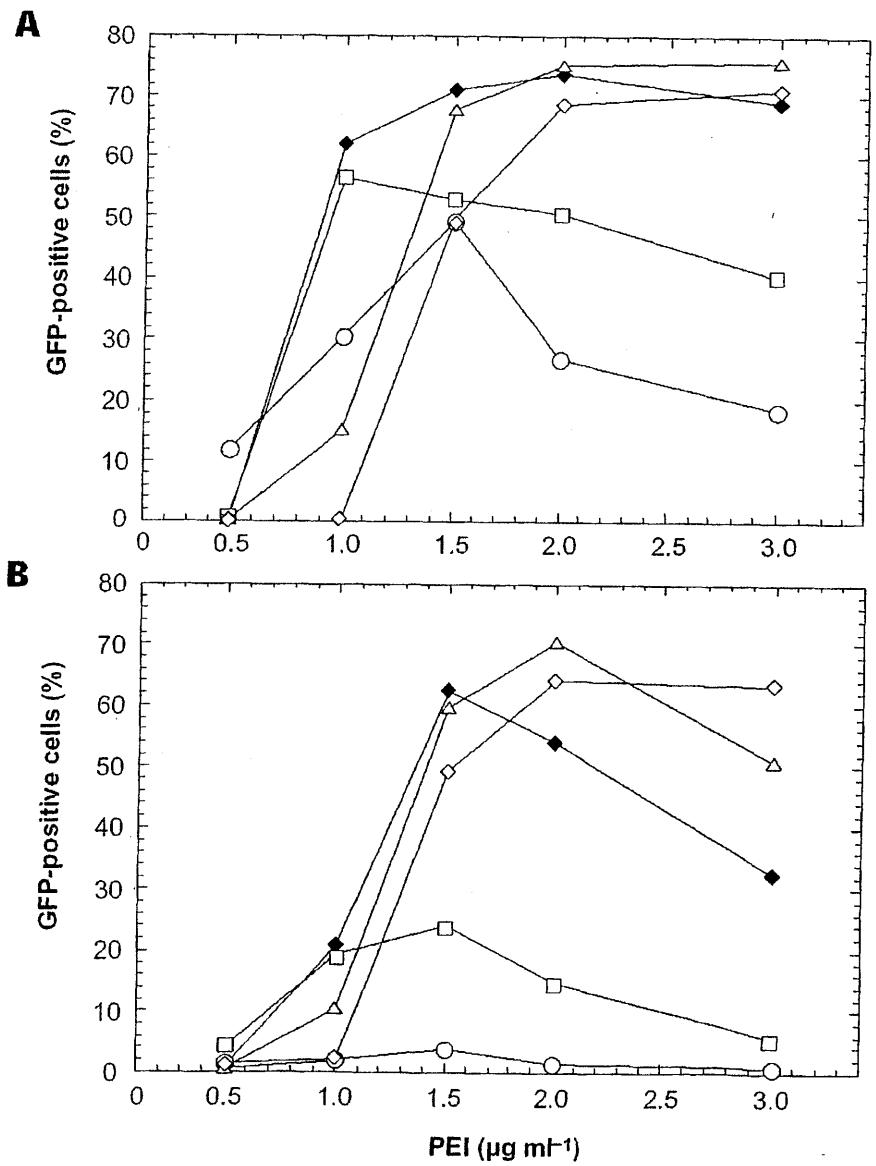

A
B
GFP-positive cells (%)
GFP-positive cells (%)
0
10
20
30
40
50
60
70
80
0
0.5
1.0
1.5
2.0
2.5
3.0
PEI (µg ml-1)

FIG. 1

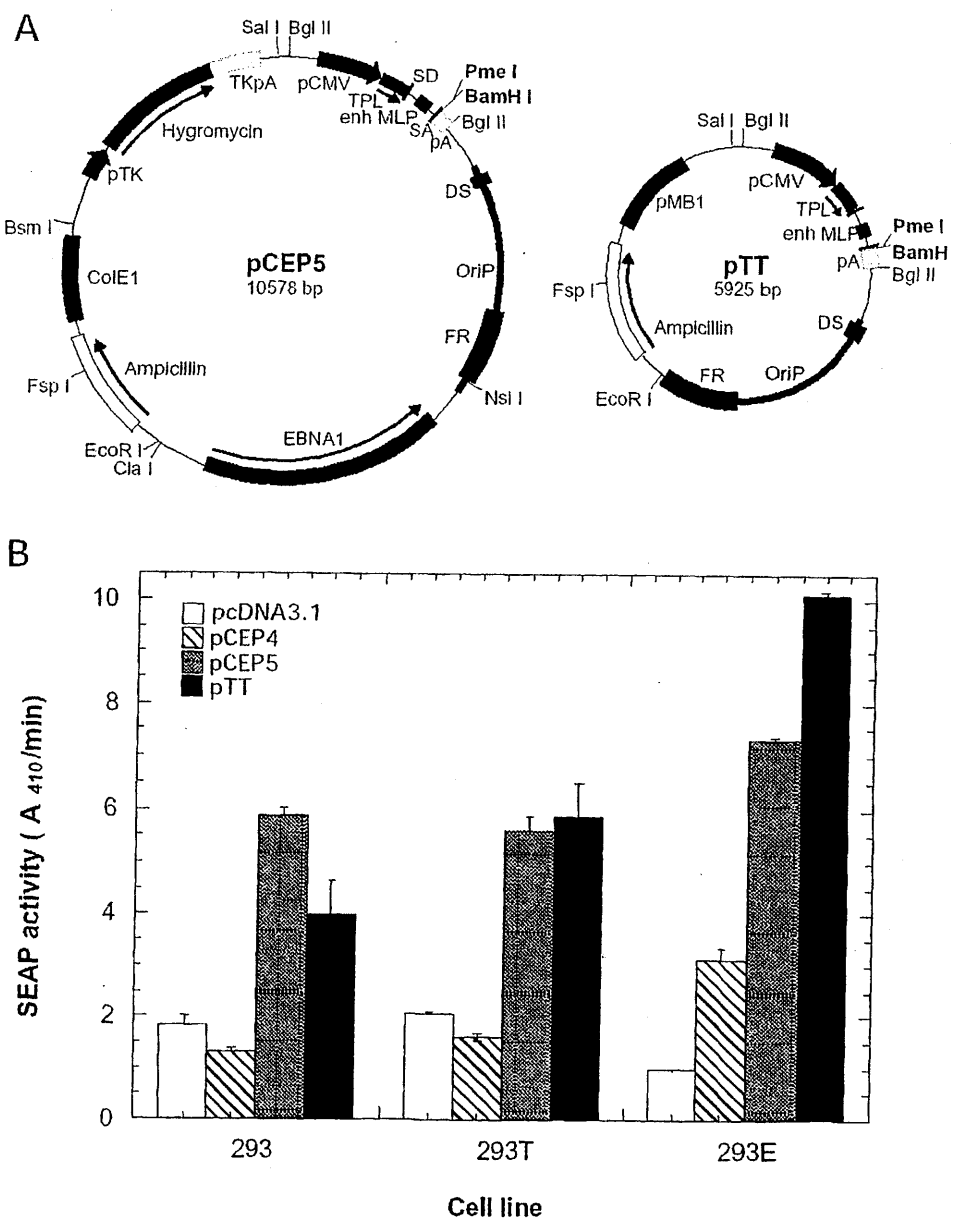

A
Sal I
Bgl II
TKpA
pCMV
SD
Pme I
BamH I
TPL
enh MLP
SA
Bgl II
pA
Hygromycin
pTK
DS
Bsm I
pCEP5
10578 bp
ColE1
OriP
FR
Ampicillin
Fsp I
NsI I
EBNA1
EcoR I
Cla I
Sal I
Bgl II
pMB1
pCMV
TPL
enh MLP
Pme I
BamH
pA
Bgl II
Fsp I
pTT
5925 bp
Ampicillin
DS
FR
OriP
EcoR I
B
pcDNA3.1
pCEP4
pCEP5
pTT
SEAP activity (A 410/min)
0
2
4
6
8
10
293
293T
293E
Cell line

