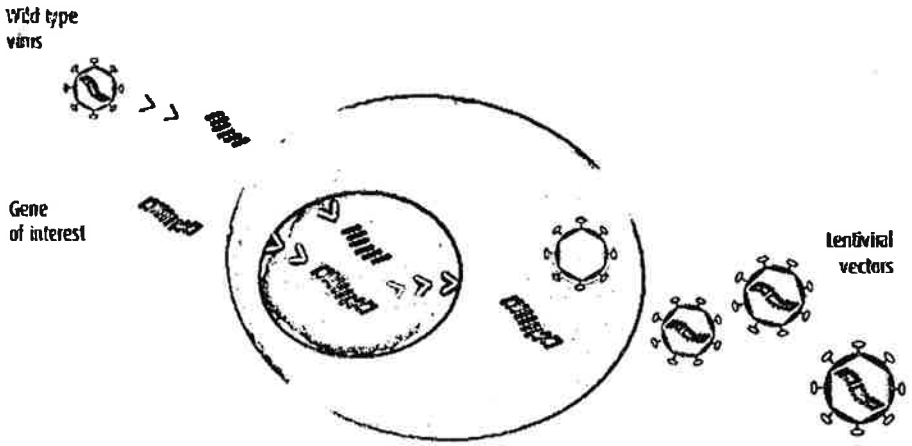
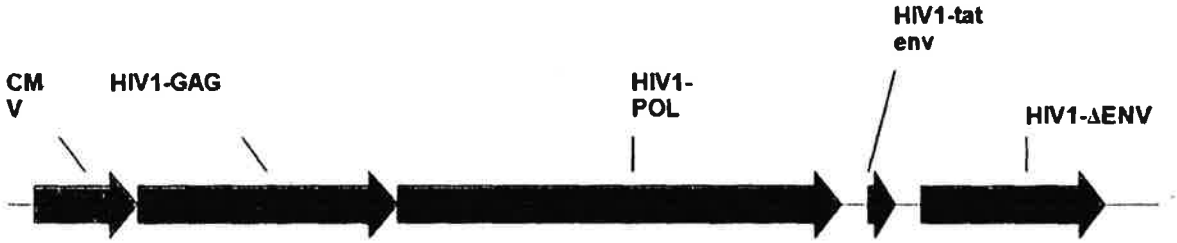


Figure 1



pGag Pol

FEATURES	Length (bp)	
promoter	602	/label=CMV
CDS	1502	/label=HIV1_gag
CDS	2737	/label=HIV1-pol
CDS	1173	/label=HIV1-env deleted
protein_bind	204	/label=HIV1_RRE

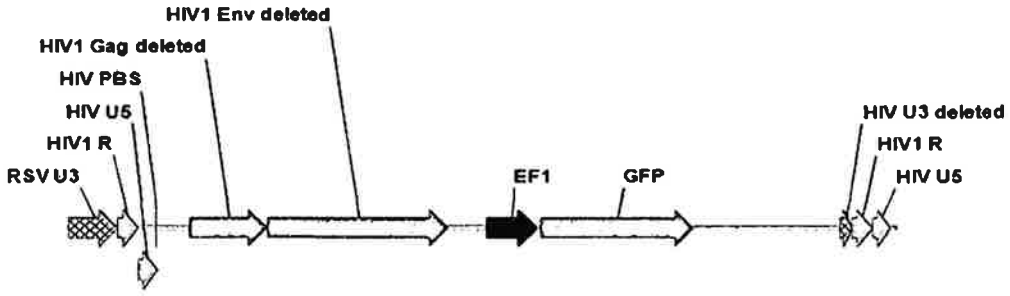
Figure 2A



pEnv

FEATURES	Length (bp)	
Promoter	677	/label=CMV
CDS	1642	/label=VSV_glycoprotein_G
polyA_signal	768	/label=HBB2

Figure 2B



pLV.EF1-GFP

Transgene expression plasmid without cDNA

FEATURES		Length (bp)
repeat_region	230	/label=RSV_U3
Feature	119	/label= packaging signal
CDS	362	/label=HIV1_gag deleted
Promoter	236	/label=EF1_promoter
CDS	719	/label=EGFP
Feature	18	/label=Primer Binding Site PBS
repeat_unit	55	/label=HIV_U3 deleted
repeat_unit	95	/label=HIV_R
repeat_unit	85	/label=HIV_U5

