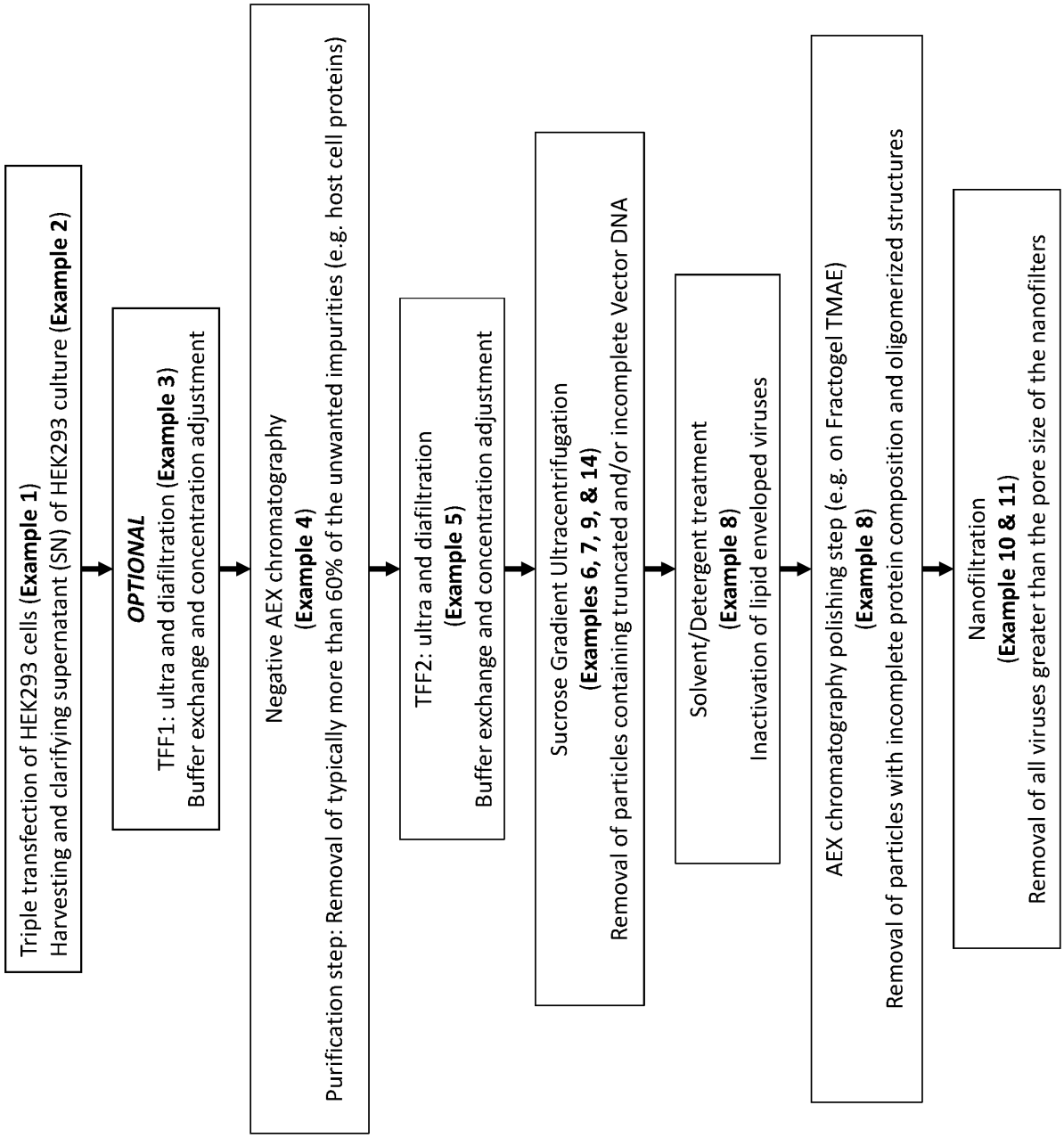

Triple transfection of HEK293 cells (Example 1)
Harvesting and clarifying supernatant (SN) of HEK293 culture (Example 2)
OPTIONAL
TFF1: ultra and diafiltration (Example 3)
Buffer exchange and concentration adjustment
Negative AEX chromatography
(Example 4)
Purification step: Removal of typically more than 60% of the unwanted impurities (e.g. host cell proteins)
TFF2: ultra and diafiltration
(Example 5)
Buffer exchange and concentration adjustment
Sucrose Gradient Ultracentrifugation
(Examples 6, 7, 9, & 14)
Removal of particles containing truncated and/or incomplete Vector DNA
Solvent/Detergent treatment
(Example 8)
Inactivation of lipid enveloped viruses
AEX chromatography polishing step (e.g. on Fractogel TMAE)
(Example 8)
Removal of particles with incomplete protein composition and oligomerized structures
Nanofiltration
(Example 10 & 11)
Removal of all viruses greater than the pore size of the nanofilters

FIGURE 1

FIGURE 2

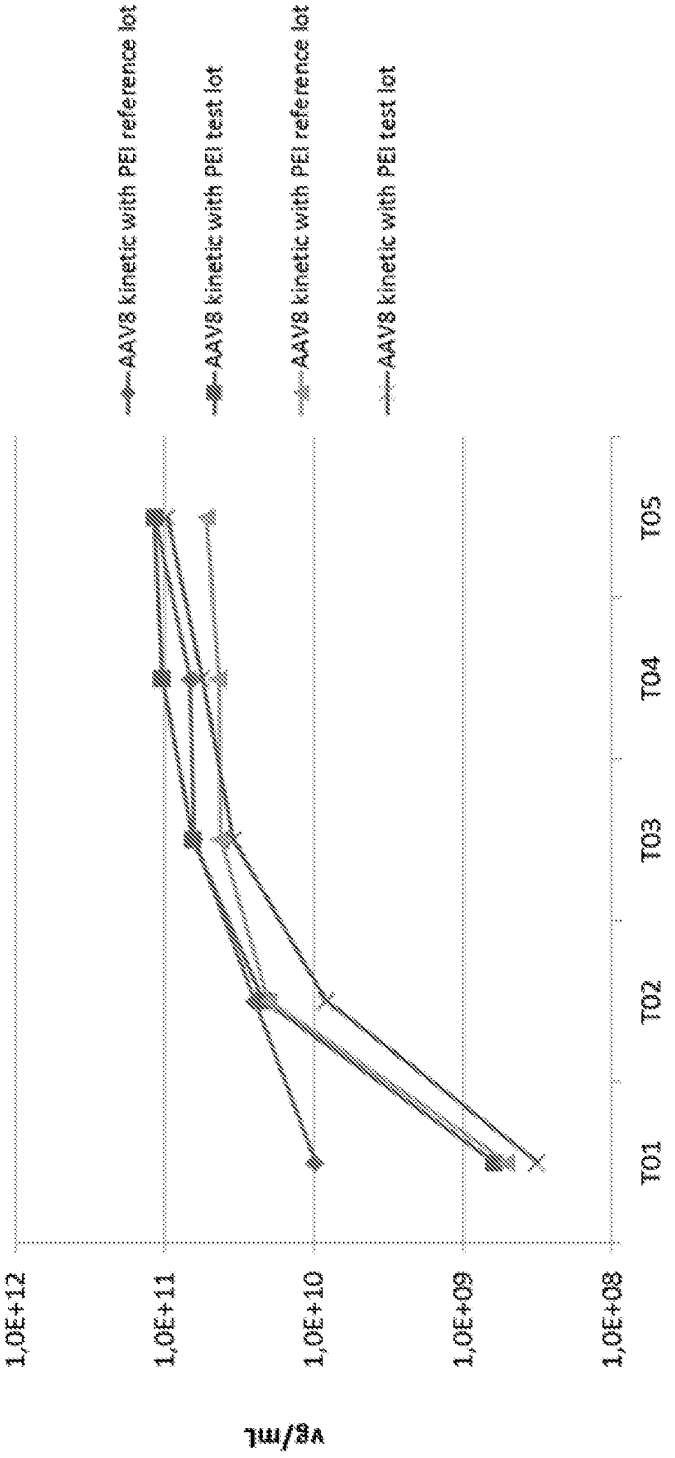

Vector genomes (supernatant): FIX qPCR
AAV8 kinetic with PEI reference lot
AAV8 kinetic with PEI test lot
AAV8 kinetic with PEI reference lot
AAV8 kinetic with PEI test lot
1,0E+12
1,0E+11
1,0E+10
1,0E+09
1,0E+08
vg/mL
T01
T02
T03
T04
T05

