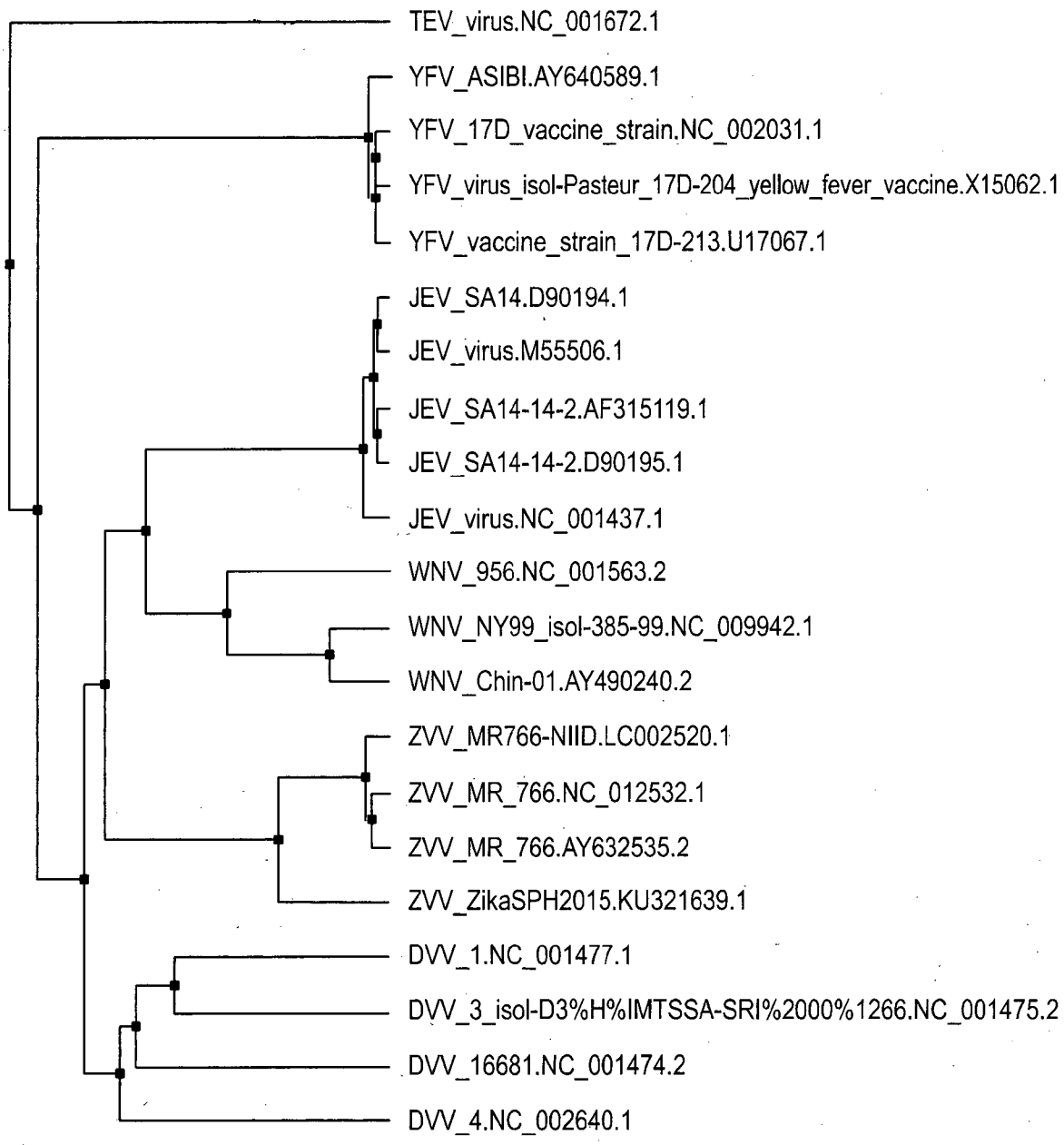

TEV_virus.NC_001672.1
YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
YFV_virus_isol-Pasteur_17D-204_yellow_fever_vaccine.X15062.1
YFV_vaccine_strain_17D-213.U17067.1
JEV_SA14.D90194.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
JEV_virus.NC_001437.1
WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2
ZVV_MR766-NIID.LC002520.1
ZVV_MR_766.NC_012532.1
ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
DVV_1.NC_001477.1
DVV_3_isol-D3%H%IMTSSA-SRI%2000%1266.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1

Figure 1

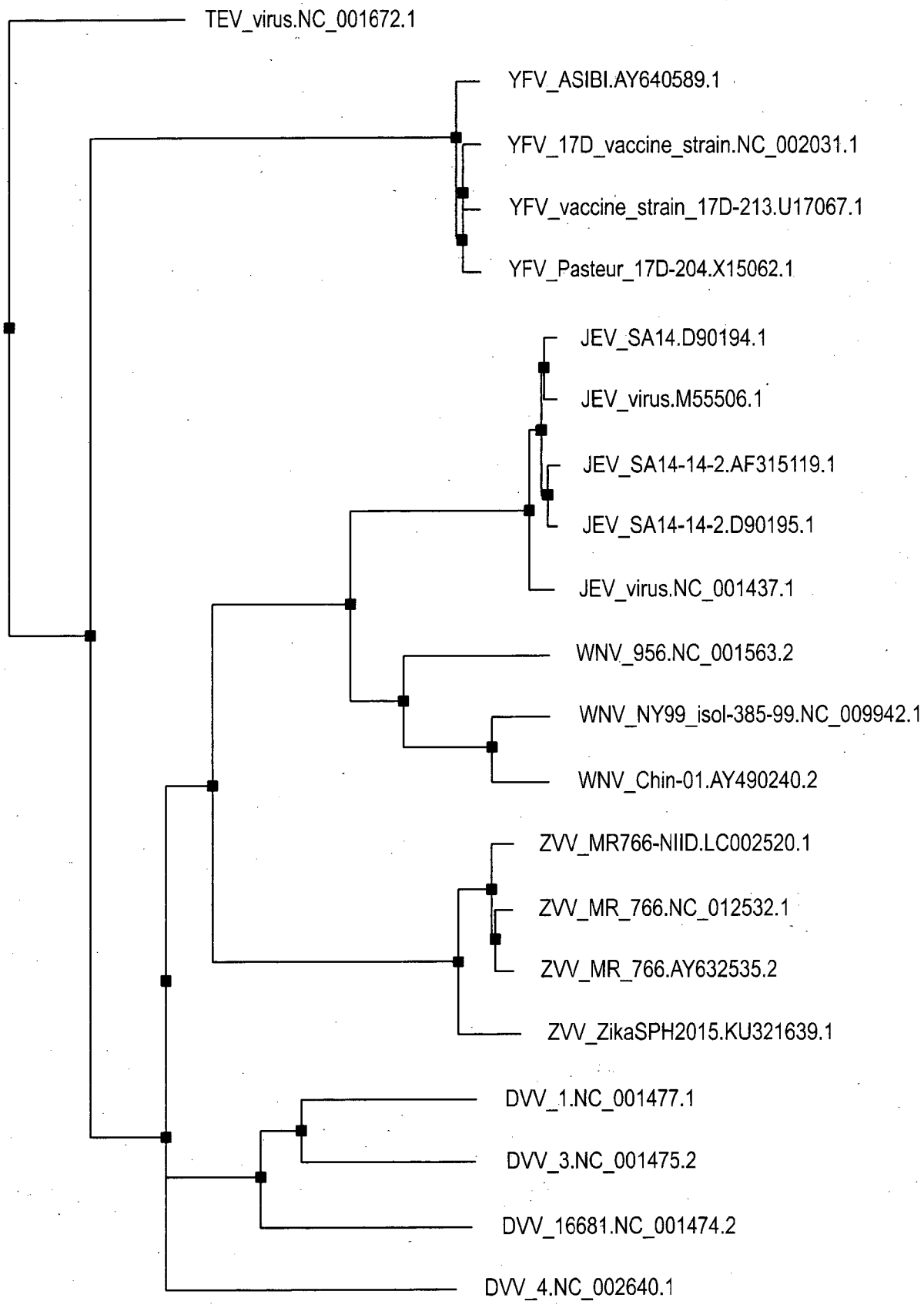

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YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
YFV_vaccine_strain_17D-213.U17067.1
YFV_Pasteur_17D-204.X15062.1
JEV_SA14.D90194.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
JEV_virus.NC_001437.1
WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2
ZVV_MR766-NIID.LC002520.1
ZVV_MR_766.NC_012532.1
ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
DVV_1.NC_001477.1
DVV_3.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1

Figure 2

		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DVV_1.NC_001477.1	0	100.0	70.1	73.0	67.8	60.5	60.4	60.5	60.5	60.7	55.8	60.6	60.3	60.6	58.0	57.9	57.9	58.0	62.3	62.3	62.3	61.3
DVV_16681.NC_001474.2	1	70.1	100.0	70.8	68.6	60.5	60.5	60.5	60.6	60.7	54.9	60.9	60.3	60.4	58.3	58.3	58.3	58.3	62.7	62.8	62.8	62.2
DVV_3.NC_001475.2	2	73.0	70.8	100.0	68.9	60.2	60.1	60.1	60.1	60.1	55.8	60.3	60.0	60.1	57.5	57.6	57.5	57.5	62.6	62.7	62.7	62.5
DVV_4.NC_002640.1	3	67.8	68.6	68.9	100.0	60.5	60.6	60.6	60.6	60.4	55.4	60.8	60.3	60.5	58.0	57.9	57.9	57.9	62.1	62.0	62.0	62.1
JEV_SA14-14-2.AF315119.1	4	60.5	60.5	60.2	60.5	100.0	99.8	99.4	99.5	98.1	56.5	70.6	70.3	70.3	57.9	58.0	58.0	58.0	61.9	61.8	61.8	61.4
JEV_SA14-14-2.D90195.1	5	60.4	60.5	60.1	60.6	99.8	100.0	99.4	99.5	98.2	56.6	70.6	70.4	70.3	57.9	58.0	57.9	58.0	61.9	61.8	61.8	61.5
JEV_SA14.D90194.1	6	60.5	60.5	60.1	60.6	99.4	99.4	100.0	99.8	98.6	56.6	70.8	70.5	70.4	58.0	58.0	58.0	58.0	61.9	61.8	61.8	61.6
JEV_virus.M55506.1	7	60.5	60.6	60.1	60.6	99.5	99.5	99.8	100.0	98.6	56.7	70.8	70.4	70.3	58.0	58.1	58.0	58.1	61.9	61.8	61.8	61.6
JEV_virus.NC_001437.1	8	60.7	60.7	60.1	60.4	98.1	98.2	98.6	98.6	100.0	56.6	70.6	70.5	70.6	58.3	58.3	58.4	58.3	62.1	62.0	62.0	61.7
TEV_virus.NC_001672.1	9	55.8	54.9	55.8	55.4	56.5	56.6	56.6	56.7	56.6	100.0	56.2	56.7	56.6	56.9	57.0	57.0	57.0	56.6	56.7	56.7	56.7
WNV_956.NC_001563.2	10	60.6	60.9	60.3	60.8	70.6	70.6	70.8	70.8	70.6	56.2	100.0	79.6	79.6	58.1	58.0	58.1	58.2	62.4	62.3	62.3	62.2
WNV_Chin-01.AY490240.2	11	60.3	60.3	60.0	60.3	70.3	70.4	70.5	70.4	70.5	56.7	79.6	100.0	95.3	58.2	58.2	58.2	58.2	62.0	62.1	62.1	62.7
WNV_NY99_isol-385-99.NC_009942.1	12	60.6	60.4	60.1	60.5	70.3	70.3	70.4	70.3	70.6	56.6	79.6	95.3	100.0	57.8	57.8	57.8	57.8	62.0	62.0	62.0	62.4
YFV_17D_vaccine_strain.NC_002031.1	13	58.0	58.3	57.5	58.0	57.9	57.9	58.0	58.0	58.3	56.9	58.1	58.2	57.8	100.0	99.4	99.9	99.9	58.5	58.5	58.5	58.2
YFV_ASIB.AY640589.1	14	57.9	58.3	57.6	57.9	58.0	58.0	58.0	58.1	58.3	57.0	58.0	58.2	57.8	99.4	100.0	99.4	99.4	58.4	58.4	58.4	58.1
YFV_Pasteur_17D-204.X15062.1	15	57.9	58.3	57.5	57.9	58.0	57.9	58.0	58.0	58.4	57.0	58.1	58.2	57.8	99.9	99.4	100.0	99.9	58.4	58.4	58.4	58.2
YFV_vaccine_strain_17D-213.U17067.1	16	58.0	58.3	57.5	57.9	58.0	58.0	58.0	58.1	58.3	57.0	58.2	58.2	57.8	99.9	99.4	99.9	100.0	58.4	58.4	58.4	58.1
ZVV_MR766-NIID.LC002520.1	17	62.3	62.7	62.6	62.1	61.9	61.9	61.9	61.9	62.1	56.6	62.4	62.0	62.0	58.5	58.4	58.4	58.4	100.0	99.7	99.7	88.9
ZVV_MR_766.AY632535.2	18	62.3	62.8	62.7	62.0	61.8	61.8	61.8	61.8	62.0	56.7	62.3	62.1	62.0	58.5	58.4	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_MR_766.NC_012532.1	19	62.3	62.8	62.7	62.0	61.8	61.8	61.8	61.8	62.0	56.7	62.3	62.1	62.0	58.5	58.4	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_ZikaSPH2015.KU321639.1	20	61.3	62.2	62.5	62.1	61.4	61.5	61.6	61.6	61.7	56.7	62.2	62.7	62.4	58.2	58.1	58.2	58.1	88.9	88.8	88.8	100.0

