Figure 1

A

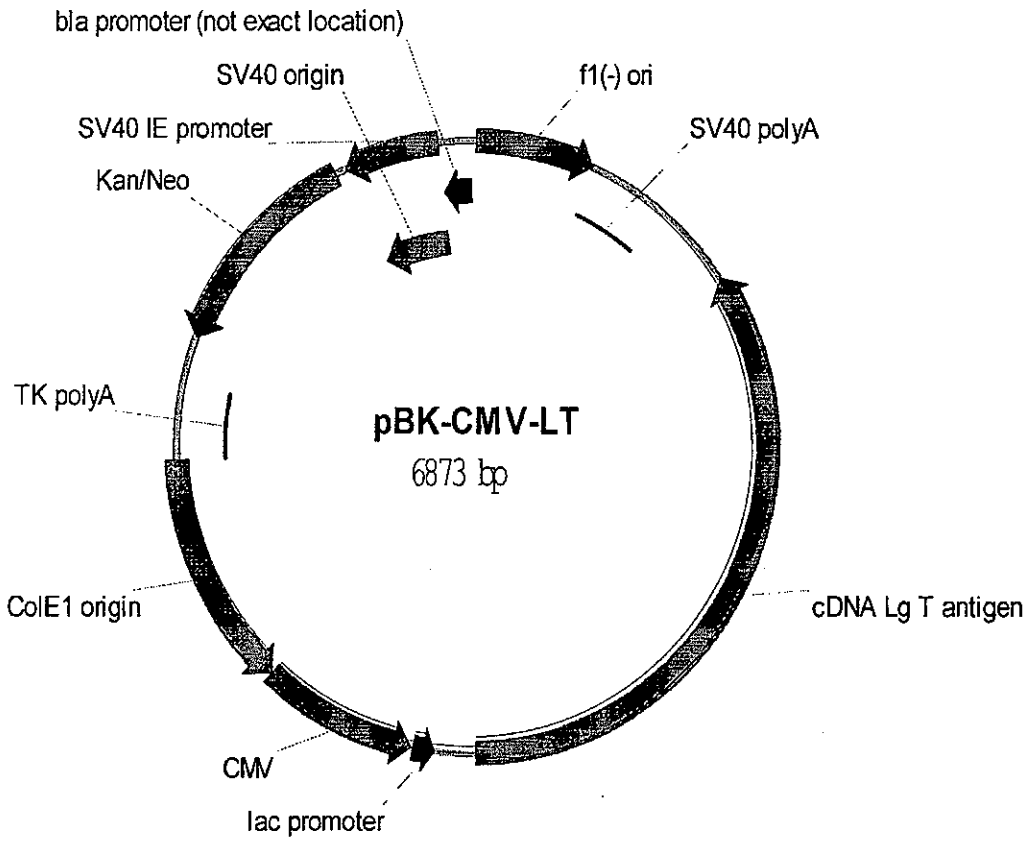

bla promoter (not exact location)
SV40 origin
f1(-) ori
SV40 IE promoter
SV40 polyA
Kan/Neo
TK polyA
pBK-CMV-LT
6873 bp
ColE1 origin
cDNA Lg T antigen
CMV
lac promoter

Figure 1

B

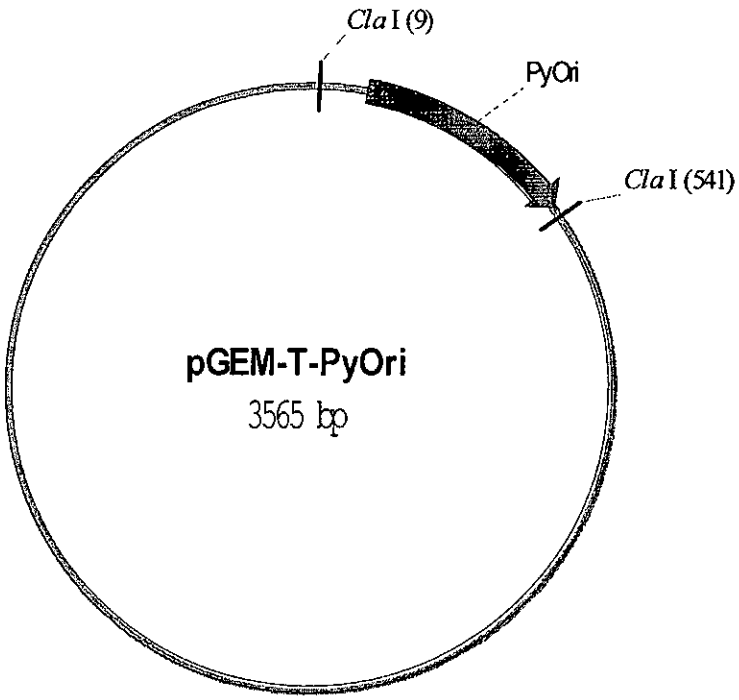
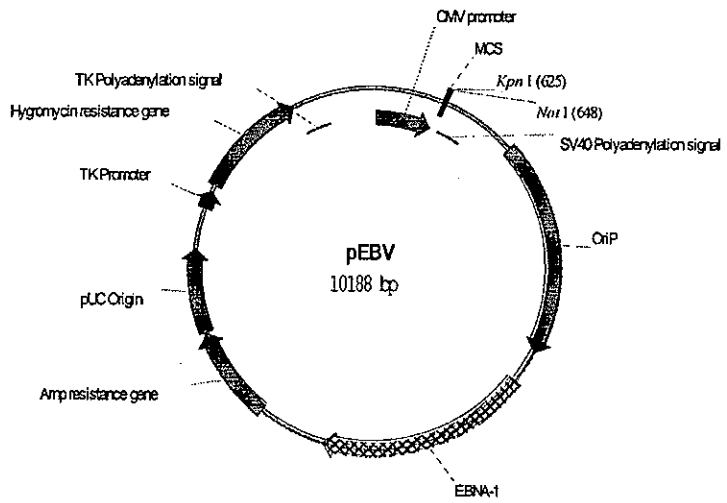


Figure 1

C


CMV promoter
MCS
Kpn I (625)
Not I (648)
SV40 Polyadenylation signal
OriP
EBNA-1
Amp resistance gene
pUC Origin
TK Promoter
Hygromycin resistance gene
TK Polyadenylation signal
pEBV
10188 bp

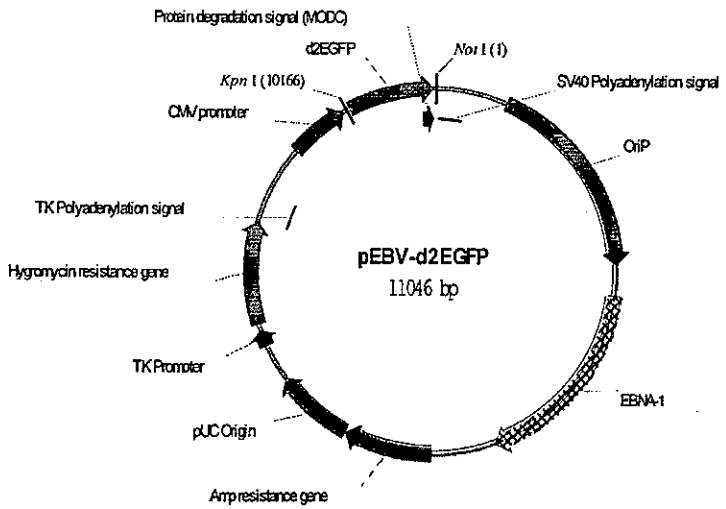
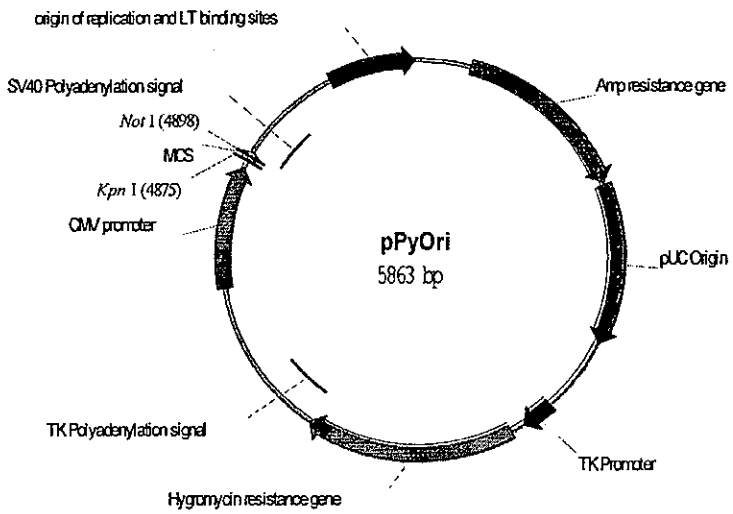

