FIGURE 1

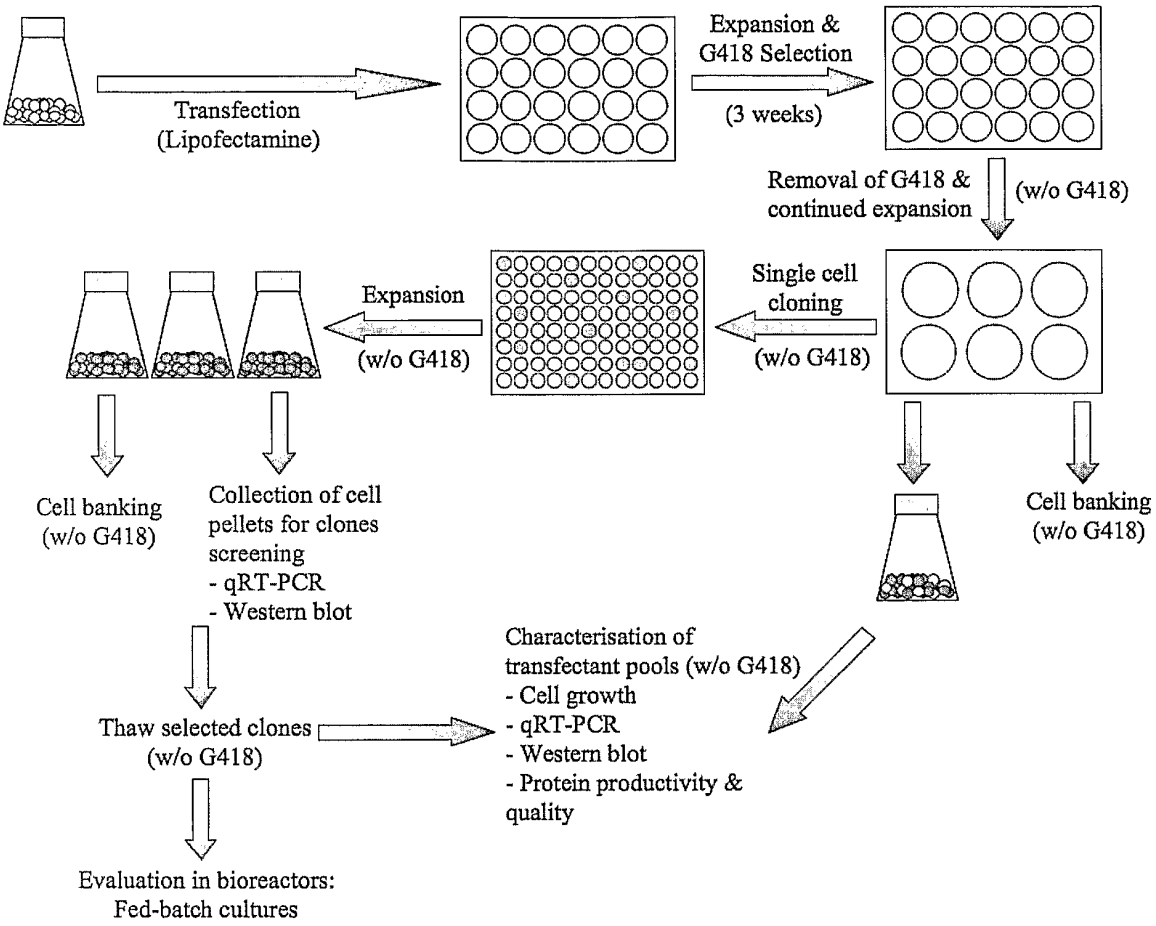

Transfection
(Lipofectamine)
Expansion &
G418 Selection
(3 weeks)
Removal of G418 &
continued expansion
(w/o G418)
Single cell
cloning
(w/o G418)
Expansion
(w/o G418)
Cell banking
(w/o G418)
Collection of cell
pellets for clones
screening
- qRT-PCR
- Western blot
Cell banking
(w/o G418)
Characterisation of
transfectant pools (w/o G418)
- Cell growth
- qRT-PCR
- Western blot
- Protein productivity &
quality
Thaw selected clones
(w/o G418)
Evaluation in bioreactors:
Fed-batch cultures

FIGURE 2

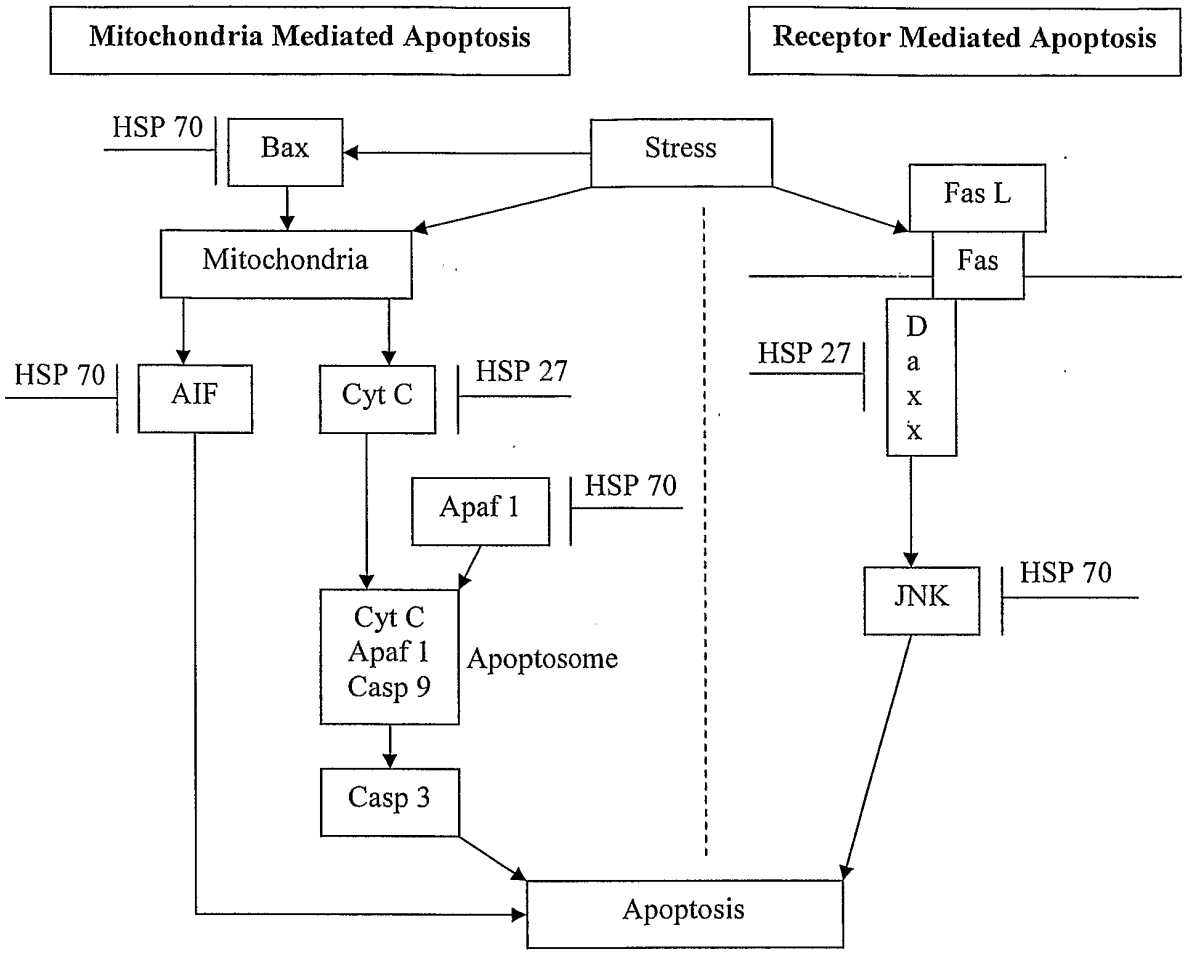

Mitochondria Mediated Apoptosis
Receptor Mediated Apoptosis
HSP 70
Bax
Stress
Fas L
Mitochondria
Fas
HSP 70
AIF
Cyt C
HSP 27
HSP 27
D
a
x
x
Apaf 1
HSP 70
JNK
HSP 70
Cyt C
Apaf 1
Casp 9
Apoptosome
Casp 3
Apoptosis

