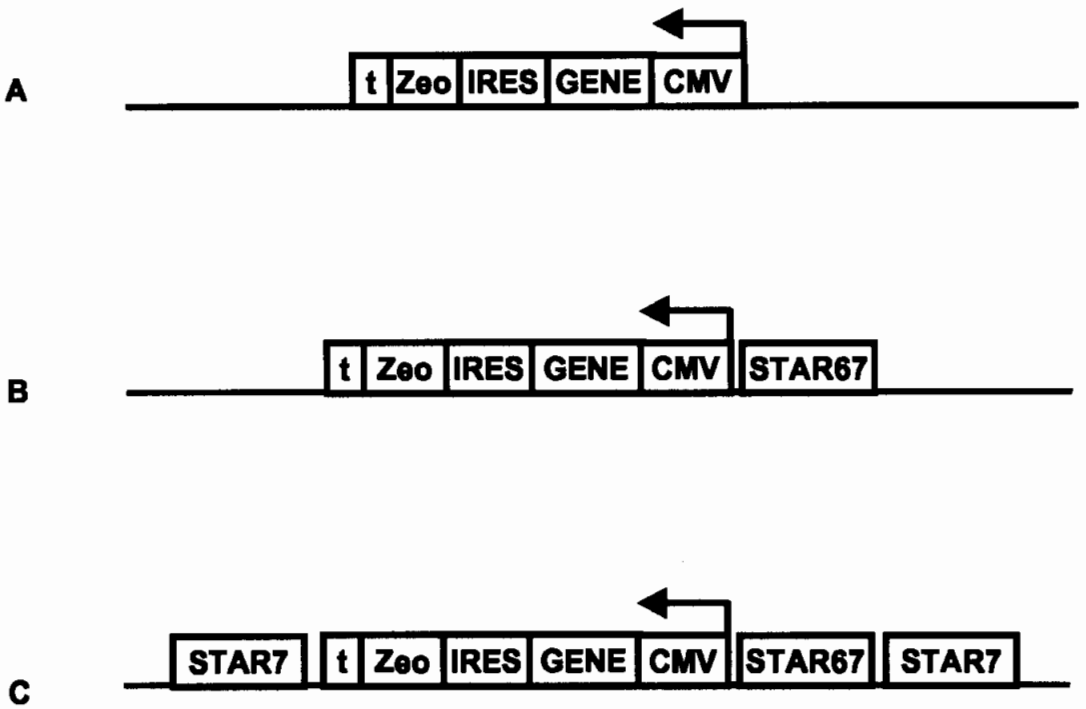
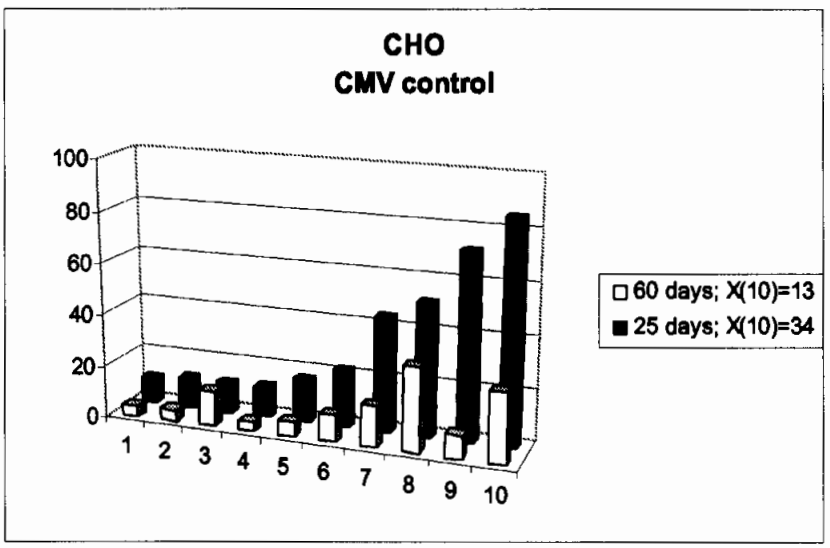
# Fig 1


A
t
Zeo
IRES
GENE
CMV
B
t
Zeo
IRES
GENE
CMV
STAR67
C
STAR7
t
Zeo
IRES
GENE
CMV
STAR67
STAR7

# Fig 2

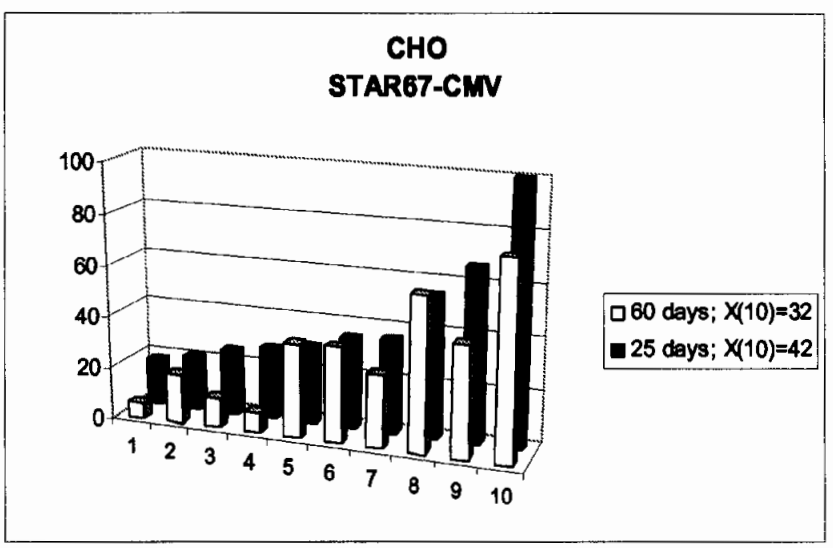
A


t
Zeo
IRES
d2EGFP
CMV


CHO
CMV control
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
60 days; X(10)=13
25 days; X(10)=34

B

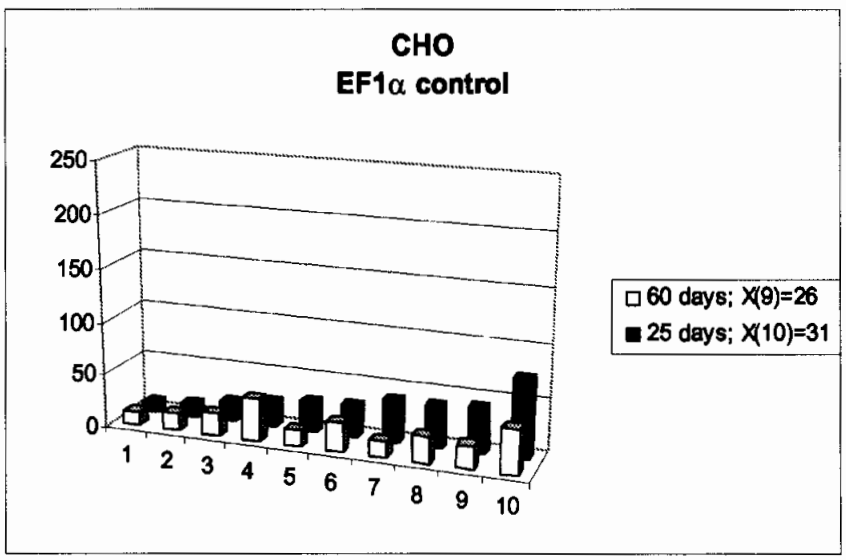

t
Zeo
IRES
d2EGFP
CMV
STAR67


CHO
STAR67-CMV
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
60 days; X(10)=32
25 days; X(10)=42

# Fig 3

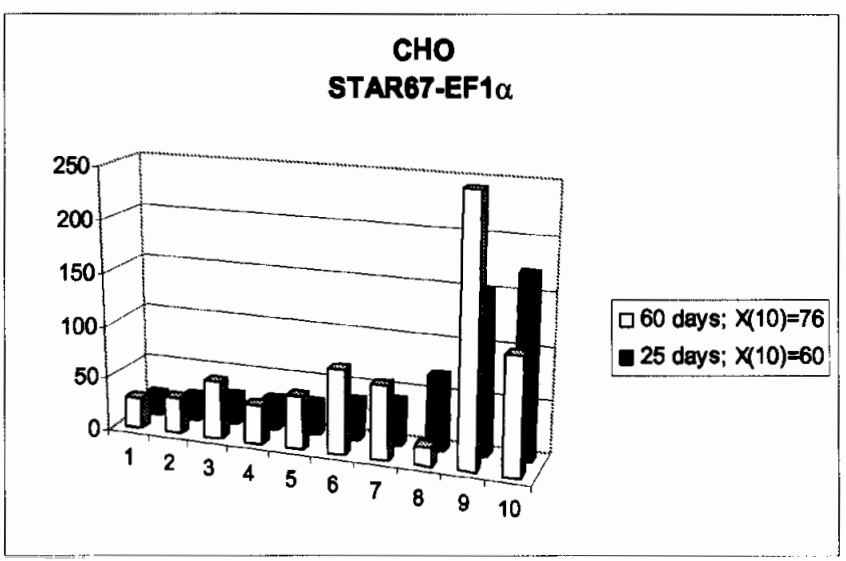
A


t
Zeo
IRES
d2EGFP
EF1α


CHO
EF1α control
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10
60 days; X(9)=26
25 days; X(10)=31

B

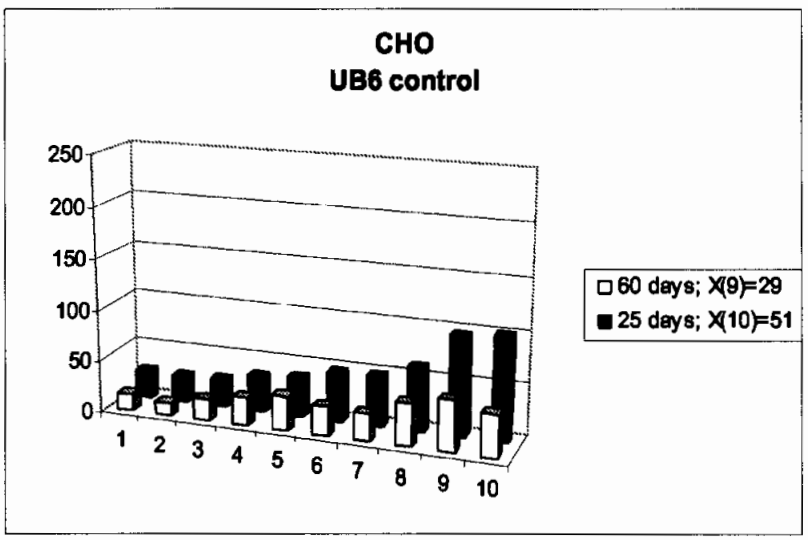

t
Zeo
IRES
d2EGFP
EF1α
STAR67


CHO
STAR67-EF1α
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10
60 days; X(10)=76
25 days; X(10)=60

# Fig 4

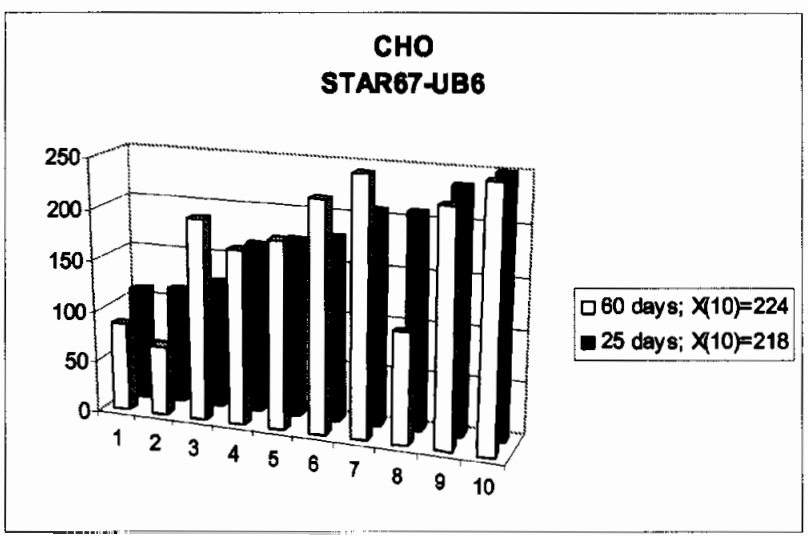
A


t
Zeo
IRES
d2EGFP
UB6


CHO
UB6 control
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10
60 days; X(9)=29
25 days; X(10)=51

B

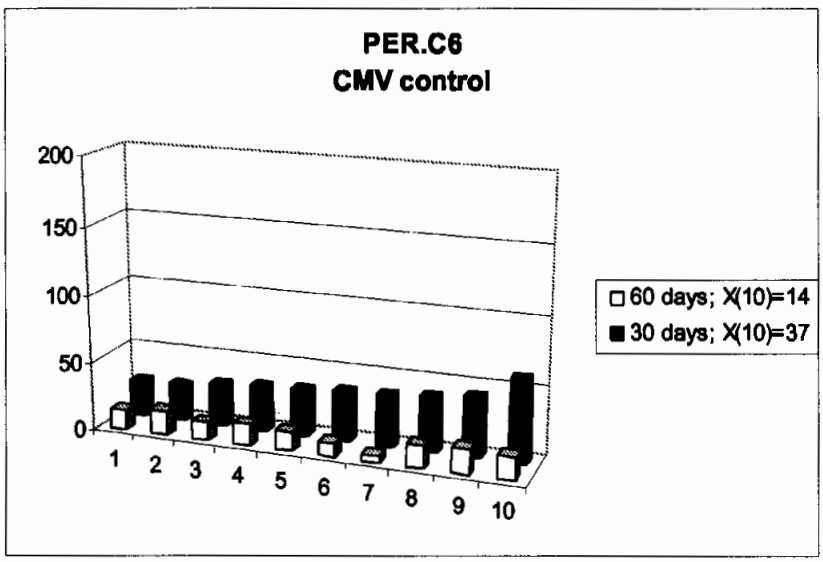

t
Zeo
IRES
d2EGFP
UB6
STAR67


CHO
STAR67-UB6
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10
60 days; X(10)=224
25 days; X(10)=218

# Fig 5

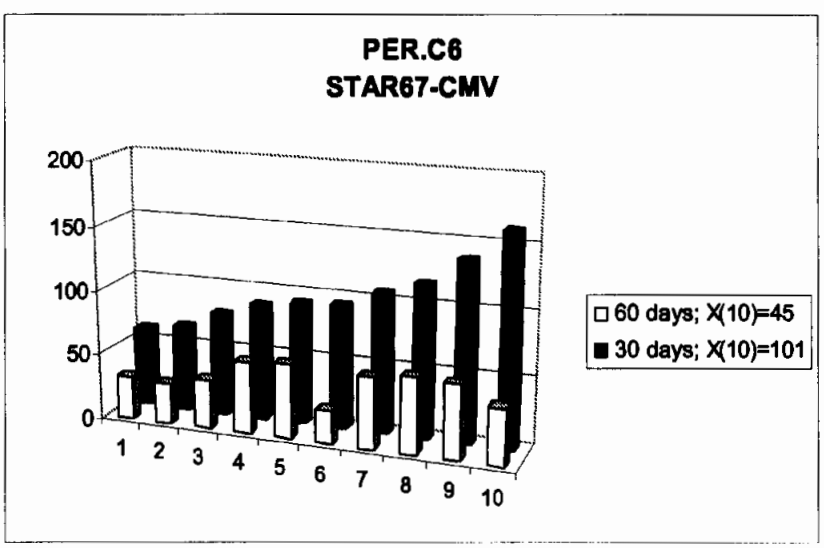
A


t
Zeo
IRES
d2EGFP
CMV


PER.C6
CMV control
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
60 days; X(10)=14
30 days; X(10)=37

B

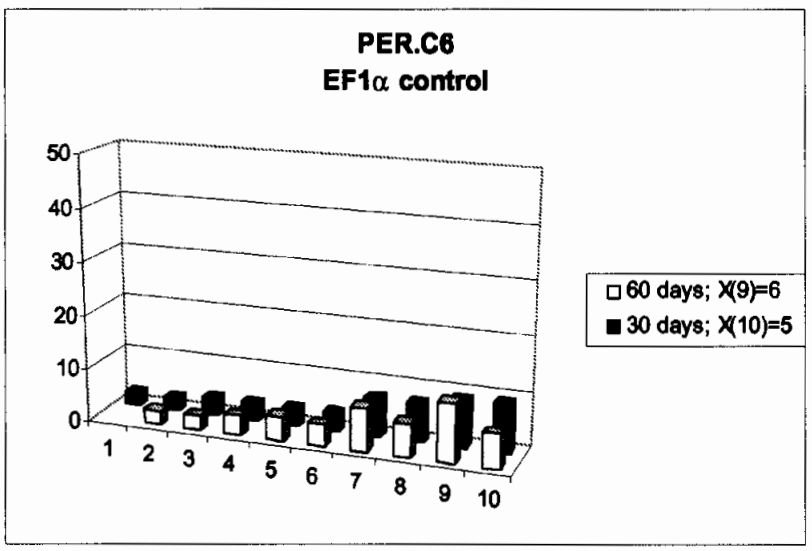

t
Zeo
IRES
d2EGFP
CMV
STAR67


PER.C6
STAR67-CMV
200
150
100
50
0
1
2
3
4
5
6
7
8
9
10
60 days; X(10)=45
30 days; X(10)=101

# Fig 6

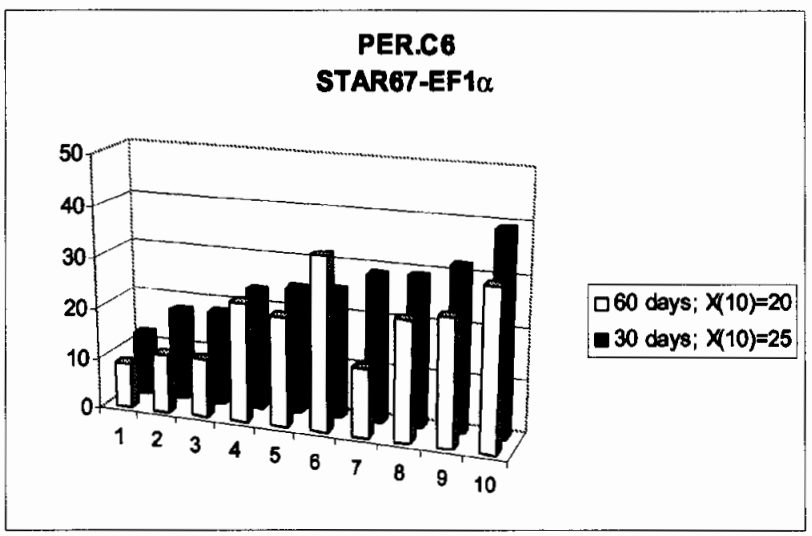
A


t
Zeo
IRES
d2EGFP
EF1α


PER.C6
EF1α control
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10
60 days; X(9)=6
30 days; X(10)=5

