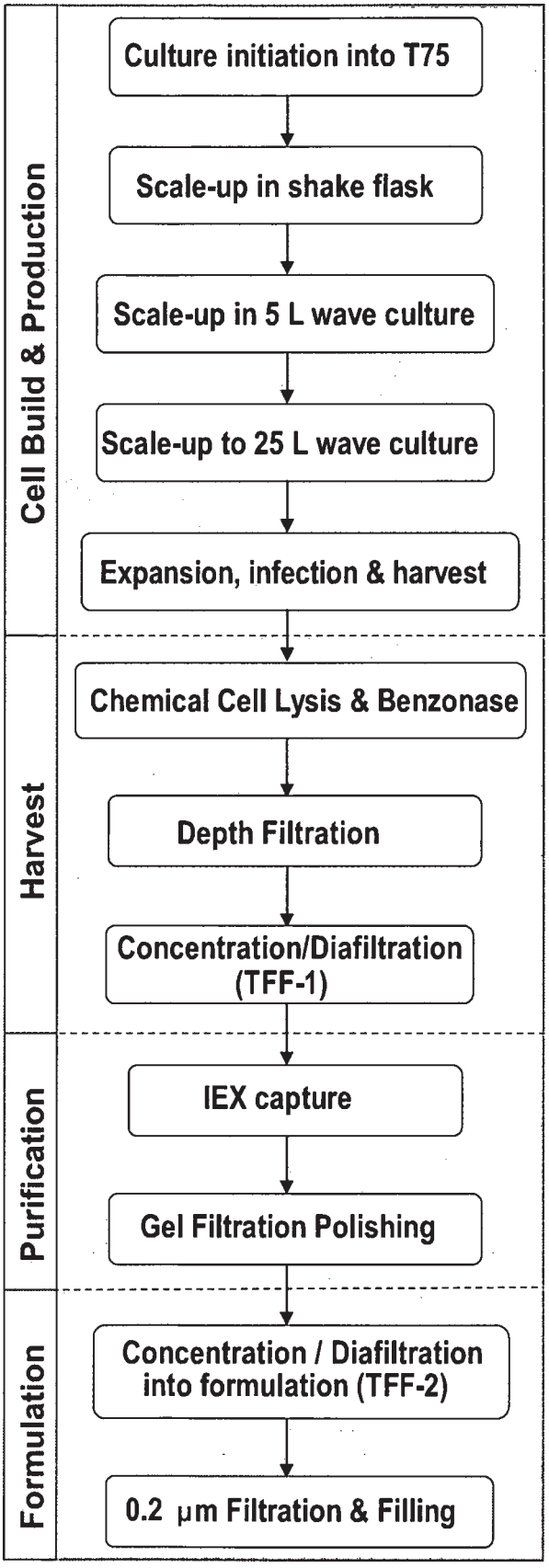
FIG. 1


Cell Build & Production
Culture initiation into T75
Scale-up in shake flask
Scale-up in 5 L wave culture
Scale-up to 25 L wave culture
Expansion, infection & harvest
Harvest
Chemical Cell Lysis & Benzonase
Depth Filtration
Concentration/Diafiltration (TFF-1)
Purification
IEX capture
Gel Filtration Polishing
Formulation
Concentration / Diafiltration into formulation (TFF-2)
0.2 µm Filtration & Filling