60 % Identity and higher
70 % Identity and higher
80 % Identity and higher

Figure 3

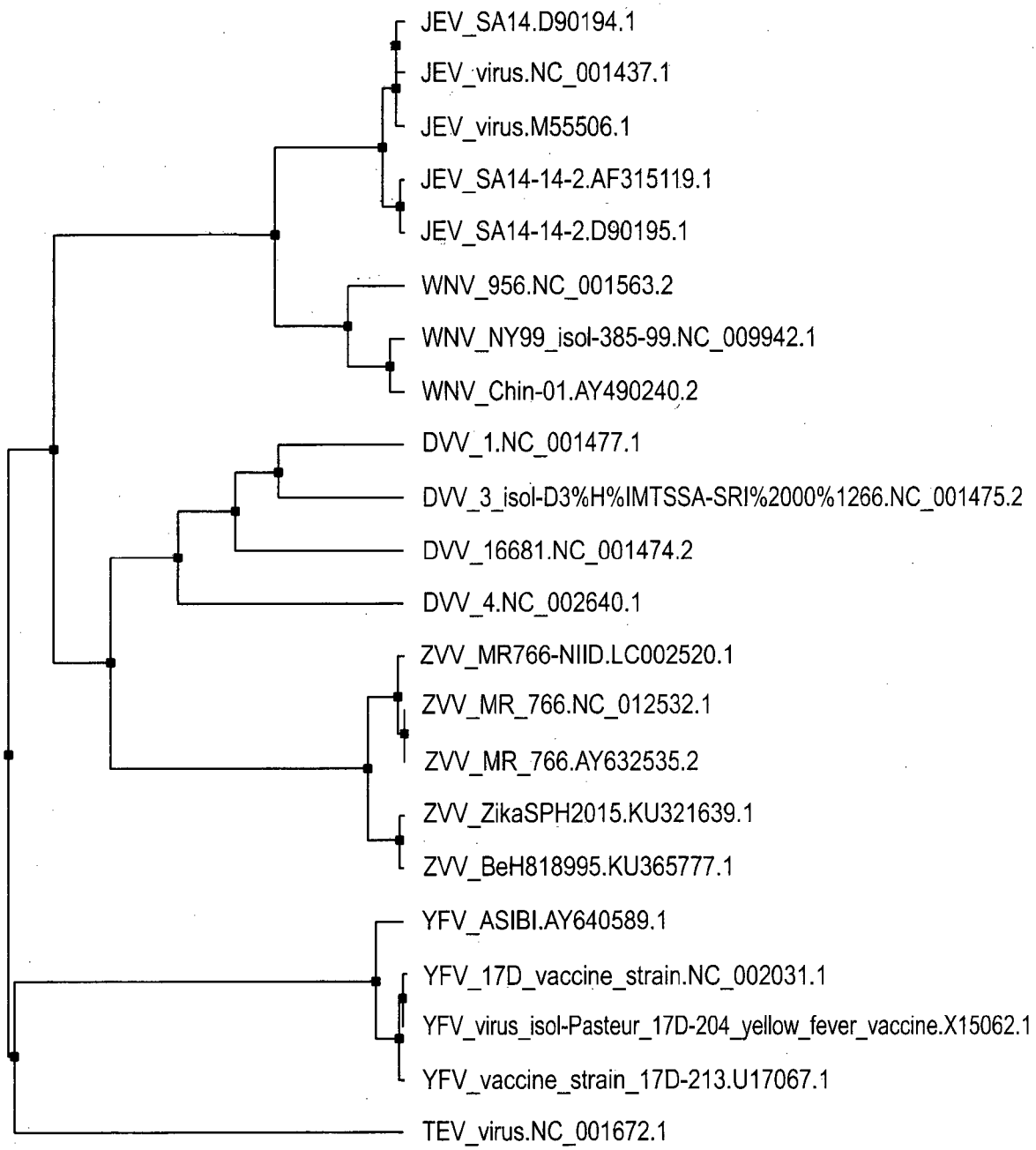

JEV_SA14.D90194.1
JEV_virus.NC_001437.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2
DVV_1.NC_001477.1
DVV_3_isol-D3%H%IMTSSA-SRI%2000%1266.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1
ZVV_MR766-NIID.LC002520.1
ZVV_MR_766.NC_012532.1
ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
ZVV_BeH818995.KU365777.1
YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
YFV_virus_isol-Pasteur_17D-204_yellow_fever_vaccine.X15062.1
YFV_vaccine_strain_17D-213.U17067.1
TEV_virus.NC_001672.1

Figure 4

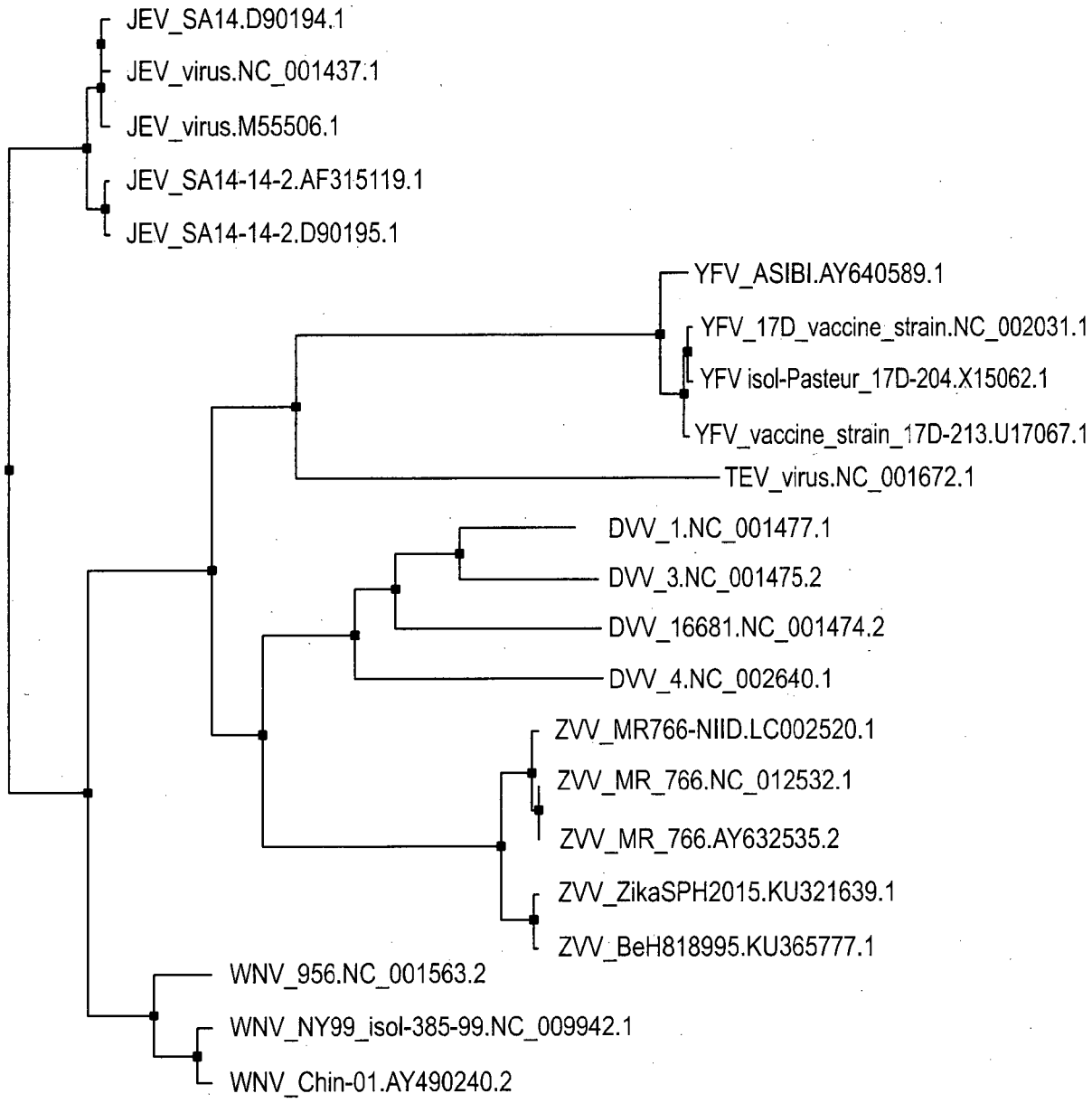

JEV_SA14.D90194.1
JEV_virus.NC_001437.1
JEV_virus.M55506.1
JEV_SA14-14-2.AF315119.1
JEV_SA14-14-2.D90195.1
YFV_ASIBI.AY640589.1
YFV_17D_vaccine_strain.NC_002031.1
YFV isol-Pasteur_17D-204.X15062.1
YFV_vaccine_strain_17D-213.U17067.1
TEV_virus.NC_001672.1
DVV_1.NC_001477.1
DVV_3.NC_001475.2
DVV_16681.NC_001474.2
DVV_4.NC_002640.1
ZVV_MR766-NIID.LC002520.1
ZVV_MR_766.NC_012532.1
ZVV_MR_766.AY632535.2
ZVV_ZikaSPH2015.KU321639.1
ZVV_BeH818995.KU365777.1
WNV_956.NC_001563.2
WNV_NY99_isol-385-99.NC_009942.1
WNV_Chin-01.AY490240.2