Figure 2C

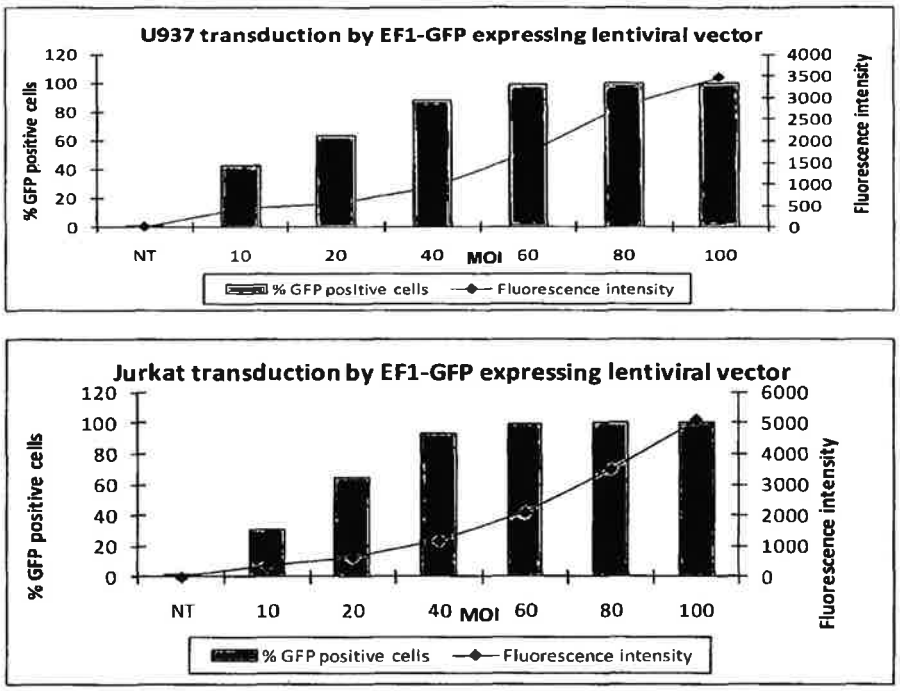


Figure 3A

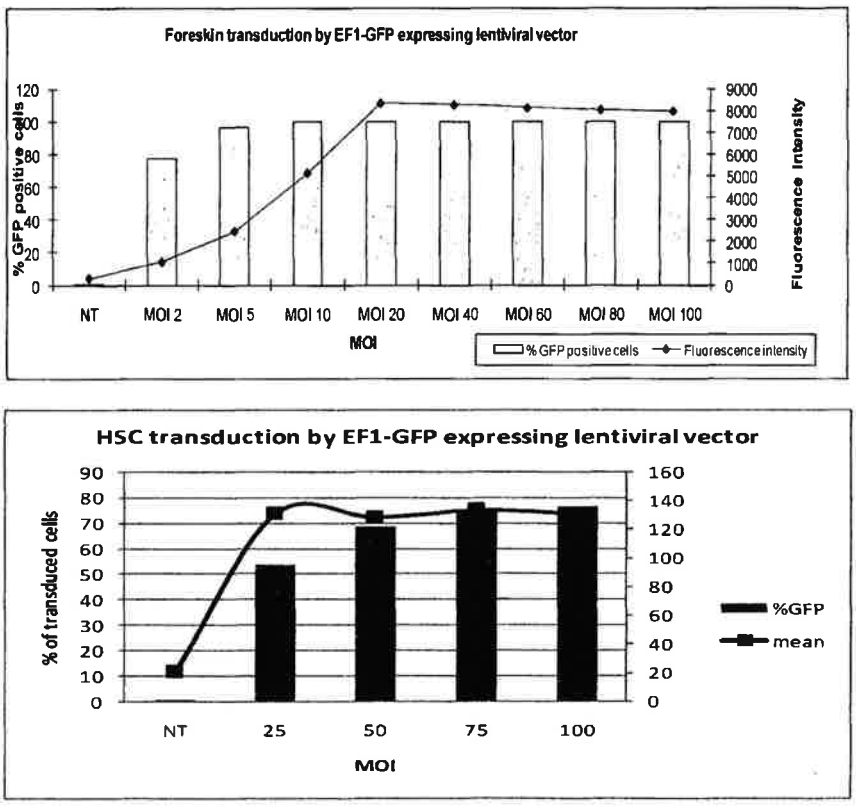


Figure 3B

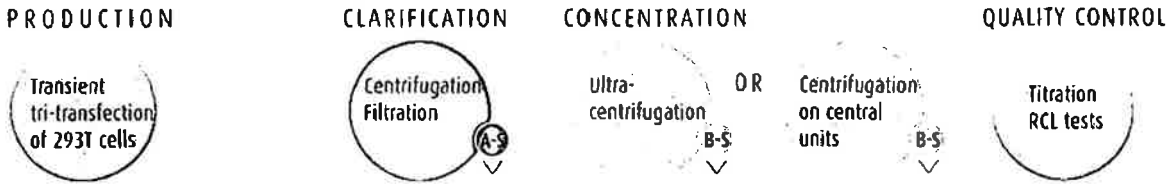


Figure 4A

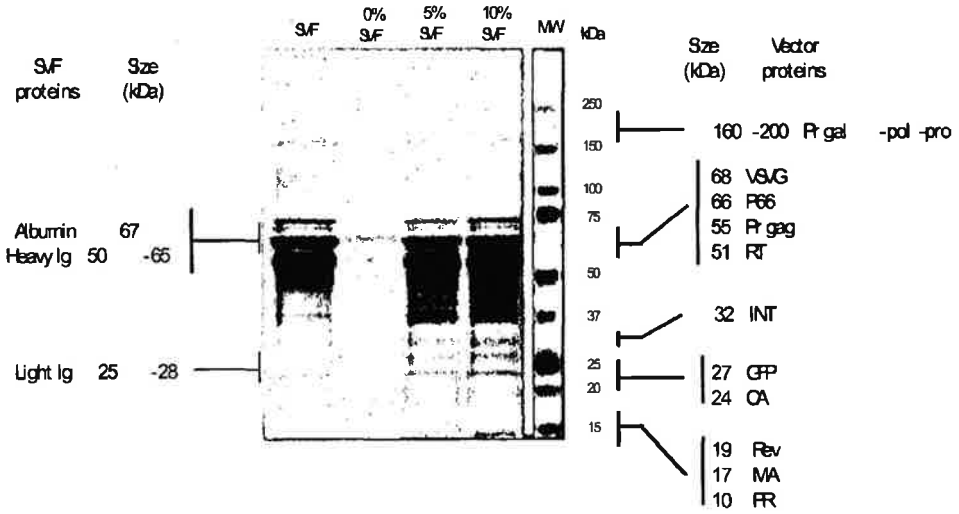


Figure 4B

% SVF	Titer (TU/mL)(1)	PP/TU (2)	TU/tot prot (TU/mg) (3)
10%	**1,20E+06**	192	**3,90E+05**
5%	**8,00E+05**	263	**3,90E+05**
0%	**1,80E+06**	161	**1,90E+06**

Figure 4C

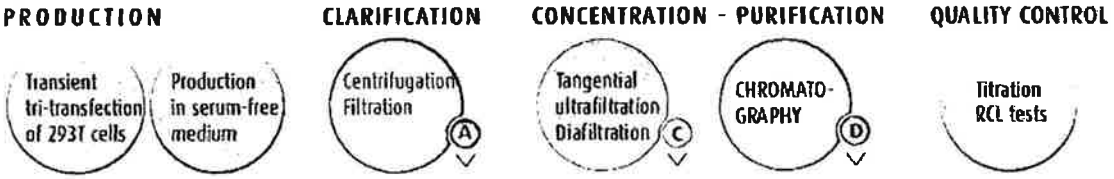


Figure 5A

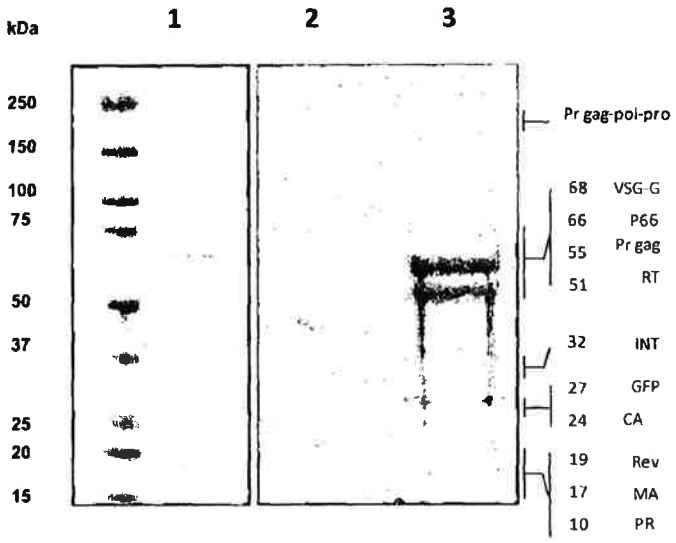


Figure 5B

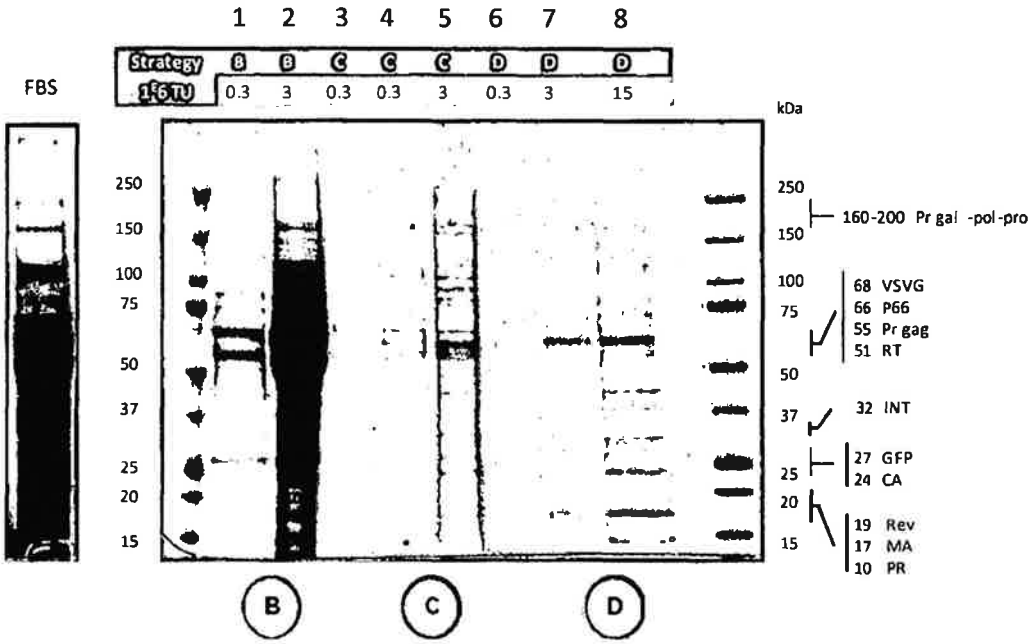

1 2 3 4 5 6 7 8
Strategy
1E5 TU
B B C C C D D D
0.3 3 0.3 0.3 3 0.3 3 15
FBS
kDa
250
150
100
75
50
37
25
20
15
160-200 Pr gal -pol-pro
68 VSVG
66 P66
55 Pr gag
51 RT
32 INT
27 GFP
24 CA
19 Rev
17 MA
10 PR
B
C
D