Adaptation summary flow-chart

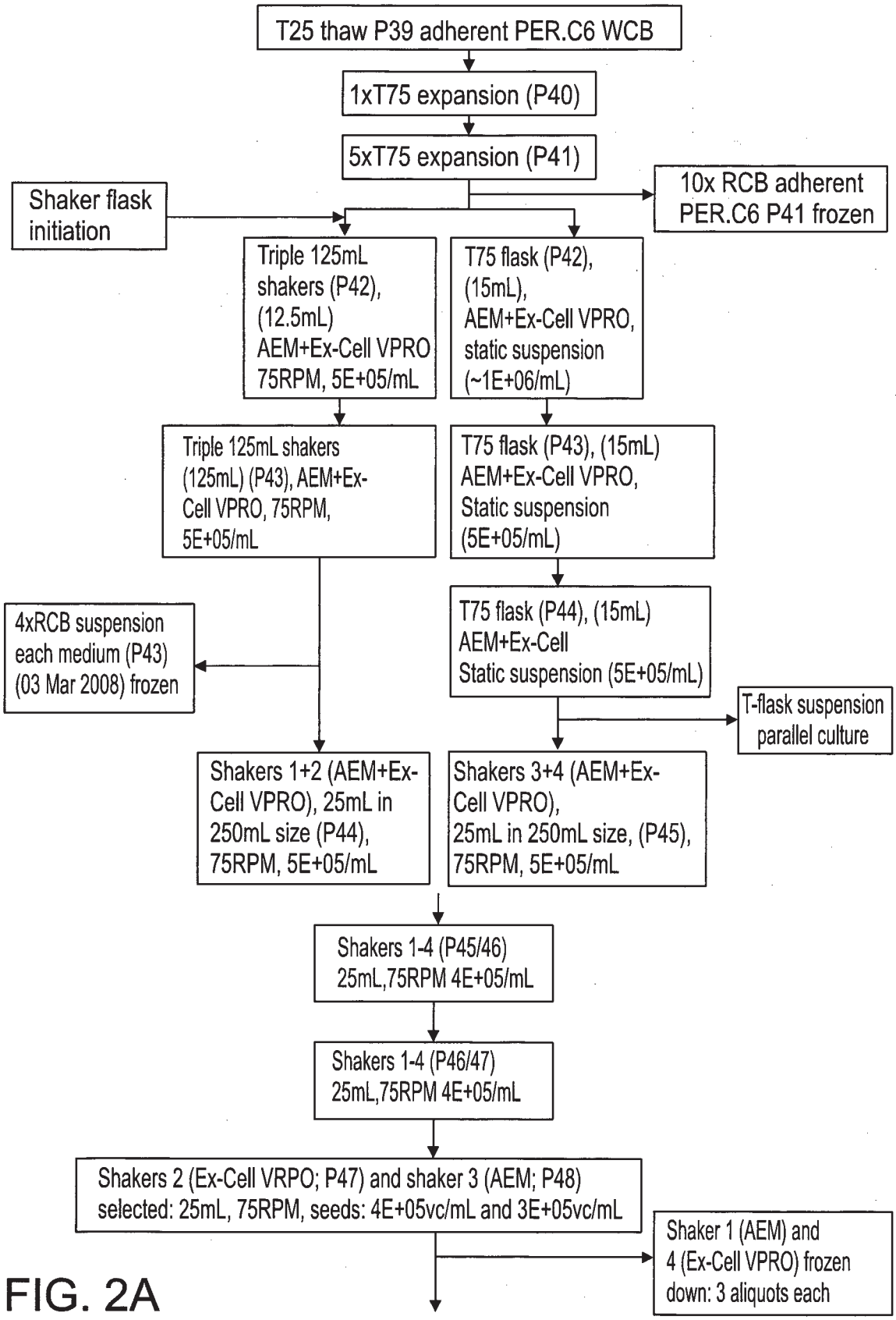


FIG. 2A

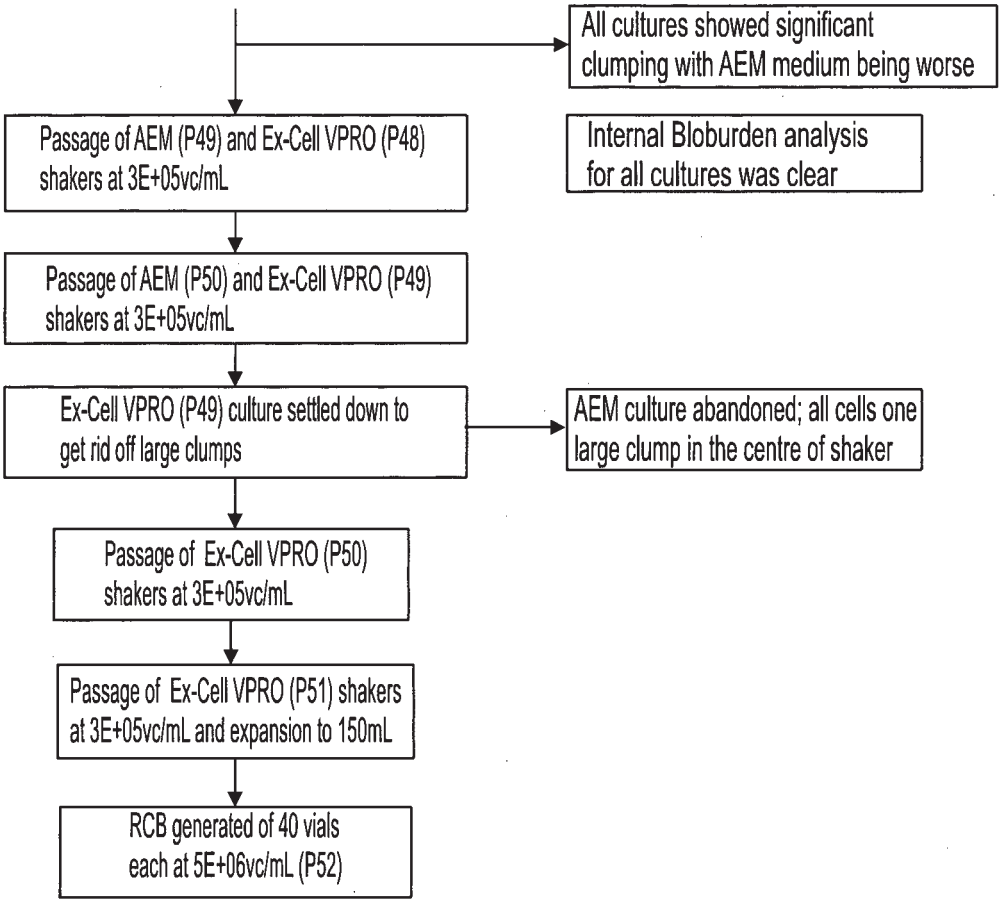

All cultures showed significant clumping with AEM medium being worse
Passage of AEM (P49) and Ex-Cell VPRO (P48) shakers at 3E+05vc/mL
Internal Bloburden analysis for all cultures was clear
Passage of AEM (P50) and Ex-Cell VPRO (P49) shakers at 3E+05vc/mL
Ex-Cell VPRO (P49) culture settled down to get rid off large clumps
AEM culture abandoned; all cells one large clump in the centre of shaker
Passage of Ex-Cell VPRO (P50) shakers at 3E+05vc/mL
Passage of Ex-Cell VPRO (P51) shakers at 3E+05vc/mL and expansion to 150mL
RCB generated of 40 vials each at 5E+06vc/mL (P52)

FIG. 2B

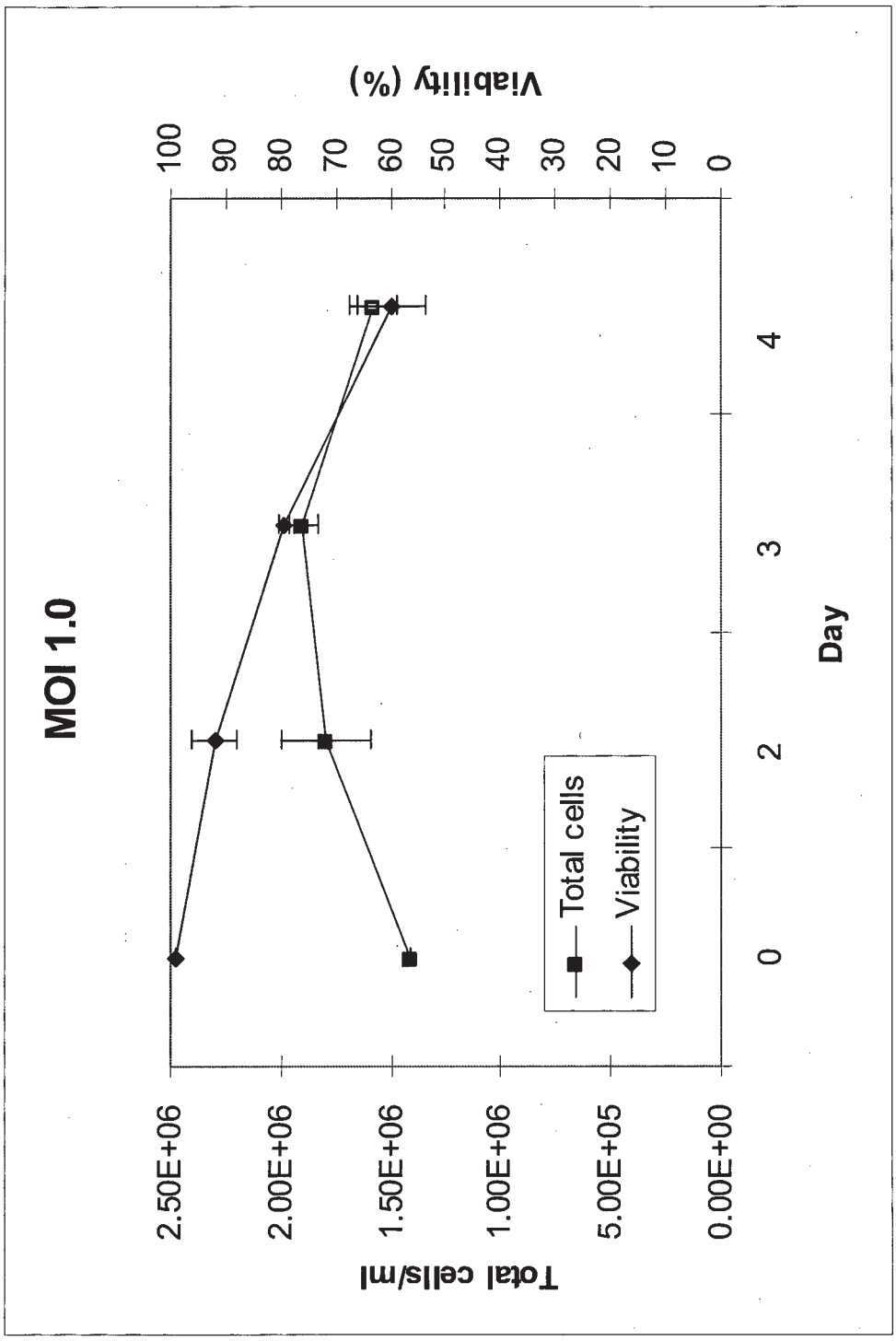

MOI 1.0
Viability (%)
100
90
80
70
60
50
40
30
20
10
0
Total cells/ml
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Day
0
2
3
4
Total cells
Viability