B

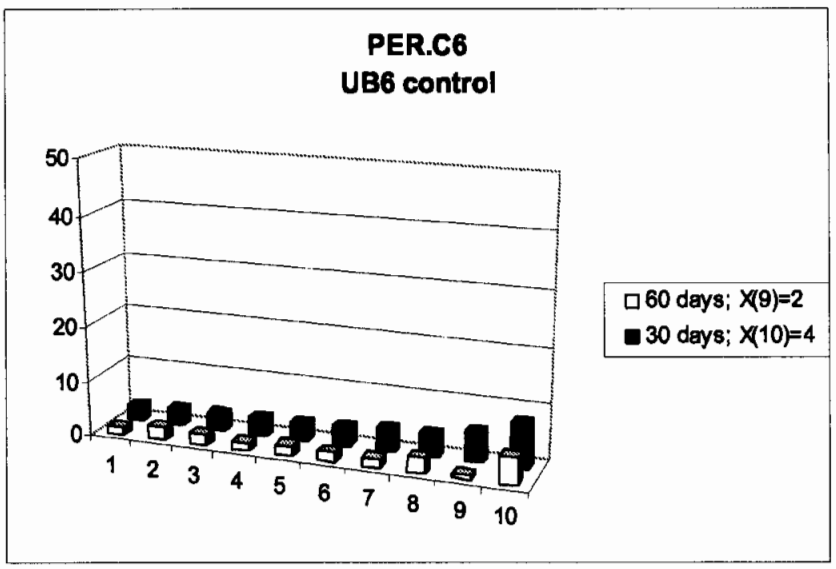

t
Zeo
IRES
d2EGFP
EF1α
STAR67


PER.C6
STAR67-EF1α
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10
60 days; X(10)=20
30 days; X(10)=25

# Fig 7

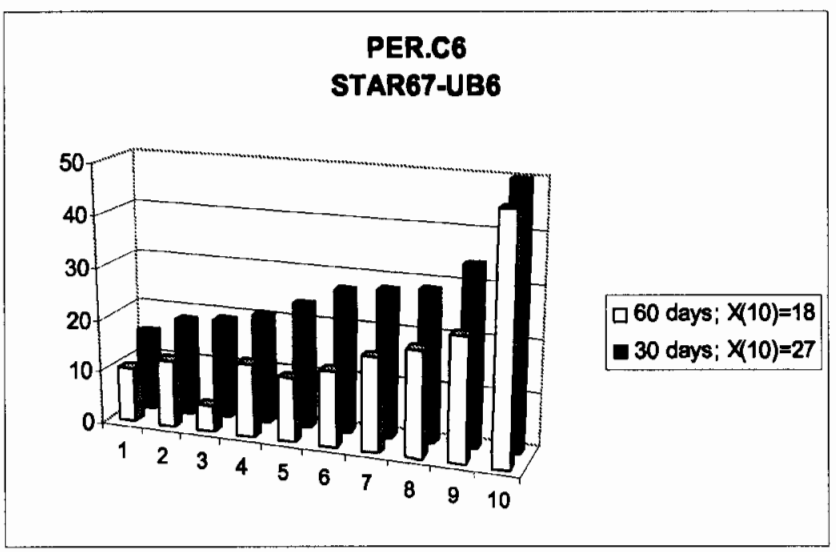
A


t
Zeo
IRES
d2EGFP
UB6

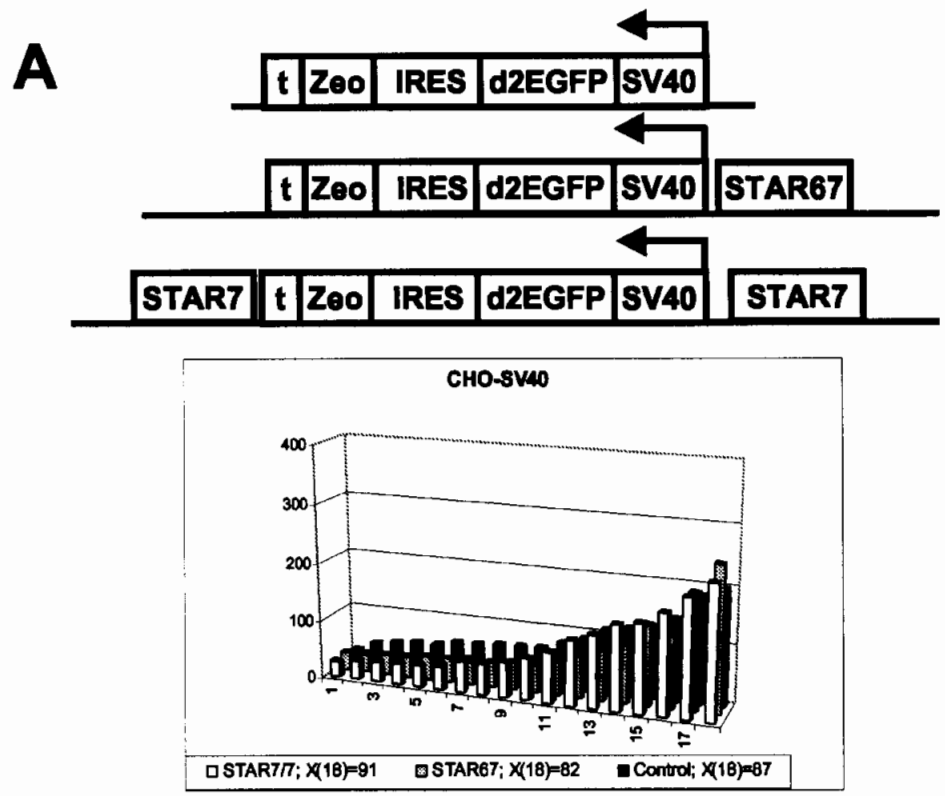

PER.C6
UB6 control
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10
60 days; X(9)=2
30 days; X(10)=4

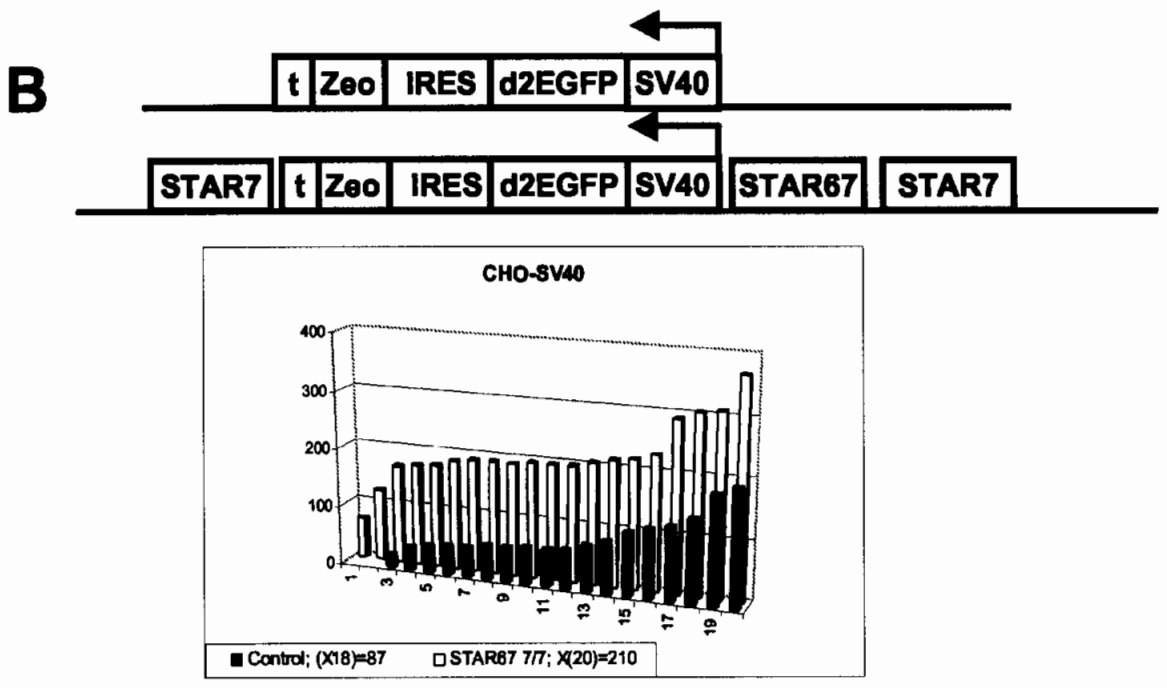
B


t
Zeo
IRES
d2EGFP
UB6
STAR67


PER.C6
STAR67-UB6
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10
60 days; X(10)=18
30 days; X(10)=27

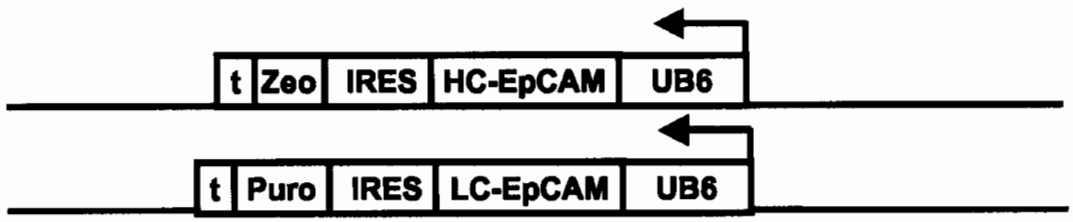
# Fig 8

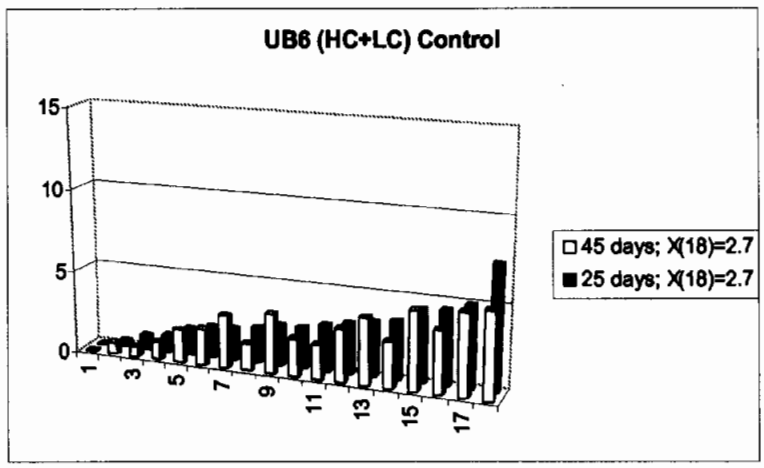

A
t
Zeo
IRES
d2EGFP
SV40
STAR67
STAR7
CHO-SV40
400
300
200
100
0
1
3
5
7
9
11
13
15
17
STAR7/7; X(18)=91
STAR67; X(18)=82
Control; X(18)=87


B
t
Zeo
IRES
d2EGFP
SV40
STAR7
STAR67
CHO-SV40
400
300
200
100
0
1
3
5
7
9
11
13
15
17
19
Control; (X18)=87
STAR67 7/7; X(20)=210

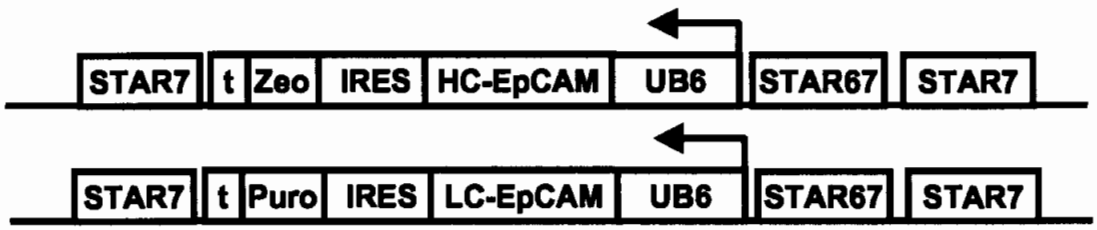
# Fig 9

A


t
Zeo
IRES
HC-EpCAM
UB6
t
Puro
IRES
LC-EpCAM
UB6


UB6 (HC+LC) Control
15
10
5
0
1
3
5
7
9
11
13
15
17
45 days; X(18)=2.7
25 days; X(18)=2.7

B


STAR7
t
Zeo
IRES
HC-EpCAM
UB6
STAR67
STAR7
STAR7
t
Puro
IRES
LC-EpCAM
UB6
STAR67
STAR7

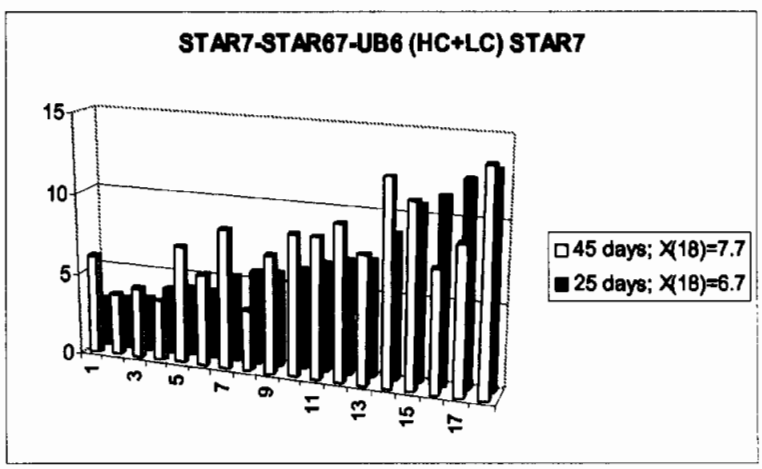
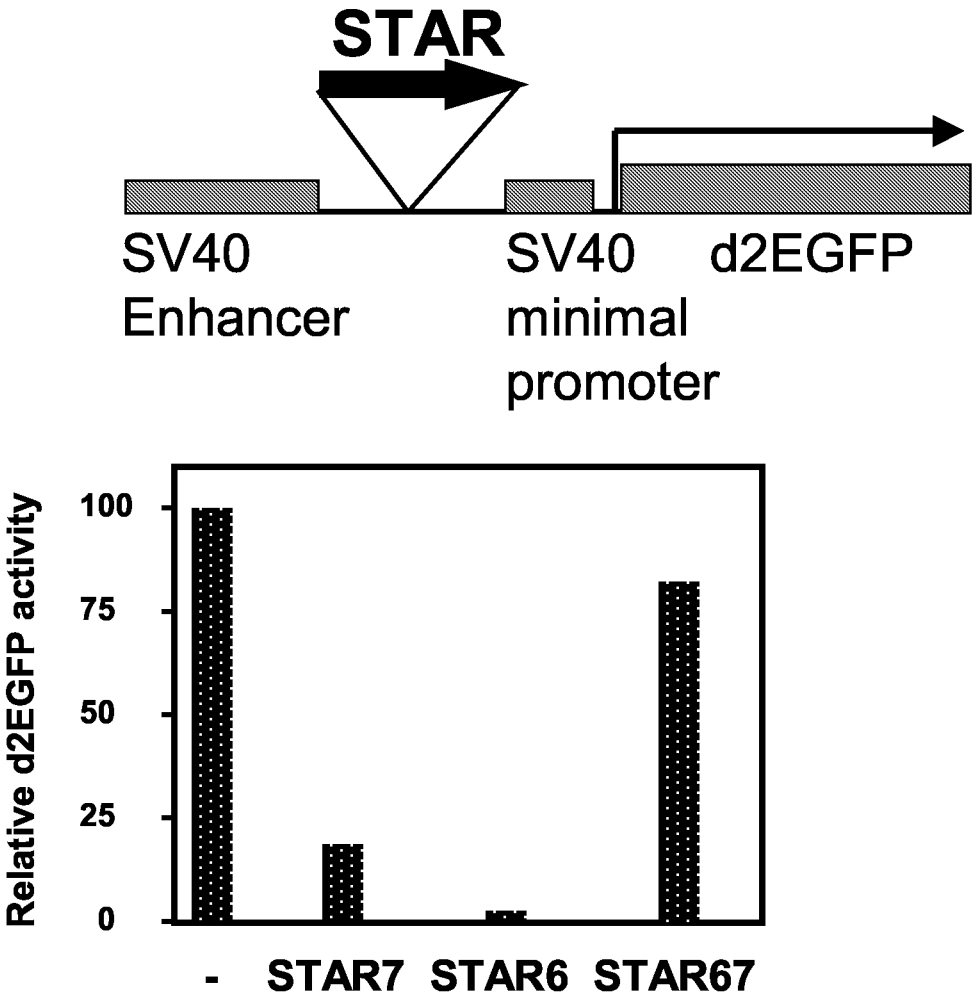
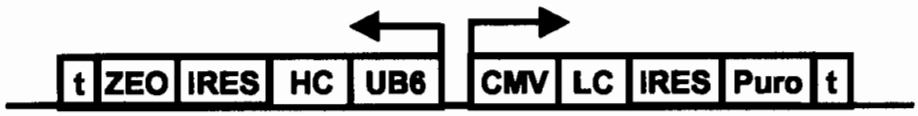

STAR7-STAR67-UB6 (HC+LC) STAR7
15
10
5
0
1
3
5
7
9
11
13
15
17
45 days; X(18)=7.7
25 days; X(18)=6.7

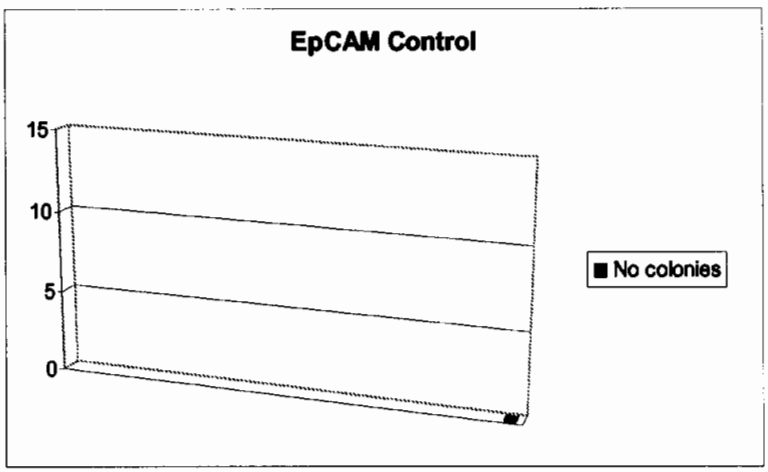
Fig 10


STAR
SV40
Enhancer
SV40
minimal
promoter
d2EGFP
Relative d2EGFP activity
100
75
50
25
0
-
STAR7
STAR6
STAR67