Figure 5C

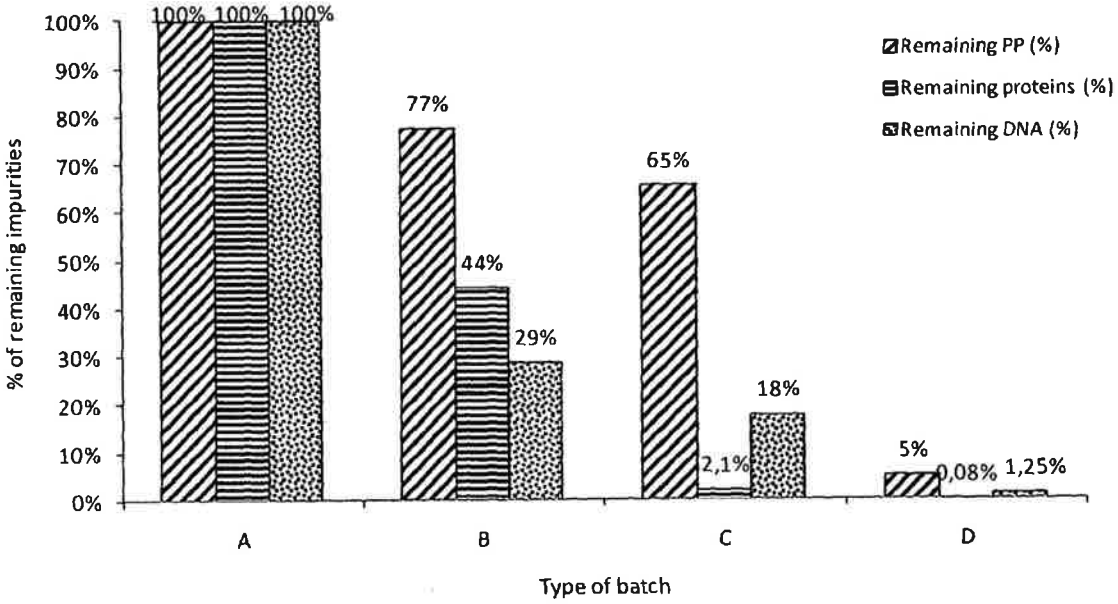


Figure 6

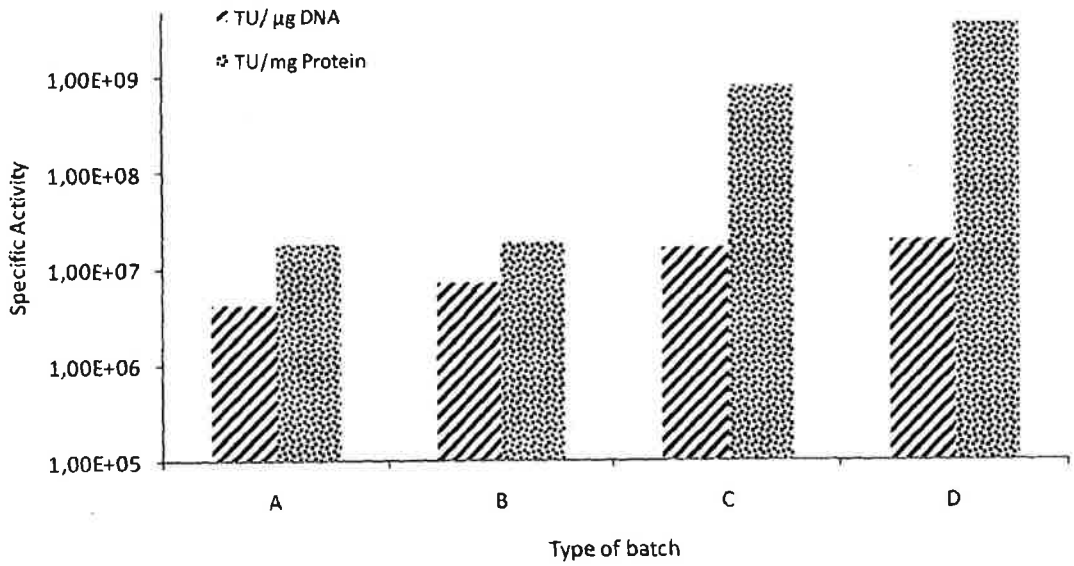


Figure 7

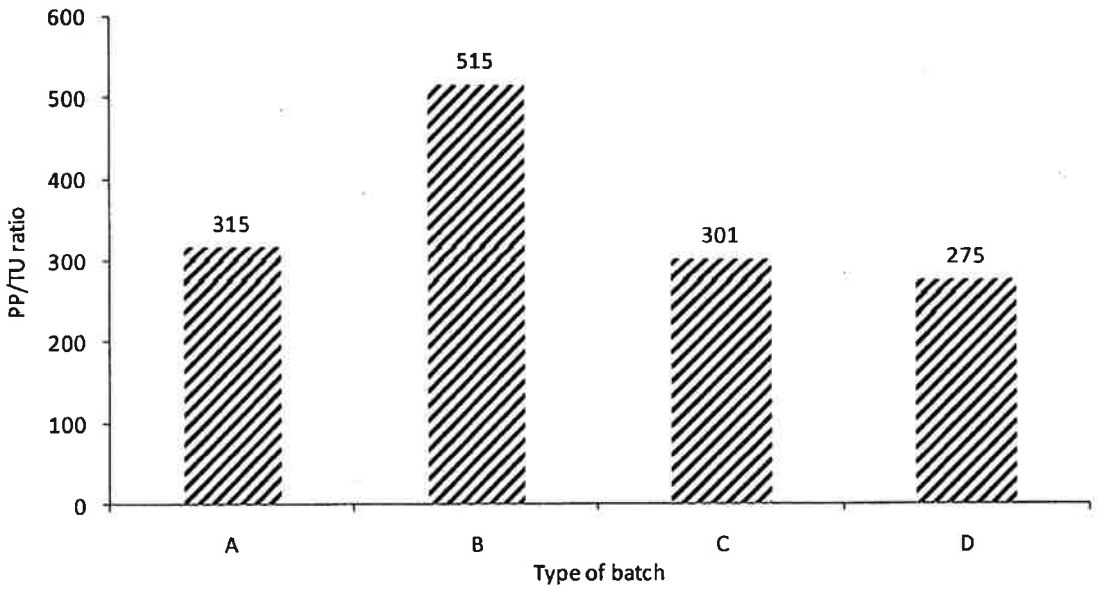


Figure 8

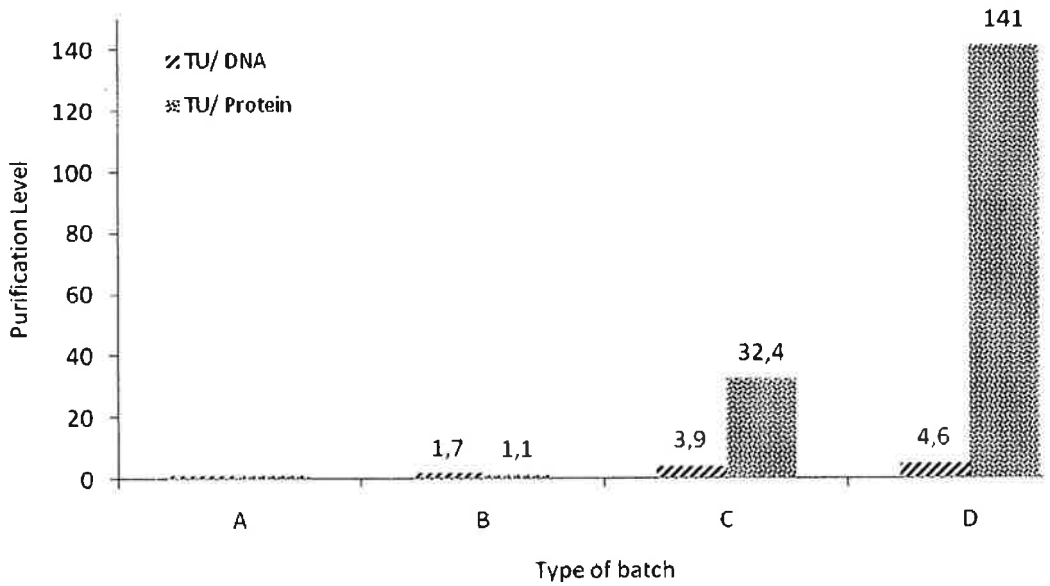


Figure 9

Figure 10

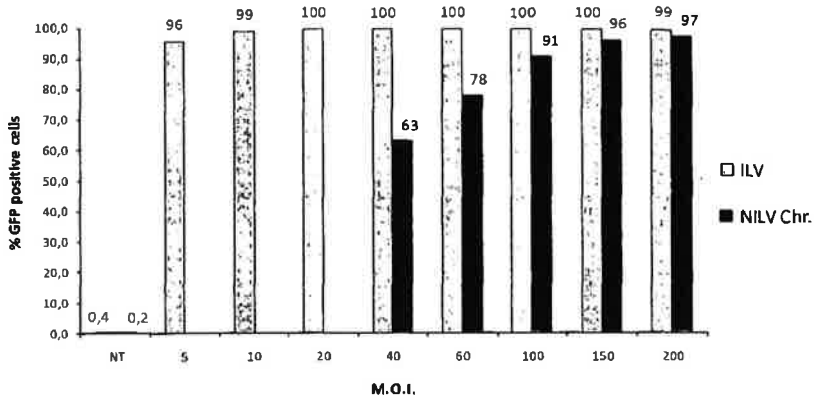