Protein degradation signal (MODC)
d2EGFP
Not I (1)
Kpn I (10166)
SV40 Polyadenylation signal
CMV promoter
OriP
TK Polyadenylation signal
Hygromycin resistance gene
pEBV-d2EGFP
11046 bp
TK Promoter
EBNA-1
pUC Origin
Amp resistance gene

Figure 1

D


origin of replication and LT binding sites
SV40 Polyadenylation signal
Not I (4898)
MCS
Kpn I (4875)
CMV promoter
Amp resistance gene
pUC Origin
TK Promoter
Hygromycin resistance gene
TK Polyadenylation signal
pPyOri
5863 bp

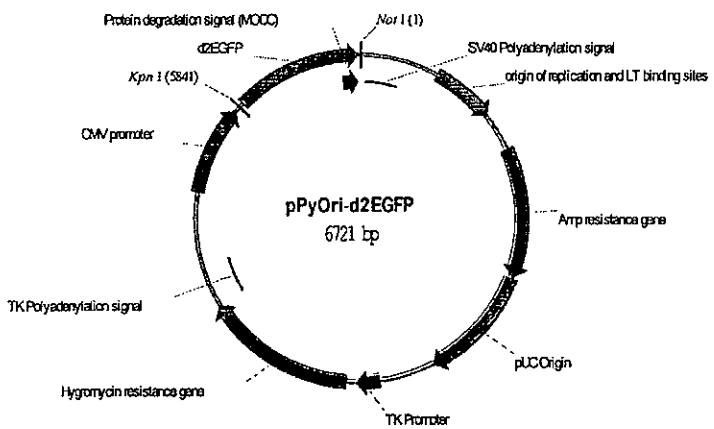

Protein degradation signal (MODC)
d2EGFP
Kpn I (5841)
CMV promoter
Not I (1)
SV40 Polyadenylation signal
origin of replication and LT binding sites
Amp resistance gene
pUC Origin
TK Promoter
Hygromycin resistance gene
TK Polyadenylation signal
pPyOri-d2EGFP
6721 bp

Figure 1

E

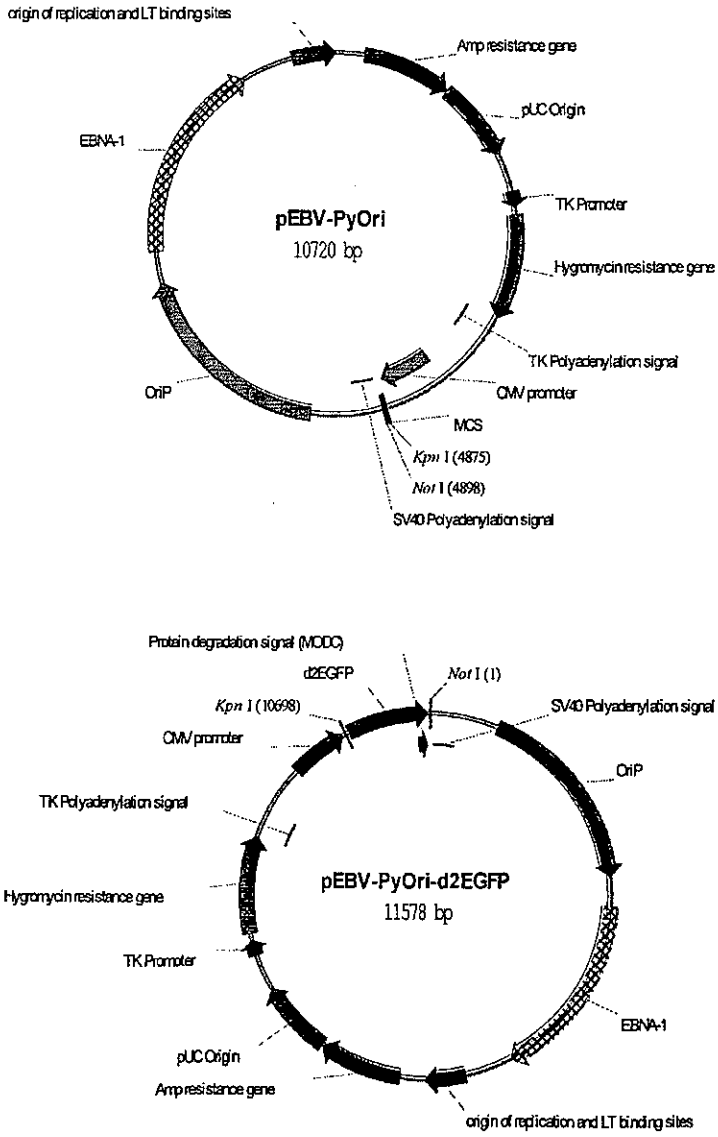
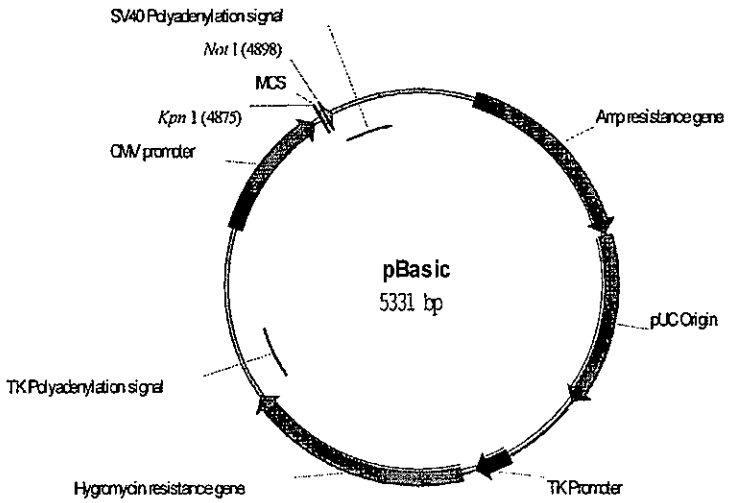

origin of replication and LT binding sites
Amp resistance gene
pUC Origin
EBNA-1
TK Promoter
pEBV-PyOri
10720 bp
Hygromycin resistance gene
TK Polyadenylation signal
OriP
CMV promoter
MCS
Kpn I (4875)
Not I (4898)
SV40 Polyadenylation signal
Protein degradation signal (MODC)
d2EGFP
Not I (1)
Kpn I (10698)
SV40 Polyadenylation signal
CMV promoter
OriP
TK Polyadenylation signal
pEBV-PyOri-d2EGFP
11578 bp
Hygromycin resistance gene
TK Promoter
EBNA-1
pUC Origin
Amp resistance gene
origin of replication and LT binding sites

Figure 1

F


SV40 Polyadenylation signal
Not I (4898)
MCS
Kpn I (4875)
CMV promoter
Amp resistance gene
pBasic
5331 bp
pUC Origin
TK Polyadenylation signal
Hygromycin resistance gene
TK Promoter

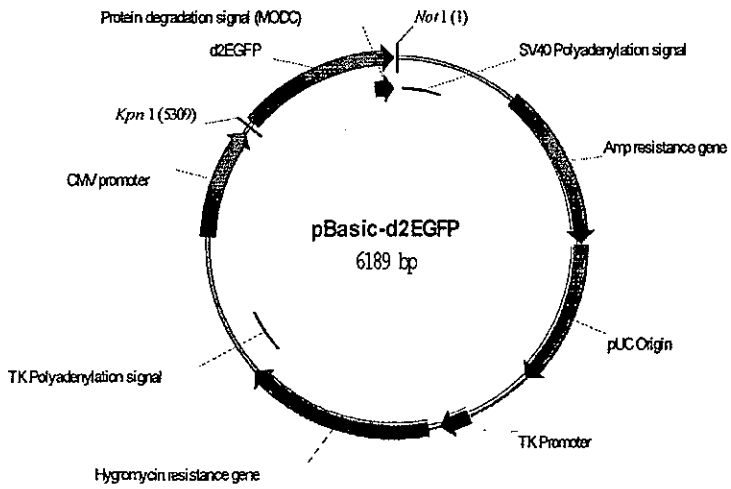

Protein degradation signal (MODC)
Not I (1)
d2EGFP
SV40 Polyadenylation signal
Kpn I (5309)
Amp resistance gene
CMV promoter
pBasic-d2EGFP
6189 bp
pUC Origin
TK Polyadenylation signal
TK Promoter
Hygromycin resistance gene