# Fig 11

A


t
ZEO
IRES
HC
UB6
CMV
LC
IRES
Puro
t


EpCAM Control
15
10
5
0
No colonies

B


t
ZEO
IRES
HC
UB6
STAR67
CMV
LC
IRES
Puro
t

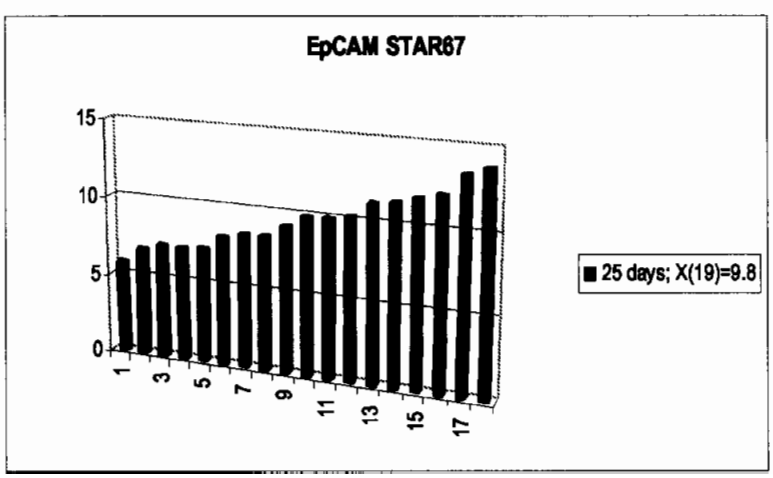

EpCAM STAR67
15
10
5
0
1
3
5
7
9
11
13
15
17
25 days; X(19)=9.8

FIG 12

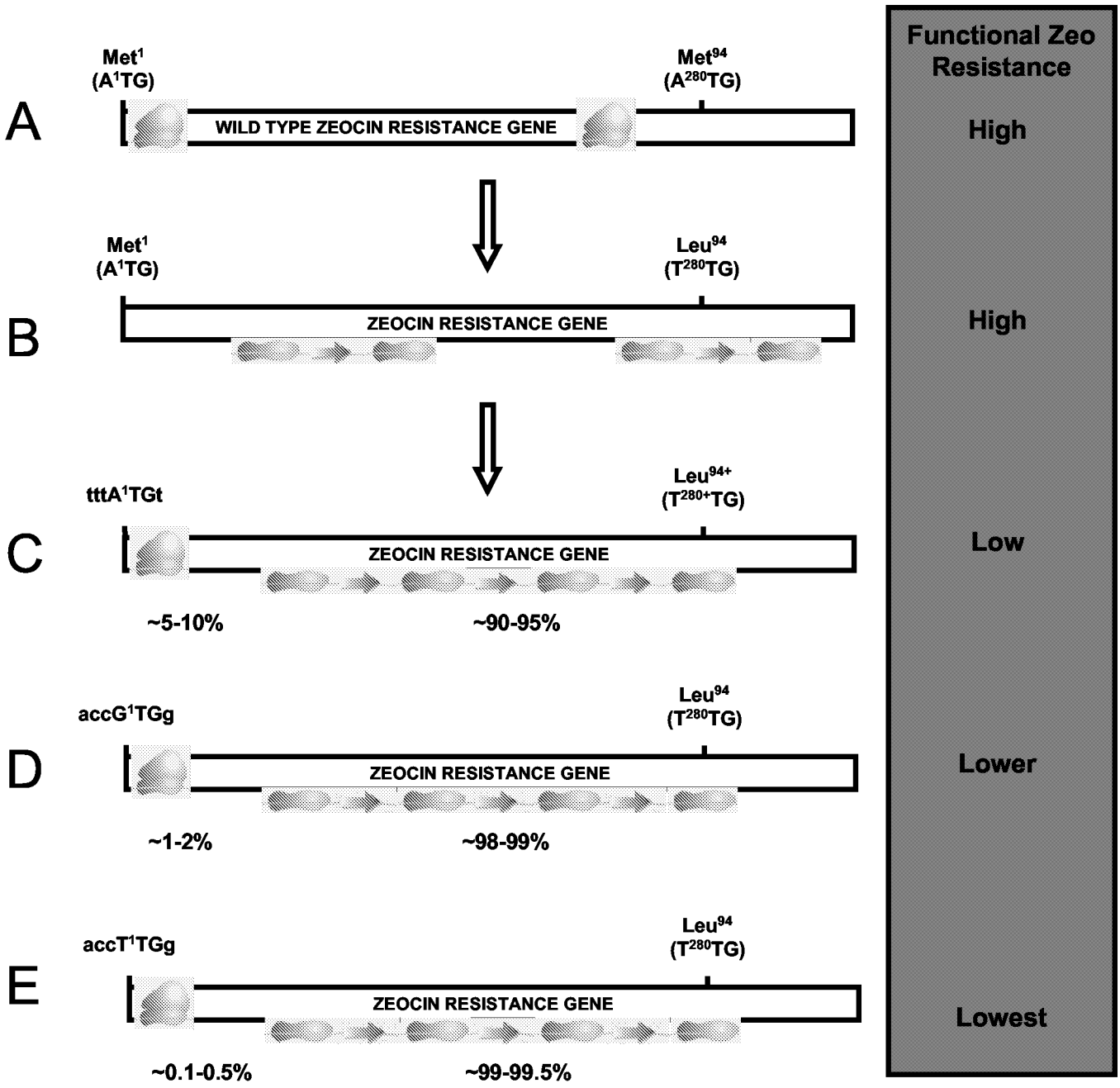

Functional Zeo Resistance
A
Met1 (A1TG)
Met94 (A280TG)
WILD TYPE ZEOCIN RESISTANCE GENE
High
B
Met1 (A1TG)
Leu94 (T280TG)
ZEOCIN RESISTANCE GENE
High
C
tttA1TGt
Leu94+ (T280+TG)
ZEOCIN RESISTANCE GENE
~5-10%
~90-95%
Low
D
accG1TGg
Leu94 (T280TG)
ZEOCIN RESISTANCE GENE
~1-2%
~98-99%
Lower
E
accT1TGg
Leu94 (T280TG)
ZEOCIN RESISTANCE GENE
~0.1-0.5%
~99-99.5%
Lowest

FIG 13

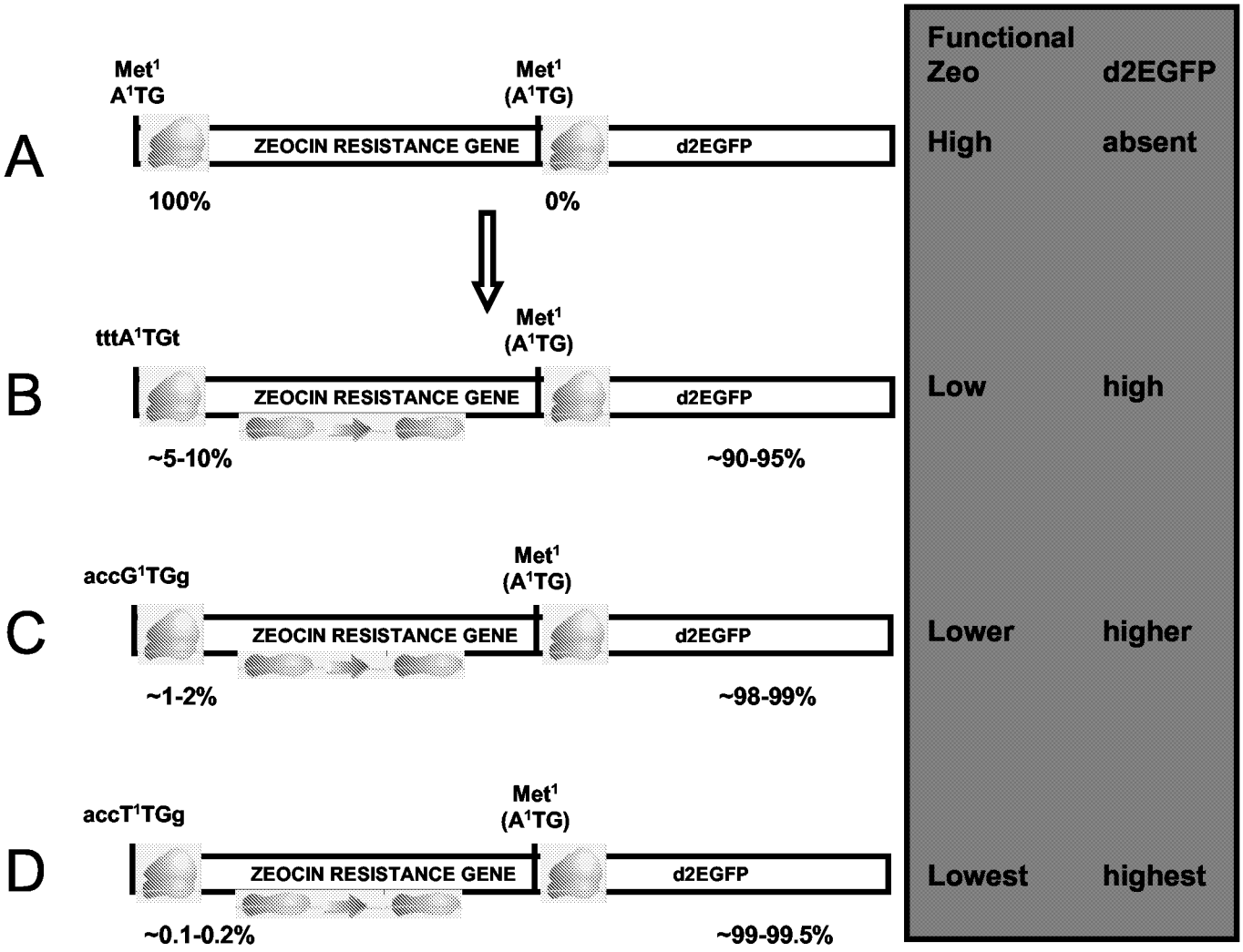
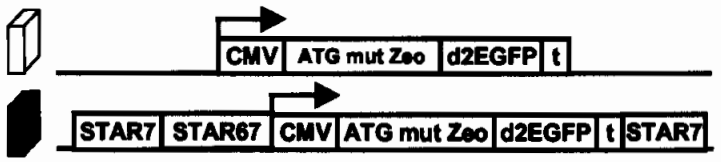
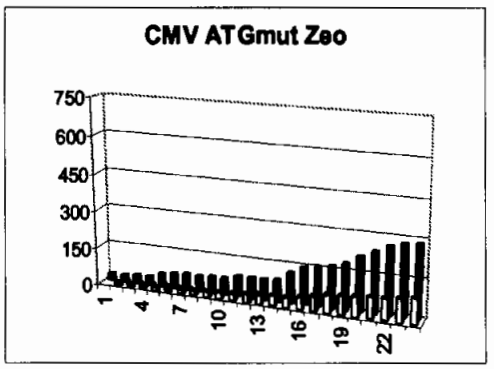

Functional
Zeo
d2EGFP
A
Met1
A1TG
Met1
(A1TG)
ZEOCIN RESISTANCE GENE
d2EGFP
High
absent
100%
0%
B
tttA1TGt
Met1
(A1TG)
ZEOCIN RESISTANCE GENE
d2EGFP
Low
high
~5-10%
~90-95%
C
accG1TGg
Met1
(A1TG)
ZEOCIN RESISTANCE GENE
d2EGFP
Lower
higher
~1-2%
~98-99%
D
accT1TGg
Met1
(A1TG)
ZEOCIN RESISTANCE GENE
d2EGFP
Lowest
highest
~0.1-0.2%
~99-99.5%

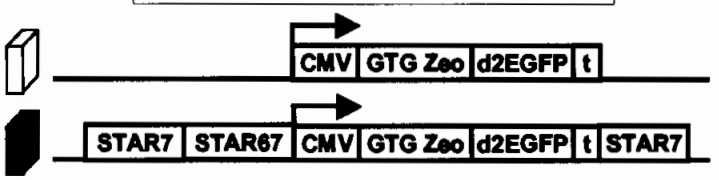
FIG 14

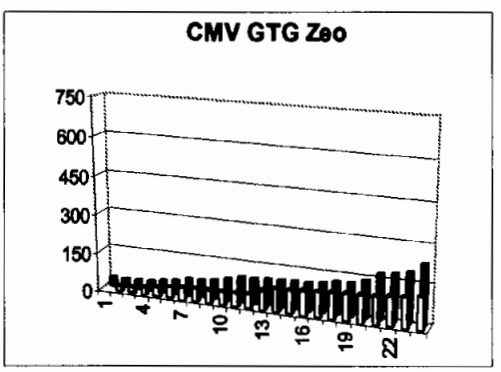
A


CMV
ATG mut Zeo
d2EGFP
t
STAR7
STAR67
CMV
ATG mut Zeo
d2EGFP
t
STAR7

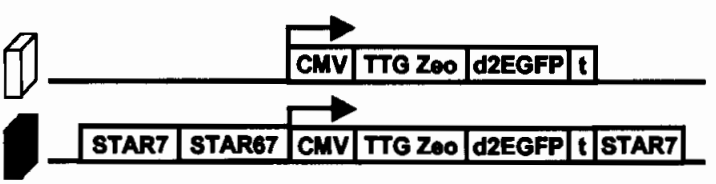

CMV ATGmut Zeo
750
600
450
300
150
0
1
4
7
10
13
16
19
22

B


CMV
GTG Zeo
d2EGFP
t
STAR7
STAR67
CMV
GTG Zeo
d2EGFP
t
STAR7


CMV GTG Zeo
750
600
450
300
150
0
1
4
7
10
13
16
19
22

C


CMV
TTG Zeo
d2EGFP
t
STAR7
STAR67
CMV
TTG Zeo
d2EGFP
t
STAR7

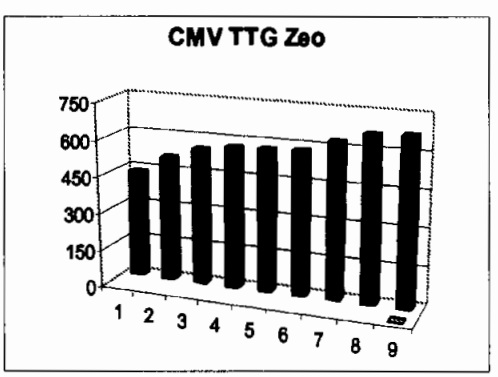
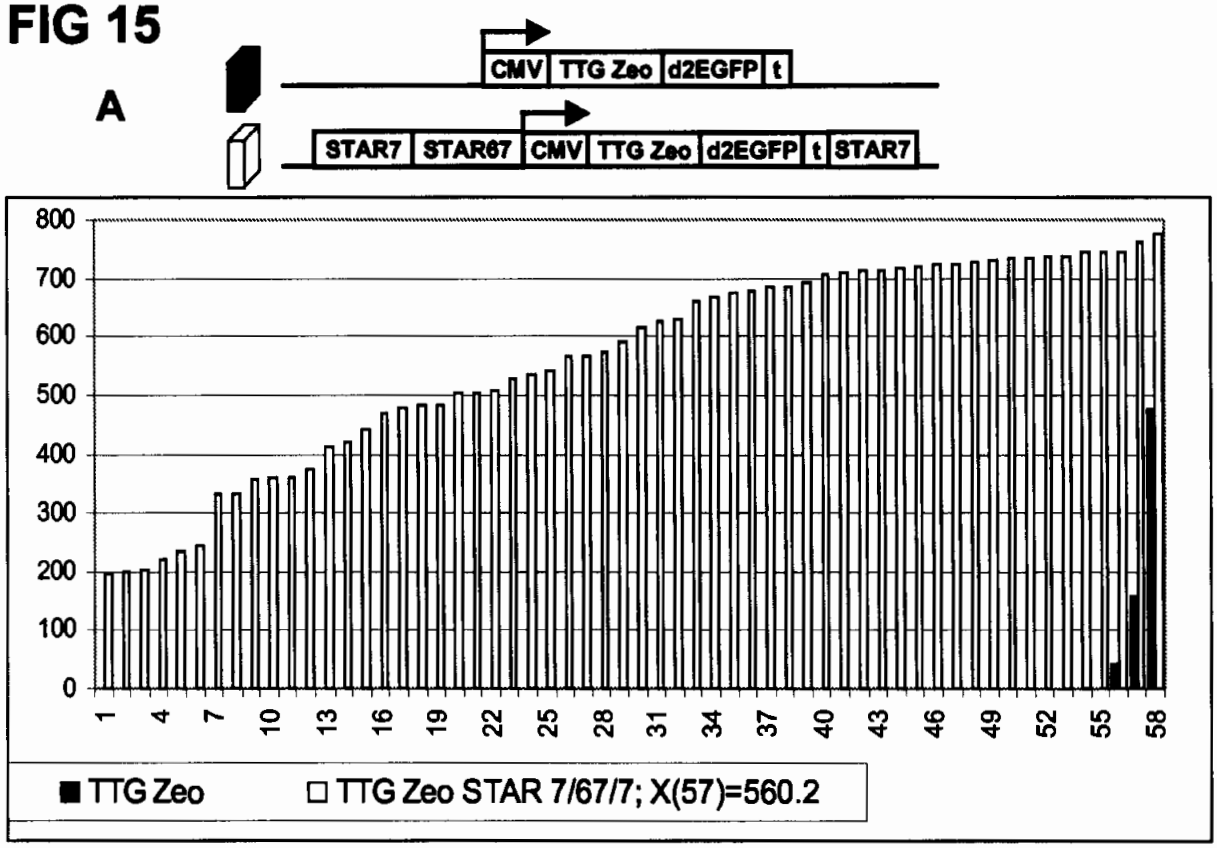

CMV TTG Zeo
750
600
450
300
150
0
1
2
3
4
5
6
7
8
9

FIG 15

A


CMV
TTG Zeo
d2EGFP
t
STAR7
STAR67
CMV
TTG Zeo
d2EGFP
t
STAR7
800
700
600
500
400
300
200
100
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58
TTG Zeo
TTG Zeo STAR 7/67/7; X(57)=560.2