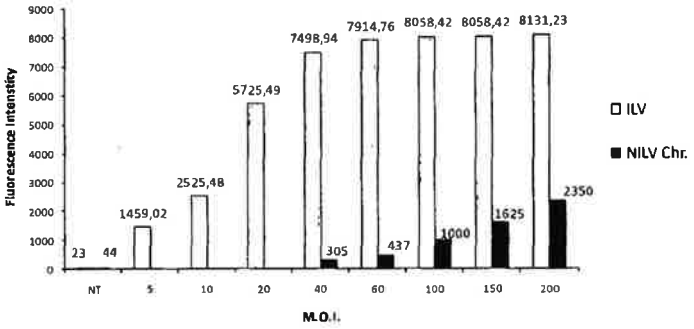


Figure 10A



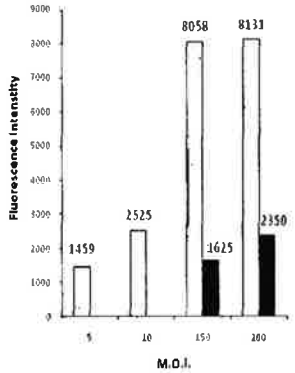


Figure 10B

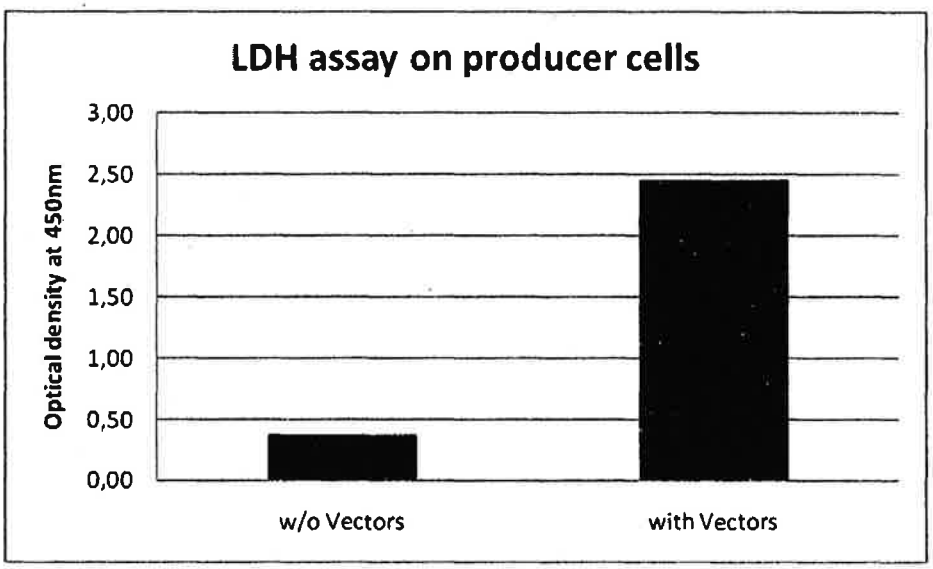

LDH assay on producer cells
Optical density at 450nm
3,00
2,50
2,00
1,50
1,00
0,50
0,00
w/o Vectors
with Vectors