Figure 1

G

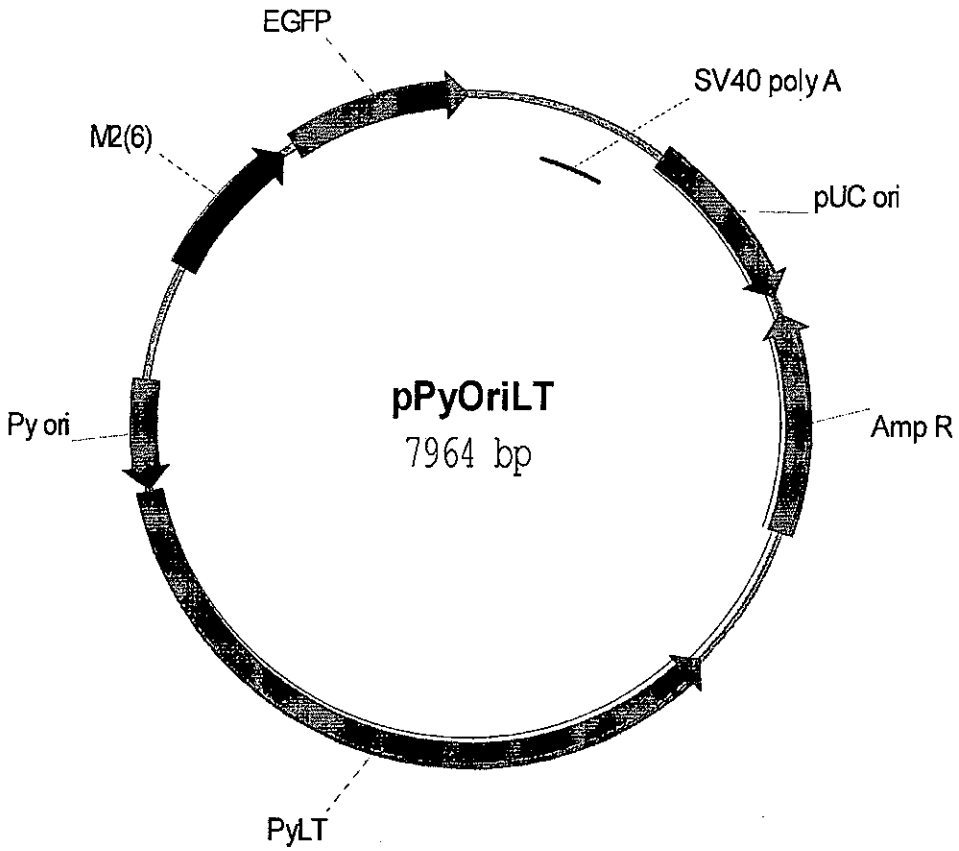
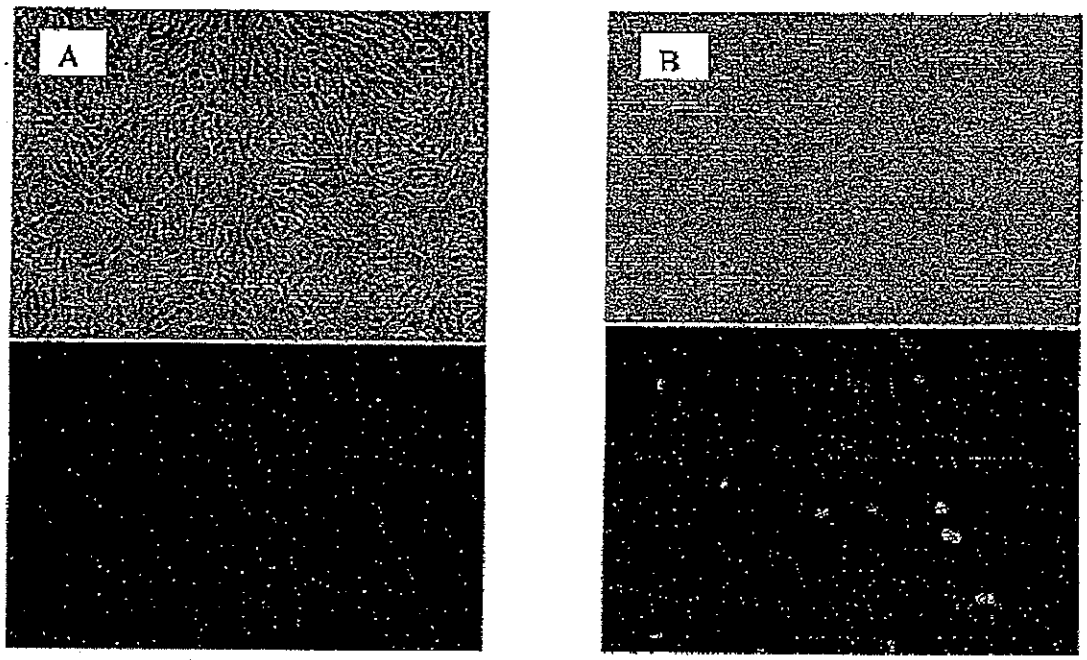


Figure 2



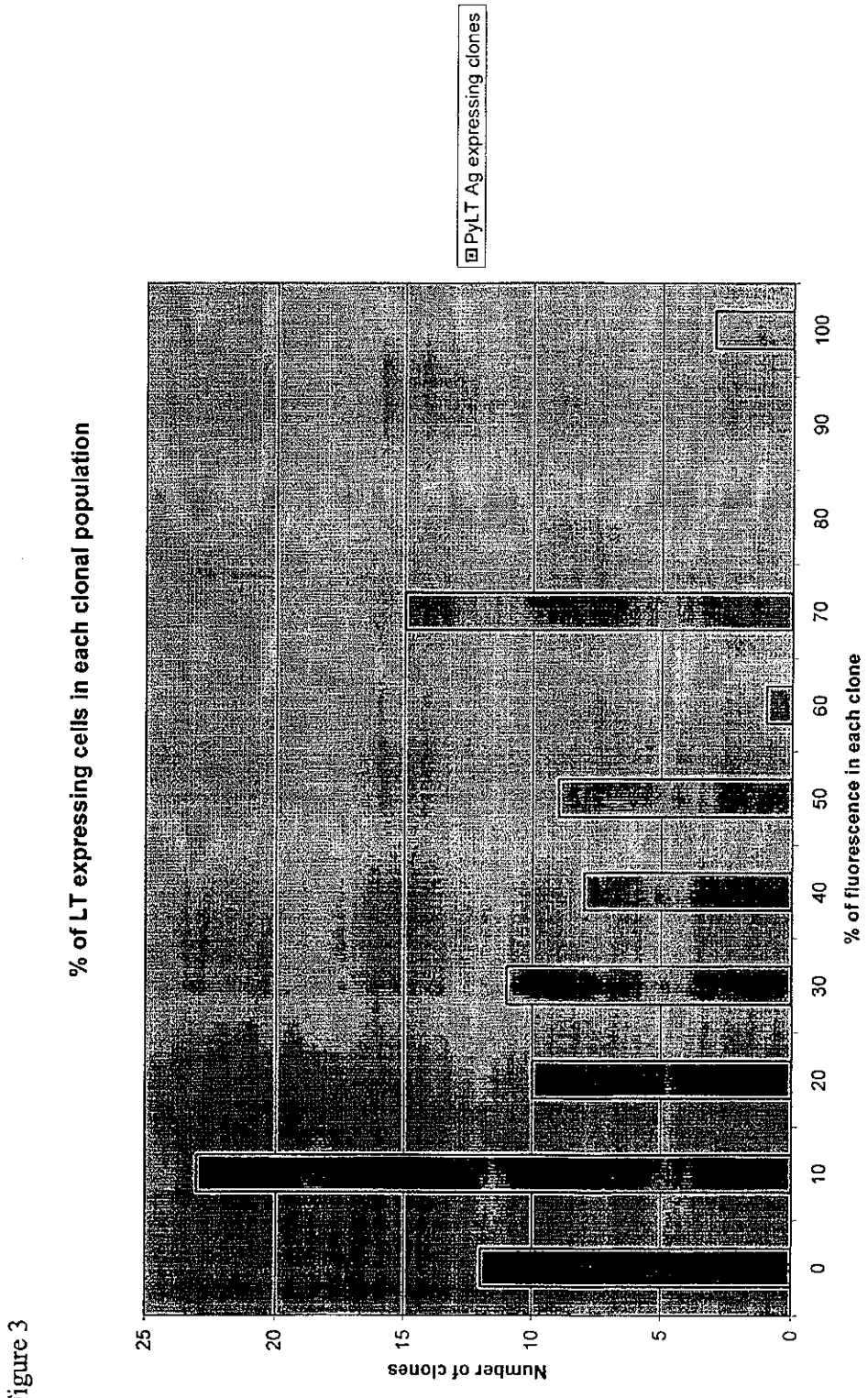

PyLT Ag expressing clones
% of LT expressing cells in each clonal population
Number of clones
% of fluorescence in each clone
0
5
10
15
20
25
0
10
20
30
40
50
60
70
80
90
100