FIG. 2

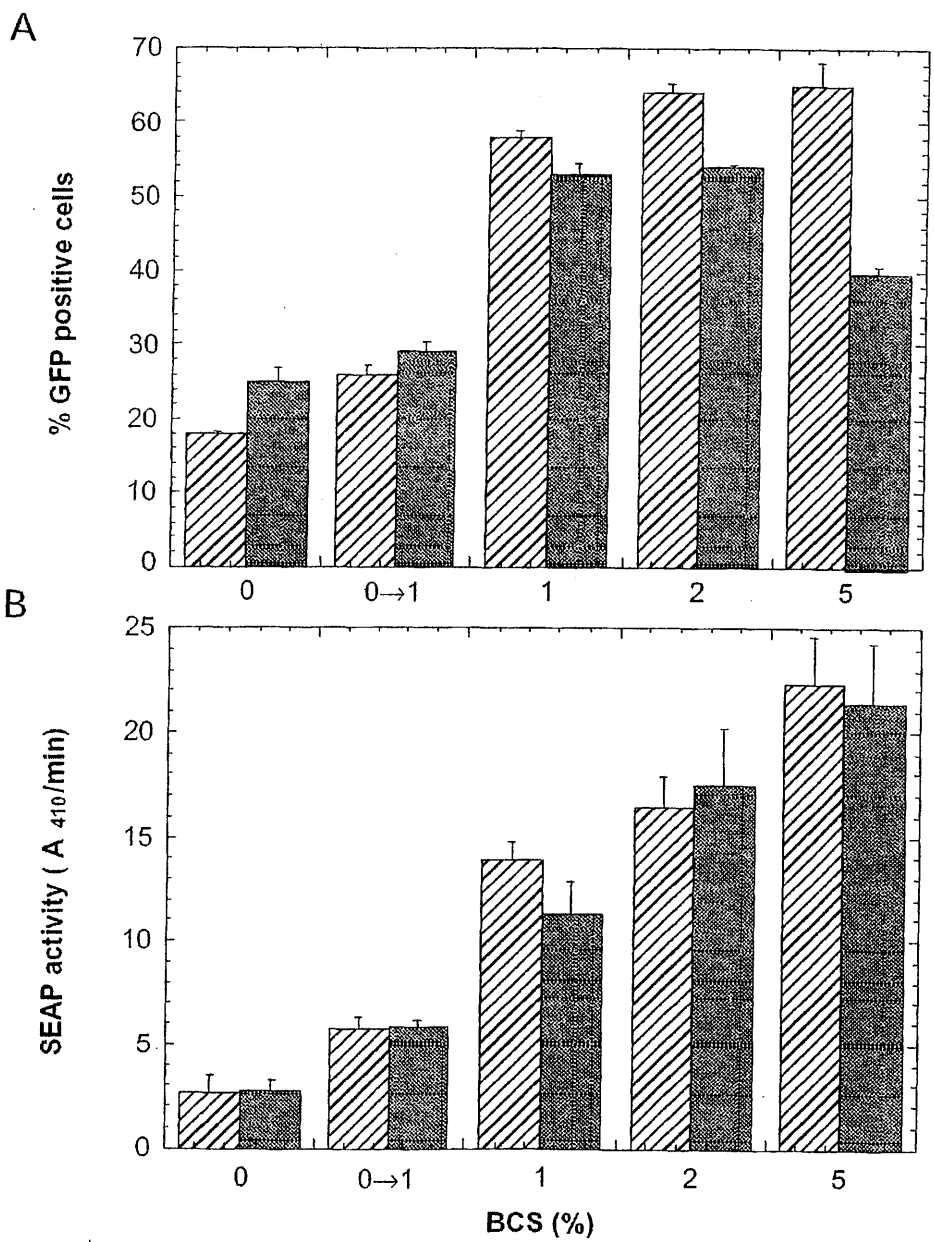

A
% GFP positive cells
70
60
50
40
30
20
10
0
0
0→1
1
2
5
B
SEAP activity (A 410/min)
25
20
15
10
5
0
0
0→1
1
2
5
BCS (%)

FIG. 3

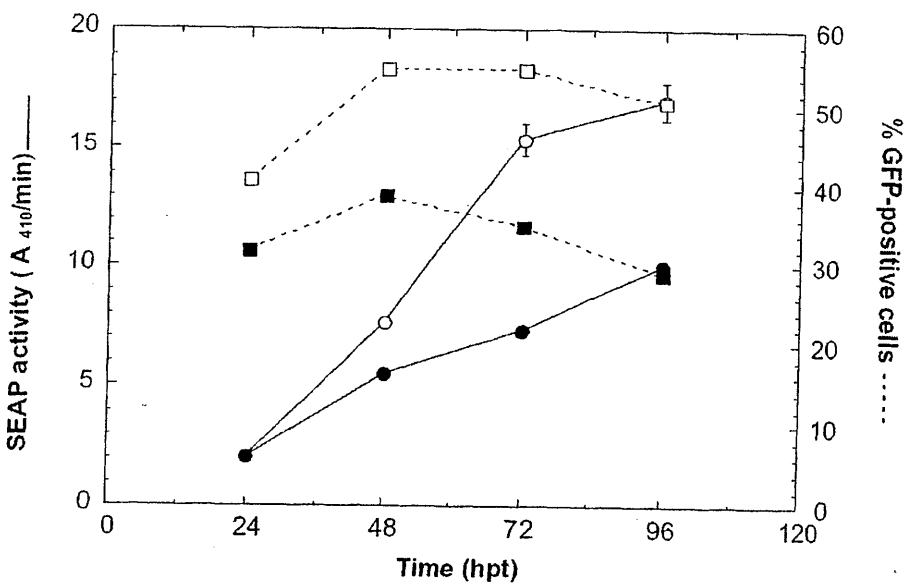

SEAP activity (A 410/min) ——
% GFP-positive cells .....
Time (hpt)
0
5
10
15
20
0
10
20
30
40
50
60
0
24
48
72
96
120