Figure 5

		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
DVV_1.NC_001477.1	0	100.0	68.7	77.4	63.8	49.7	49.7	50.1	49.9	50.5	38.3	51.5	50.5	50.9	43.0	43.8	43.0	43.2	57.3	57.9	58.4	58.4	57.1
DVV_16681.NC_001474.2	1	68.7	100.0	68.7	64.0	46.7	46.7	47.1	46.9	47.5	38.9	48.4	47.6	47.6	43.6	44.4	43.6	43.6	53.6	53.8	54.2	54.2	53.8
DVV_3.NC_001475.2	2	77.4	68.7	100.0	62.8	48.0	48.0	48.2	48.2	48.6	38.2	46.8	46.7	46.3	41.6	42.2	41.6	41.7	57.3	57.7	58.2	58.2	57.1
DVV_4.NC_002640.1	3	63.8	64.0	62.8	100.0	47.5	47.5	47.7	47.5	48.1	39.7	49.6	49.6	49.8	40.0	40.6	40.0	40.0	55.8	56.4	56.4	56.4	55.8
JEV_SA14-14-2.AF315119.1	4	49.7	46.7	48.0	47.5	100.0	99.8	98.0	98.6	97.8	39.6	76.1	76.5	76.7	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV_SA14-14-2.D90195.1	5	49.7	46.7	48.0	47.5	99.8	100.0	98.2	98.8	98.0	39.8	76.3	76.7	76.9	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV_SA14.D90194.1	6	50.1	47.1	48.2	47.7	98.0	98.2	100.0	99.4	99.4	40.0	77.5	77.8	78.0	43.3	43.3	43.3	43.3	54.4	55.4	55.2	55.2	54.4
JEV_virus.M55506.1	7	49.9	46.9	48.2	47.5	98.6	98.8	99.4	100.0	99.2	40.0	77.3	77.6	77.8	43.3	43.3	43.3	43.3	54.2	55.2	55.0	55.0	54.2
JEV_virus.NC_001437.1	8	50.5	47.5	48.6	48.1	97.8	98.0	99.4	99.2	100.0	40.4	78.0	78.4	78.6	43.5	43.5	43.5	43.5	54.8	55.4	55.2	55.2	54.8
TEV_virus.NC_001672.1	9	38.3	38.9	38.2	39.7	39.6	39.8	40.0	40.0	40.4	100.0	41.3	41.6	41.0	42.3	41.9	42.3	42.3	39.9	39.9	39.6	39.6	39.9
WNV_956.NC_001563.2	10	51.5	48.4	46.8	49.6	76.1	76.3	77.5	77.3	78.0	41.3	100.0	94.6	94.0	43.8	43.6	43.8	43.8	55.0	55.0	54.8	54.8	55.0
WNV_Chin-01.AY490240.2	11	50.5	47.6	46.7	49.6	76.5	76.7	77.8	77.6	78.4	41.6	94.6	100.0	98.8	43.9	43.5	43.9	43.9	54.4	54.2	54.0	54.0	54.4
WNV_NY99_isol-385-99.NC_009942.1	12	50.9	47.6	45.3	49.8	76.7	76.9	78.0	77.8	78.6	41.0	94.0	98.8	100.0	43.9	43.5	43.9	43.9	54.0	53.8	53.8	53.8	54.0
YFV_17D_vaccine_strain.NC_002031.1	13	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	100.0	97.6	100.0	99.8	42.0	43.4	43.5	43.5	42.0
YFV_ASIB.AY640589.1	14	43.8	44.4	42.2	40.6	42.9	42.9	43.3	43.3	43.5	41.9	43.6	43.5	43.5	97.6	100.0	97.6	97.4	42.4	43.8	43.9	43.9	42.4
YFV_isol-Pasteur_17D-204.X15062.1	15	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	100.0	97.6	100.0	99.8	42.0	43.4	43.5	43.5	42.0
YFV_vaccine_strain_17D-213.U17067.1	16	43.2	43.6	41.7	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	99.8	97.4	99.8	100.0	42.0	43.4	43.5	43.5	42.0
ZVVBeH818995.KU365777.1	17	57.3	53.6	57.3	55.8	54.0	54.0	54.4	54.2	54.8	39.9	55.0	54.4	54.0	42.0	42.4	42.0	42.0	100.0	96.8	96.4	96.4	99.8
ZVV_MR766-NIID.LC002520.1	18	57.9	53.8	57.7	56.4	54.8	54.8	55.4	55.2	55.4	39.9	55.0	54.2	53.8	43.4	43.8	43.4	43.4	96.8	100.0	98.8	98.8	96.6
ZVV_MR_766.AY632535.2	19	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.0	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	96.4	98.8	100.0	100.0	96.2
ZVV_MR_766.NC_012532.1	20	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.0	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	96.4	98.8	100.0	100.0	96.2
ZVV_ZikaSPH2015.KU321639.1	21	57.1	53.8	57.1	55.8	54.0	54.0	54.4	54.2	54.8	39.9	55.0	54.4	54.0	42.0	42.4	42.0	42.0	99.8	96.6	96.2	96.2	100.0

50 % Identity and higher
60 % Identity and higher
70 % Identity and higher

Figure 6

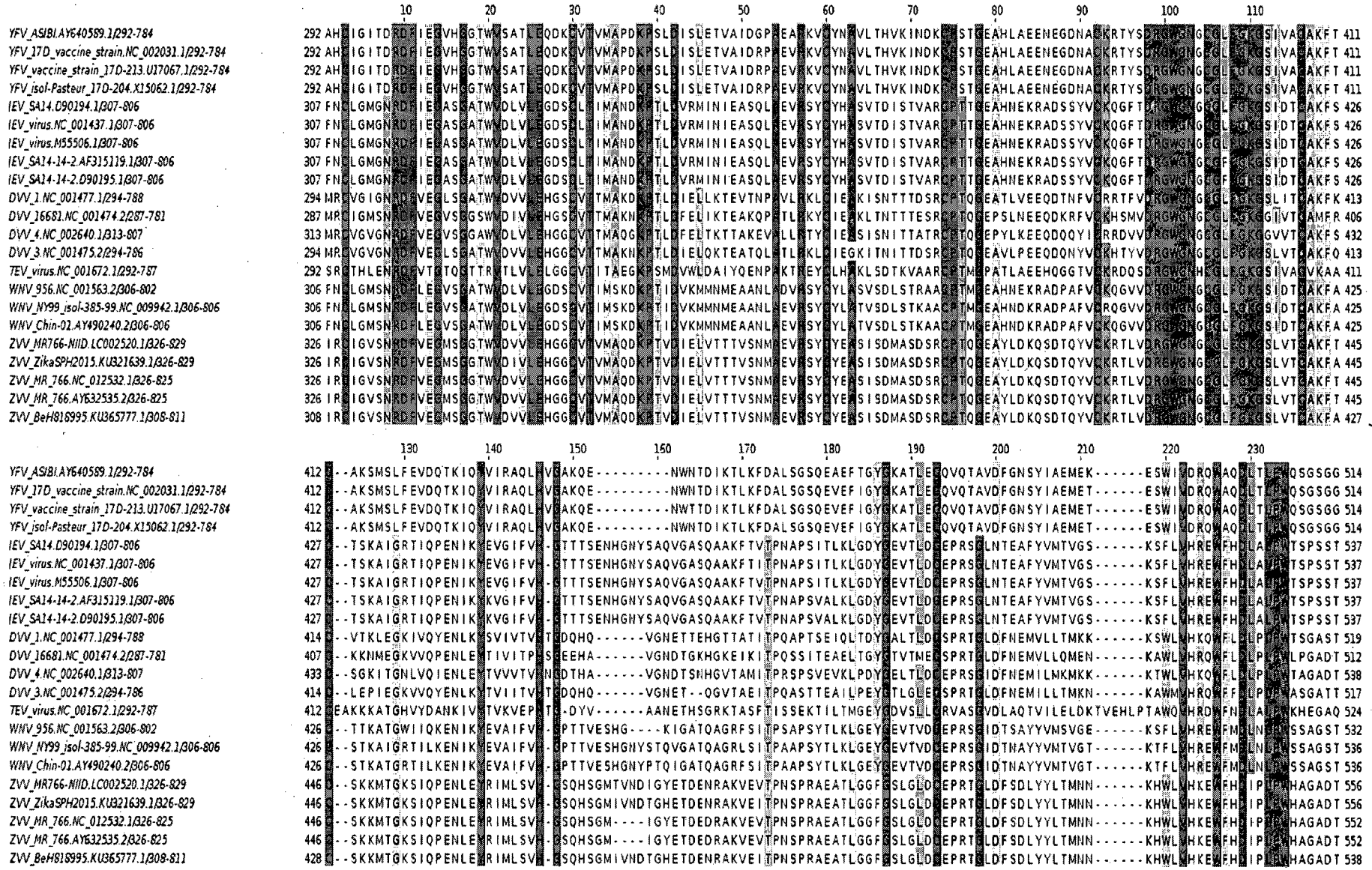


Figure 7 (Part 1 of 3)

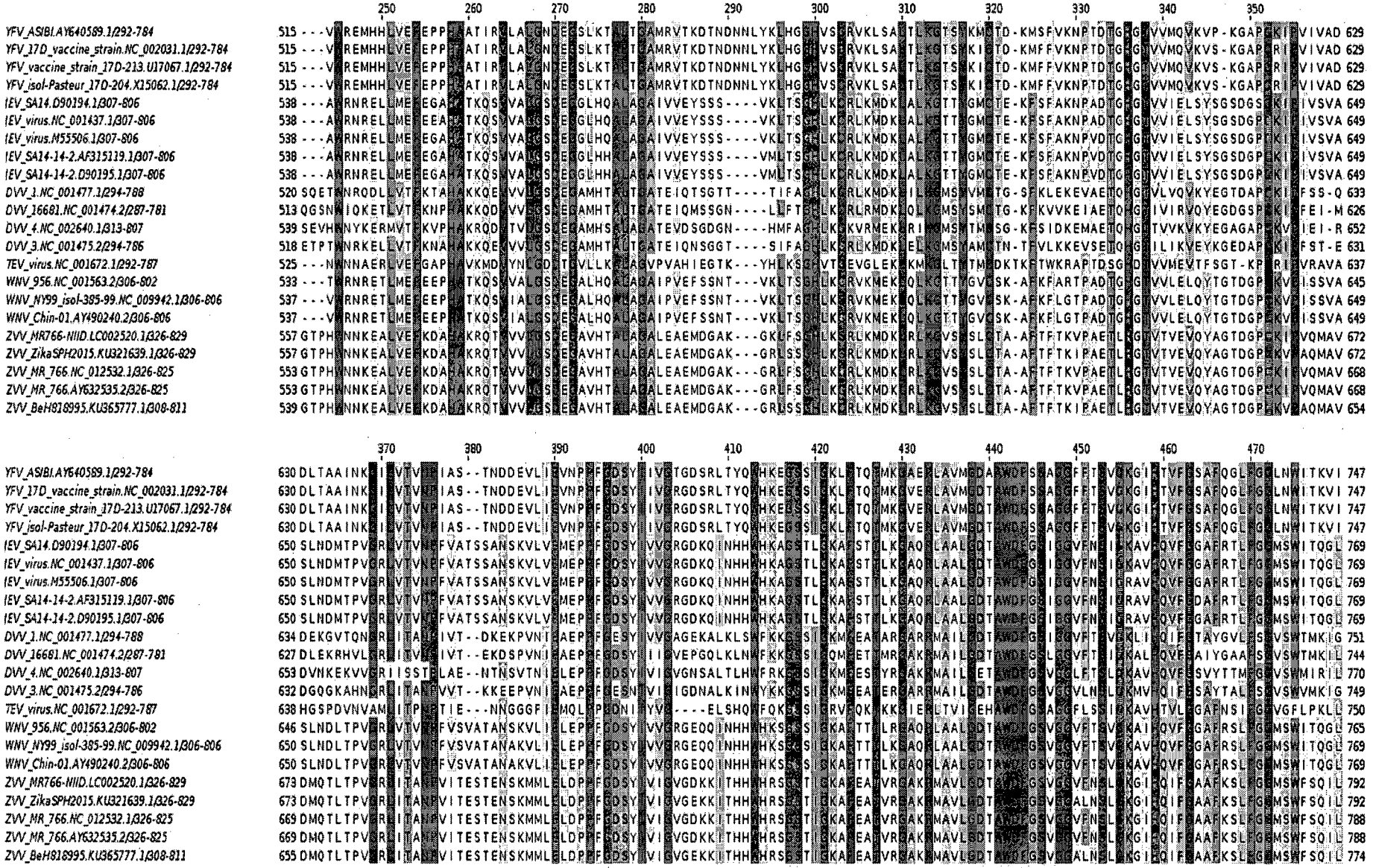


Figure 7 (Part 2 of 3)