Figure 11

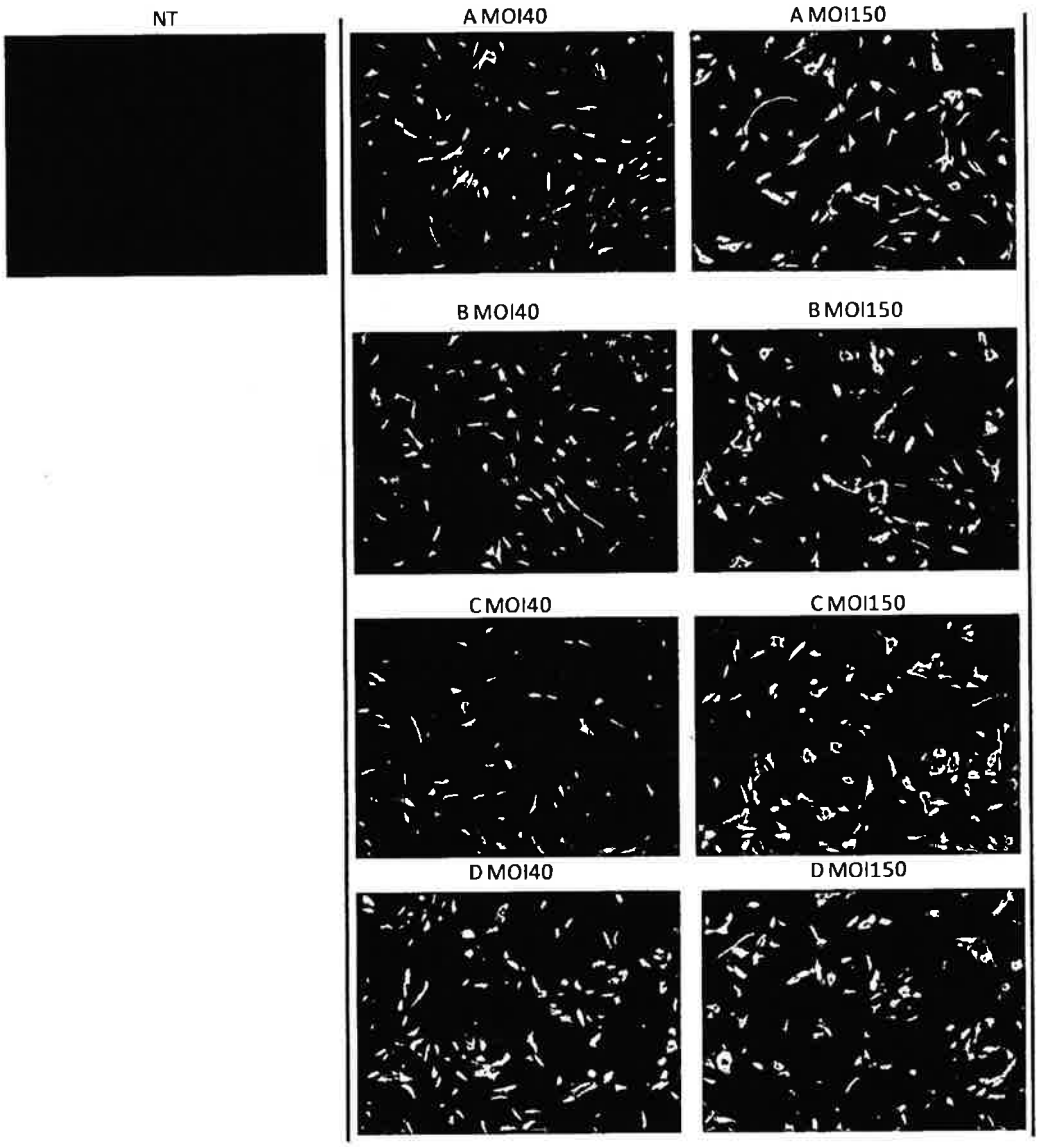

NT
A MOI40
A MOI150
B MOI40
B MOI150
C MOI40
C MOI150
D MOI40
D MOI150

Figure 12A

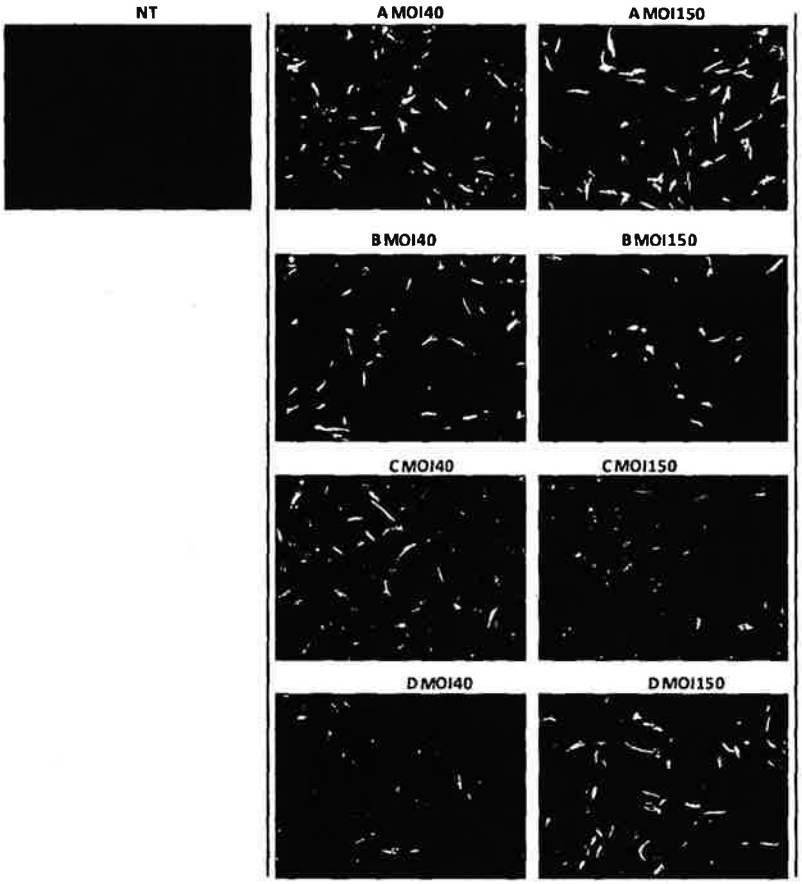


Figure 12B

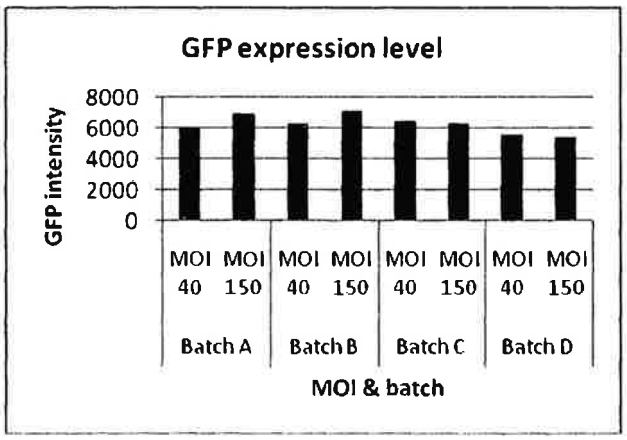


Figure 12C

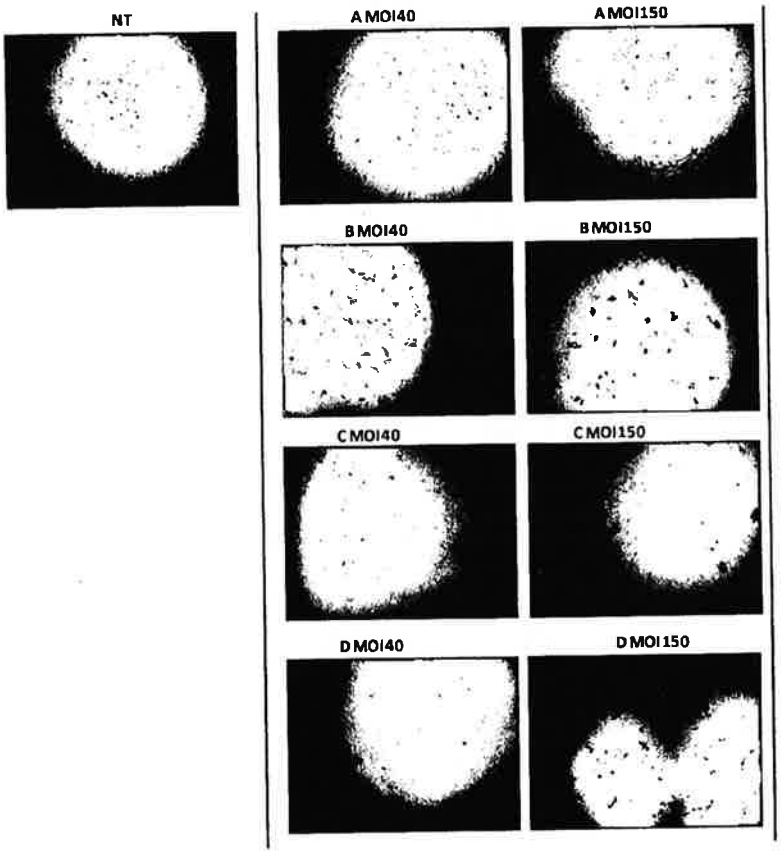
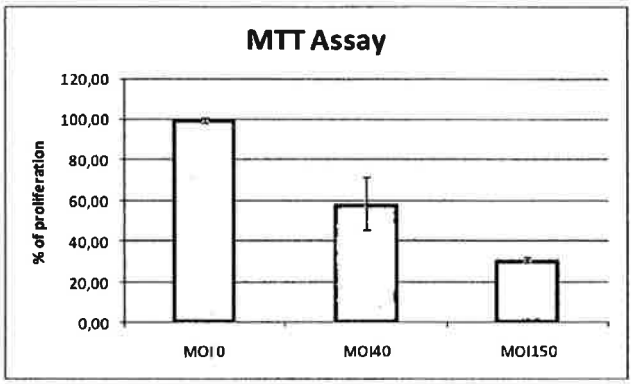