Figure 3

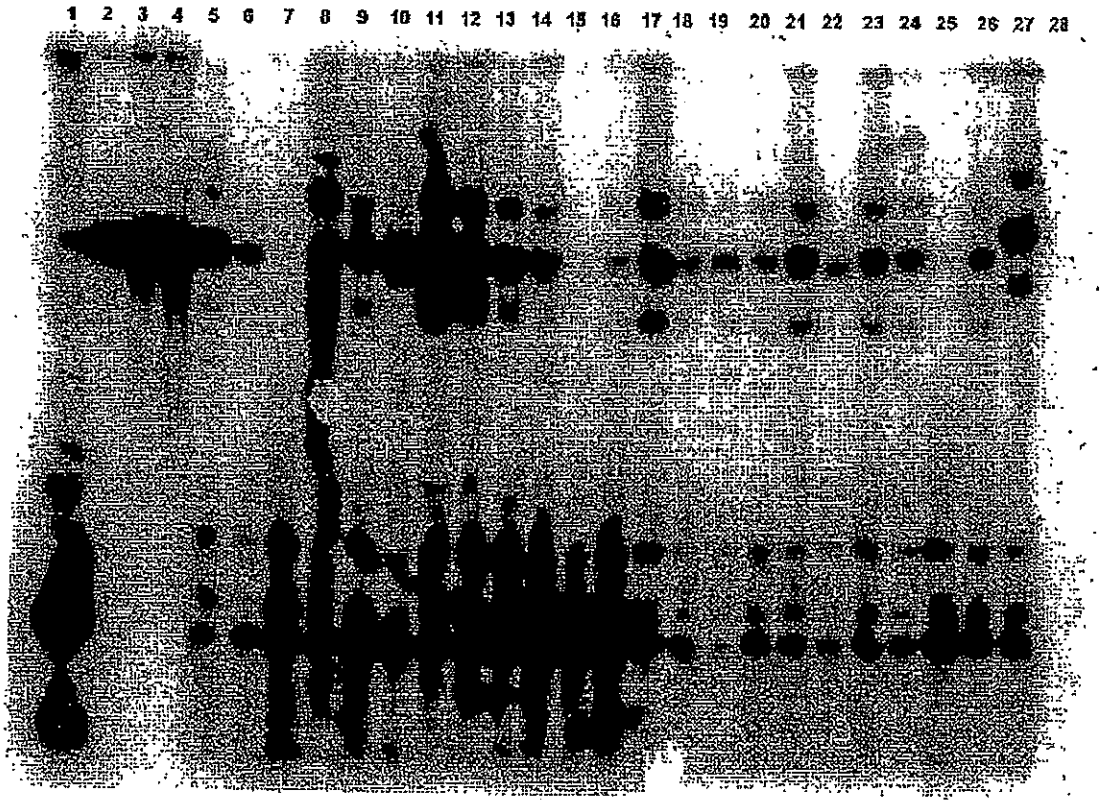


Figure 4

Figure 5 (1)

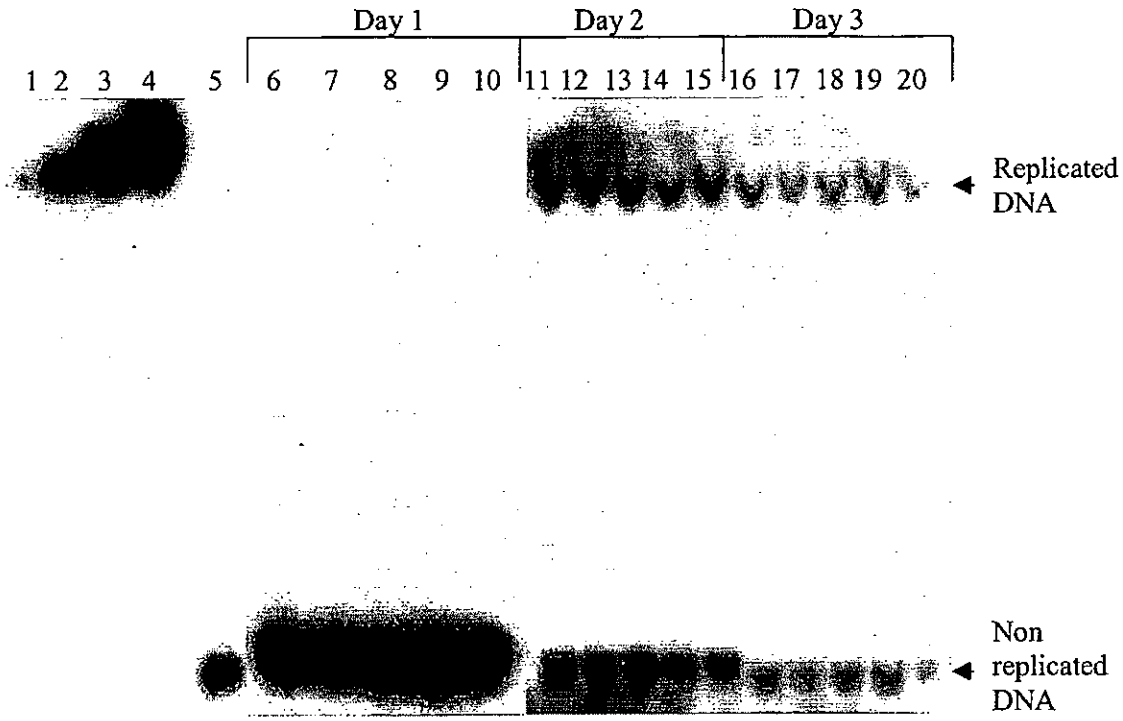
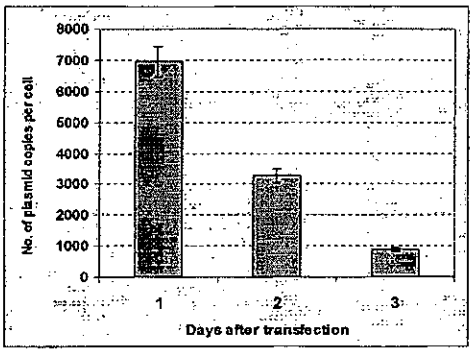
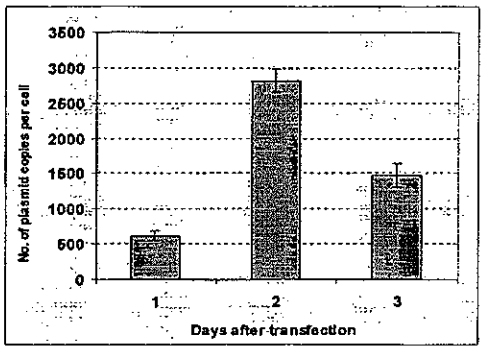

Day 1
Day 2
Day 3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Replicated DNA
Non replicated DNA

Figure 5 (2)