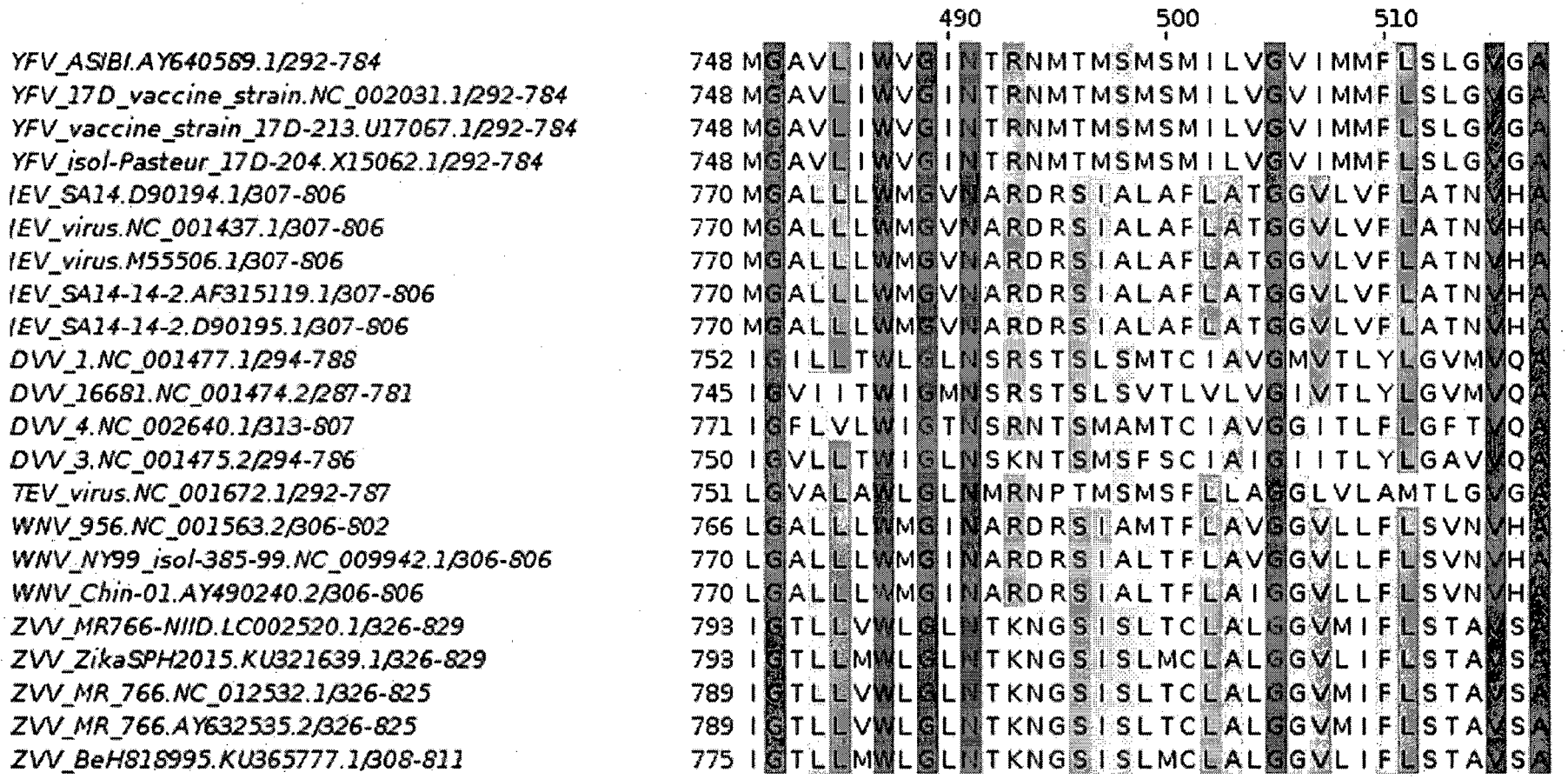


Figure 7 (Part 3 of 3)

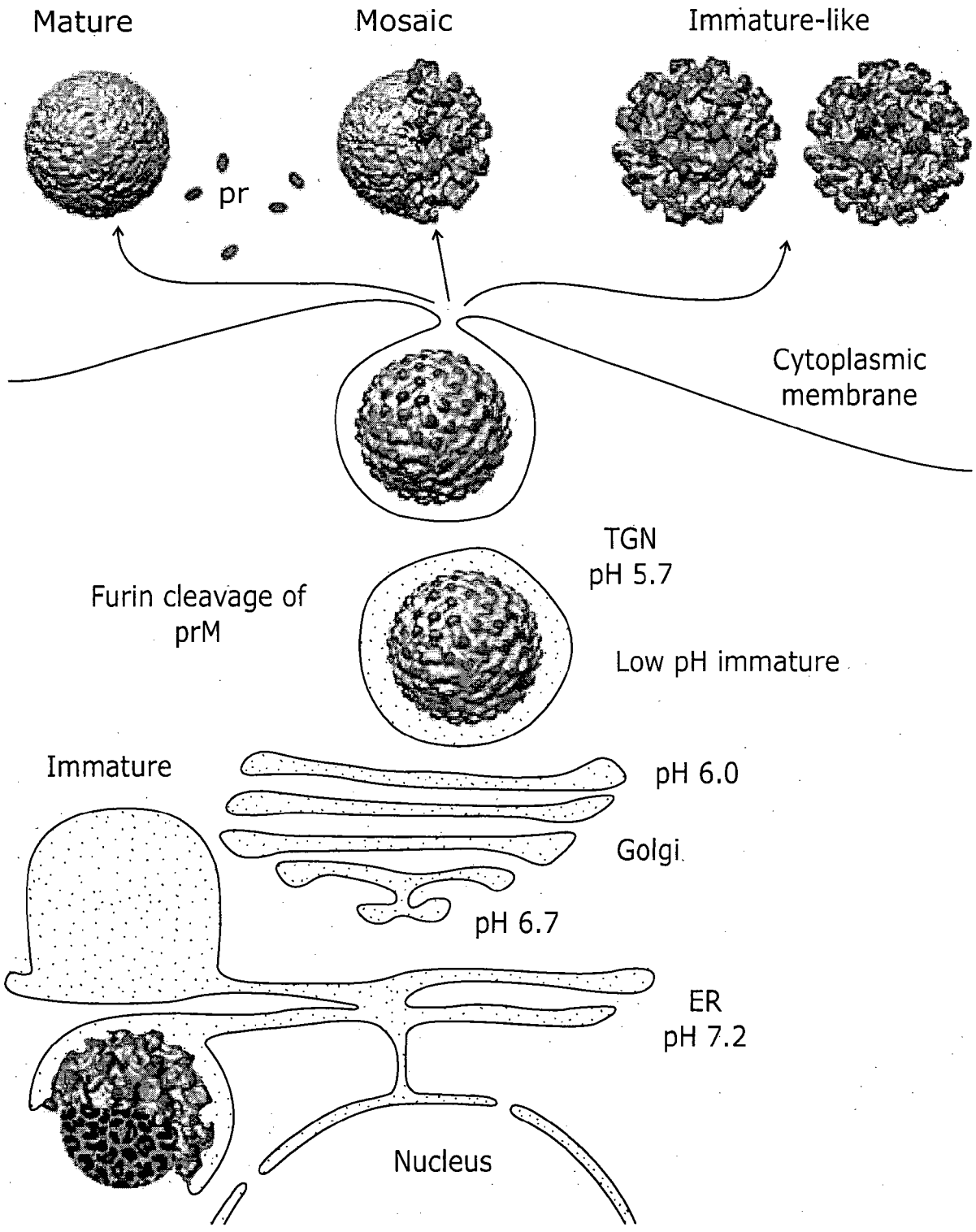

Mature
Mosaic
Immature-like
pr
Cytoplasmic membrane
TGN
pH 5.7
Furin cleavage of prM
Low pH immature
Immature
pH 6.0
Golgi
pH 6.7
ER
pH 7.2
Nucleus