NT
A MOI40
A MOI150
B MOI40
B MOI150
C MOI40
C MOI150
D MOI40
D MOI150

Figure 13



	MOI 0	MOI40	MOI150
simplicate	98,03	44,24	32,46
duplicate	100,75	55,63	29,63
triplicate	100,24	75,83	30,73
mean	99,67	58,57	30,94
SD	1,18	13,06	1,17

Figure 14A

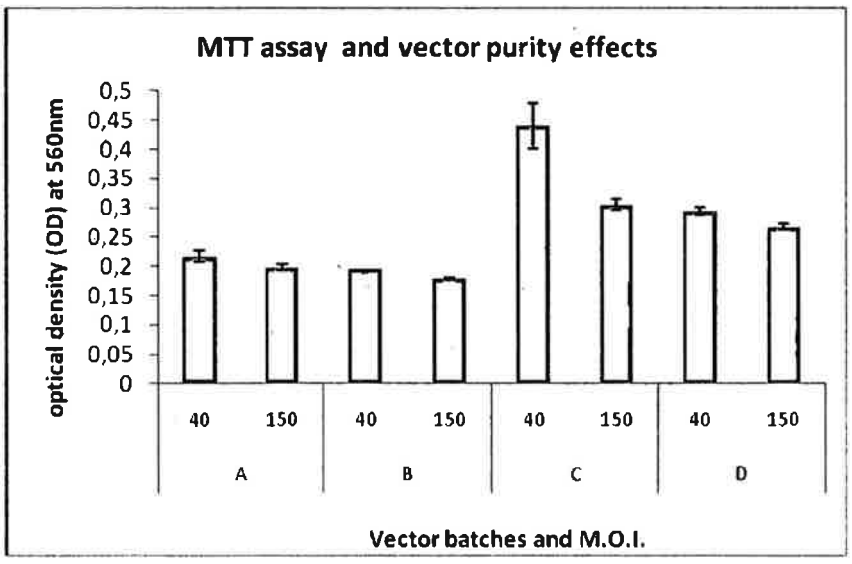


Figure 14B

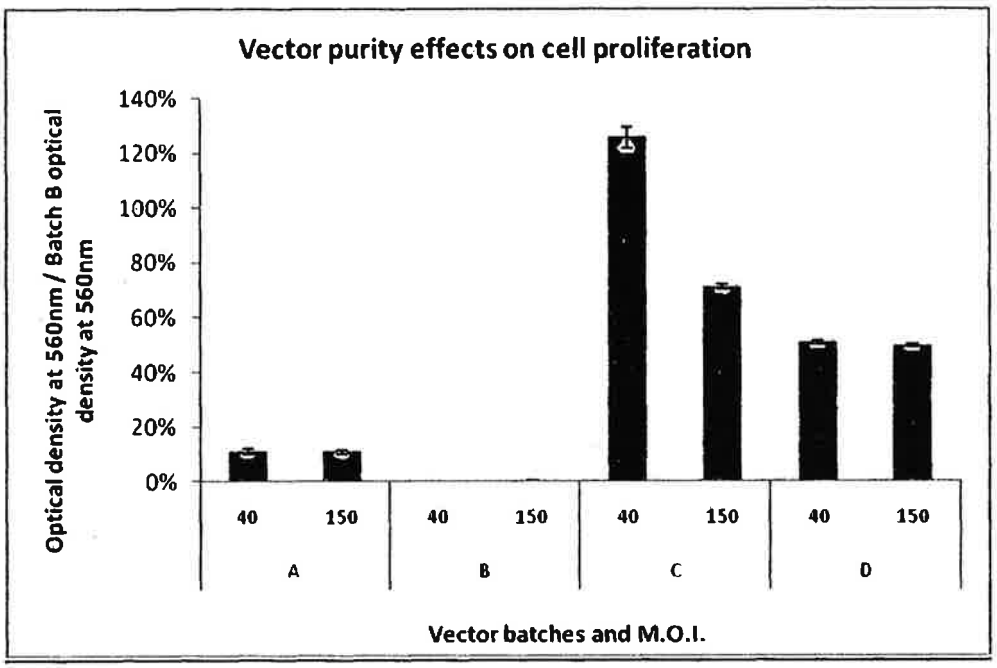

Vector purity effects on cell proliferation
Optical density at 560nm / Batch B optical density at 560nm
140%
120%
100%
80%
60%
40%
20%
0%
40
150
40
150
40
150
40
150
A
B
C
D
Vector batches and M.O.I.

Figure 14C

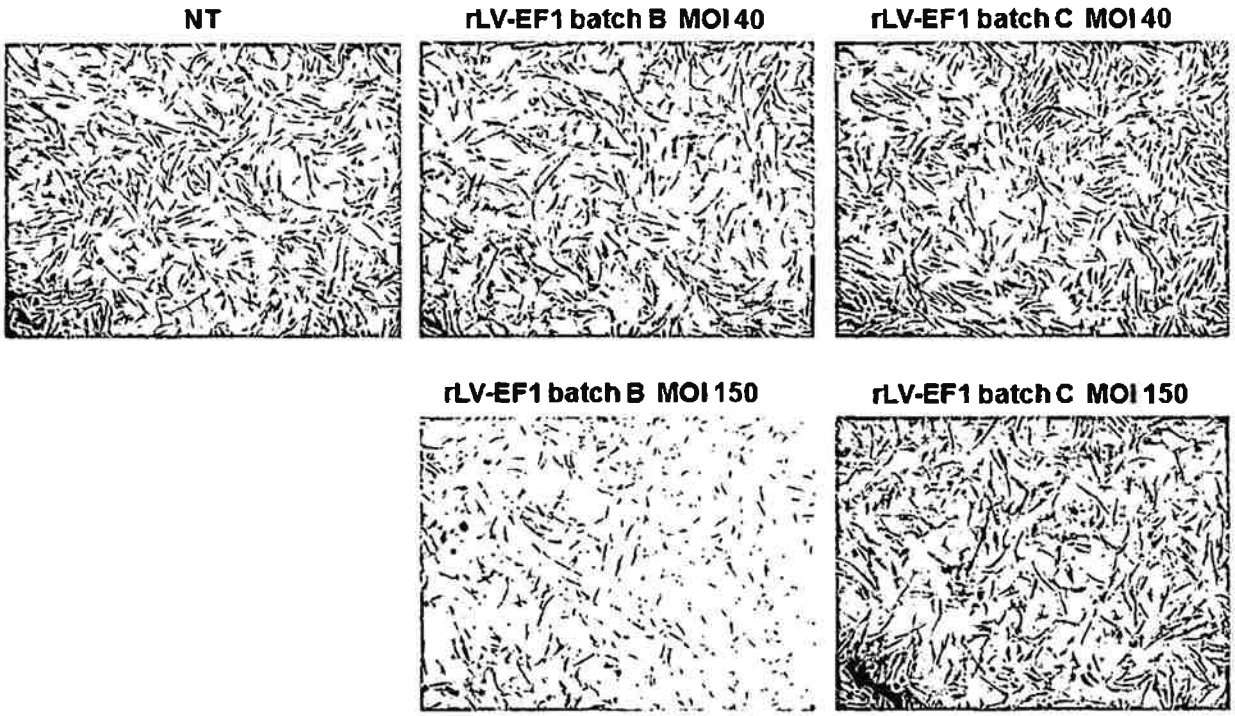


Figure 15A

	Titer (TU/mL) (1)	TU / total DNA (TU/µg) (2)	TU / total proteins (TU/mg) (3)	PP/TU (4)
rLV-EF1 batch B	4,7E+08	9,1E+06	4,3E+07	104
rLV-EF1 batch C	8,9E+09	2,0E+07	3,7E+08	67