Non replicated DNA



Replicated DNA

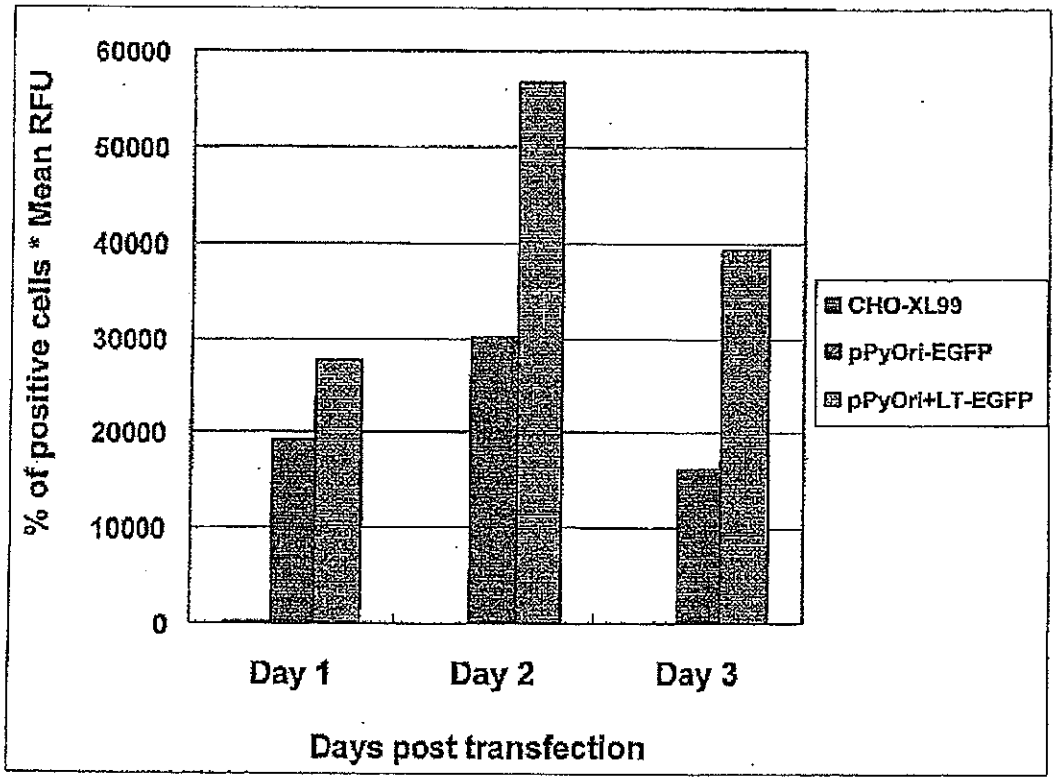
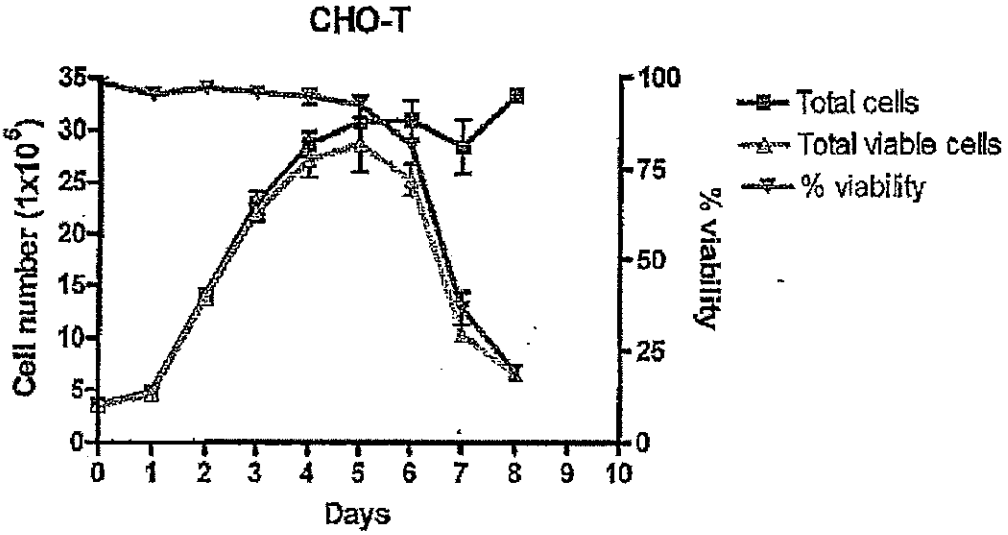

% of positive cells * Mean RFU
60000
50000
40000
30000
20000
10000
0
Day 1
Day 2
Day 3
Days post transfection
CHO-XL99
pPyOri-EGFP
pPyOri+LT-EGFP