Figure 8

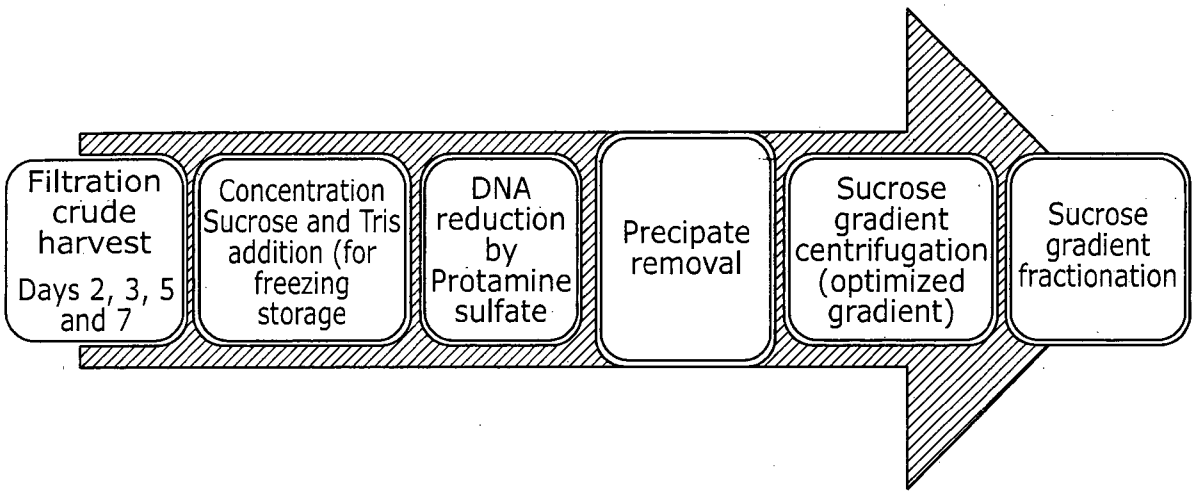


Figure 9A

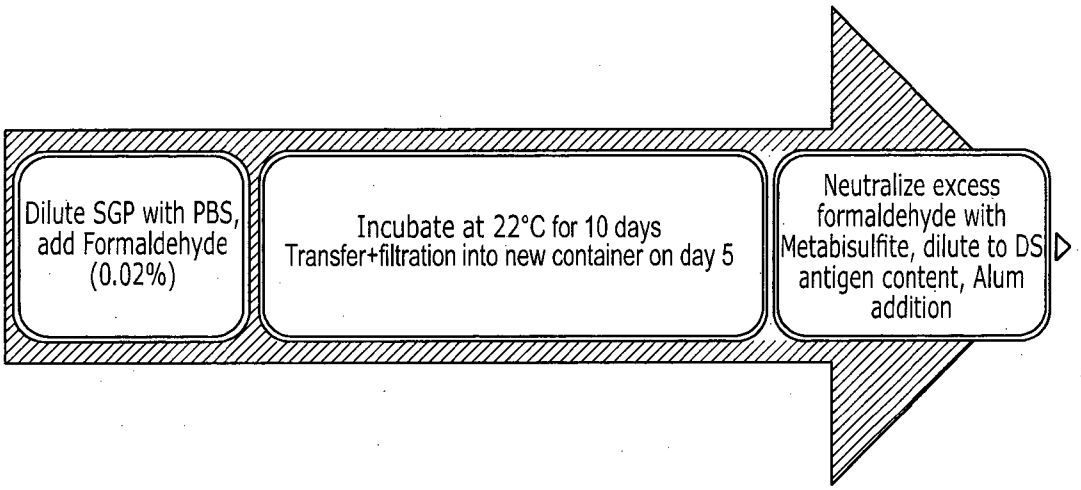


Figure 9B

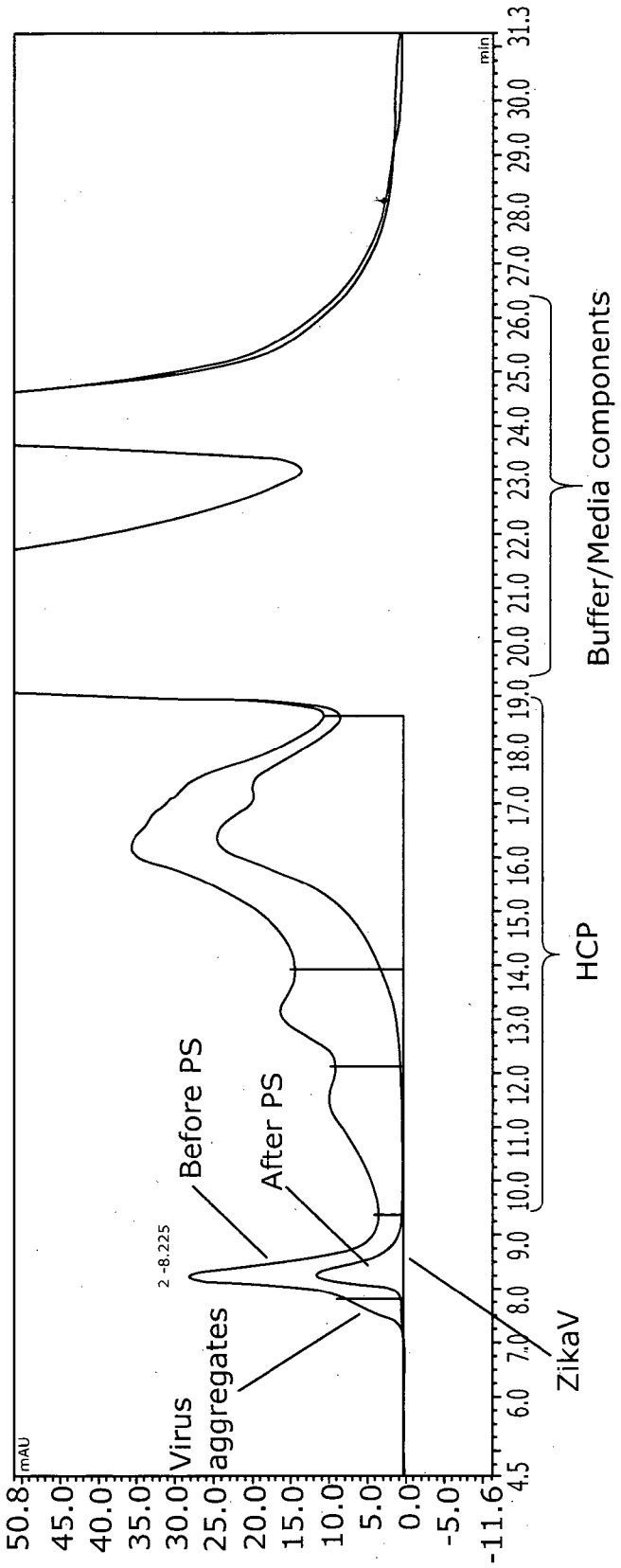
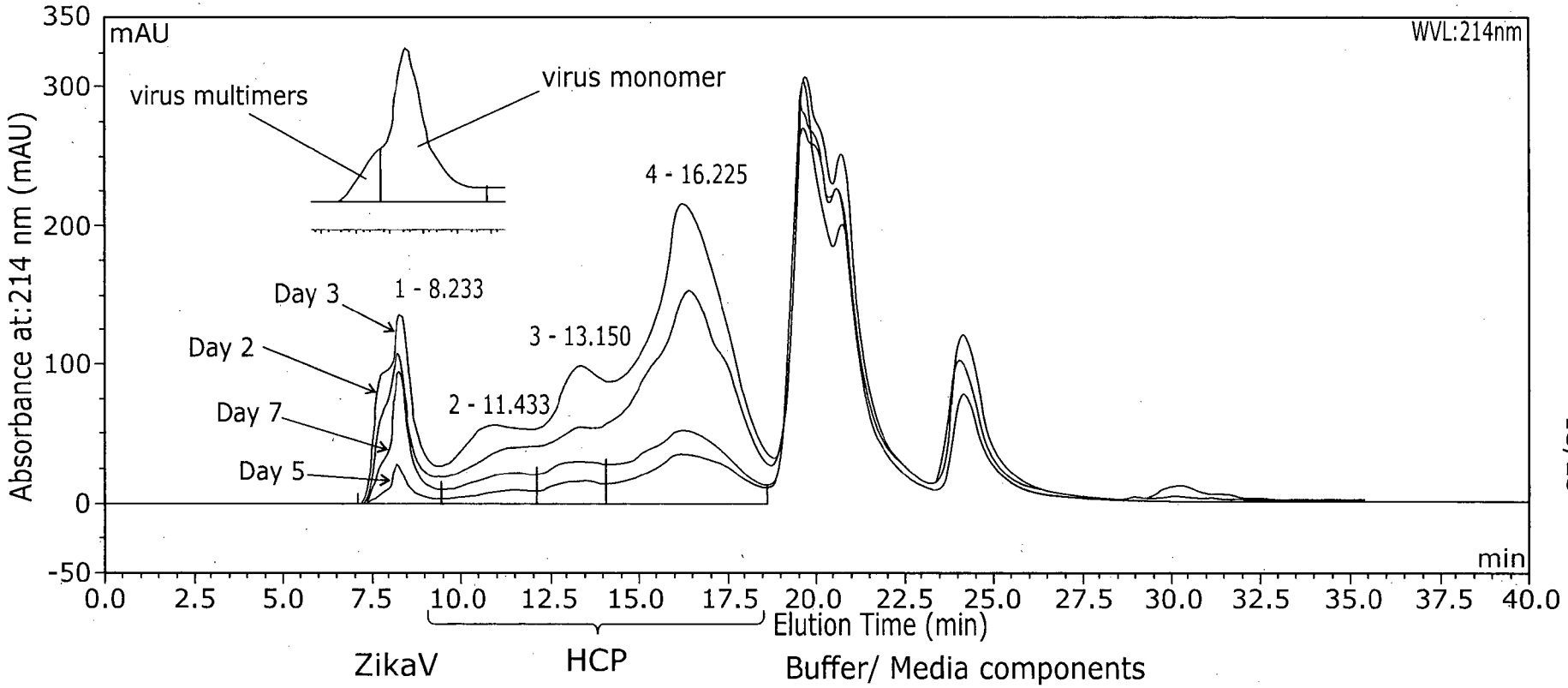
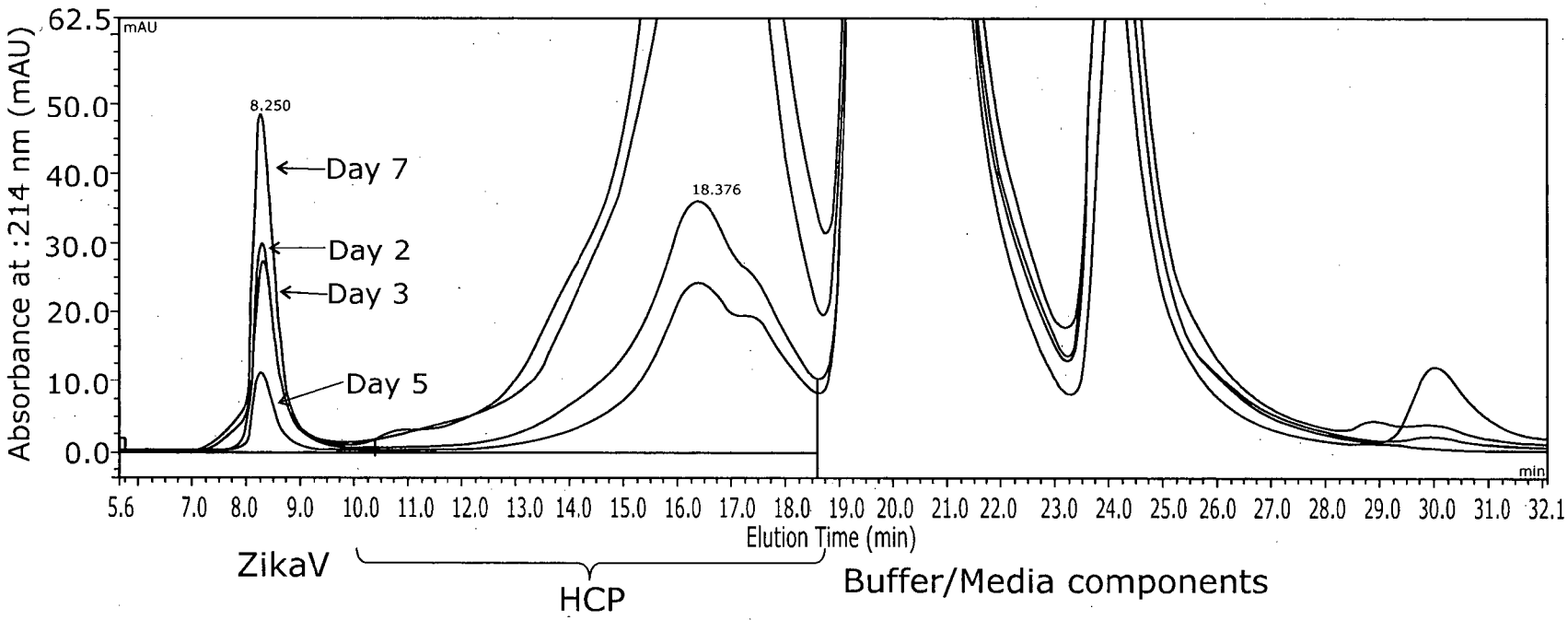

mAU
50.8
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min
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24.0
25.0
26.0
27.0
28.0
29.0
30.0
31.3
2 -8.225
Virus aggregates
Before PS
After PS
ZikaV
HCP
Buffer/Media components

Figure 10



+ Similar virus concentration in harvests Day 2, Day3, Day 7; lower virus concentration in harvest Day 5
+ Day 2 and Day 3 harvests contained more HCP than Days 5/7 (correlates with observed CPE)
+ Minor amount of virus multimers observed

Figure 11



+ PS removes virus aggregates and partially host cell proteins (HCP)
+ Infectoius virus titer is not affected by PS treatment
+ Strong cytopathic effect (CPE) on day 3,drop in productivity
+ Remaining cells seem to recover well and increase in Zika productivity