B

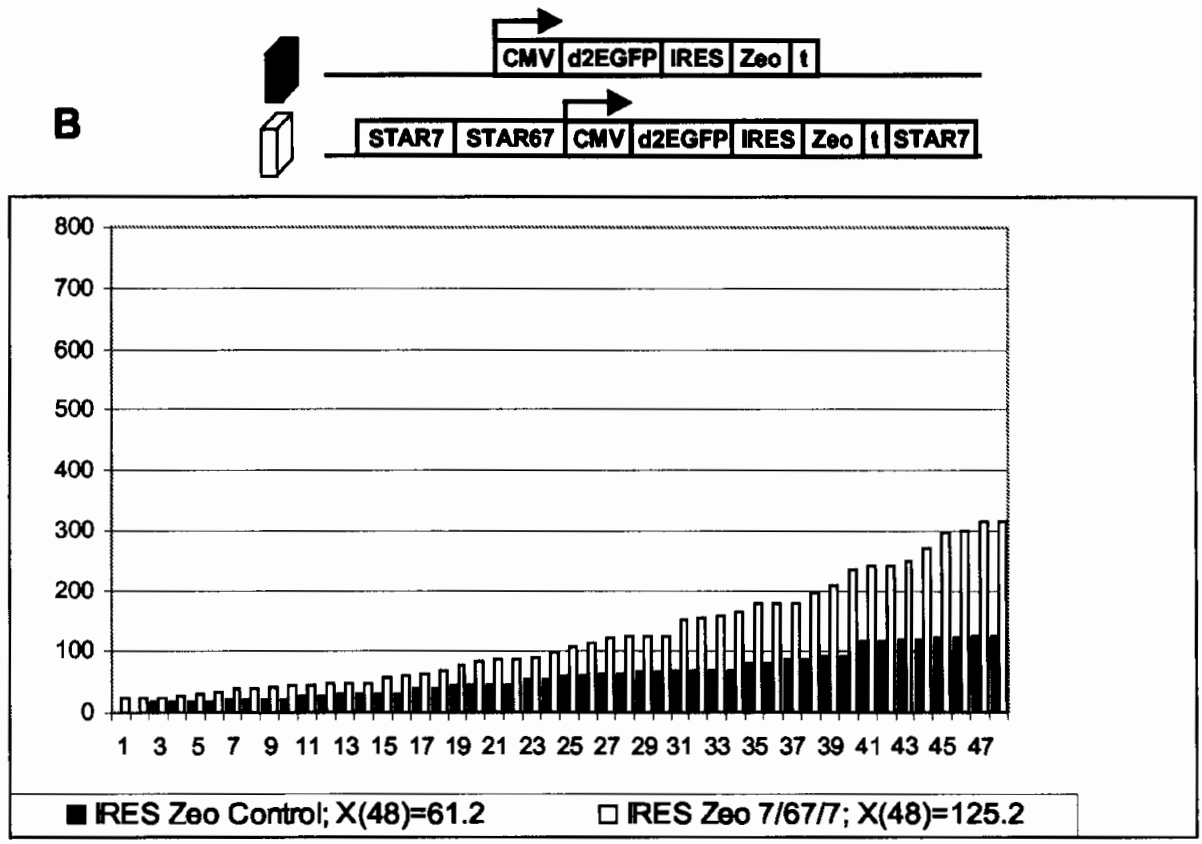
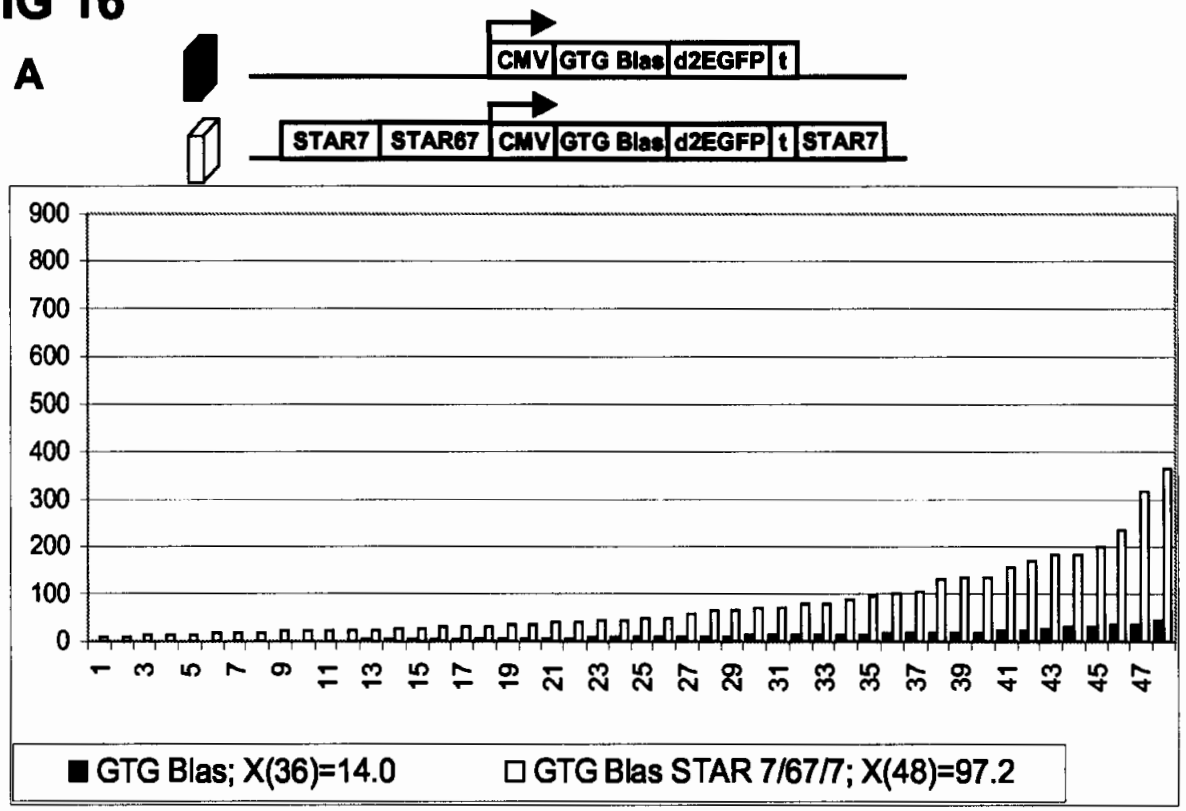

CMV
d2EGFP
IRES
Zeo
t
STAR7
STAR67
CMV
d2EGFP
IRES
Zeo
t
STAR7
800
700
600
500
400
300
200
100
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
IRES Zeo Control; X(48)=61.2
IRES Zeo 7/67/7; X(48)=125.2

FIG 16

A


CMV
GTG Bias
d2EGFP
t
STAR7
STAR67
CMV
GTG Bias
d2EGFP
t
STAR7
900
800
700
600
500
400
300
200
100
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
GTG Blas; X(36)=14.0
GTG Blas STAR 7/67/7; X(48)=97.2

B

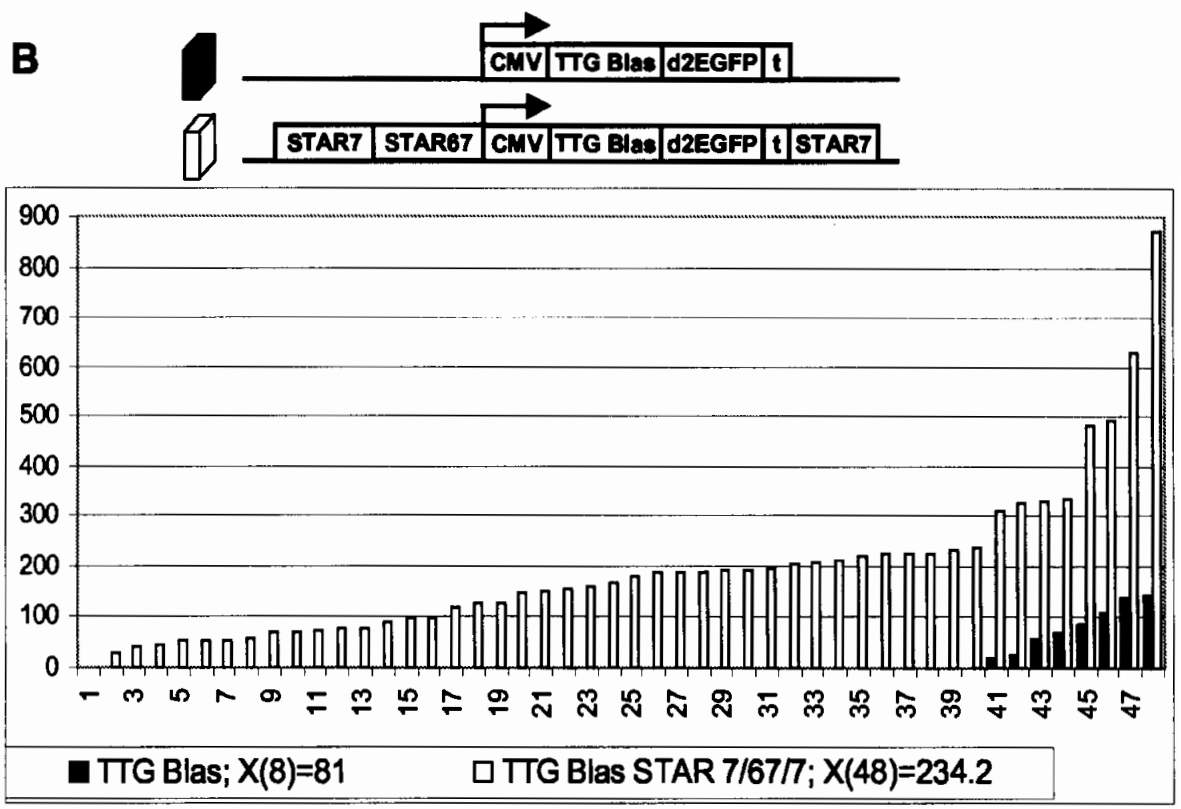

CMV
TTG Bias
d2EGFP
t
STAR7
STAR67
CMV
TTG Bias
d2EGFP
t
STAR7
900
800
700
600
500
400
300
200
100
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
TTG Blas; X(8)=81
TTG Blas STAR 7/67/7; X(48)=234.2

FIG 17


STAR7
STAR67
CMV
TTG Zeo
d2EGFP
t
STAR7

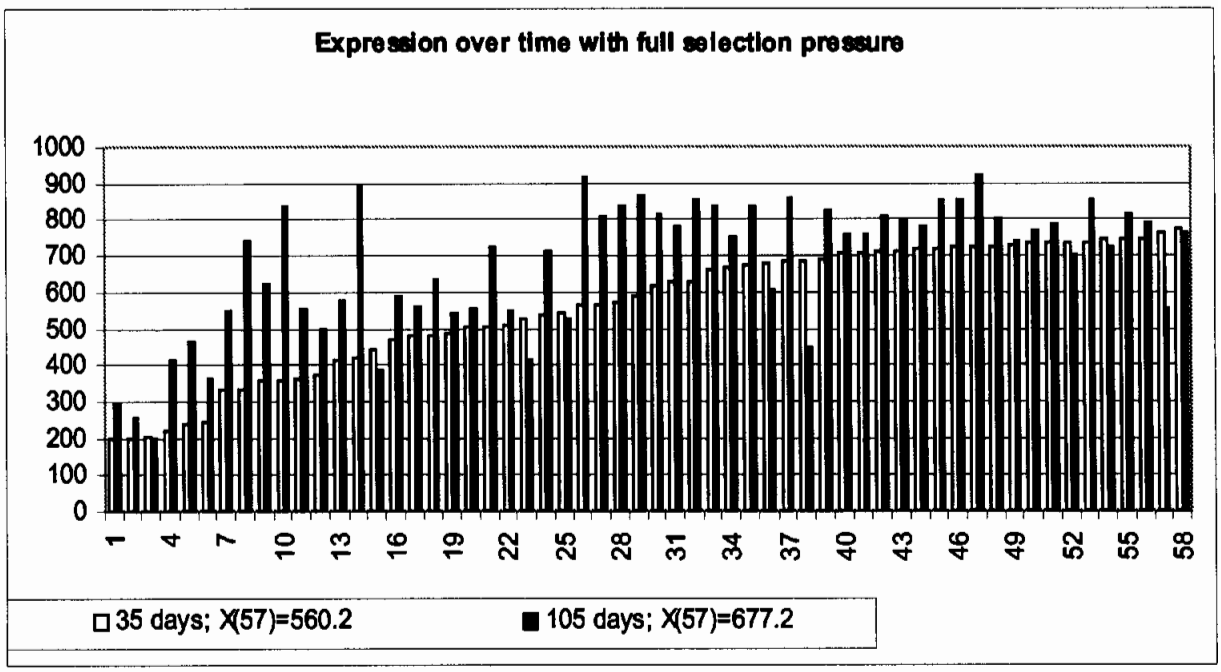

Expression over time with full selection pressure
1000
900
800
700
600
500
400
300
200
100
0
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
35 days; X(57)=560.2
105 days; X(57)=677.2

FIG 18


STAR7
STAR67
CMV
TTG Zeo
d2EGFP
t
STAR7

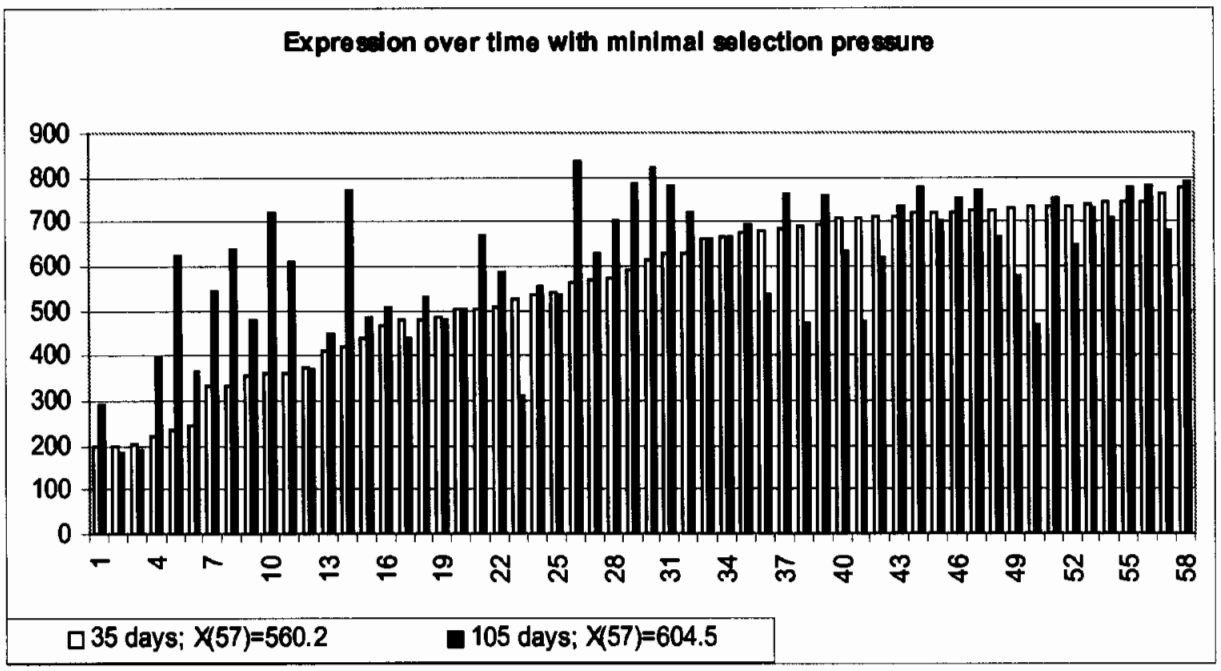

Expression over time with minimal selection pressure
900
800
700
600
500
400
300
200
100
0
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
35 days; X(57)=560.2
105 days; X(57)=604.5

FIG 19


STAR7
STAR67
CMV
TTG Zeo
d2EGFP
t
STAR7

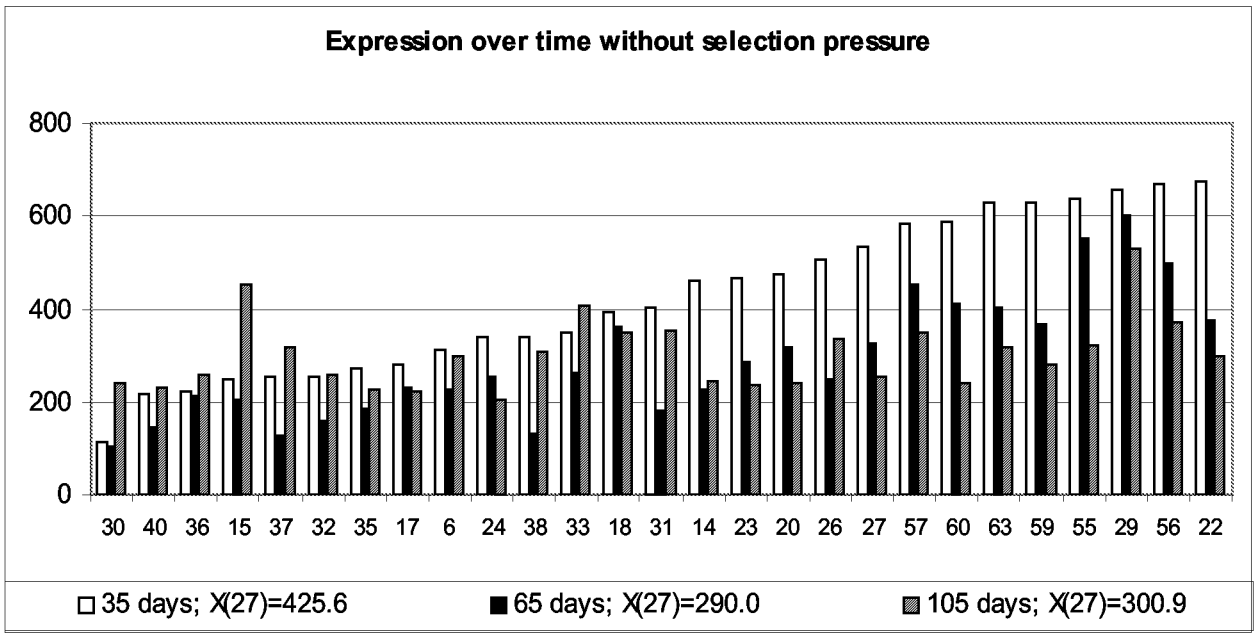
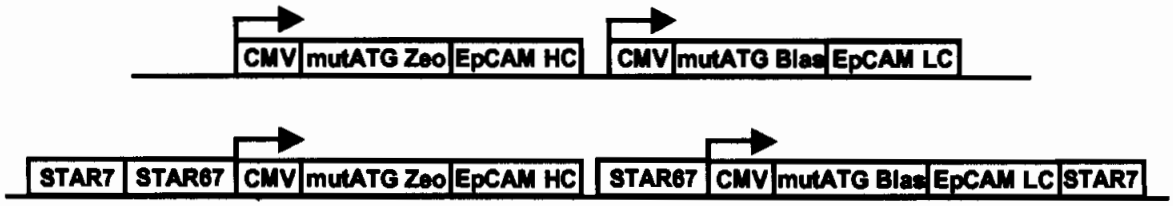

Expression over time without selection pressure
800
600
400
200
0
30 40 36 15 37 32 35 17 6 24 38 33 18 31 14 23 20 26 27 57 60 63 59 55 29 56 22
35 days; X(27)=425.6
65 days; X(27)=290.0
105 days; X(27)=300.9

FIG 20


CMV
mutATG Zeo
EpCAM HC
CMV
mutATG Blas
EpCAM LC
STAR7
STAR67
CMV
mutATG Zeo
EpCAM HC
STAR67
CMV
mutATG Blas
EpCAM LC
STAR7