FIG. 3A

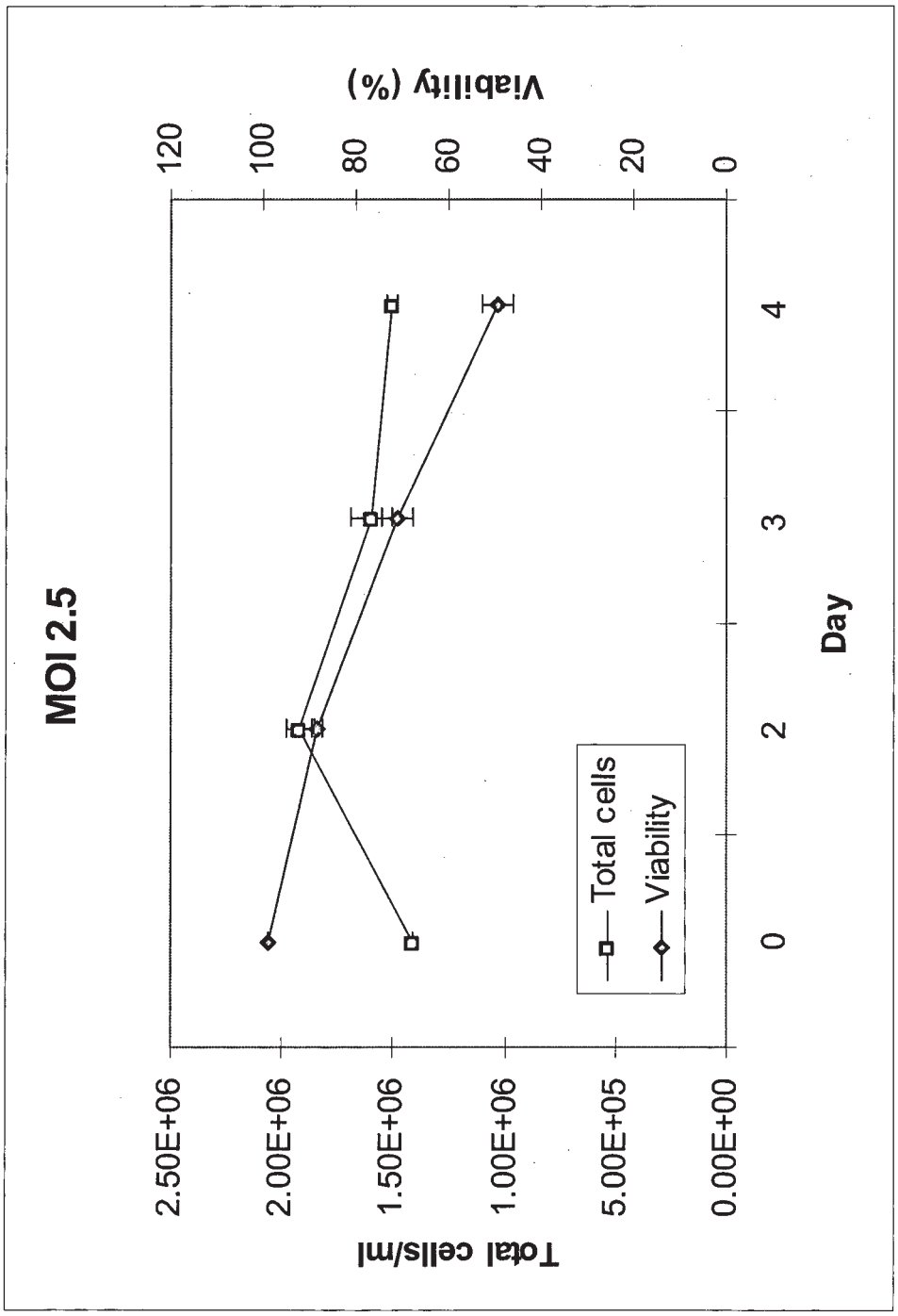

MOI 2.5
Viability (%)
120
100
80
60
40
20
0
Total cells/ml
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
0
2
3
4
Day
Total cells
Viability

FIG. 3B

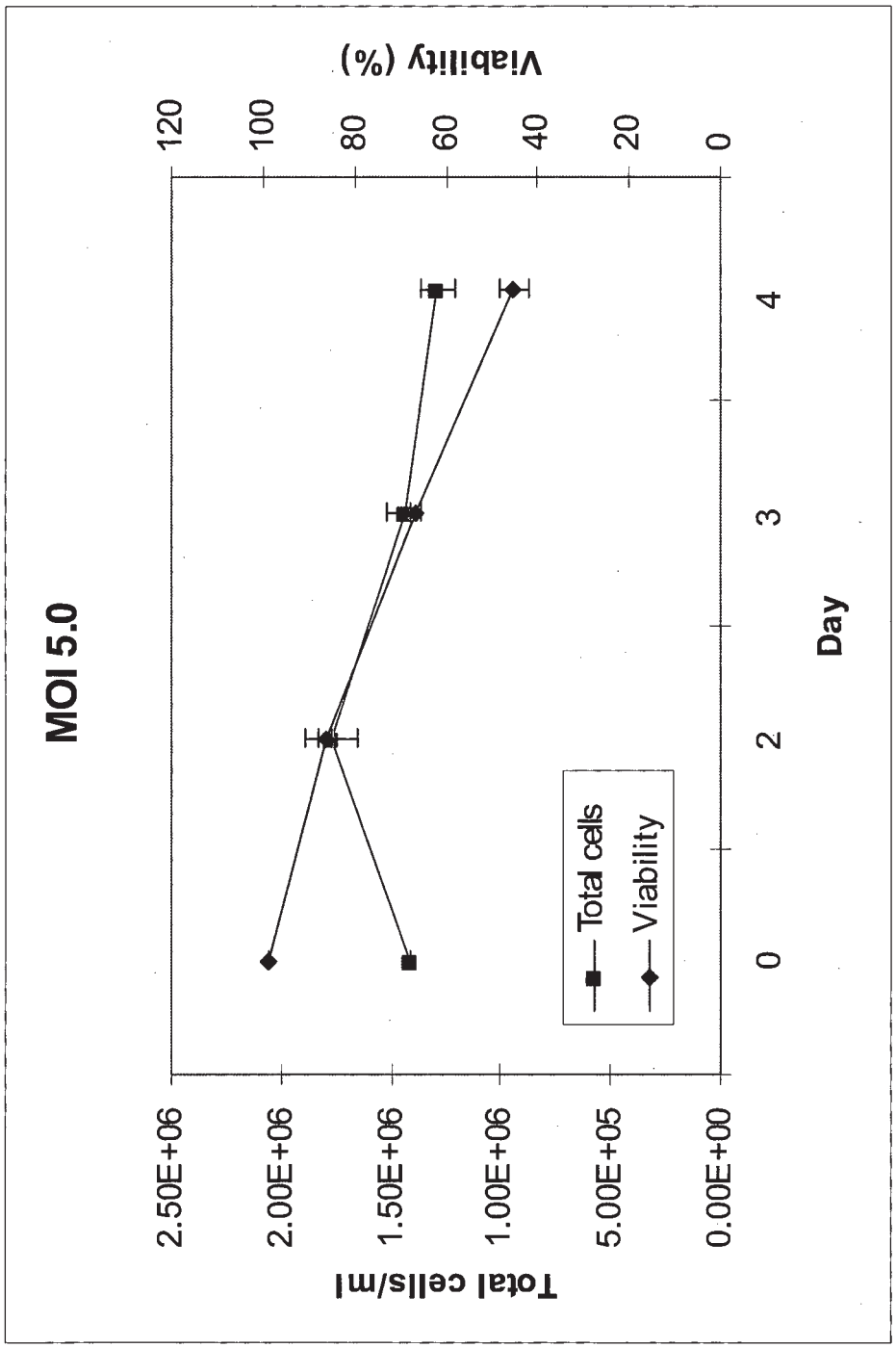

MOI 5.0
Viability (%)
120
100
80
60
40
20
0
Total cells/ml
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Day
0
2
3
4
Total cells
Viability