FIGURE 3

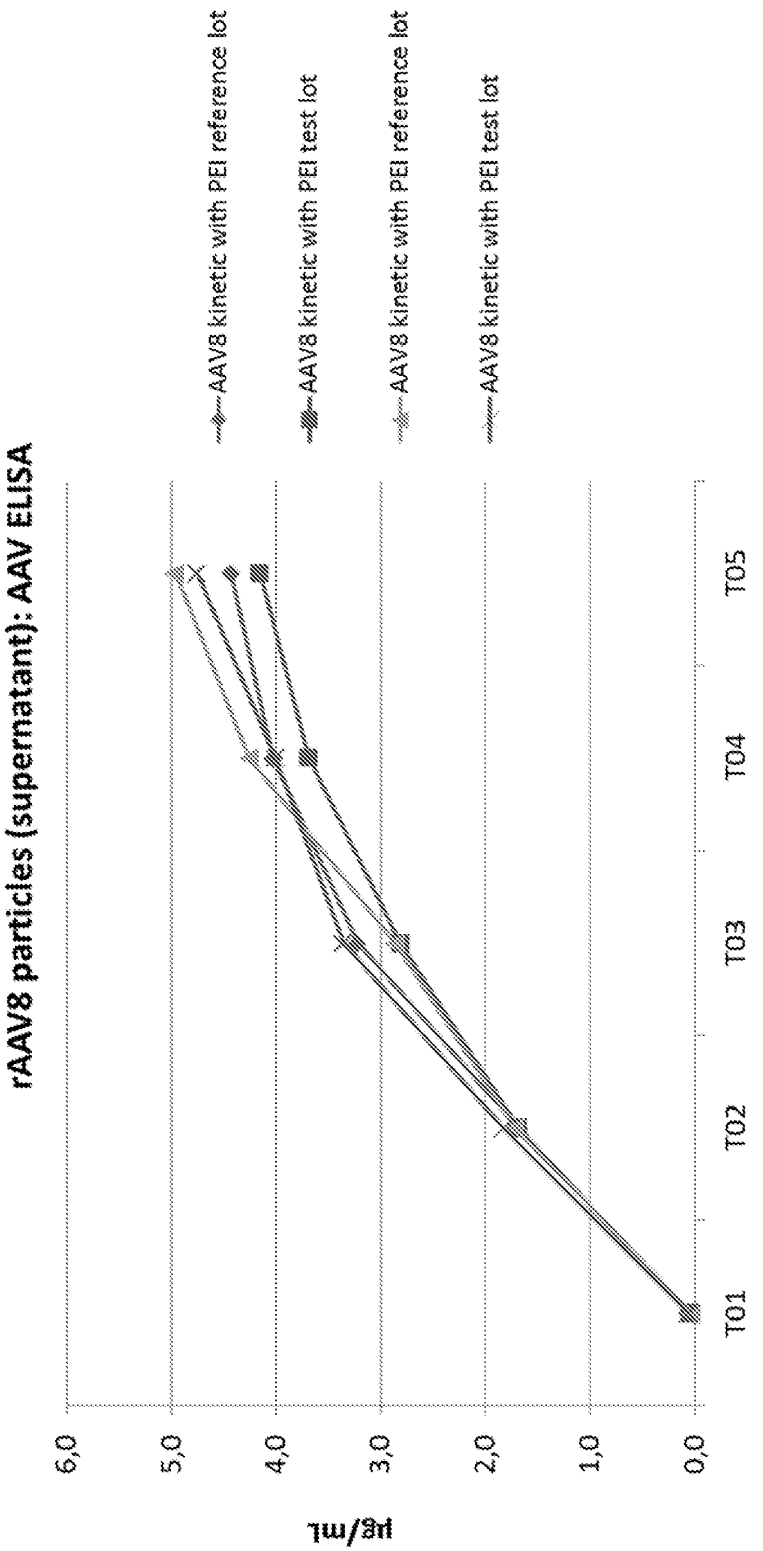

rAAV8 particles (supernatant): AAV ELISA
AAV8 kinetic with PEI reference lot
AAV8 kinetic with PEI test lot
AAV8 kinetic with PEI reference lot
AAV8 kinetic with PEI test lot
6,0
5,0
4,0
3,0
2,0
1,0
0,0
pg/mL
T01
T02
T03
T04
T05