FIG. 4

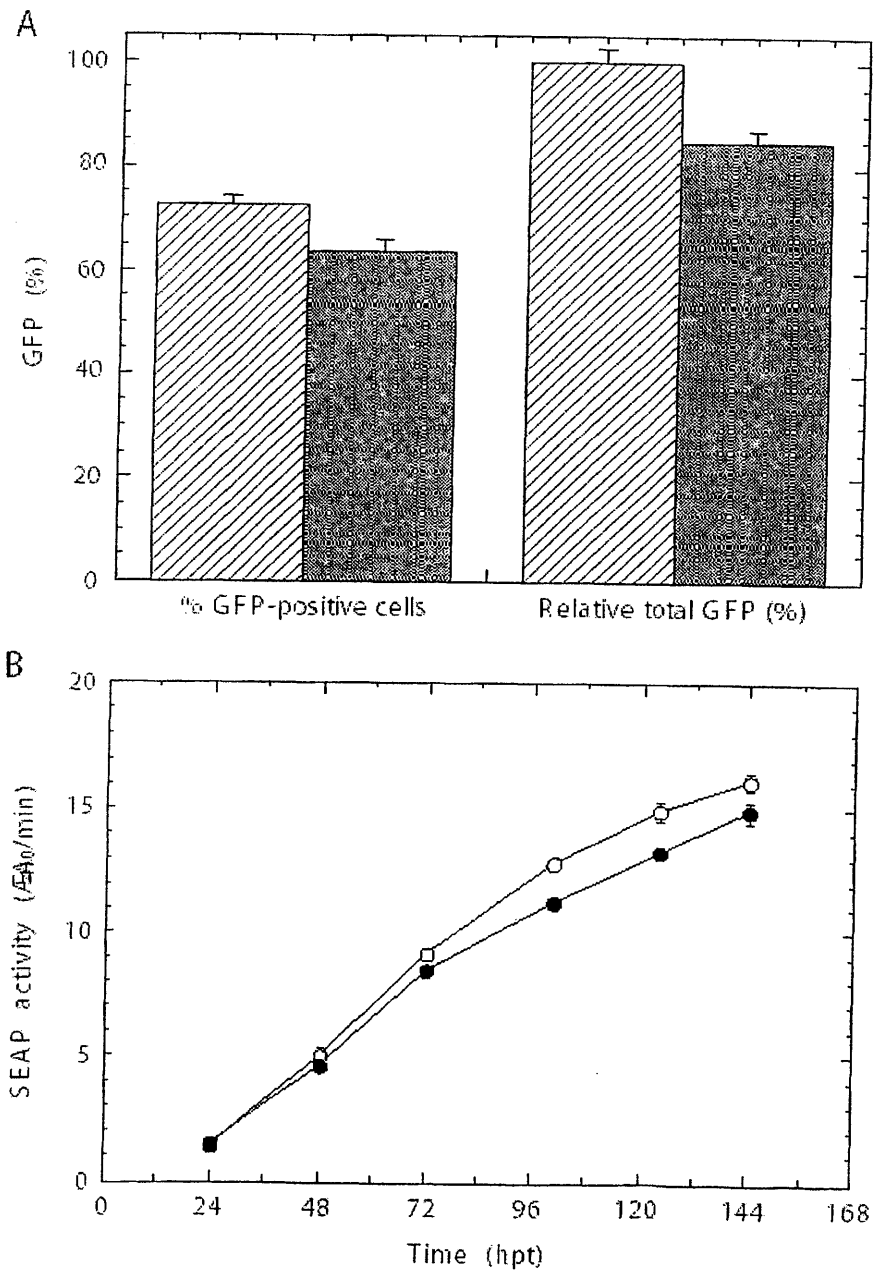
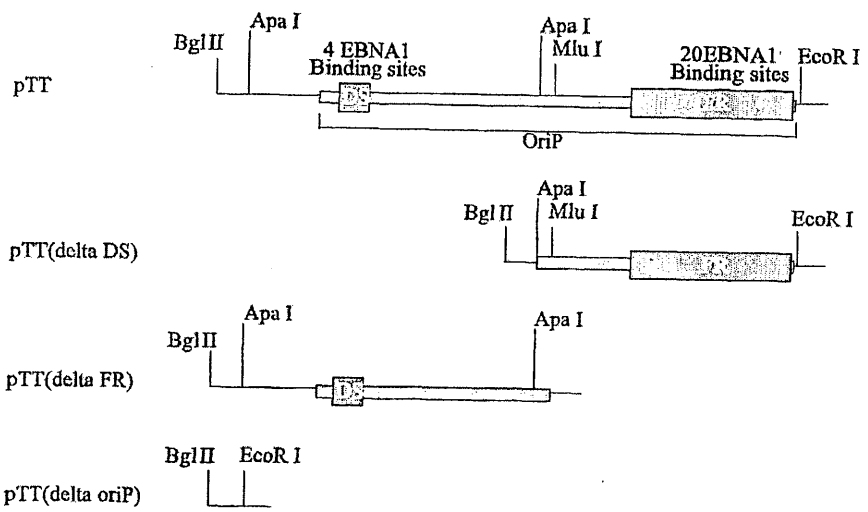

A
100
80
60
40
20
0
GFP (%)
% GFP-positive cells
Relative total GFP (%)
B
20
15
10
5
0
SEAP activity (ΔA/min)
0
24
48
72
96
120
144
168
Time (hpt)

FIG. 5


pTT
Bgl II
Apa I
4 EBNA1 Binding sites
Apa I
Mlu I
20EBNA1 Binding sites
EcoR I
DS
FR
OriP
pTT(delta DS)
Bgl II
Apa I
Mlu I
EcoR I
pTT(delta FR)
Bgl II
Apa I
Apa I
DS
pTT(delta oriP)
Bgl II
EcoR I

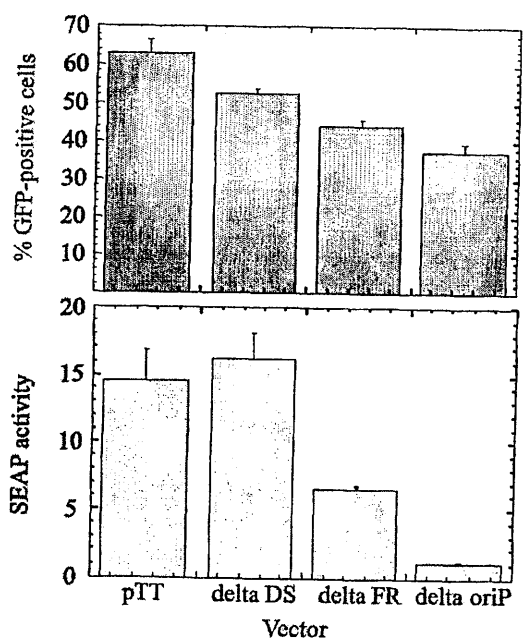

% GFP-positive cells
70
60
50
40
30
20
10
SEAP activity
20
15
10
5
0
pTT
delta DS
delta FR
delta oriP
Vector