FIG. 3C

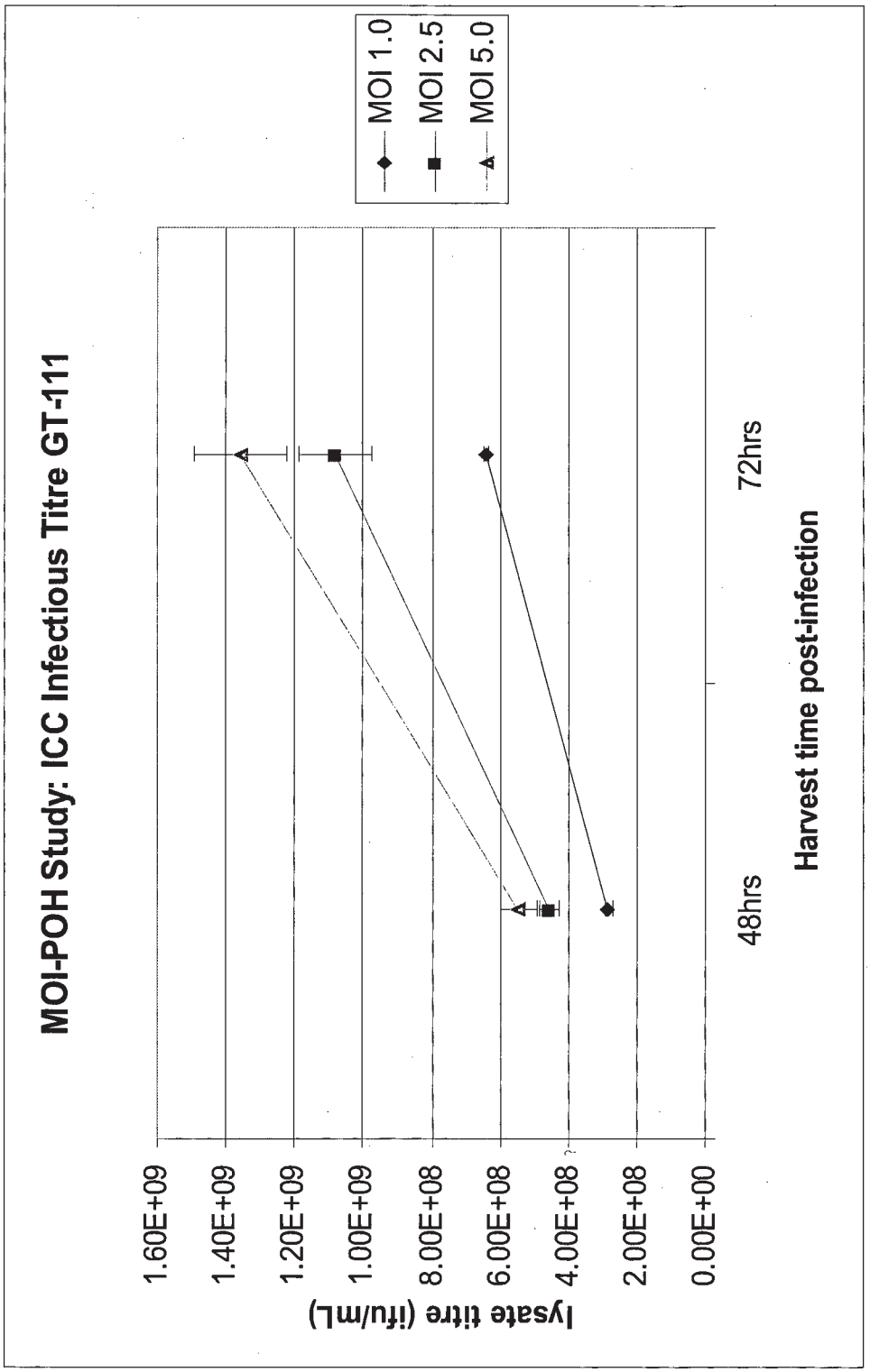

MOI-POH Study: ICC Infectious Titre GT-111
MOI 1.0
MOI 2.5
MOI 5.0
lysate titre (ifu/mL)
1.60E+09
1.40E+09
1.20E+09
1.00E+09
8.00E+08
6.00E+08
4.00E+08
2.00E+08
0.00E+00
48hrs
72hrs
Harvest time post-infection

FIG. 4

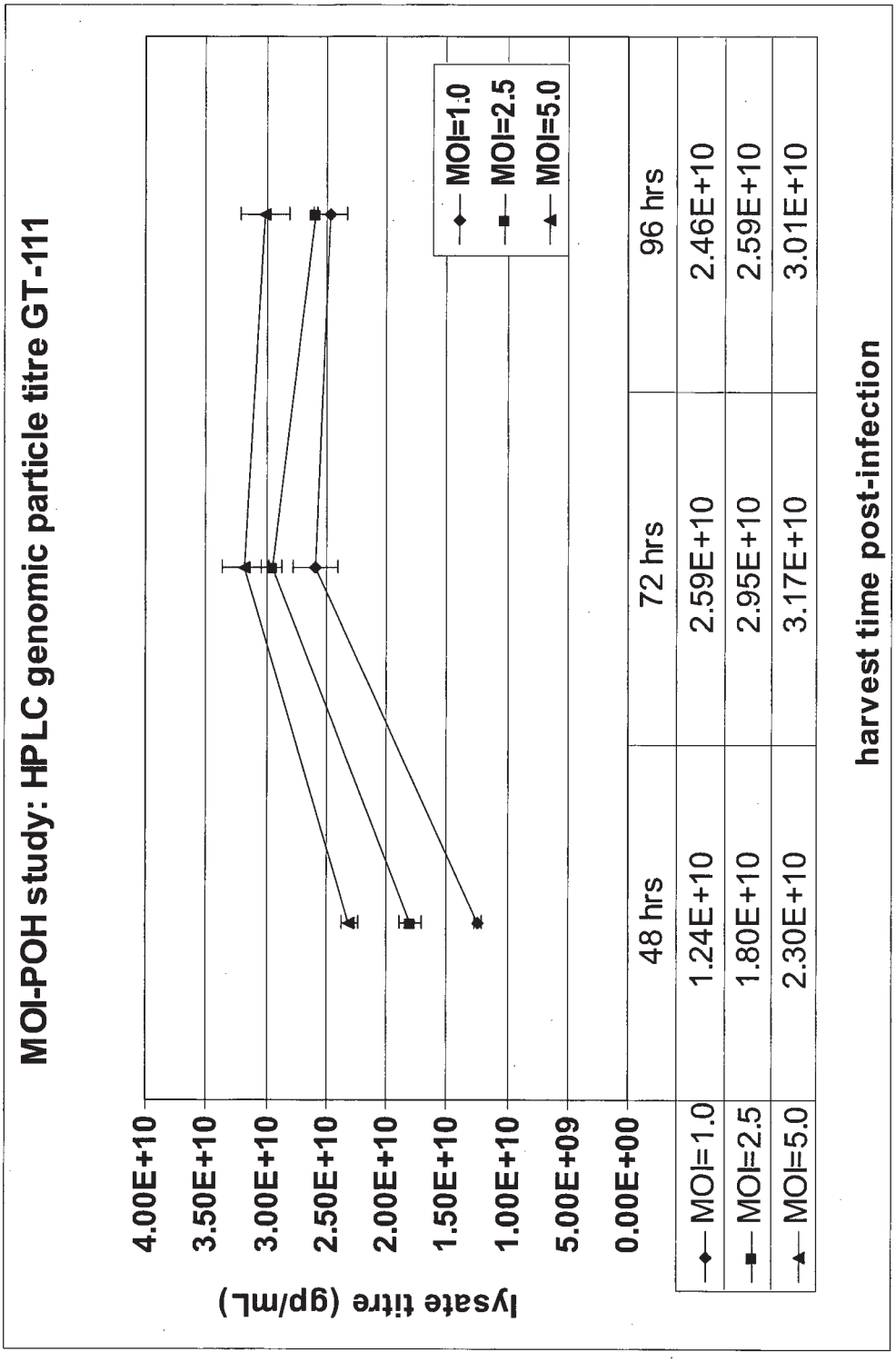

MOI-POH study: HPLC genomic particle titre GT-111
lysate titre (gp/mL)
4.00E+10
3.50E+10
3.00E+10
2.50E+10
2.00E+10
1.50E+10
1.00E+10
5.00E+09
0.00E+00
MOI=1.0
MOI=2.5
MOI=5.0
48 hrs
72 hrs
96 hrs
MOI=1.0 1.24E+10 2.59E+10 2.46E+10
MOI=2.5 1.80E+10 2.95E+10 2.59E+10
MOI=5.0 2.30E+10 3.17E+10 3.01E+10
harvest time post-infection