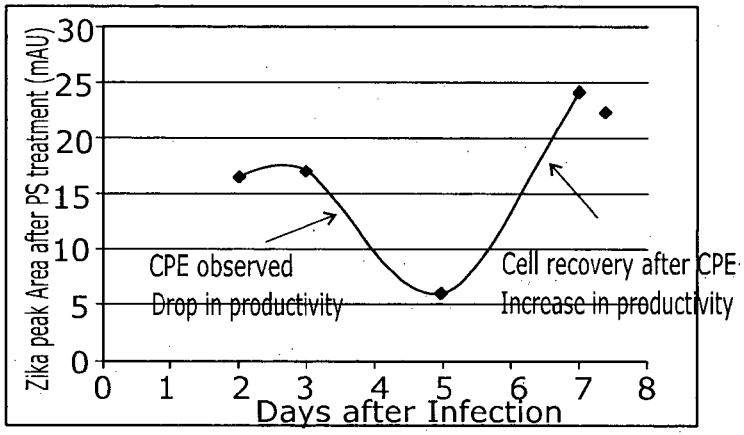


Figure 12

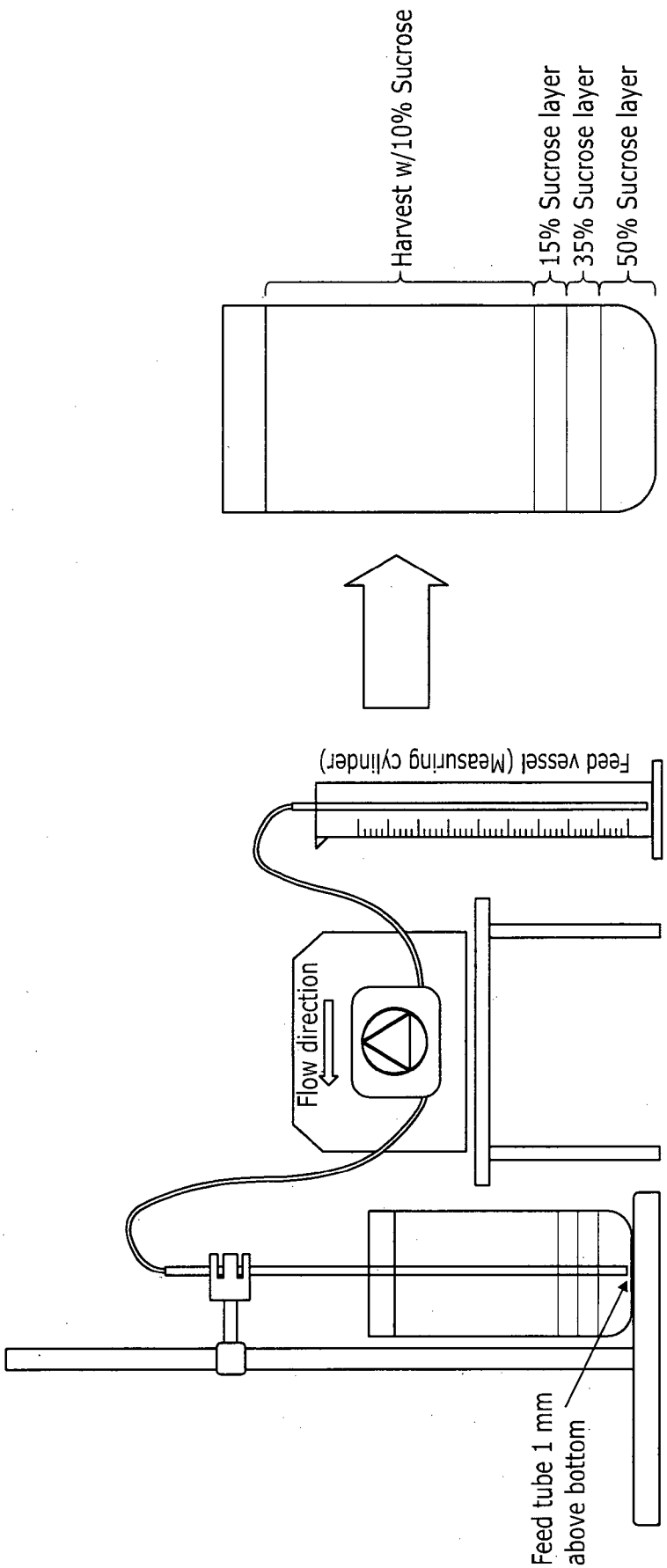
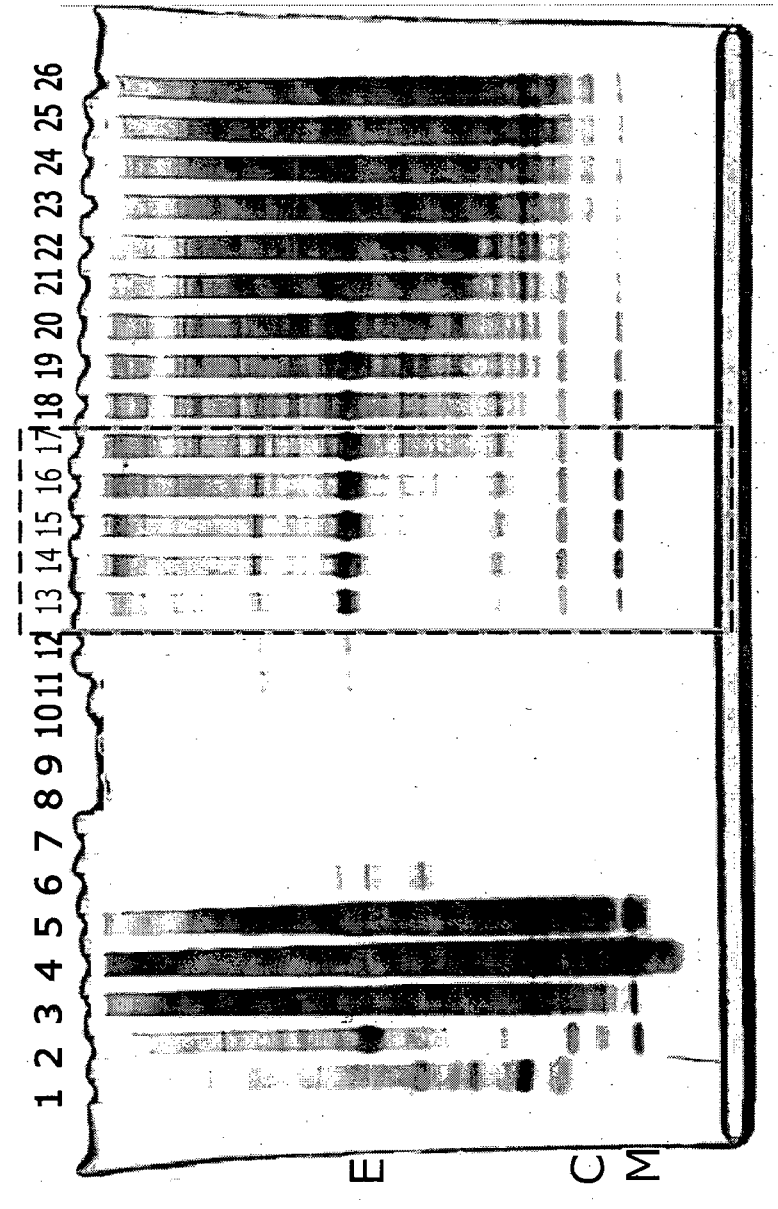

Harvest w/10% Sucrose
15% Sucrose layer
35% Sucrose layer
50% Sucrose layer
Feed vessel (Measuring cylinder)
Flow direction
Feed tube 1 mm
above bottom

Figure 13



Lane	Sample
1	Molecular weight Marker
2	JEV Sucrose Gradient Pool (Reference)
3	Zika Harvest pool (day 2,3,5,7)
4	Zika Harvest pool (day 2,3,5,7)concentrated 300x
5	Zika Harvest pool (day 2,3,5,7)concentrated 300x + Protamine sulfate
6	Ultrafiltration Permeate
7	SGC Bottle 1, Fraction 4
8	SGC Bottle 1, Fraction 5
9	SGC Bottle 1, Fraction 6
10	SGC Bottle 1, Fraction 7
11	SGC Bottle 1, Fraction 8
12	SGC Bottle 1, Fraction 9
13	SGC Bottle 1, Fraction 10
14	SGC Bottle 1, Fraction 11
15	SGC Bottle 1, Fraction 12
16	SGC Bottle 1, Fraction 13
17	SGC Bottle 1, Fraction 14
18	SGC Bottle 1, Fraction 15
19	SGC Bottle 1, Fraction 16
20	SGC Bottle 1, Fraction 17
21	SGC Bottle 1, Fraction 18
22	SGC Bottle 1, Fraction 19
23	SGC Bottle 1, Fraction 20
24	SGC Bottle 1, Fraction 21
25	SGC Bottle 1, Fraction 22
26	SGC Bottle 1, Fraction 23

Pooled fractions F10-F14
(10mL total volume pere bottle)

Figure 14

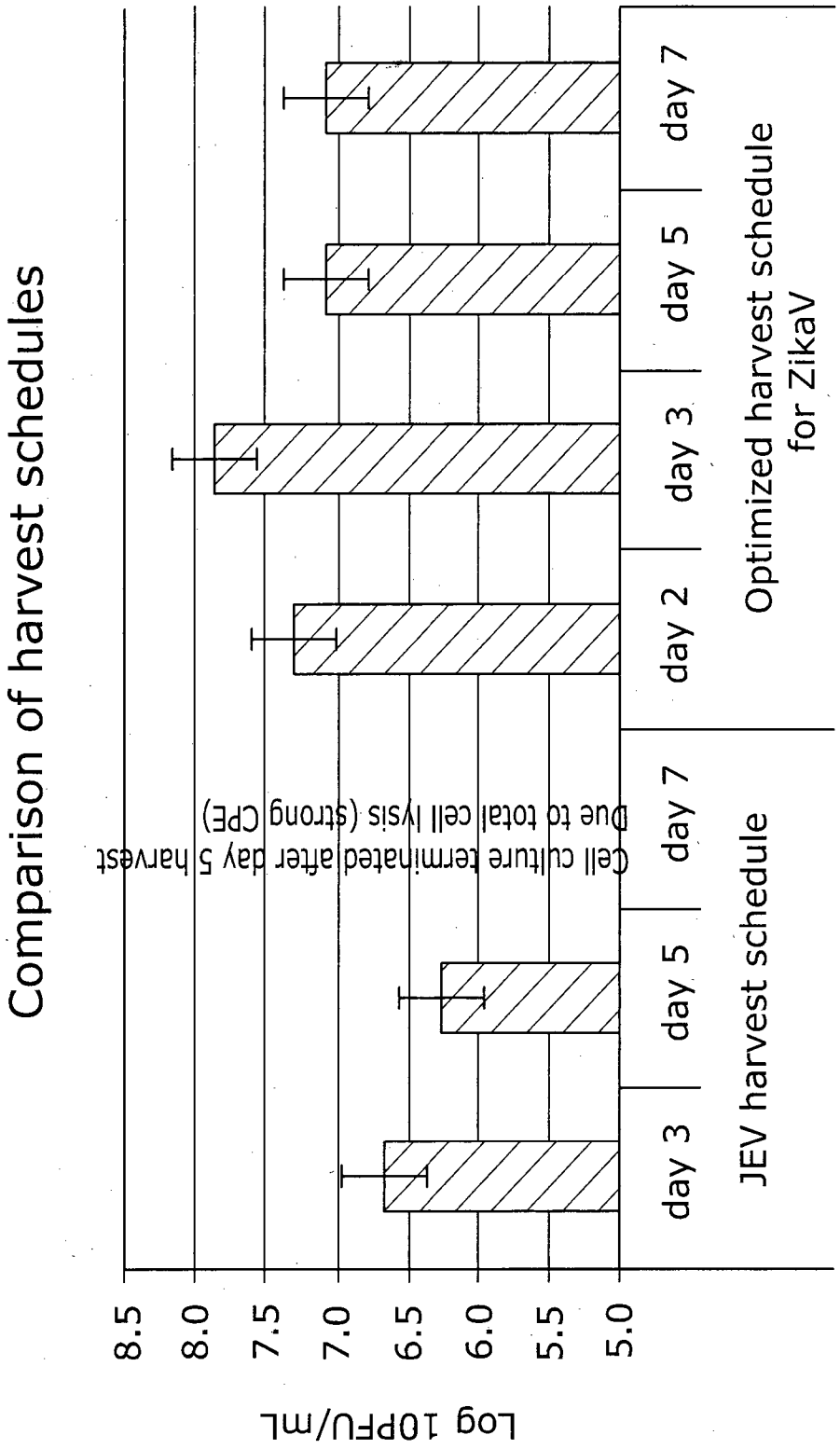

Comparison of harvest schedules
Log 10PFU/mL
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
day 3
day 5
day 7
day 2
day 3
day 5
day 7
JEV harvest schedule
Optimized harvest schedule for ZikaV
Cell culture terminated after day 5 harvest
Due to total cell lysis (strong CPE)