FIG. 6

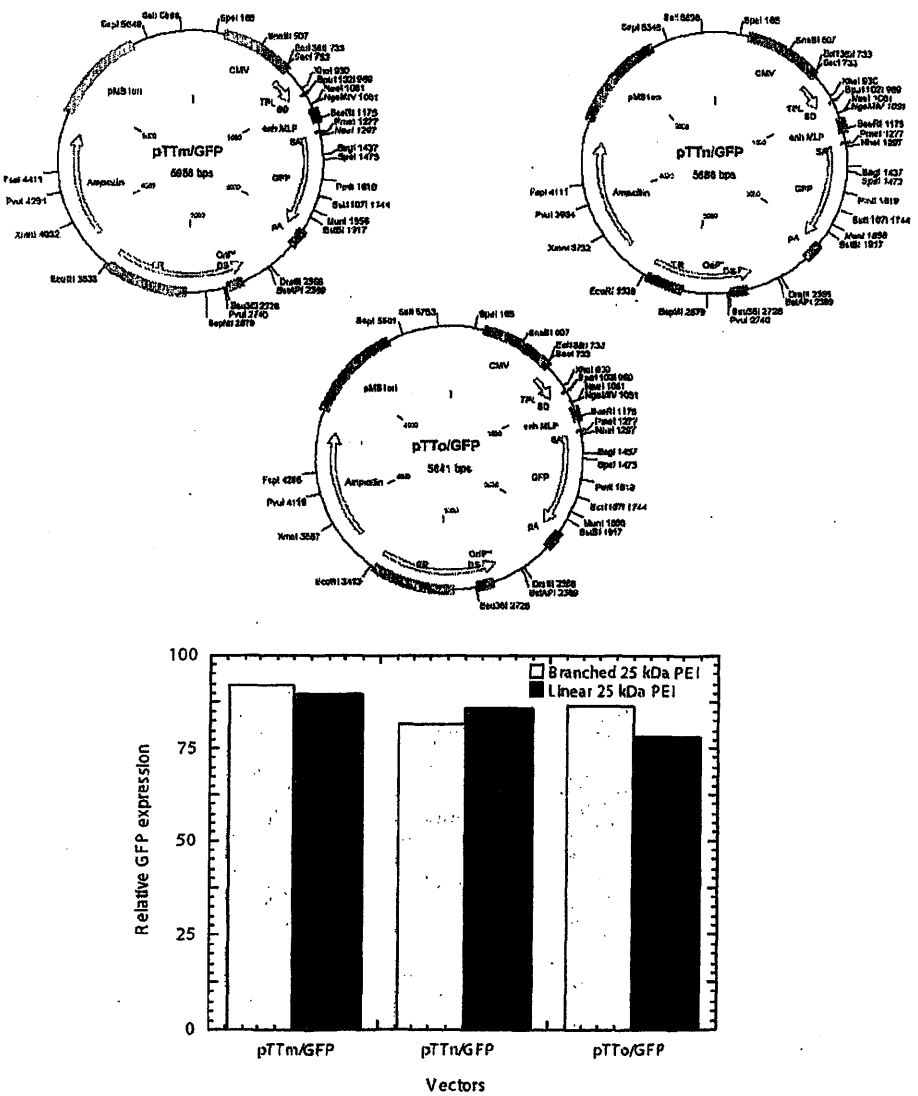

pTTm/GFP
5656 bps
pTTn/GFP
5686 bps
pTTo/GFP
5641 bps
Relative GFP expression
100
75
50
25
0
Branched 25 kDa PEI
Linear 25 kDa PEI
pTTm/GFP
pTTn/GFP
pTTo/GFP
Vectors

FIG. 7

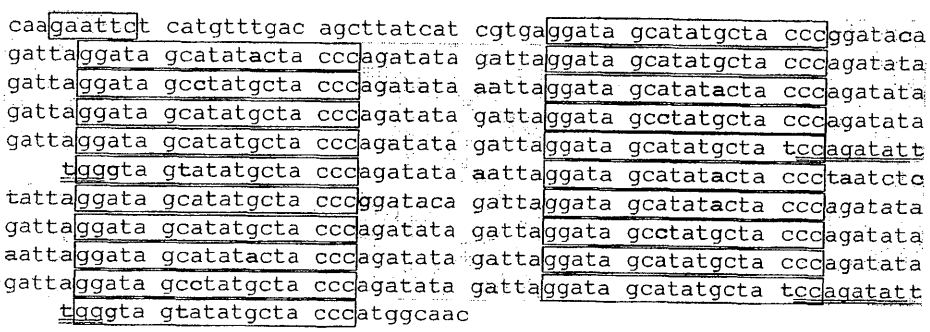
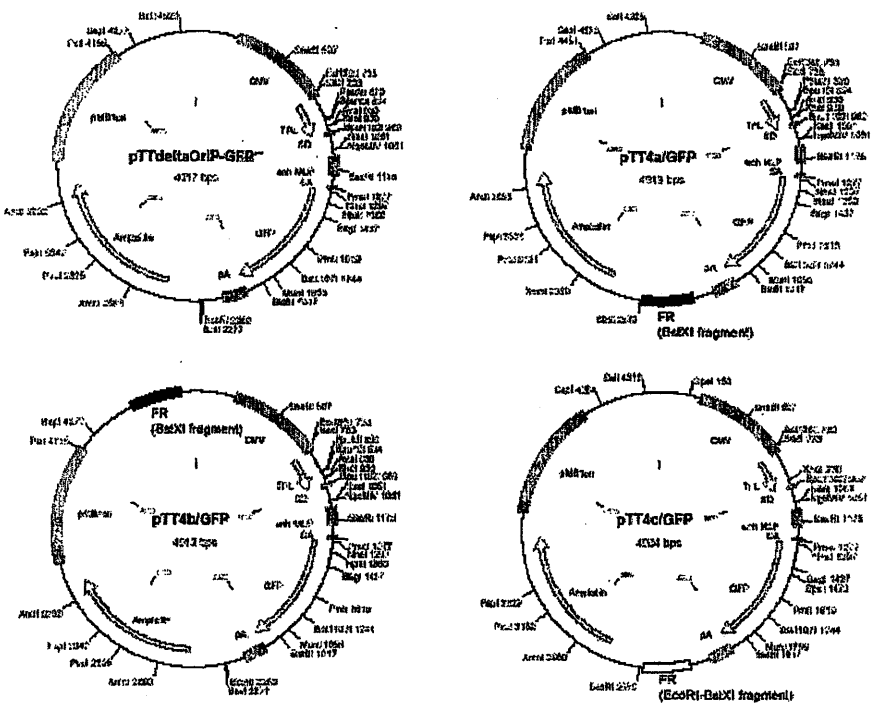

caagaattct catgtttgac agcttatcat cgtgaggata gcatatgcta cccggataca
gattaggata gcatatacta cccagatata gattaggata gcatatgcta cccagatata
gattaggata gcctatgcta cccagatata aattaggata gcatatacta cccagatata
gattaggata gcatatgcta cccagatata gattaggata gcctatgcta cccagatata
gattaggata gcatatgcta cccagatata gattaggata gcatatgcta tccagatatt
tgggta gtatatgcta cccagatata aattaggata gcatatacta ccctaatctc
tattaggata gcatatgcta cccggataca gattaggata gcatatacta cccagatata
gattaggata gcatatgcta cccagatata gattaggata gcctatgcta cccagatata
aattaggata gcatatacta cccagatata gattaggata gcatatgcta cccagatata
gattaggata gcctatgcta cccagatata gattaggata gcatatgcta tccagatatt
tgggta gtatatgcta cccatggcaac

FIG. 8

A


pTTdeltaOriP-GRR
pTT4a/GFP
FR
(BstXI fragment)
pTT4b/GFP
pTT4c/GFP
(EcoRI-BstXI fragment)