FIGURE 3

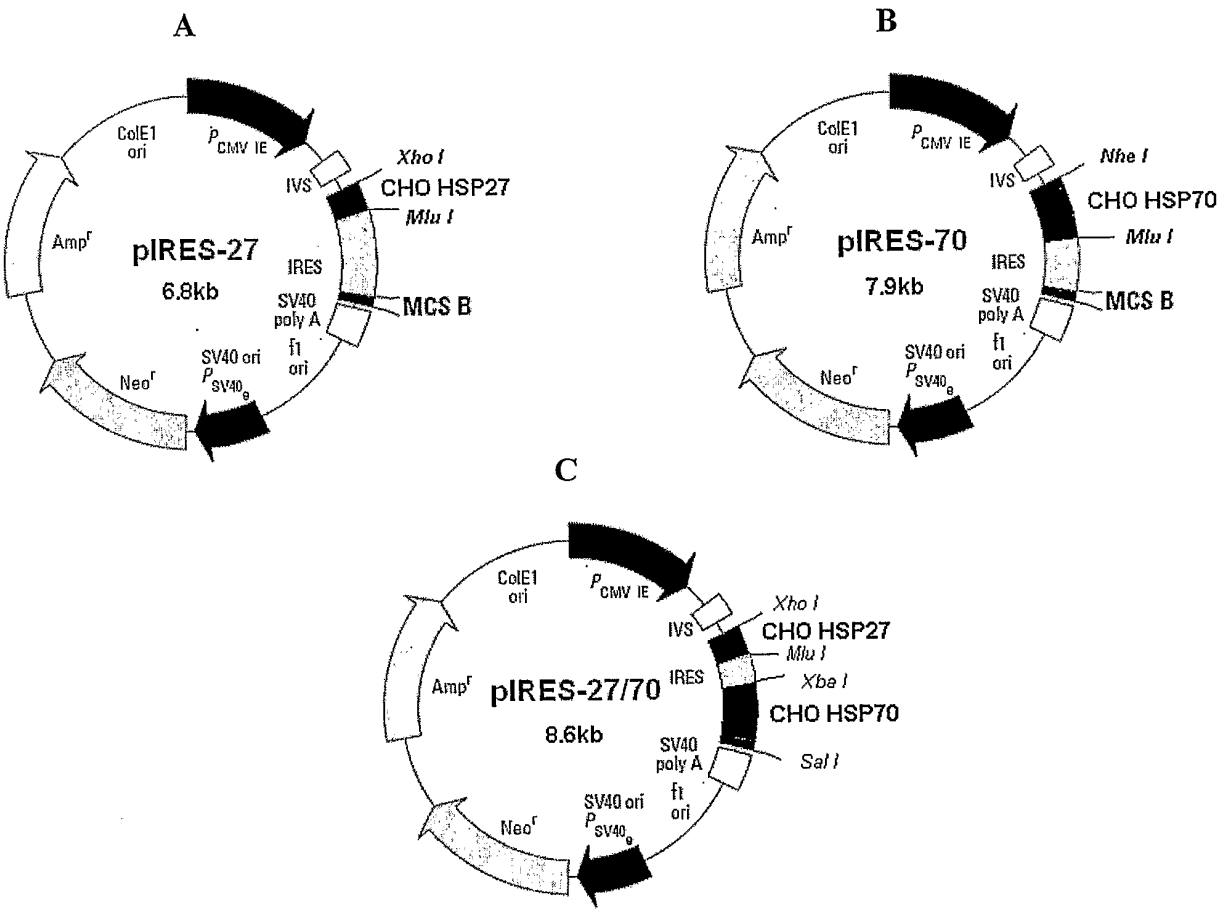
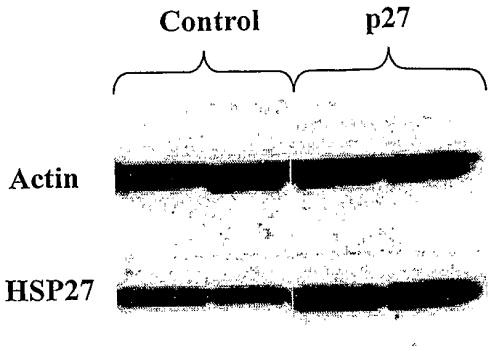
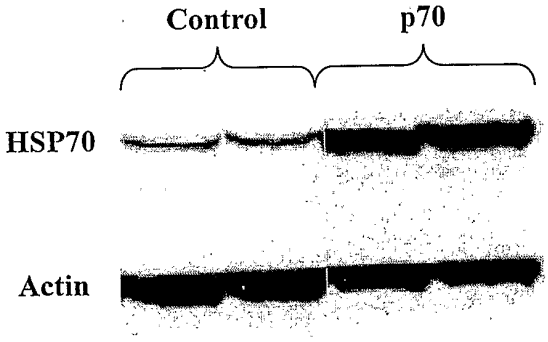

A
ColE1 ori
P CMV IE
IVS
Xho I
CHO HSP27
Mlu I
MCS B
IRES
SV40 poly A
f1 ori
SV40 ori
P SV40
Neo r
Amp r
pIRES-27
6.8kb
B
ColE1 ori
P CMV IE
IVS
Nhe I
CHO HSP70
Mlu I
MCS B
IRES
SV40 poly A
f1 ori
SV40 ori
P SV40
Neo r
Amp r
pIRES-70
7.9kb
C
ColE1 ori
P CMV IE
IVS
Xho I
CHO HSP27
Mlu I
IRES
Xba I
CHO HSP70
SV40 poly A
Sal I
f1 ori
SV40 ori
P SV40
Neo r
Amp r
pIRES-27/70
8.6kb

FIGURE 4

A.


Control
p27
Actin
HSP27

B.


Control
p70
HSP70
Actin

C.