Figure 6

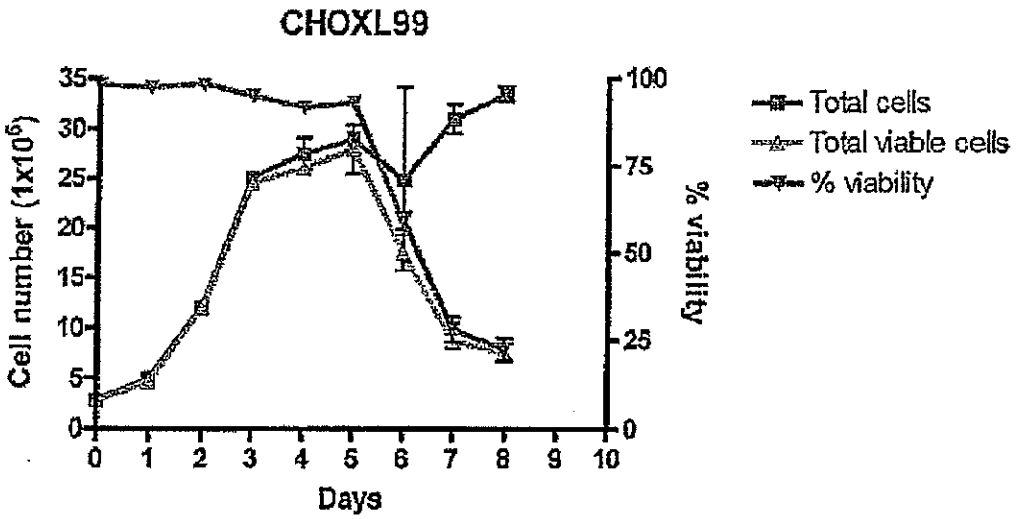
Figure 7

A



Doubling time: 21.23hr
Peak viable cell denstiy: 2.85x10^6 Cells/ml

B



Doubling time: 20.01hr
Peak viable cell diensity: 2.75x10^6 cells/ml


pEBV
pEBV/PyOri
pPyOri
pBasic
EBNA1
OriP
Hyg
CMV
GFP/HGH
Py-Ori

Figure 8

Figure 9A

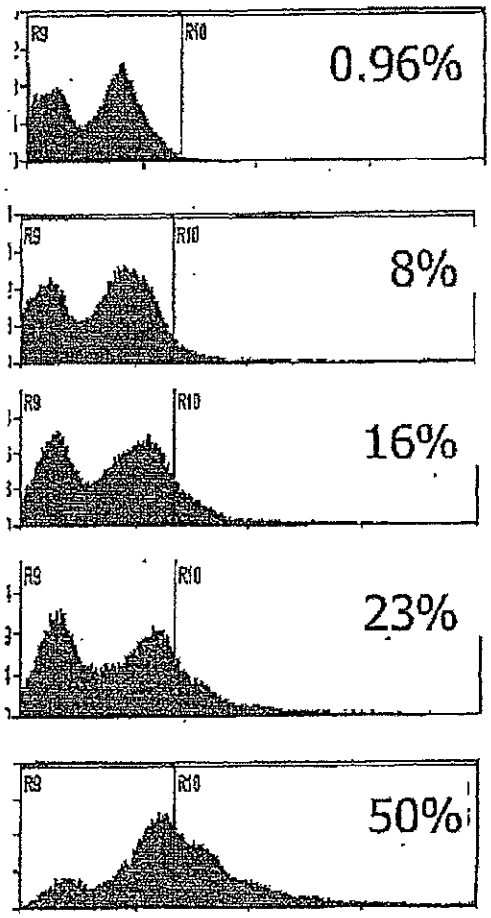
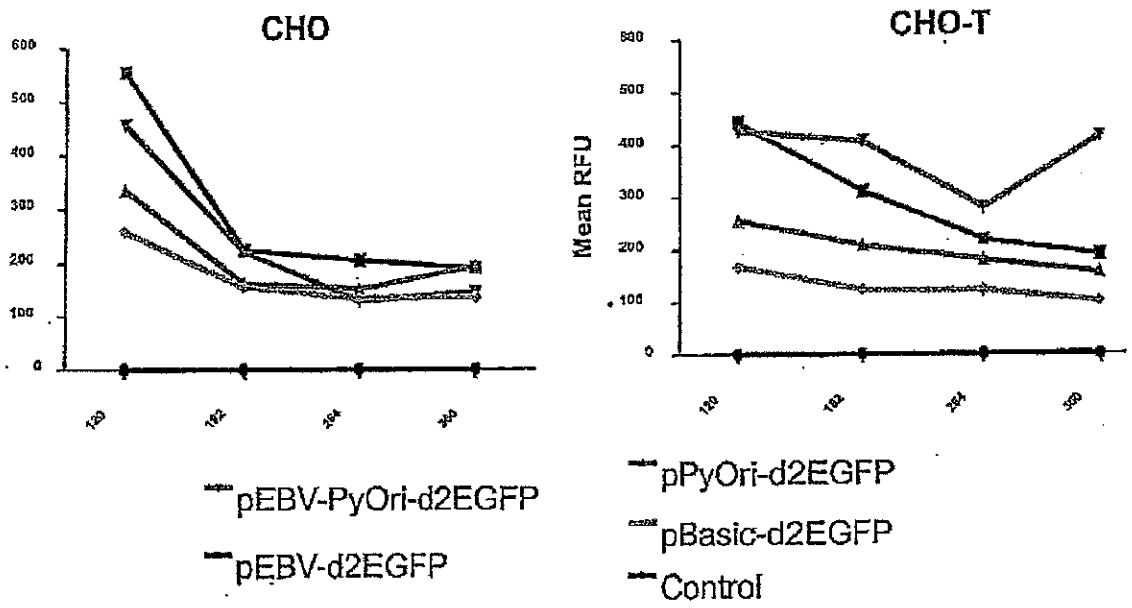
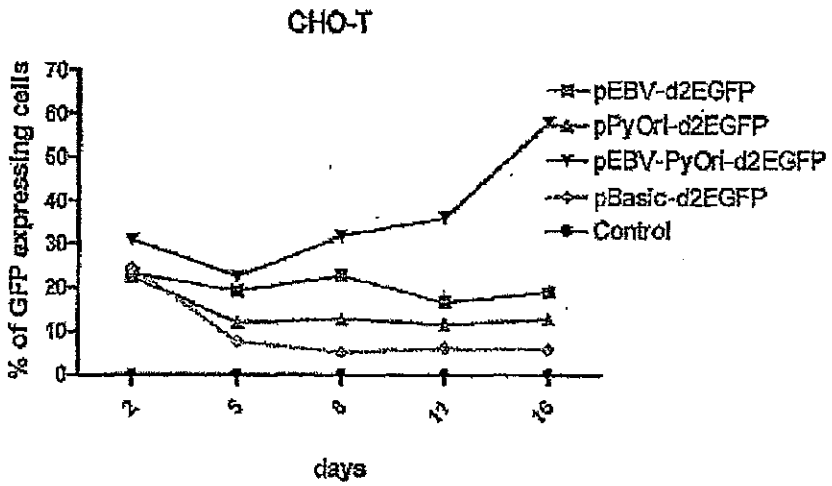

R9
R10
0.96%
untransfected
R9
R10
8%
pBasic EGFP
R9
R10
16%
pPyori EGFP
R9
R10
23%
pEBV EGFP
R9
R10
50%
pEBV/PyOri EGFP

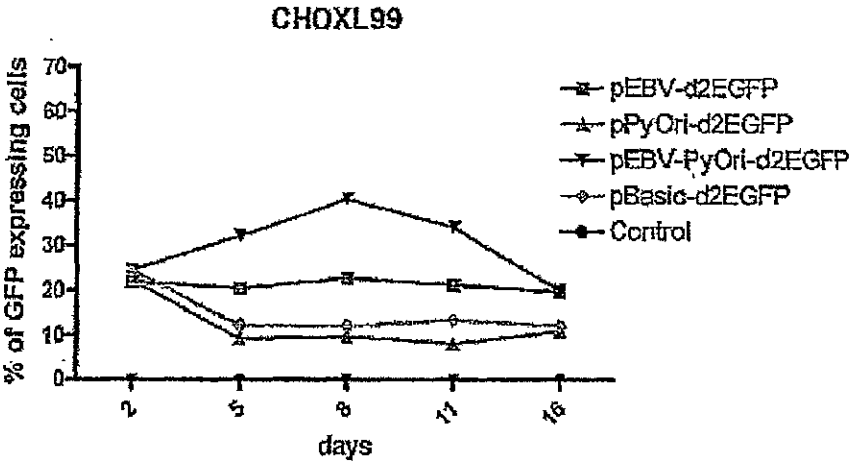
Figure 9B


CHO
CHO-T
Mean RFU
pEBV-PyOri-d2EGFP
pEBV-d2EGFP
pPyOri-d2EGFP
pBasic-d2EGFP
Control

A



B



C

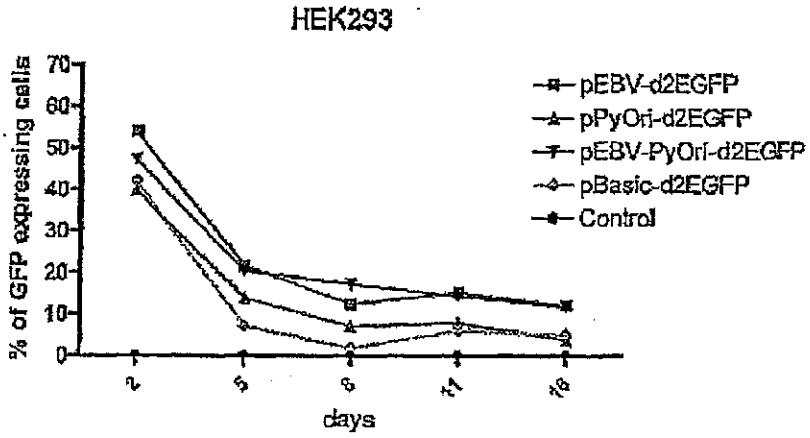


Figure 10

Figure 11 (A)

