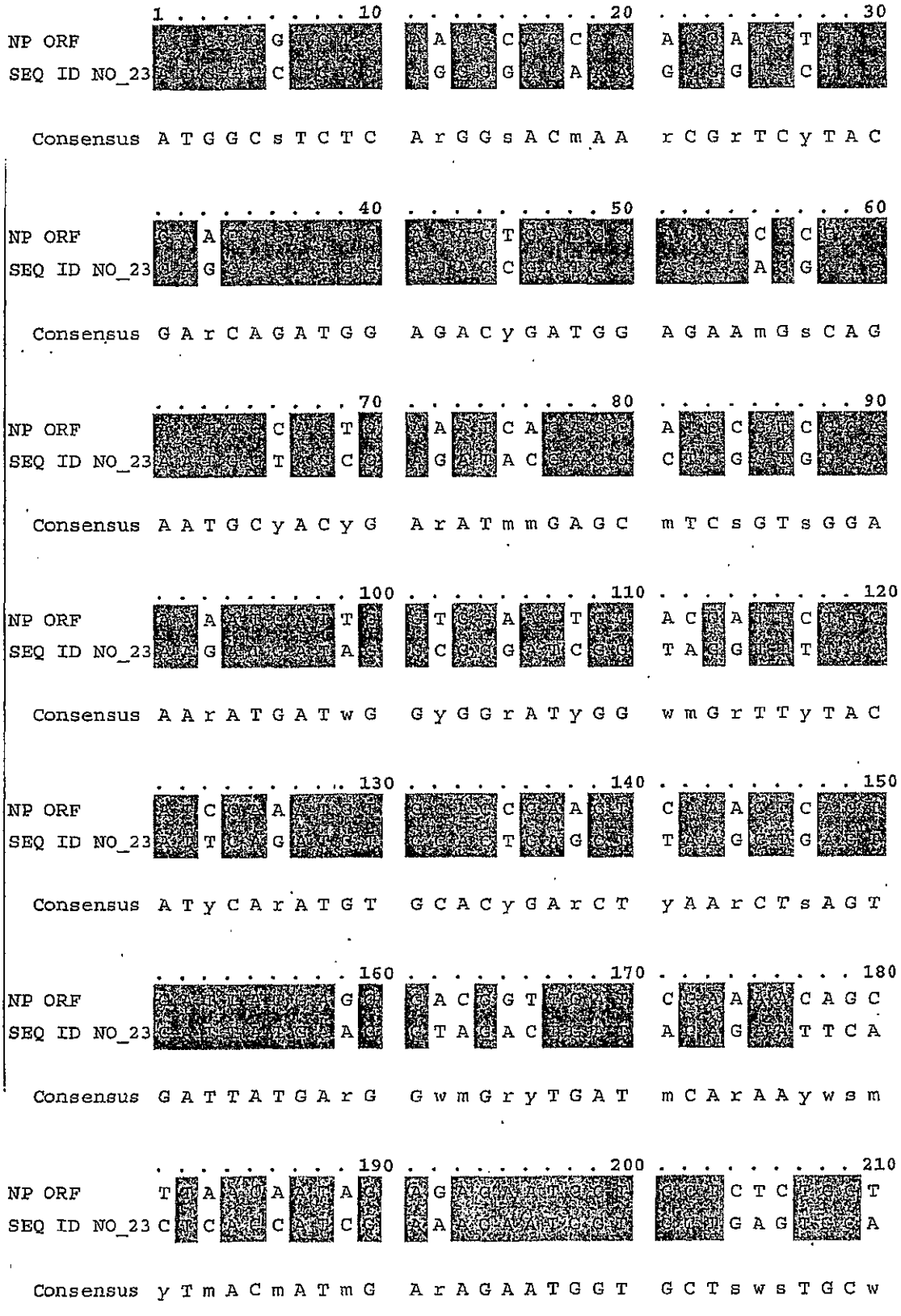
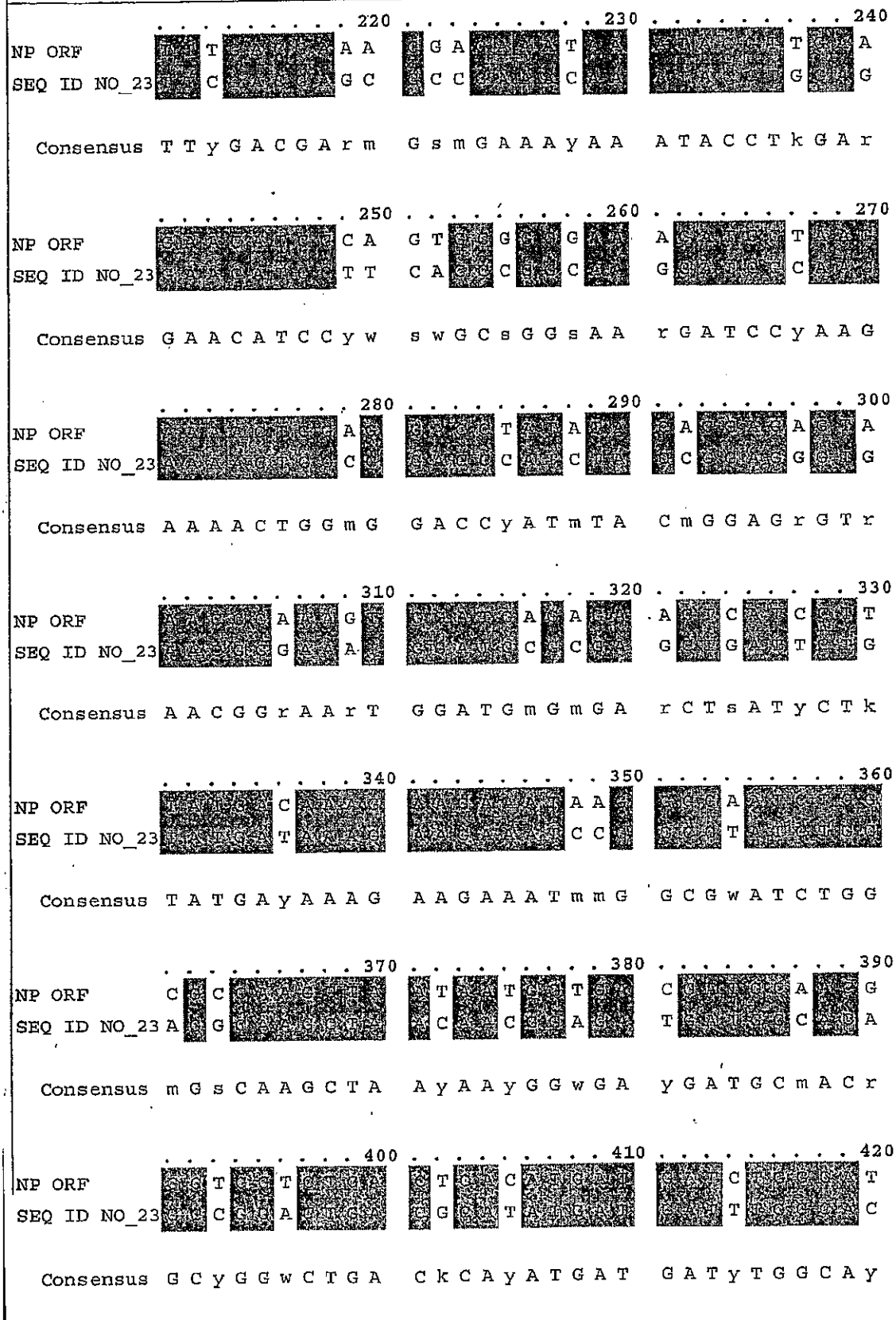
Alignment View Print




OMIGA (2.0)