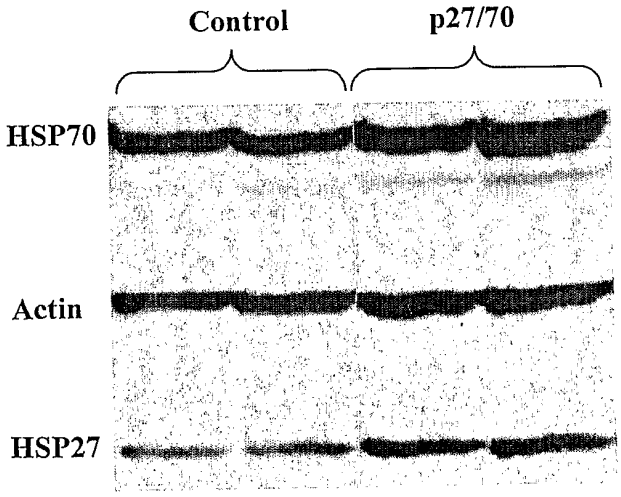

Control
p27/70
HSP70
Actin
HSP27

FIGURE 5

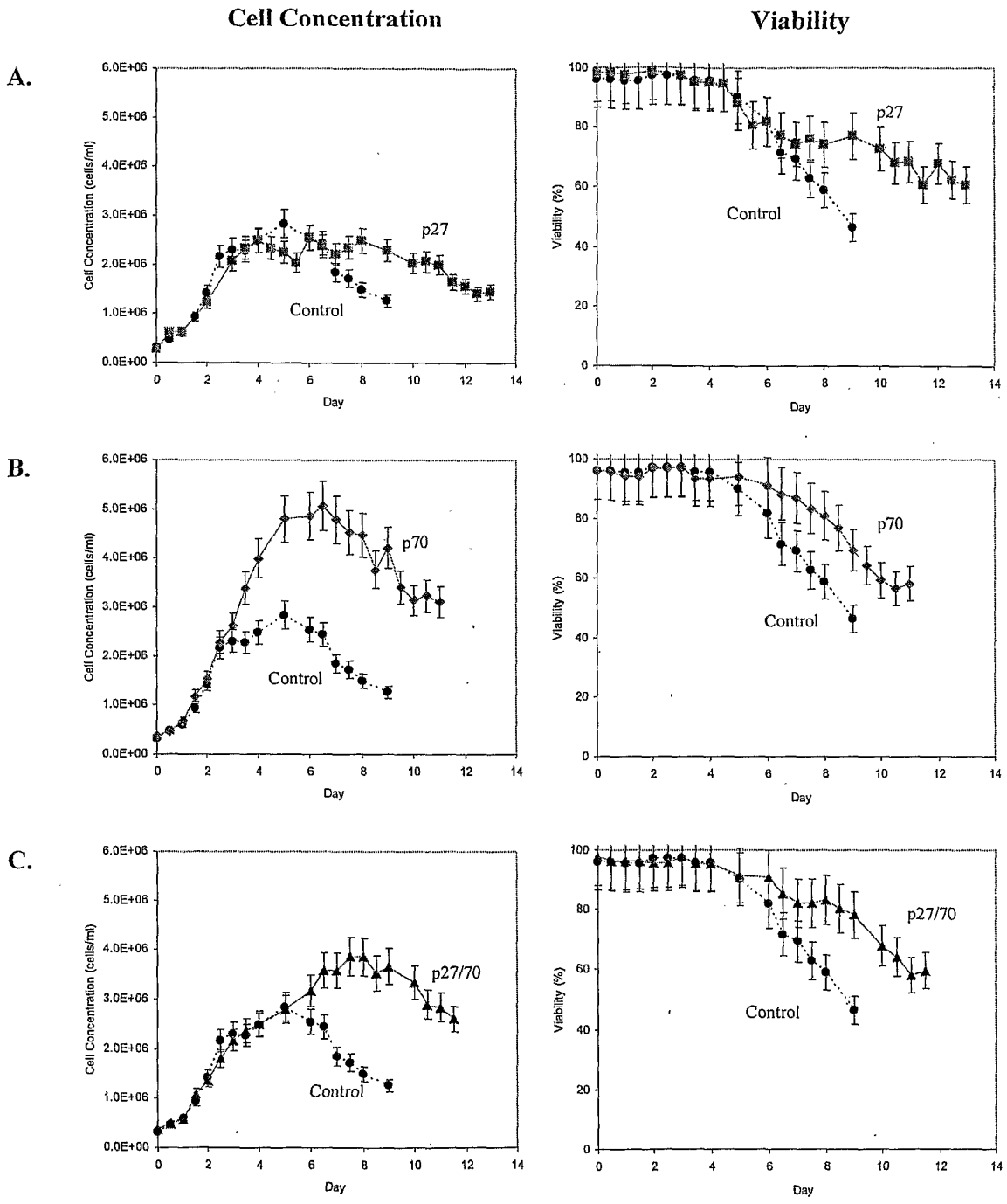

Cell Concentration
Viability
A.
B.
C.
Cell Concentration (cells/ml)
Viability (%)
Day
p27
p70
p27/70
Control
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
100
80
60
40
20
0
0
2
4
6
8
10
12
14

FIGURE 6

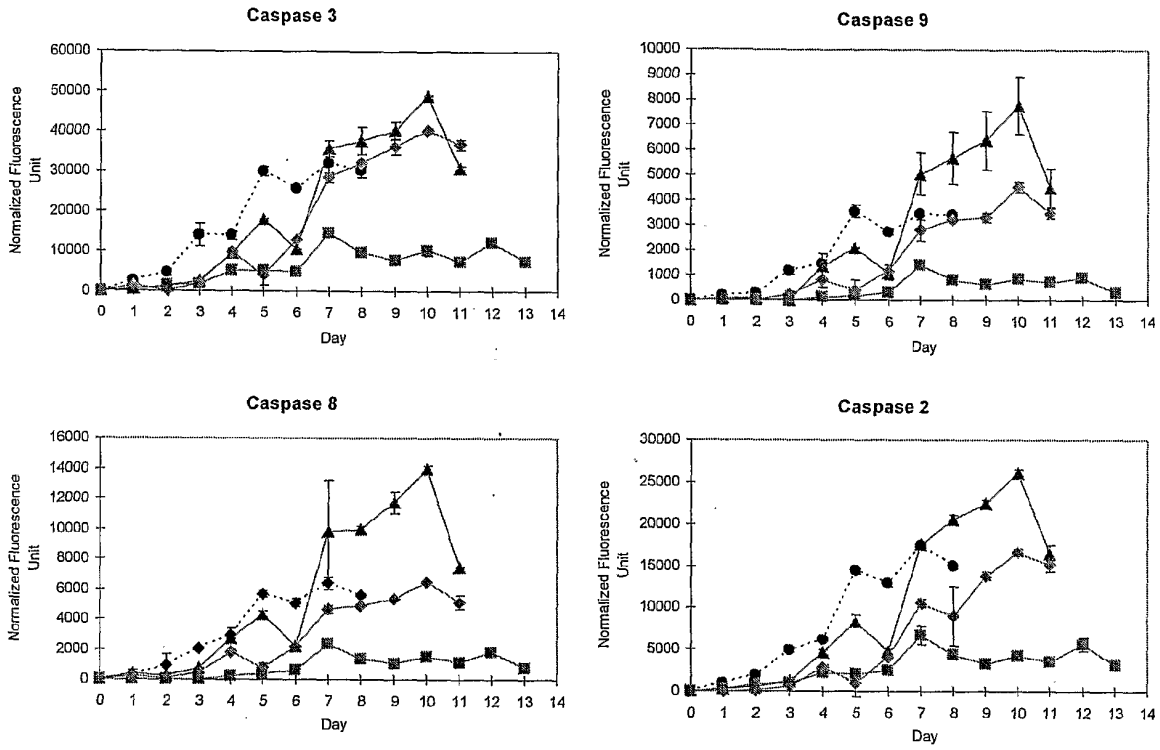

Caspase 3
Normalized Fluorescence Unit
60000
50000
40000
30000
20000
10000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day
Caspase 9
Normalized Fluorescence Unit
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day
Caspase 8
Normalized Fluorescence Unit
16000
14000
12000
10000
8000
6000
4000
2000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day
Caspase 2
Normalized Fluorescence Unit
30000
25000
20000
15000
10000
5000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day