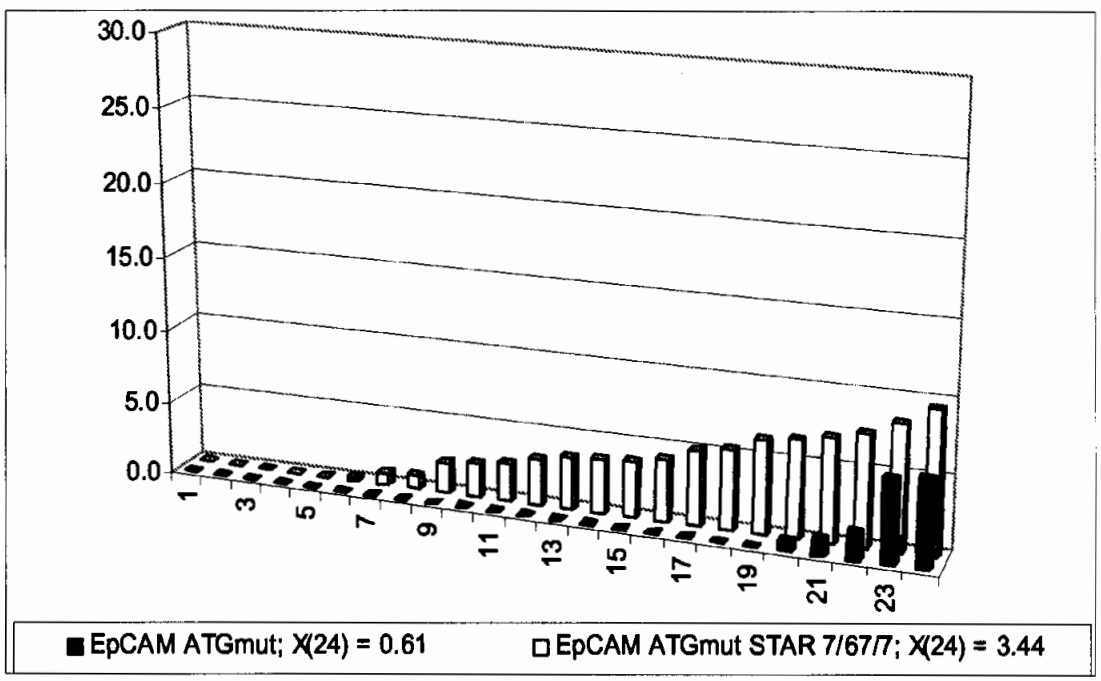
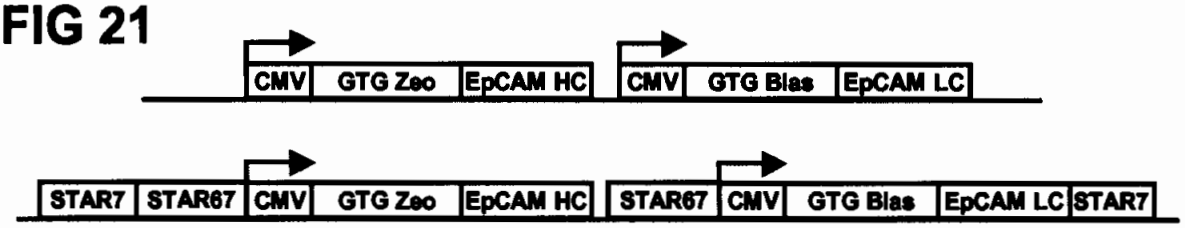

30.0
25.0
20.0
15.0
10.0
5.0
0.0
1
3
5
7
9
11
13
15
17
19
21
23
EpCAM ATGmut; X(24) = 0.61
EpCAM ATGmut STAR 7/67/7; X(24) = 3.44

FIG 21


CMV
GTG Zeo
EpCAM HC
CMV
GTG Bias
EpCAM LC
STAR7
STAR67
CMV
GTG Zeo
EpCAM HC
STAR67
CMV
GTG Bias
EpCAM LC
STAR7

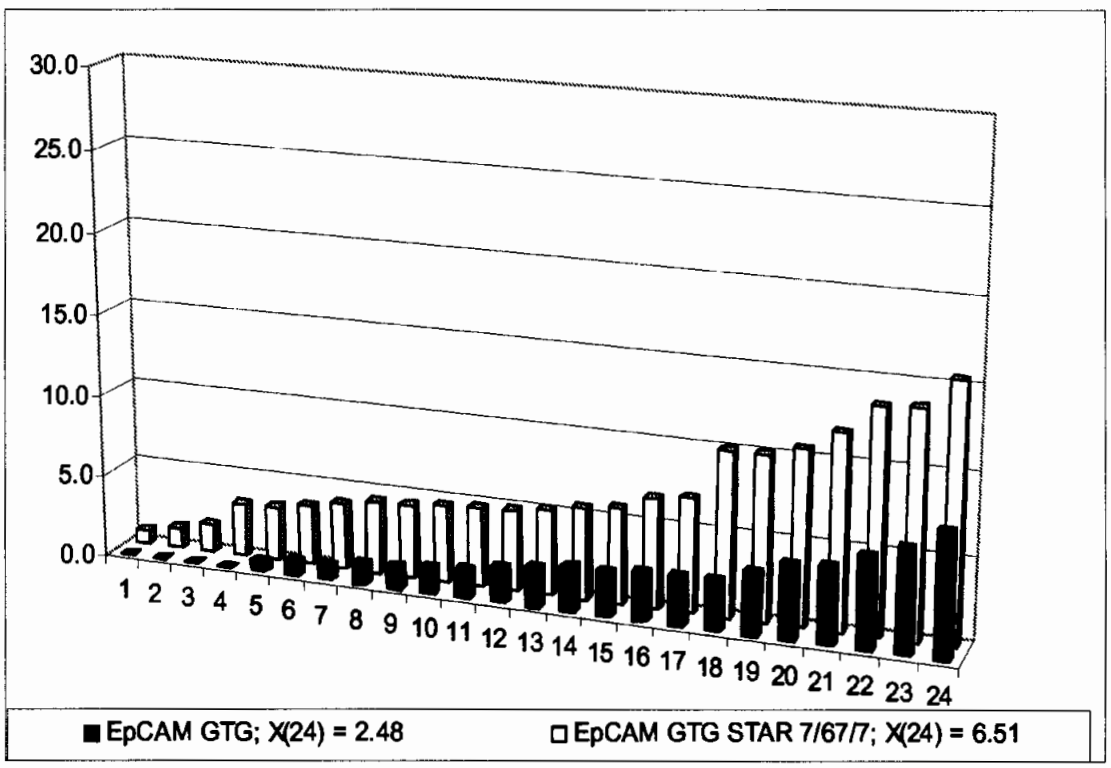
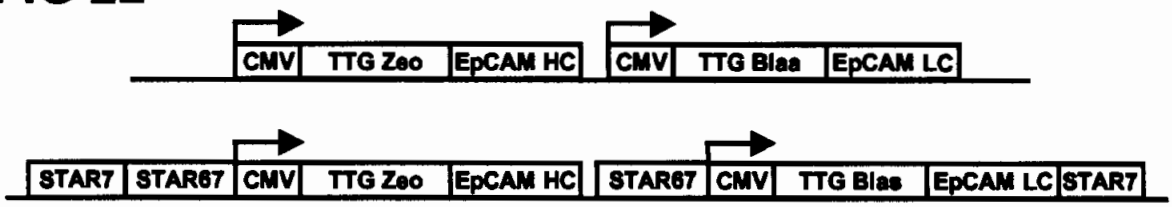

30.0
25.0
20.0
15.0
10.0
5.0
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
EpCAM GTG; X(24) = 2.48
EpCAM GTG STAR 7/67/7; X(24) = 6.51

FIG 22


CMV
TTG Zeo
EpCAM HC
CMV
TTG Blaa
EpCAM LC
STAR7
STAR67
CMV
TTG Zeo
EpCAM HC
STAR67
CMV
TTG Blas
EpCAM LC
STAR7

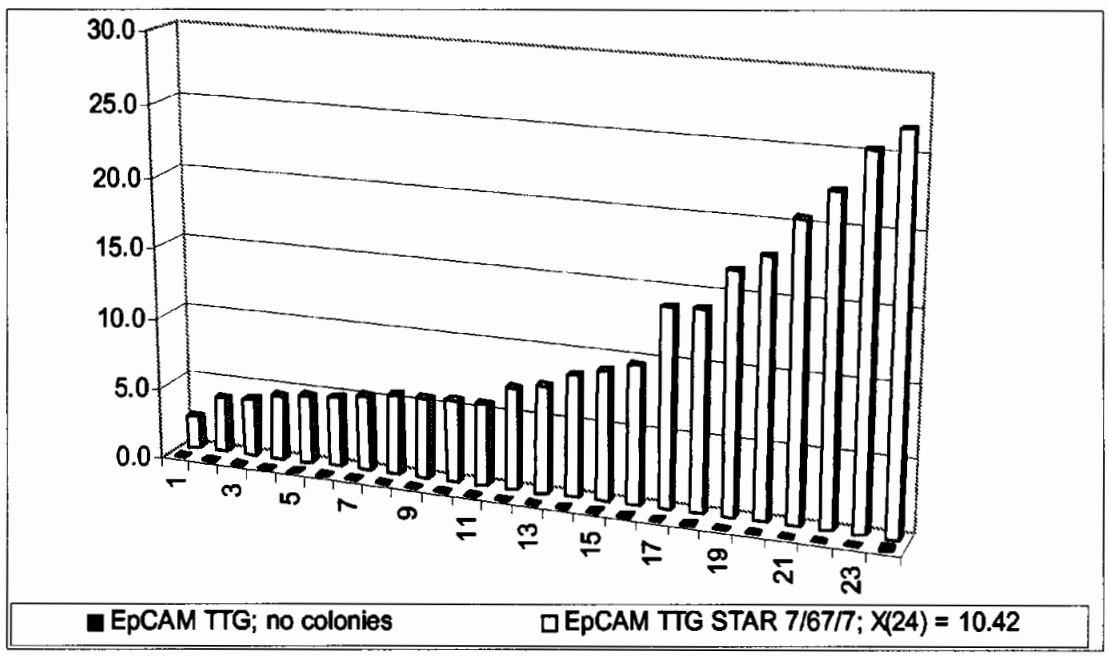

30.0
25.0
20.0
15.0
10.0
5.0
0.0
1
3
5
7
9
11
13
15
17
19
21
23
EpCAM TTG; no colonies
EpCAM TTG STAR 7/67/7; X(24) = 10.42

FIG 23

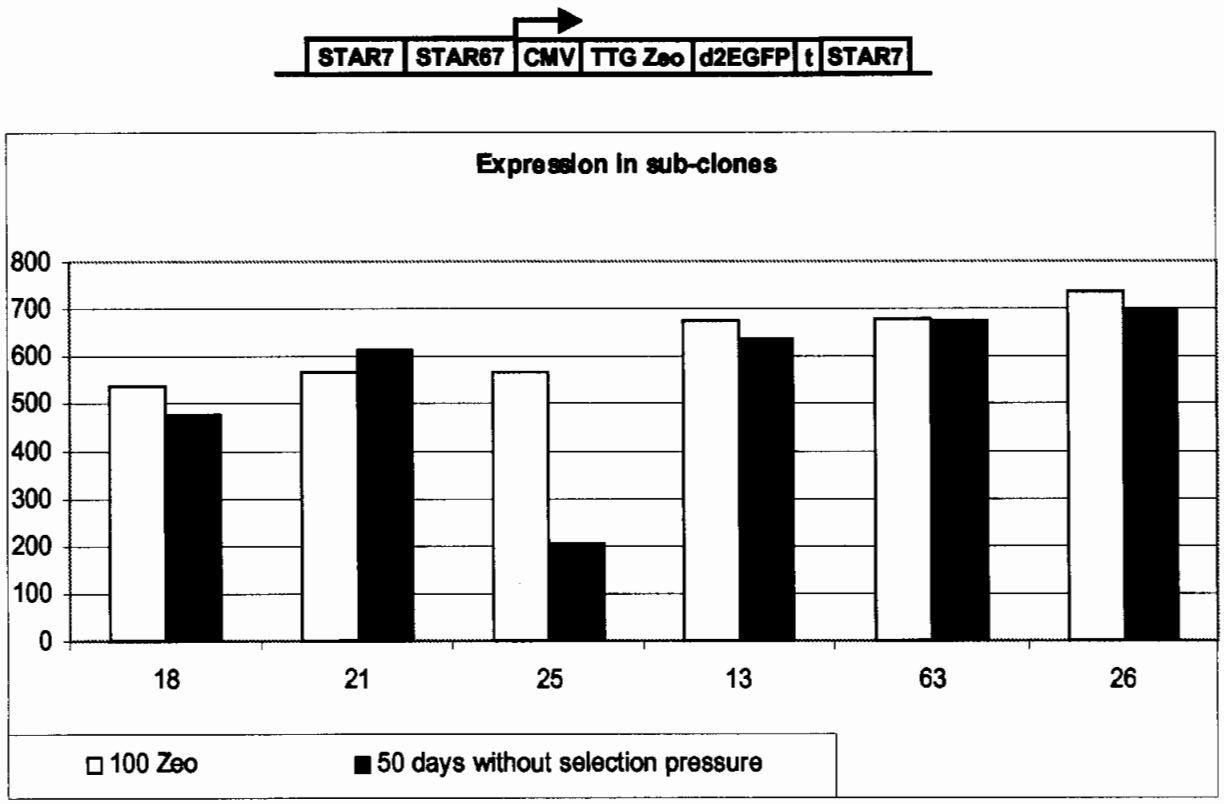

STAR7
STAR67
CMV
TTG Zeo
d2EGFP
t
STAR7
Expression in sub-clones
800
700
600
500
400
300
200
100
0
18
21
25
13
63
26
100 Zeo
50 days without selection pressure

FIG 24

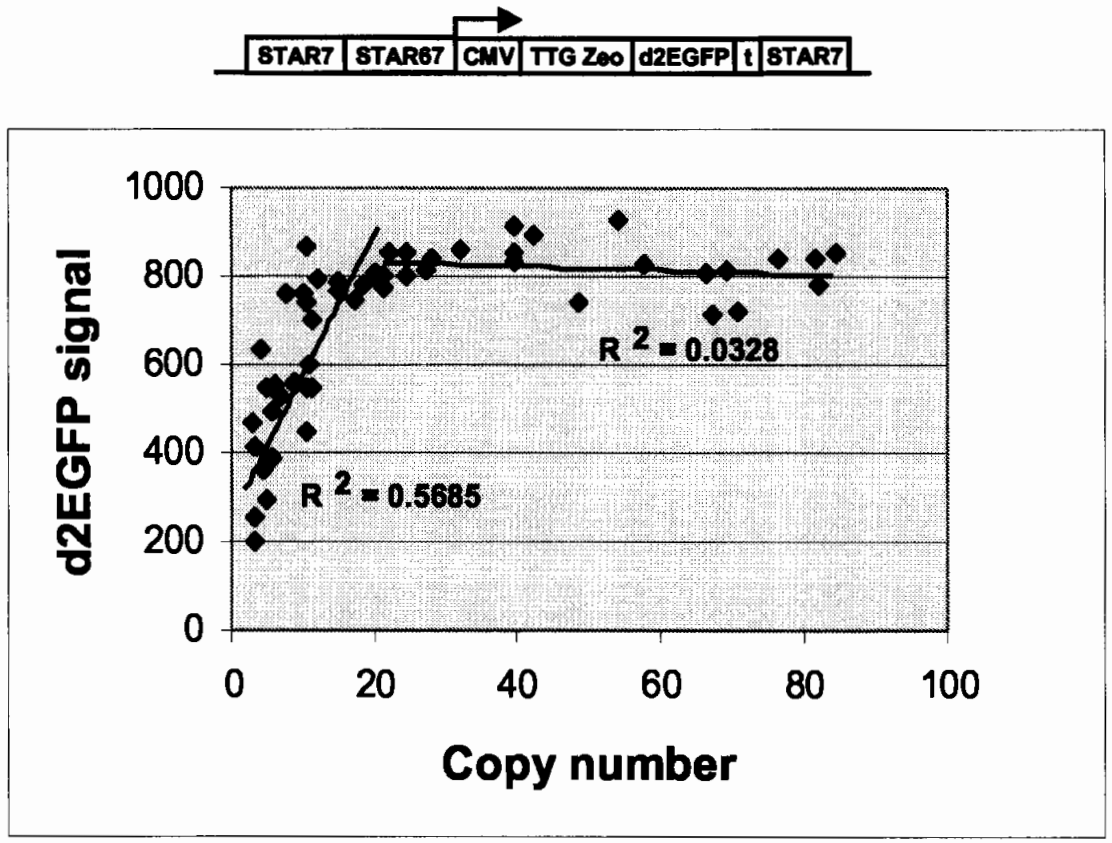

STAR7
STAR67
CMV
TTG Zeo
d2EGFP
t
STAR7
d2EGFP signal
1000
800
600
400
200
0
0
20
40
60
80
100
Copy number
R 2 = 0.0328
R 2 = 0.5685

FIG 25

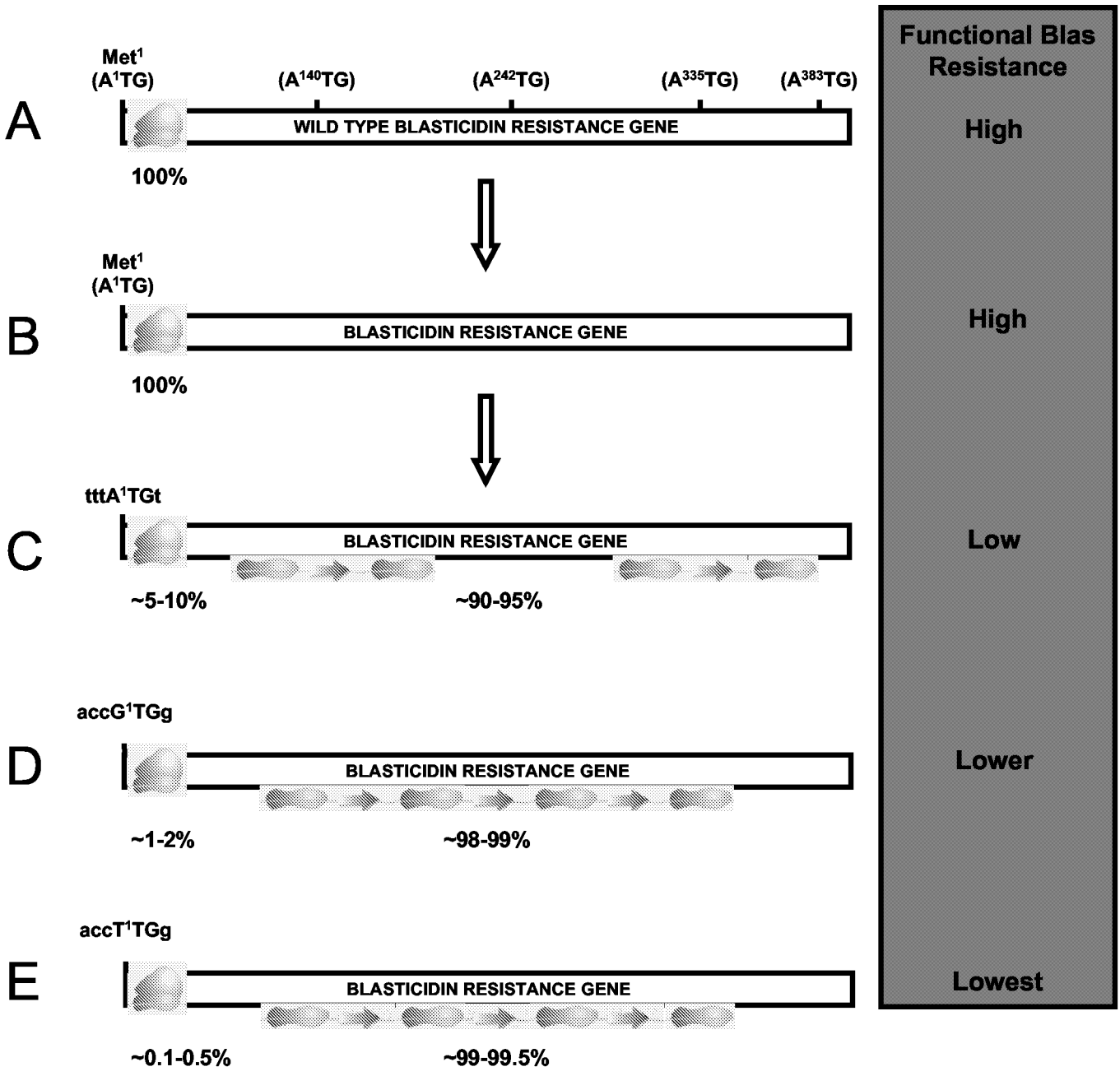

Functional Blas Resistance
A
Met1 (A1TG)
(A140TG)
(A242TG)
(A335TG)
(A383TG)
WILD TYPE BLASTICIDIN RESISTANCE GENE
100%
High
B
Met1 (A1TG)
BLASTICIDIN RESISTANCE GENE
100%
High
C
tttA1TGt
BLASTICIDIN RESISTANCE GENE
~5-10%
~90-95%
Low
D
accG1TGg
BLASTICIDIN RESISTANCE GENE
~1-2%
~98-99%
Lower
E
accT1TGg
BLASTICIDIN RESISTANCE GENE
~0.1-0.5%
~99-99.5%
Lowest

