```
ATG GTA CGT AGC TCC TCT CGC ACT CCG TCC GAT AAG CCG GTT GCT
M   V   R   S   S   S   R   T   P   S   D   K   P   V   A

CAT GTA GTT GCT AAC CCT CAG GCA GAA GGT CAG CTG CAG TGG CTG
H   V   V   A   N   P   Q   A   E   G   Q   L   Q   W   L

AAC CGT CGC GCT AAC GCC CTG CTG GCA AAC GGC GTT GAG CTC CGT
N   R   R   A   N   A   L   L   A   N   G   V   E   L   R

GAT AAC CAG CTC GTG GTA CCT TCT GAA GGT CTG TAC CTG ATC TAT
D   N   Q   L   V   V   P   S   E   G   L   Y   L   I   Y

TCT CAA GTA CTG TTC AAG GGT CAG GGC TGC CCG TCG ACT CAT GTT
S   Q   V   L   F   K   G   Q   G   C   P   S   T   H   V

CTG CTG ACT CAC ACC ATC AGC CGT ATT GCT GTA TCT TAC CAG ACC
L   L   T   H   T   I   S   R   I   A   V   S   Y   Q   T

AAA GTT AAC CTG CTG AGC GCT ATC AAG TCT CCG TGC CAG CGT GAA
K   V   N   L   L   S   A   I   K   S   P   C   Q   R   E

ACT CCC GAG GGT GCA GAA GCG AAA CCA TGG TAT GAA CCG ATC TAC
T   P   E   G   A   E   A   K   P   W   Y   E   P   I   Y

CTG GGT GGC GTA TTT CAA CTG GAG AAA GGT GAC CGT CTG TCC GCA
L   G   G   V   F   Q   L   E   K   G   D   R   L   S   A

GAA ATC AAC CGT CCT GAC TAT CTA GAT TTC GCT GAA TCT GGC CAG
E   I   N   R   P   D   Y   L   D   F   A   E   S   G   Q

GTG TAC TTC GGT ATT ATC GCA CTG TAA
V   Y   F   G   I   I   A   L   *
```

FIG.1

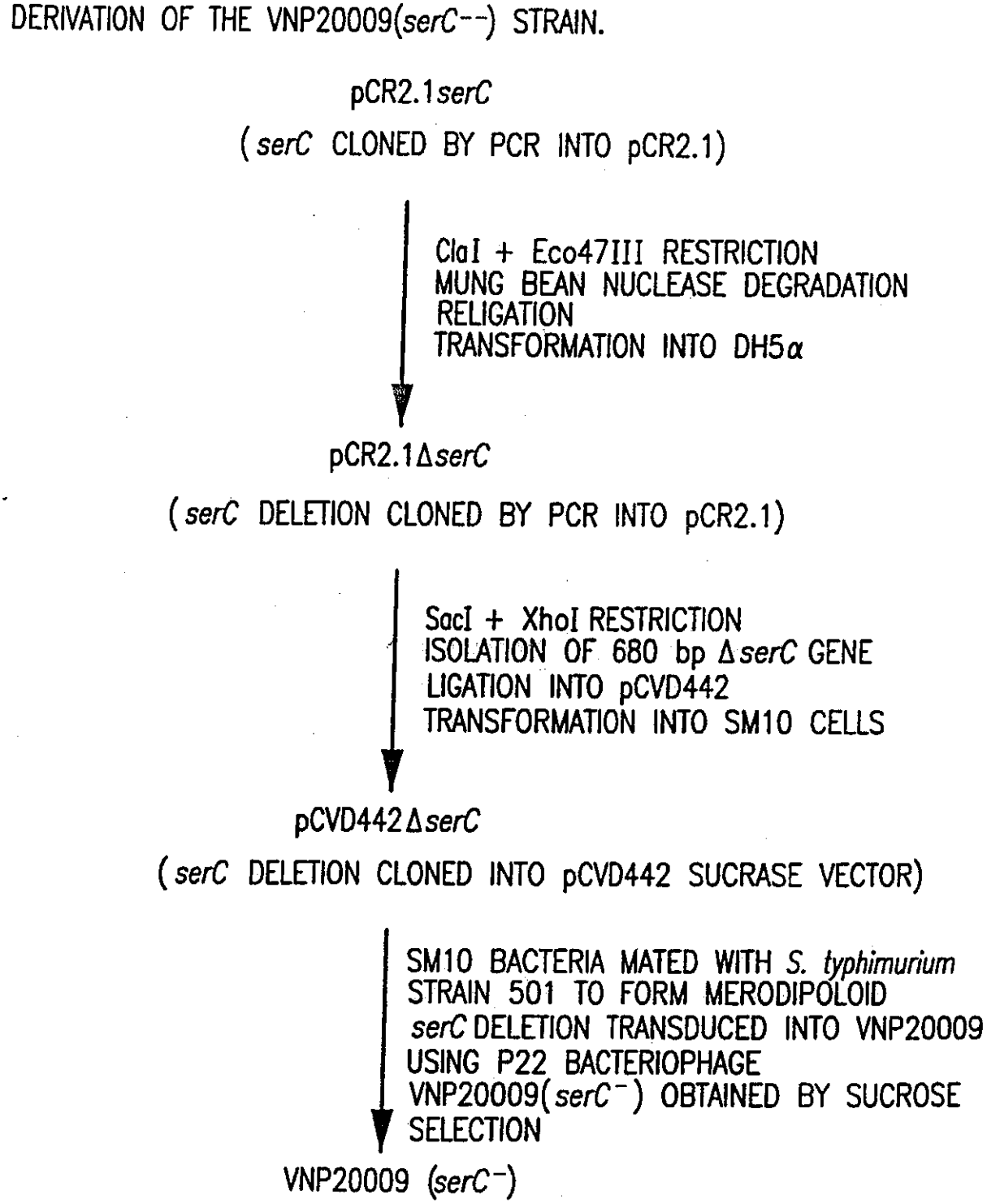

DERIVATION OF THE VNP20009(serC--) STRAIN.
pCR2.1serC
(serC CLONED BY PCR INTO pCR2.1)
ClaI + Eco47III RESTRICTION
MUNG BEAN NUCLEASE DEGRADATION
RELIGATION
TRANSFORMATION INTO DH5α
pCR2.1ΔserC
(serC DELETION CLONED BY PCR INTO pCR2.1)
SacI + XhoI RESTRICTION
ISOLATION OF 680 bp ΔserC GENE
LIGATION INTO pCVD442
TRANSFORMATION INTO SM10 CELLS
pCVD442ΔserC
(serC DELETION CLONED INTO pCVD442 SUCRASE VECTOR)
SM10 BACTERIA MATED WITH S. typhimurium
STRAIN 501 TO FORM MERODIPOLOID
serC DELETION TRANSDUCED INTO VNP20009
USING P22 BACTERIOPHAGE
VNP20009(serC-) OBTAINED BY SUCROSE
SELECTION
VNP20009 (serC-)

FIG.2

Quantitation of TNFα expression by pTS-BrpTNFα Clone 2.

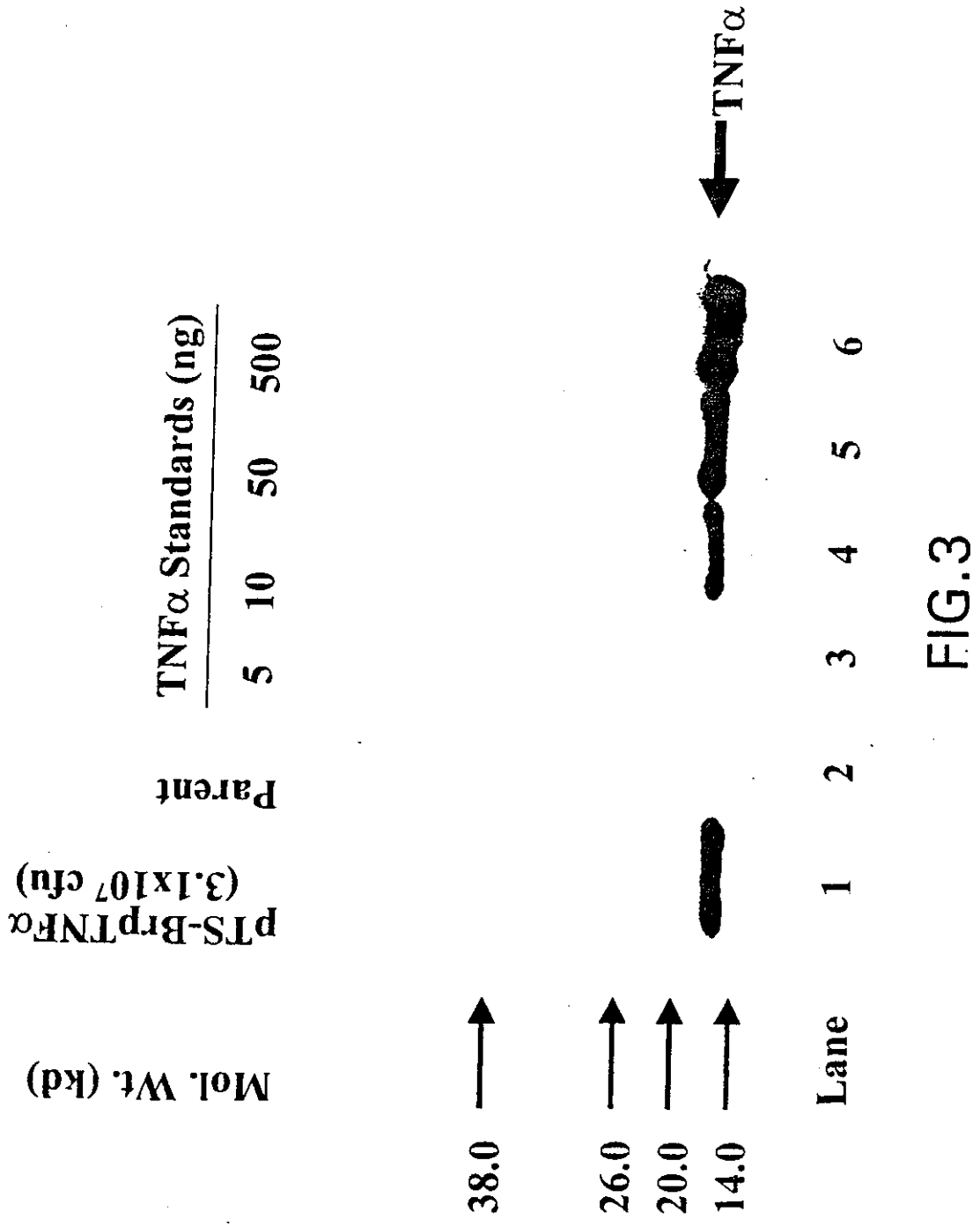


FIG.3

```
ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC
M   K   K   T   A   I   A   I   A   V   A   L   A   G   F

GCT ACC GTA GCG CAG GCC CAT ATG GTA CGT AGC TCC TCT CGC ACT
A   T   V   A   Q   A   H   M   V   R   S   S   S   R   T

CCG TCC GAT AAG CCG GTT GCT CAT GTA GTT GCT AAC CCT CAG GCA
P   S   D   K   P   V   A   H   V   V   A   N   P   Q   A

GAA GGT CAG CTG CAG TGG CTG AAC CGT CGC GCT AAC GCC CTG CTG
E   G   Q   L   Q   W   L   N   R   R   A   N   A   L   L

GCA AAC GGC GTT GAG CTC CGT GAT AAC CAG CTC GTG GTA CCT TCT
A   N   G   V   E   L   R   D   N   Q   L   V   V   P   S

GAA GGT CTG TAC CTG ATC TAT TCT CAA GTA CTG TTC AAG GGT CAG
E   G   L   Y   L   I   Y   S   Q   V   L   F   K   G   Q

GGC TGC CCG TCG ACT CAT GTT CTG CTG ACT CAC ACC ATC AGC CGT
G   C   P   S   T   H   V   L   L   T   H   T   I   S   R

ATT GCT GTA TCT TAC CAG ACC AAA GTT AAC CTG CTG AGC GCT ATC
I   A   V   S   Y   Q   T   K   V   N   L   L   S   A   I

AAG TCT CCG TGC CAG CGT GAA ACT CCC GAG GGT GCA GAA GCG AAA
K   S   P   C   Q   R   E   T   P   E   G   A   E   A   K

CCA TGG TAT GAA CCG ATC TAC CTG GGT GGC GTA TTT CAA CTG GAG
P   W   Y   E   P   I   Y   L   G   G   V   F   Q   L   E

AAA GGT GAC CGT CTG TCC GCA GAA ATC AAC CGT CCT GAC TAT CTA
K   G   D   R   L   S   A   E   I   N   R   P   D   Y   L

GAT TTC GCT GAA TCT GGC CAG GTG TAC TTC GGT ATT ATC GCA CTG
D   F   A   E   S   G   Q   V   Y   F   G   I   I   A   L

TAA
*
```

FIG.4

Expression and processing of a trc promoter-driven ompA-TRAIL fusion gene product in JM109 bacteria.
Standards
ompA-TRAIL
(Nco1 junction)
(Nde1 junction)
Unprocessed (30.6 kd)
Processed (28.6 kd)
37.6 kd
25.4 kd
1 2 3 4 5 6 7

FIG.5

```
ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC
M   K   K   T   A   I   A   I   A   V   A   L   A   G   F

GCT ACC GTA GCG CAG GCC CAT ATG GCT AAC GAG CTG AAG CAG ATG
A   T   V   A   Q   A   H   M   A   N   E   L   K   Q   M

CAG GAC AAG TAC TCC AAA AGT GGC ATT GCT TGT TTC TTA AAA GAA
Q   D   K   Y   S   K   S   G   I   A   C   F   L   K   E

GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA GAG AGT ATG AAC AGC
D   D   S   Y   W   D   P   N   D   E   E   S   M   N   S

CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG CTC GTT AGA AAG
P   C   W   Q   V   K   W   Q   L   R   Q   L   V   R   K

ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA
M   I   L   R   T   S   E   E   T   I   S   T   V   Q   E

AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG
K   Q   Q   N   I   S   P   L   V   R   E   R   G   P   Q

AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA
R   V   A   A   H   I   T   G   T   R   G   R   S   N   T

TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA
L   S   S   P   N   S   K   N   E   K   A   L   G   R   K

ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC
I   N   S   W   E   S   S   R   S   G   H   S   F   L   S

AAC TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG
N   L   H   L   R   N   G   E   L   V   I   H   E   K   G

TTT TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA
F   Y   Y   I   Y   S   Q   T   Y   F   R   F   Q   E   E

ATA AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT
I   K   E   N   T   K   N   D   K   Q   M   V   Q   Y   I

TAC AAA TAC ACA AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT
Y   K   Y   T   S   Y   P   D   P   I   L   L   M   K   S

GCT AGA AAT AGT TGT TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT
A   R   N   S   C   W   S   K   D   A   E   Y   G   L   Y

TCC ATC TAT CAA GGG GGA ATA TTT GAG CTT AAG GAA AAT GAC AGA
S   I   Y   Q   G   G   I   F   E   L   K   E   N   D   R

ATT TTT GTT TCT GTA ACA AAT GAG CAC TTG ATA GAC ATG GAC CAT
I   F   V   S   V   T   N   E   H   L   I   D   M   D   H

GAA GCC AGT TTT TTC GGG GCC TTT TTA GTT GGC TAA
E   A   S   F   F   G   A   F   L   V   G   *
```

FIG.6

Expression and processing of a *trc* promoter-driven *ompA*-TNFα fusion gene product in JM109 bacteria.