Figure 15

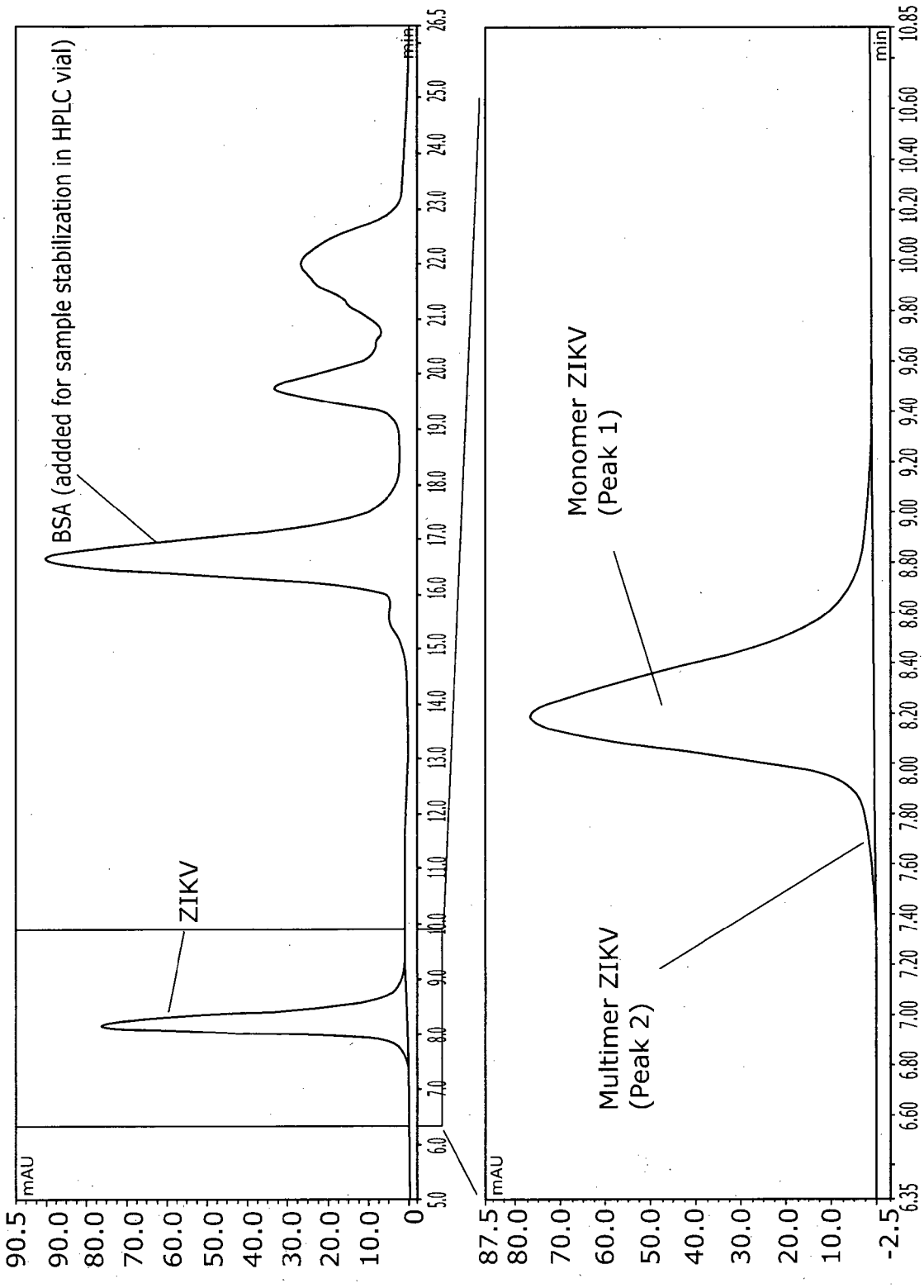

BSA (addded for sample stabilization in HPLC vial)
ZIKV
Monomer ZIKV
(Peak 1)
Multimer ZIKV
(Peak 2)
mAU
min

Figure 16

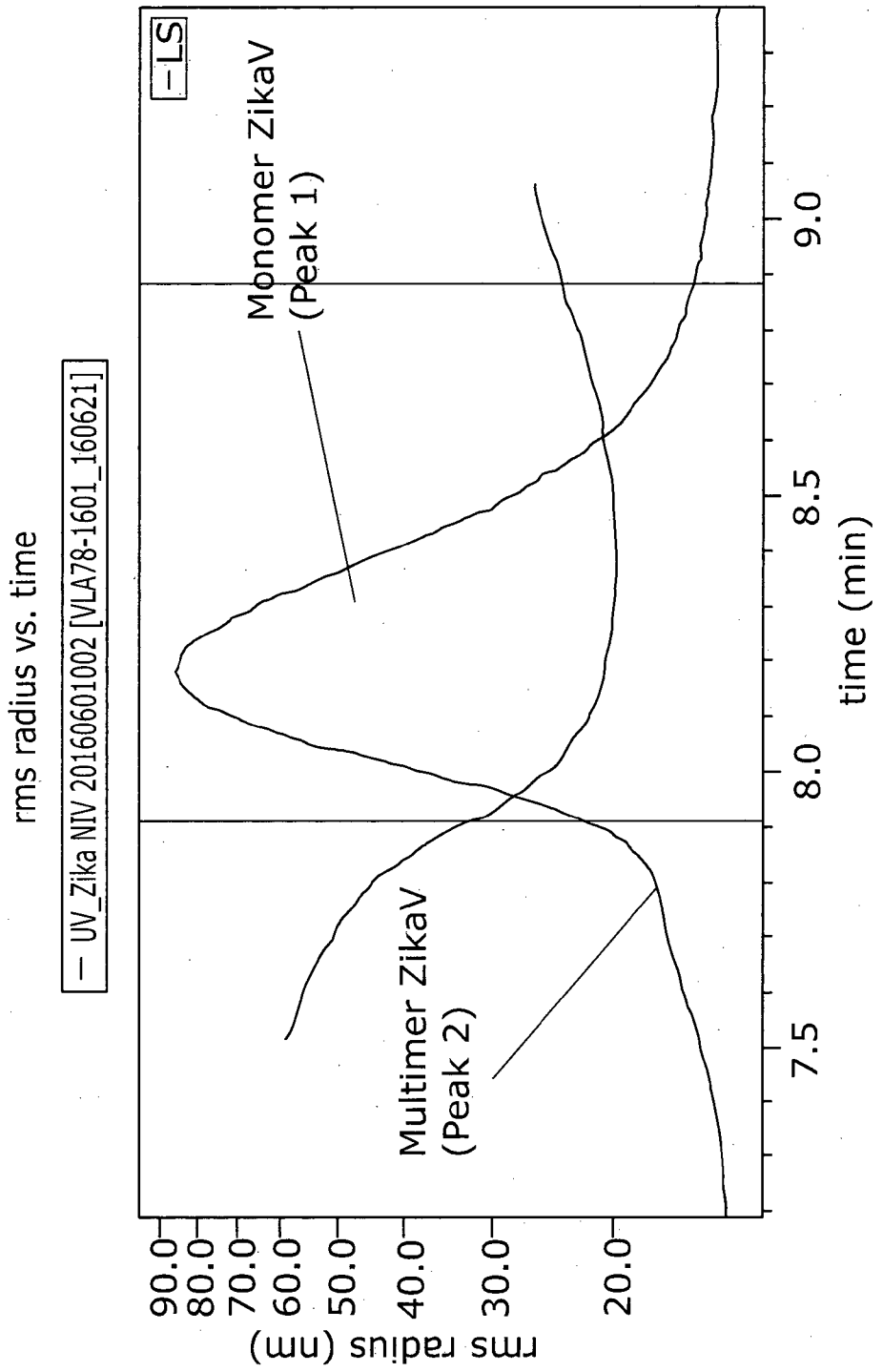

rms radius vs. time
UV_Zika NIV 20160601002 [VLA78-1601_160621]
LS
Monomer ZikaV
(Peak 1)
Multimer ZikaV
(Peak 2)
rms radius (nm)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
7.5
8.0
8.5
9.0
time (min)