Fig. 26

**ATG**GCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGA
CGTCGCCGGAGCGGTCGAGTTCTGGACCGACCGGCTCGGGTTCT
CCCGGGACTTCGTGGAGGACGACTTCGCCGGTGTGGTCCGGGAC
GACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTGGTGCC
GGACAACACCCTGGCCTGGGTGTGGGTGCGCGGCCTGGACGAGC
TGTACGCCGAGTGGTCGGAGGTCGTGTCCACGAACTTCCGGGAC
GCCTCCGGGCCGGCC**ATG**ACCGAGATCGGCGAGCAGCCGTGGGG
GCGGGAGTTCGCCCTGCGCGACCCGGCCGGCAACTGCGTGCACT
TCGTGGCCGAGGAGCAGGACTGA

Fig. 27

**ATG**GCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAAC
GGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCG
CAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCAT
TTTACTGGGGGACCTTGTGCAGAACTCGTGGTGCTGGGCACTGCTGCTGC
TGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAACA
GGGGCATCTTGAGCCCCTGCGGACGGTGCCGACAGGTGCTTCTCGATCTG
CATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGC
AGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAA

Fig. 28

**ATG**ACCGAGTACAAGCCCACGGTGCGCCTCGCCACCCGCGACGACGTCCCC
AGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCGCCACGCGC
CACACCGTCGATCCGGACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAA
CTCTTCCTCACGCGCGTCGGGCTCGACATCGGCAAGGTGTGGGTCGCGGAC
GACGGCGCCGCGGTGGCGGTCTGGACCACGCCGGAGAGCGTCGAAGCGGGG
GCGGTGTTCGCCGAGATCGGCCCGCGC**ATG**GCCGAGTTGAGCGGTTCCCGG
CTGGCCGCGCAGCAACAG**ATG**GAAGGCCTCCTGGCGCCGCACCGGCCCAAG
GAGCCCGCGTGGTTCCTGGCCACCGTCGGCGTCTCGCCCGACCACCAGGGC
AAGGGTCTGGGCAGCGCCGTCGTGCTCCCCGGAGTGGAGGCGGCCGAGCGC
GCCGGGGTGCCCGCCTTCCTGGAGACCTCCGCGCCCCGCAACCTCCCCTTC
TACGAGCGGCTCGGCTTCACCGTCACCGCCGACGTCGAGTGCCCGAAGGAC
CGCGCGACCTGGTGC**ATG**ACCCGCAAGCCCGGTGCCTGA

Fig. 29

**ATG**GTTCGACCATTGAACTGCATCGTCGCCGTGTCCCAAAAT**ATG**GGGATT
GGCAAGAACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTCAAGTAC
TTCCAAAGA**ATG**ACCACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTG
ATT**ATG**GGTAGGAAAACCTGGTTCTCCATTCCTGAGAAGAATCGACCTTTA
AAGGACAGAATTAATATAGTTCTCAGTAGAGAACTCAAAGAACCACCACGA
GGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGACTTATTGAA
CAACCGGAATTGGCAAGTAAAGTAGAC**ATG**GTTTGGATAGTCGGAGGCAGT
TCTGTTTACCAGGAAGCC**ATG**AATCAACCAGGCCACCTCAGACTCTTTGTG
ACAAGGATC**ATG**CAGGAATTTGAAAGTGACACGTTTTTCCCAGAAATTGAT
TTGGGGAAATATAAACTTCTCCCAGAATACCCAGGCGTCCTCTCTGAGGTC
CAGGAGGAAAAAGGCATCAAGTATAAGTTTGAAGTCTACGAGAAGAAAGAC
TAA

Fig. 30

**ATG**AAAAAGCCTGAACTCACCGCGACGTCTGTCGAGAAGTTTCTGATCGAA
AAGTTCGACAGCGTCTCCGACCTG**ATG**CAGCTCTCGGAGGGCGAAGAATCT
CGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAAT
AGCTGCGCCGATGGTTTCTACAAAGATCGTTATGTTTATCGGCACTTTGCA
TCGGCCGCGCTCCCGATTCCGGAAGTGCTTGACATTGGGGAATTCAGCGAG
AGCCTGACCTATTGCATCTCCCGCCGTGCACAGGGTGTCACGTTGCAAGAC
CTGCCTGAAACCGAACTGCCCGCTGTTCTGCAGCCGGTCGCGGAGGCC**ATG**
GATGCGATCGCTGCGGCCGATCTTAGCCAGACGAGCGGGTTCGGCCCATTC
GGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCG
ATTGCTGATCCCCATGTGTATCACTGGCAAACTGTG**ATG**GACGACACCGTC
AGTGCGTCCGTCGCGCAGGCTCTCGATGAGCTG**ATG**CTTTGGGCCGAGGAC
TGCCCCGAAGTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTC
CTGACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCG**ATG**
TTCGGGGATTCCCAATACGAGGTCGCCAACATCTTCTTCTGGAGGCCGTGG
TTGGCTTGT**ATG**GAGCAGCAGACGCGCTACTTCGAGCGGAGGCATCCGGAG
CTTGCAGGATCGCCGCGGCTCCGGGCGTAT**ATG**CTCCGCATTGGTCTTGAC
CAACTCTATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCG
CAGGGTCGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGT
ACACAAATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGTAGAA
GTACTCGCCGATAGTGGAAACCGACGCCCCAGCACTCGTCCGGAGGCAAAG
GAATTCGGGAGATGGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCG
GAAGGAACCCGCGCT**ATG**ACGGCAATAAAAAGACAGAATAAAACGCACGGG
TGTTGGGTCGTTTGTTCATAA

Fig. 31

**ATG**GGATCGGCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCT
TGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGC
TCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTT
GTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCG
CGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGAC
GTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGG
CAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATC**ATG**
GCTGATGCA**ATG**CGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTC
GACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGG**ATG**GAAGCC
GGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCA
GCCGAACTGTTCGCCAGGCTCAAGGCGCGC**ATG**CCCGACGGCGATGATCTC
GTCGTGACCCATGGCGATGCCTGCTTGCCGAATATC**ATG**GTGGAAAATGGC
CGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTAT
CAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAA
TGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAG
CGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA

Fig. 32

**ATG**ACCACCTCAGCAAGTTCCCACTTAAATAAAGGCATCAAGCAGGTGTAC
**ATG**TCCCTGCCTCAGGGTGAGAAAGTCCAGGCC**ATG**TATATCTGGATCGAT
GGTACTGGAGAAGGACTGCGCTGCAAGACCCGGACCCTGGACAGTGAGCCC
AAGTGTGTGGAAGAGTTGCCTGAGTGGAATTTCGATGGCTCCAGTACTTTA
CAGTCTGAGGGTTCCAACAGTGAC**ATG**TATCTCGTGCCTGCTGCC**ATG**TTT
CGGGACCCCTTCCGTAAGGACCCTAACAAGCTGGTGTTATGTGAAGTTTTC
AAGTACAATCGAAGGCCTGCAGAGACCAATTTGAGGCACACCTGTAAACGG
ATA**ATG**GAC**ATG**GTGAGCAACCAGCACCCCTGGTTTGGC**ATG**GAGCAGGAG
TATACCCTC**ATG**GGGACAGATGGGCACCCCTTTGGTTGGCCTTCCAACGGC
TTCCCAGGGCCCCAGGGTCCATATTACTGTGGTGTGGGAGCAGACAGAGCC
TATGGCAGGGACATCGTGGAGGCCCATTACCGGGCCTGCTTGTATGCTGGA
GTCAAGATTGCGGGGACTAATGCCGAGGTC**ATG**CCTGCCCAGTGGGAATTT
CAGATTGGACCTTGTGAAGGAATCAGC**ATG**GGAGATCATCTCTGGGTGGCC
CGTTTCATCTTGCATCGTGTGTGTGAAGACTTTGGAGTGATAGCAACCTTT
GATCCTAAGCCCATTCCTGGGAACTGGAATGGTGCAGGCTGCCATACCAAC
TTCAGCACCAAGGCC**ATG**CGGGAGGAGAATGGTCTGAAGTACATCGAGGAG
GCCATTGAGAAACTAAGCAAGCGGCACCAGTACCACATCCGTGCCTATGAT
CCCAAGGGAGGCCTGGACAATGCCCGACGTCTAACTGGATTCCATGAAACC
TCCAACATCAACGACTTTTCTGGTGGTGTAGCCAATCGTAGCGCCAGCATA
CGCATTCCCCGGACTGTTGGCCAGGAGAAGAAGGGTTACTTTGAAGATCGT
CGCCCCTCTGCCAACTGCGACCCCTTTTCGGTGACAGAAGCCCTCATCCGC
ACGTGTCTTCTCAATGAAACCGGCGATGAGCCCTTCCAGTACAAAAATTA

FIG 33

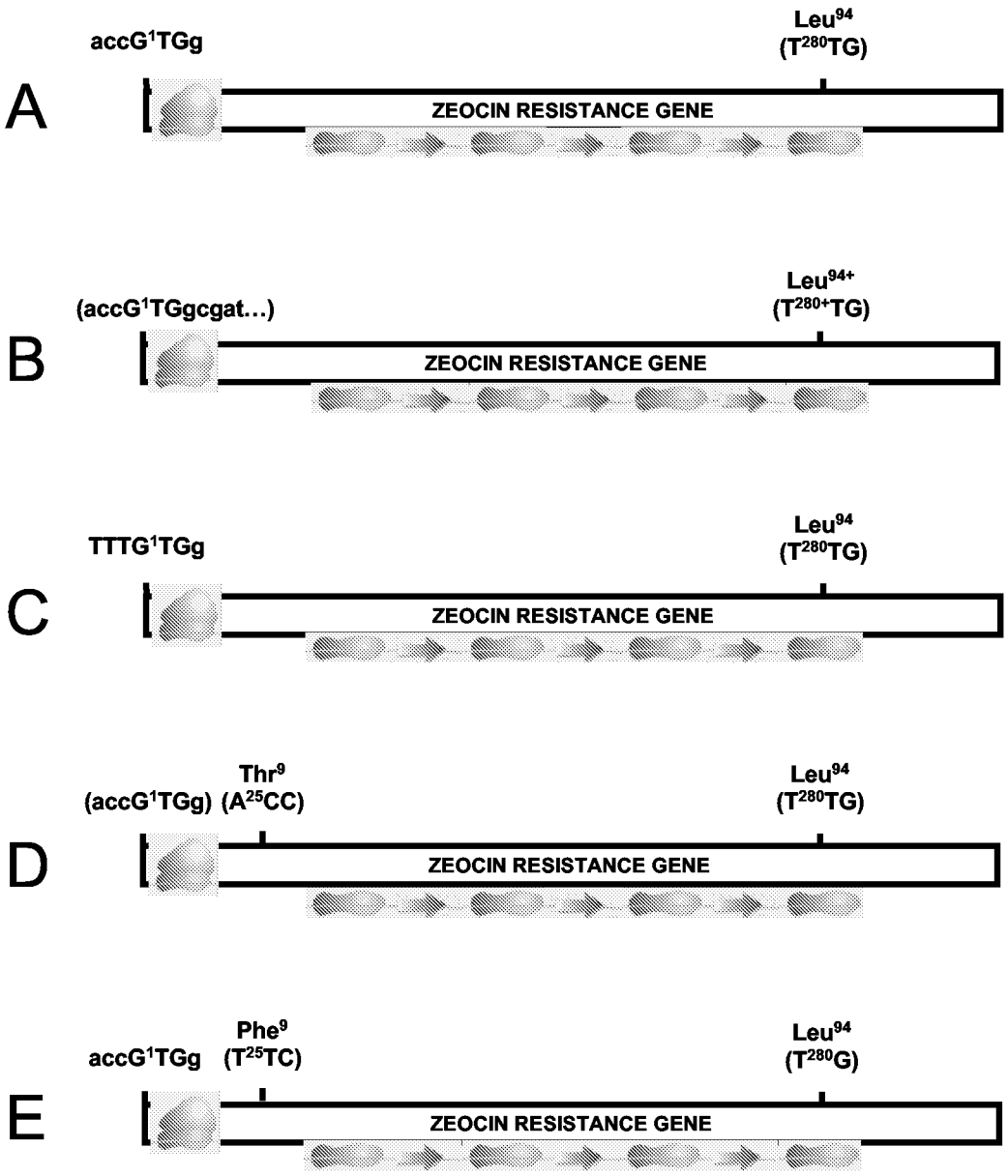
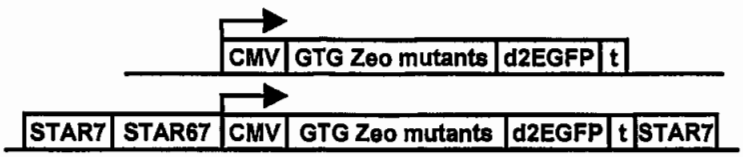

A
accG1TGg
Leu94
(T280TG)
ZEOCIN RESISTANCE GENE
B
(accG1TGgcgat…)
Leu94+
(T280+TG)
ZEOCIN RESISTANCE GENE
C
TTTG1TGg
Leu94
(T280TG)
ZEOCIN RESISTANCE GENE
D
(accG1TGg)
Thr9
(A25CC)
Leu94
(T280TG)
ZEOCIN RESISTANCE GENE
E
accG1TGg
Phe9
(T25TC)
Leu94
(T280G)
ZEOCIN RESISTANCE GENE

FIG 34


CMV
GTG Zeo mutants
d2EGFP
t
STAR7
STAR67
CMV
GTG Zeo mutants
d2EGFP
t
STAR7

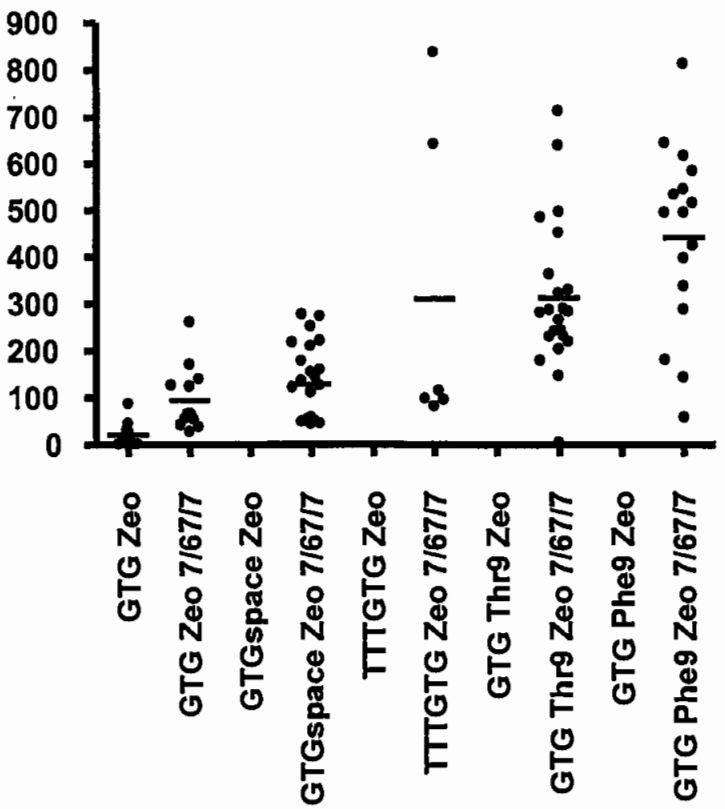

900
800
700
600
500
400
300
200
100
0
GTG Zeo
GTG Zeo 7/67/7
GTGspace Zeo
GTGspace Zeo 7/67/7
TTTGTG Zeo
TTTGTG Zeo 7/67/7
GTG Thr9 Zeo
GTG Thr9 Zeo 7/67/7
GTG Phe9 Zeo
GTG Phe9 Zeo 7/67/7

FIG 35

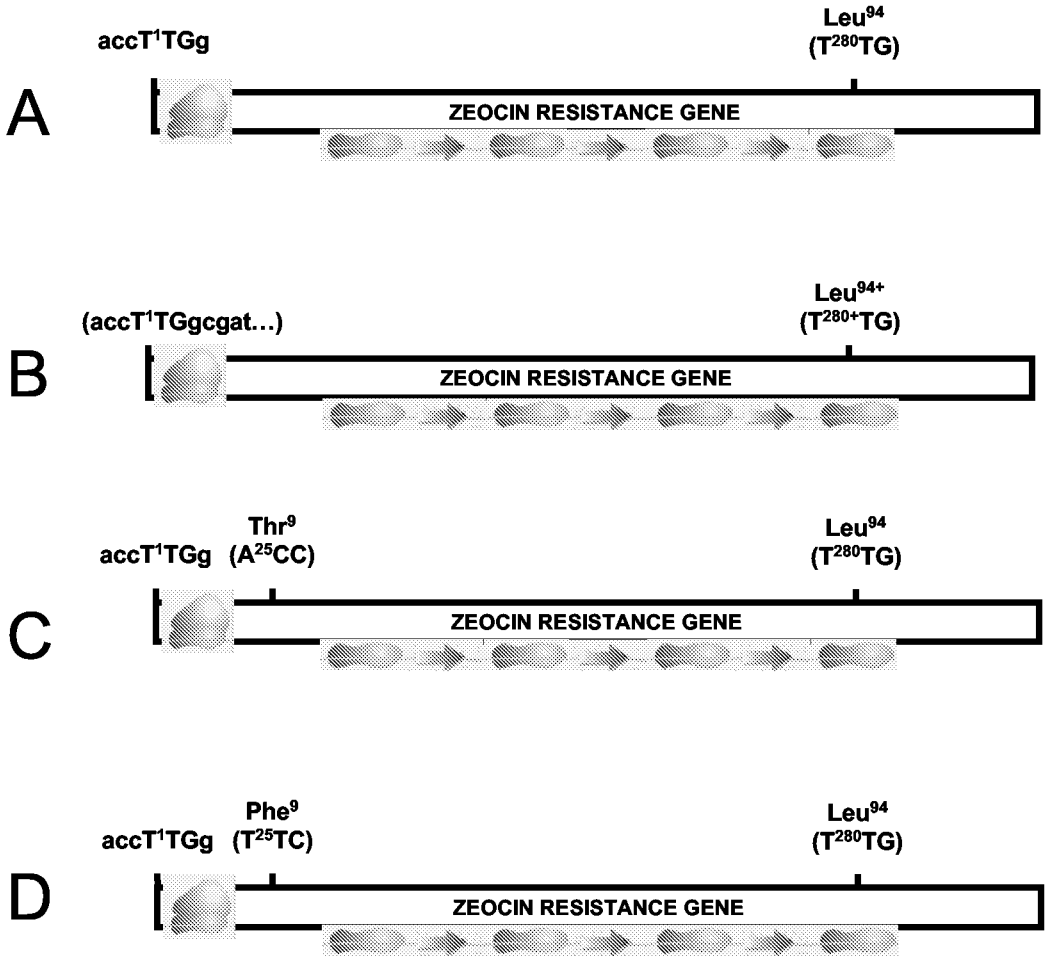
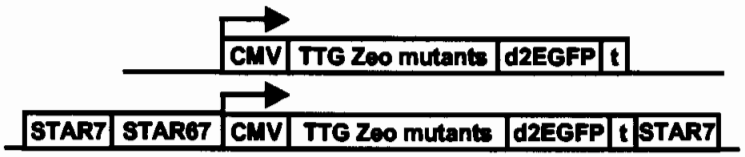