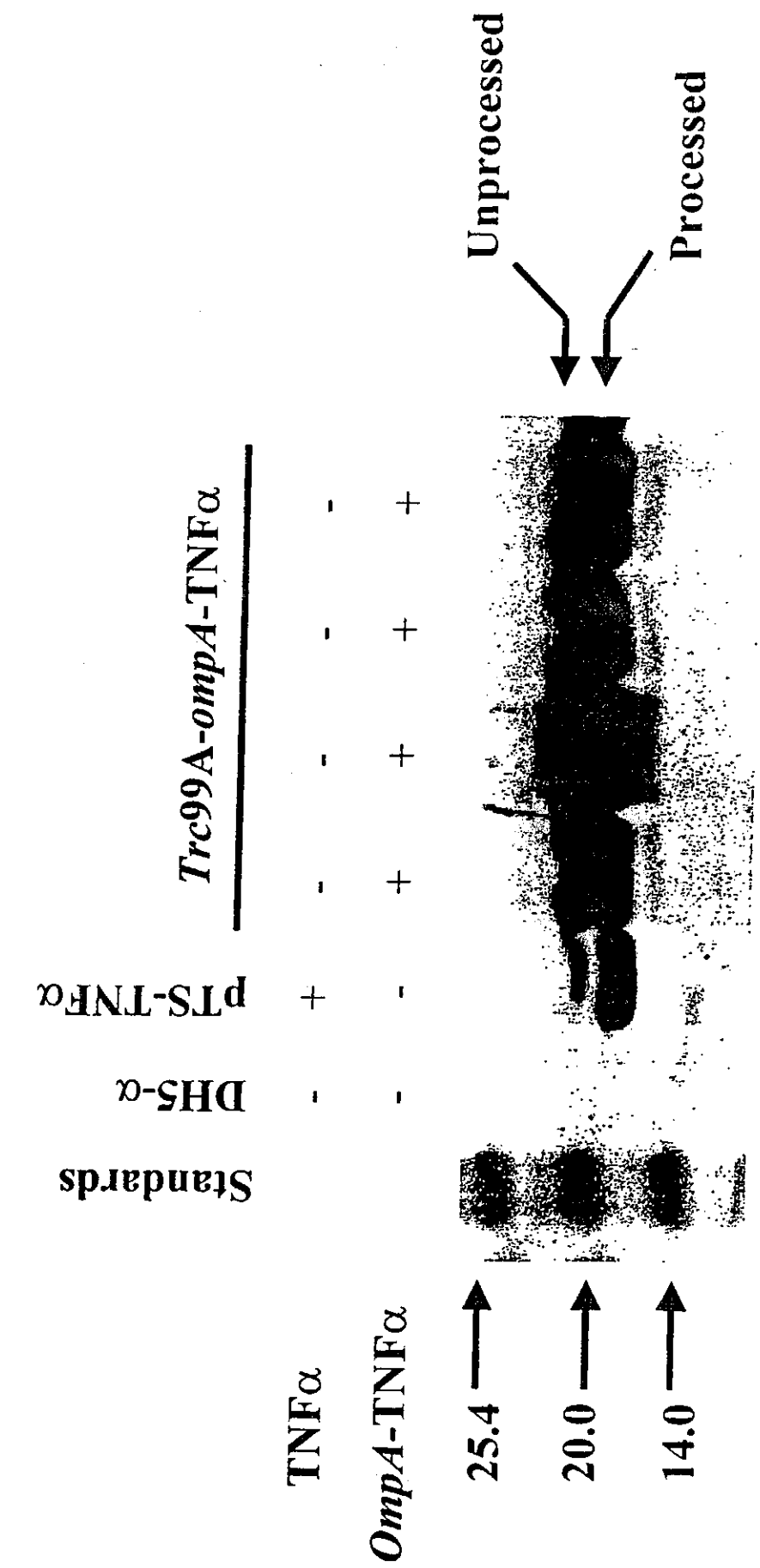


FIG.7

```
ATG AAA AAG ACG GCT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG
M   K   K   T   A   L   A   L   L   L   L   L   L   A   L

ACT AGT GTA GCG CAG GCC GCT CCT ACT AGC TCG AGC ACT AAG AAA
T   S   V   A   Q   A   A   P   T   S   S   S   T   K   K

ACT CAA CTG CAA TTG GAG CAT CTG CTG CTG GAT CTG CAG ATG ATT
T   Q   L   Q   L   E   H   L   L   L   D   L   Q   M   I

CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG
L   N   G   I   N   N   Y   K   N   P   K   L   T   R   M

CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA
L   T   F   K   F   Y   M   P   K   K   A   T   E   L   K

CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA
H   L   Q   C   L   E   E   E   L   K   P   L   E   E   V

CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC
L   N   L   A   Q   S   K   N   F   H   L   R   P   R   D

CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC
L   I   S   N   I   N   V   I   V   L   E   L   K   G   S

GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT
E   T   T   F   M   C   E   Y   A   D   E   T   A   T   I

GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT
V   E   F   L   N   R   W   I   T   F   A   Q   S   I   I

AGC ACG TTA ACT TAA
S   T   L   T   *
```

FIG.8

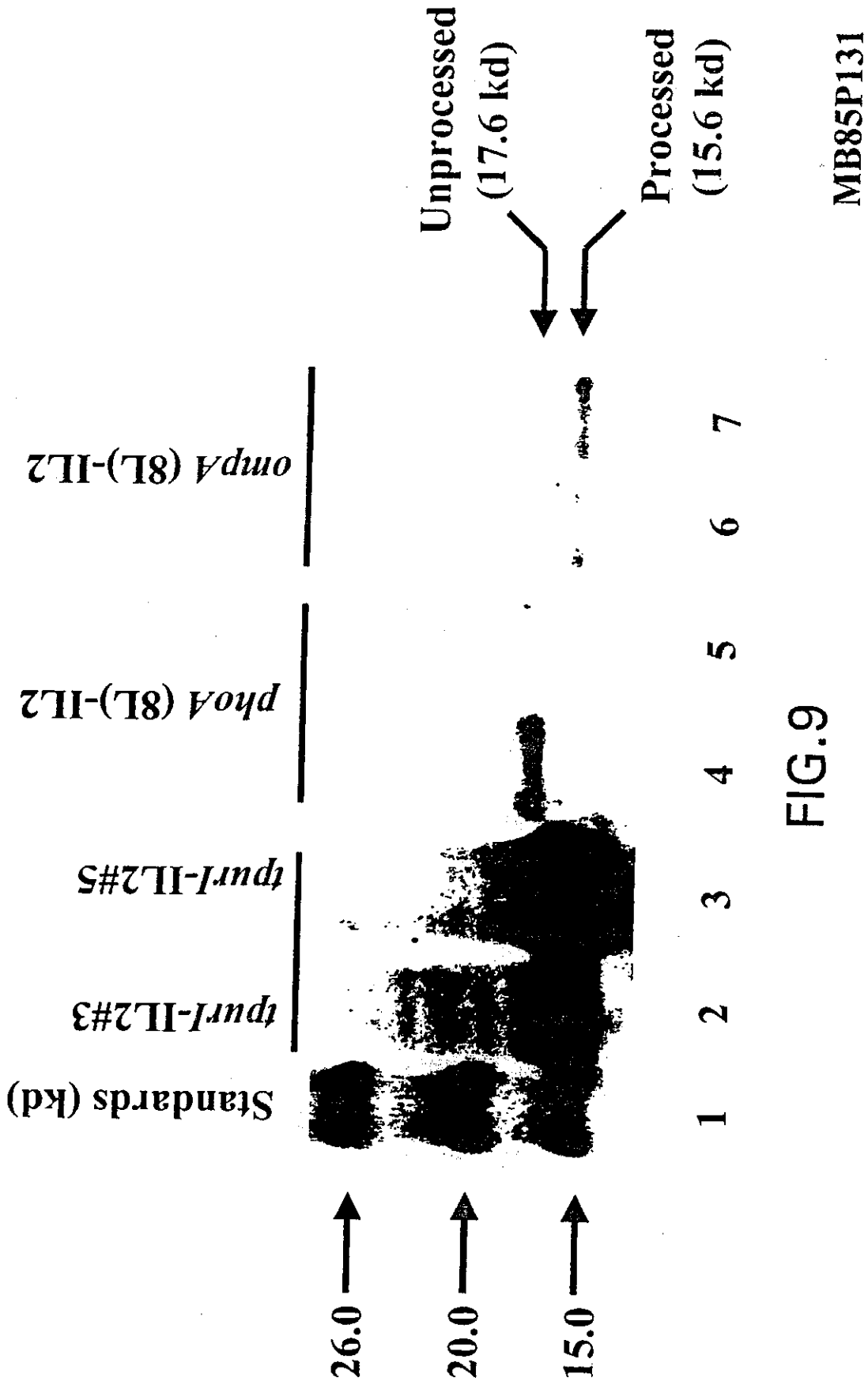

Periplasmic localization and processing of ompA-IL2 fusion proteins.
Standards (kd)
tpurI-IL2#3
tpurI-IL2#5
phoA (8L)-IL2
ompA (8L)-IL2
Unprocessed (17.6 kd)
Processed (15.6 kd)
MB85P131
1 2 3 4 5 6 7
26.0
20.0
15.0

FIG.9

```
ATG AAA CAG TCG ACT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG
M   K   Q   S   T   L   A   L   L   L   L   L   L   A   L

ACT AGT GTG GCC AAA GCG GCT CCT ACT AGC TCG AGC ACT AAG AAA
T   S   V   A   K   A   A   P   T   S   S   S   T   K   K

ACT CAA CTG CAA TTG GAG CAT CTG CTG CTG GAT CTG CAG ATG ATT
T   Q   L   Q   L   E   H   L   L   L   D   L   Q   M   I

CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG
L   N   G   I   N   N   Y   K   N   P   K   L   T   R   M

CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA
L   T   F   K   F   Y   M   P   K   K   A   T   E   L   K

CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA
H   L   Q   C   L   E   E   E   L   K   P   L   E   E   V

CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC
L   N   L   A   Q   S   K   N   F   H   L   R   P   R   D

CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC
L   I   S   N   I   N   V   I   V   L   E   L   K   G   S

GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT
E   T   T   F   M   C   E   Y   A   D   E   T   A   T   I

GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT
V   E   F   L   N   R   W   I   T   F   A   Q   S   I   I

AGC ACG TTA ACT TAA
S   T   L   T   *
```

FIG.10

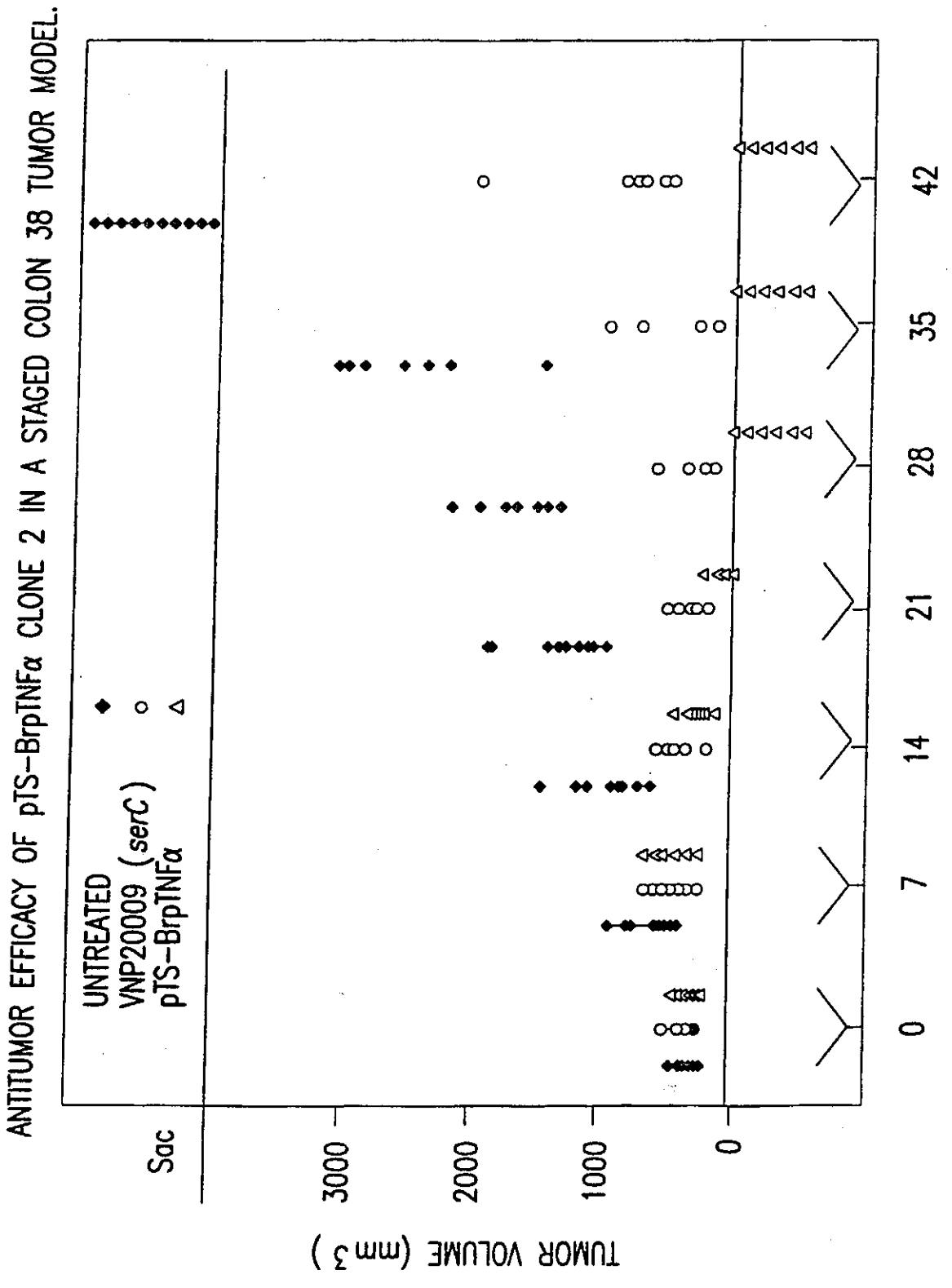

ANTITUMOR EFFICACY OF pTS-BrpTNFα CLONE 2 IN A STAGED COLON 38 TUMOR MODEL.
UNTREATED
VNP20009 (serC)
pTS-BrpTNFα
Sac
3000
2000
1000
0
TUMOR VOLUME (mm3)
0
7
14
21
28
35
42
DAYS AFTER INOCULATION

FIG.11

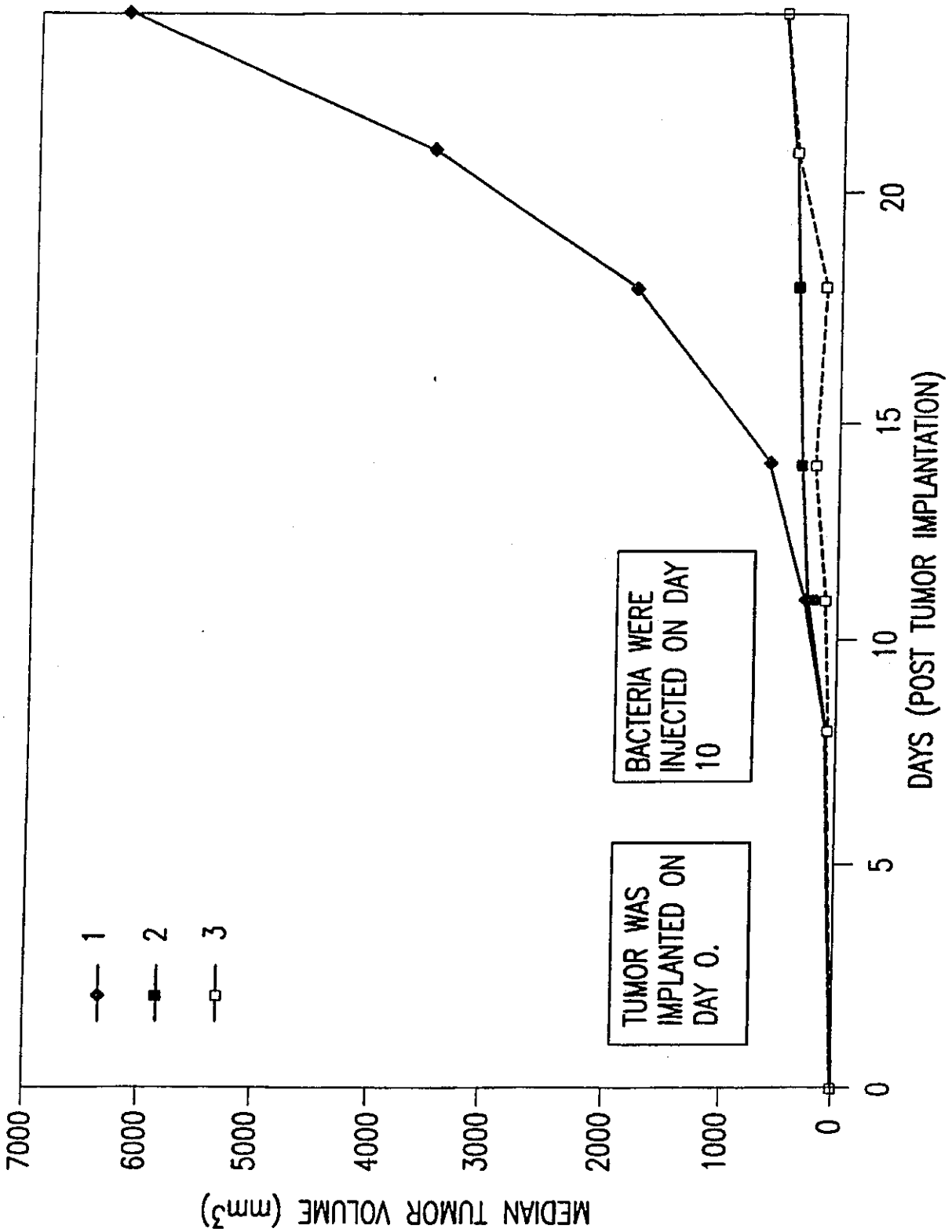

MEDIAN TUMOR VOLUME (mm3)
7000
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
DAYS (POST TUMOR IMPLANTATION)
1
2
3
TUMOR WAS IMPLANTED ON DAY 0.
BACTERIA WERE INJECTED ON DAY 10