FIGURE 4

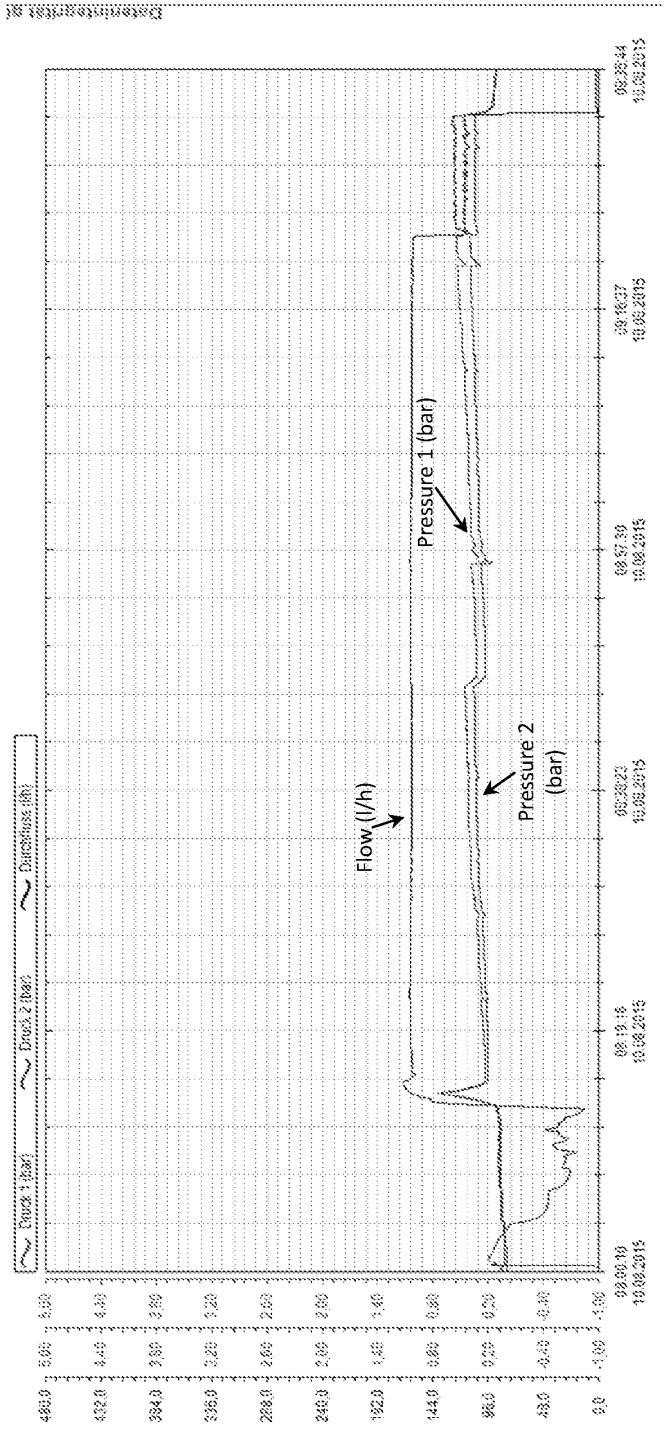


FIGURE 5

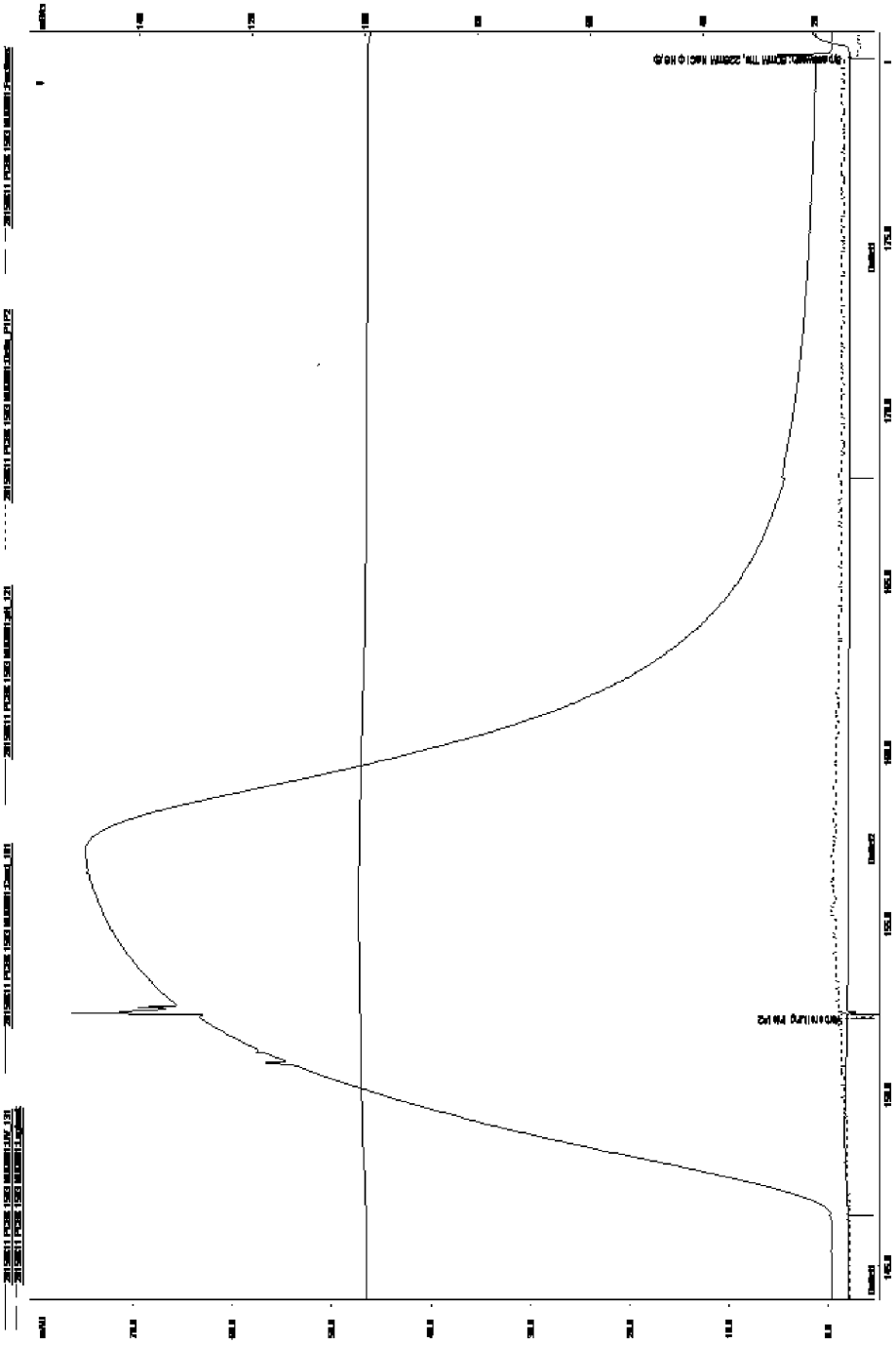
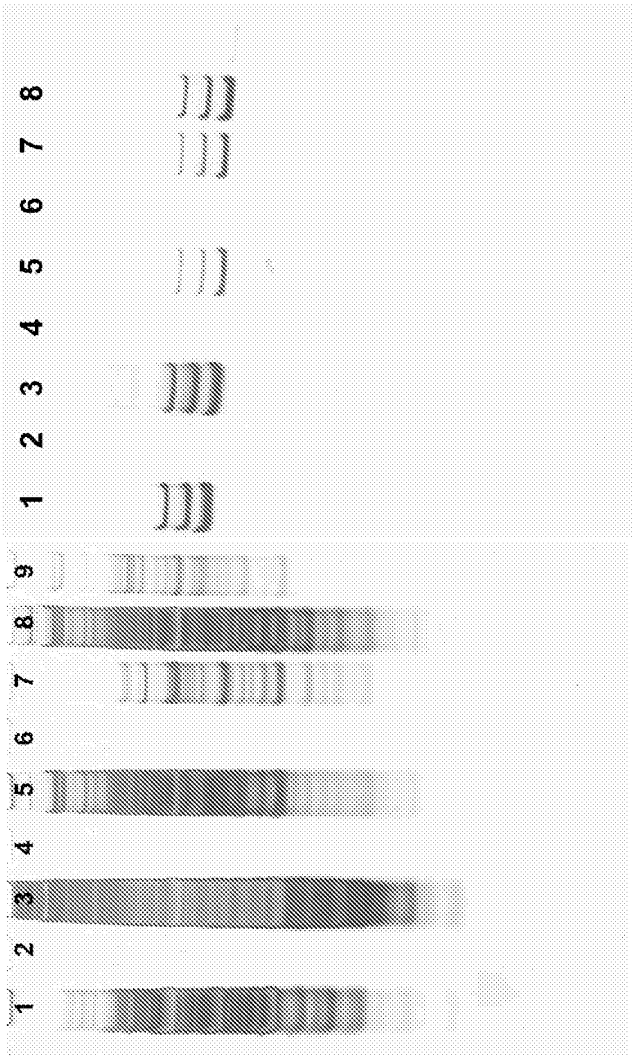


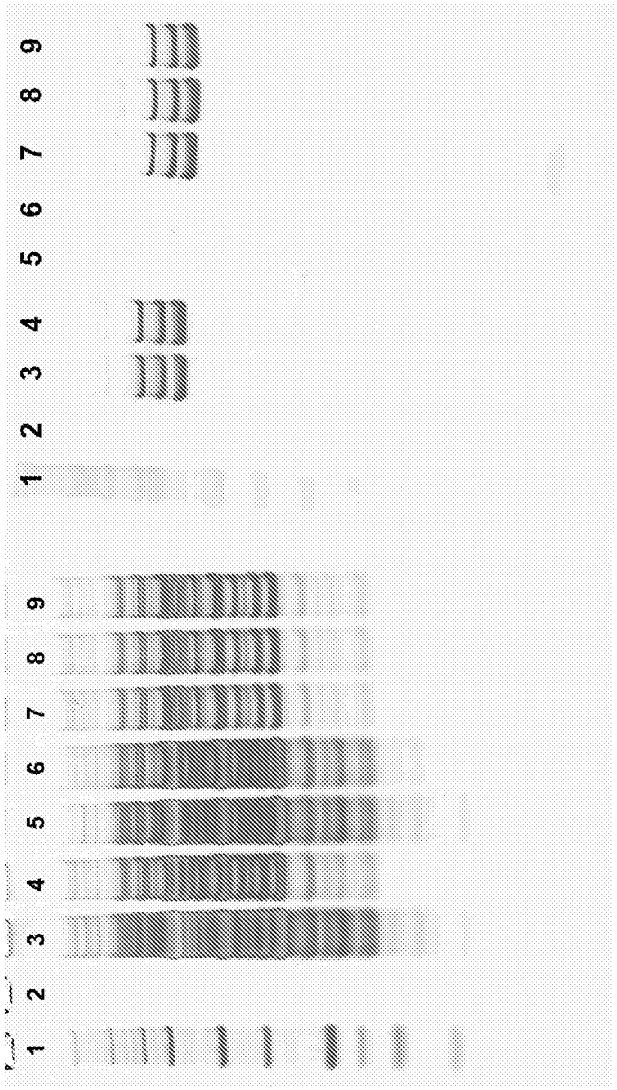
FIGURE 6



Description of Samples by Lane Numbers

1.Mustang Q Flow through (Product fraction)
2.Blank
3. Mustang Q Load (UF/DF concentrate)
4.Blank
5.Mustang Q Eluate
6.Blank
7 Mustang Q Flow through 1:10
8.Start Material (UF/DF concentrate) 1:10
9. Mustang Q Eluate 1:10