FIGURE 7

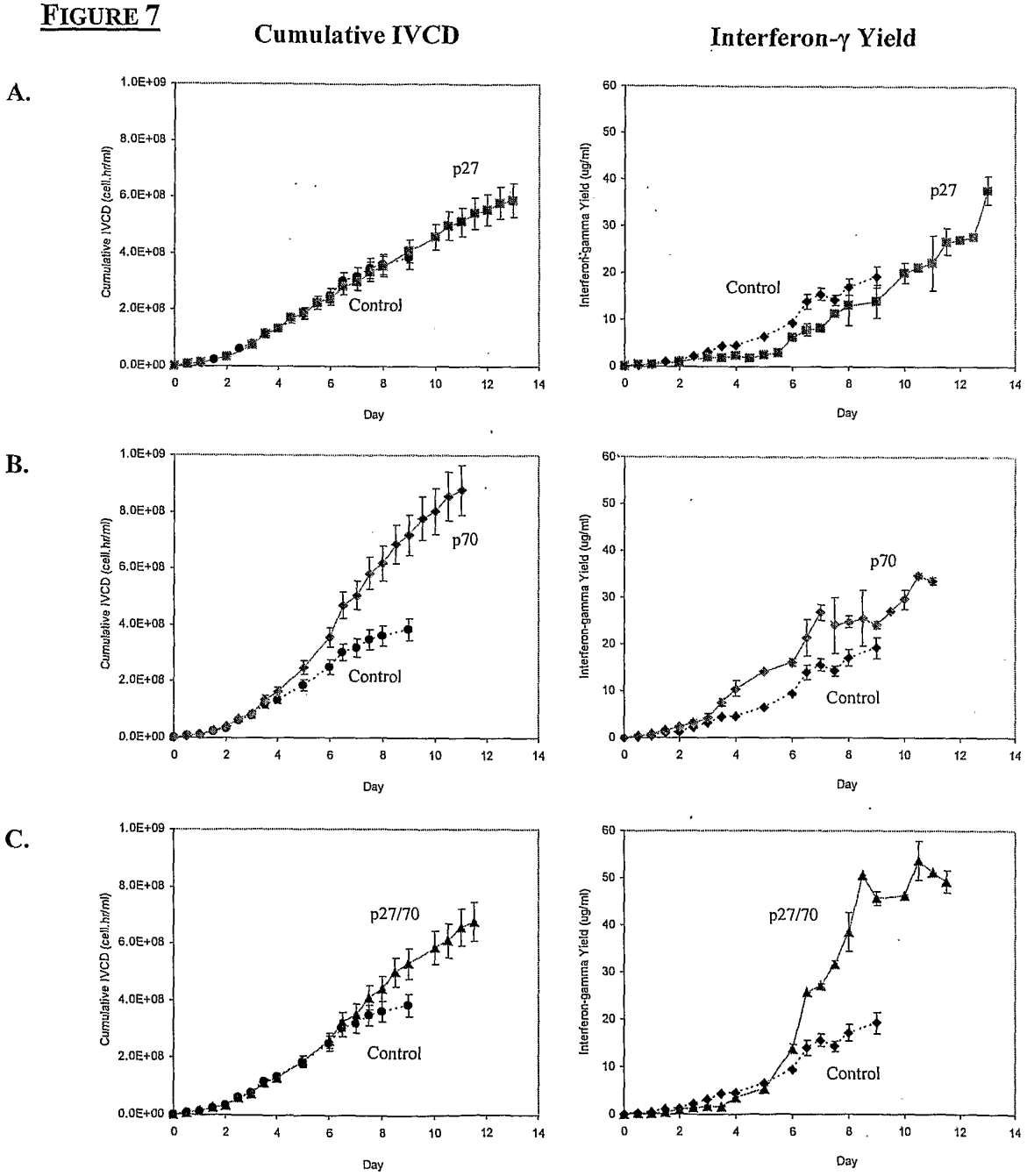
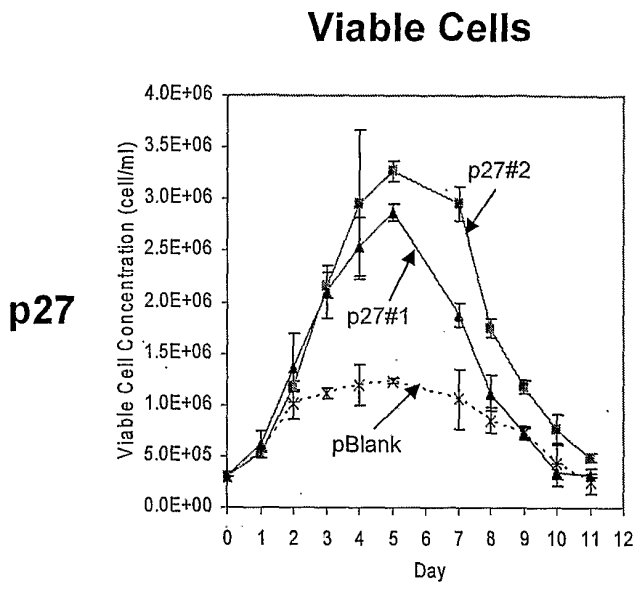
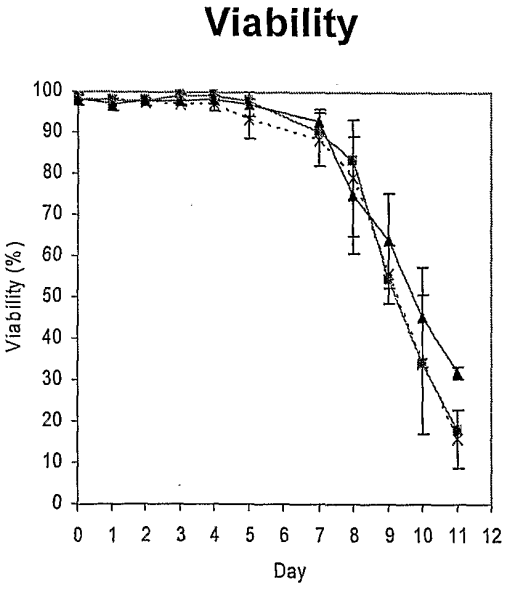

Cumulative IVCD
Interferon-γ Yield
A.
B.
C.
Cumulative IVCD (cell.hr/ml)
Interferon-gamma Yield (ug/ml)
Day
p27
Control
p70
p27/70
1.0E+09
8.0E+08
6.0E+08
4.0E+08
2.0E+08
0.0E+00
0
2
4
6
8
10
12
14
60
50
40
30
20
10

FIGURE 8

p27


Viable Cells
Viable Cell Concentration (cell/ml)
4.0E+06
3.5E+06
3.0E+06
2.5E+06
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
p27#2
p27#1
pBlank
0 1 2 3 4 5 6 7 8 9 10 11 12
Day