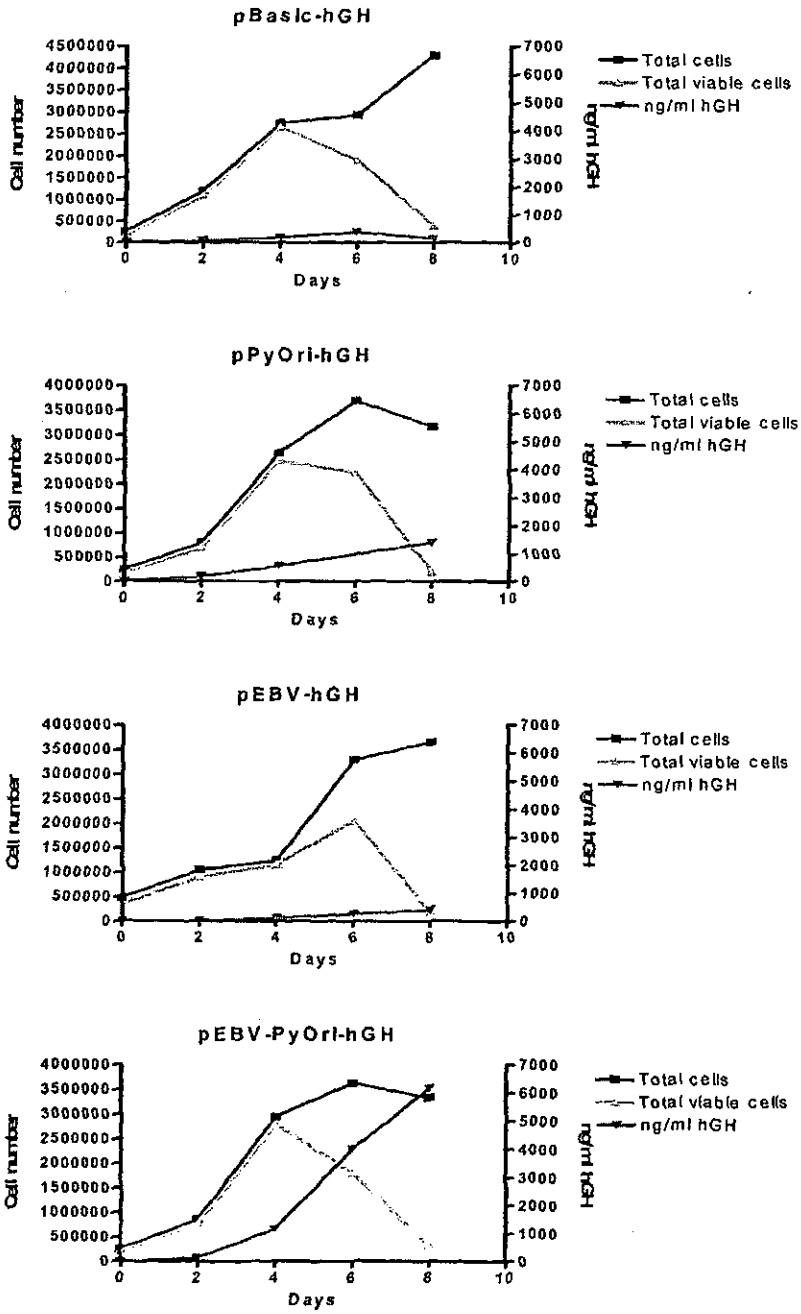

pBasic-hGH
Cell number
ng/ml hGH
Days
Total cells
Total viable cells
ng/ml hGH
pPyOri-hGH
Cell number
ng/ml hGH
Days
Total cells
Total viable cells
ng/ml hGH
pEBV-hGH
Cell number
ng/ml hGH
Days
Total cells
Total viable cells
ng/ml hGH
pEBV-PyOri-hGH
Cell number
ng/ml hGH
Days
Total cells
Total viable cells
ng/ml hGH

Figure 11 (B)

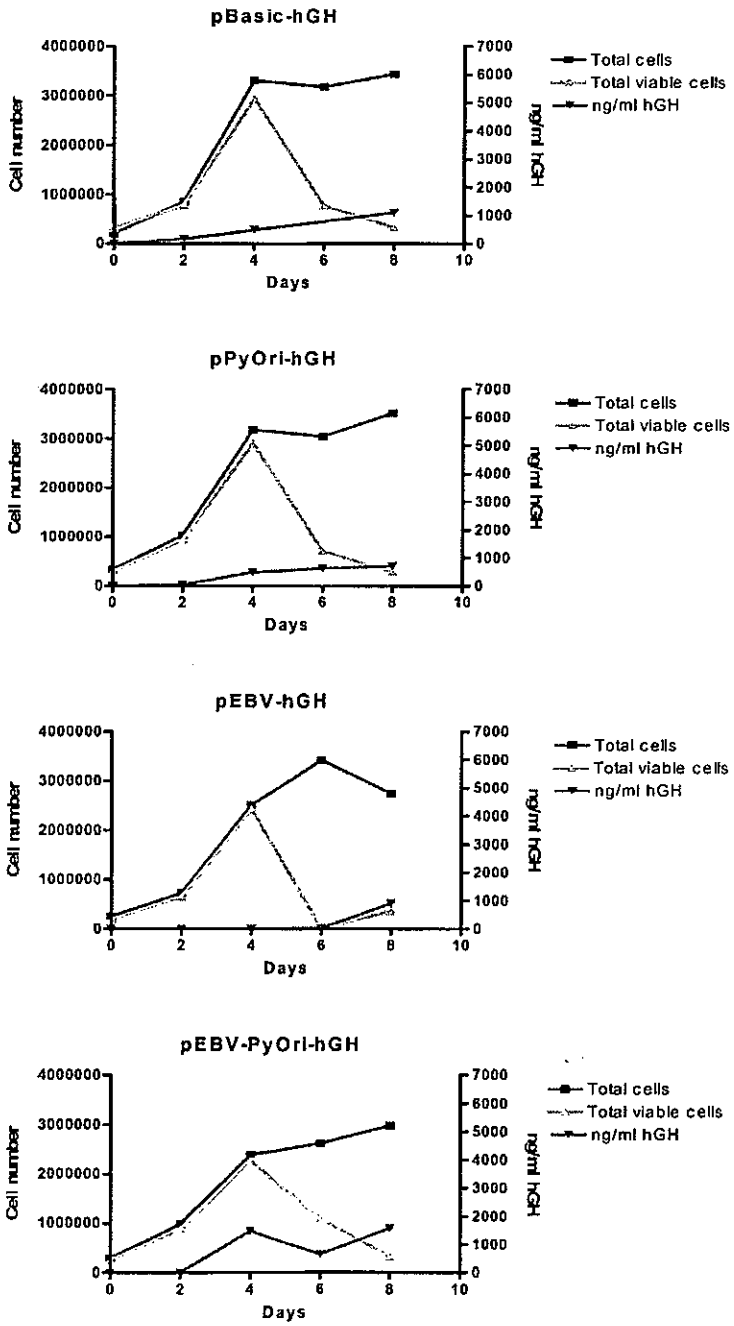
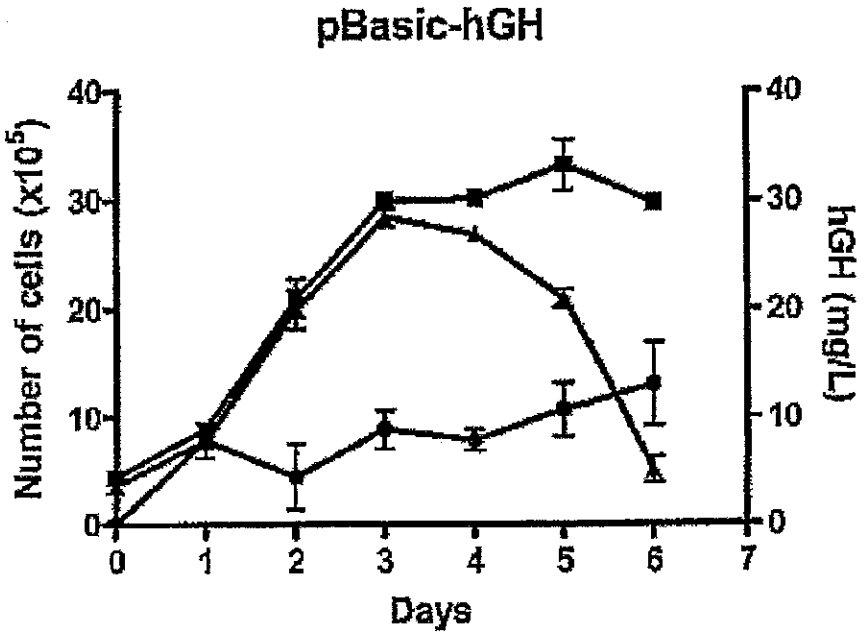
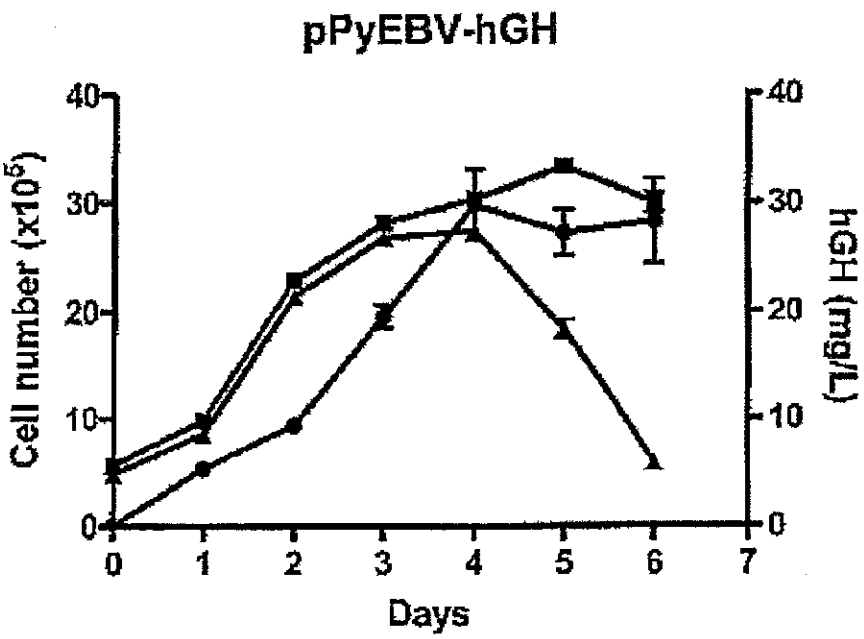

pBasic-hGH
pPyOri-hGH
pEBV-hGH
pEBV-PyOri-hGH
Cell number
ng/ml hGH
Days
Total cells
Total viable cells
ng/ml hGH
0
1000000
2000000
3000000
4000000
1000
2000
3000
4000
5000
6000
7000
2
4
6
8
10