Figure 1A

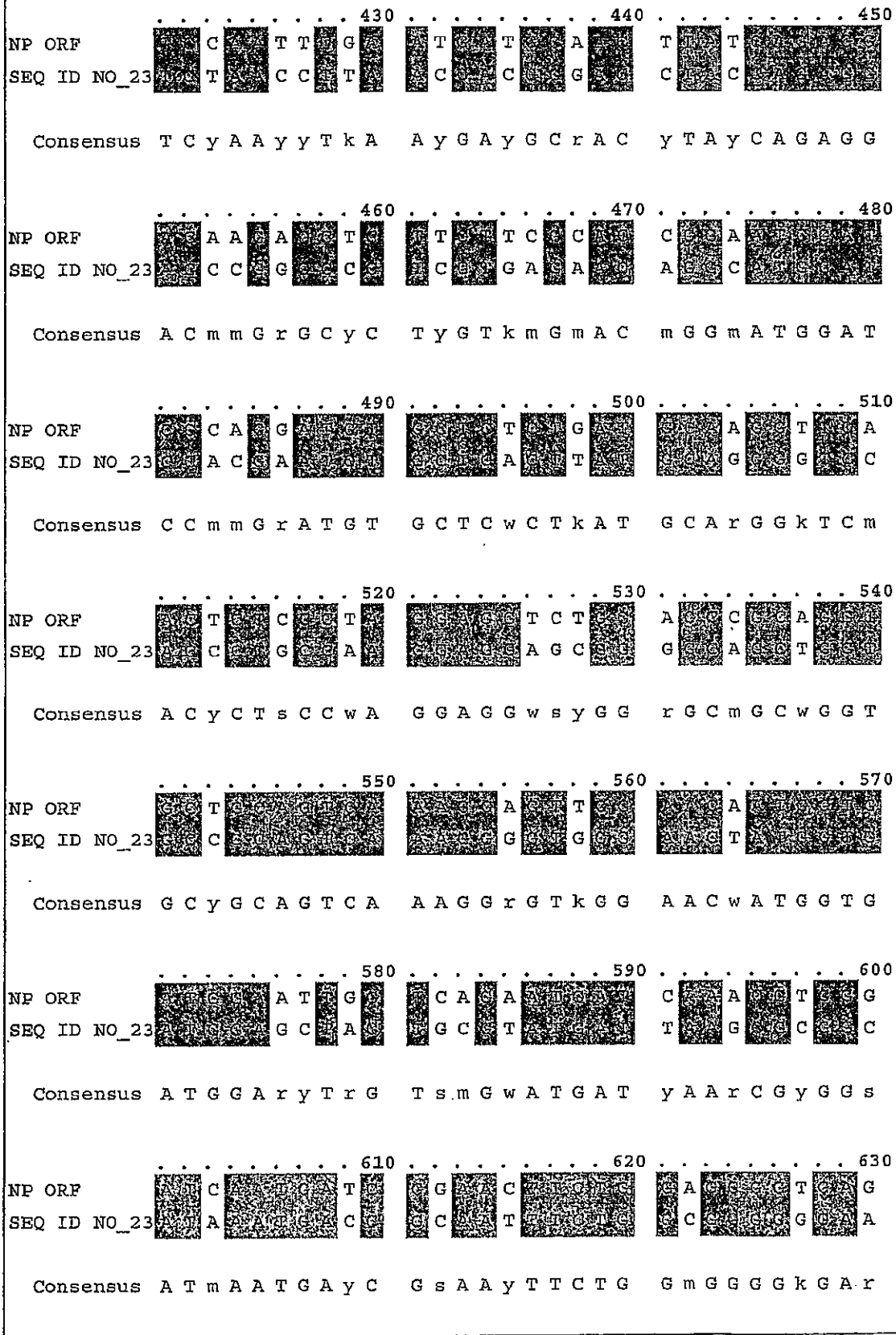
## Alignment View Print




OMIGA (2.0)

Figure 1B

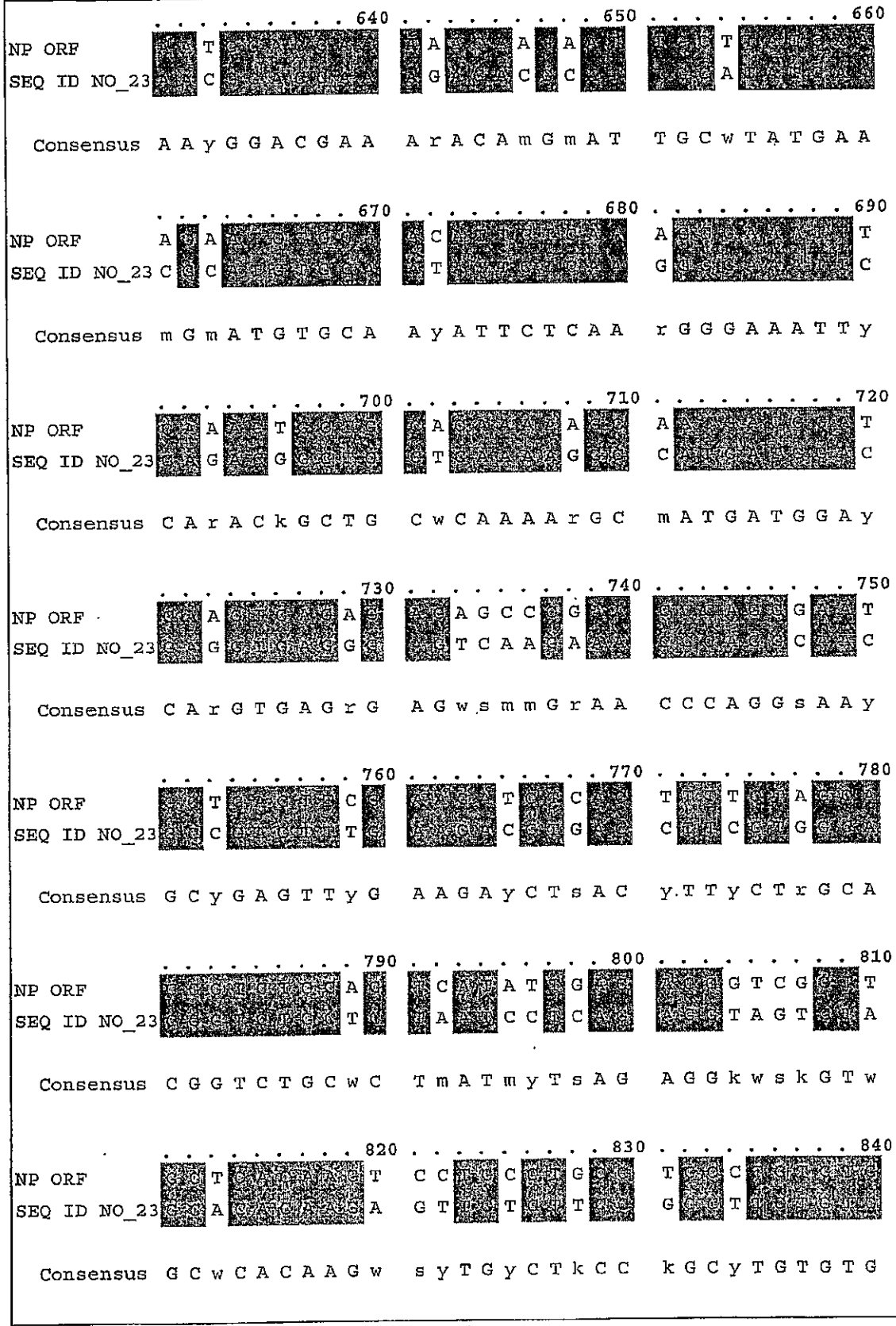
Alignment View Print




OMIGA (2.0)

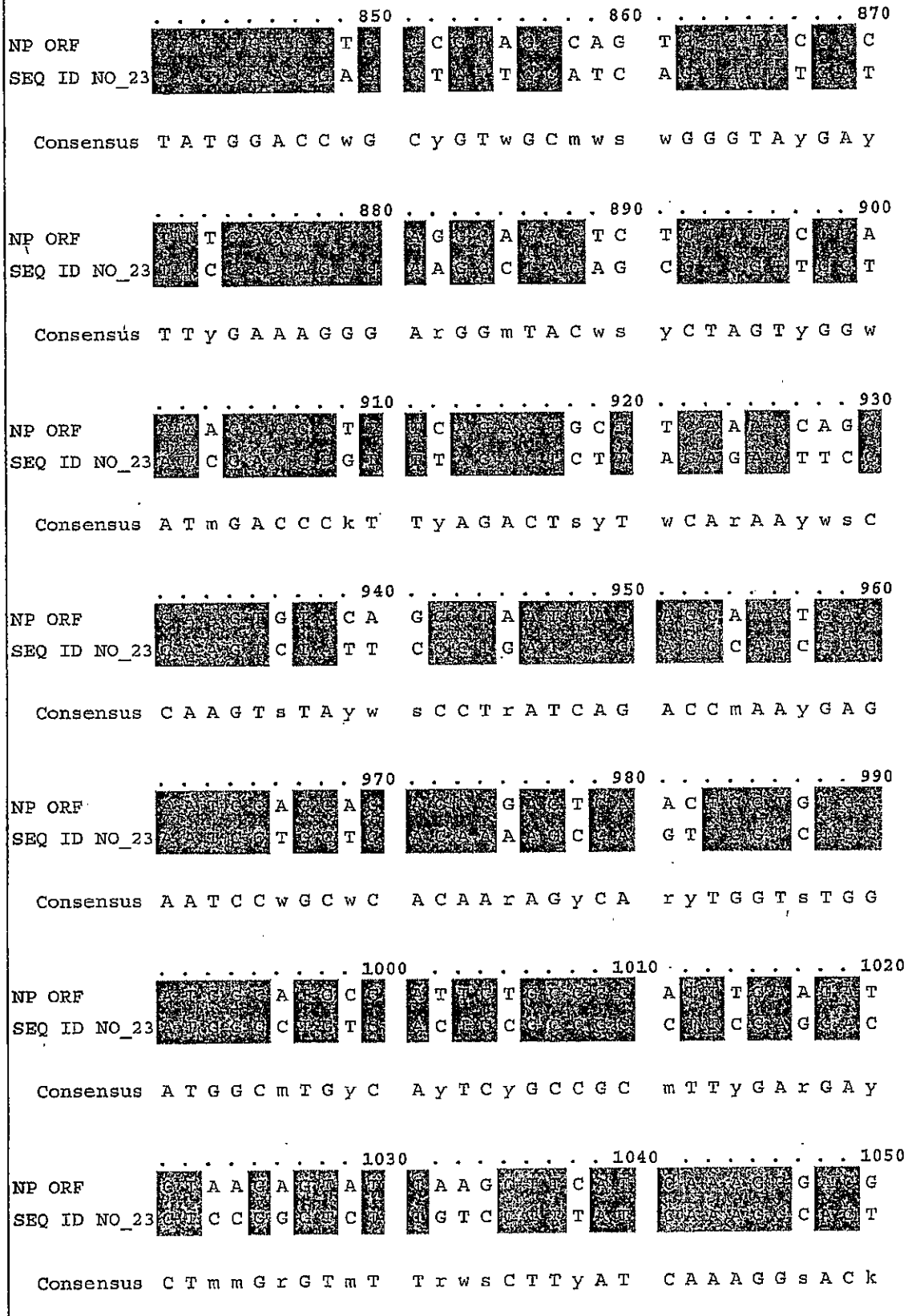
Figure 1C

Alignment View Print




OMIGA (2.0)

Figure 1D




OMIGA (2.0)