FIG.12

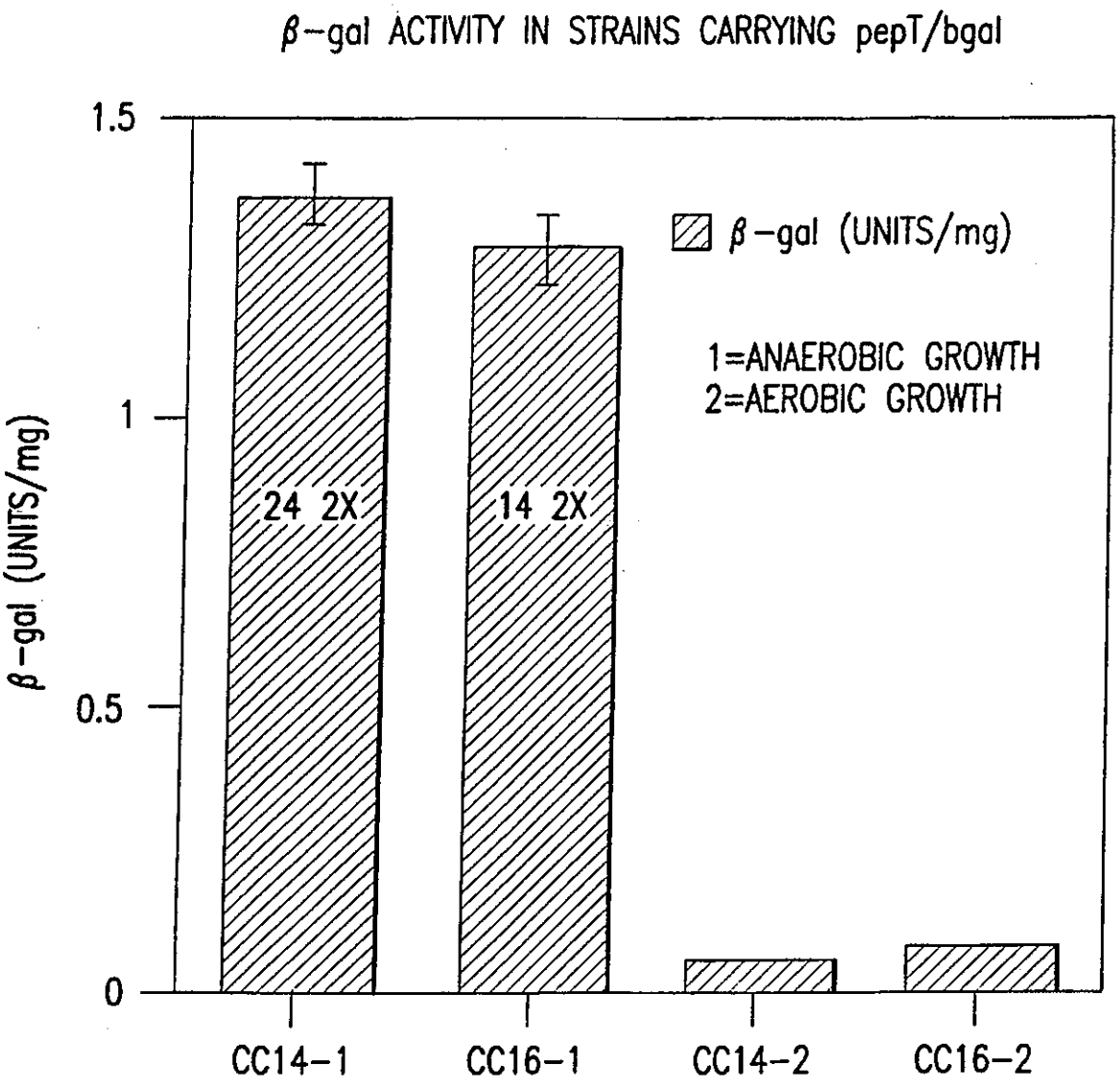

β-gal ACTIVITY IN STRAINS CARRYING pepT/bgal
β-gal (UNITS/mg)
1.5
1
0.5
0
β-gal (UNITS/mg)
1=ANAEROBIC GROWTH
2=AEROBIC GROWTH
24 2X
14 2X
CC14-1
CC16-1
CC14-2
CC16-2

FIG.13A

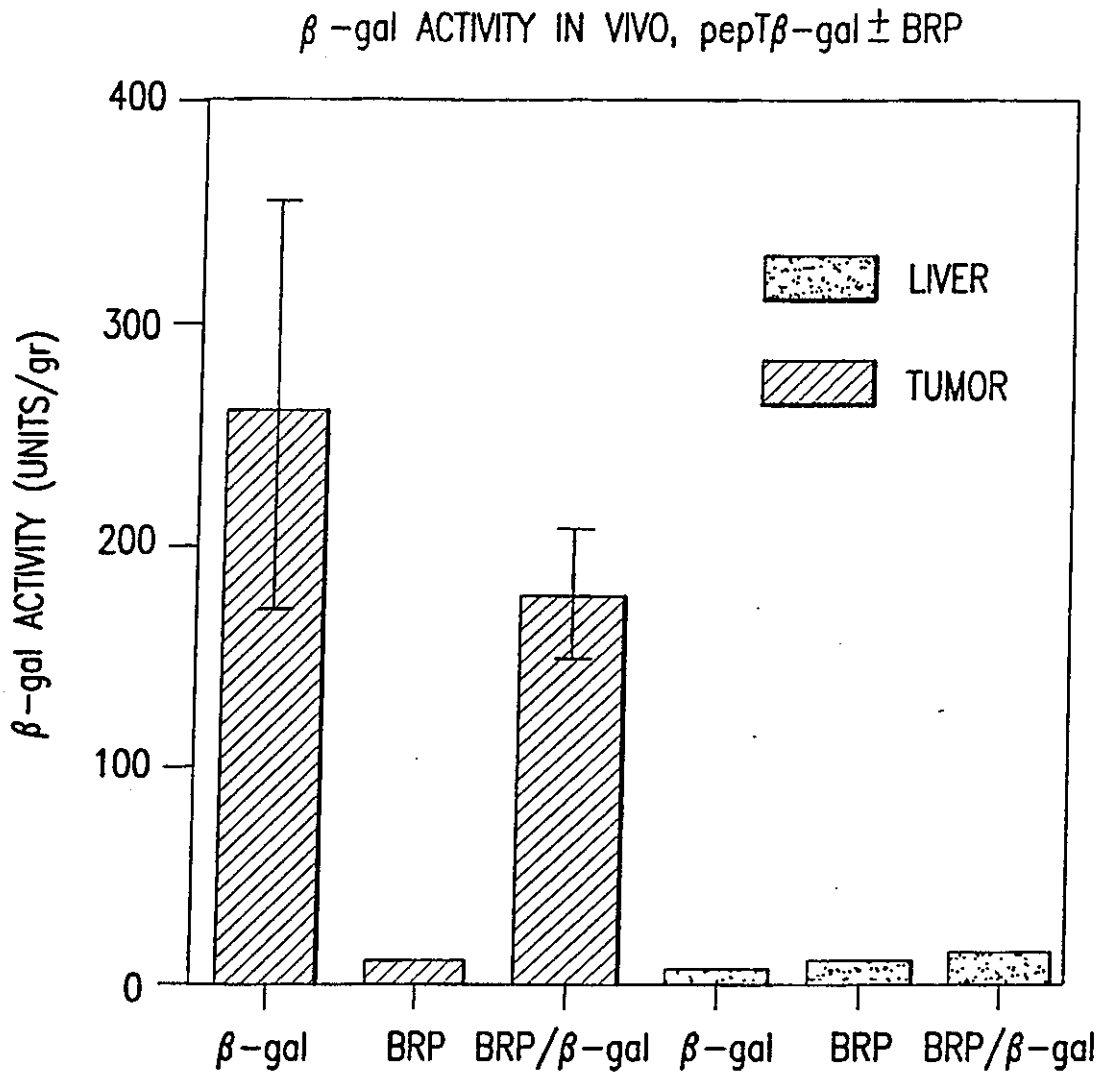

β -gal ACTIVITY IN VIVO, pepTβ-gal ± BRP
β-gal ACTIVITY (UNITS/gr)
400
300
200
100
0
LIVER
TUMOR
β-gal
BRP
BRP/β-gal
β-gal
BRP
BRP/β-gal

FIG.13B

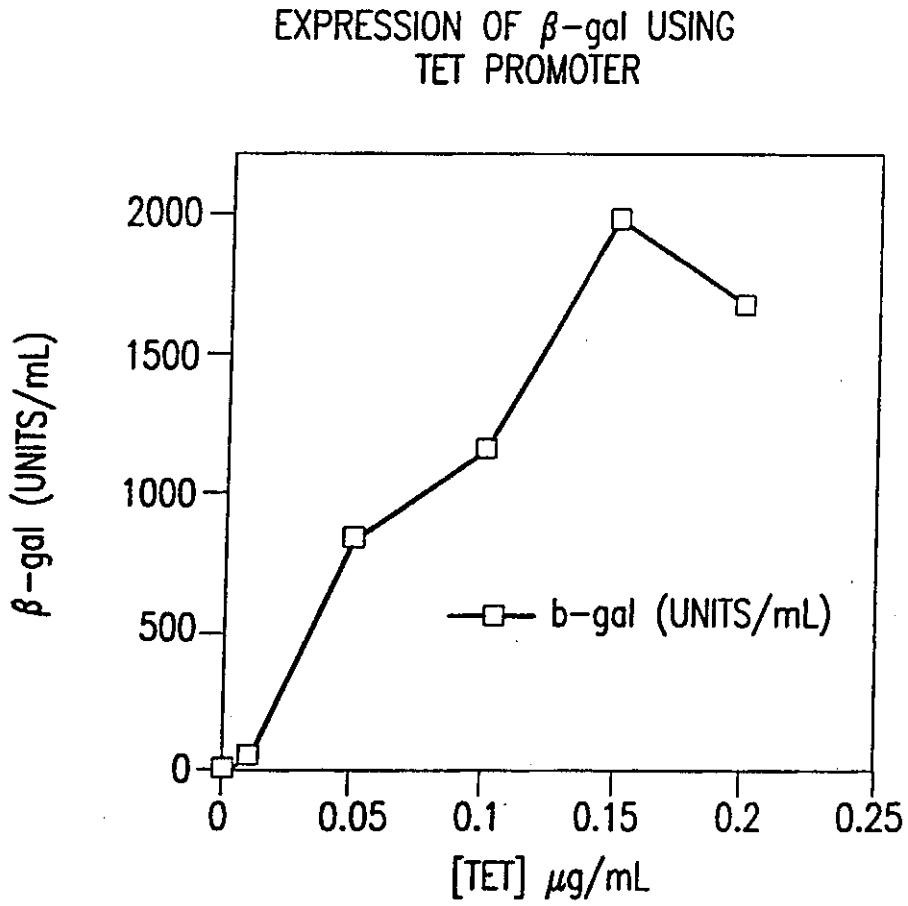

EXPRESSION OF β-gal USING
TET PROMOTER
β-gal (UNITS/mL)
2000
1500
1000
500
0
b-gal (UNITS/mL)
0
0.05
0.1
0.15
0.2
0.25
[TET] μg/mL

FIG.14

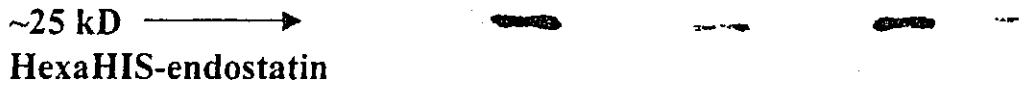


FIG.15A



FIG.15B

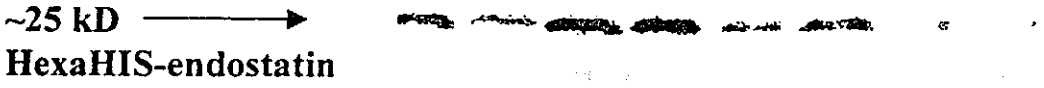


FIG. 16

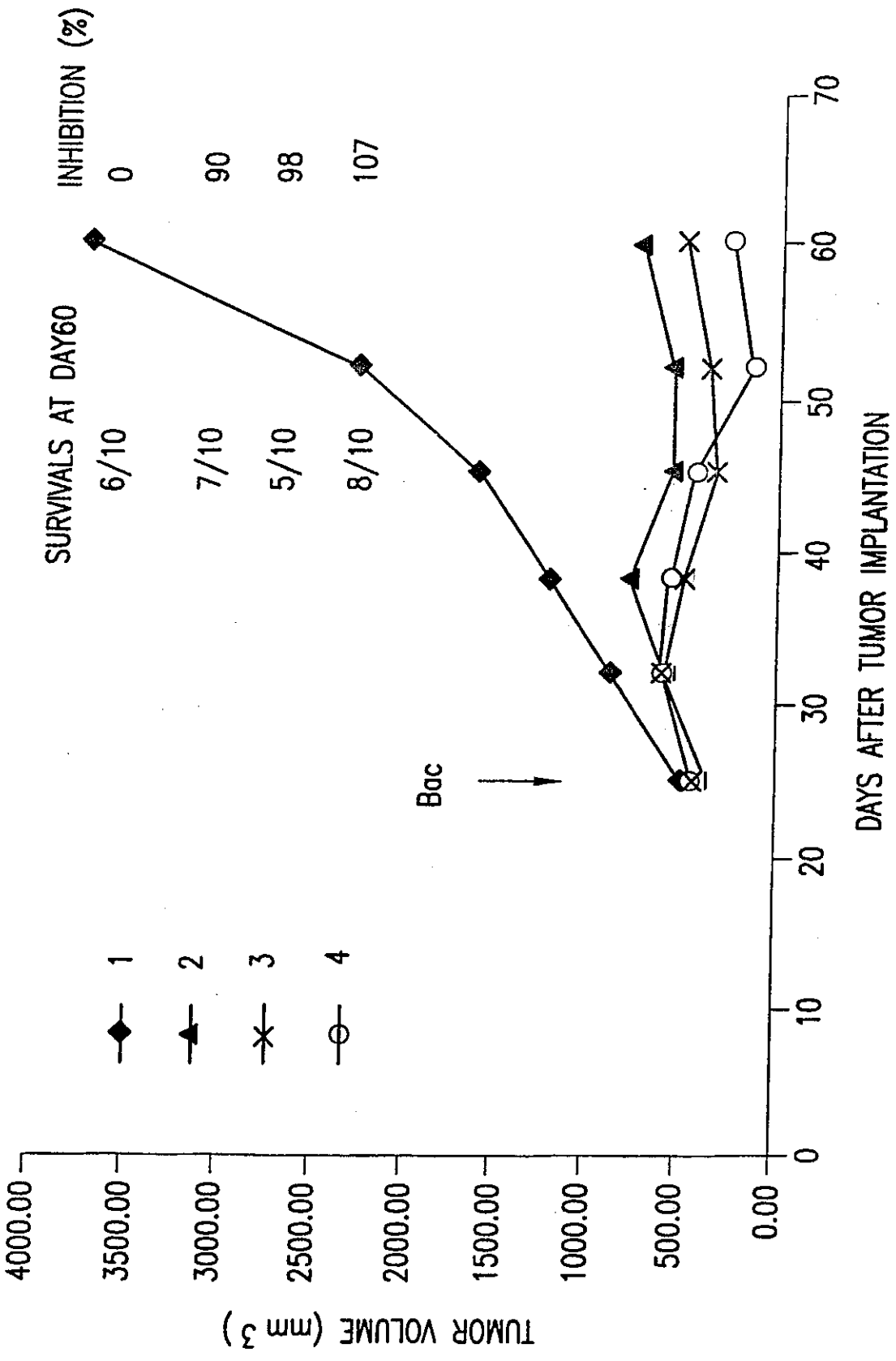

INHIBITION (%)
0
90
98
107
SURVIVALS AT DAY60
6/10
7/10
5/10
8/10
Bac
1
2
3
4
TUMOR VOLUME (mm 3)
4000.00
3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
0
10
20
30
40
50
60
70
DAYS AFTER TUMOR IMPLANTATION