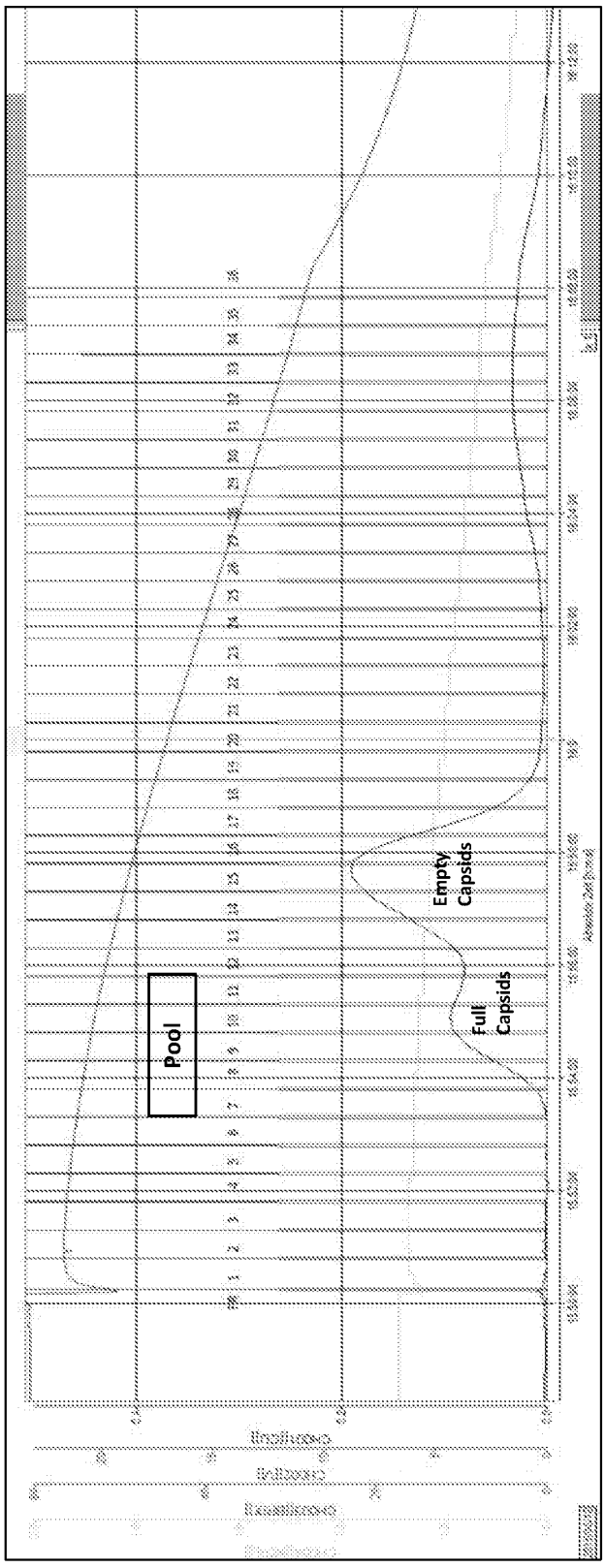
FIGURE 7



Description of Samples by Lane Numbers

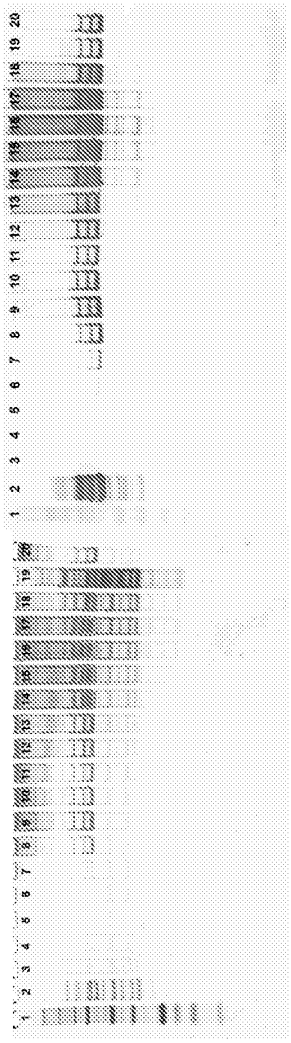
1. Marker
2. Blank
3. TFF_LOAD (Mustang Q Flow through)
4. TFF – Retentate diluted 1:14
5. TFF – Filtrate
6. TFF - Diafiltrate
7. TFF – retentate diafiltrated diluted 1:14
8. TFF – retentate diafiltrated diluted 1:14 prior 0.2µm filtration
9. TFF - retentate diafiltrated diluted 1:14 post 0.2µm filtration

FIGURE 8



Elution profile ultracentrifugation

FIGURE 9



1.Marker
2.Ultracentrifugation LOAD diluted 1:50
3.Ultracentrifugation FR
4.Ultracentrifugation F01
5.Ultracentrifugation F02
6.Ultracentrifugation F03
7.Ultracentrifugation F04
8.Ultracentrifugation F05
9.Ultracentrifugation F06
10.Ultracentrifugation F07
11.Ultracentrifugation F08
12.Ultracentrifugation F09
13.Ultracentrifugation F10
14.Ultracentrifugation F11
15.Ultracentrifugation F12
16.Ultracentrifugation F13
17.Ultracentrifugation F14
18.Ultracentrifugation F15
19.Ultracentrifugation F1
20.Ultracentrifugation Pool 04 - F08

