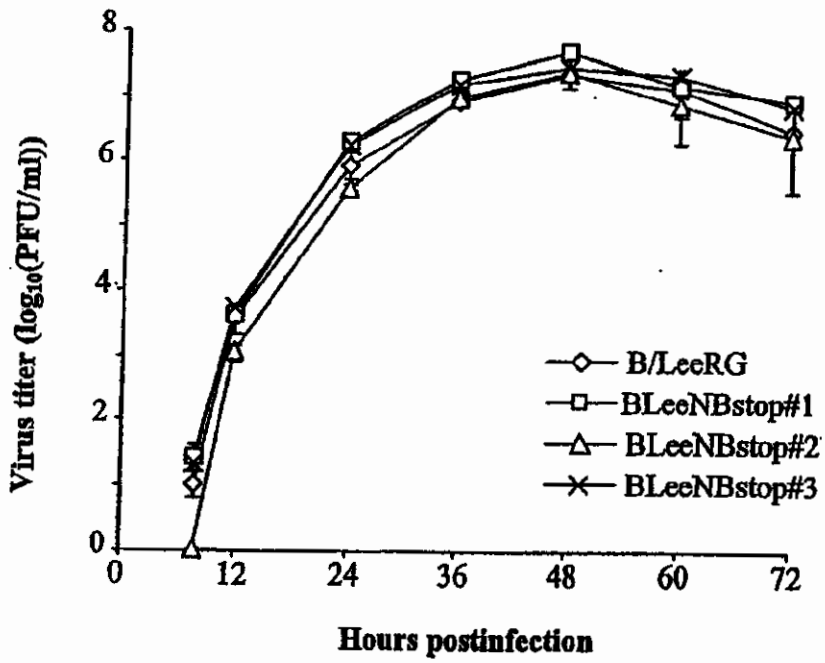

Virus titer (log_{10}(PFU/ml))
8
6
4
2
0
0
12
24
36
48
60
72
Hours postinfection
B/LeeRG
BLeeNBstop#1
BLeeNBstop#2
BLeeNBstop#3

Fig 6

B/Lee

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Figure 7

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SEQUENCE LISTING

<110> Kawaoka, Yoshihiro
WARF - Wisconsin Alumni Research Foundation

<120> Viruses Encoding Mutant Membrane Protein

<130> 800.036US1

<150> US 60/464,776
<151> 2003-04-23

<150> US 60/465,328
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<213> Influenza virus B/Lee/40

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<211> 2368
<212> DNA
<213> Influenza virus B/Lee/40

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<212> DNA
<213> Influenza virus B/Lee/40

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35<211> 1882
<212> DNA
<213> Influenza virus B/Lee/40

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<213> Influenza virus B/Lee/40

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<211> 1841
<212> DNA
<213> Influenza virus B/Lee/40

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gacatggaaa ggaatctaat ccaaaatgca caagctgtgg agagaatcct attggctgca 420
actgatgaca agaaaactga ataccaaaag aaaaggaatg ccagagatgt caaagaaggg 480
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aggtggaaga attgcaatga gcccaatttt cactgtattt cttactatgc atttaagcaa 1140

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<210> 8
<211> 1096
<212> DNA
<213> Influenza virus B/Lee/40

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attctggagt gctatgaaag gttttcatgg caaagagccc ttgactatcc tggtcaagac 180
cgcctacaca gactaaaacg aaaattagaa tcaagaataa agactcacaa caagagtgag 240
cctgagaata aaaggatgtc tcttgaagag agaaaagcaa ttggggtaaa aatgatgaaa 300
gtgcttctgt ttatggatcc ctctgctgga attgaagggt ttgagccata ctgtgtgaaa 360
aatccctcaa ctagcaaatg tccaaattac gattggaccg attaccctcc aaccccagga 420
aagtaccttg atgacataga agaagagccg gaaaatgtcg atcacccaat tgaggtagta 480
ttaagggaca tgaacaataa agatgcacga caaaagataa aggatgaagt aaacactcag 540
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gtgttggtga acggaacctt cctcaagcac cctaatggag acaagtcctt atcaactctt 660
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agaactttat ctcttgagta aaagaattga tgatagtata ttgttccaca aaacagtaat 960
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<212> DNA
<213> Artificial Sequence

<220>
<223> A synthetic oligonucleotide.

<400> 10
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<210> 11
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<220>
<223> A synthetic oligonucleotide.

<400> 11
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<210> 12
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<210> 13
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<220>
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<220>
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<223> A synthetic oligonucleotide.

<400> 19
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<210> 20
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<400> 20
gccaaaaatg aacaatgcta cc 22

<210> 21
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<213> Influenza virus B/Lee/40

<400> 21
ctaaaatttt a 11

<210> 22
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<220>
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<400> 22
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<210> 23
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<212> DNA
<213> Artificial Sequence

<220>
<223> A synthetic oligonucleotide.

<400> 23
cttaaatttt a 11

<210> 24
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<212> DNA
<213> Artificial Sequence

<220>
<223> A synthetic oligonucleotide.

<400> 24
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<210> 25
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> A synthetic oligonucleotide.

<400> 25
cttaaatttt a 11

<210> 26
<211> 23
<212> DNA
<213> Artificial Sequence

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<400> 26
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