B

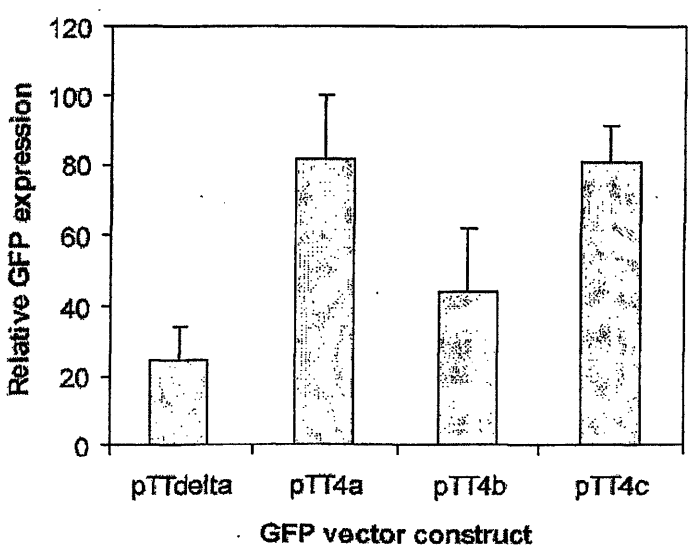
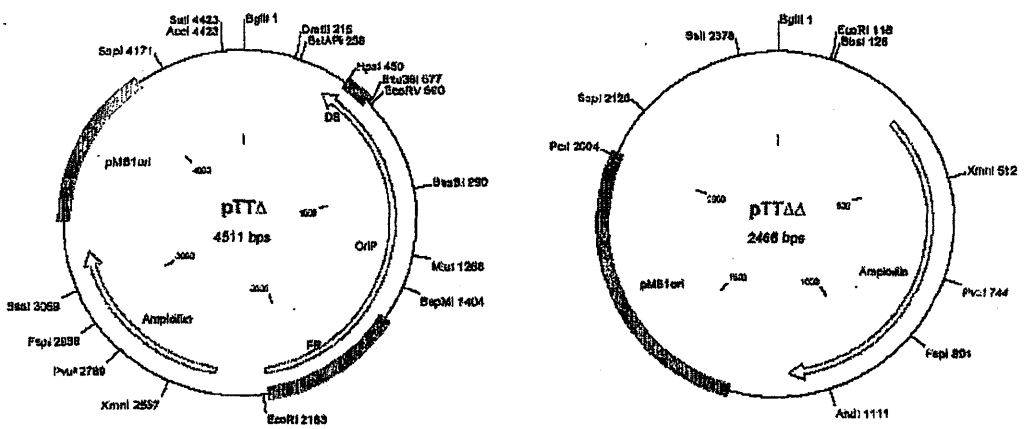

120
100
80
60
40
20
0
Relative GFP expression
pTTdelta
pTT4a
pTT4b
pTT4c
GFP vector construct

FIG. 9


pTTΔ
4511 bps
pTTΔΔ
2466 bps
pMB1ori
Ampicillin
OriP
EBNA1
DS
SalI 4423
AccI 4423
BglII 1
SapI 4171
NruI 450
Bsu36I 577
EcoRV 660
BssSI 290
MluI 1266
BspMI 1404
SalI 3068
FspI 2898
PvuI 2789
XmnI 2557
EcoRI 2169
SalI 2378
BglII 1
EcoRI 118
BsaI 126
SapI 2120
PciI 2004
XmnI 512
PvuI 744
FspI 801
AseI 1111

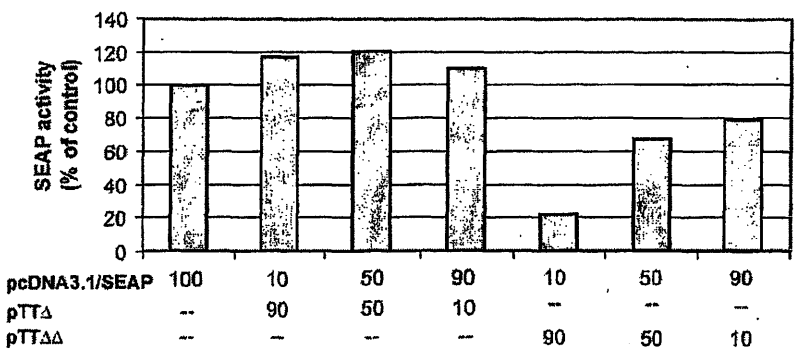

SEAP activity
(% of control)
140
120
100
80
60
40
20
0
pcDNA3.1/SEAP 100 10 50 90 10 50 90
pTTΔ -- 90 50 10 -- -- --
pTTΔΔ -- -- -- -- 90 50 10

FIG. 10

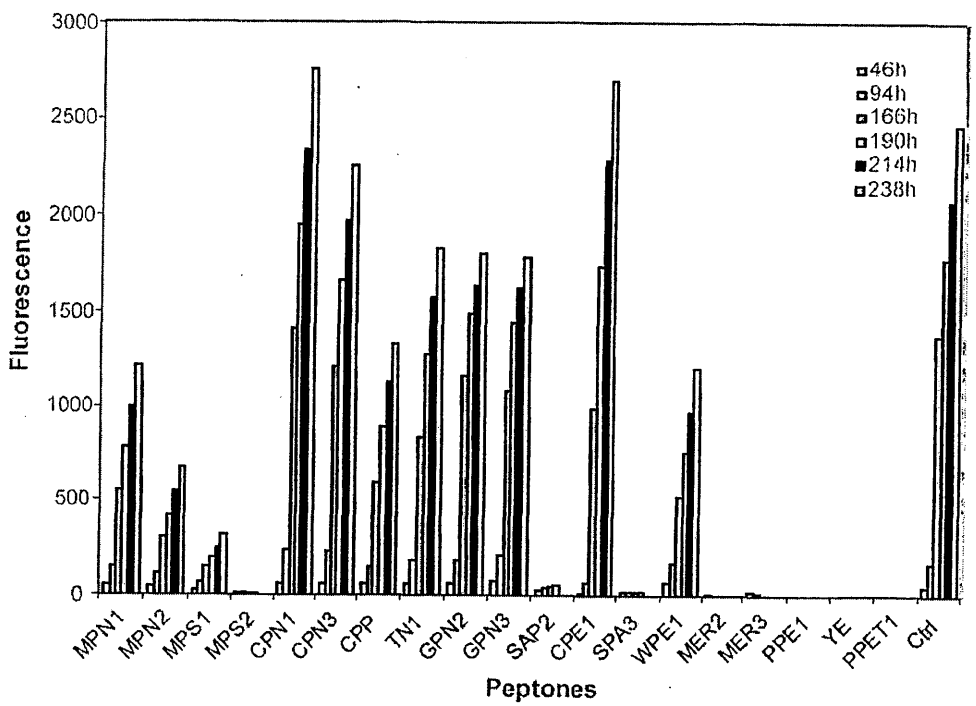

3000
2500
2000
1500
1000
500
0
Fluorescence
46h
94h
166h
190h
214h
238h
MPN1
MPN2
MPS1
MPS2
CPN1
CPN3
CPP
TN1
GPN2
GPN3
SAP2
CPE1
SPA3
WPE1
MER2
MER3
PPE1
YE
PPET1
Ctrl
Peptones