Viability
Viability (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Day

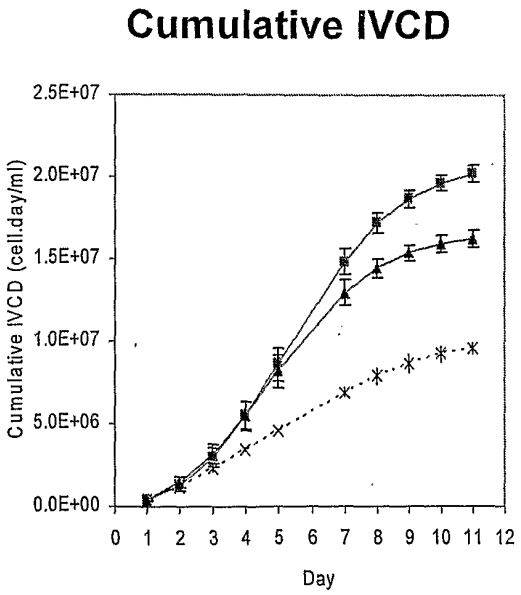

Cumulative IVCD
Cumulative IVCD (cell.day/ml)
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
0 1 2 3 4 5 6 7 8 9 10 11 12
Day

FIGURE 9

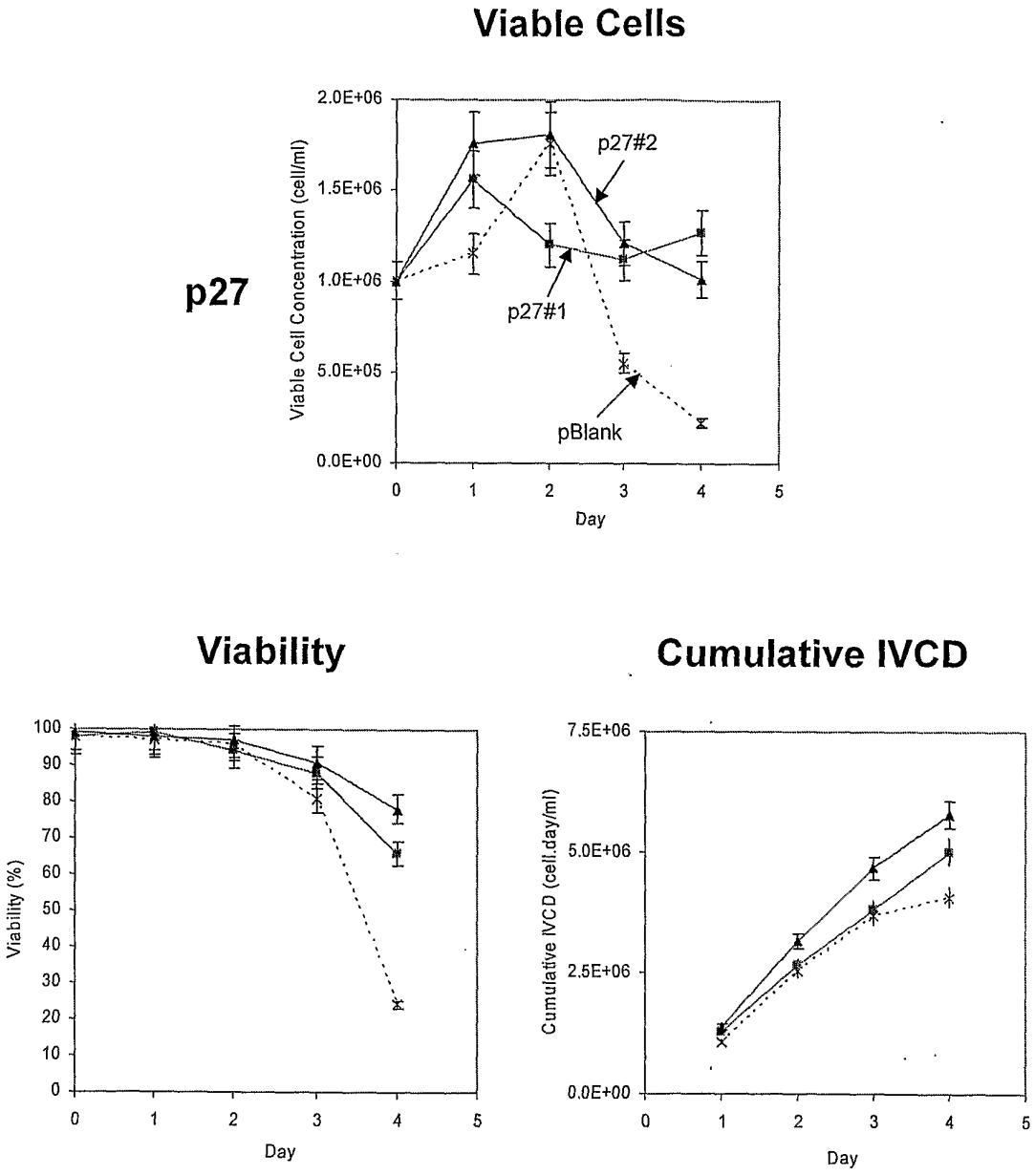

Viable Cells
p27
Viable Cell Concentration (cell/ml)
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
p27#2
p27#1
pBlank
0
1
2
3
4
5
Day
Viability
Viability (%)
100
90
80
70
60
50
40
30
20
10
0
Day
Cumulative IVCD
Cumulative IVCD (cell.day/ml)
7.5E+06
5.0E+06
2.5E+06
0.0E+00
Day