A
accT1TGg
Leu94
(T280TG)
ZEOCIN RESISTANCE GENE
B
(accT1TGgcgat...)
Leu94+
(T280+TG)
ZEOCIN RESISTANCE GENE
C
accT1TGg
Thr9
(A25CC)
Leu94
(T280TG)
ZEOCIN RESISTANCE GENE
D
accT1TGg
Phe9
(T25TC)
Leu94
(T280TG)
ZEOCIN RESISTANCE GENE

FIG 36


CMV
TTG Zeo mutants
d2EGFP
t
STAR7
STAR67
CMV
TTG Zeo mutants
d2EGFP
t
STAR7

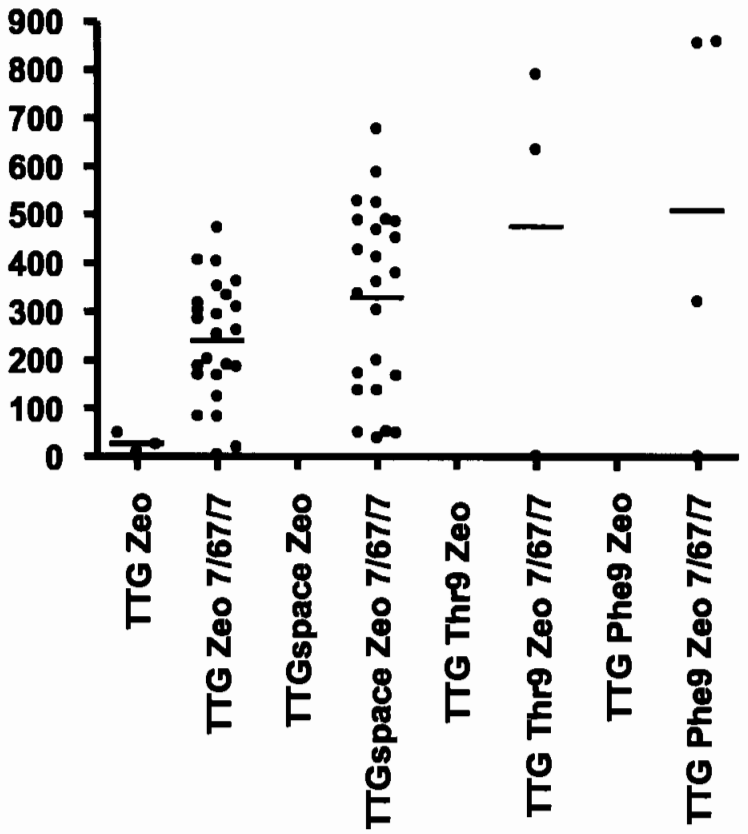

900
800
700
600
500
400
300
200
100
0
TTG Zeo
TTG Zeo 7/67/7
TTGspace Zeo
TTGspace Zeo 7/67/7
TTG Thr9 Zeo
TTG Thr9 Zeo 7/67/7
TTG Phe9 Zeo
TTG Phe9 Zeo 7/67/7

FIG 37

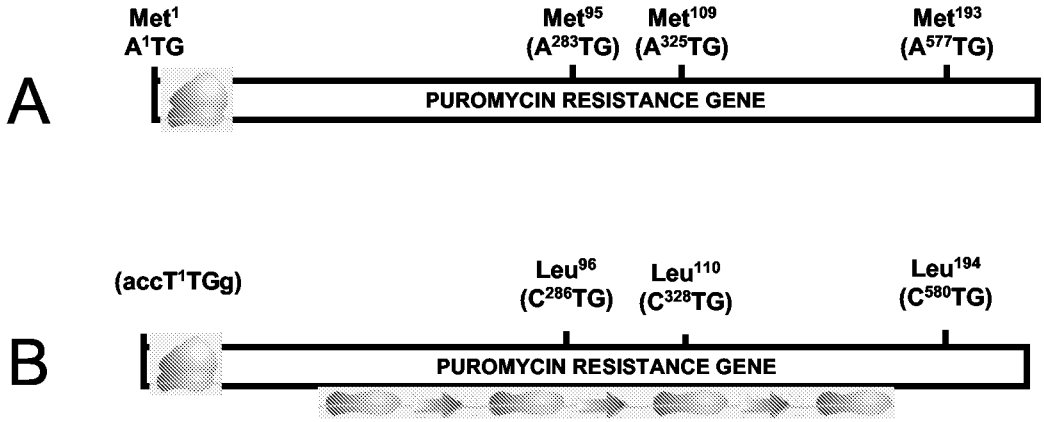
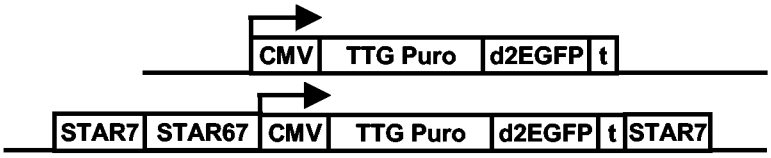

A
Met1
A1TG
Met95
(A283TG)
Met109
(A325TG)
Met193
(A577TG)
PUROMYCIN RESISTANCE GENE
B
(accT1TGg)
Leu96
(C286TG)
Leu110
(C328TG)
Leu194
(C580TG)
PUROMYCIN RESISTANCE GENE

FIG 38


CMV
TTG Puro
d2EGFP
t
STAR7
STAR67
CMV
TTG Puro
d2EGFP
t
STAR7

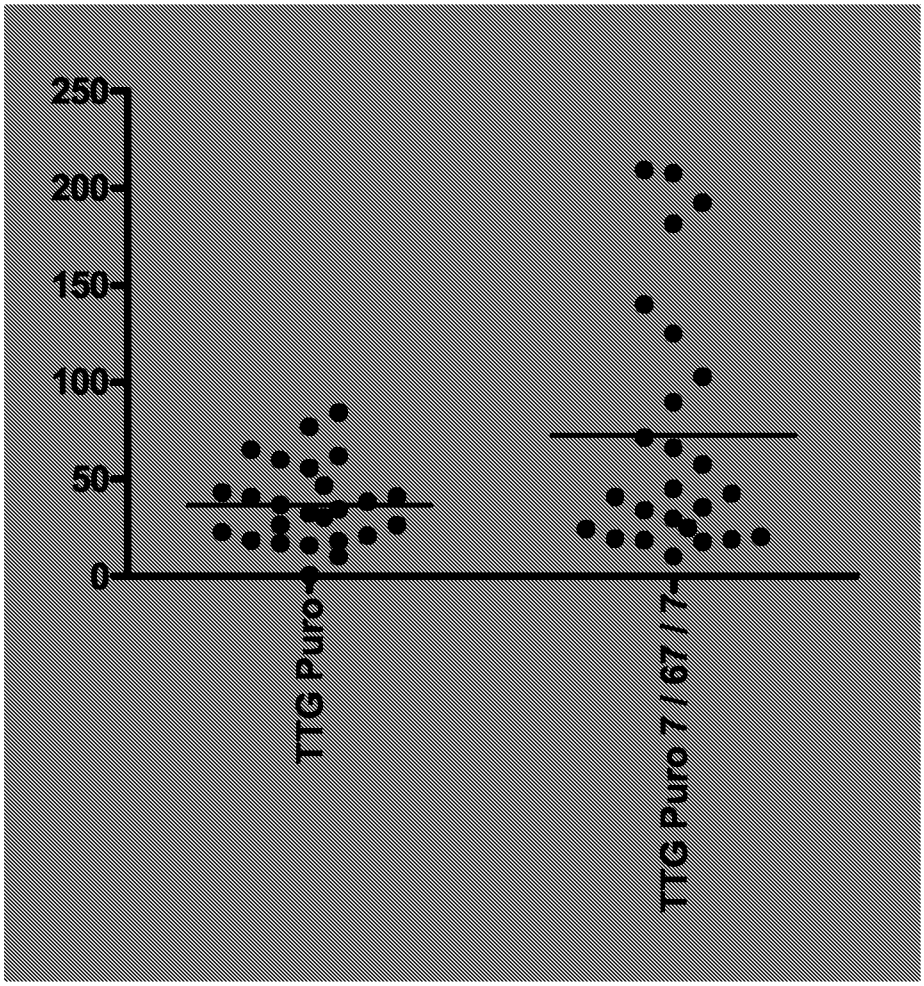

250
200
150
100
50
0
TTG Puro
TTG Puro 7 / g7 / 7

FIG 39

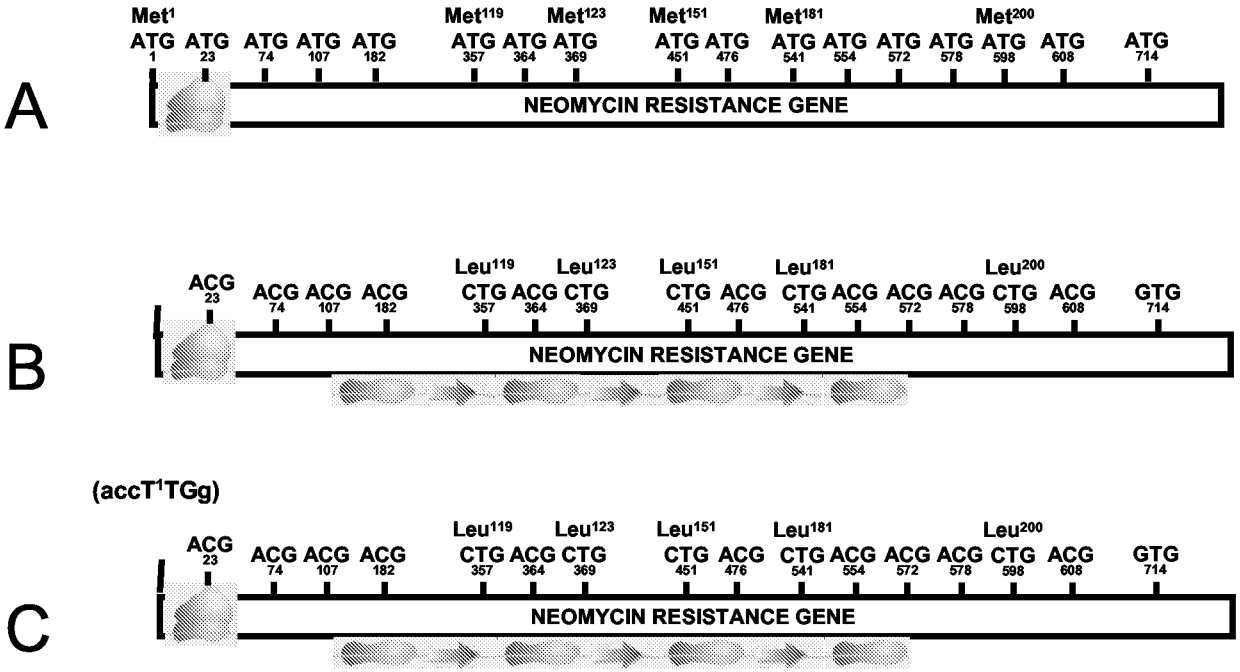

A
Met1
ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG
1 23 74 107 182 357 364 369 451 476 541 554 572 578 598 608 714
Met119 Met123 Met151 Met181 Met200
NEOMYCIN RESISTANCE GENE
B
ACG ACG ACG ACG CTG ACG CTG CTG ACG CTG ACG ACG ACG CTG ACG GTG
23 74 107 182 357 364 369 451 476 541 554 572 578 598 608 714
Leu119 Leu123 Leu151 Leu181 Leu200
NEOMYCIN RESISTANCE GENE
(accT1TGg)
C
ACG ACG ACG ACG CTG ACG CTG CTG ACG CTG ACG ACG ACG CTG ACG GTG
23 74 107 182 357 364 369 451 476 541 554 572 578 598 608 714
Leu119 Leu123 Leu151 Leu181 Leu200
NEOMYCIN RESISTANCE GENE

FIG 40

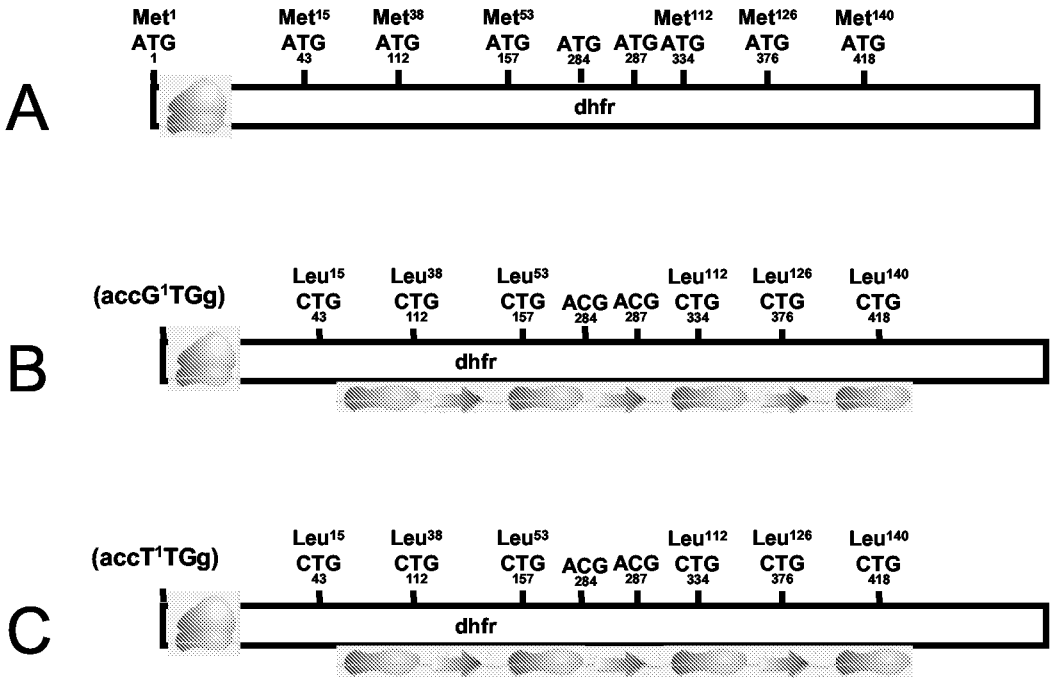
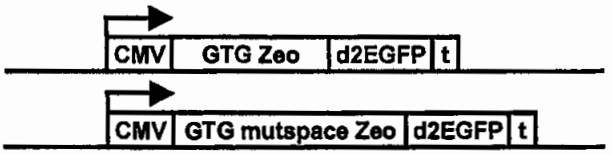

A
Met1 ATG 1
Met15 ATG 43
Met38 ATG 112
Met53 ATG 157
ATG 284
ATG 287
Met112 ATG 334
Met126 ATG 376
Met140 ATG 418
dhfr
B
(accG1TGg)
Leu15 CTG 43
Leu38 CTG 112
Leu53 CTG 157
ACG 284
ACG 287
Leu112 CTG 334
Leu126 CTG 376
Leu140 CTG 418
dhfr
C
(accT1TGg)
Leu15 CTG 43
Leu38 CTG 112
Leu53 CTG 157
ACG 284
ACG 287
Leu112 CTG 334
Leu126 CTG 376
Leu140 CTG 418
dhfr

FIG 41


CMV
GTG Zeo
d2EGFP
t
CMV
GTG mutspace Zeo
d2EGFP
t

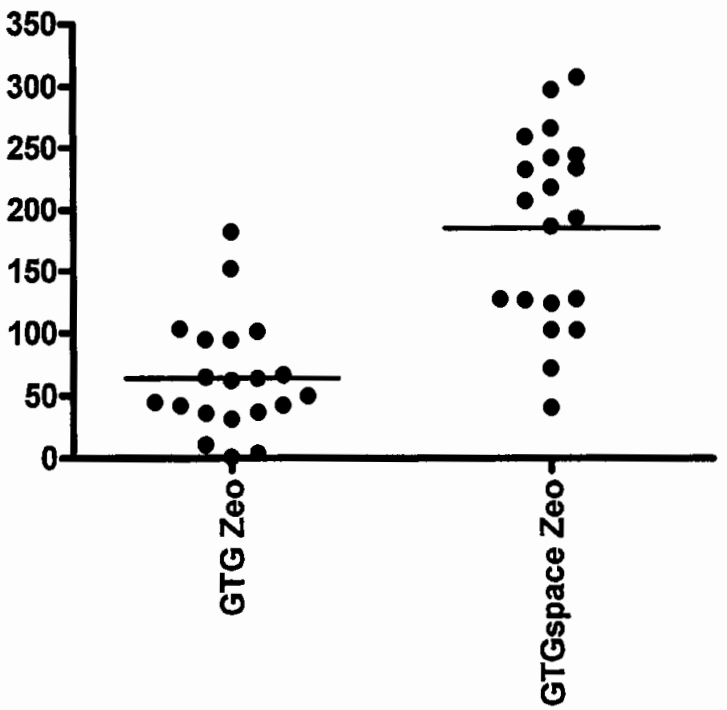

350
300
250
200
150
100
50
0
GTG Zeo
GTGspace Zeo

FIG 42

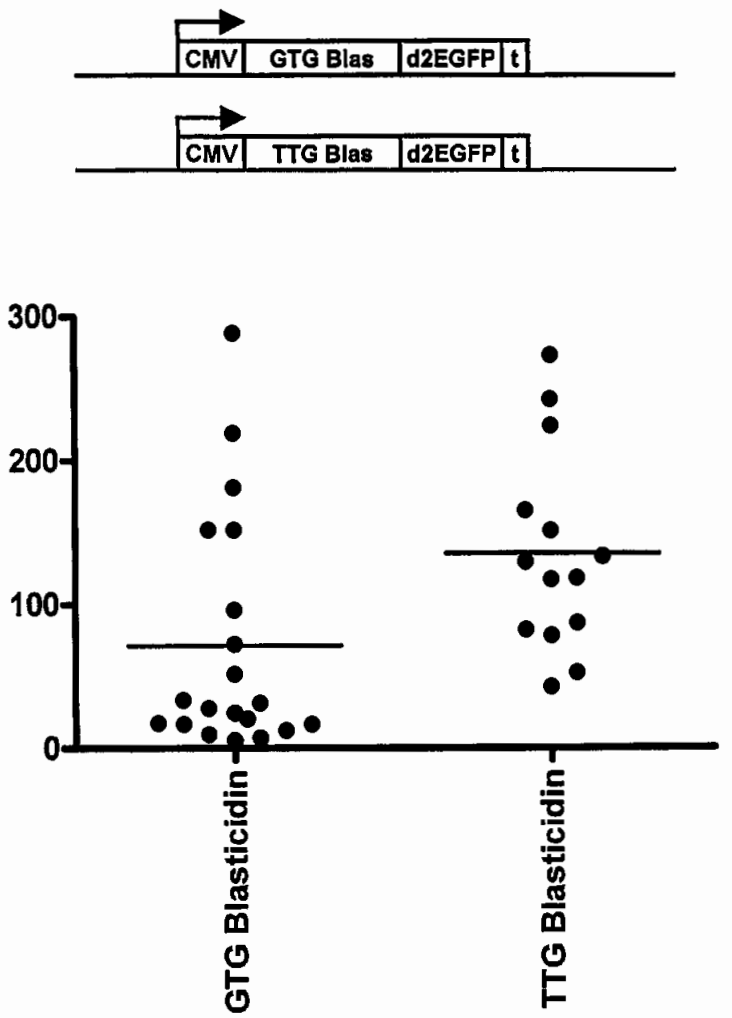
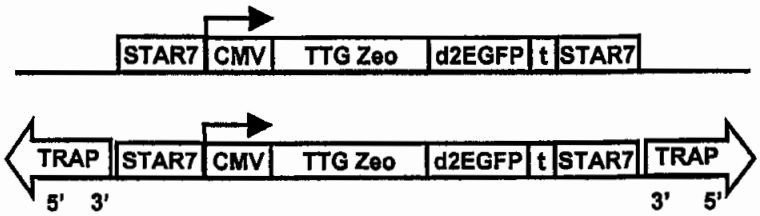