Figure 12

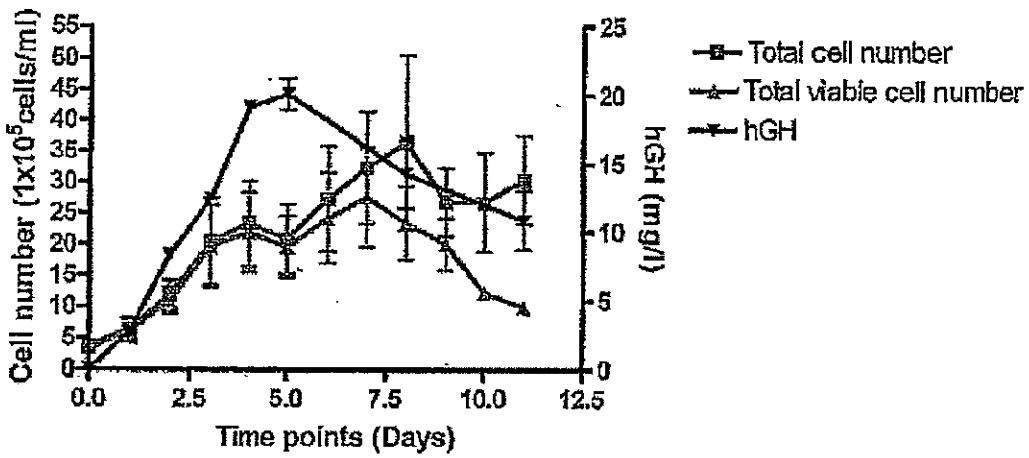
A


pBasic-hGH
Number of cells (x10^5)
hGH (mg/L)
Days
0
10
20
30
40
0
1
2
3
4
5
6
7

B


pPyEBV-hGH
Cell number (x10^5)
hGH (mg/L)
Days
0
10
20
30
40
0
1
2
3
4
5
6
7

A


Cell number (1x10^{5}cells/ml)
hGH (mg/l)
Time points (Days)
Total cell number
Total viable cell number
hGH

B

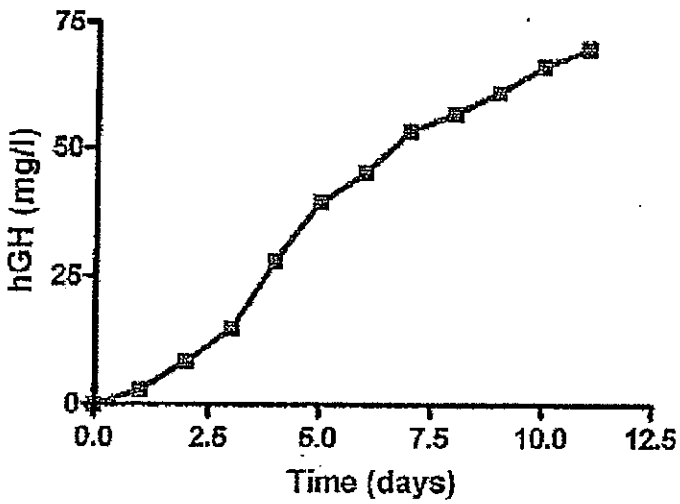

hGH (mg/l)
Time (days)

Figure 13

Figure 13

C

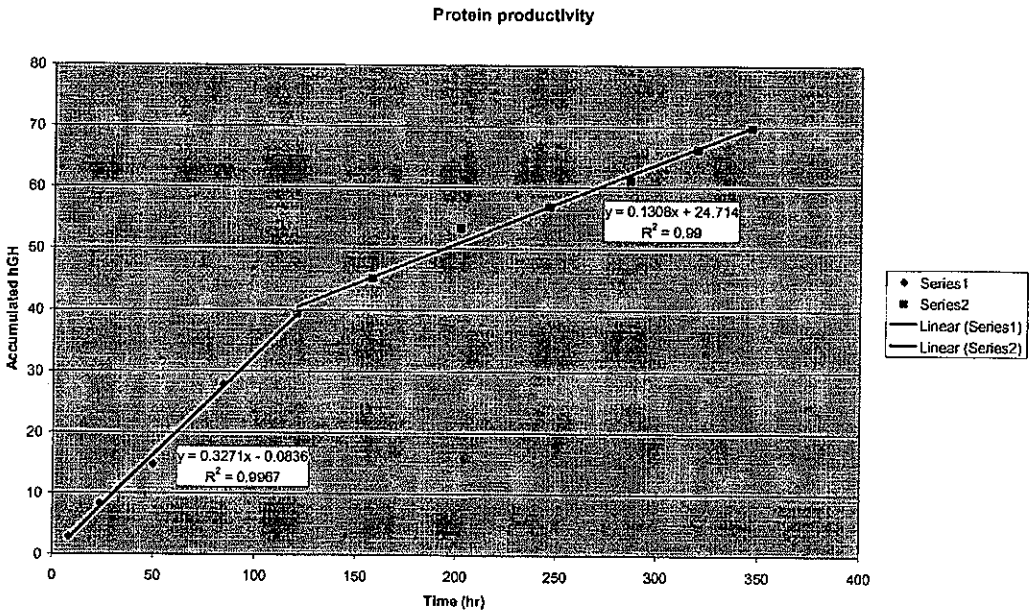
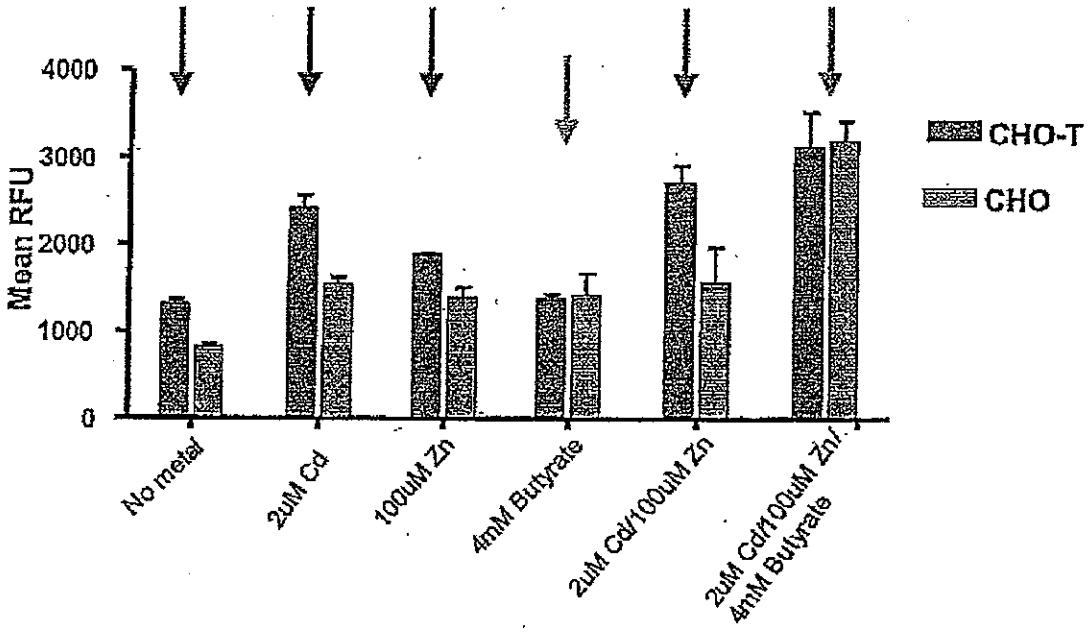

Protein productivity
Accumulated hGH
Time (hr)
y = 0.1308x + 24.714
R² = 0.99
y = 0.3271x - 0.0836
R² = 0.9967
Series1
Series2
Linear (Series1)
Linear (Series2)
0
10
20
30
40
50
60
70
80
0
50
100
150
200
250
300
350
400

Figure 14


4000
3000
2000
1000
0
Mean RFU
CHO-T
CHO
No metal
2uM Cd
100uM Zn
4mM Butyrate
2uM Cd/100uM Zn
2uM Cd/100uM Zn/
4mM Butyrate