Figure 1E

Alignment View Print

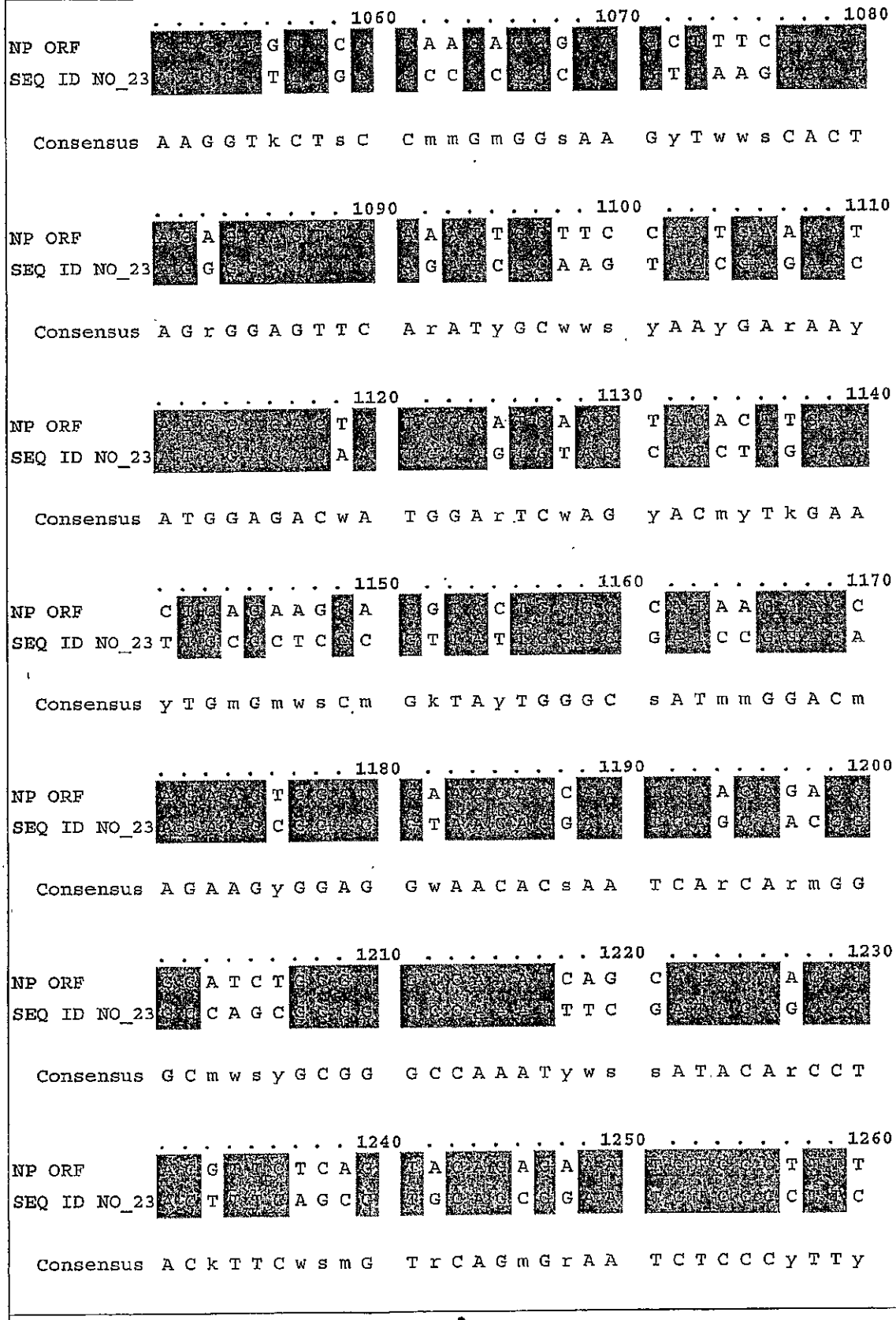
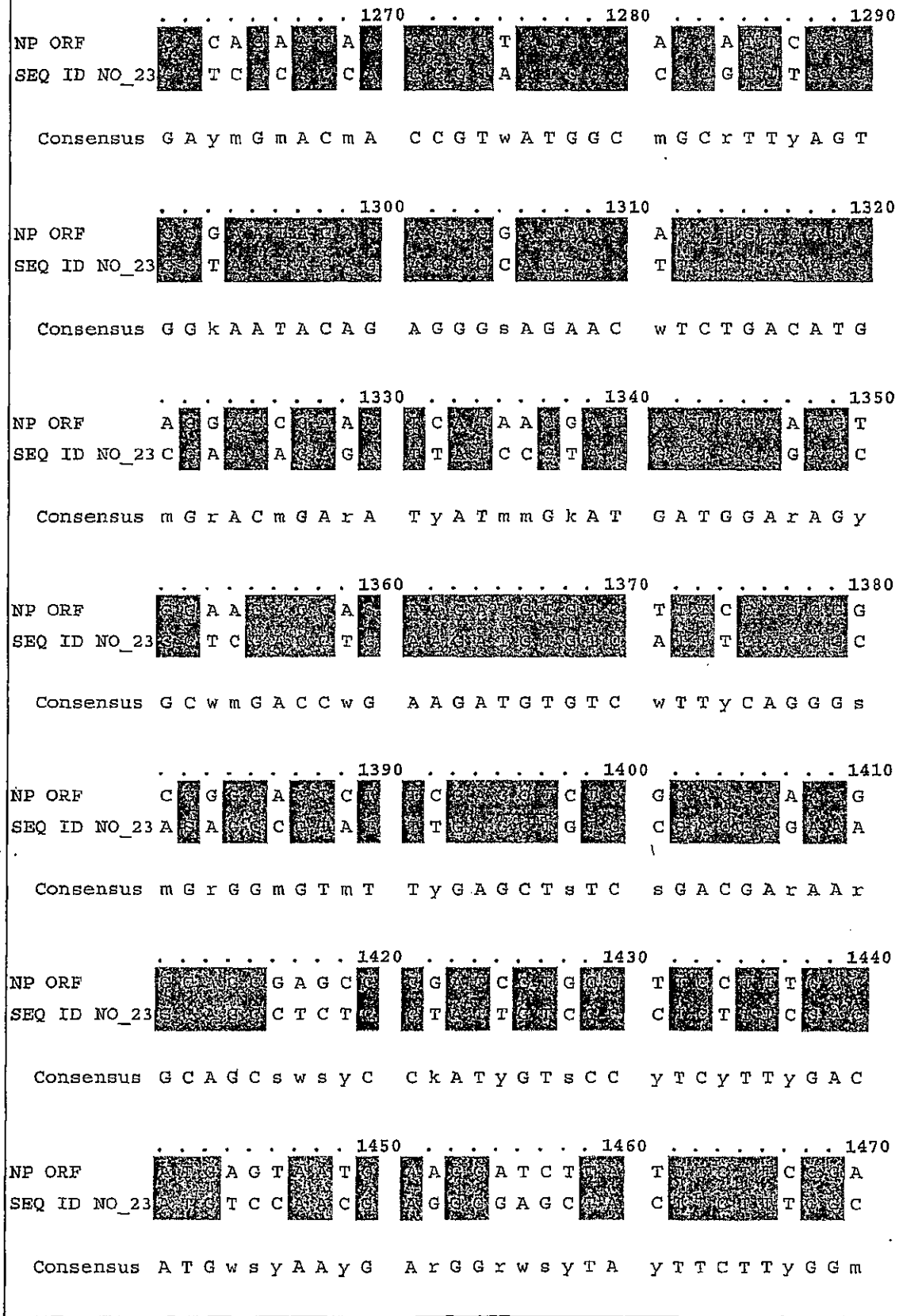





Figure 1F

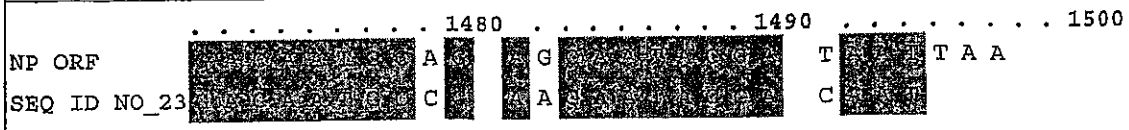
Alignment View Print




OMIGA (2.0)