FIGURE 10

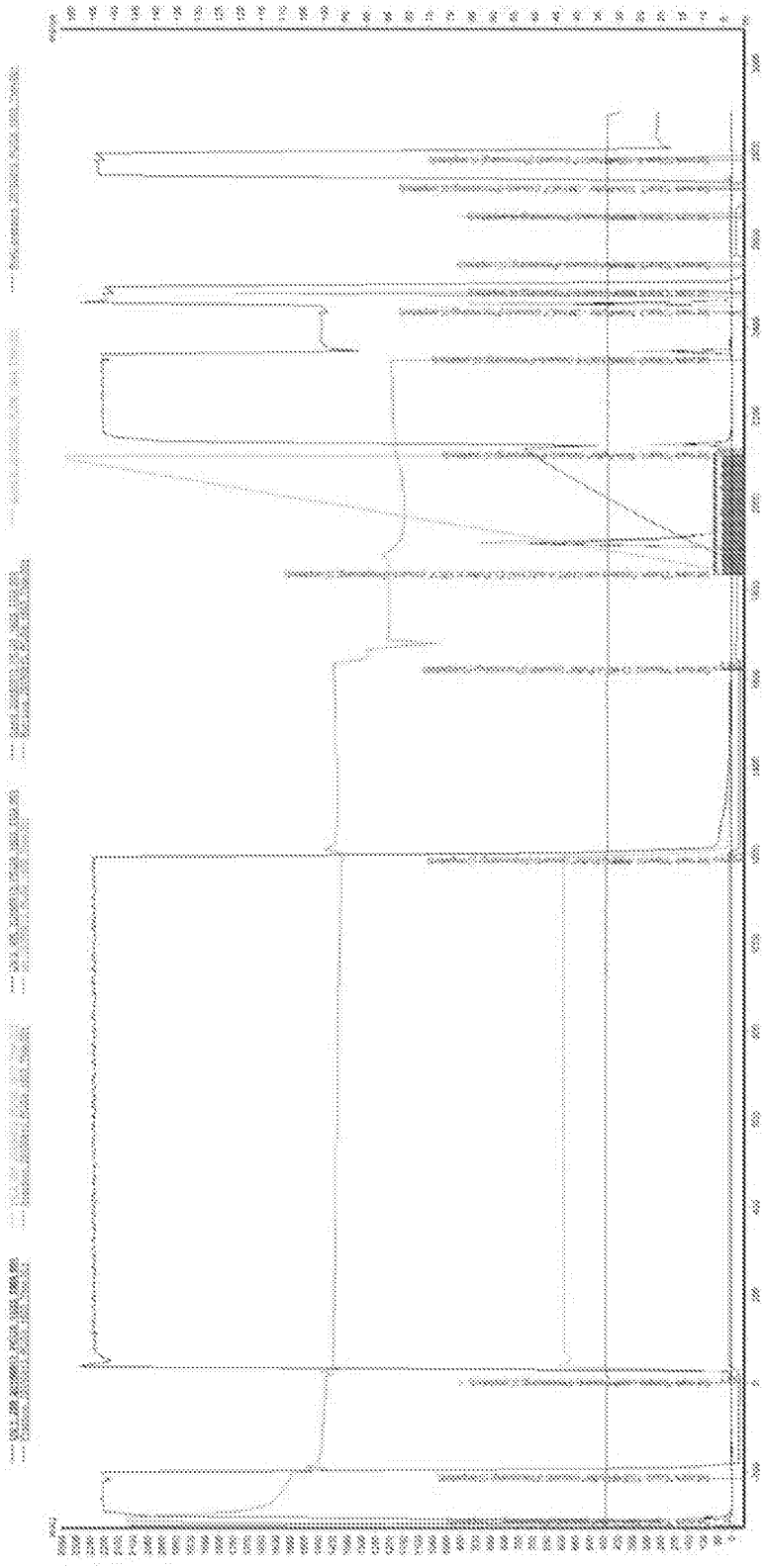


FIGURE 11

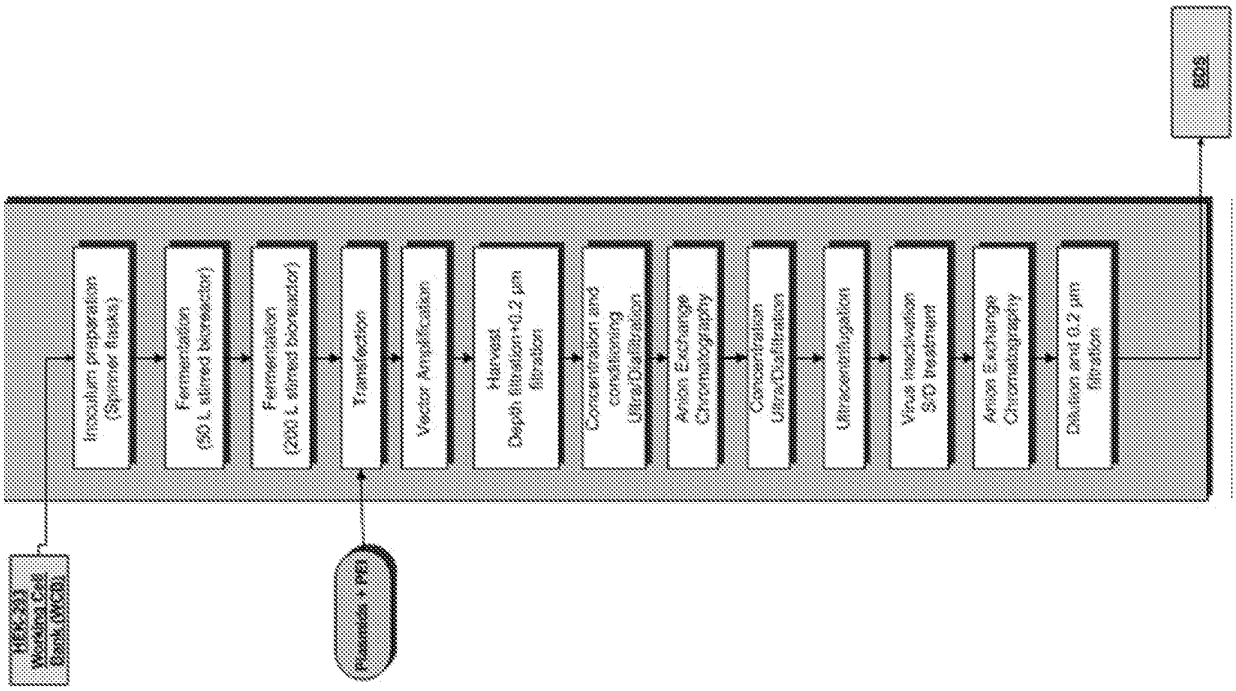

Inoculum preparation (Spinner flasks)
Fermentation (50 L stirred bioreactor)
Fermentation (200 L stirred bioreactor)
Transfection
Vector Amplification
Harvest Depth filtration+0.2 µm filtration
Concentration and conditioning Ultrafiltration
Anion Exchange Chromatography
Concentration Ultrafiltration
Ultracentrifugation
Virus inactivation S/D treatment
Anion Exchange Chromatography
Diafiltration and 0.2 µm filtration
DS
Plasmids + PEI

FIGURE 12

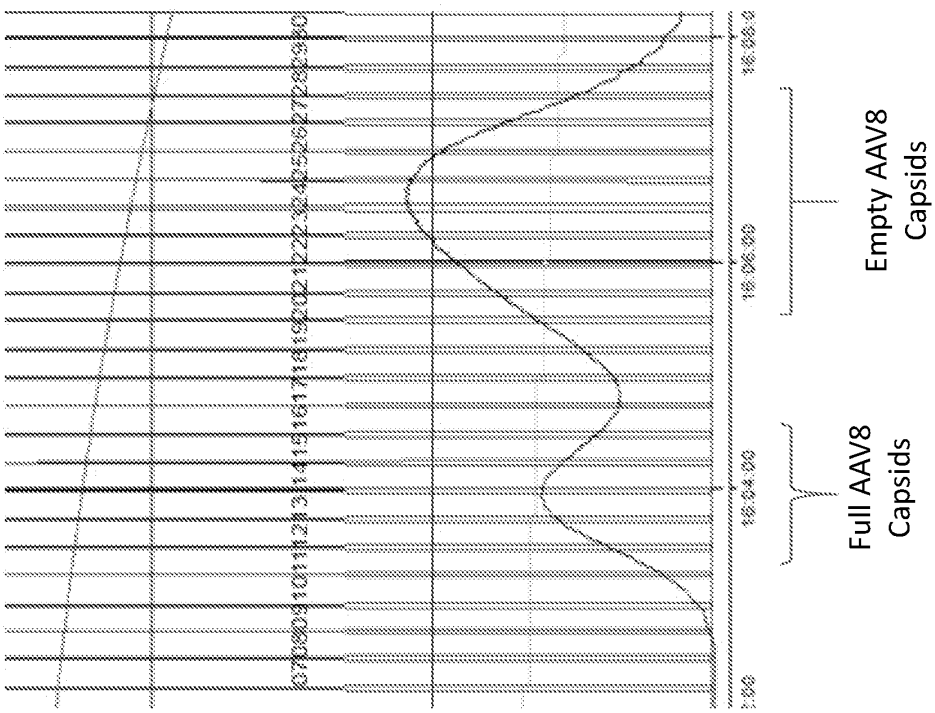
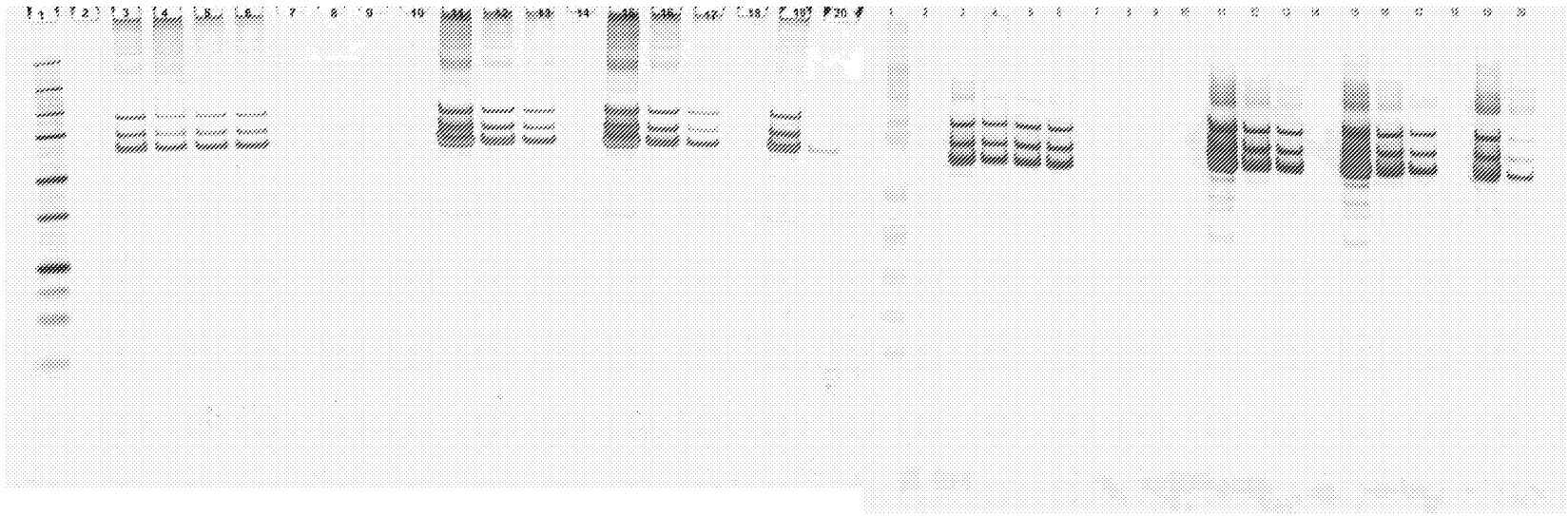


FIGURE 13



Description of Samples by Lane Number

1.Mol weight marker
2.Blank
3.Start Material (Ultracentrifugation pool)
4.Start Material (Ultracentrifugation pool) filtrated
5.Start Material diluted prior SD Treatment
6.TMAE_LOAD post SD Treatment
7.TMAE_Flow through
8.TMAE_Wash
9.TMEA_Wash2
10.Blank
11.TMAE_BDS_Bulk conc (Main eluate from TMAE diluted 1:2)
12.TMAE_BDS_Bulk diluted 1:4
13.TMAE_BDS_Bulk diluted 1:8
14.Blank
15.TMAE_FDP_FC conc (Main eluate from TMAE diluted to final volume)
16.TMAE_FDP_FC diluted 1:4
17.TMAE_FDP_FC diluted 1:8
18.Blank
19.TMAE_NE1 (Eluate 2)
20.TMAE_NE2 (Eluate 3)