FIG.17

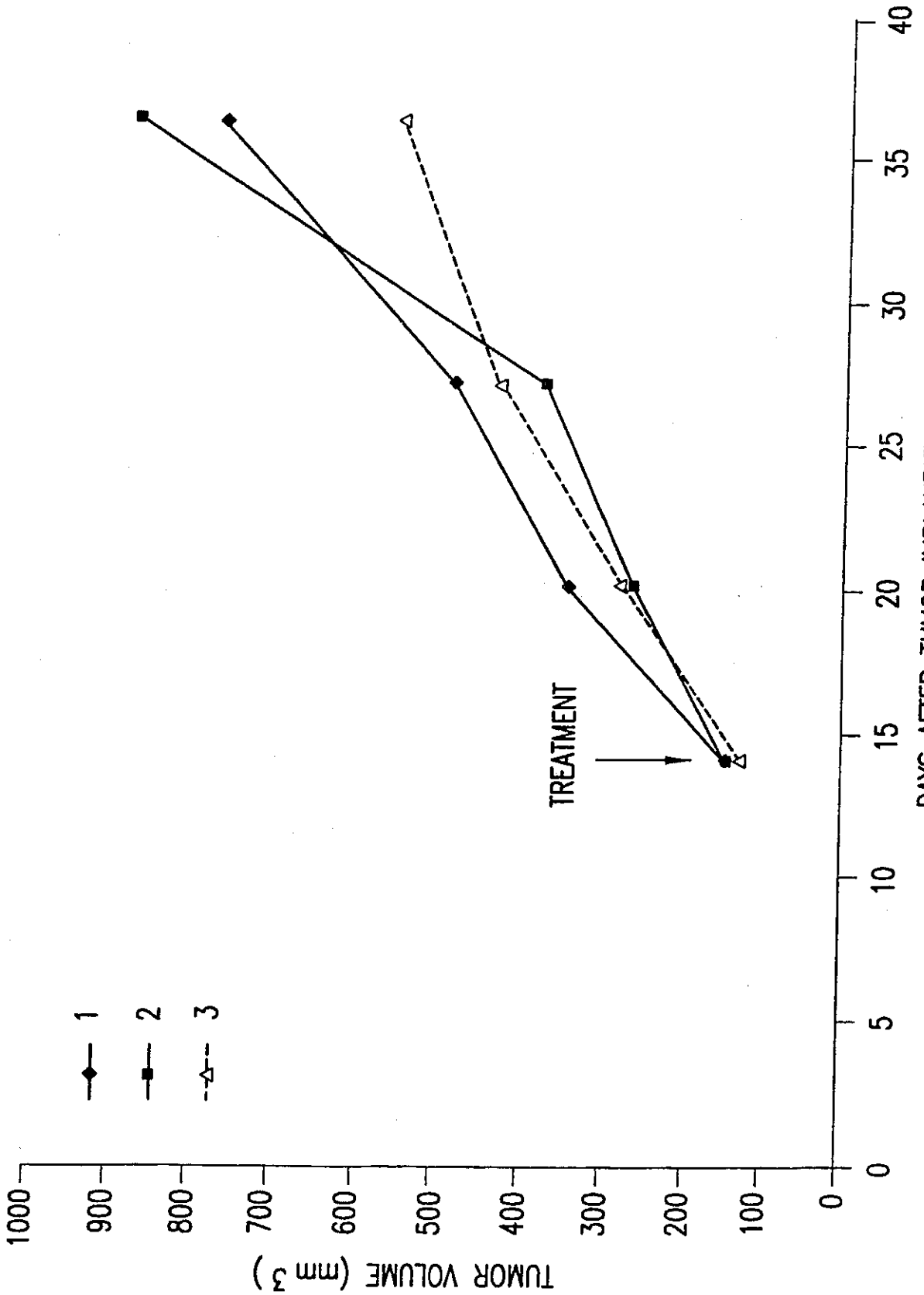

TUMOR VOLUME (mm 3)
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
DAYS AFTER TUMOR IMPLANTATION
TREATMENT
1
2
3

FIG.18

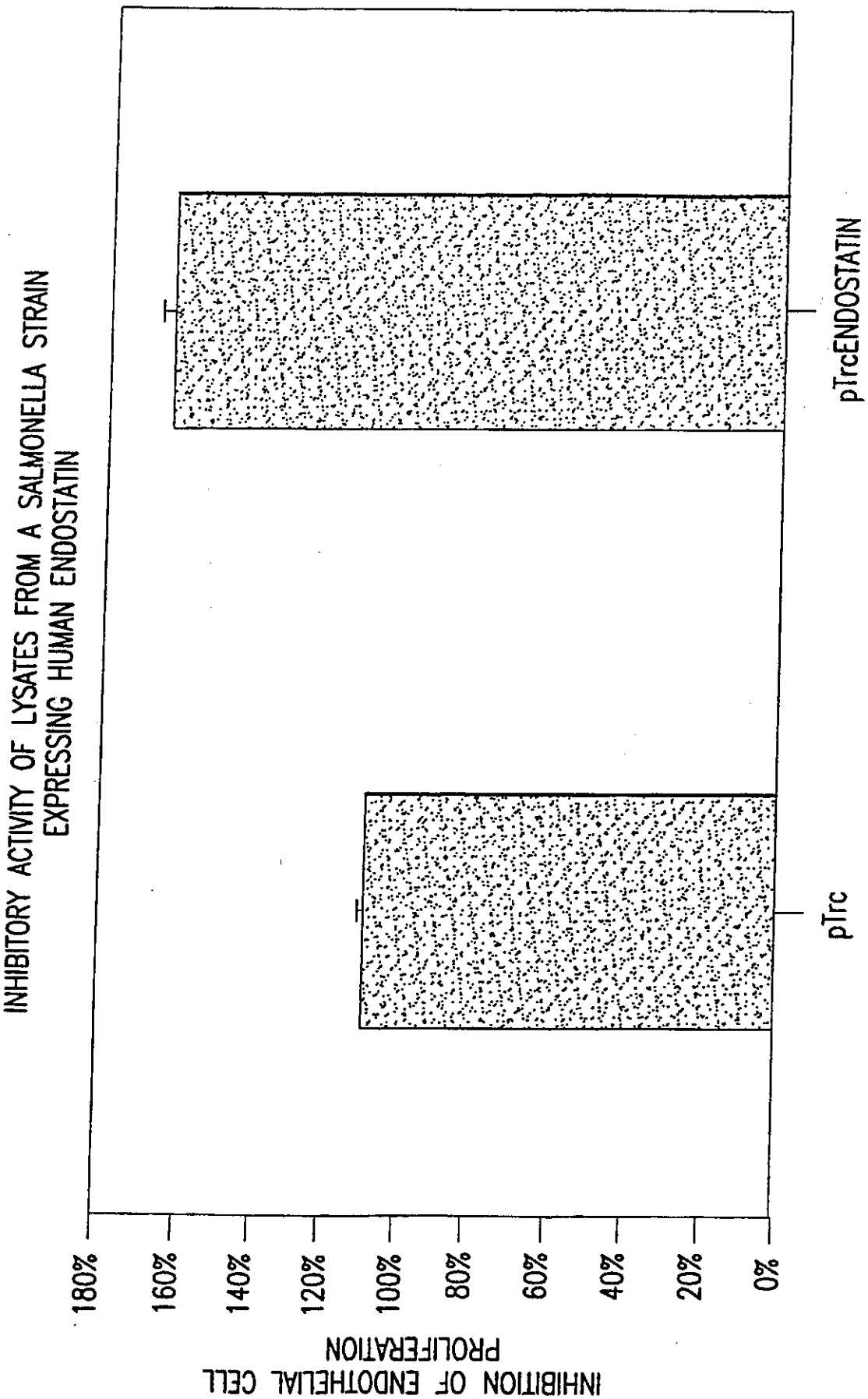

INHIBITORY ACTIVITY OF LYSATES FROM A SALMONELLA STRAIN EXPRESSING HUMAN ENDOSTATIN
pTrcENDOSTATIN
pTrc
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
INHIBITION OF ENDOTHELIAL CELL PROLIFERATION

FIG.19

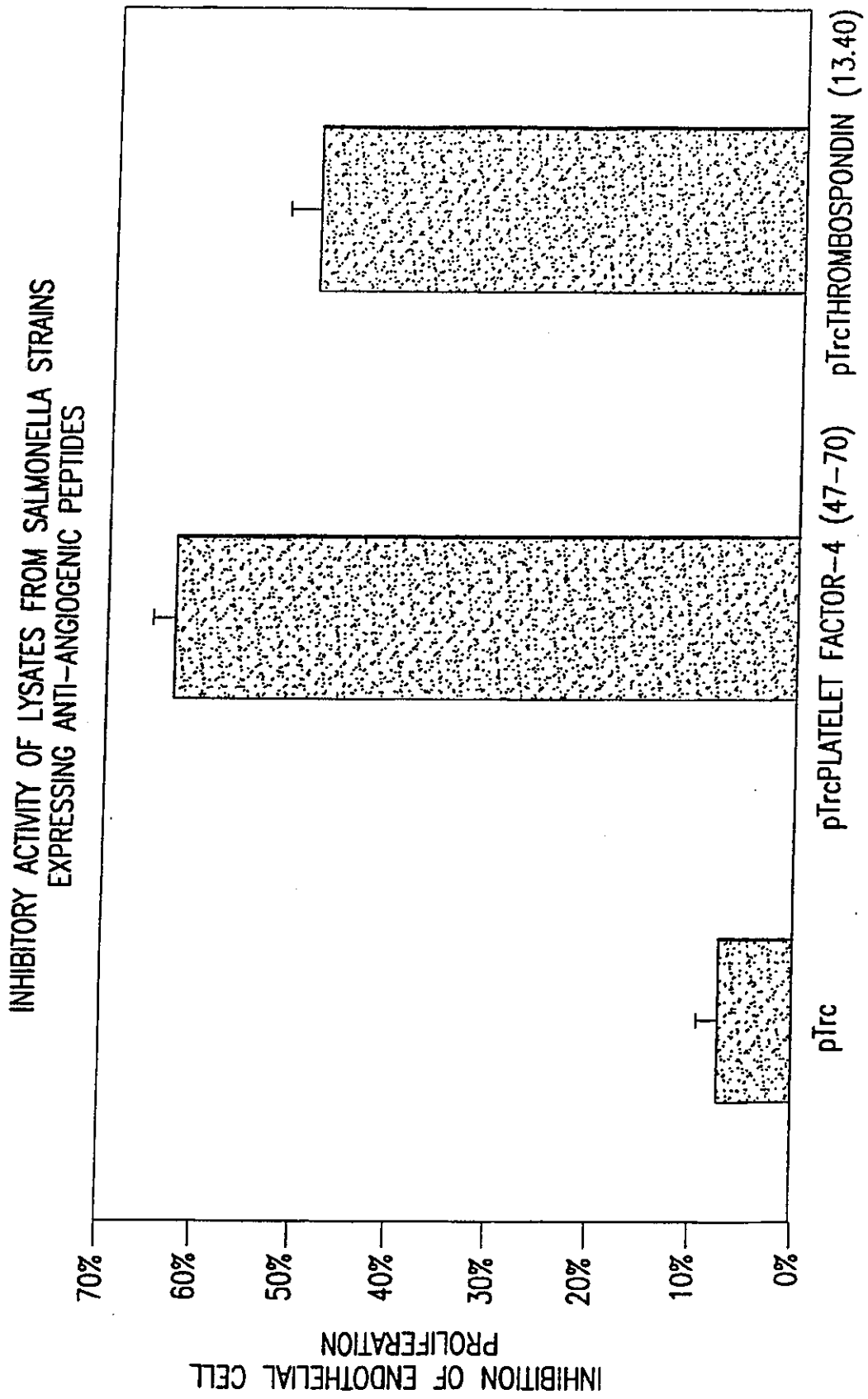

INHIBITORY ACTIVITY OF LYSATES FROM SALMONELLA STRAINS
EXPRESSING ANTI-ANGIOGENIC PEPTIDES
INHIBITION OF ENDOTHELIAL CELL
PROLIFERATION
70%
60%
50%
40%
30%
20%
10%
0%
pTrc
pTrcPLATELET FACTOR-4 (47-70)
pTrcTHROMBOSPONDIN (13.40)

FIG.20

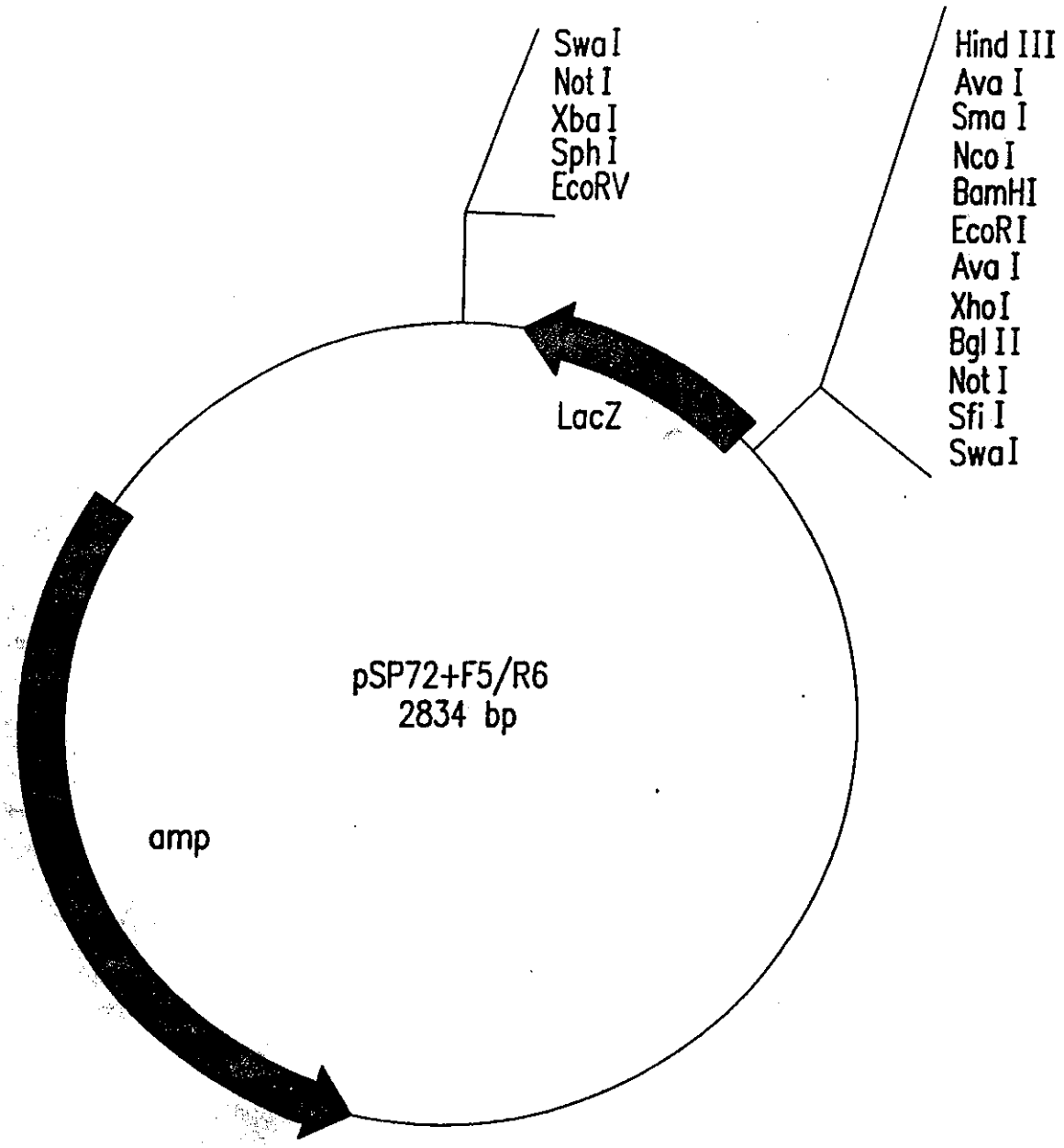

Swa I
Not I
Xba I
Sph I
EcoRV
Hind III
Ava I
Sma I
Nco I
BamHI
EcoR I
Ava I
Xho I
Bgl II
Not I
Sfi I
Swa I
LacZ
pSP72+F5/R6
2834 bp
amp

FIG.21

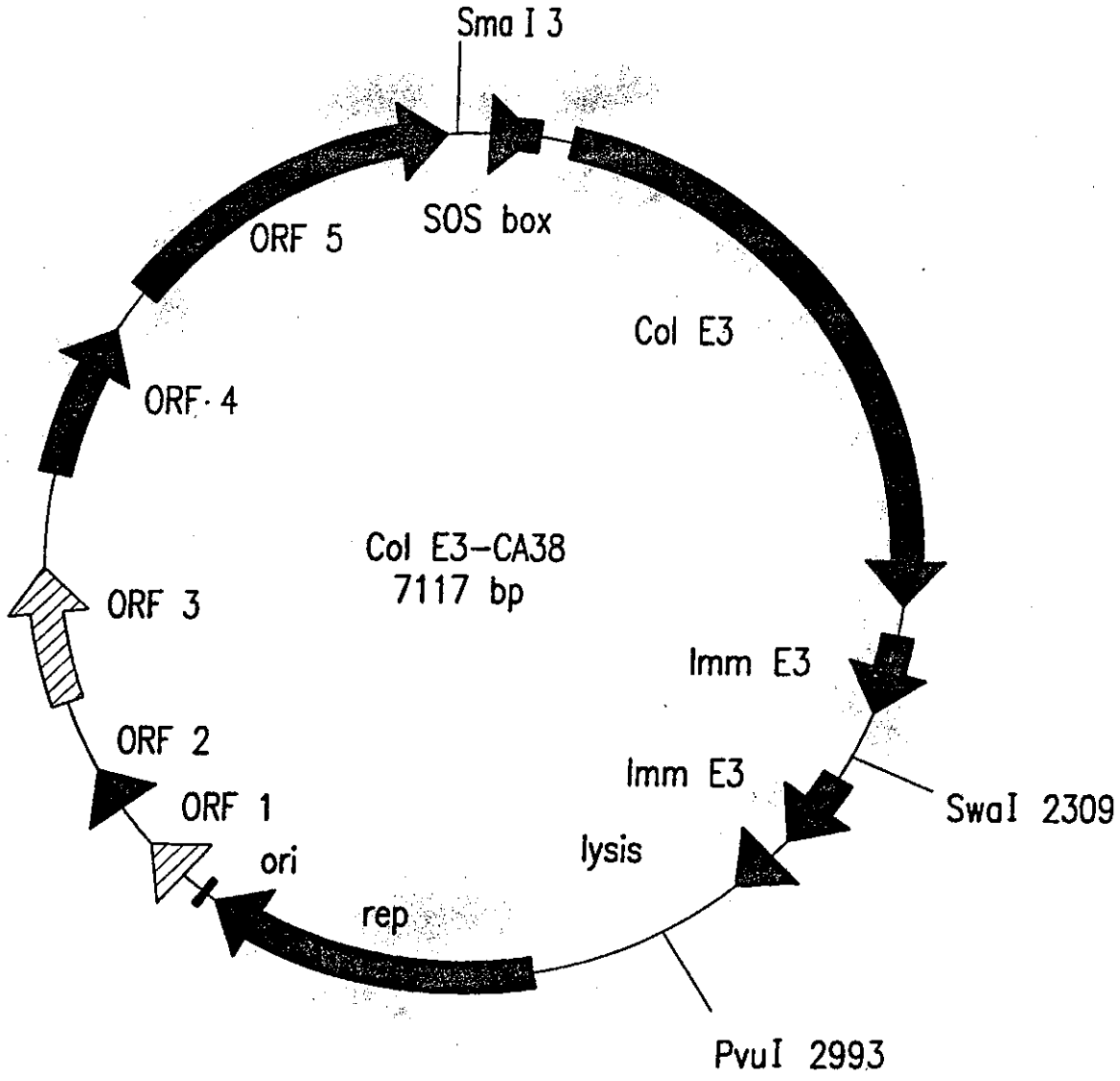

Sma I 3
SOS box
Col E3
Imm E3
Imm E3
SwaI 2309
lysis
PvuI 2993
rep
ori
ORF 1
ORF 2
ORF 3
ORF 4
ORF 5
Col E3-CA38
7117 bp