Figure 1G

Alignment View Print



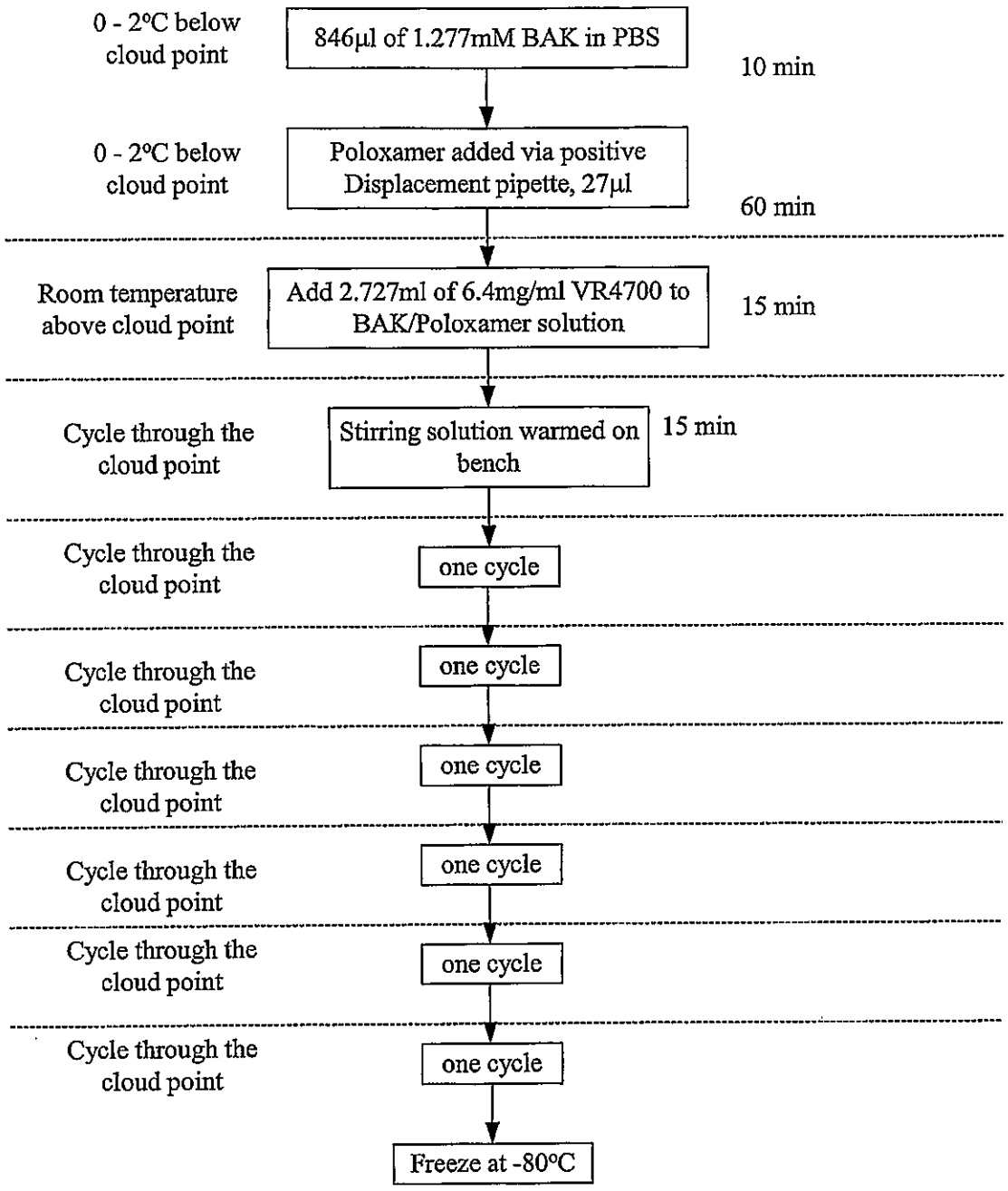

0 - 2°C below cloud point
846µl of 1.277mM BAK in PBS
10 min
0 - 2°C below cloud point
Poloxamer added via positive Displacement pipette, 27µl
60 min
Room temperature above cloud point
Add 2.727ml of 6.4mg/ml VR4700 to BAK/Poloxamer solution
15 min
Cycle through the cloud point
Stirring solution warmed on bench
15 min
Cycle through the cloud point
one cycle
Cycle through the cloud point
one cycle
Cycle through the cloud point
one cycle
Cycle through the cloud point
one cycle
Cycle through the cloud point
one cycle
Cycle through the cloud point
one cycle
Freeze at -80°C

**FIG. 2**

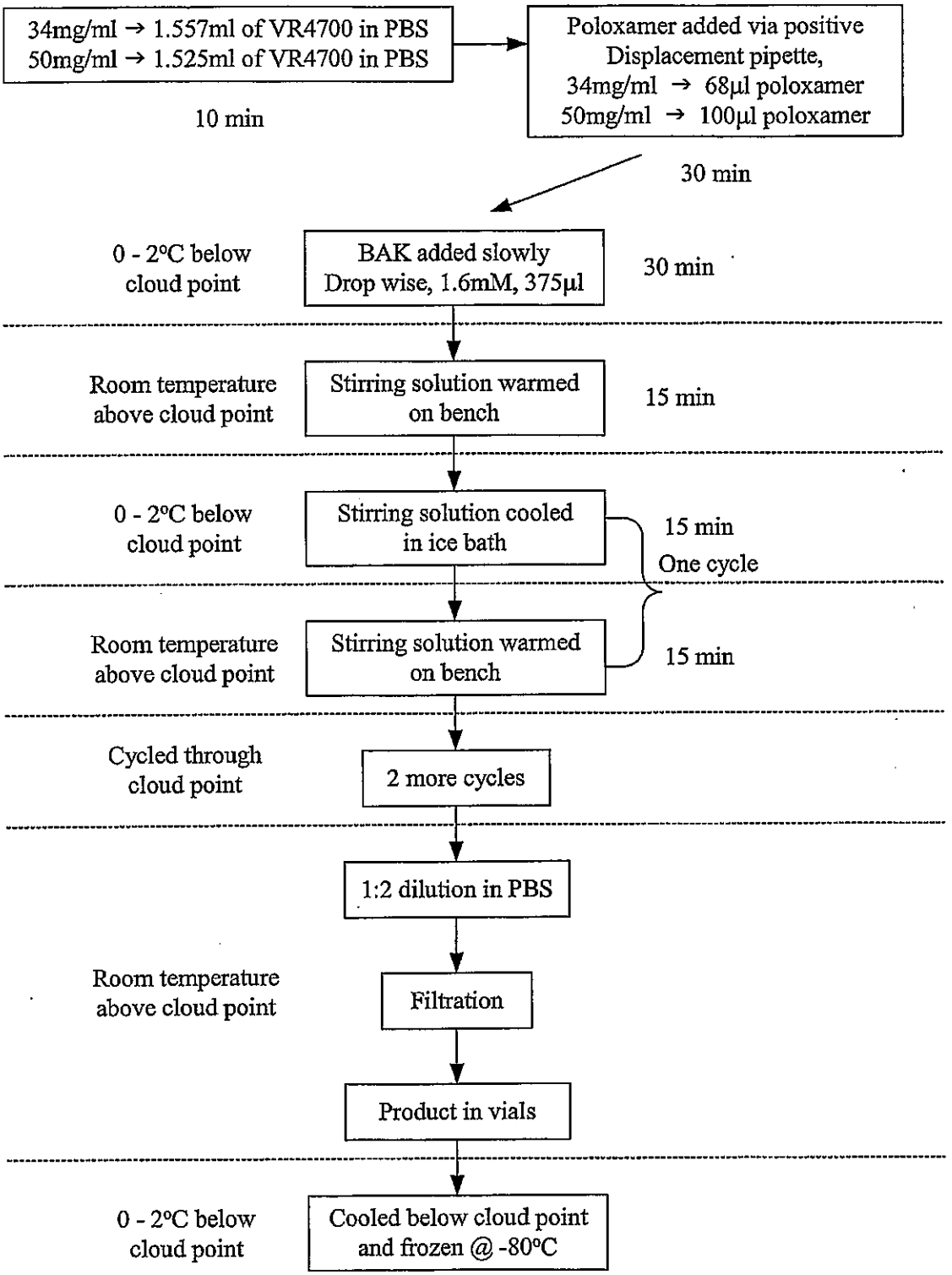

34mg/ml → 1.557ml of VR4700 in PBS
50mg/ml → 1.525ml of VR4700 in PBS
10 min
Poloxamer added via positive Displacement pipette,
34mg/ml → 68µl poloxamer
50mg/ml → 100µl poloxamer
30 min
0 - 2°C below cloud point
BAK added slowly Drop wise, 1.6mM, 375µl
30 min
Room temperature above cloud point
Stirring solution warmed on bench
15 min
0 - 2°C below cloud point
Stirring solution cooled in ice bath
15 min
One cycle
Room temperature above cloud point
Stirring solution warmed on bench
15 min
Cycled through cloud point
2 more cycles
1:2 dilution in PBS
Room temperature above cloud point
Filtration
Product in vials
0 - 2°C below cloud point
Cooled below cloud point and frozen @ -80°C

**FIG. 3**

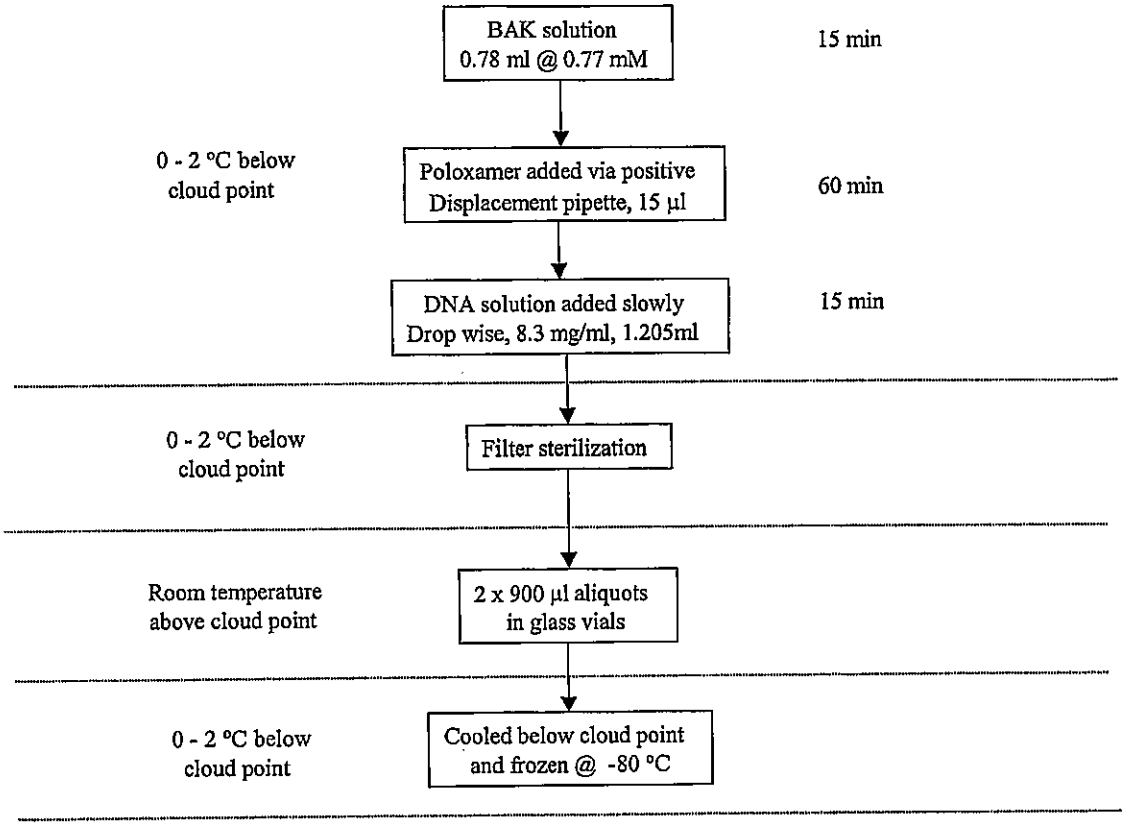

BAK solution
0.78 ml @ 0.77 mM
15 min
0 - 2 °C below
cloud point
Poloxamer added via positive
Displacement pipette, 15 µl
60 min
DNA solution added slowly
Drop wise, 8.3 mg/ml, 1.205ml
15 min
0 - 2 °C below
cloud point
Filter sterilization
Room temperature
above cloud point
2 x 900 µl aliquots
in glass vials
0 - 2 °C below
cloud point
Cooled below cloud point
and frozen @ -80 °C