Figure 15B

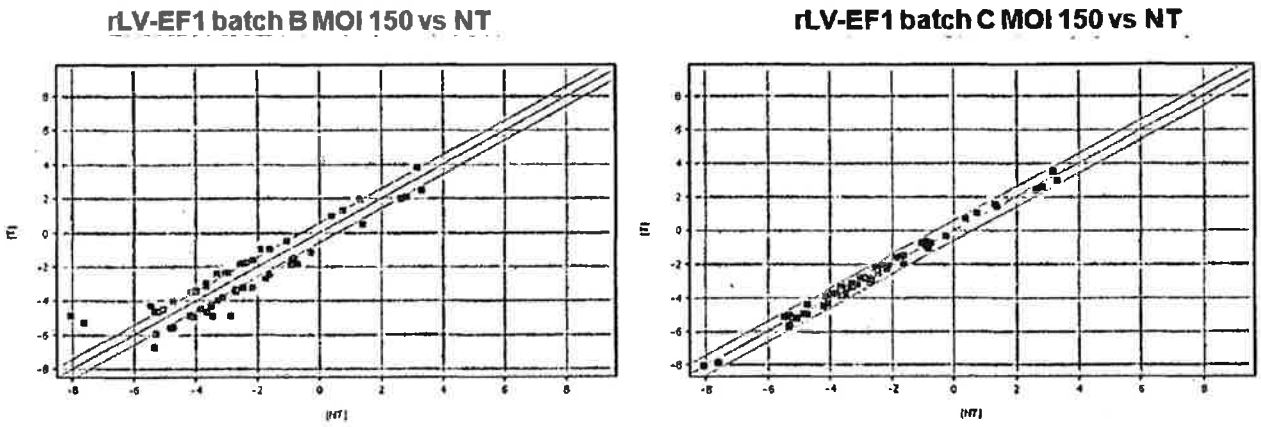


Figure 16A

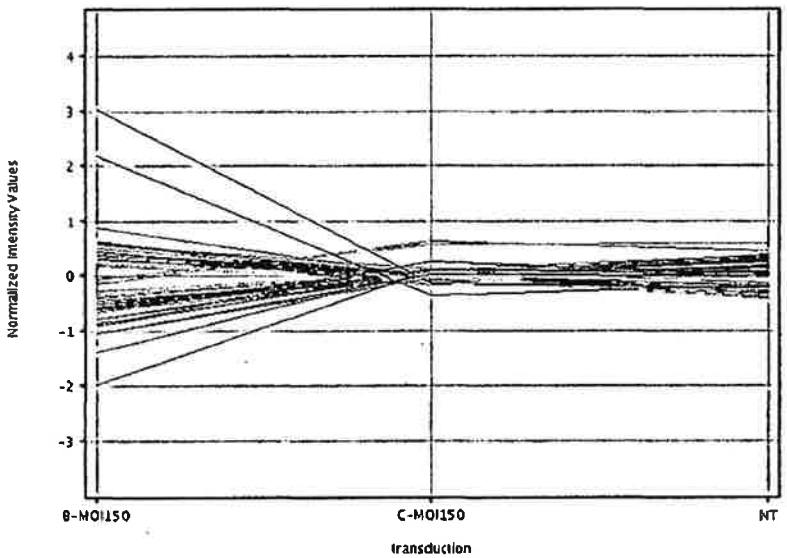


Figure 16B

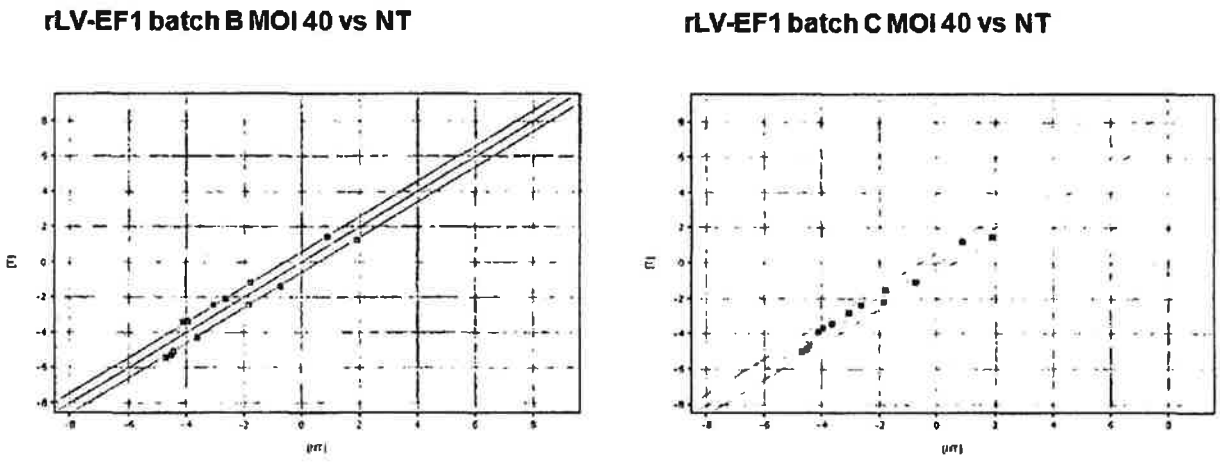


Figure 16C

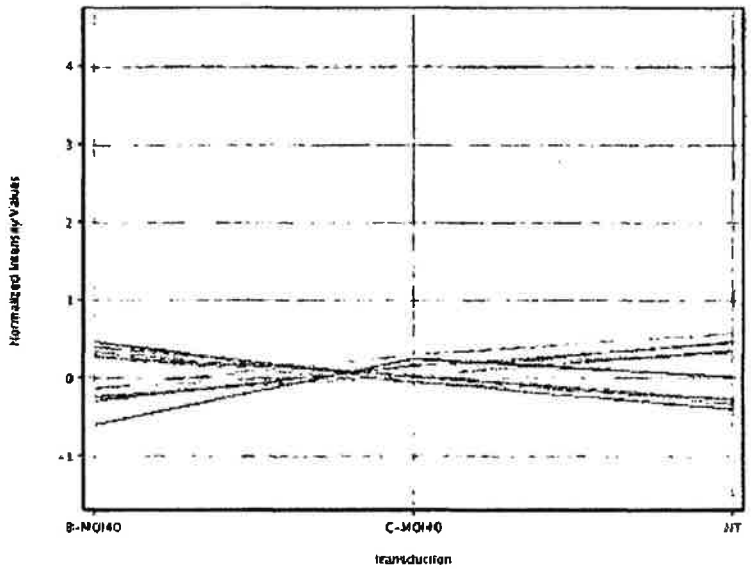


Figure 16 D

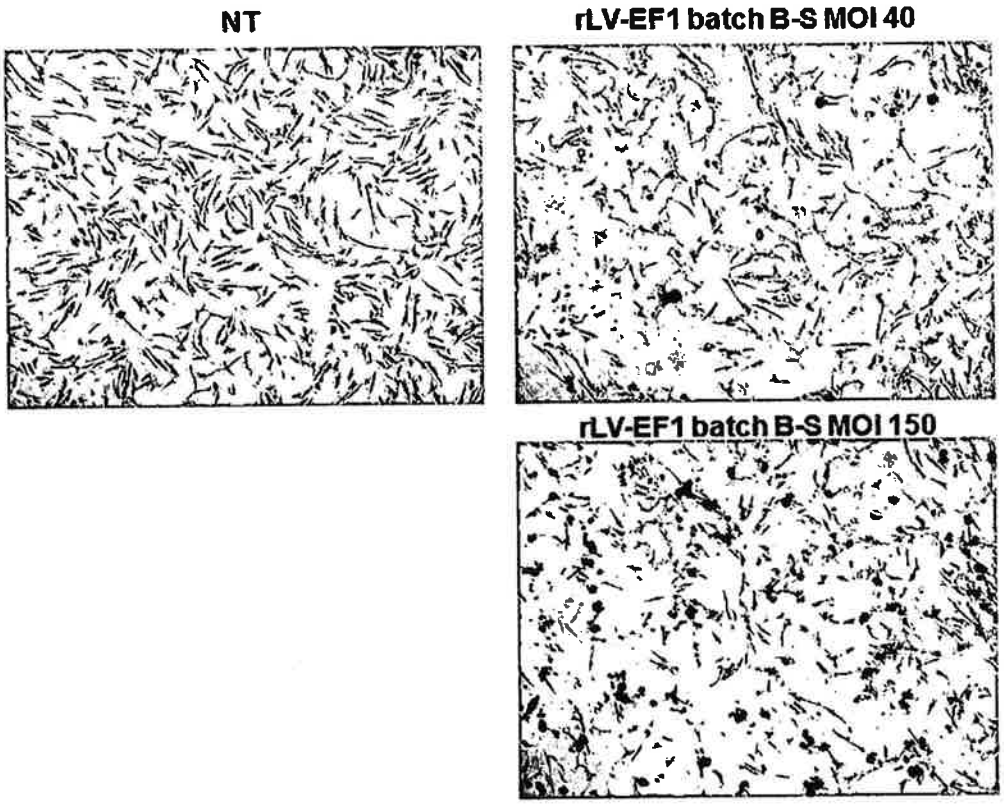


Figure 17A

	Titer (TU/mL) (1)	TU / total DNA (TU/µg) (2)	TU / total proteins (TU/mg) (3)	PP/TU (4)
rLV-EF1 batch B-S	3,3E+07	4,7E+05	1,1E+05	1017

Figure 17B

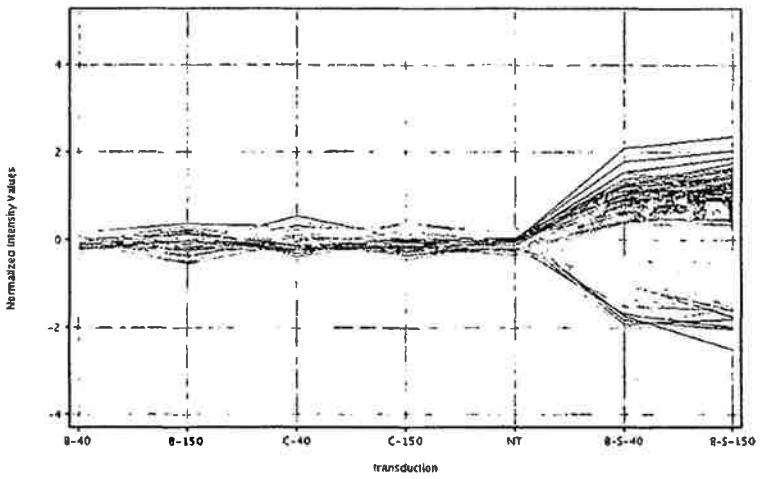

Normalized intensity Values
4
2
0
-2
-4
B-40
B-150
C-40
C-150
NT
B-S-40
B-S-150
transduction

Figure 18

Process	A	B	C	D
Titer (TU/ml)	$1x10^6$	$1x10^7$ - $1x10^8$	$1x10^7$ - $1x10^8$	$1x10^7$ - $1x10^8$
Process recovery (%)	100%	45-55%	55-70%	10-20%
Concentration factor	N/A	90-110	20-40	5-20
Proteins removal (%)	N/A	55-65%	up to 98%	up to 99,9%
DNA removal (%)	N/A	60-75%	70-90%	up to 98,8%
PP/TU ratio	200-900	up to 600	200-600	100-400
Applications	Immortalized cell lines	Immortalized cell lines	Primary and stem cells	*in vivo* injection

Table 1

	Vectalys Batch	A	B	C	D
	Volume (ml)	5200	1,3	171	40
Transduction Unit	Titre (TU/ml)	2,46E+06	2,33E+08	4,86E+07	1,74E+07
	Total TU	1,3E+10	3,0E+08	8,3E+09	6,9E+08
	Recovery (%)	100%	47%	69%	6%
	Step Yield (%)	100%	47%	69%	24%
Physical particles	Titre p24 (PP/ml)	7,75E+08	1,20E+11	1,46E+10	4,77E+09
	Total PP	4,03E+12	1,56E+11	2,50E+12	1,91E+11
	Recovery (%)	100%	77%	65%	5%
Residual DNA	[DNA] (ng/ml)	589,6	33845,33	3010,44	909,03
	Total DNA (µg)	3066	44	515	36
	Recovery (%)	100%	29%	18%	1,25%
	Removal Yield (%)	100%	71%	82%	68%
Total Protein	[Protein] (mg/ml)	0,14	12,4	0,061	0,01