FIG. 5

FIG. 6A - 5L Cultibag exhaustion growth

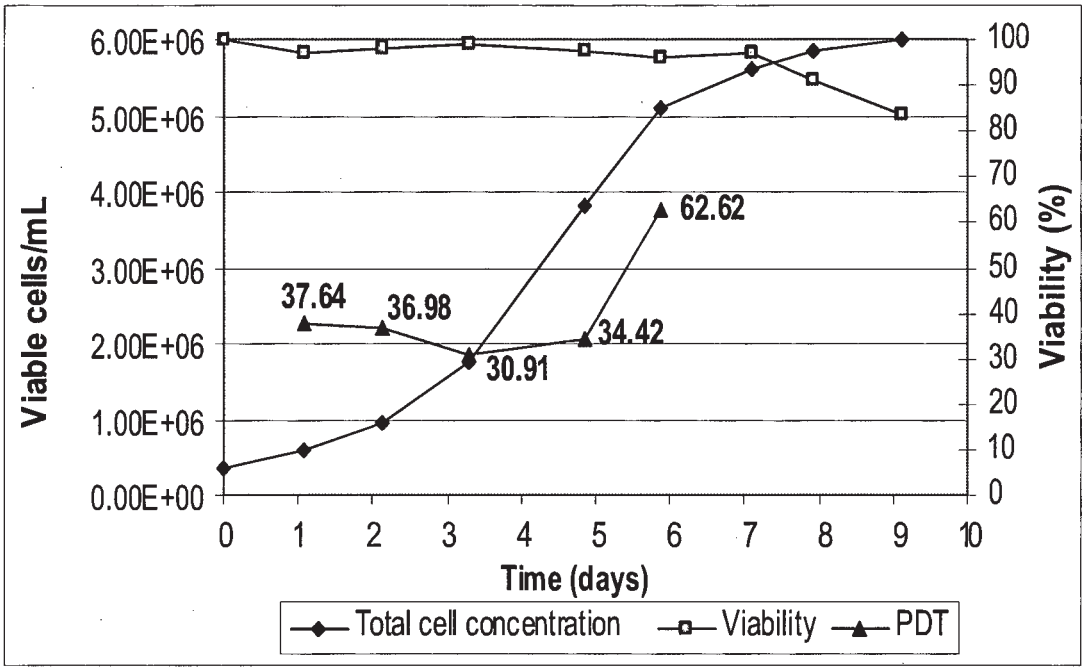
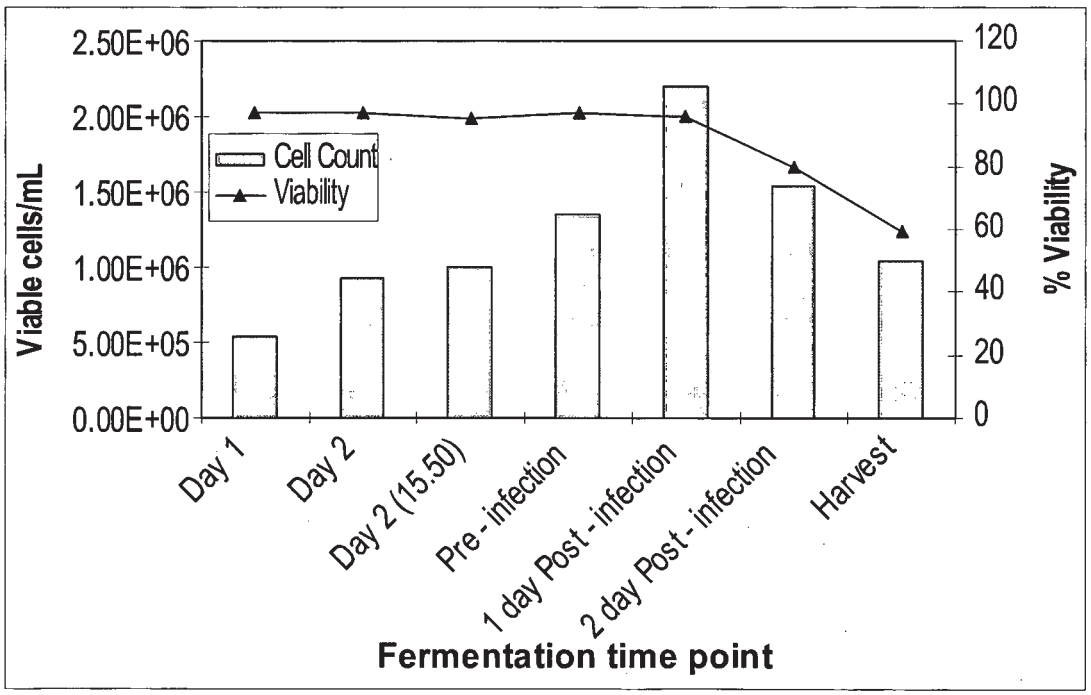


FIG. 6B - 25L Cultibag exhaustion growth



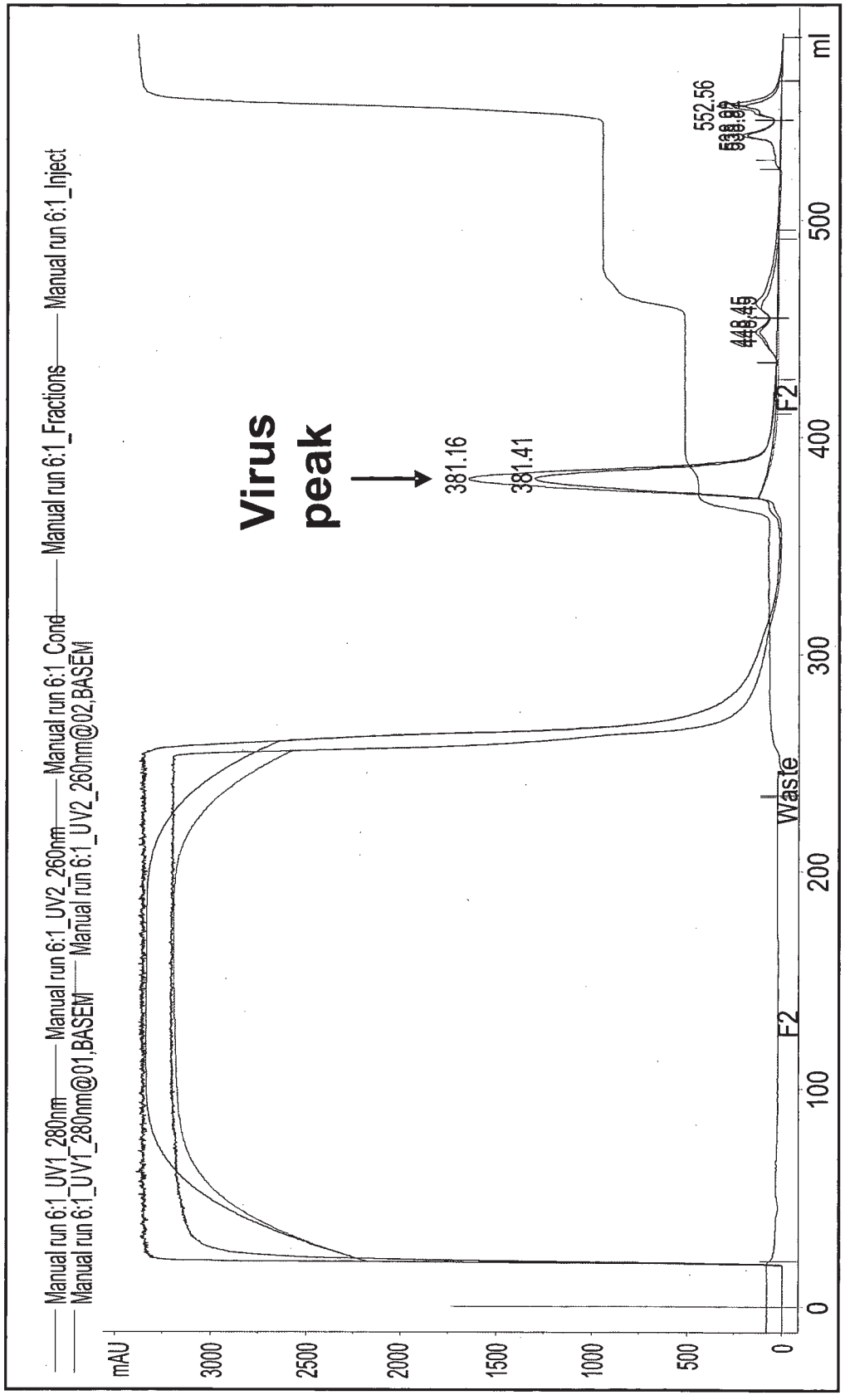

Manual run 6:1_UV1_280nm
Manual run 6:1_UV2_260nm
Manual run 6:1_Cond
Manual run 6:1_Fractions
Manual run 6:1_Inject
Manual run 6:1_UV1_280nm@01,BASEM
Manual run 6:1_UV2_260nm@02,BASEM
mAU
3000
2500
2000
1500
1000
500
0
0
100
200
300
400
500
ml
Virus peak
381.16
381.41
552.56
F2
F2
Waste