FIG.22

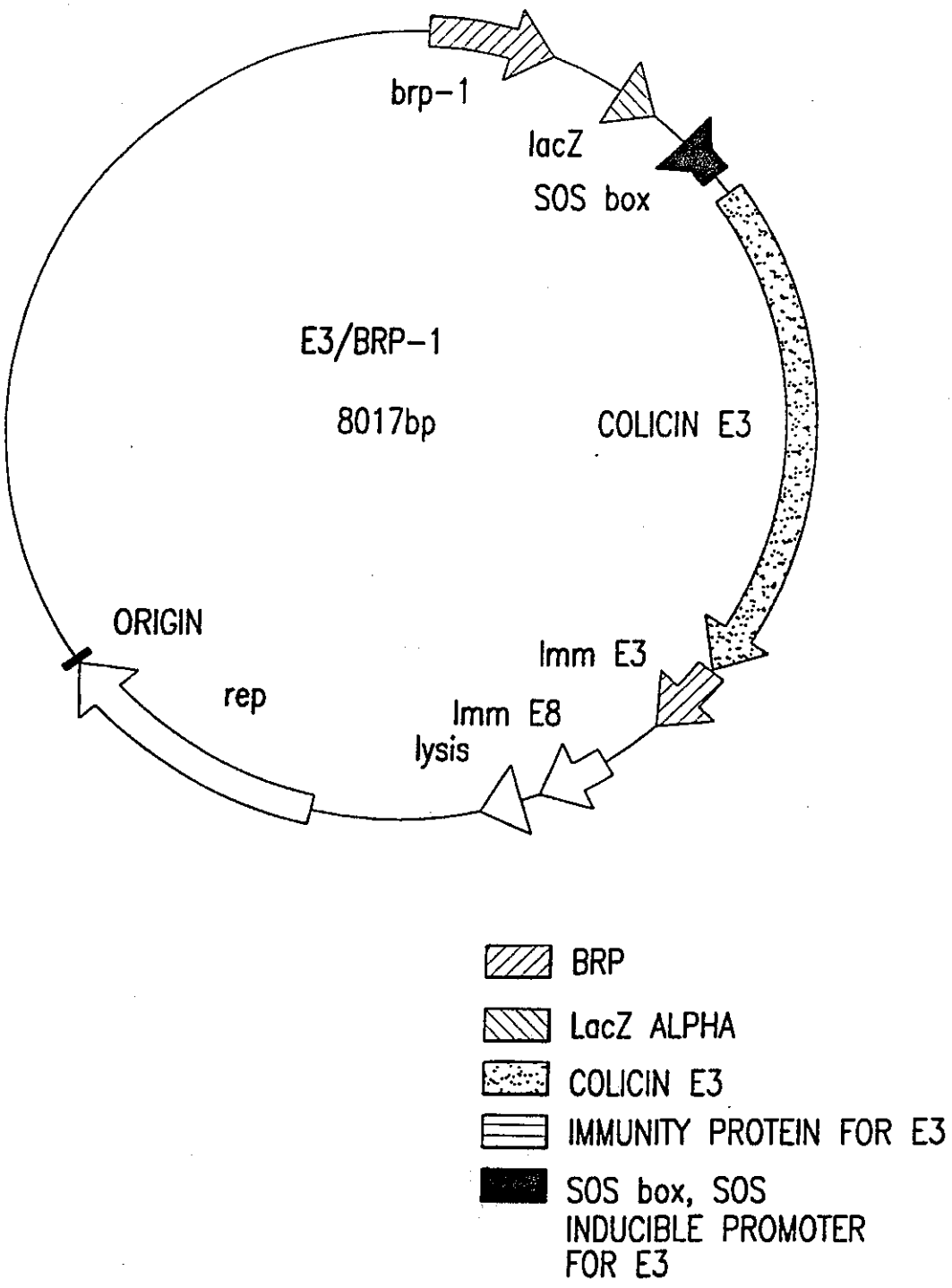

brp-1
lacZ
SOS box
E3/BRP-1
8017bp
COLICIN E3
ORIGIN
rep
Imm E3
Imm E8
lysis
BRP
LacZ ALPHA
COLICIN E3
IMMUNITY PROTEIN FOR E3
SOS box, SOS INDUCIBLE PROMOTER FOR E3

FIG.23

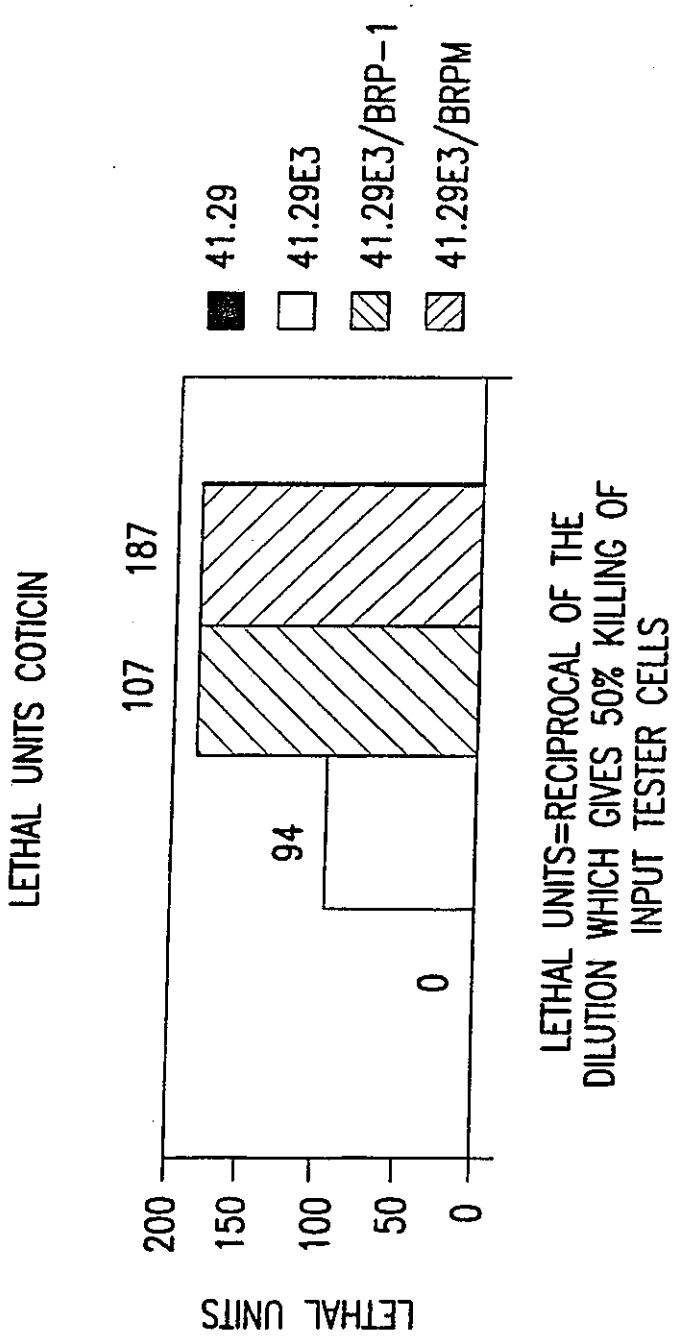

LETHAL UNITS COTICIN
41.29
41.29E3
41.29E3/BRP-1
41.29E3/BRPM
107
187
94
0
200
150
100
50
0
LETHAL UNITS
LETHAL UNITS=RECIPROCAL OF THE DILUTION WHICH GIVES 50% KILLING OF INPUT TESTER CELLS

FIG.24

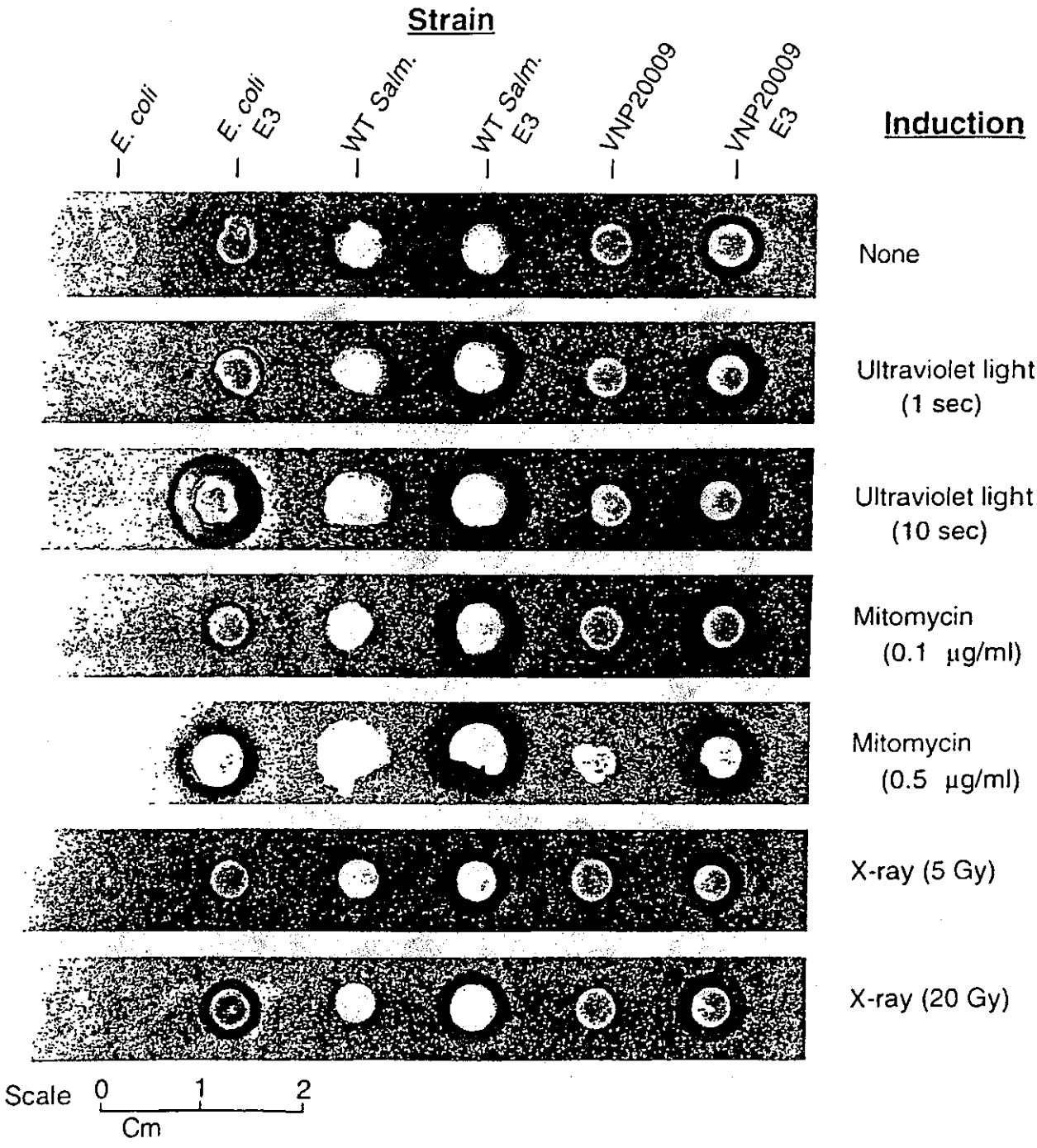

Strain
E. coli
E. coli E3
WT Salm.
WT Salm. E3
VNP20009
VNP20009 E3
Induction
None
Ultraviolet light (1 sec)
Ultraviolet light (10 sec)
Mitomycin (0.1 µg/ml)
Mitomycin (0.5 µg/ml)
X-ray (5 Gy)
X-ray (20 Gy)
Scale 0 1 2
Cm

FIG.25

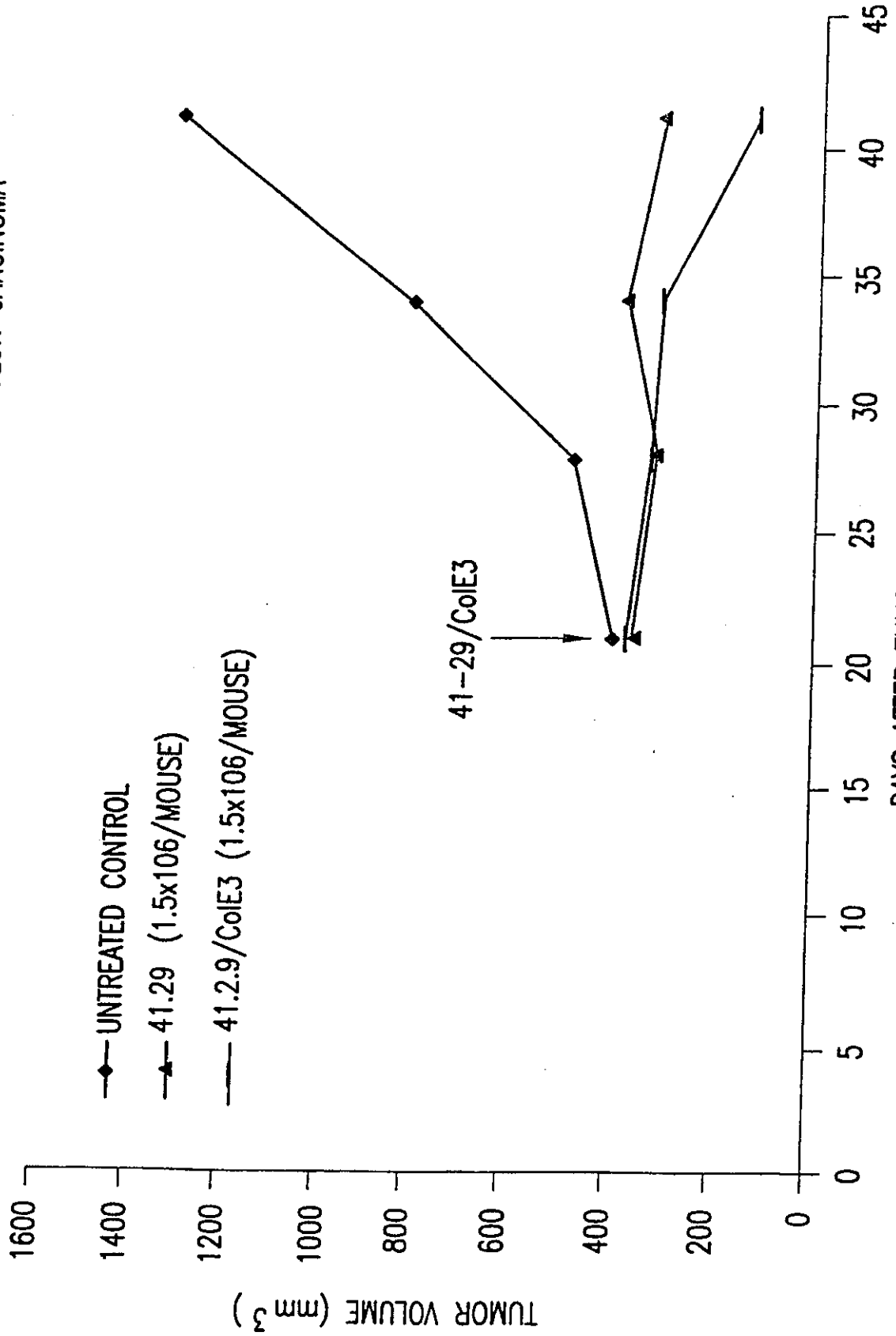

EFFICACY OF 41.2.9/ColE3 ON C38 MURINE COLON CARCINOMA
UNTREATED CONTROL
41.29 (1.5x106/MOUSE)
41.2.9/ColE3 (1.5x106/MOUSE)
41-29/ColE3
TUMOR VOLUME (mm 3)
DAYS AFTER TUMOR IMPLANTATION
0
200
400
600
800
1000
1200
1400
1600
0
5
10
15
20
25
30
35
40
45