FIG. 11

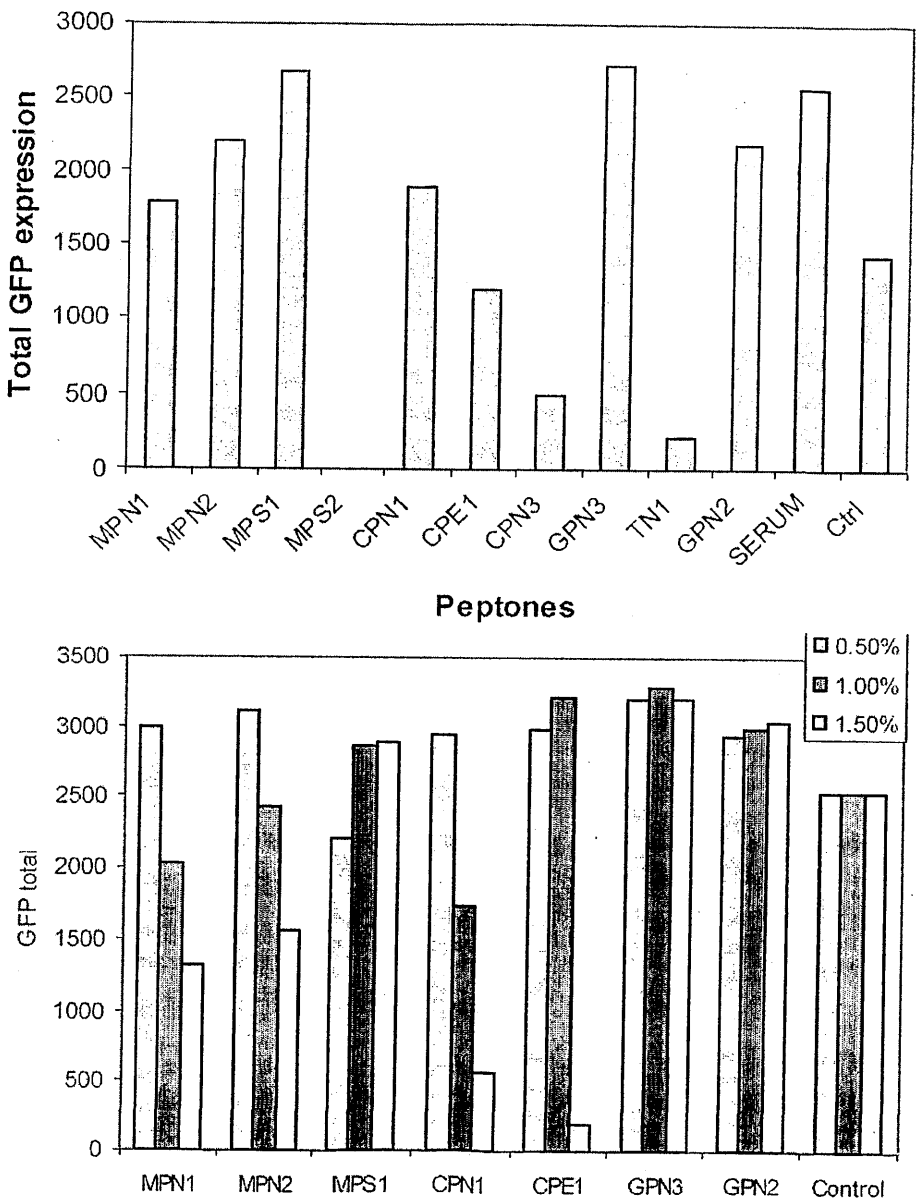

Total GFP expression
3000
2500
2000
1500
1000
500
0
MPN1
MPN2
MPS1
MPS2
CPN1
CPE1
CPN3
GPN3
TN1
GPN2
SERUM
Ctrl
Peptones
0.50%
1.00%
1.50%
GFP total
3500
3000
2500
2000
1500
1000
500
0
MPN1
MPN2
MPS1
CPN1
CPE1
GPN3
GPN2
Control

FIG. 12

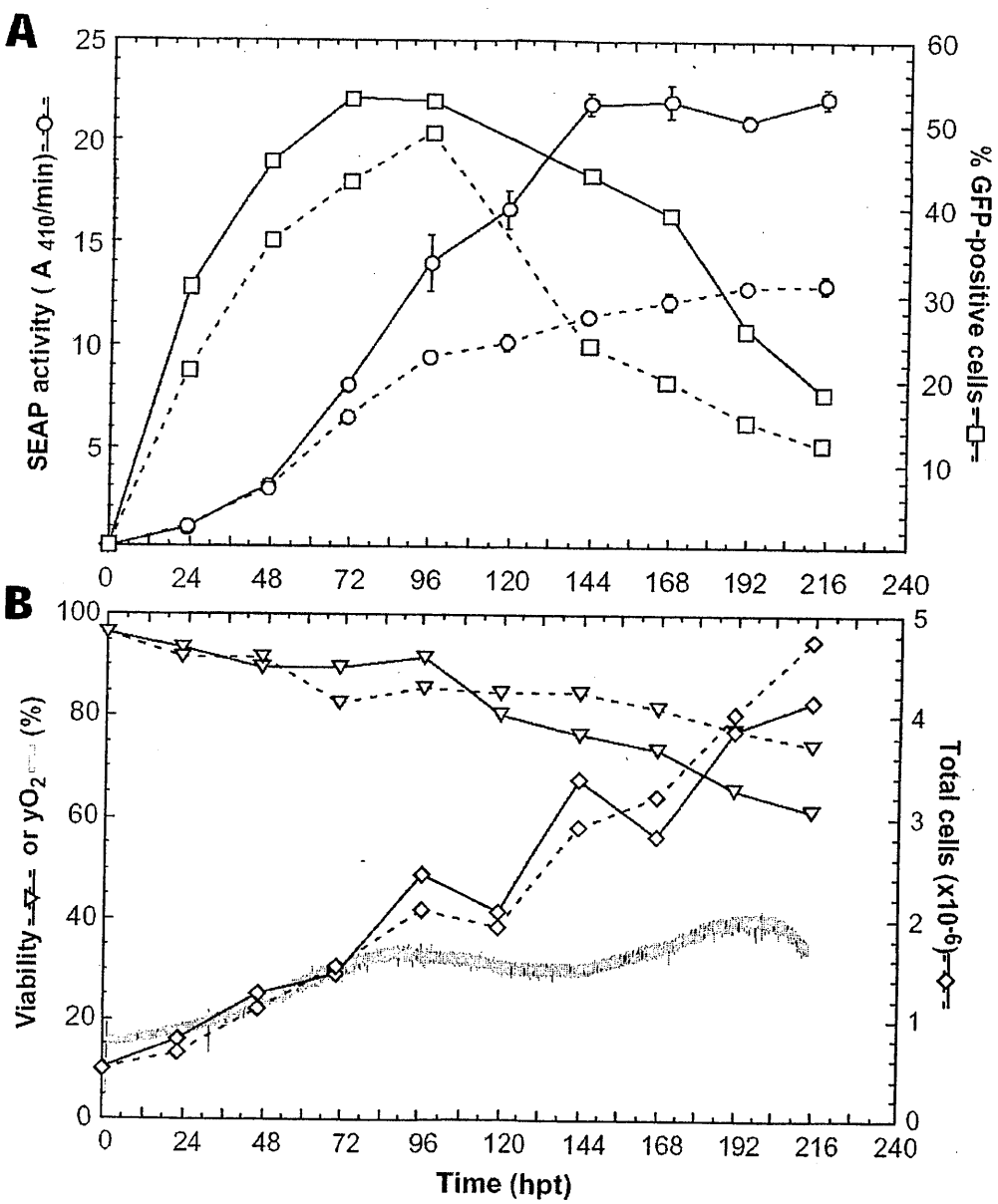

A
B
SEAP activity (A 410/min)
% GFP-positive cells
Viability or yO2 (%)
Total cells (x10-6)
Time (hpt)
0
24
48
72
96
120
144
168
192
216
240