Figure 17

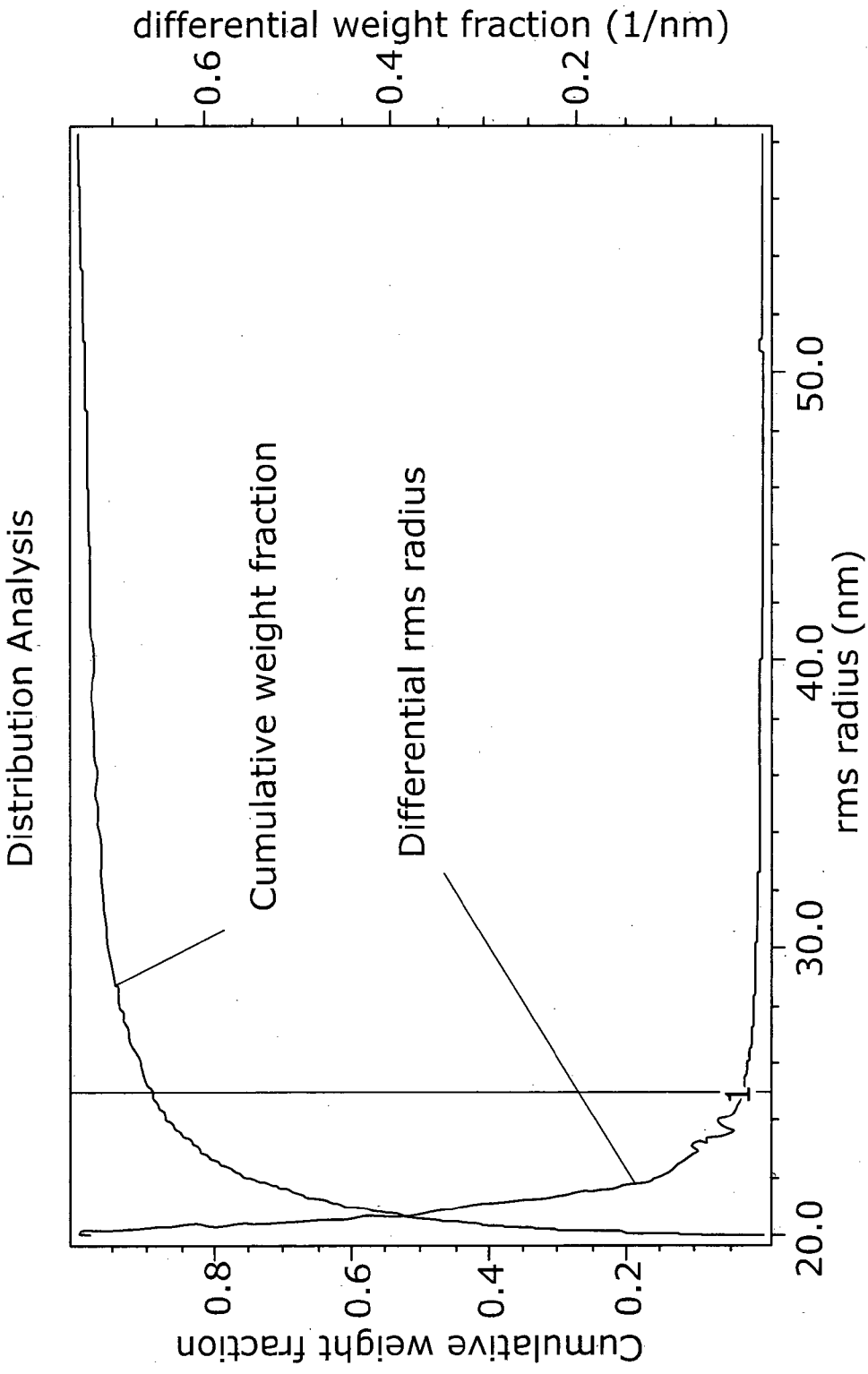
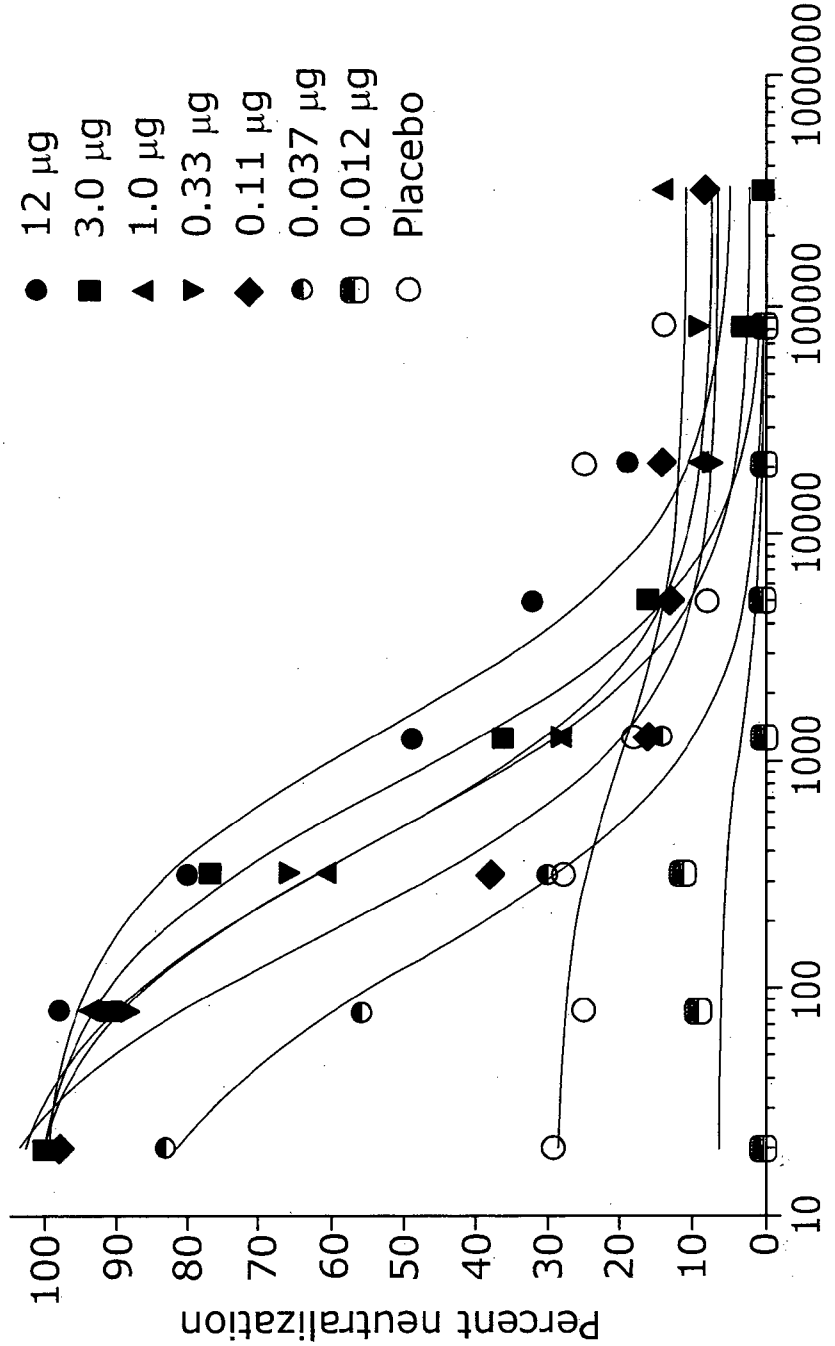
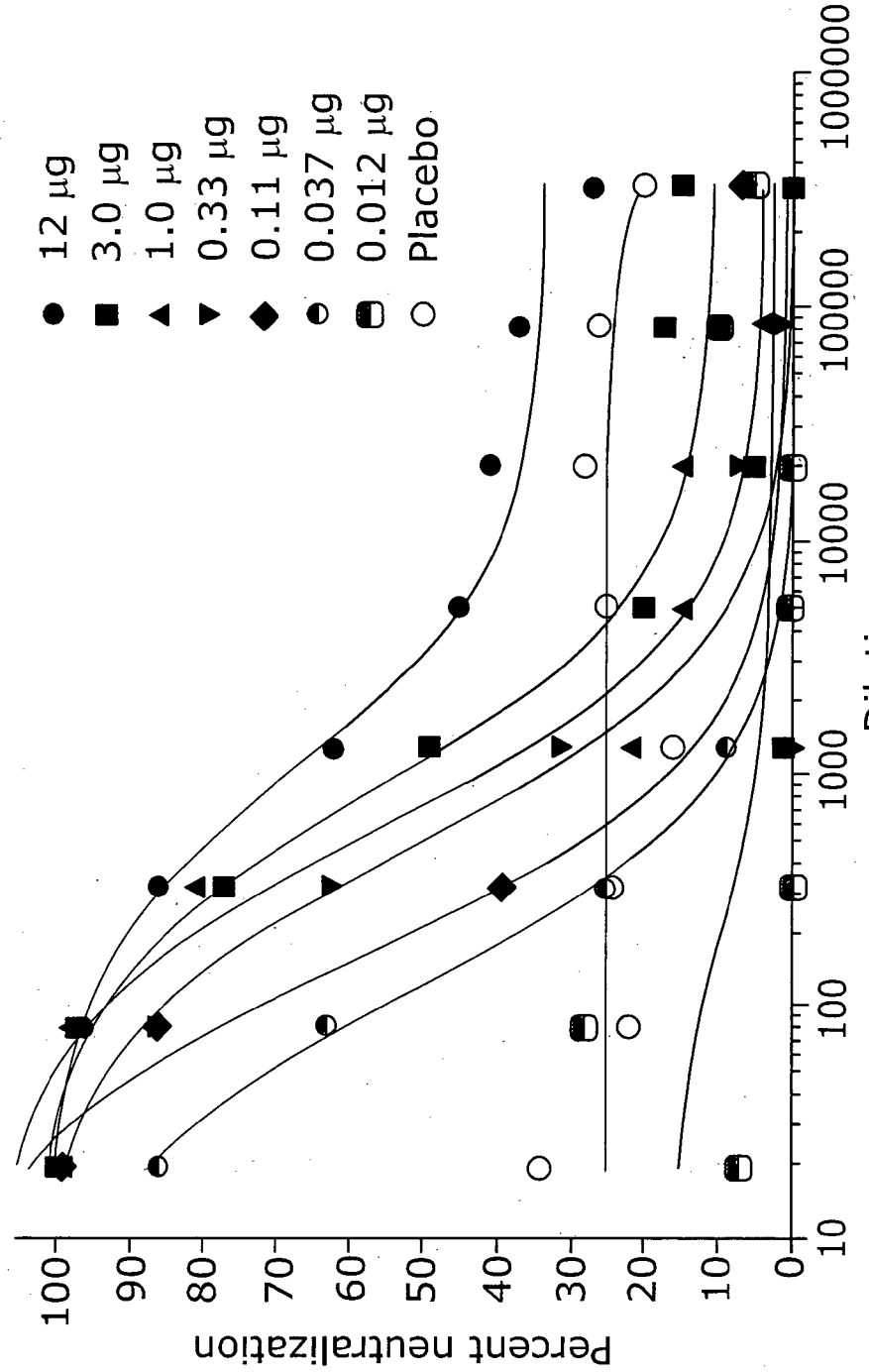

Distribution Analysis
Cumulative weight fraction
Differential rms radius
Cumulative weight fraction
0.2
0.4
0.6
0.8
differential weight fraction (1/nm)
0.2
0.4
0.6
rms radius (nm)
20.0
30.0
40.0
50.0

Figure 18



	12 µg	3.0 µg	1.0 µg	0.33 µg	0.11 µg	0.037 µg
EC50	1371	807.6	380.7	456.1	179.9	142.0

Figure 19



	12 µg	3.0 µg	1.0 µg	0.33 µg	0.11 µg	0.037 µg
EC50	1029	819.1	556.9	477.3	158.1	118.0

Figure 20

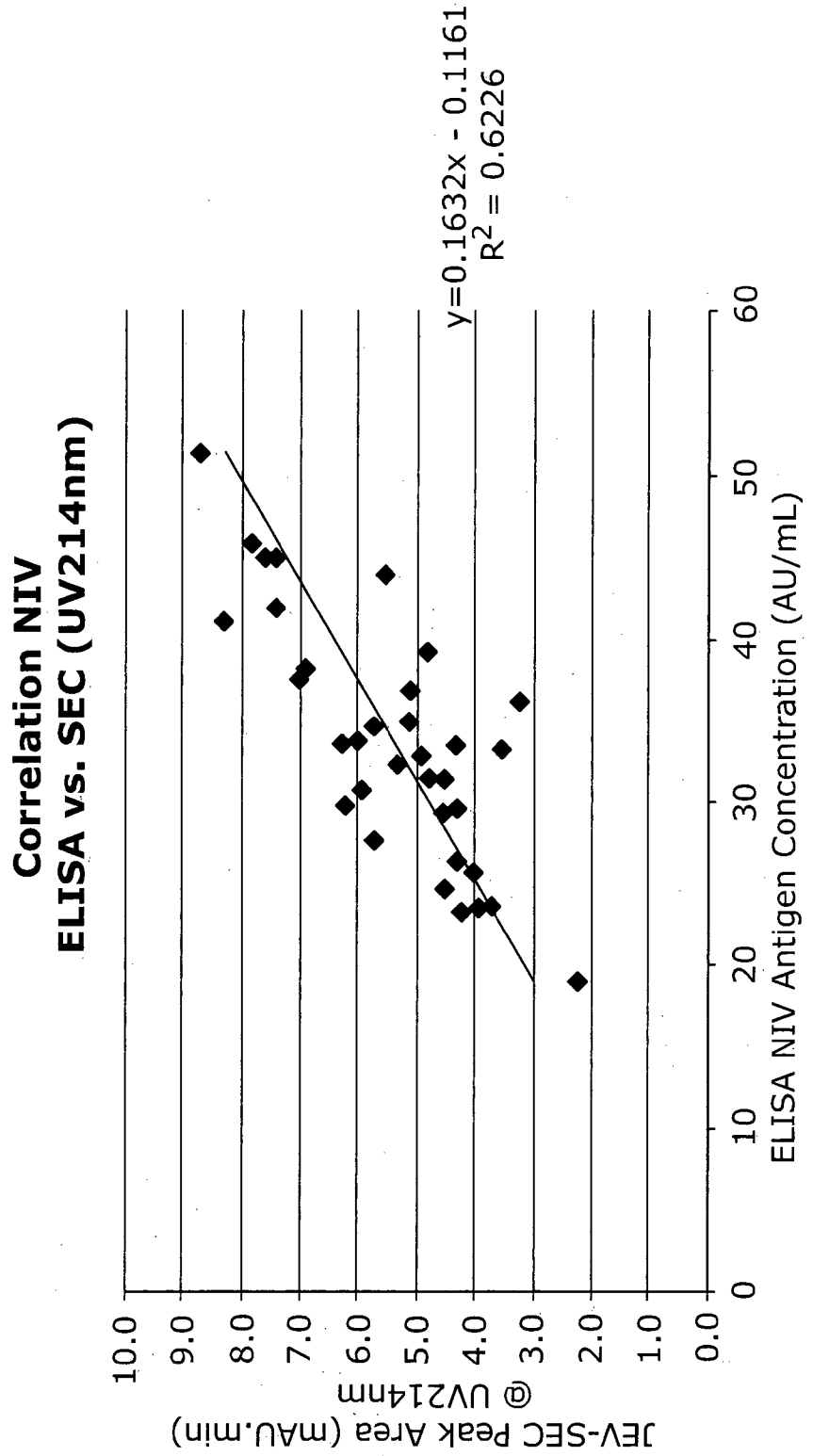

Correlation NIV
ELISA vs. SEC (UV214nm)
y=0.1632x - 0.1161
R2 = 0.6226
JEV-SEC Peak Area (mAU.min)
@ UV214nm
ELISA NIV Antigen Concentration (AU/mL)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
0
10
20
30
40
50
60

Figure 21