	Total Protein (mg)	728	16,1	10,4	0,4
	Recovery (%)	100%	44%	2,1%	0,08%
	Removal Yield (%)	100%	56%	98%	97%
Specific activity	TU/ µg DNA	4,17E+06	6,88E+06	1,61E+07	1,91E+07
	TU/mg Protein	1,76E+07	1,88E+07	7,96E+08	3,47E+09
		5,70E+00	5,33E+00	1,26E-01	2,88E-02
	PP/TU	315	515	301	275
Purification Level	TU/ DNA	1	1,7	3,9	4,6
	TU/ Protein	1	1,1	32,4	141
	PP/TU	1	1,6	1	0,9

Table 2

	Harvests times after transfection(H)	40	48	64	72	96	40	48	64	72	96	64	88	64	88
	Harvests times after medium removal (H)	16	24	40	48	64	16	24	40	48	64	40	64	40	64
	Production time/harvest (H)	16	8	16	8	16	16	8	16	8	16	40	24	40	24
	Conditions	Without sodium butyrate induction					With sodium butyrate induction					Without sodium butyrate induction		Without sodium butyrate induction	
	Harvest	R1-32h	R2-48h	R3-56h	R4-72h	R5-96h	R1-32h	R2-48h	R3-56h	R4-72h	R5-96h	R1-64h	R2-88h	R1-64h	R2-88h
	Volume (ml)	7,5	7,5	7,5	7,5	7,5	7,5	7,5	7,5	7,5	7,5	7,5	7,5	7,5	7,5
Transduction Unit	Titre (TU/ml)	1,40E+07	8,80E+06	8,80E+06	3,30E+06	3,60E+06	2,30E+07	3,50E+06	4,50E+06	2,30E+06	2,80E+06	1,10E+07	3,60E+06	1,50E+07	3,30E+06
	TU/ Production	2,69E+08					2,71E+08					1,10E+08		1,37E+08	
Total DNA	[DNA] (µg/ml)	0,43	0,57	1,36	1,12	1,74	3,819	3,364	3,878	2,155	2,127	2,44	3,36	9,03	5,39
	DNA average	1,05					3,07					2,90		7,21	
LDH	% Cytotoxicité	7,8%	6,7%	25,4%	16,4%	NA	40,7%	18,3%	41,8%	22,2%	NA	81,9%	48,4%	97,4%	52,4%
Specific activity	TU/ µg DNA	3,28E+07	1,53E+07	6,45E+06	2,94E+06	5,53E+05	6,02E+06	1,04E+06	1,16E+06	1,07E+06	1,32E+06	4,51E+06	1,07E+06	1,66E+06	2E+05
	PP/TU	119	40	100	63	48	118	77	102	43	40,3571429	262	141	90	187

Table 3