FIGURE 10

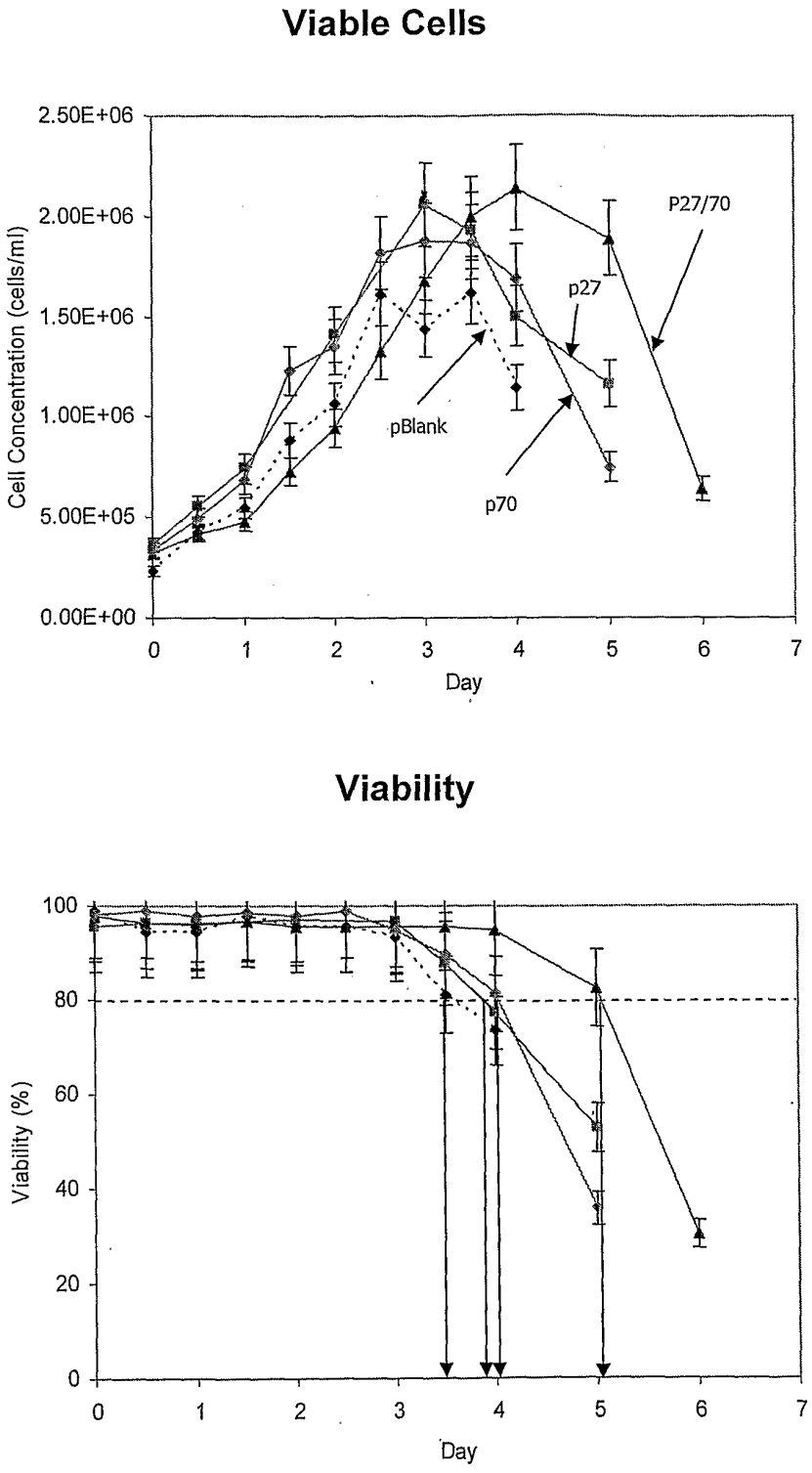

Viable Cells
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell Concentration (cells/ml)
0
1
2
3
4
5
6
7
Day
P27/70
p27
pBlank
p70
Viability
100
80
60
40
20
0
Viability (%)
0
1
2
3
4
5
6
7
Day

FIGURE 11A

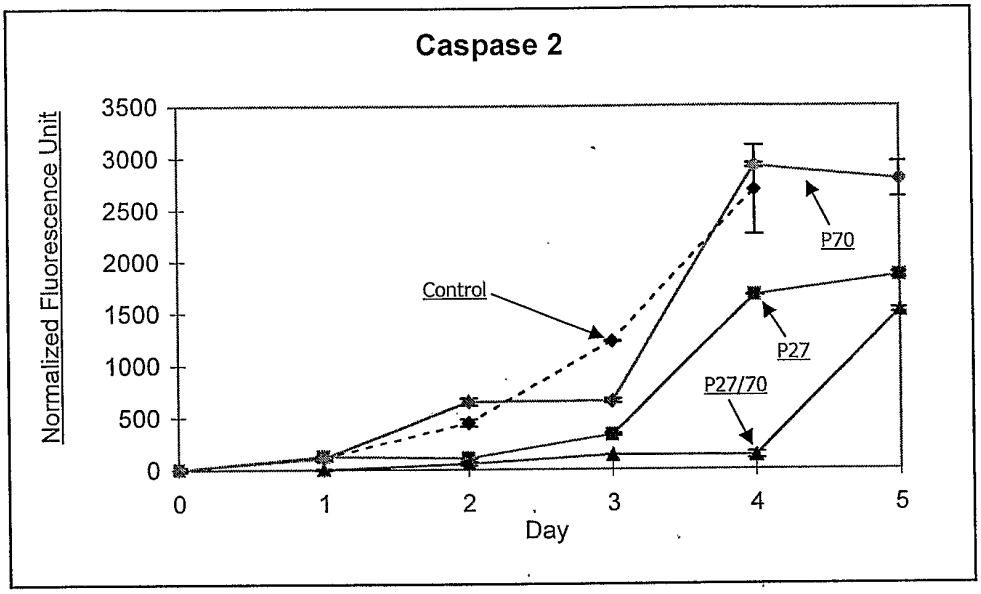

Caspase 2
Normalized Fluorescence Unit
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
Day
Control
P70
P27
P27/70

FIGURE 11B

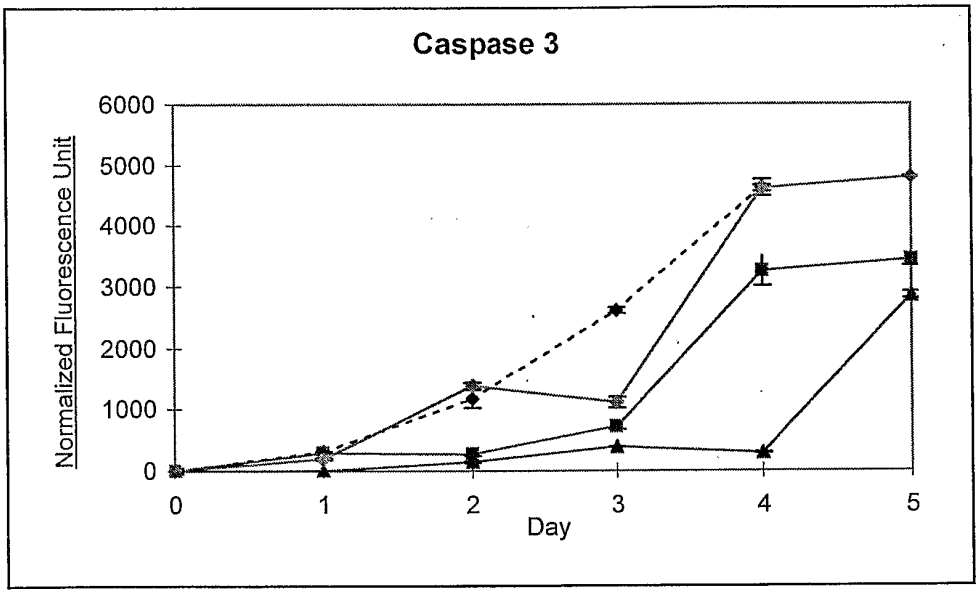

Caspase 3
Normalized Fluorescence Unit
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
5
Day

FIGURE 11C

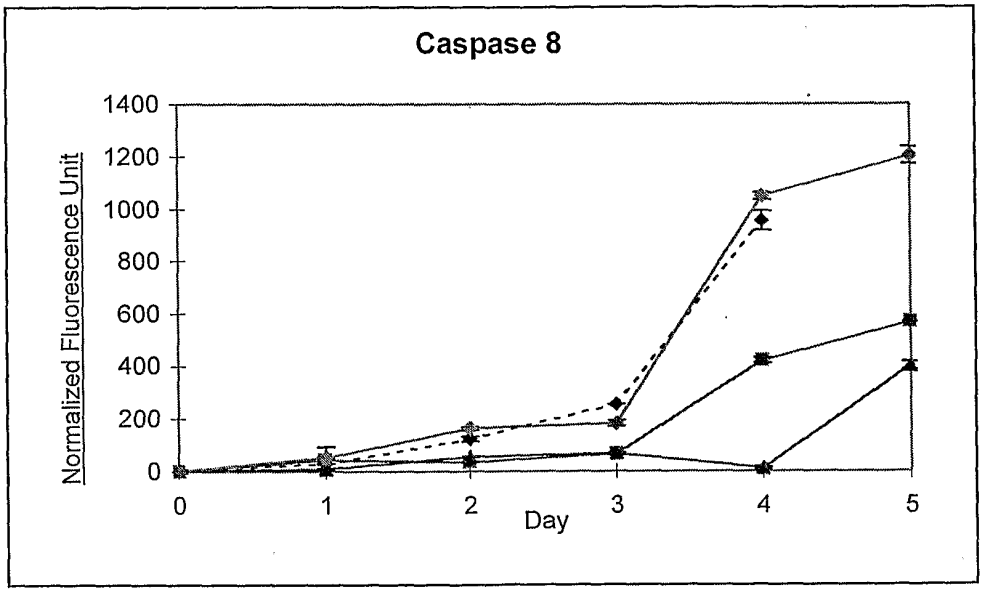

Caspase 8
Normalized Fluorescence Unit
1400
1200
1000
800
600
400
200
0
0
1
2
3
4
5
Day