**FIG. 4**

Figure 5

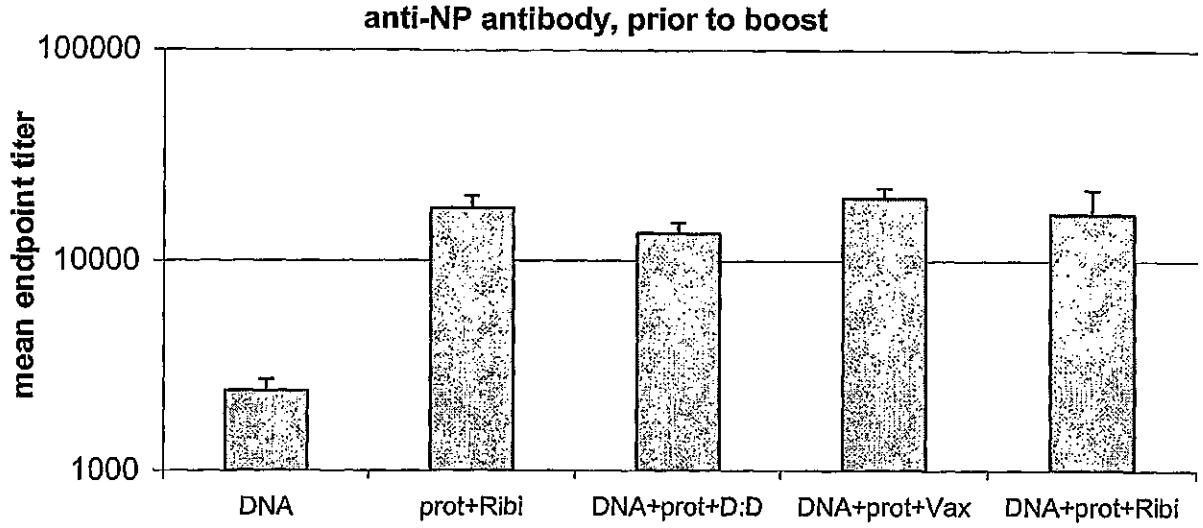



Figure 6

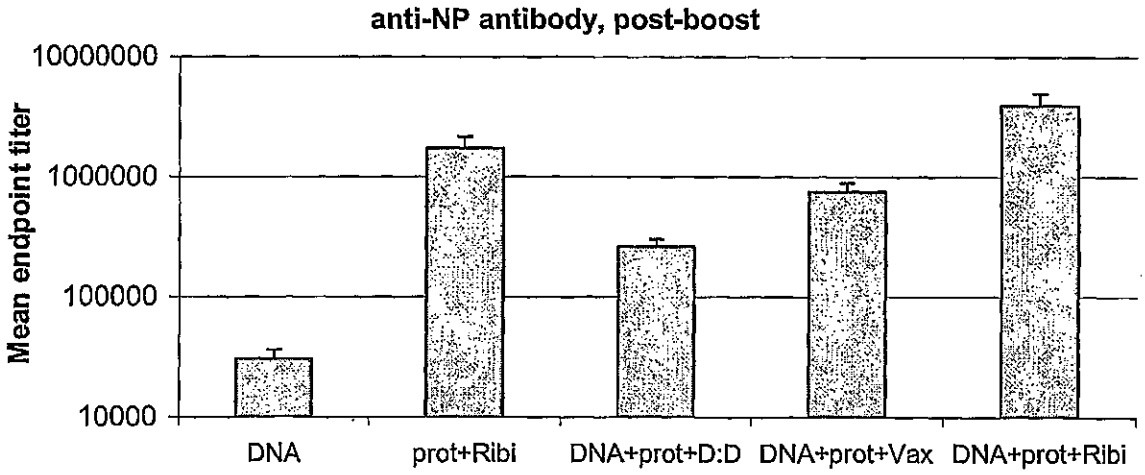


**Figure 7**

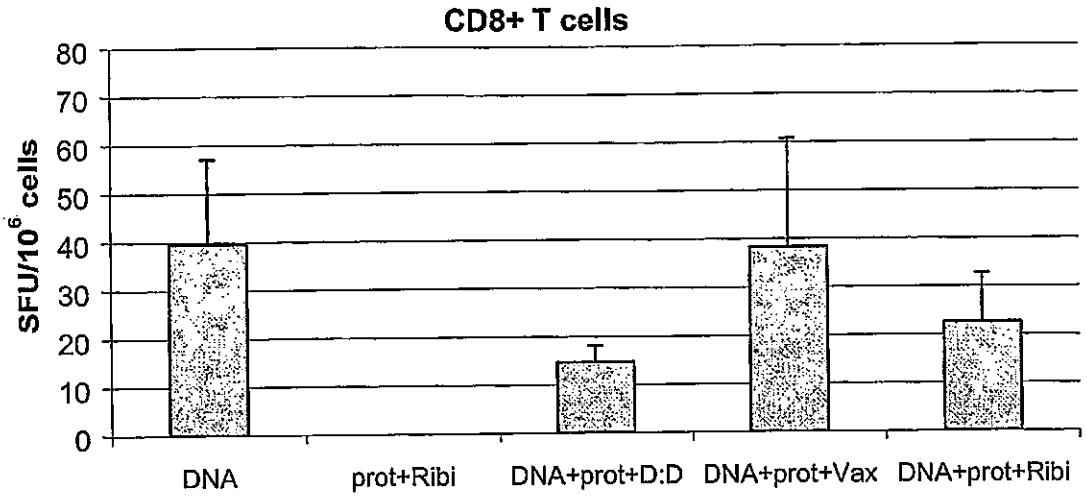



**Figure 8**

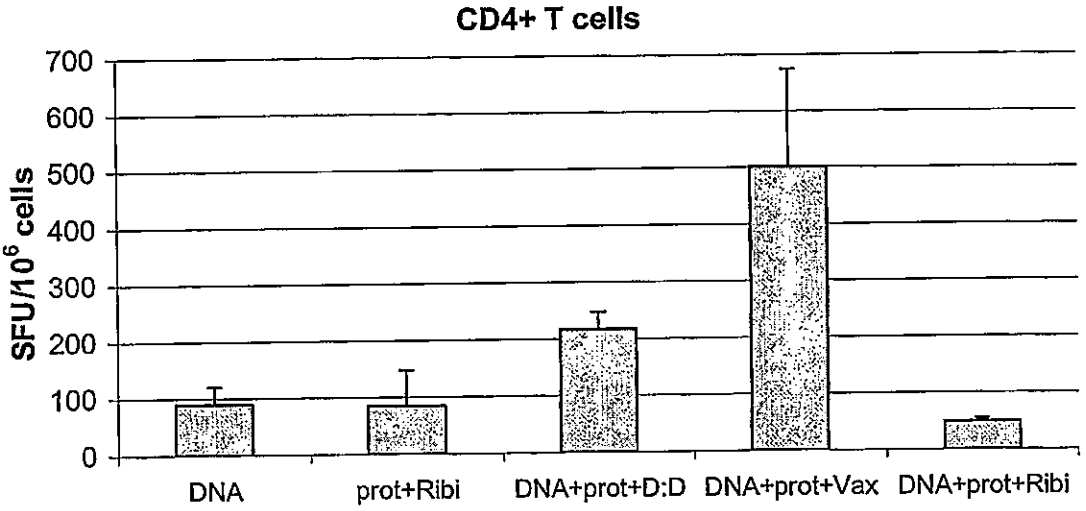


Figure 9A

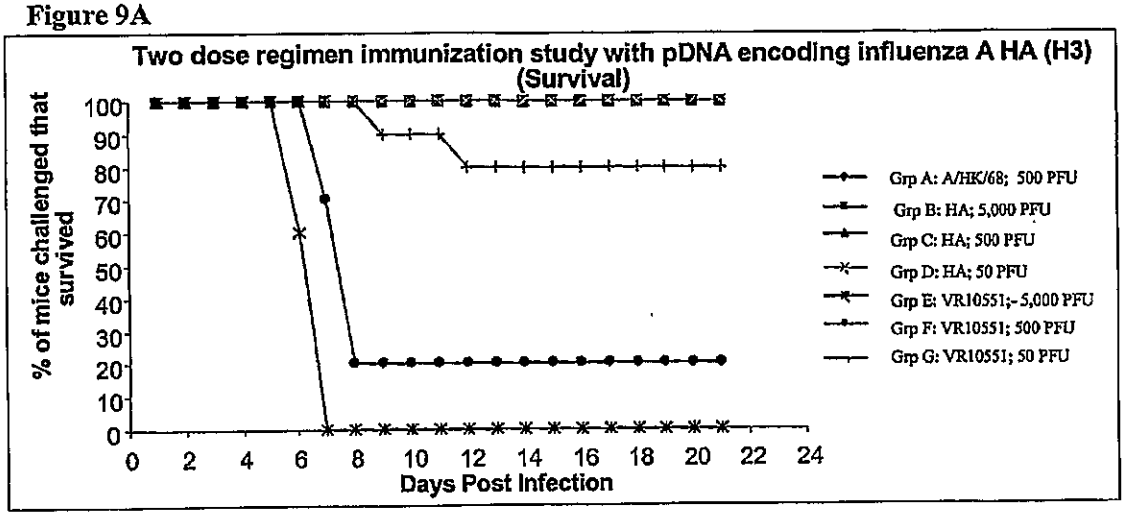



Figure 9B

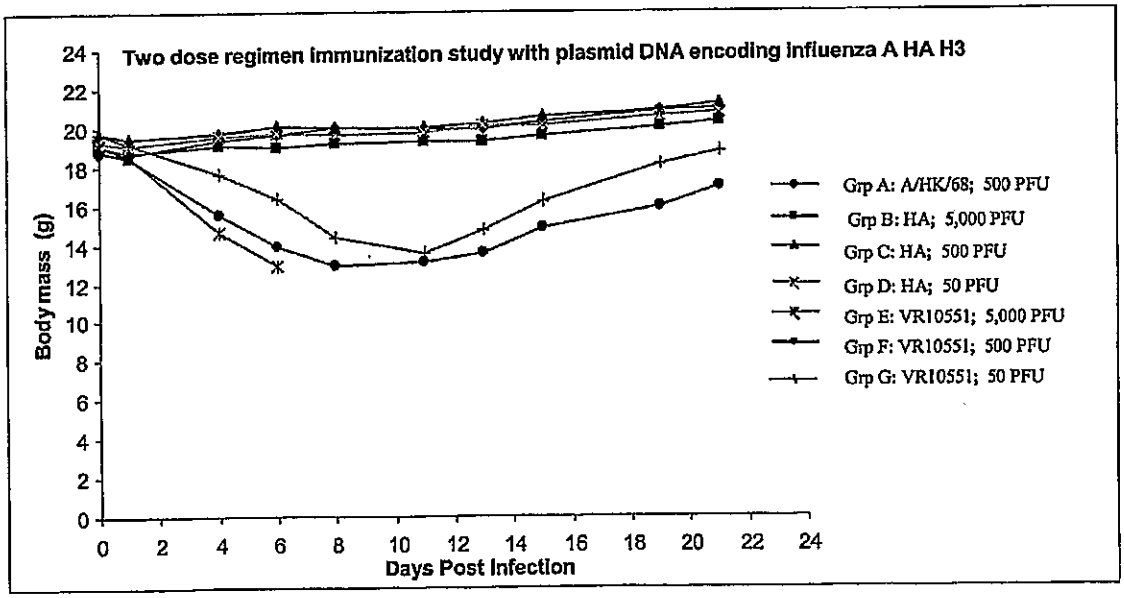


**Figure 10A**

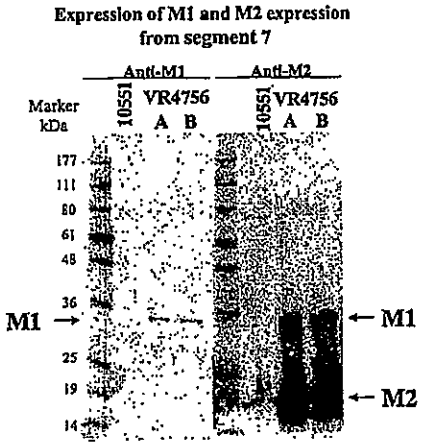



**Figure 10B**

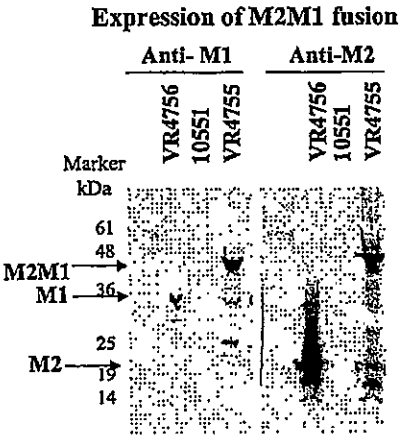


**Figure 11A**

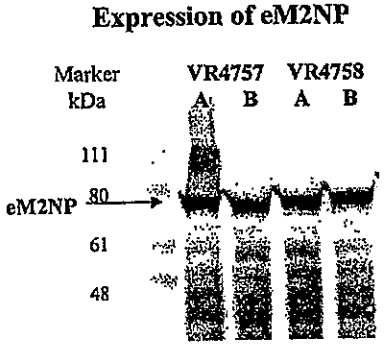
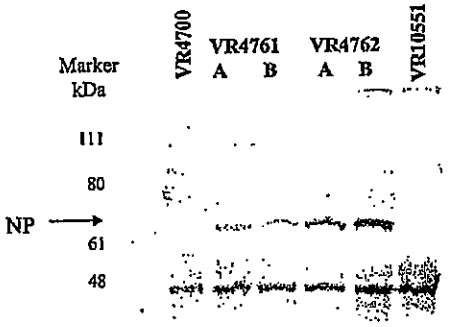
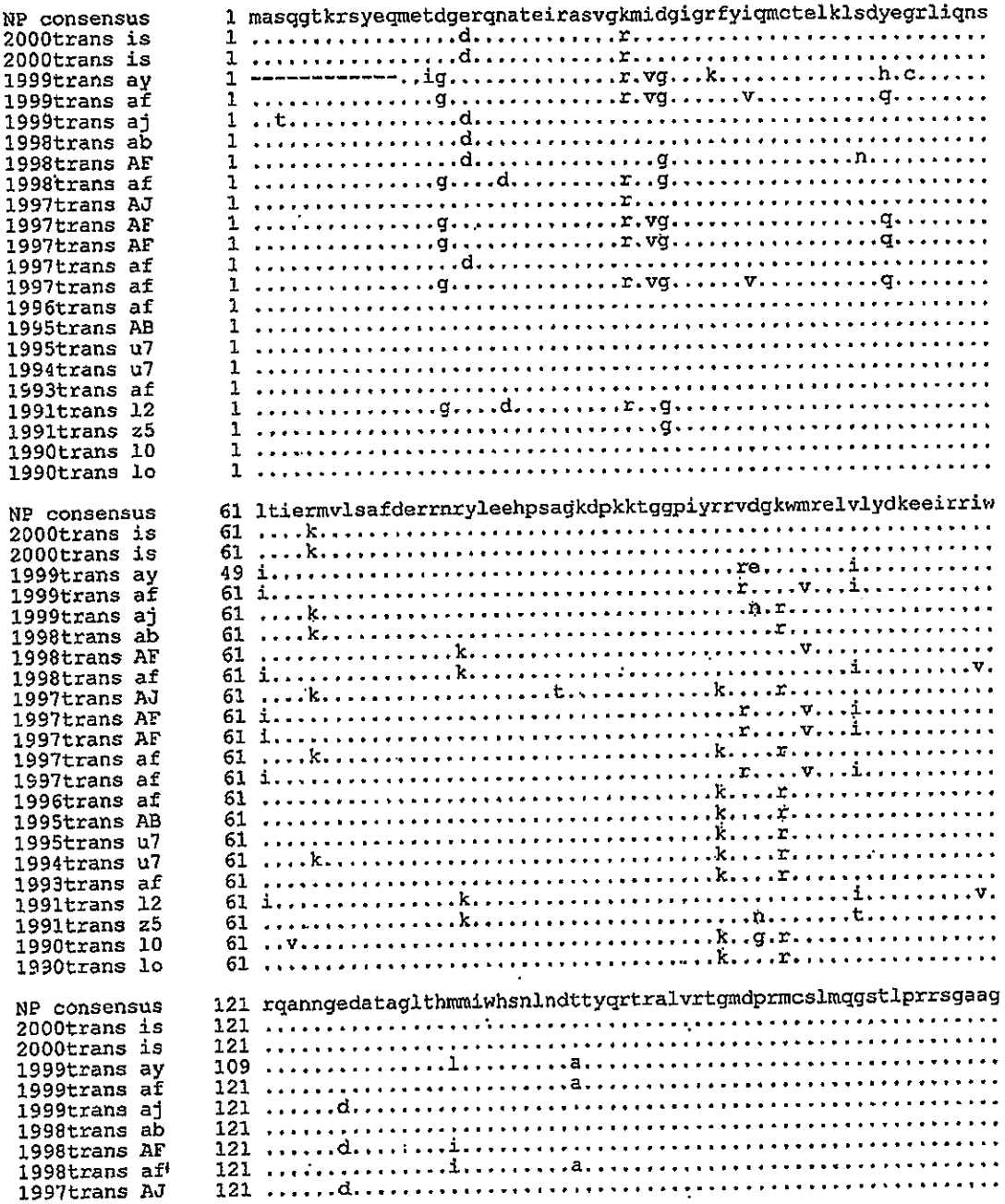



**Figure 11B**



# Figure 12: NP consensus vs. 1990-2000 strains



# NP consensus vs. 1990-2000 strains



```
1997trans AF    121 .....a.....
1997trans AF    121 .....a.....
1997trans af    121 ...........
1997trans af    121 .....a.....
1996trans af    121 .....d.....
1995trans AB    121 .....d.....
1995trans u7    121 .....d.....
1994trans u7    121 ...........
1993trans af    121 .....d.....
1991trans l2    121 .....i.....a.....
1991trans z5    121 .....d.....a.....
1990trans l0    121 .....d...r.....
1990trans lo    121 .....d...r.....

NP consensus    181 aavkgigtmvmelirmikrgindrnfwrgengrktrsayermcnilkgkfqtaaqrammd
2000trans is    181 .............v.....t.....v.
2000trans is    181 .............v.....v.
1999trans ay    169 ..i.....r..i.....v.
1999trans af    181 ..i..v.....d...r..i.....
1999trans aj    181 .............v.....v.
1998trans ab    181 .....v.....v.
1998trans AF    181 .....v....l.....i.....
1998trans af    181 .....v..ia.....r..i.....