FIG. 7A

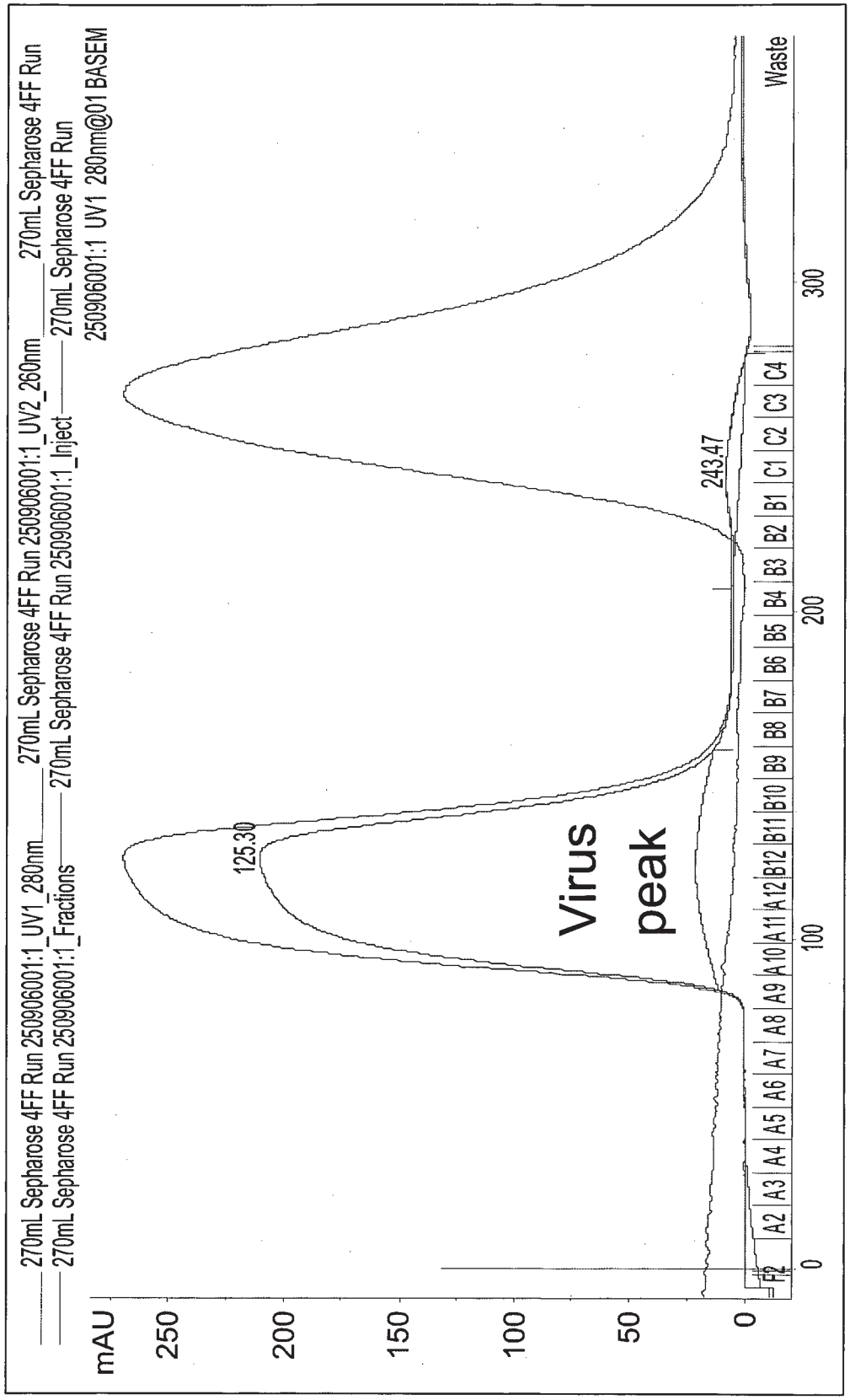

270mL Sepharose 4FF Run 250906001:1 UV1_280nm
270mL Sepharose 4FF Run 250906001:1 UV2_260nm
270mL Sepharose 4FF Run
270mL Sepharose 4FF Run 250906001:1_Fractions
270mL Sepharose 4FF Run 250906001:1_Inject
270mL Sepharose 4FF Run
250906001:1 UV1 280nm@01 BASEM
mAU
250
200
150
100
50
0
125.30
243.47
Virus
peak
F2
A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B12 B11 B10 B9 B8 B7 B6 B5 B4 B3 B2 B1 C1 C2 C3 C4
Waste
0
100
200
300

FIG. 7B

Lane	Sample
1	MW marker
2	Post Benzonase CVL
3	Post clarification
4	Post TFF(1)
5	Pre-IEX
6	Post IEX
7	Pre GPC
8	Post GPC
9	Post TFF(2) rinse
10	Drug Substance
11	TFF(2) rinse
12	CsCl purified reference material

FIG. 8

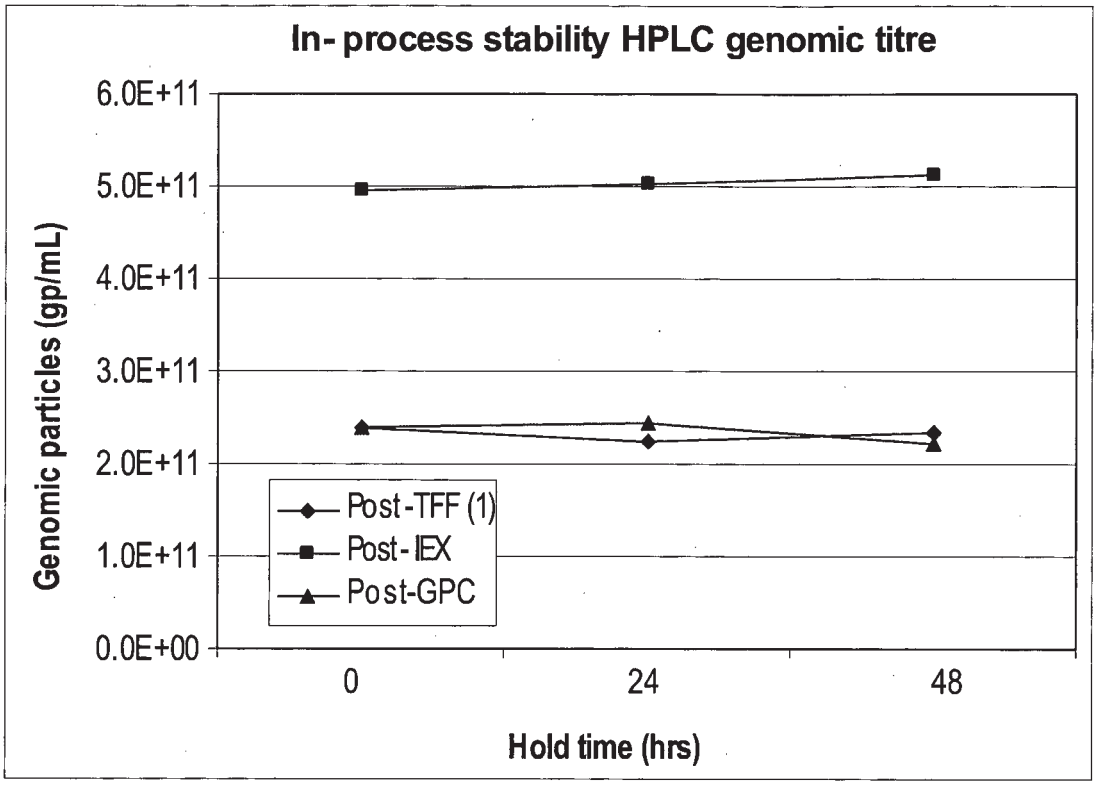

In- process stability HPLC genomic titre
6.0E+11
5.0E+11
4.0E+11
3.0E+11
2.0E+11
1.0E+11
0.0E+00
Genomic particles (gp/mL)
Post-TFF (1)
Post-IEX
Post-GPC
0
24
48
Hold time (hrs)