FIGURE 14

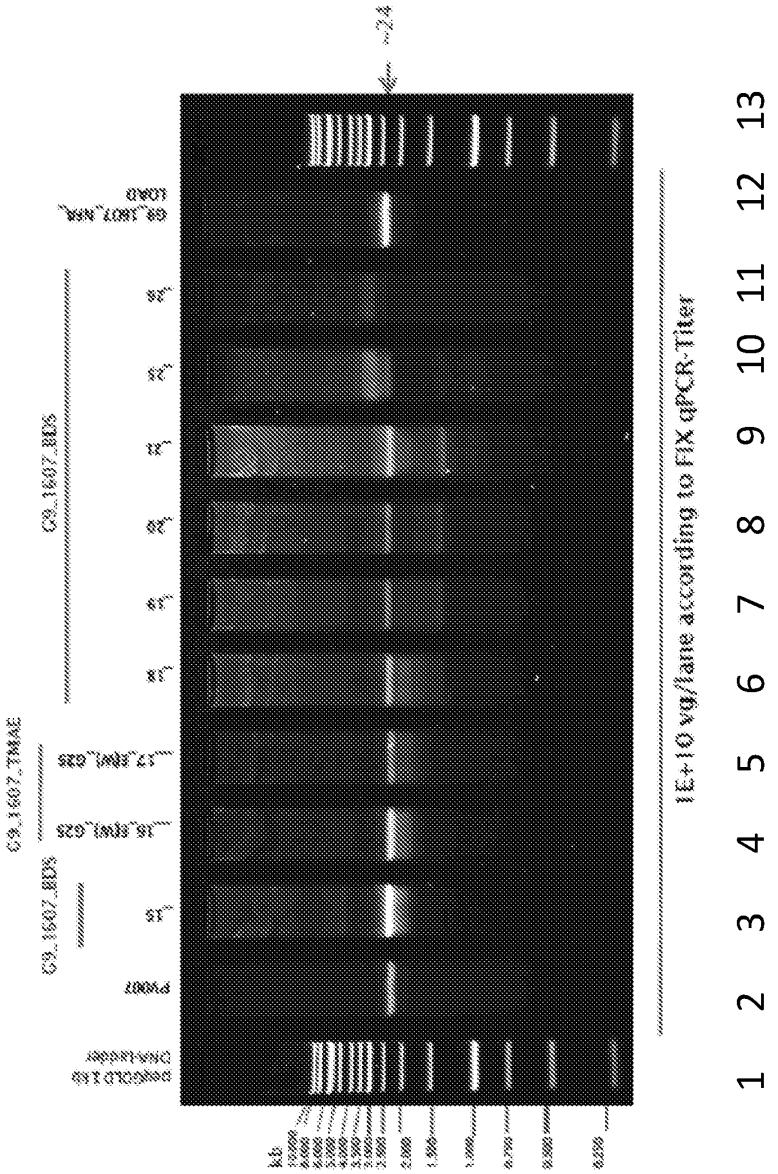

~24
C9_1607_BDS
C9_1607_TMAE
1E+10 vg/lane according to FIX qPCR-Titer
1 2 3 4 5 6 7 8 9 10 11 12 13

FIGURE 15

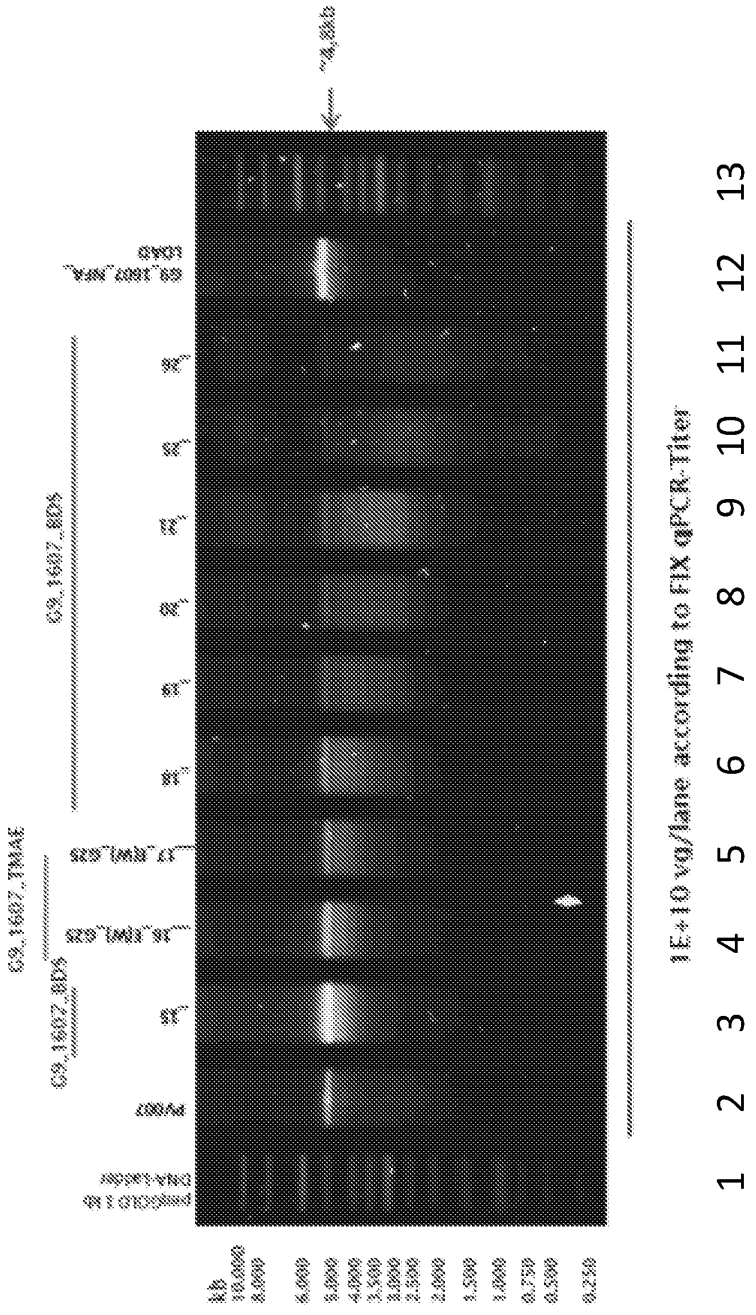

~4.8kb
C9_1607_BDS
C9_1607_TMAE
1E+10 vg/lane according to FIX qPCR-Titer
1 2 3 4 5 6 7 8 9 10 11 12 13