FIG.26

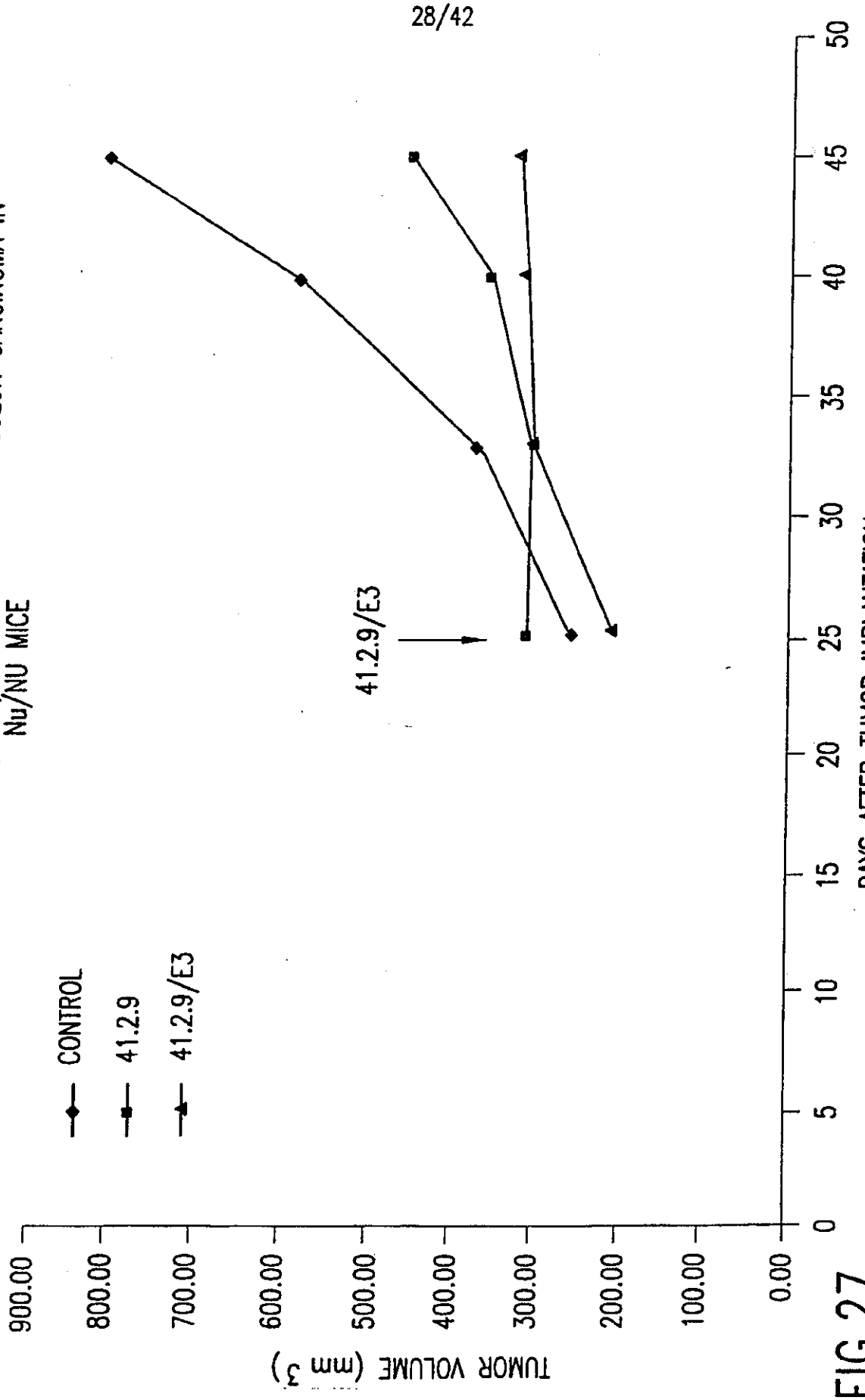

ANTI-TUMOR ACTIVITY OF 41.2.9 / Col/E3 ON DLD1 HUMAN COLON CARCINOMA IN Nu/NU MICE
CONTROL
41.2.9
41.2.9/E3
41.2.9/E3
TUMOR VOLUME (mm 3)
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
0
5
10
15
20
25
30
35
40
45
50
DAYS AFTER TUMOR IMPLANTATION

FIG.27

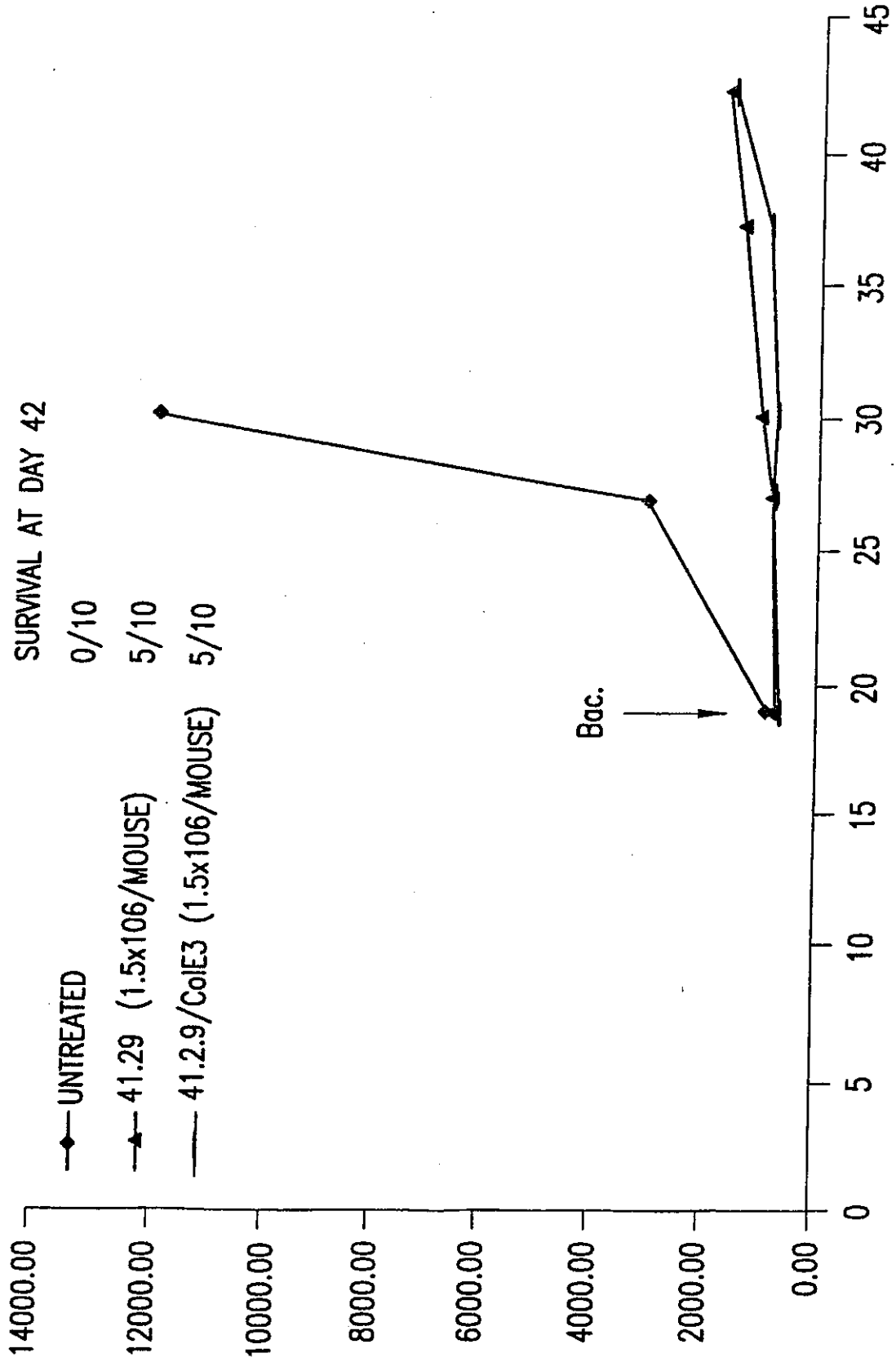

EFFICACY OF 41.2.9/ColE3 ON B16 MURINE MELANOMA
SURVIVAL AT DAY 42
UNTREATED 0/10
41.29 (1.5x106/MOUSE) 5/10
41.2.9/ColE3 (1.5x106/MOUSE) 5/10
Bac.
TUMOR VOLUME (mm 3)
14000.00
12000.00
10000.00
8000.00
6000.00
4000.00
2000.00
0.00
0
5
10
15
20
25
30
35
40
45
DAYS AFTER TUMOR IMPLANTATION

FIG.28

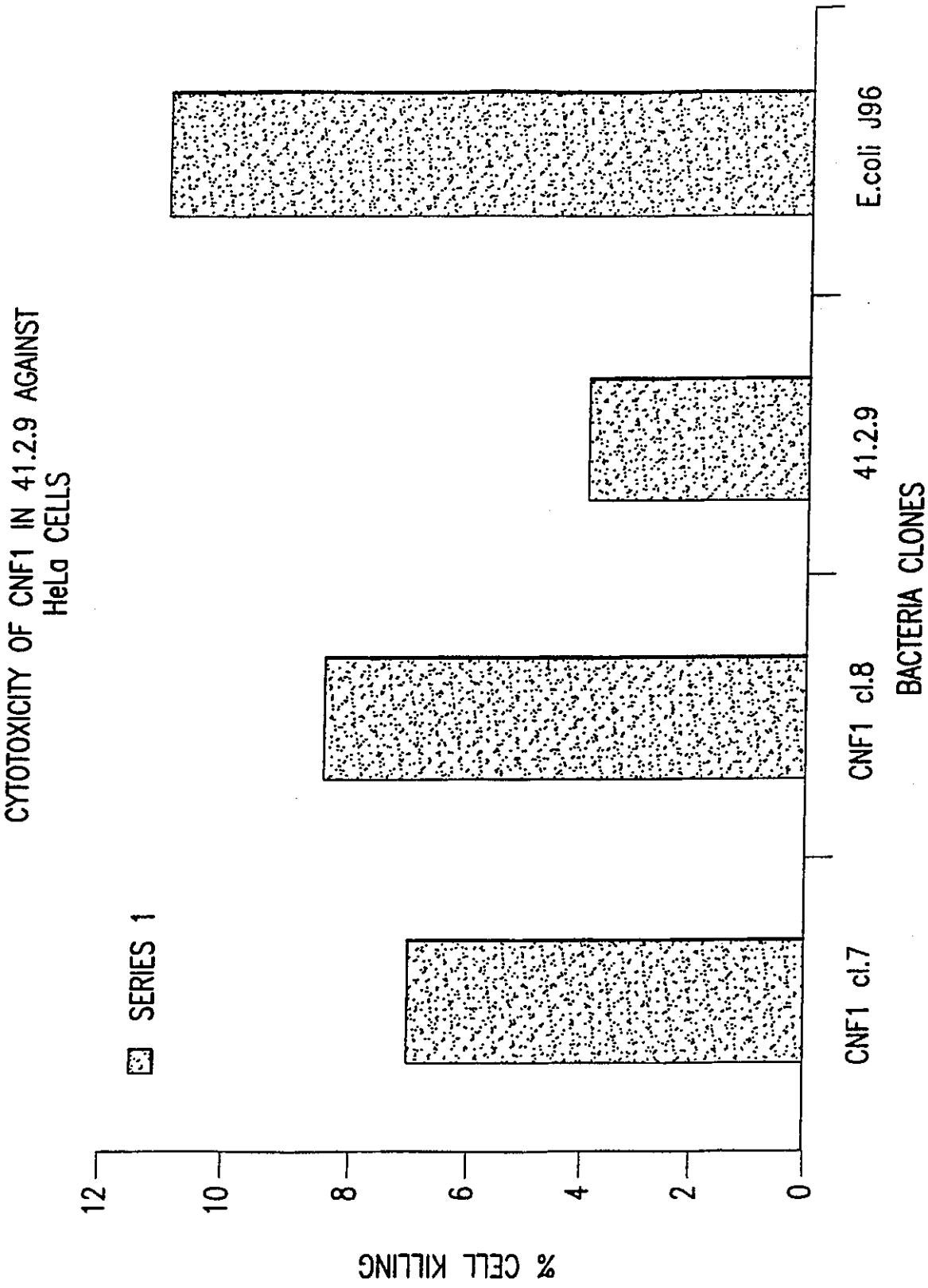
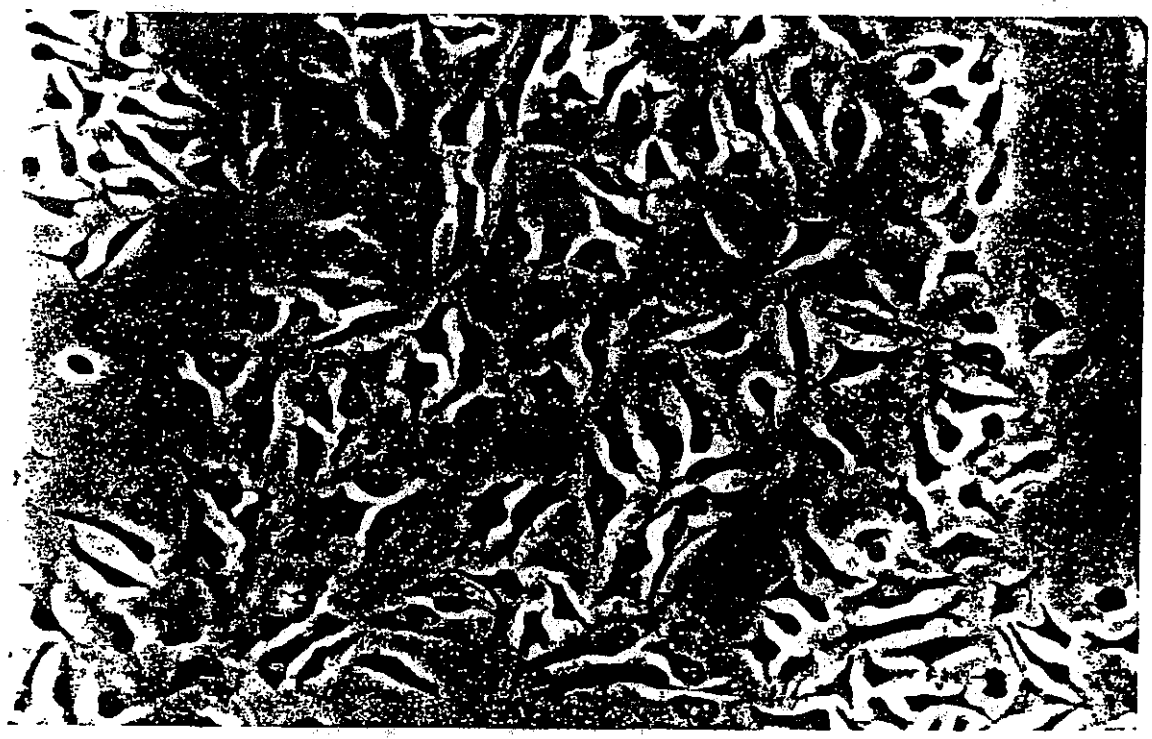

CYTOTOXICITY OF CNF1 IN 41.2.9 AGAINST HeLa CELLS
SERIES 1
% CELL KILLING
0
2
4
6
8
10
12
E.coli J96
41.2.9
CNF1 cl.8
CNF1 cl.7
BACTERIA CLONES

FIG.29



HeLa untreated (20x Obj.)

FIG.30A



HeLa CNF1 in DH5 cl.15 (20x Obj.)

FIG.30B

GATATCATTC TGGCCTCTGA CGTTGTGATG GTCGCACGTG GCGATCTGGG CGTTGAAATC GGCGATCCGG 70
AGCTGGTTGG TATCCAGAAA GCGCTGATTC GCCGTGCGCG TCAGCTAAAC CGCGCAGTCA TCACCGCAAC 140
GCAAATGATG GAGTCGATGA TCACCAACCC GATGCCGACC CGTGCGGAAG TGATGGACGT GGCGAACGCC 210
GTCCTGGATG GCACGGATGC GGTTATGCTG TCTGCCGAAA CCGCAGCCGG TCAGTATCCT TCTGAAACCG 280
TTGCCGCAAT GGCGCGCGTC TGCCTGGGCG CAGAAAAAAT CCCCAGCATC AATGTGTCTA AACACCGTCT 350
CGACGTGCAG TTCGACAACG TTGAAGAAGC CATTGCCATG TCTGCGATGT ATGCGGCAAA CCATCTGAAA 420
GGCGTTACCG CGATCATCAC CATGACGGAA TCCGGTCGTA CCGCGCTAAT GACTTCCCGT ATCAGCTCCG 490
GCCTGCCGAT TTTCGCCATG TCGCGCCATG AACGCACGCT GAACCTGACC GCGCTCTATC GCGGAGTAAC 560
GCCGGTGCAT TTTGATAGCG CGGCTGATGG CGTTGTCGCG GCACATGAAG CTGTTAATCT GCTGCGCGAT 630
AAAGGGTATC TGGTTTCCGG CGACCTGGTT ATCGTGACCC AGGGCGATGT CATGAGCACC GTCGGTTCAA 700
CCAATACCAC GCGGCCGCCC CCTTAATTAA CCCCGCATGC GGGGGGCCAT ATAGGCCGGG GATTTAAATG 770
CAAACGTCCG CCGAAACGCC GACGCACTGT GTTCCAGATA TAGTCAAAAA CCGGATTACC CTGATTATGA 840
AACATCGCCG CCATTTTTTG CCCCTGAGAG GCCATCAGCA TGGCTGGAAT GTCGACGCCC CAGCCATGCG 910
GTACGAGAAA AATGACTTTT TCGTCGTTAC GACGCATCTC CTCGATAATC TCCAGACCTT CCCAGTCAAC 980
ACGCTGTTGA ATTTTTTTCG GACCGCGCAT CGCCAACTCA GCCATCATCG CCATTGCCTG TGGCGCGGTG 1050
GCGAACATCT CATCGACAAT CGCTTCGCGC TCAGCTTCGC TACGCTGCGG AAAGCACAAC GACAGATTAA 1120
TTAGCGCCCG GCGACGAGAA CTCTTCCCCA GCCGTCCGGC AAAACGCCCC AGCGTCGCCA GCAAAGGGTC 1190
GCGGAATGAT GCCGGTGTTA ATGCGATCCC CGCCATTGCC GCCGCGCCCA ACCAGGCGCC CCAATACTGT 1260
GGATAGCGAA AGGATTTTTC GAATTCAGGG ATATACTCAC TATTATTTTT TTTGGTTTCC ATGCTTTTCC 1330
AGGGTCTGCT GACGCGAAAA GGAATTGTGA ATAGTGTAGC GACGTCTGCG TCTCACACAA AACAAAAAAG 1400
CCGGCACACA TCGCGTACCG GCTCTGTCAG CGCATTTGTT AATCGAAGCG CAGTTGCGGC AGAACCTCTT 1470
TCACCTGTGC CAGGTATTCA CGACGATCTG ACCCCGTCAG ACCTTCCGTG CGCGGCAATT TTGCTGTCAG 1530
AGGGTTAACG GCTTGCTGGT TGATC 1555