CMV
GTG Blas
d2EGFP
t
CMV
TTG Blas
d2EGFP
t
300
200
100
0
GTG Blasticidin
TTG Blasticidin

FIG 43


STAR7
CMV
TTG Zeo
d2EGFP
t
STAR7
TRAP
STAR7
CMV
TTG Zeo
d2EGFP
t
STAR7
TRAP
5'
3'
3'
5'

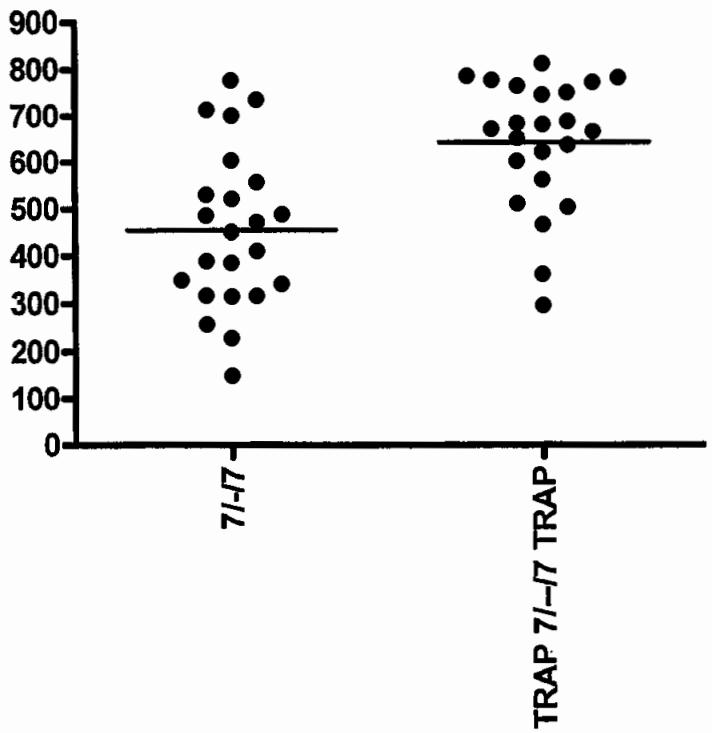

900
800
700
600
500
400
300
200
100
0
7I-/7
TRAP 7I-/7 TRAP

FIG 44


STAR7
STAR67
CMV
TTG Zeo
EpCAM LC
STAR67
CMV
TTG Blas
EpCAM HC
STAR7

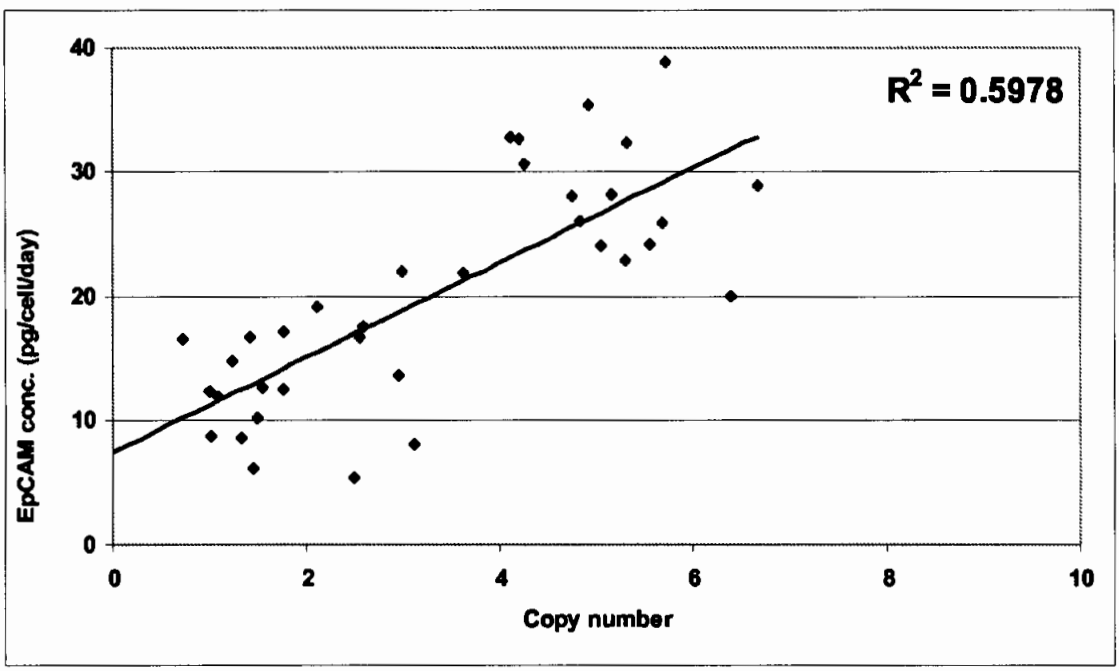
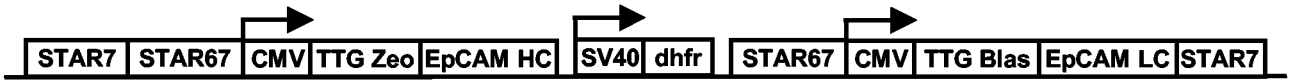

$R^2$ = 0.5978
EpCAM conc. (pg/cell/day)
Copy number
0
10
20
30
40
0
2
4
6
8
10

FIG 45


STAR7
STAR67
CMV
TTG Zeo
EpCAM HC
SV40
dhfr
STAR67
CMV
TTG Blas
EpCAM LC
STAR7

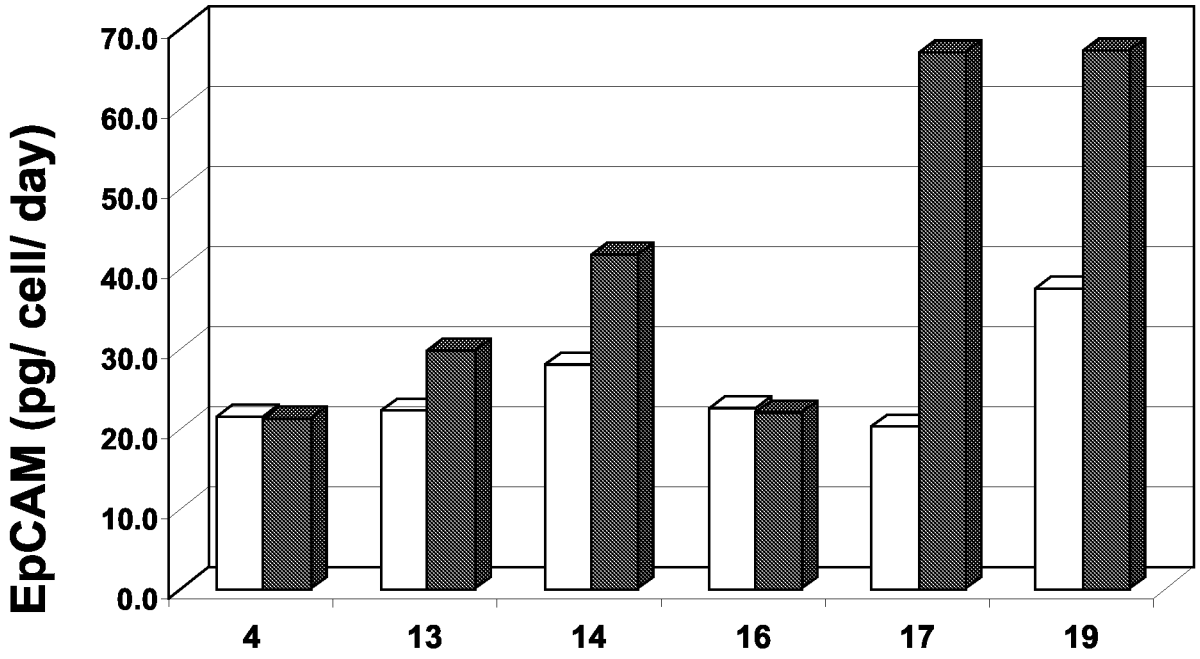
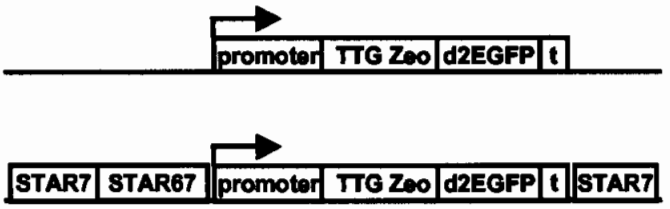

EpCAM (pg/ cell/ day)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
4
13
14
16
17
19

FIG 46


promoter
TTG Zeo
d2EGFP
t
STAR7
STAR67
promoter
TTG Zeo
d2EGFP
t
STAR7

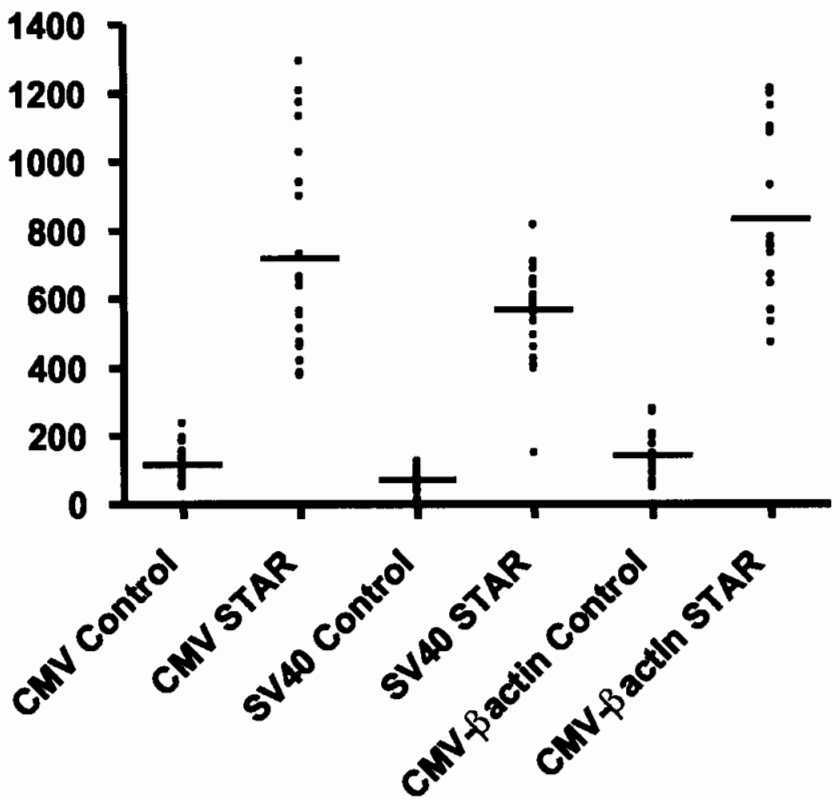
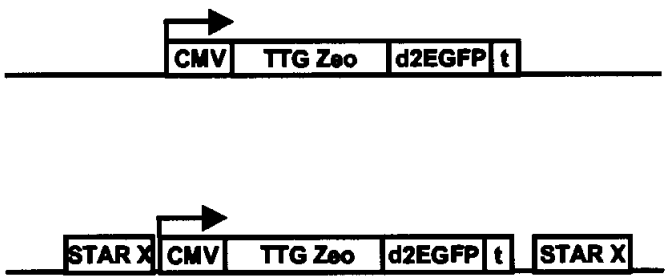

1400
1200
1000
800
600
400
200
0
CMV Control
CMV STAR
SV40 Control
SV40 STAR
CMV-βactin Control
CMV-βactin STAR

FIG 47


CMV
TTG Zeo
d2EGFP
t
STAR X
CMV
TTG Zeo
d2EGFP
t
STAR X

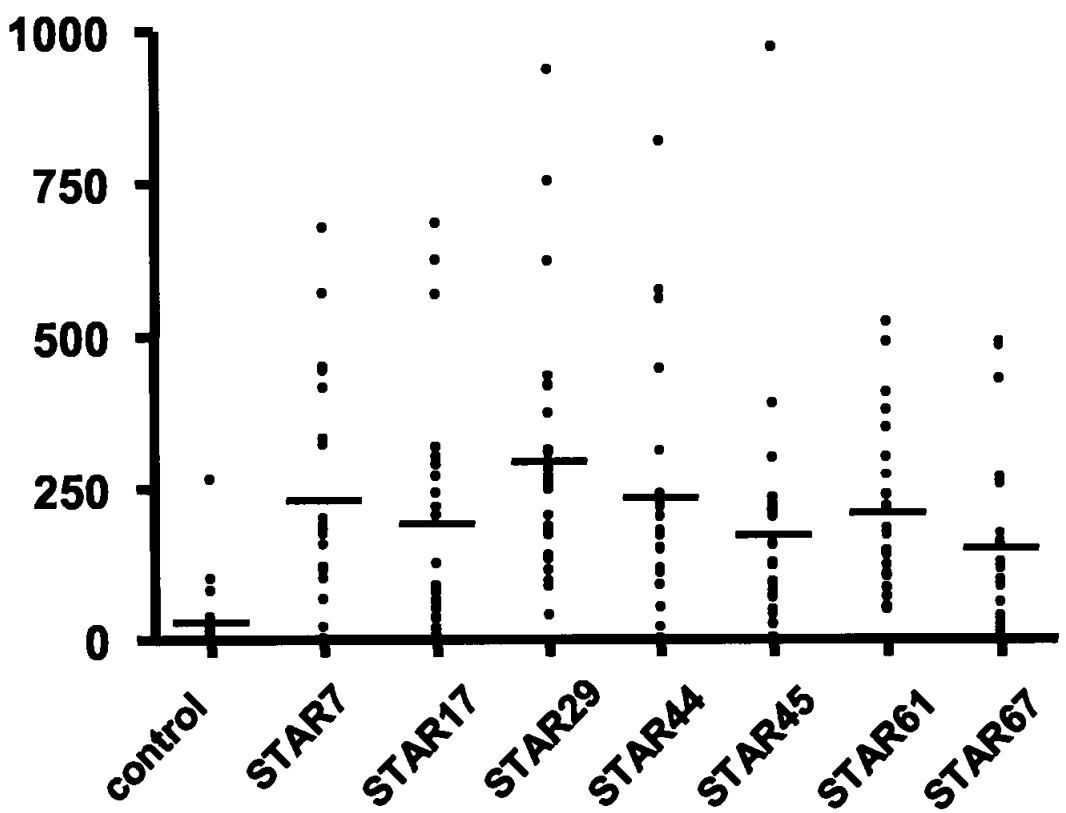
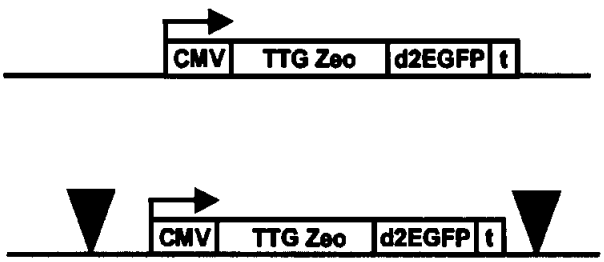
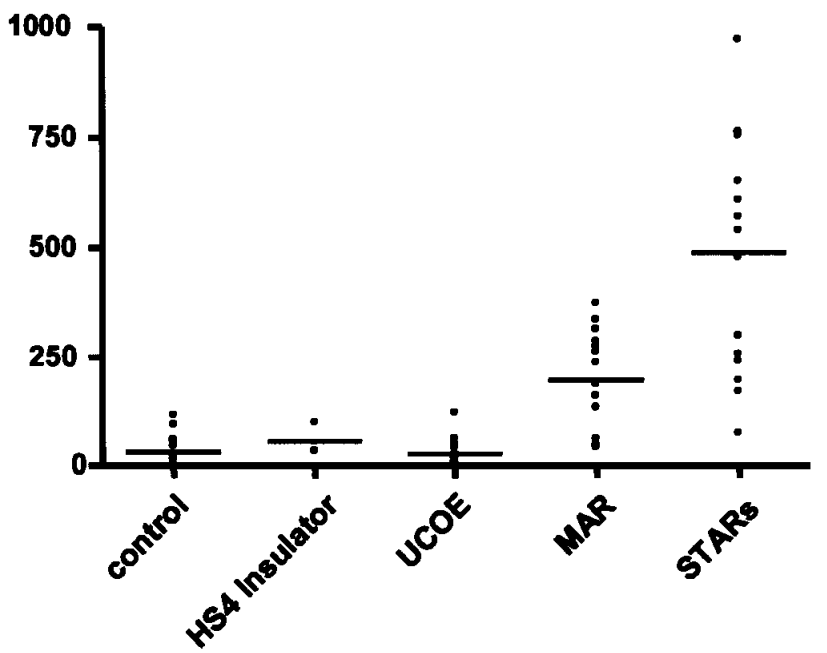

1000
750
500
250
0
control
STAR7
STAR17
STAR29
STAR44
STAR45
STAR61
STAR67

FIG 48


CMV
TTG Zeo
d2EGFP
t
CMV
TTG Zeo
d2EGFP
t


1000
750
500
250
0
control
HS4 Insulator
UCOE
MAR
STARs