FIG. 9A

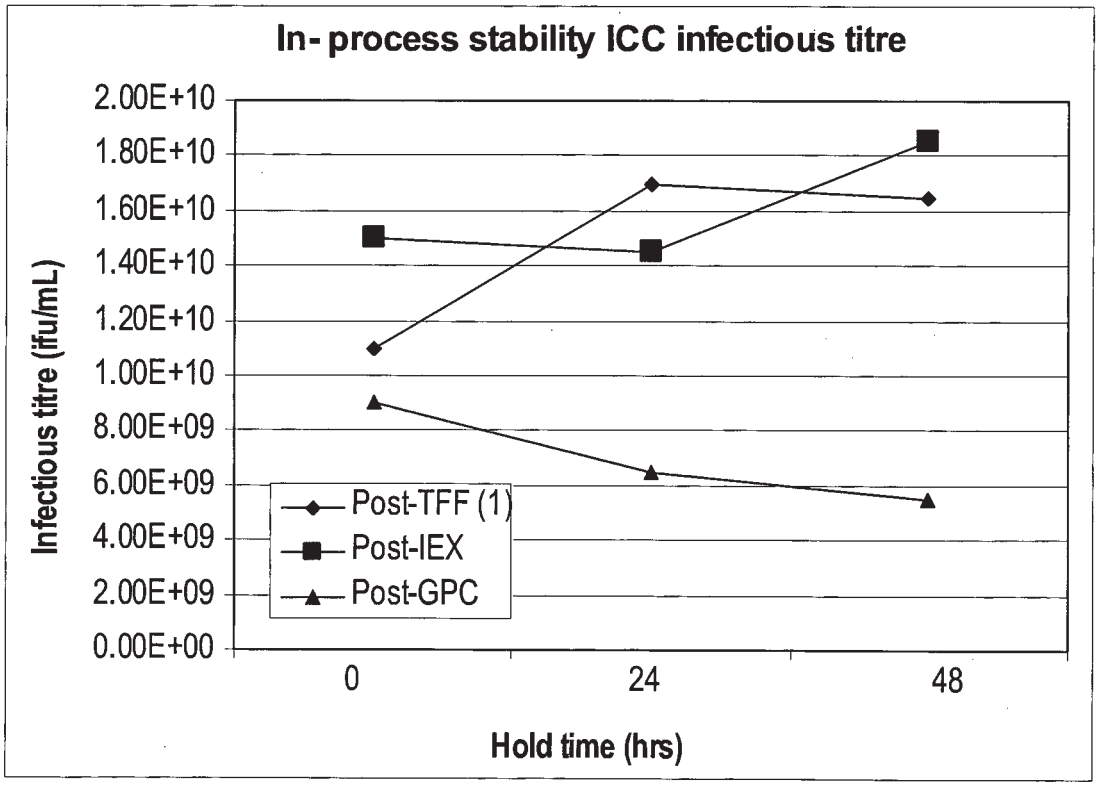

In- process stability ICC infectious titre
2.00E+10
1.80E+10
1.60E+10
1.40E+10
1.20E+10
1.00E+10
8.00E+09
6.00E+09
4.00E+09
2.00E+09
0.00E+00
Infectious titre (ifu/mL)
Post-TFF (1)
Post-IEX
Post-GPC
0
24
48
Hold time (hrs)

FIG. 9B

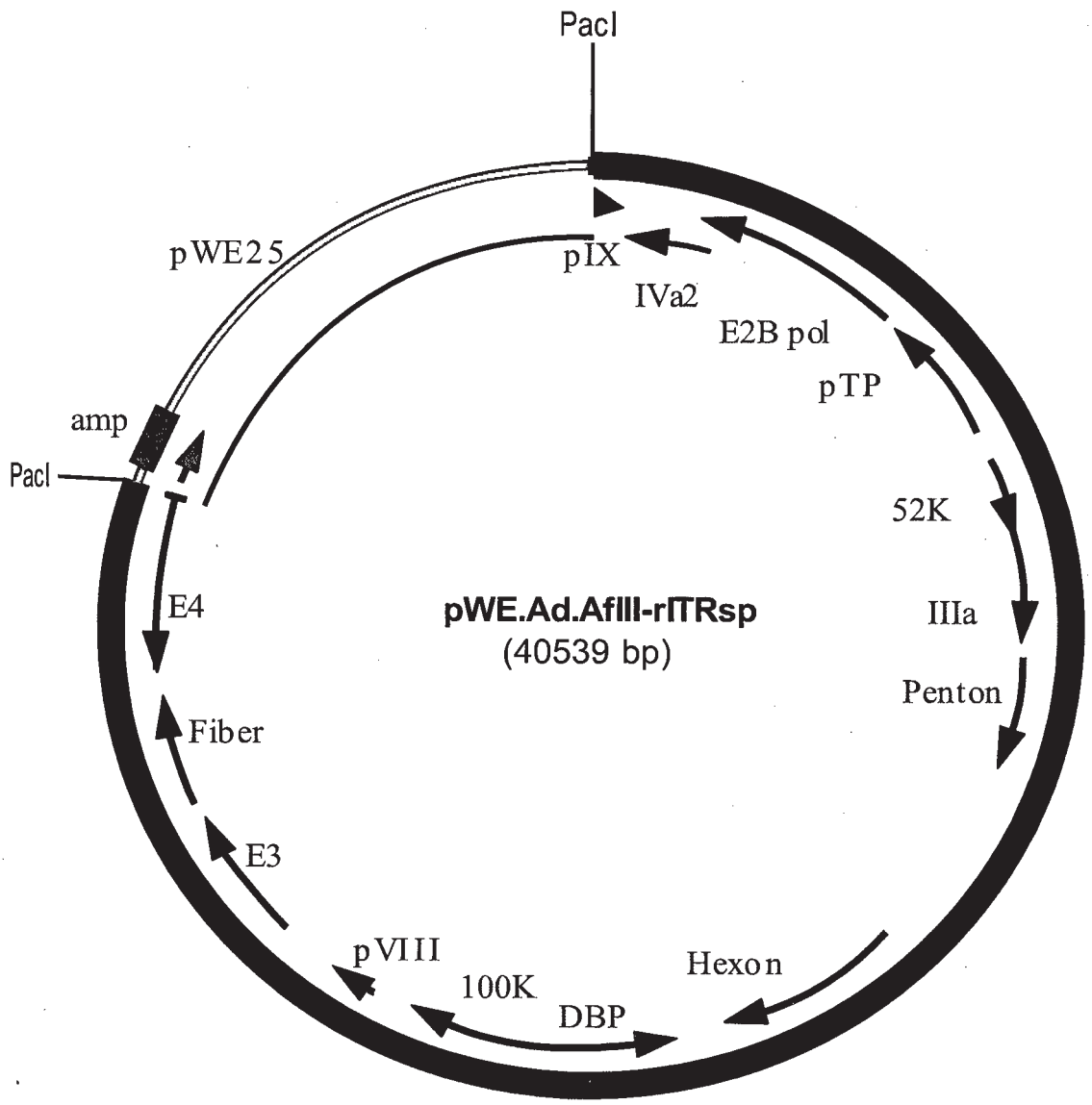

PacI
pWE25
pIX
IVa2
E2B pol
pTP
amp
PacI
52K
E4
pWE.Ad.AflII-rITRsp
(40539 bp)
IIIa
Penton
Fiber
E3
pVIII
100K
Hexon
DBP