FIG.31

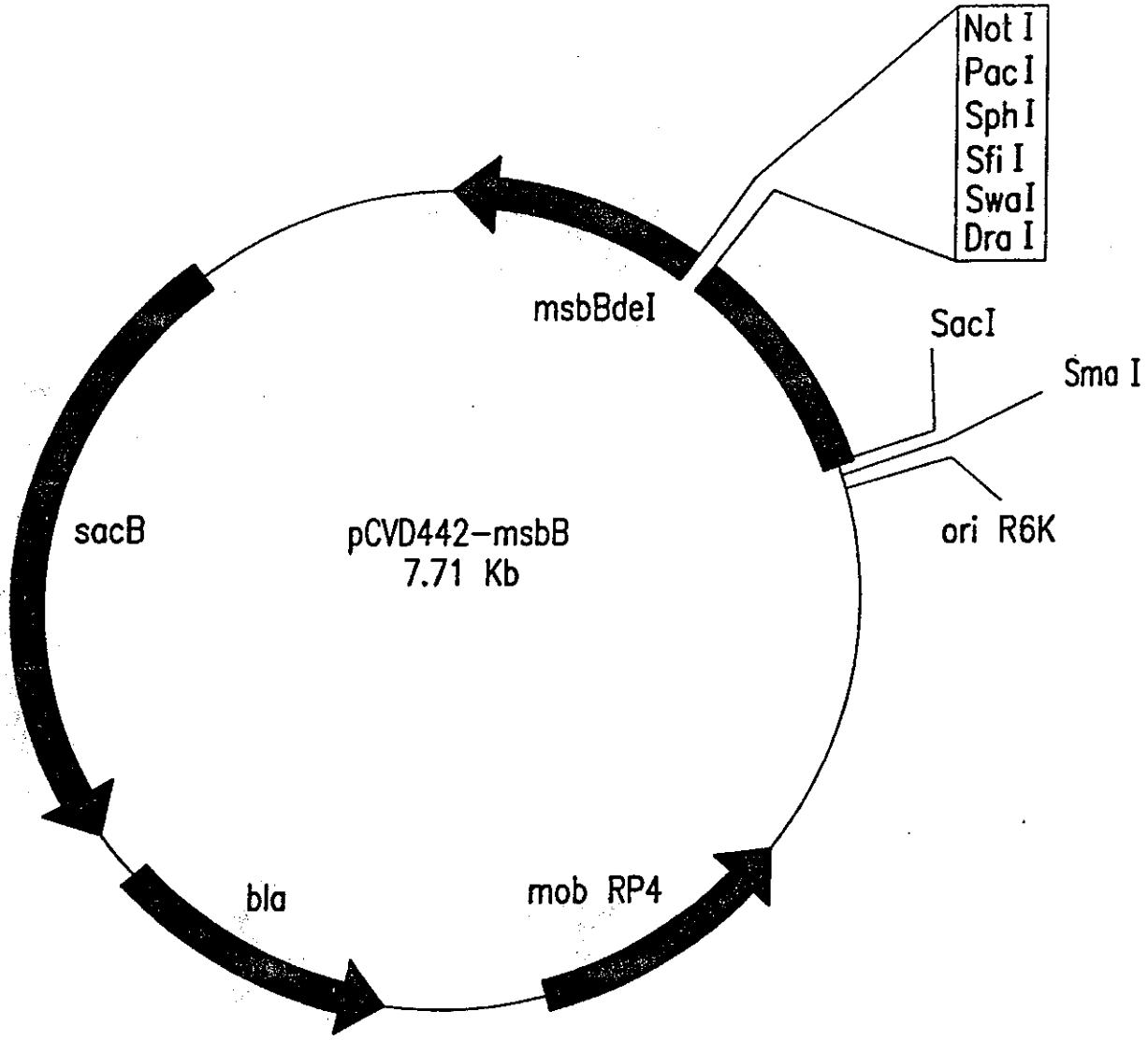

Not I
Pac I
Sph I
Sfi I
SwaI
Dra I
msbBdeI
SacI
Sma I
ori R6K
sacB
pCVD442–msbB
7.71 Kb
bla
mob RP4

FIG.32

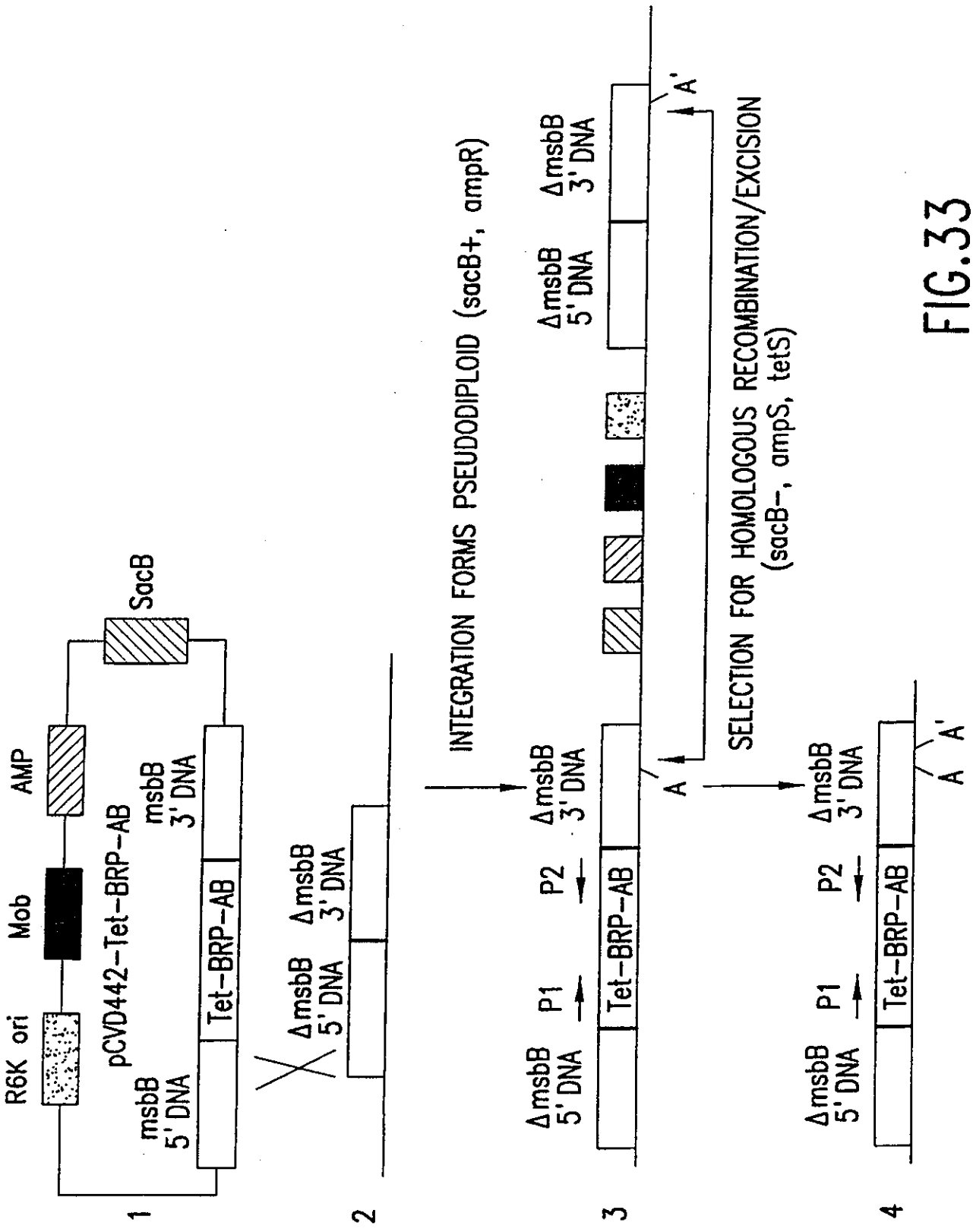

R6K ori
Mob
AMP
SacB
pCVD442-Tet-BRP-AB
msbB 5' DNA
msbB 3' DNA
Tet-BRP-AB
ΔmsbB 5' DNA
ΔmsbB 3' DNA
INTEGRATION FORMS PSEUDODIPLOID (sacB+, ampR)
P1
P2
A
A'
SELECTION FOR HOMOLOGOUS RECOMBINATION/EXCISION (sacB-, ampS, tetS)
1
2
3
4

FIG.33

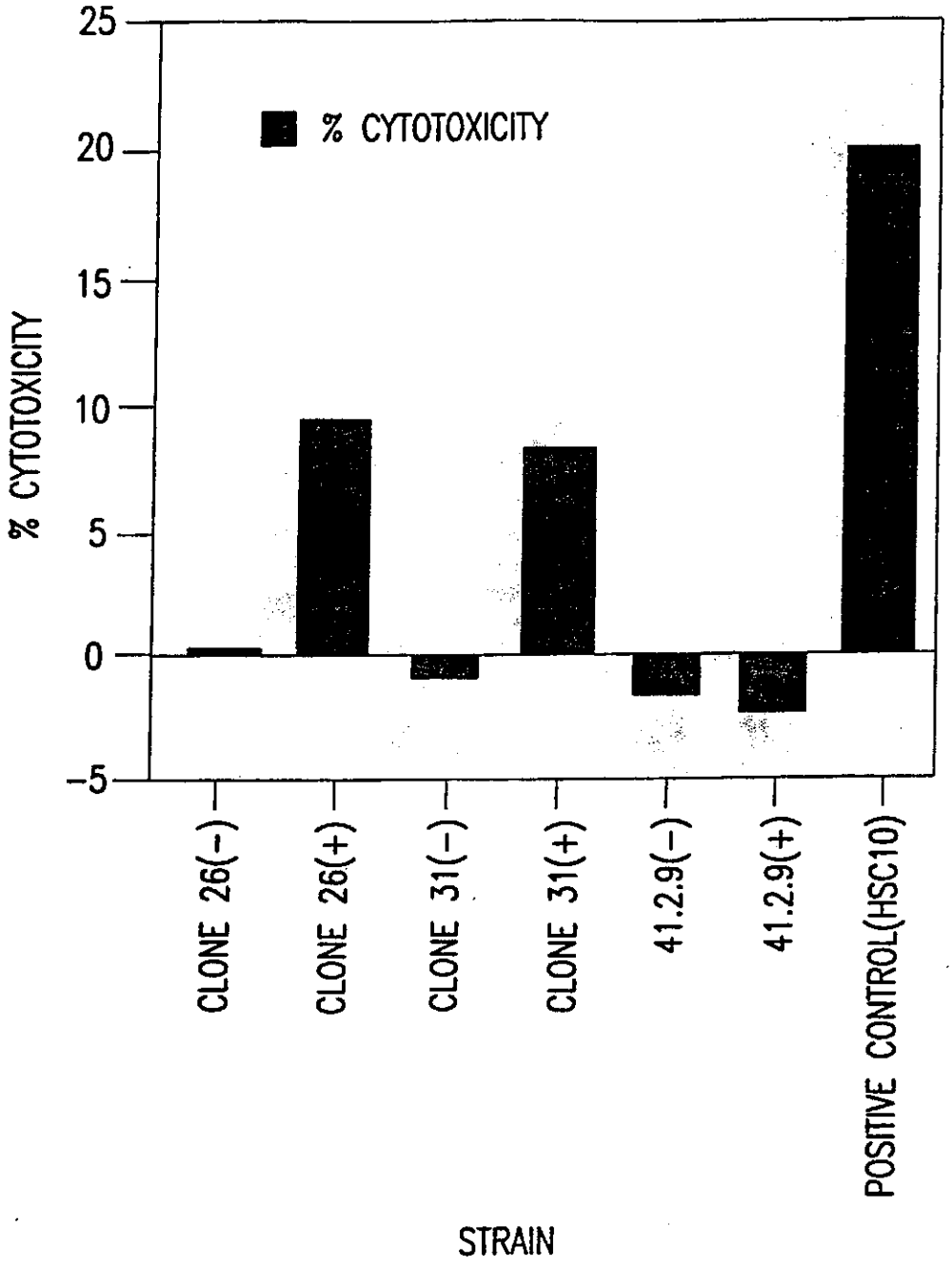

25
20
15
10
5
0
-5
% CYTOTOXICITY
% CYTOTOXICITY
CLONE 26(-)
CLONE 26(+)
CLONE 31(-)
CLONE 31(+)
41.2.9(-)
41.2.9(+)
POSITIVE CONTROL(HSC10)
STRAIN

FIG.34

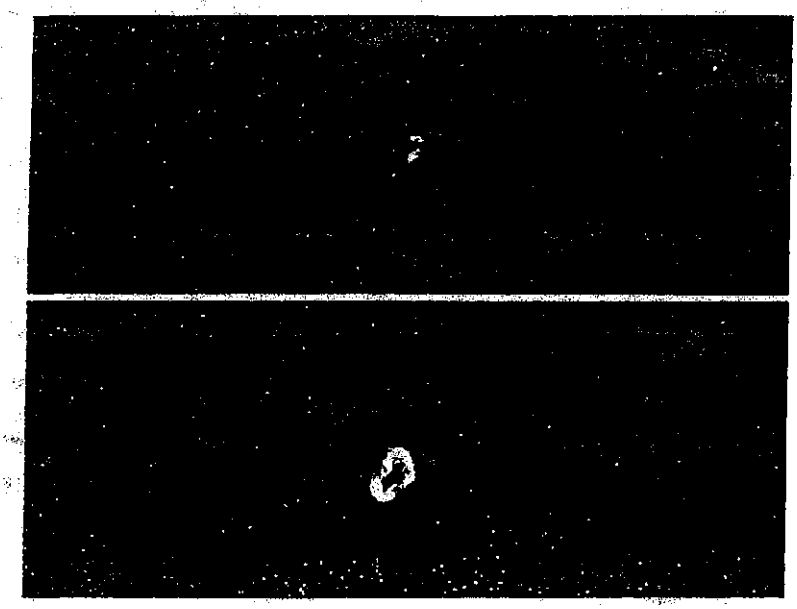


FIG.35

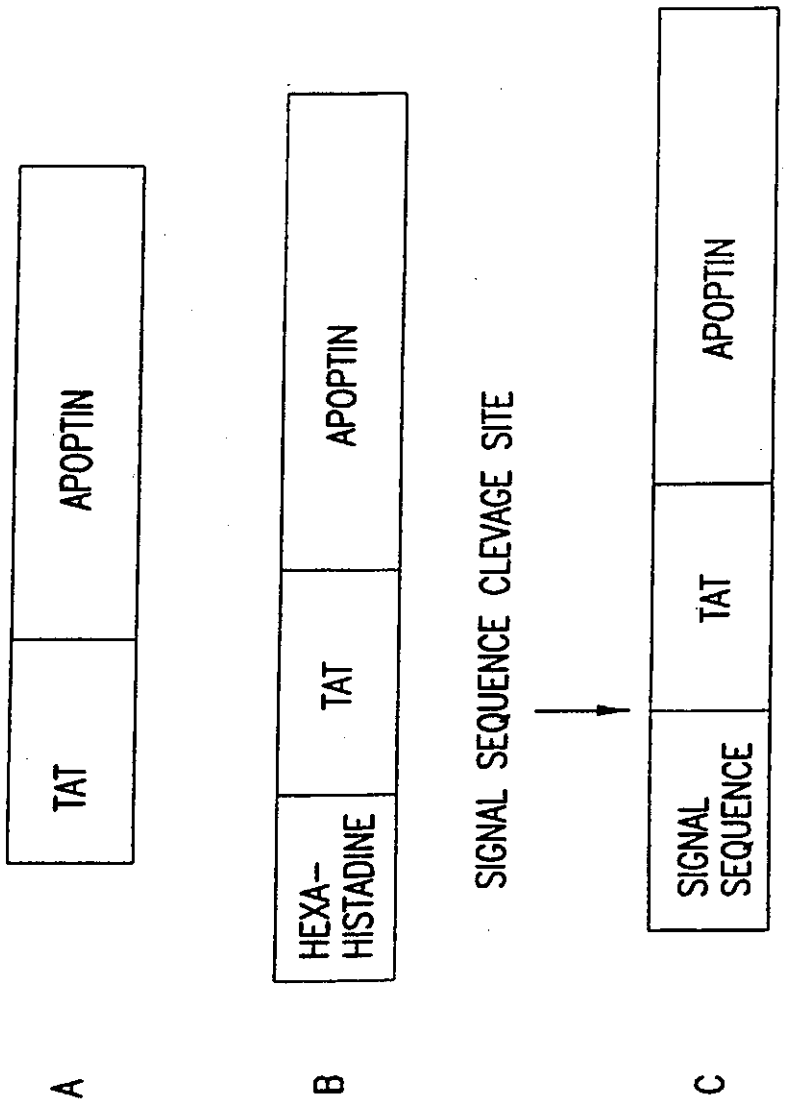

TAT
APOPTIN
HEXA-HISTADINE
TAT
APOPTIN
SIGNAL SEQUENCE CLEVAGE SITE
SIGNAL SEQUENCE
TAT
APOPTIN
A
B
C

FIG.36

Protein Sequence of 616-4 F

Length of 616-4 F: 551 bp; Listed from: 1 to: 551;
Translated from: 7 to: 409 (Entire region);
Genetic Code used: Universal; Wed, Aug 16, 2000 1:40 PM

```
Frame 1          M   A   Y   G   R   K   K   R   R   Q   R   R   R   M   N
        NAG ACC ATG GCT TAT GGC AGA AAA AAA AGA AGA CAG AGA AGA AGA ATC AAC
                  9          18          27          36          45

A   L   Q   E   D   T   P   P   G   P   S   T   V   F   R   P   P   T   S
GCG CTG CAG GAA GAT ACC CCG CCG GGC CCG TCC ACC GTG TTT CGC CCG CCG ACC TCC
        60          69          78          87          96          105

S   R   P   L   E   T   P   H   C   R   E   I   R   I   G   I   A   G   I
TCC CGC CCG CTG GAA ACC CCG CAT TGC CGC GAA ATC CGC ATC GGC ATC GCG GGC ATC
        117         126         135         144         153         162

T   I   T   L   S   L   C   G   C   A   N   A   R   A   P   T   L   R   S
ACC ATC ACC CTG TCC CTG TGC GGC TGC GCG AAC GCG CGC GCG CCG ACC CTG CGC TCC
        174         183         192         201         210         219

A   T   A   D   N   S   E   N   T   G   F   K   N   V   P   D   L   R   T
GCG ACC GCG GAT AAC TCC GAA AAC ACC GGC TTT AAA AAC GTC CCG GAT CTG CGC ACC
        231         240         249         258         267         276

D   Q   P   K   P   P   S   K   K   R   S   C   D   P   S   E   Y   R   V
GAT CAG CCG AAA CCG CCG TCC AAA AAA CGC TCC TGC GAT CCG TCC GAA TAT CGC GTC
        288         297         306         315         324         333

S   E   L   K   E   S   L   I   T   T   T   P   S   R   P   R   T   A   R
TCC GAA CTG AAA GAA TCC CTG ATC ACC ACC ACC CCG TCC CGC CCG CGC ACC GCC CGC
        345         354         363         372         381         390

R   C   I   R   L   .
CGC TGC ATC CGC CTC TGA AAG CTT GGC TGT TTT GGC GGA TGA GAG AAG ATT TTC AGC
        402         411         420         429         438         447

CTG ATA CAG ATT AAA TCA GAA CGC AGA AGC GGT CTG ATA AAA CAG AAT TTG CCT GGC
        459         468         477         486         495         504

GGC AGT AGC GCG GTG GTC CCA CCT GAC CCC ATG CCG AAC TCA GA
        516         525         534         543
```