1997trans AJ    181 .............v.....
1997trans AF    181 ..i..v.....k.....r..i.....k....
1997trans AF    181 ..i..v.....r..i.....k....
1997trans af    181 .............v.....v.
1997trans af    181 ..i..v.....v.....d...r..i.....
1996trans af    181 .....
1995trans AB    181 .....
1995trans u7    181 .....
1994trans u7    181 .............v.....
1993trans af    181 .....
1991trans l2    181 .....v..ia.....r..i.....
1991trans z5    181 .....v.....v.....i.....k....
1990trans l0    181 .....i.....
1990trans lo    181 .....

NP consensus    241 qvresrnpgnaeiedliflarsalilrgsvahksclpacvygpavssgydfekegyslvg
2000trans is    241 .....
2000trans is    241 .....
1999trans ay    229 .....i..l..a.....r.....
1999trans af    241 .....l..a.....r.....
1999trans aj    241 .....
1998trans ab    241 .....
1998trans AF    241 .....d...t.....a.....
1998trans af    241 .....l..a..h...r.....
1997trans AJ    241 .....
1997trans AF    241 .....l..a.....r.....
1997trans AF    241 .....a.....r.....
1997trans af    241 .....
1997trans af    241 .....l..a.....r.....
1996trans af    241 .....s.....
1995trans AB    241 .....s.....
1995trans u7    241 .....s.....
1994trans u7    241 .....n.....
1993trans af    241 .....s.....n.....
1991trans l2    241 .....l..a..h...r.....
1991trans z5    241 .....f...t.....t.....a.....r.....
1990trans l0    241 .....s.....
1990trans lo    241 .....s.....

NP consensus    301 idpfkllqnsqvyslrpnenpahksqlvwmachsaafedlrllsfirgtkvsprgklst
2000trans is    301 .....
2000trans is    301 .....
1999trans ay    289 ....r.....f....s.....i.....vs.....r.v...q...
```