FIG. 13

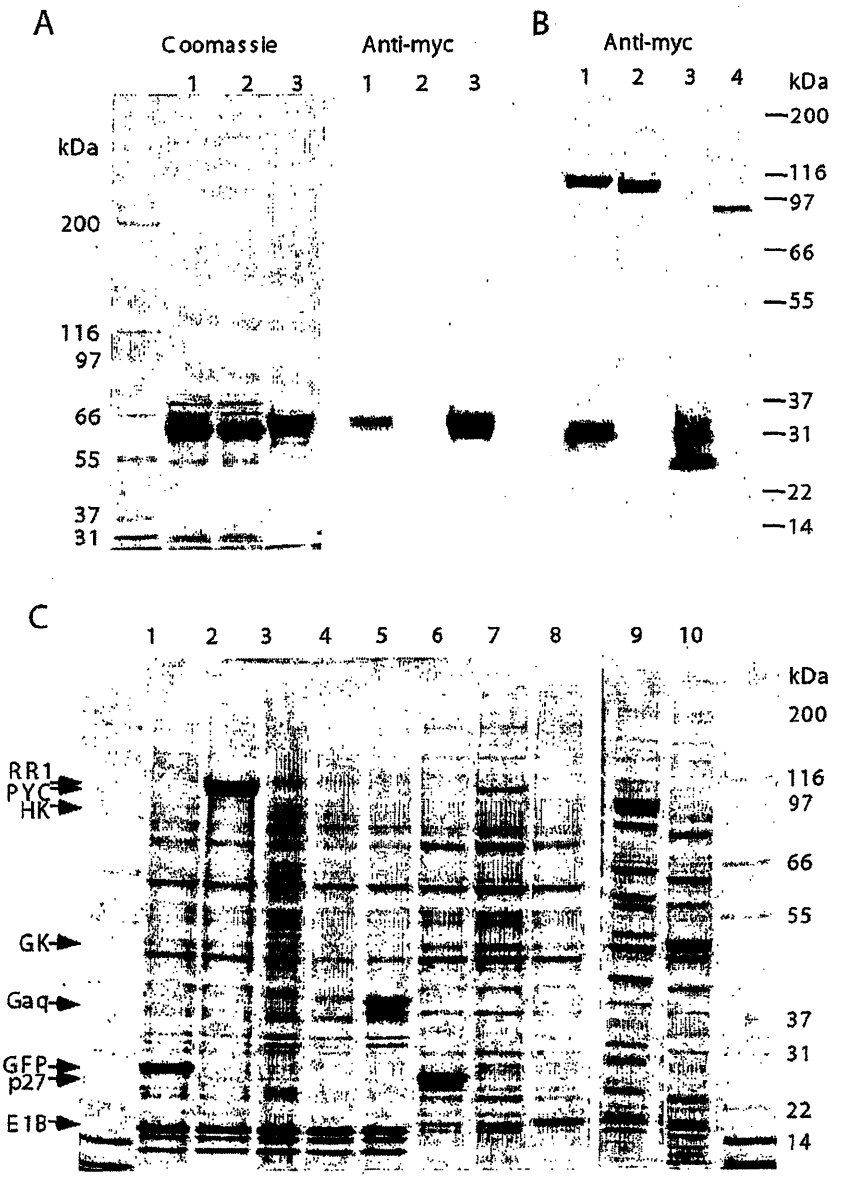
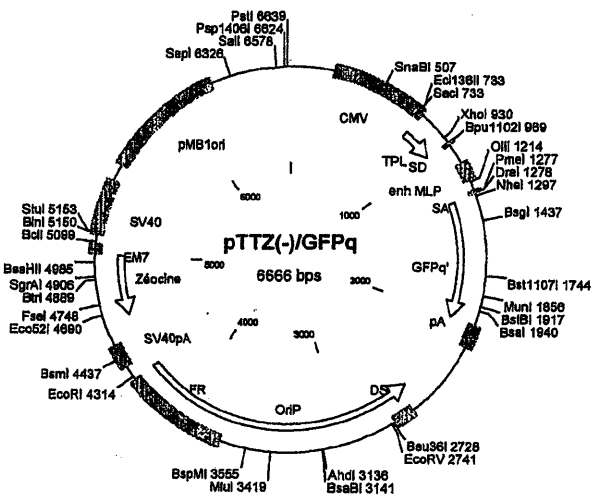

A
Coomassie
Anti-myc
1 2 3 1 2 3
kDa
200
116
97
66
55
37
31
B
Anti-myc
1 2 3 4
kDa
200
116
97
66
55
37
31
22
14
C
1 2 3 4 5 6 7 8 9 10
kDa
200
116
97
66
55
37
31
22
14
RR1
PYC
HK
GK
Gag
GFP
p27
E1B

FIG. 14


pTTZ(-)/GFPq
6666 bps
CMV
TPL
SD
enh MLP
SA
GFPq'
pA
pMB1ori
SV40
EM7
Zéocine
SV40pA
FR
OriP
DS
PstI 6639
Psp1406I 6824
SalI 6578
SspI 6326
SnaBI 507
Eci136II 733
SacI 733
XhoI 930
Bpu1102I 989
OliI 1214
PmeI 1277
DraI 1278
NheI 1297
BsgI 1437
StuI 5153
BlnI 5150
BclI 5069
BssHII 4985
SgrAI 4906
BtrI 4869
FseI 4748
Eco52I 4690
BsmI 4437
EcoRI 4314
BspMI 3555
MluI 3419
AhdI 3136
BsaBI 3141
Bsu36I 2728
EcoRV 2741
Bst1107I 1744
MunI 1856
BstBI 1917
BsaI 1940

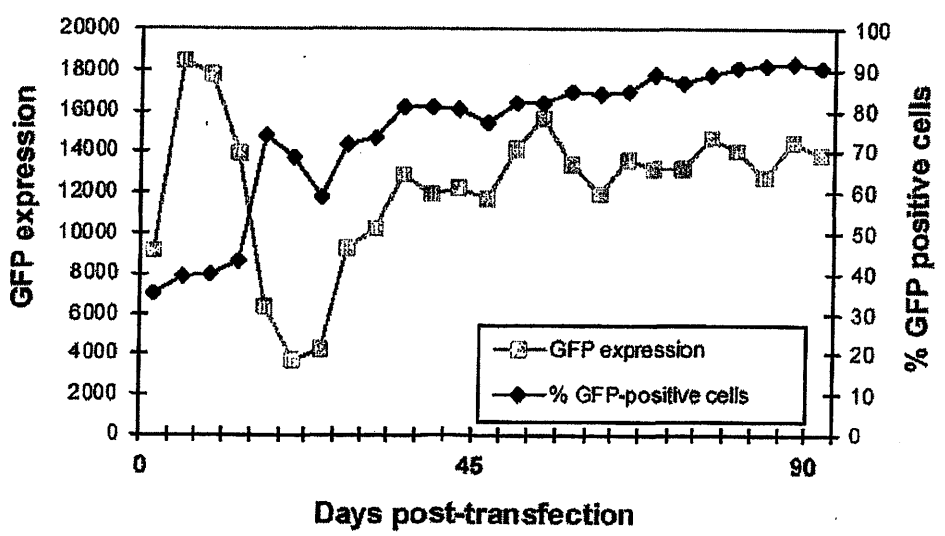

GFP expression
% GFP positive cells
Days post-transfection
GFP expression
% GFP-positive cells
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
100
90
80
70
60
50
40
30
20
10
0
0
45
90