FIGURE 16A

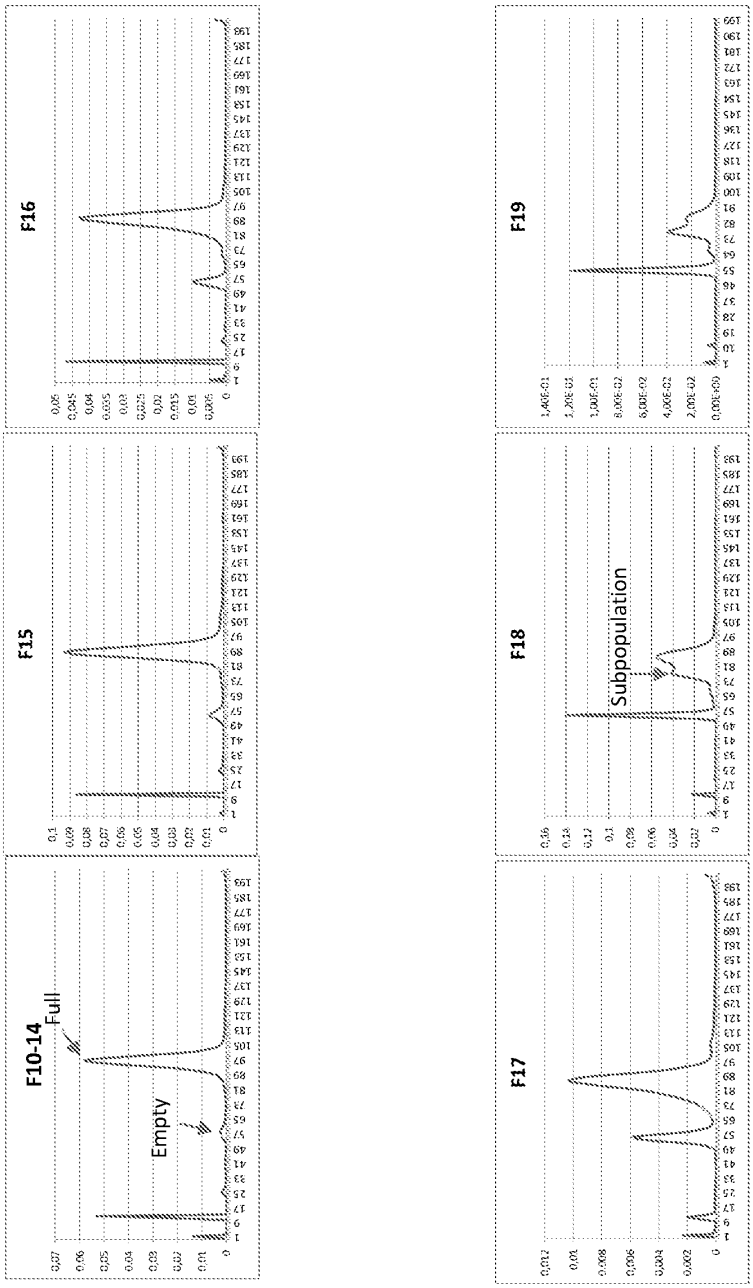


FIGURE 16B

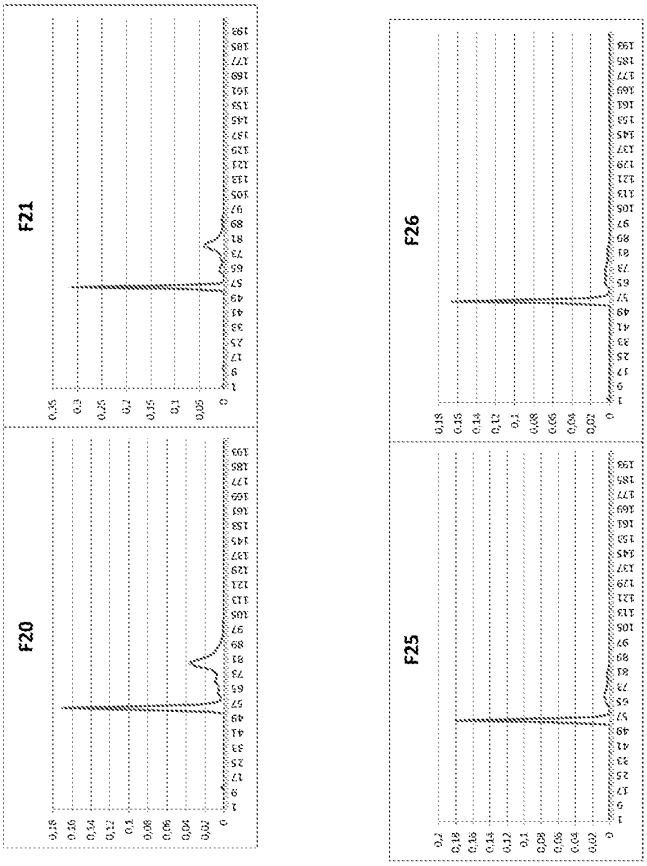


FIGURE 17

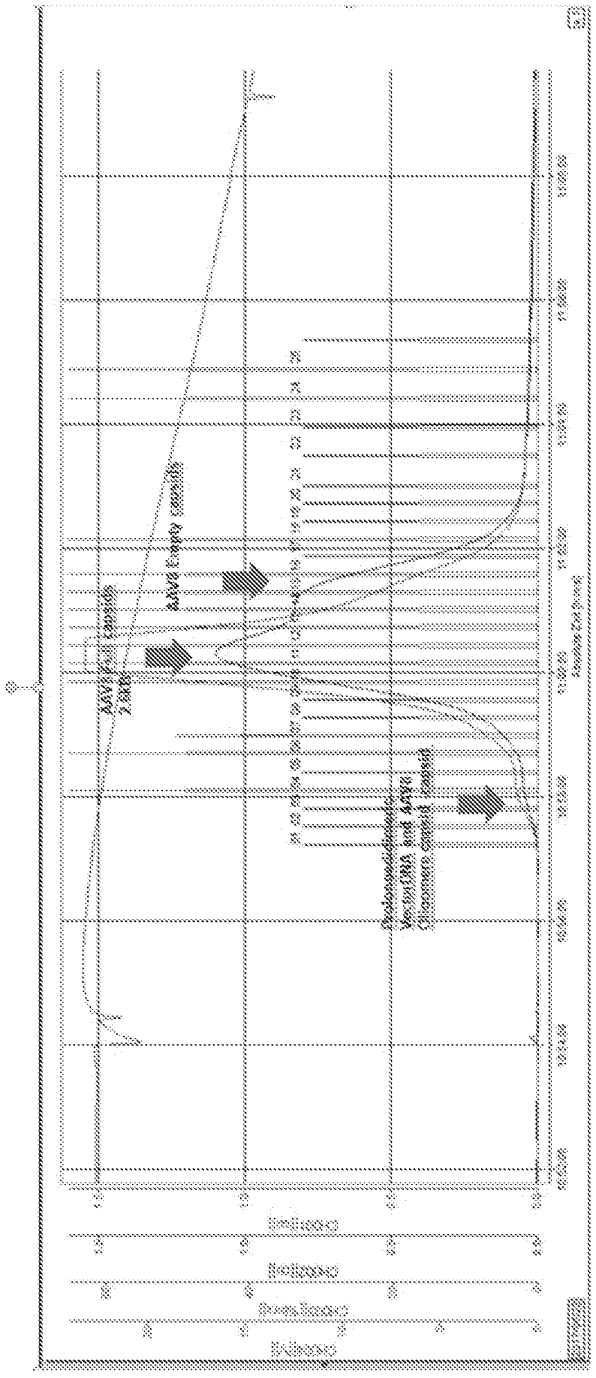


Figure 18

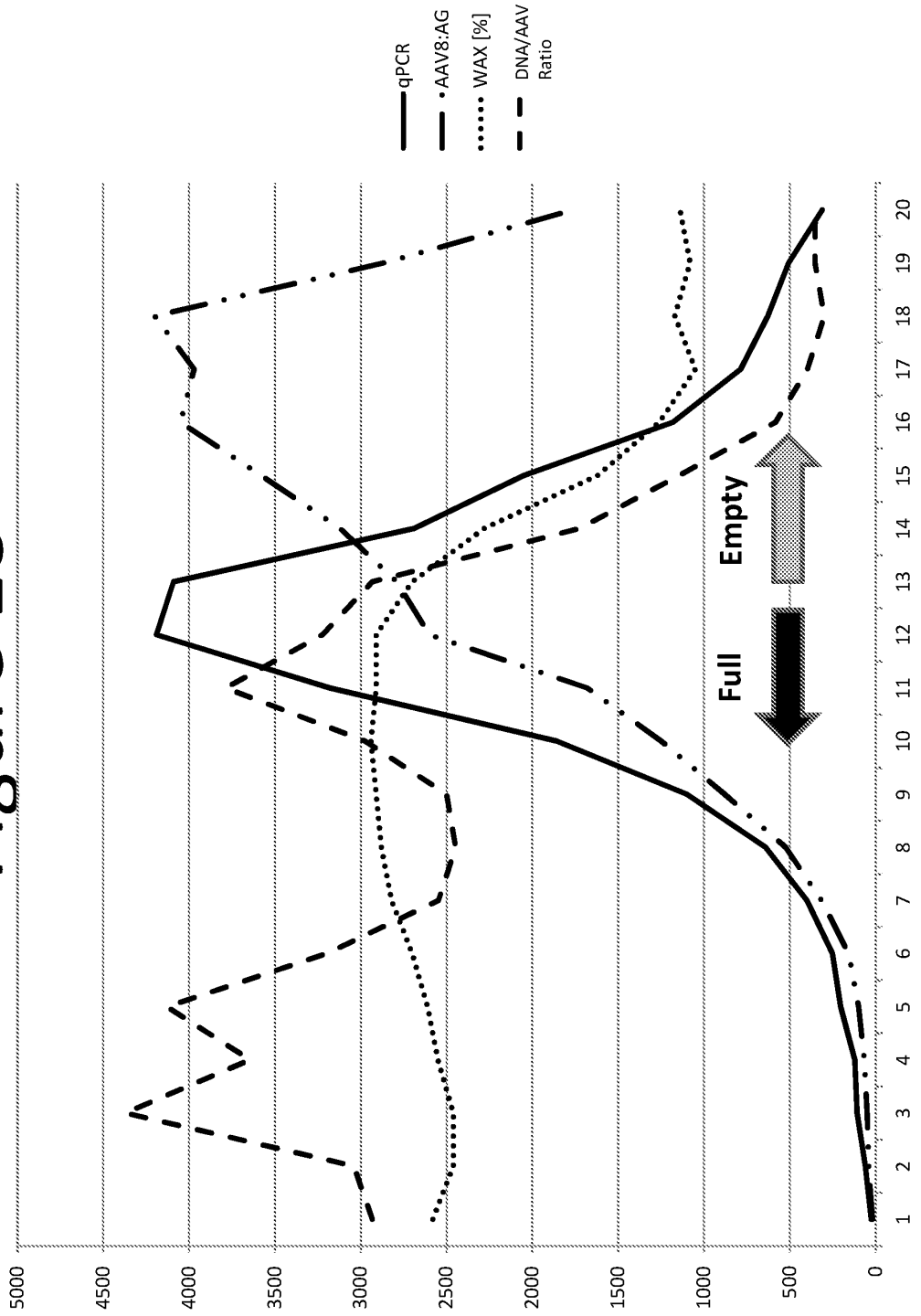

qPCR
AAV8:AG
WAX [%]
DNA/AAV Ratio
Empty
Full
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Figure 19