# NP consensus vs. 1990-2000 strains



```
1999trans af    301 ....r.......f.............................vs......r.i...q...
1999trans aj    301 ..........................................................
1998trans ab    301 ..........................................................
1998trans AF    301 v.......t.........................n........vs......r.l.......
1998trans af    301 ............f.........y.....................vs.....k..i.......
1997trans AJ    301 ..........................................................
1997trans AF    301 ....r.......f.....k.d........r.............vs......r.i...q...
1997trans AF    301 ....r.......f.....k.......................vs......r.i...q...
1997trans af    301 ..........................................................
1997trans af    301 ....r.......f.............................vs......r.i...q...
1996trans af    301 ..........................................................
1995trans AB    301 ..........................................................
1995trans u7    301 ..........................................................
1994trans u7    301 ..........................................................
1993trans af    301 ..........................................................
1991trans l2    301 ............f.............................vs.....k..v.......
1991trans z5    301 ....r.....................................v....k....l.......
1990trans l0    301 ..........................................................
1990trans lo    301 ..........................................................

NP consensus    361 rgvqiasnenmdnmgsstlelrsrywairtrsggntnqqrasaqqisvqptfsvqrnlpf
2000trans is    361 .......................g..................................
2000trans is    361 .......................g..................................
1999trans ay    349 ..........etvd.......................h.................s...
1999trans af    361 ..........vea.d.....................................
1999trans aj    361 .......................g........d.....................
1998trans ab    361 ..i.....................g.....................................
1998trans AF    361 ............aiv.....................................t.....
1998trans af    361 ..........vea.d.n.....................k.....................
1997trans AJ    361 ..i.....................g.............................a.....
1997trans AF    361 ..........vea.d.....................f.........n.....f..
1997trans AF    361 ..........vea.d.t.....................................
1997trans af    361 .......................g..................................
1997trans af    361 ..........vea.d.....................................
1996trans af    361 ............e.....................................
1995trans AB    361 ............e.....................................
1995trans u7    361 ............e.....................................
1994trans u7    361 .......................g..................................
1993trans af    361 ............e.....................................
1991trans l2    361 ..........vea.d.....................k.....................
1991trans z5    361 ...........et.e.....................................i.....
1990trans l0    361 ............e.....................................
1990trans lo    361 ............e.....................................

NP consensus    421 ekstvmaaftgntegrtsdmr-aeiirmmegakpeevsfrgrgvfelsdekatnpivpsf
2000trans is    421 .......................-..................................
2000trans is    421 .......................-..................................
1999trans ay    409 .ra.i.................-t.......s....d...q.....................
1999trans af    421 .rp.i....k............-t.......s.r..d...q.....................
1999trans aj    421 .......................-..................................
1998trans ab    421 .......................-..................................
1998trans AF    421 ..t.i.................r....k.-.s.r......q.........kr.........
1998trans af    421 .ra.......s..n.........-t.v.....s....tl..q.............s.....
1997trans AJ    421 .......................-..................................
1997trans AF    421 .rv.i....k.......r....-t.......s.r..d...q.....................
1997trans AF    421 .rv.i....k............-t.......s.r..d...q.....................
1997trans af    421 .......................-..................................
1997trans af    421 .r..i....k............-t.......s.r..d...q.....................
1996trans af    421 .......................-.........t..........................
1995trans AB    421 .......................-..................................
1995trans u7    421 .......................-.........t..........................
1994trans u7    421 .......................-..................................
1993trans af    421 .......................-..................................
1991trans l2    421 .ra....v.s..n.........-t.v.....s....dl..q.....................
1991trans z5    421 drt.i....n............-t.......s.r..d...q.............as......
1990trans l0    421 .......................-..................................
```

# NP consensus vs. 1990-2000 strains



```
1990trans lo    421 .....................-.........k.............................

NP consensus    480 dmsnegsyffgdnaeeydn
2000trans is    480 e......------------
2000trans is    480 ei.....------------
1999trans ay    468 .-------------------
1999trans af    480 ....................
1999trans aj    480 ....................
1998trans ab    480 ....................
1998trans AF    480 ..............d.....
1998trans af    480 ....................
1997trans AJ    480 ....................
1997trans AF    480 ....................
1997trans AF    480 ....................
1997trans af    480 ....................
1997trans af    480 ...:................
1996trans af    480 ....................
1995trans AB    480 ....................
1995trans u7    480 ....................
1994trans u7    480 ....................
1993trans af    480 ....................
1991trans l2    480 ....................
1991trans z5    480 ....................
1990trans l0    480 ....................
1990trans lo    480 ...:.........------
```

- Same amino acids… dotted line
- No sequence available – dashed line
- 22 NP full, or nearly full-length sequences available for comparison from www.flu.lanl.gov
- Amino acid chosen for consensus based on amino acid with the majority of the 22 sequences. In cases of a tie, the amino acid found in the 2000 strain was favored.