FIG.37

Protein Sequence of TAP6H8 trcF

Length of TAP6H8 trcF: 751 bp; Listed from: 1 to: 444;
Translated from: 7 to: 427 (Entire region);
Genetic Code used: Universal; Mon, Aug 14, 2000 3:19 PM

```
Frame 1          M   A   H   H   H   H   H   H   Y   G   R   K   K   R   R
        NAG ACC ATG GCT CAT CAC CAT CAC CAC CAT TAT GGC CGC AAA AAA CGC CGT
                  9          18          27          36          45

Q   R   R   R   M   N   A   L   Q   E   D   T   P   P   G   P   S   T   V
CAG CGC CGT CGC ATG AAC GCG CTG CAG GAA GAT ACC CCG CCG GGC CCG TCC ACC GTG

        60          69          78          87          96          105

F   R   P   P   T   S   S   R   P   L   E   T   P   H   C   R   E   I   R
TTT CGC CCG CCG ACC TCC TCC CGC CCG CTG GAA ACC CCG CAT TGC CGC GAA ATC CGC
        117         126         135         144         153         162

I   G   I   A   G   I   T   I   T   L   S   L   C   G   C   A   N   A   R
ATC GGC ATC GCG GGC ATC ACC ATC ACC CTG TCC CTG TGC GGC TGC GCG AAC GCG CGC
  174           183           192           201         210     219

A   P   T   L   R   S   A   T   A   D   N   S   E   N   T   G   F   K   N
GCG CCG ACC CTG CGC TCC GCG ACC GCG GAT AAC TCC GAA AAC ACC GGC TTT AAA AAC
        231         240         249         258         267         276

V   P   D   L   R   T   D   Q   P   K   P   P   S   K   K   R   S   C   D
GTC CCG GAT CTG CGC ACC GAT CAG CCG AAA CCG CCG TCC AAA AAA CGC TCC TGC GAT
        288         297         306         315         324         333

P   S   E   Y   R   V   S   E   L   K   E   S   L   I   T   T   T   P   S
CCG TCC GAA TAT CGC GTC TCC GAA CTG AAA GAA TCC CTG ATC ACC ACC ACC CCG TCC
        345         354         363         372         381         390

R   P   R   T   A   R   R   C   I   R   L   *
CGC CCG CGC ACC GCC CGC CGC TGC ATC CGC CTC TGA AAG CTT GGC TGT TTT
        402         411         420         429         438
```

FIG.38

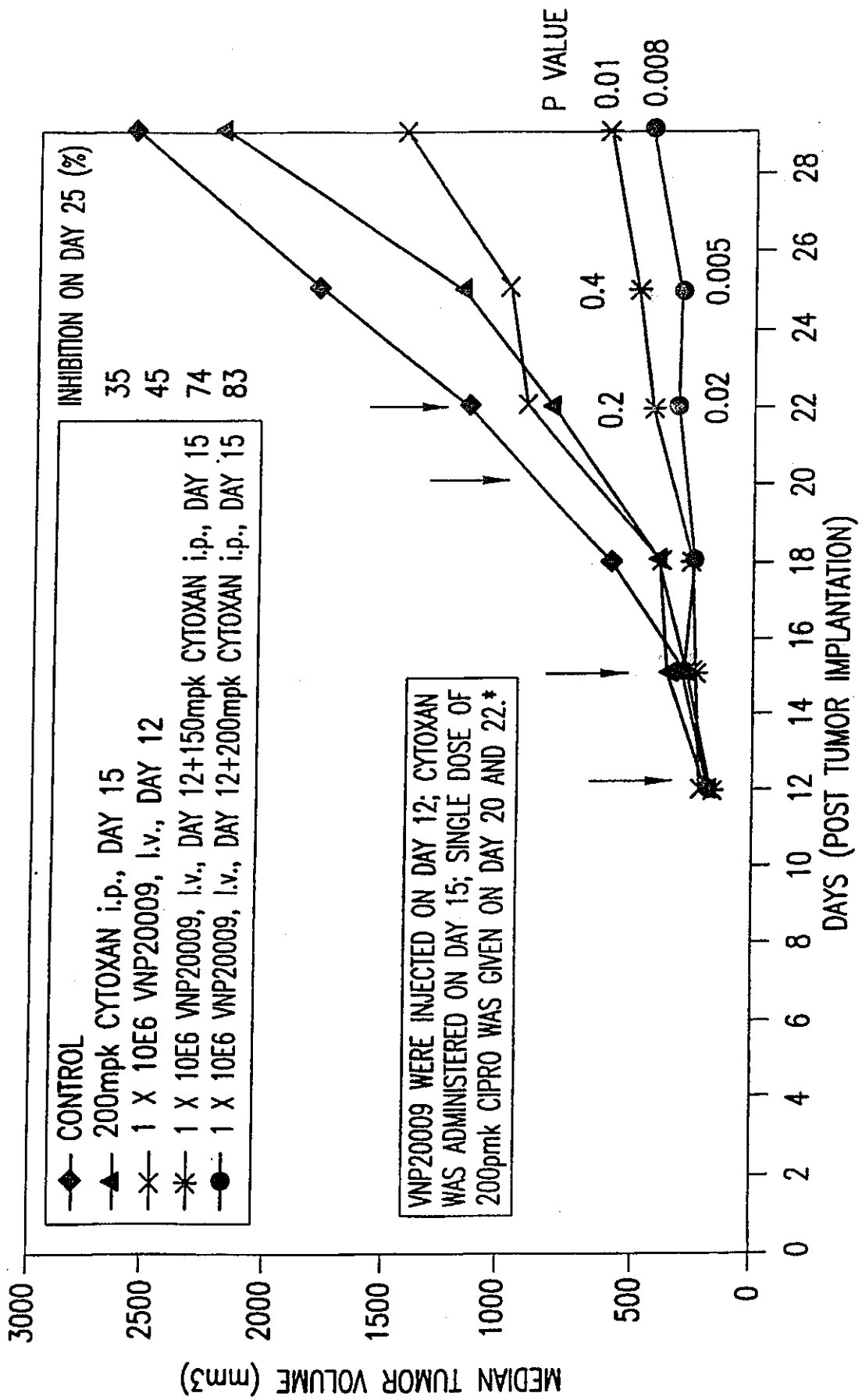

MEDIAN TUMOR VOLUME (mm3)
3000
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS (POST TUMOR IMPLANTATION)
CONTROL
200mpk CYTOXAN i.p., DAY 15
1 X 10E6 VNP20009, I.v., DAY 12
1 X 10E6 VNP20009, I.v., DAY 12+150mpk CYTOXAN i.p., DAY 15
1 X 10E6 VNP20009, I.v., DAY 12+200mpk CYTOXAN i.p., DAY 15
INHIBITION ON DAY 25 (%)
35
45
74
83
P VALUE
0.01
0.008
0.4
0.005
0.2
0.02
VNP20009 WERE INJECTED ON DAY 12; CYTOXAN WAS ADMINISTERED ON DAY 15; SINGLE DOSE OF 200pmk CIPRO WAS GIVEN ON DAY 20 AND 22.*

FIG.39

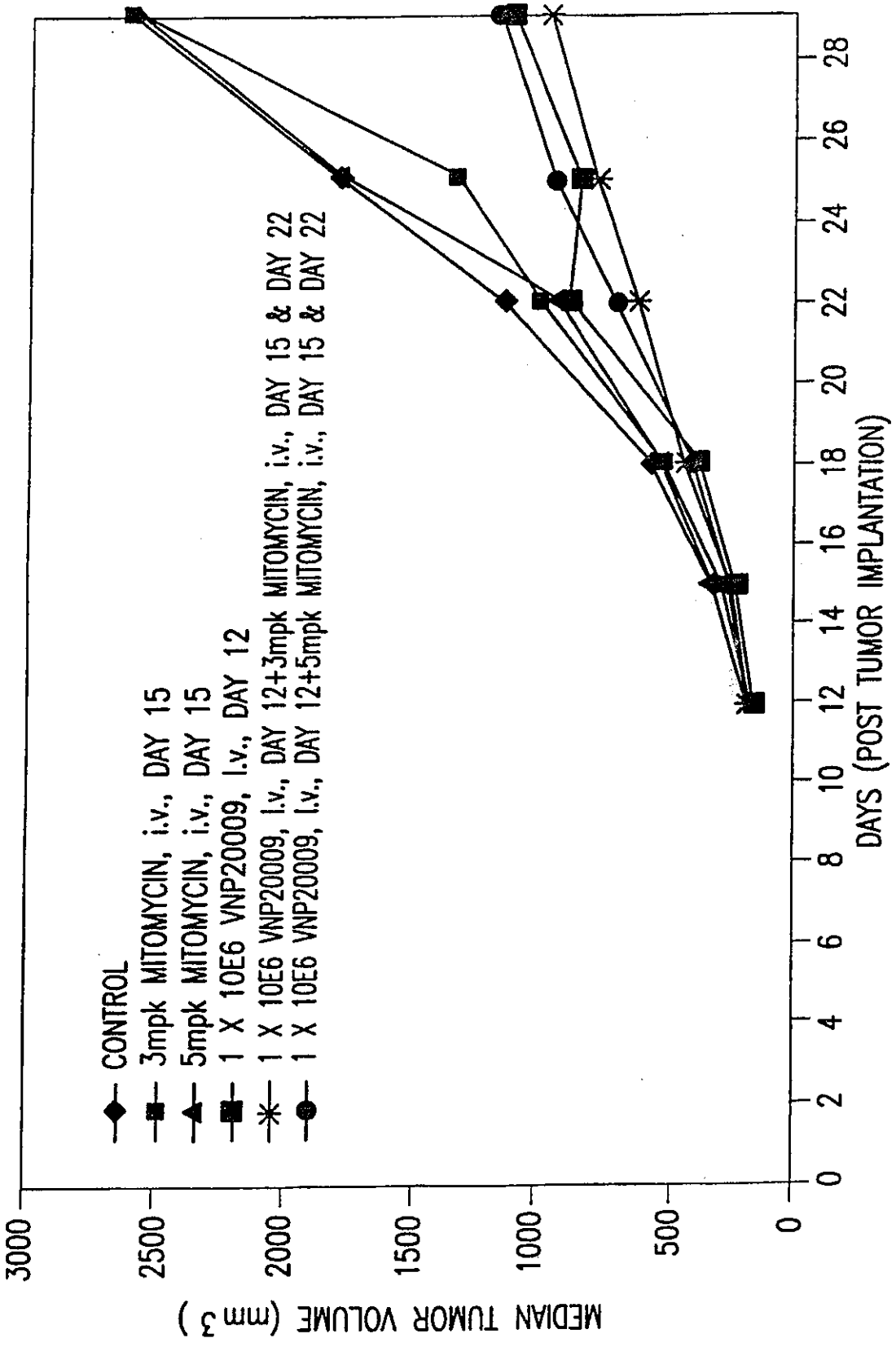

MEDIAN TUMOR VOLUME (mm 3)
3000
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS (POST TUMOR IMPLANTATION)
CONTROL
3mpk MITOMYCIN, i.v., DAY 15
5mpk MITOMYCIN, i.v., DAY 15
1 X 10E6 VNP20009, I.v., DAY 12
1 X 10E6 VNP20009, I.v., DAY 12+3mpk MITOMYCIN, i.v., DAY 15 & DAY 22
1 X 10E6 VNP20009, I.v., DAY 12+5mpk MITOMYCIN, i.v., DAY 15 & DAY 22

FIG.40

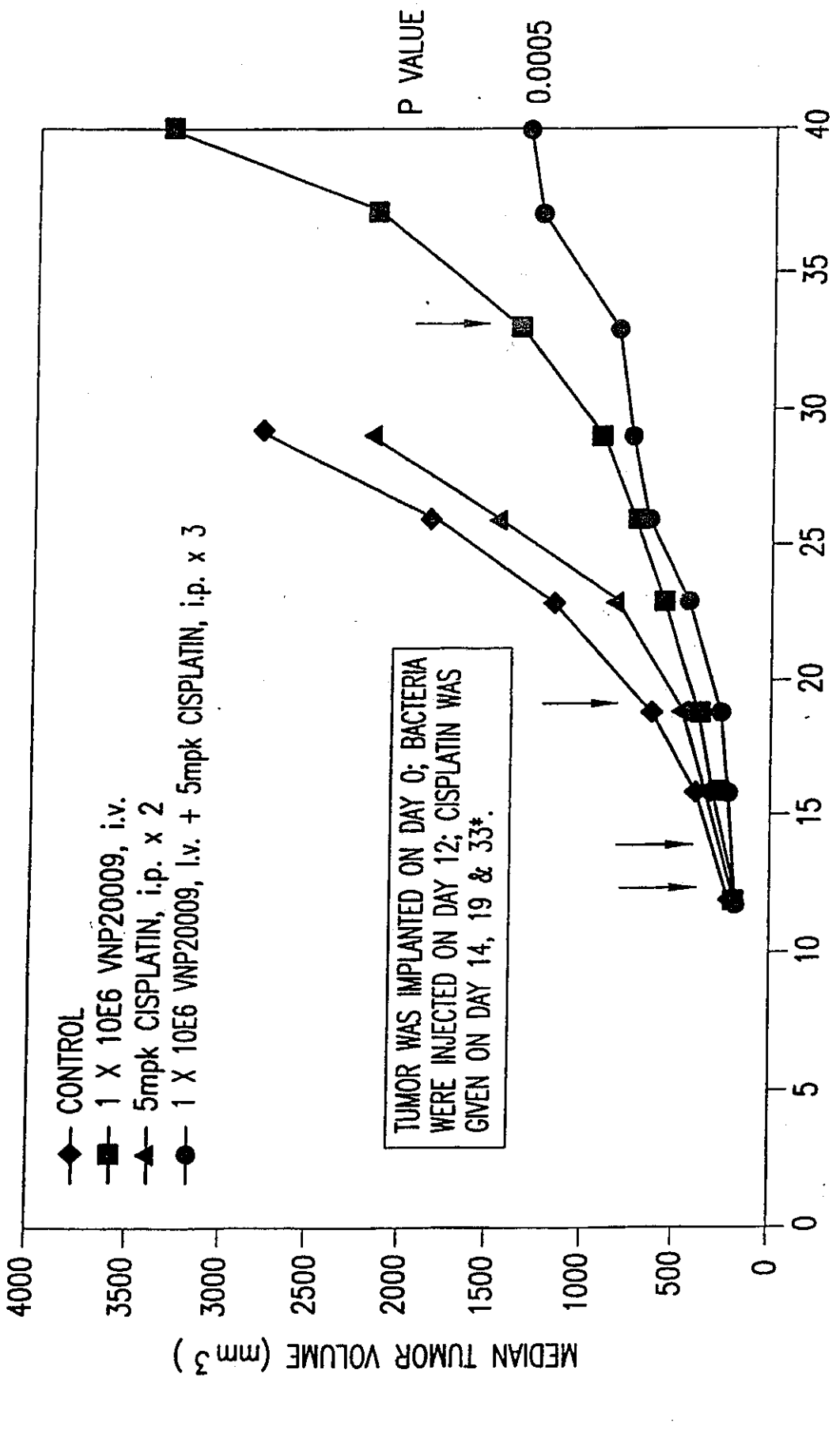

P VALUE
0.0005
CONTROL
1 X 10E6 VNP20009, i.v.
5mpk CISPLATIN, i.p. x 2
1 X 10E6 VNP20009, i.v. + 5mpk CISPLATIN, i.p. x 3
TUMOR WAS IMPLANTED ON DAY 0; BACTERIA WERE INJECTED ON DAY 12; CISPLATIN WAS GIVEN ON DAY 14, 19 & 33*.
MEDIAN TUMOR VOLUME (mm3)
DAYS (POST TUMOR IMPLANTATION)
* GROUPS OF CONTROL AND TREATED WITH CISPLATIN ONLY WERE STOPPED ON DAY 29.

FIG.41