FIG. 10

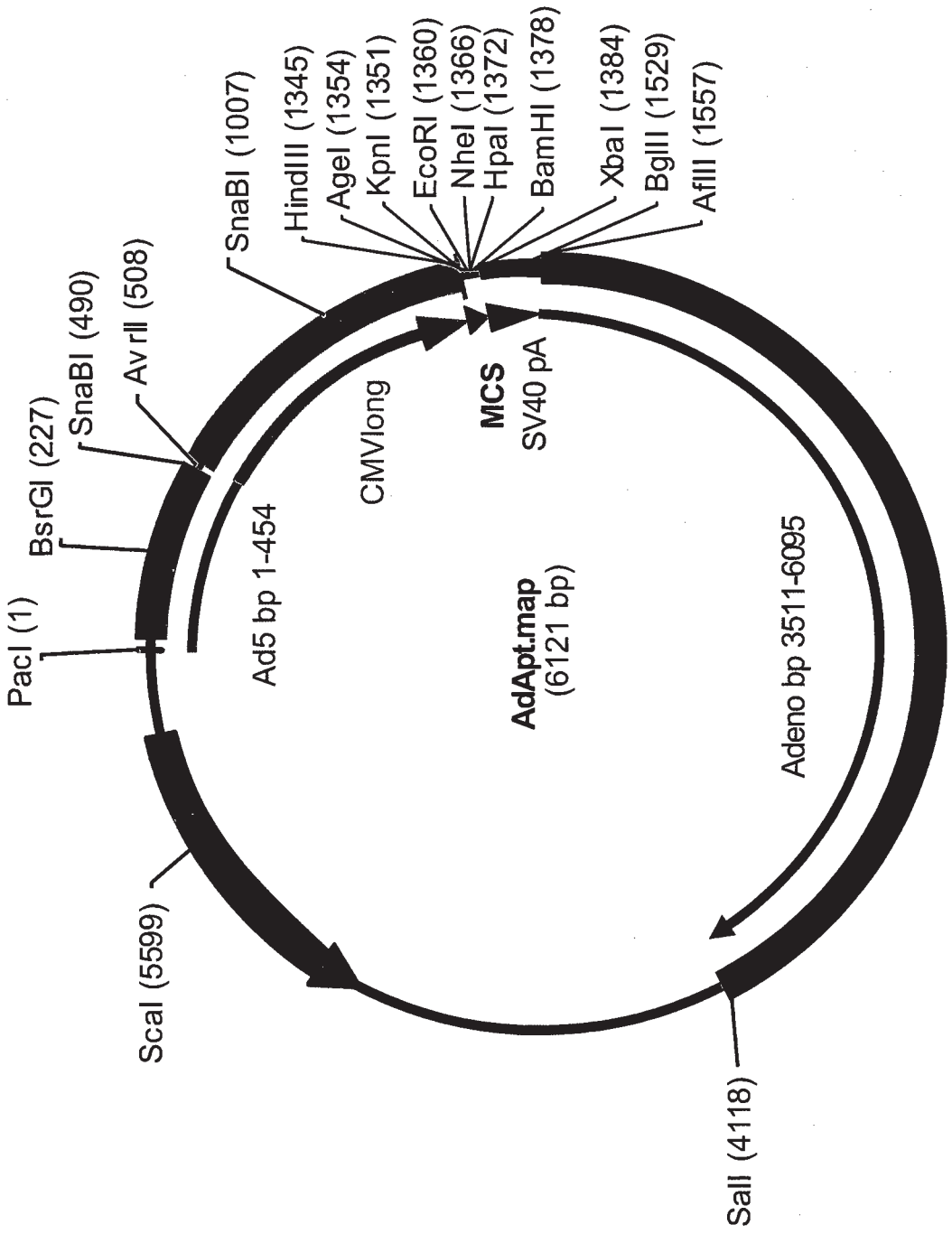

PacI (1)
BsrGI (227)
SnaBI (490)
AvrII (508)
SnaBI (1007)
HindIII (1345)
AgeI (1354)
KpnI (1351)
EcoRI (1360)
NheI (1366)
HpaI (1372)
BamHI (1378)
XbaI (1384)
BglII (1529)
AflII (1557)
Ad5 bp 1-454
CMVlong
MCS
SV40 pA
AdApt.map
(6121 bp)
Adeno bp 3511-6095
ScaI (5599)
SalI (4118)

FIG. 11

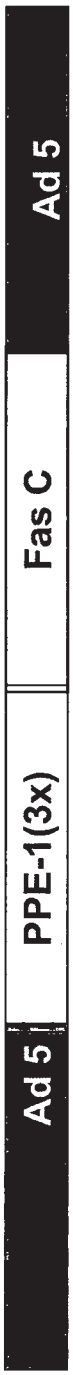


FIG. 12

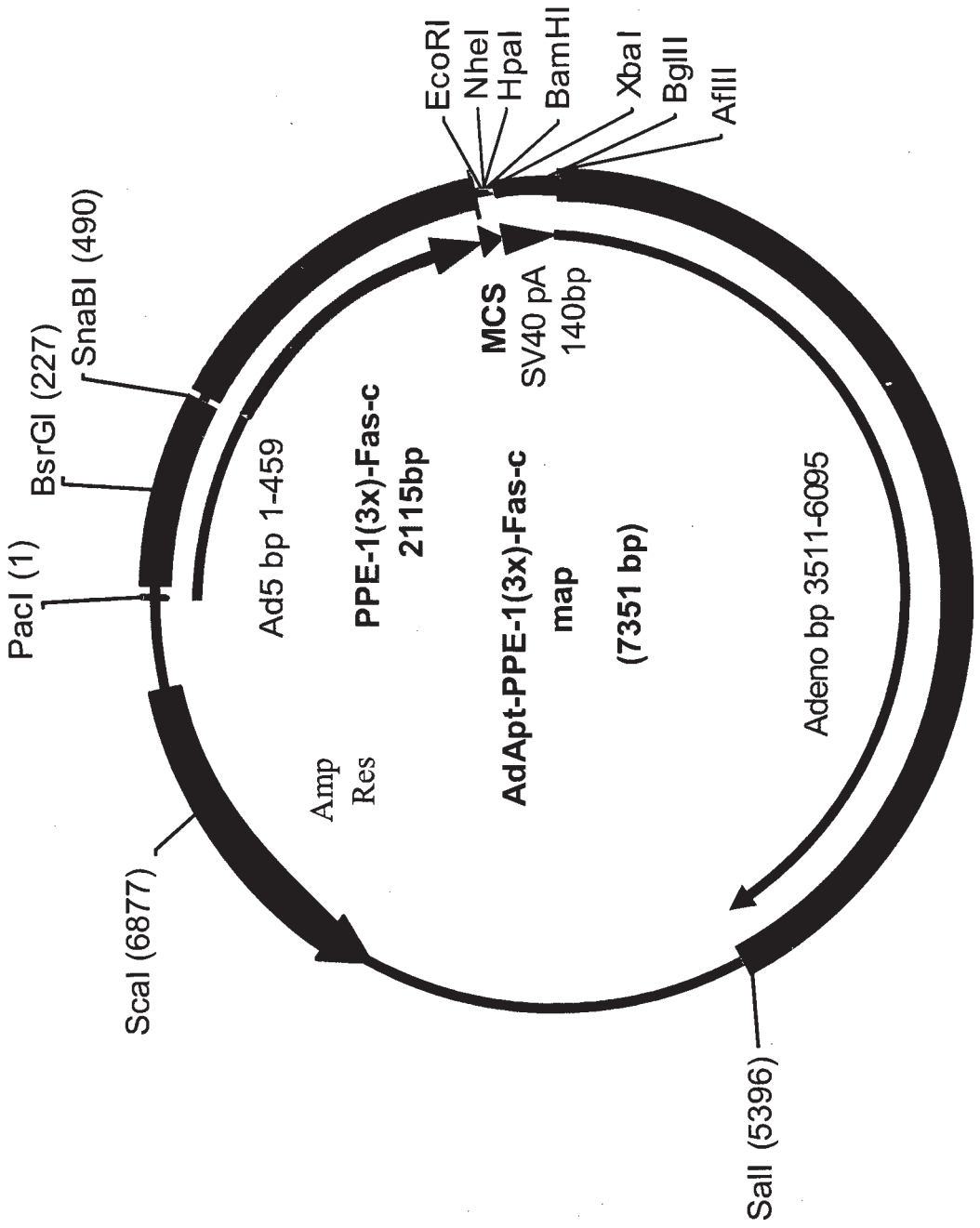
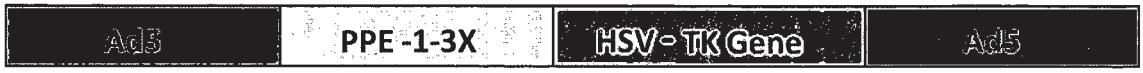

EcoRI
NheI
HpaI
BamHI
XbaI
BglII
AflII
SnaBI (490)
BsrGI (227)
PacI (1)
ScaI (6877)
SalI (5396)
MCS
SV40 pA
140bp
Ad5 bp 1-459
PPE-1(3x)-Fas-c
2115bp
AdApt-PPE-1(3x)-Fas-c
map
(7351 bp)
Amp
Res
Adeno bp 3511-6095

FIG. 13



AdPPE-1(3X)-TK construct

FIG. 14



CRAd construct

FIG. 15