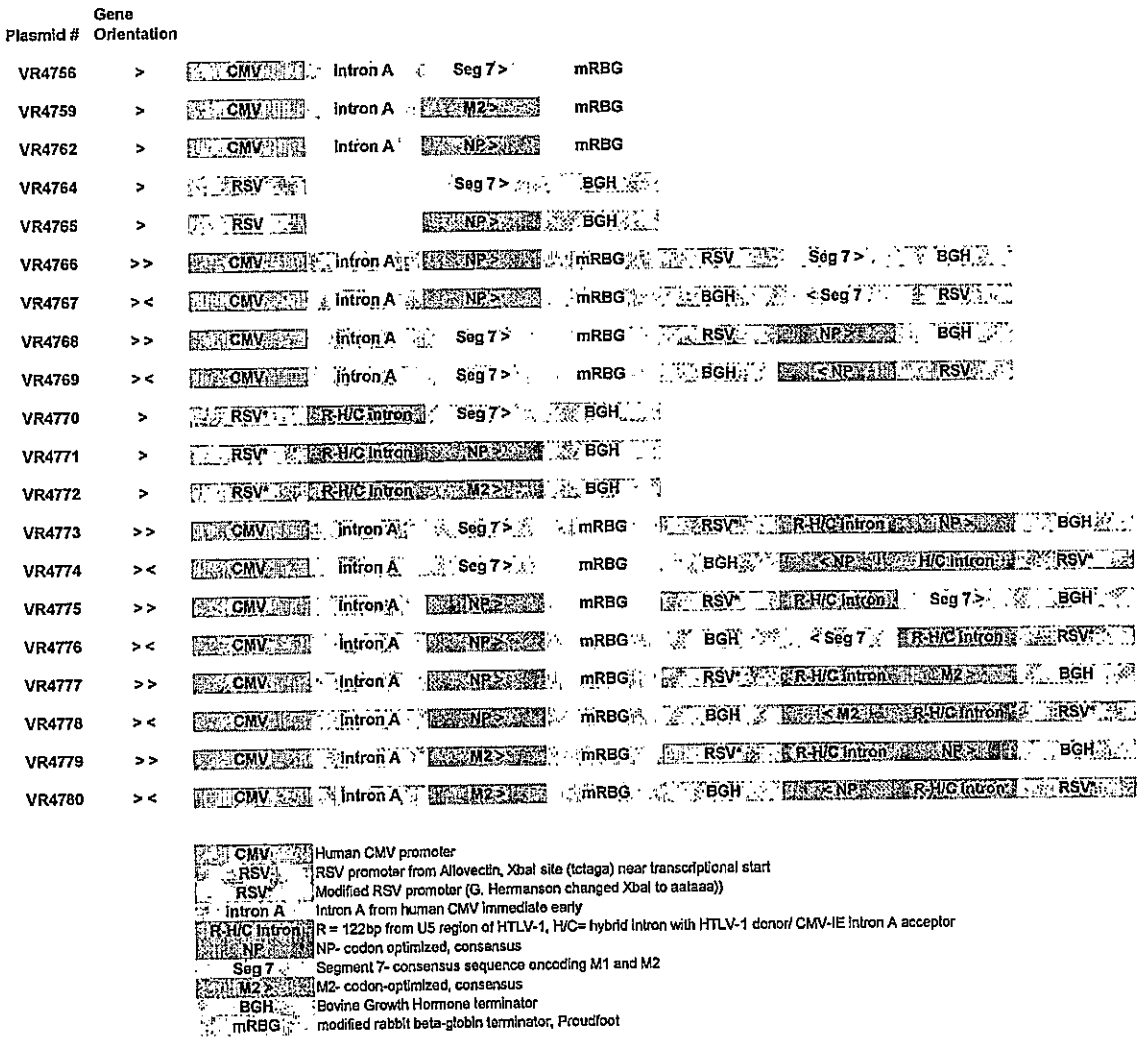
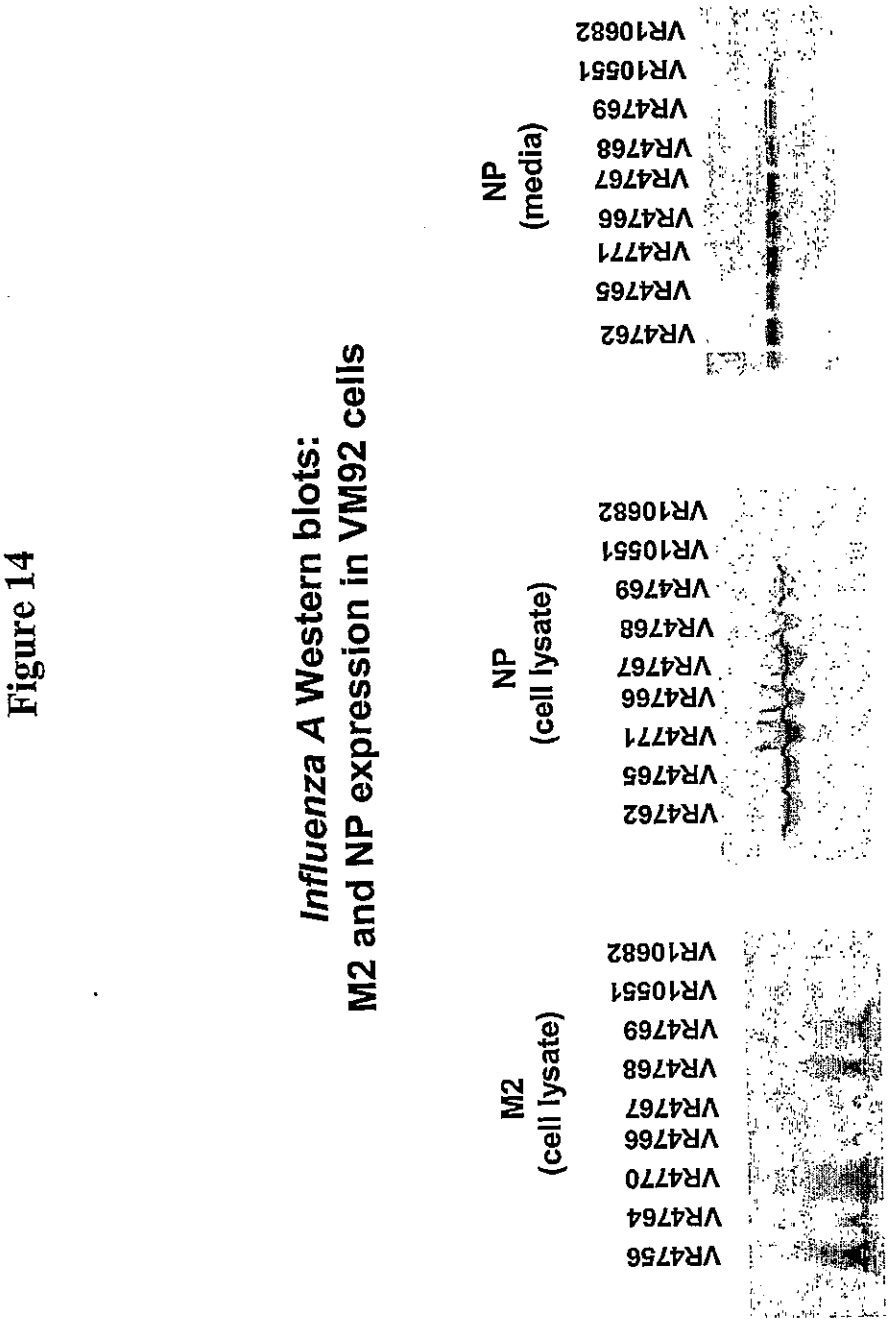
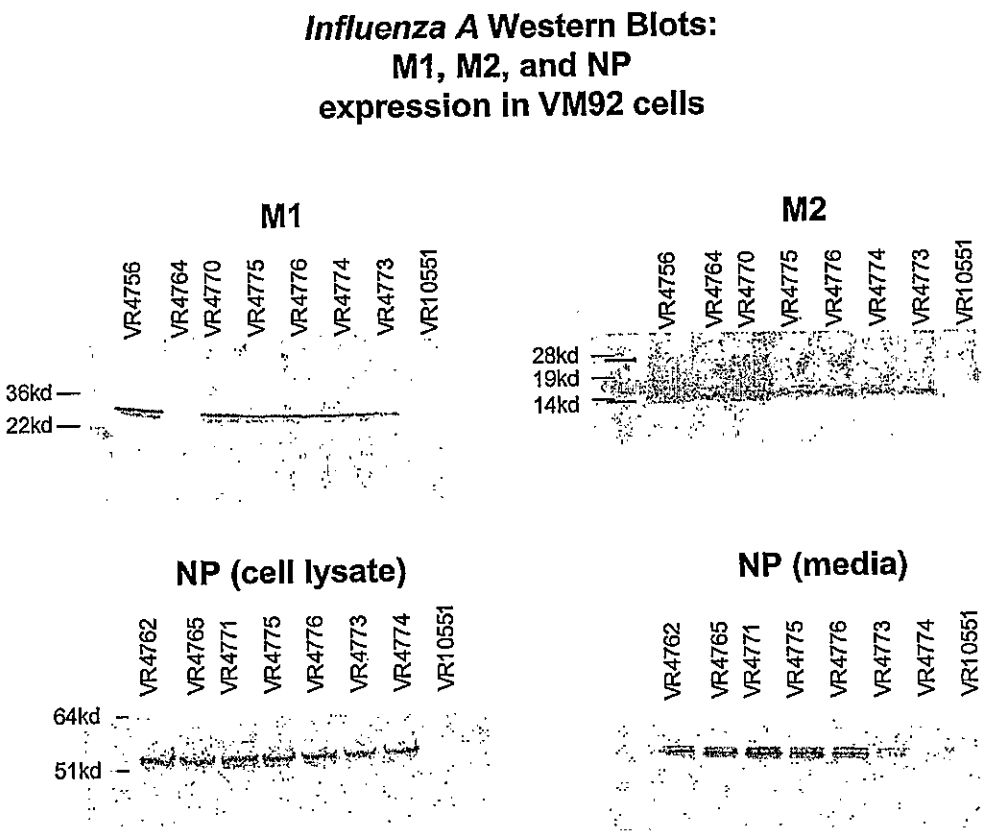

Plasmid #
Gene Orientation
VR4756 > CMV Intron A Seg 7> mRBG
VR4759 > CMV Intron A M2> mRBG
VR4762 > CMV Intron A NP> mRBG
VR4764 > RSV Seg 7> BGH
VR4765 > RSV NP> BGH
VR4766 >> CMV Intron A NP> mRBG RSV Seg 7> BGH
VR4767 >< CMV Intron A NP> mRBG BGH <Seg 7 RSV
VR4768 >> CMV Intron A Seg 7> mRBG RSV NP> BGH
VR4769 >< CMV Intron A Seg 7> mRBG BGH <NP RSV
VR4770 > RSV* R-H/C Intron Seg 7> BGH
VR4771 > RSV* R-H/C Intron NP> BGH
VR4772 > RSV* R-H/C Intron M2> BGH
VR4773 >> CMV Intron A Seg 7> mRBG RSV* R-H/C Intron NP> BGH
VR4774 >< CMV Intron A Seg 7> mRBG BGH <NP H/C Intron RSV*
VR4775 >> CMV Intron A M2> mRBG RSV* R-H/C Intron Seg 7> BGH
VR4776 >< CMV Intron A NP> mRBG BGH <Seg 7 R-H/C Intron RSV*
VR4777 >> CMV Intron A NP> mRBG RSV* R-H/C Intron M2> BGH
VR4778 >< CMV Intron A NP> mRBG BGH <M2 R-H/C Intron RSV*
VR4779 >> CMV Intron A M2> mRBG RSV* R-H/C Intron NP> BGH
VR4780 >< CMV Intron A M2> mRBG BGH <NP R-H/C Intron RSV*
CMV Human CMV promoter
RSV RSV promoter from Allovectin, XbaI site (tctaga) near transcriptional start
RSV* Modified RSV promoter (G. Hermanson changed XbaI to aataaa))
Intron A Intron A from human CMV immediate early
R-H/C Intron R = 122bp from U5 region of HTLV-1, H/C= hybrid intron with HTLV-1 donor/ CMV-IE Intron A acceptor
NP NP- codon optimized, consensus
Seg 7 Segment 7- consensus sequence encoding M1 and M2
M2 M2- codon-optimized, consensus
BGH Bovine Growth Hormone terminator
mRBG modified rabbit beta-globin terminator, Proudfoot

**Figure 13**

Figure 14


Influenza A Western blots:
M2 and NP expression in VM92 cells
M2
(cell lysate)
VR4756
VR4764
VR4770
VR4766
VR4767
VR4768
VR4769
VR10551
VR10682
NP
(cell lysate)
VR4762
VR4765
VR4771
VR4766
VR4767
VR4768
VR4769
VR10551
VR10682
NP
(media)
VR4762
VR4765
VR4771
VR4766
VR4767
VR4768
VR4769
VR10551
VR10682

# Figure 15


*Influenza A* Western Blots:
M1, M2, and NP
expression in VM92 cells
M1
VR4756
VR4764
VR4770
VR4775
VR4776
VR4774
VR4773
VR10551
36kd
22kd
M2
VR4756
VR4764
VR4770
VR4775
VR4776
VR4774
VR4773
VR10551
28kd
19kd
14kd
NP (cell lysate)
VR4762
VR4765
VR4771
VR4775
VR4776
VR4773
VR4774
VR10551
64kd
51kd
NP (media)
VR4762
VR4765
VR4771
VR4775
VR4776
VR4773
VR4774
VR10551