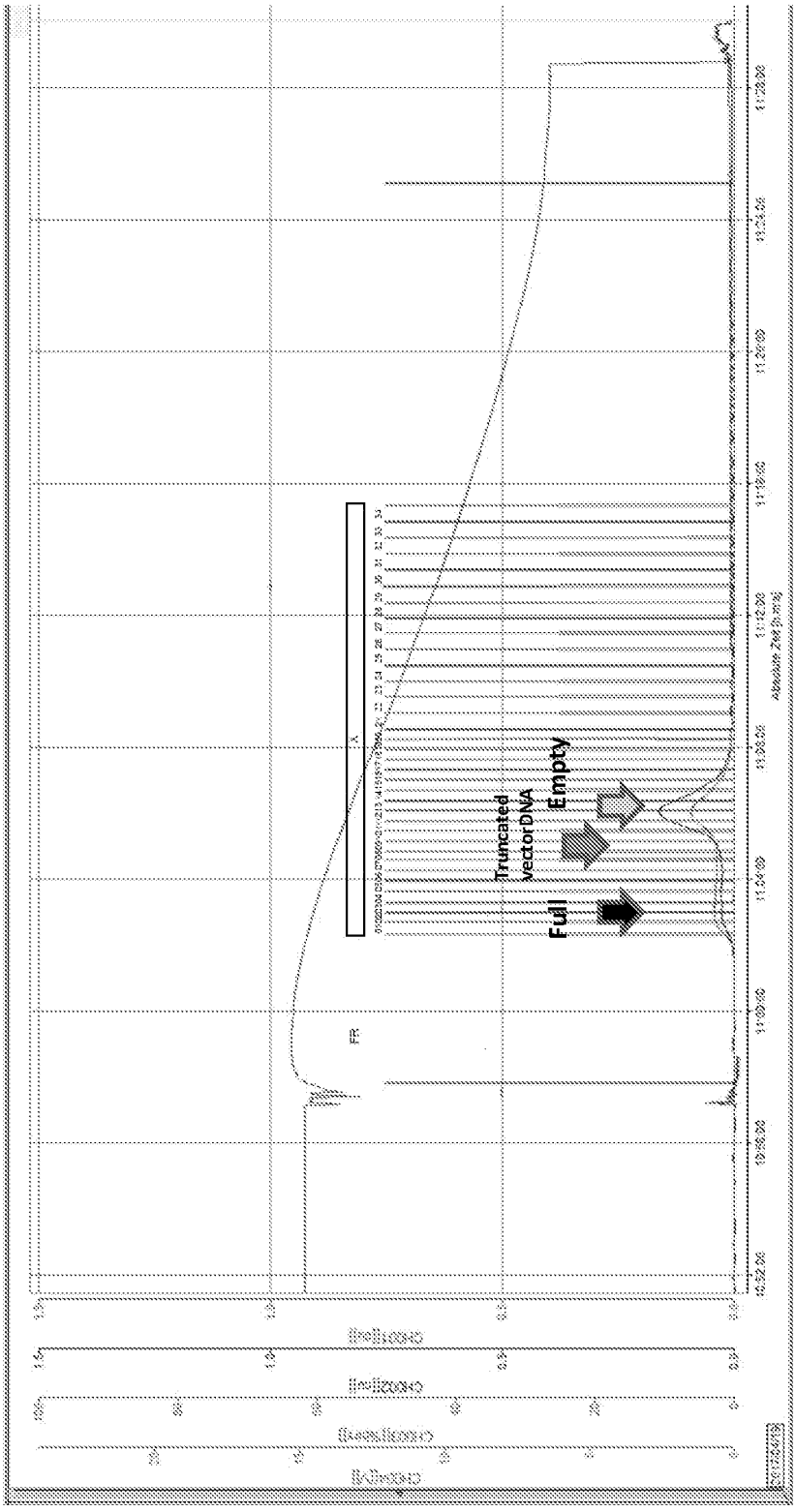


Figure 20

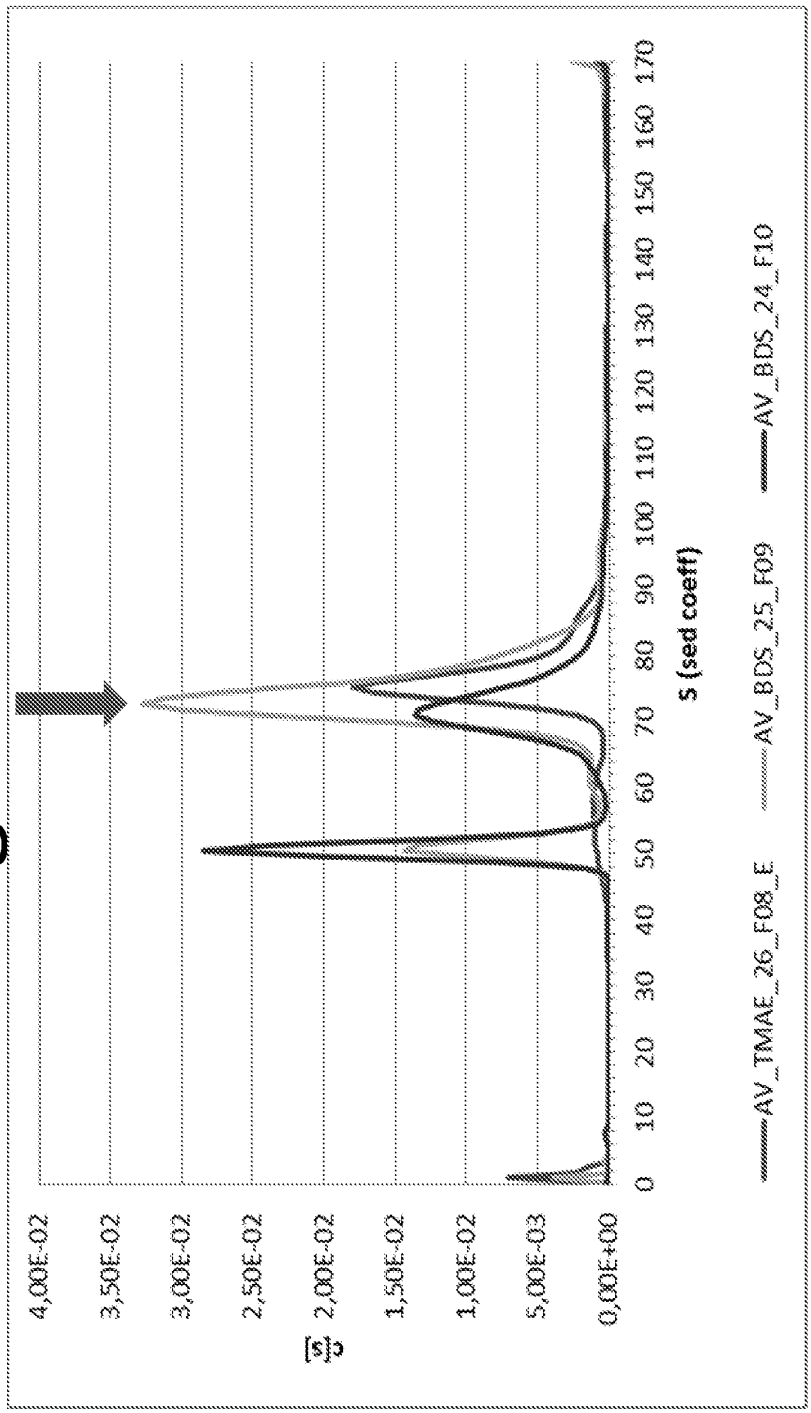

4,00E-02
3,50E-02
3,00E-02
2,50E-02
2,00E-02
1,50E-02
1,00E-02
5,00E-03
0,00E+00
c[s]
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
S (sed coeff)
AV_TMAE_26_F08_E
AV_BDS_25_F09
AV_BDS_24_F10

Figure 21

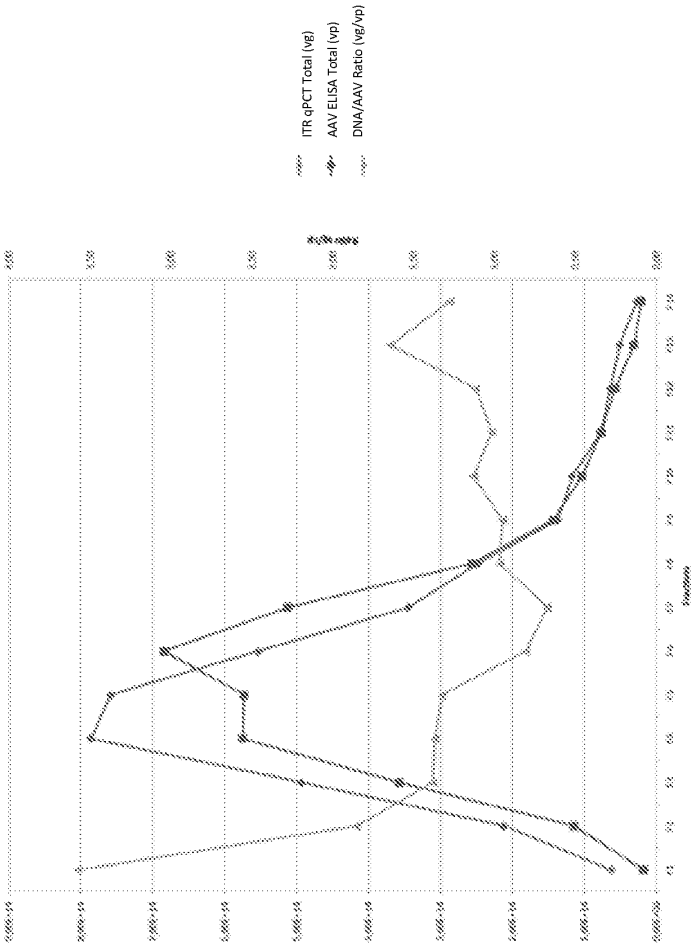


Figure 22