FIGURE 11D

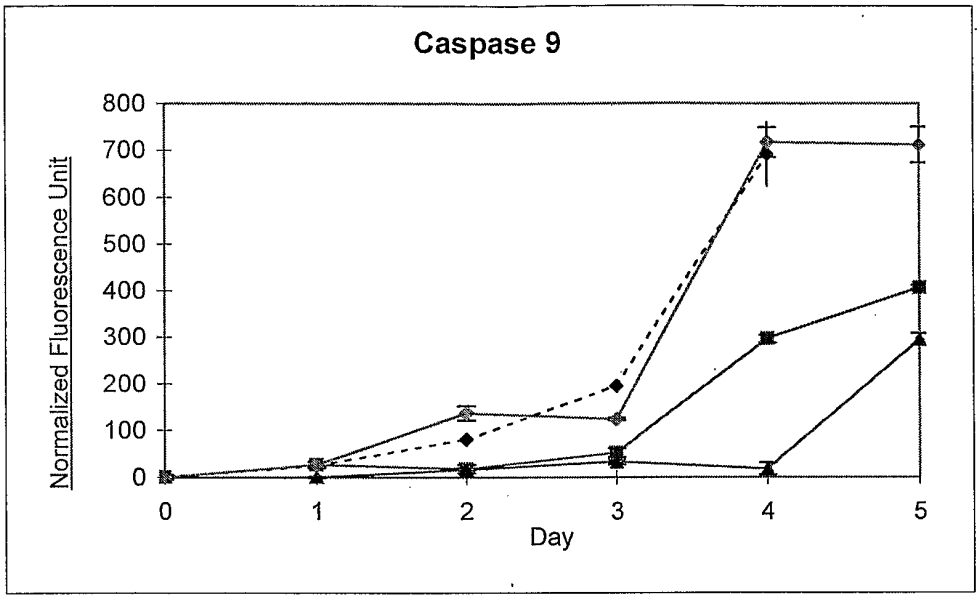
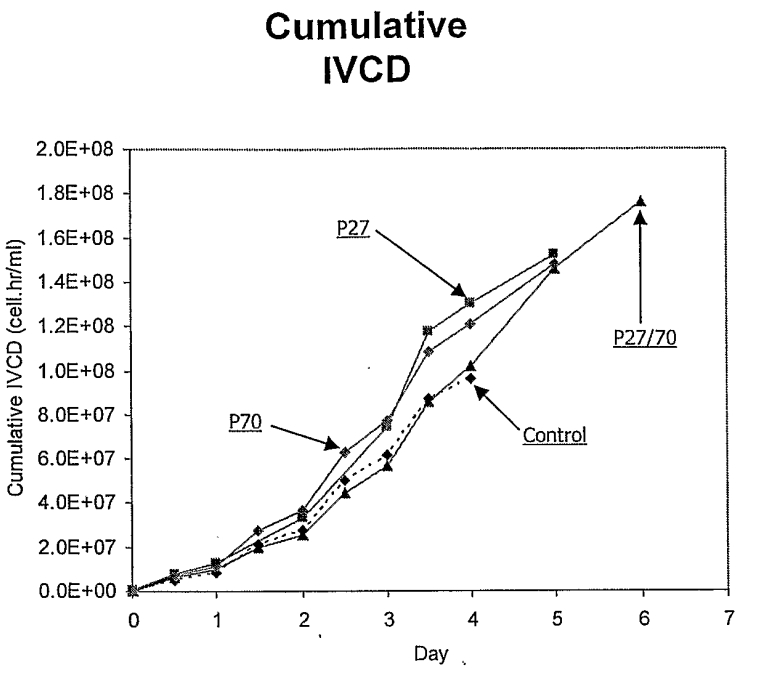

Caspase 9
Normalized Fluorescence Unit
800
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Day

FIGURE 12


Cumulative IVCD
Cumulative IVCD (cell.hr/ml)
2.0E+08
1.8E+08
1.6E+08
1.4E+08
1.2E+08
1.0E+08
8.0E+07
6.0E+07
4.0E+07
2.0E+07
0.0E+00
0
1
2
3
4
5
6
7
Day
P27
P27/70
P70
Control

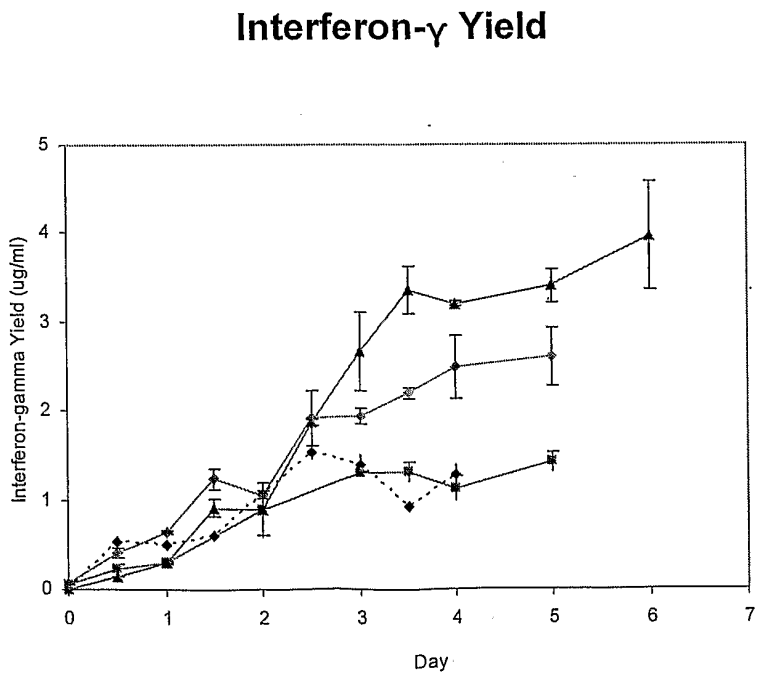

Interferon-γ Yield
Interferon-gamma Yield (ug/ml)
5
4
3
2
1
0
0
1
2
3
4
5
6
7
Day