FIG. 15

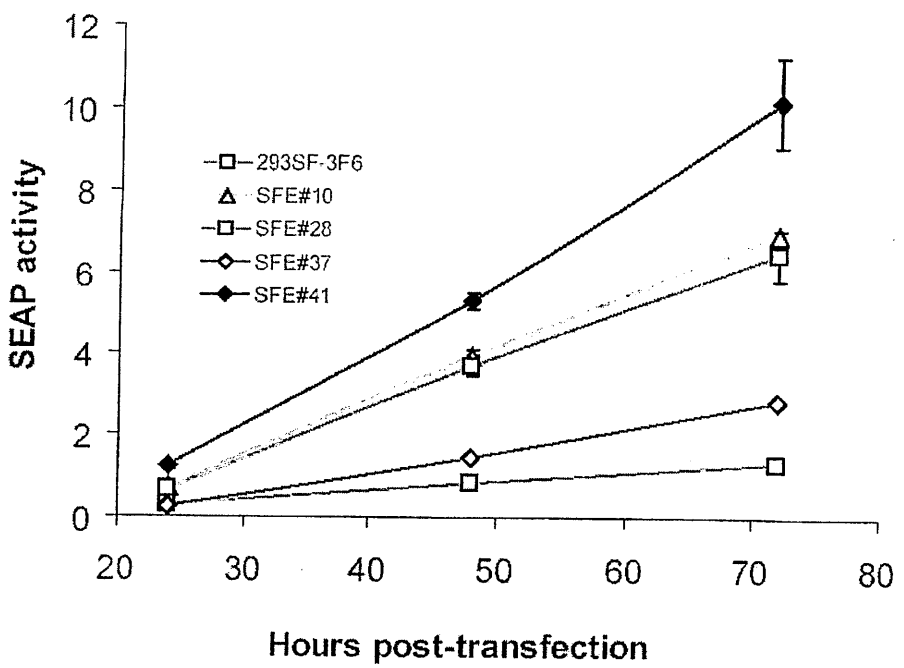

12
10
8
6
4
2
0
SEAP activity
293SF-3F6
SFE#10
SFE#28
SFE#37
SFE#41
20
30
40
50
60
70
80
Hours post-transfection

FIG. 16

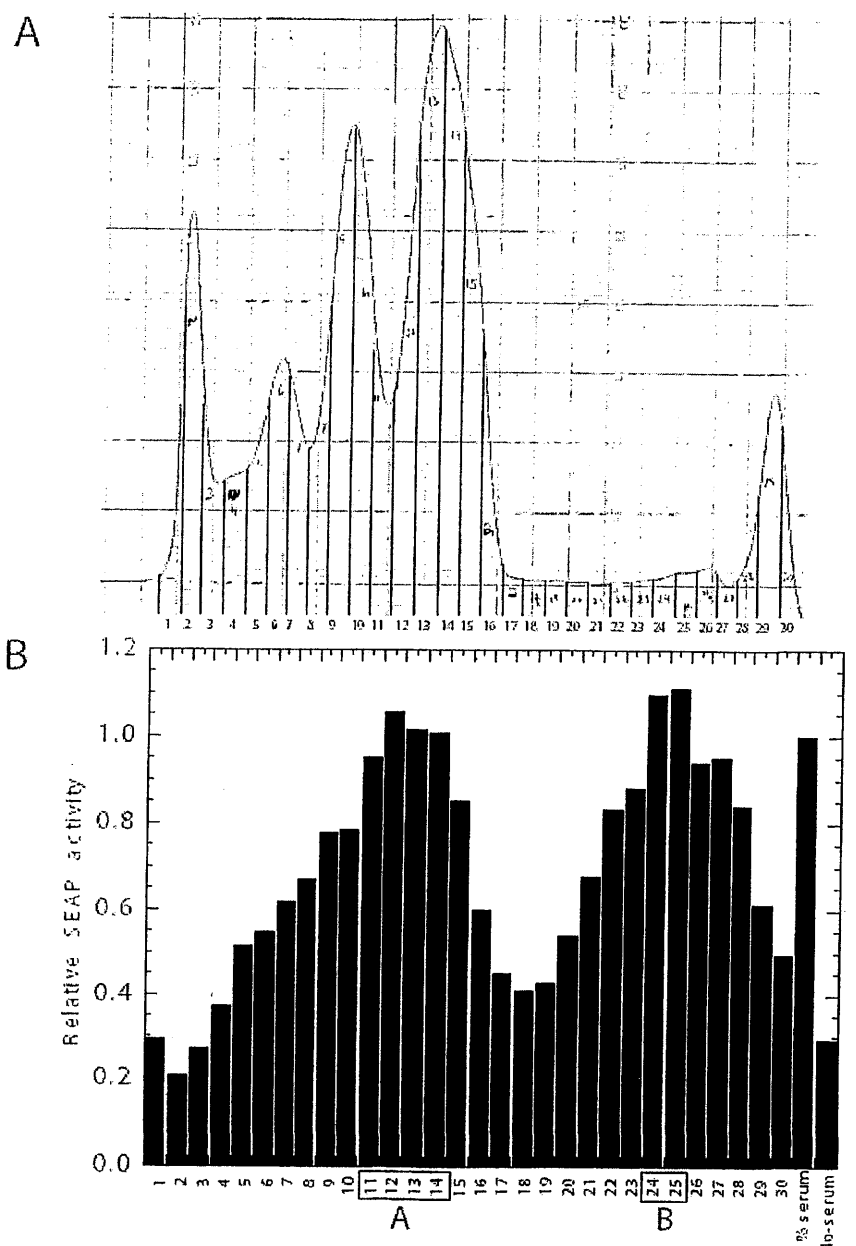

A
B
Relative SEAP activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
B
1% serum
No-serum

FIG. 17

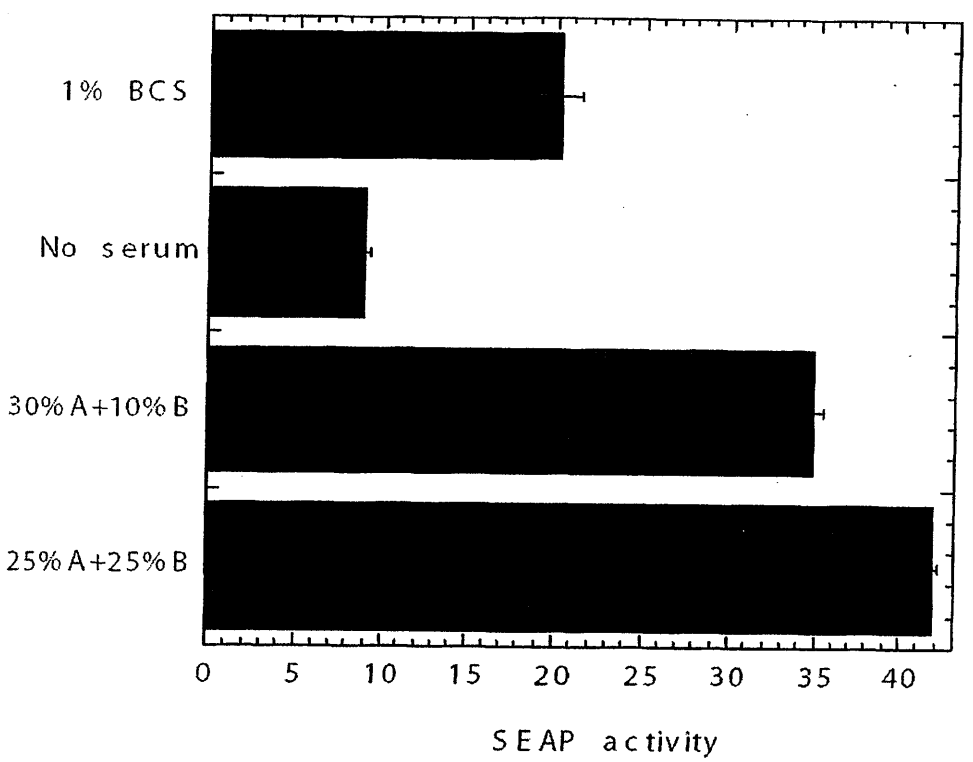

1% BCS
No serum
30% A+10% B
25% A+25% B
0
5
10
15
20
25
30
35
40
SEAP activity

FIG. 18