FIGURE 13

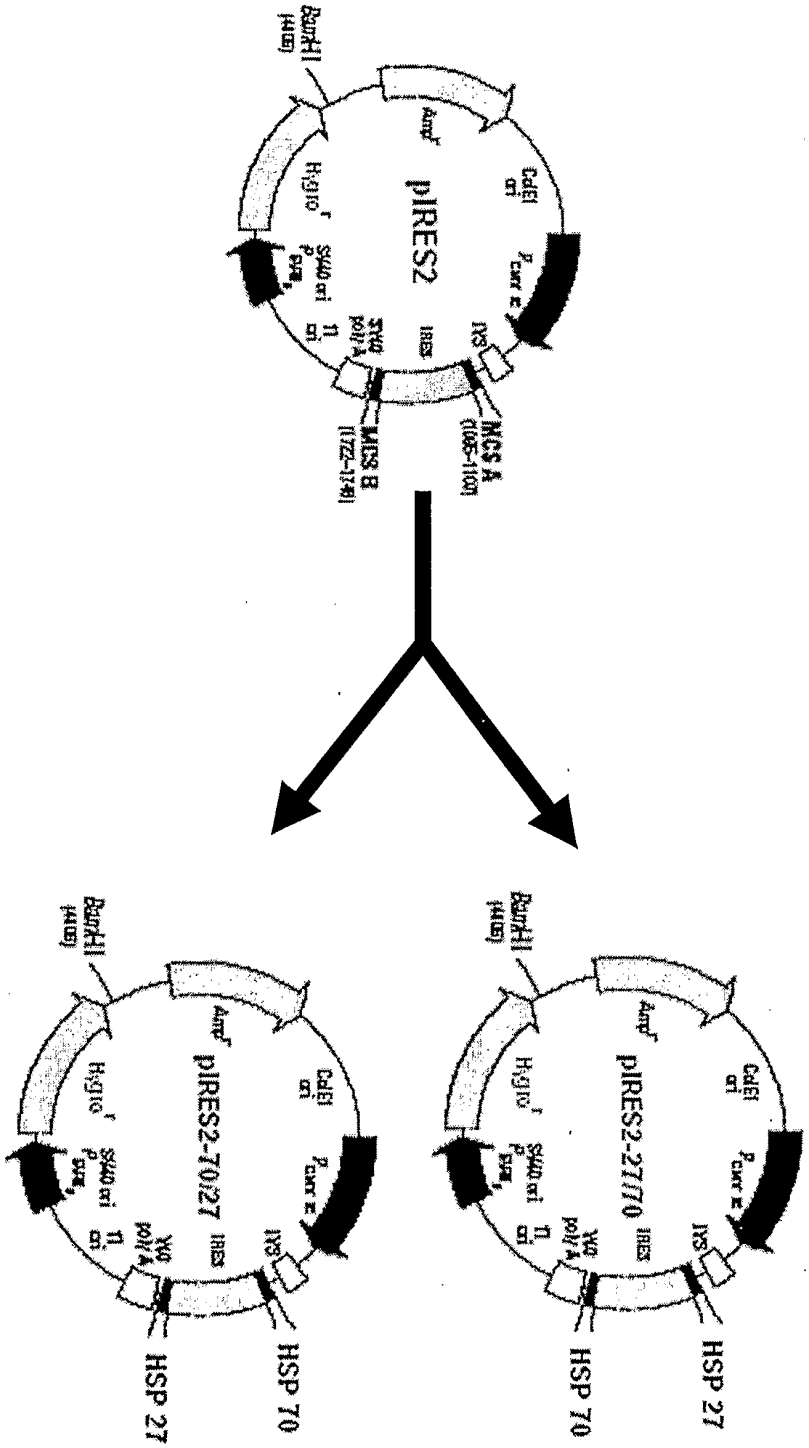
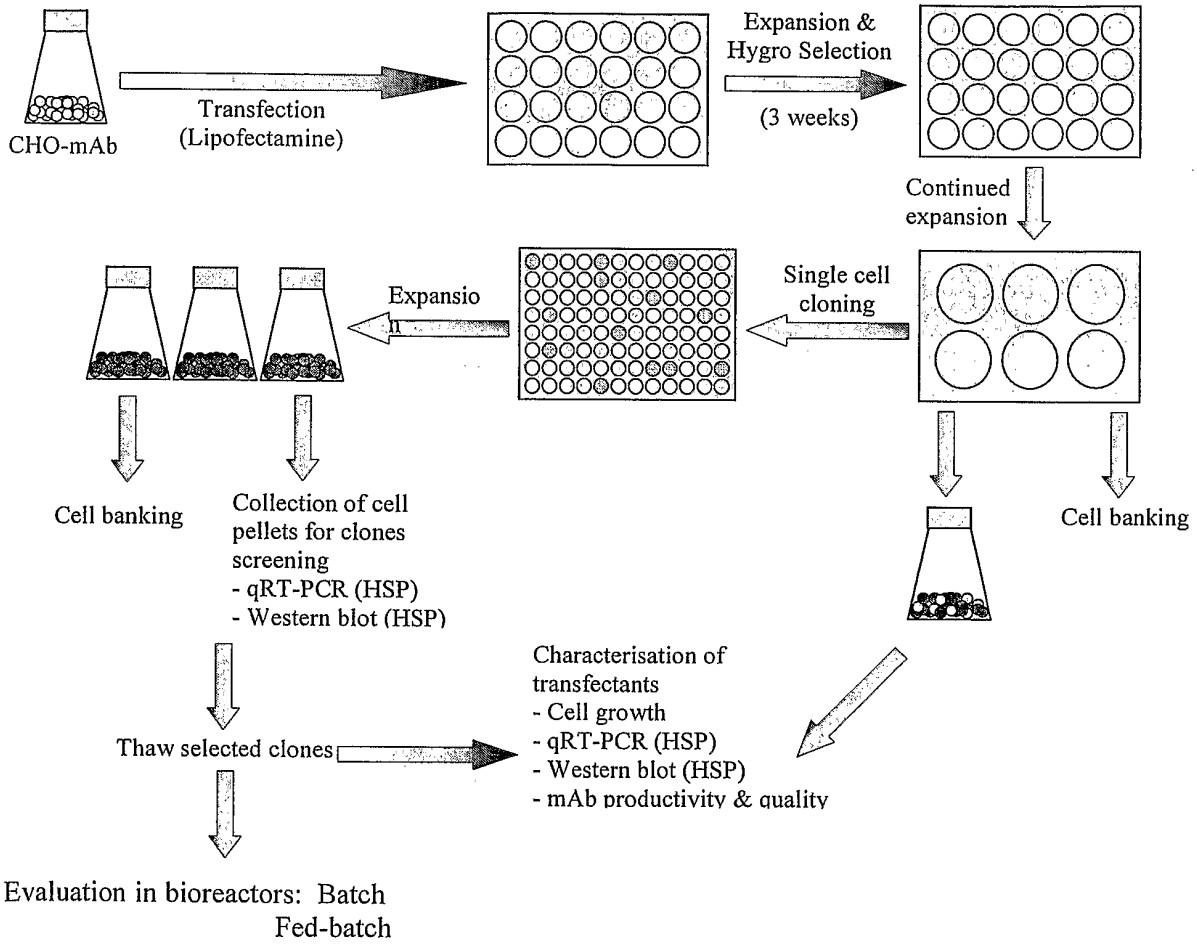


FIGURE 14


CHO-mAb
Transfection
(Lipofectamine)
Expansion &
Hygro Selection
(3 weeks)
Continued
expansion
Single cell
cloning
Expansio
Cell banking
Collection of cell
pellets for clones
screening
- qRT-PCR (HSP)
- Western blot (HSP)
Cell banking
Thaw selected clones
Characterisation of
transfectants
- Cell growth
- qRT-PCR (HSP)
- Western blot (HSP)
- mAb productivity & quality
Evaluation in bioreactors: Batch
Fed-batch