
P
FUSION PROTEIN
P
FUSION PROTEIN WITH SPACER
P
FUSION PROTEIN WITH MULTIPLE ANTIGENS
P P
FUSION PROTEIN WITH MULTIPLE PAMPS

FIG. 1

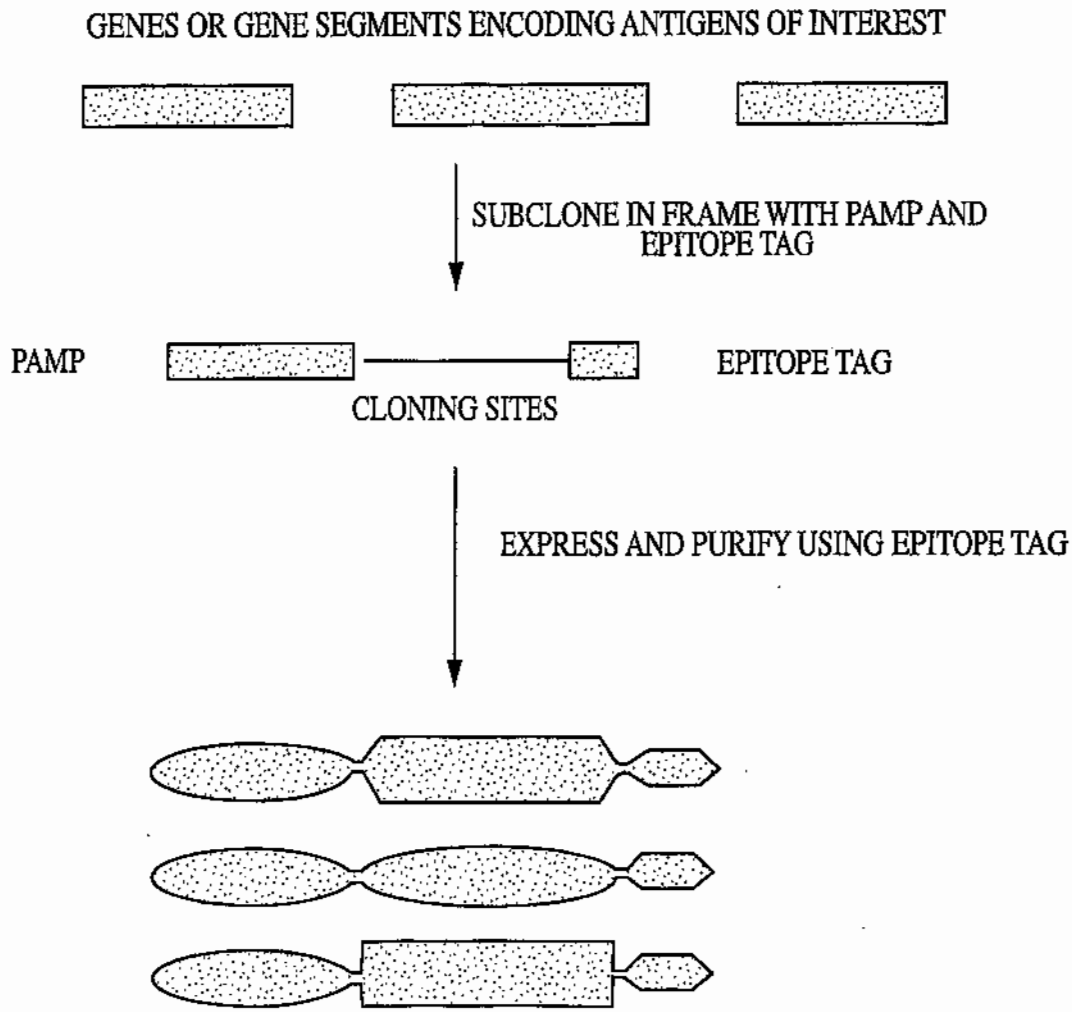

GENES OR GENE SEGMENTS ENCODING ANTIGENS OF INTEREST
SUBCLONE IN FRAME WITH PAMP AND EPITOPE TAG
PAMP
EPITOPE TAG
CLONING SITES
EXPRESS AND PURIFY USING EPITOPE TAG

FIG. 2

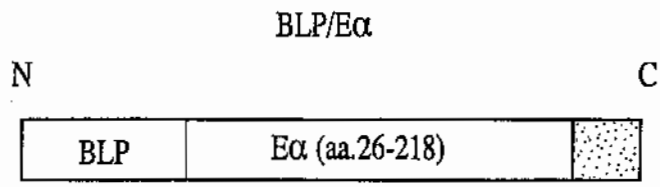


FIG. 3

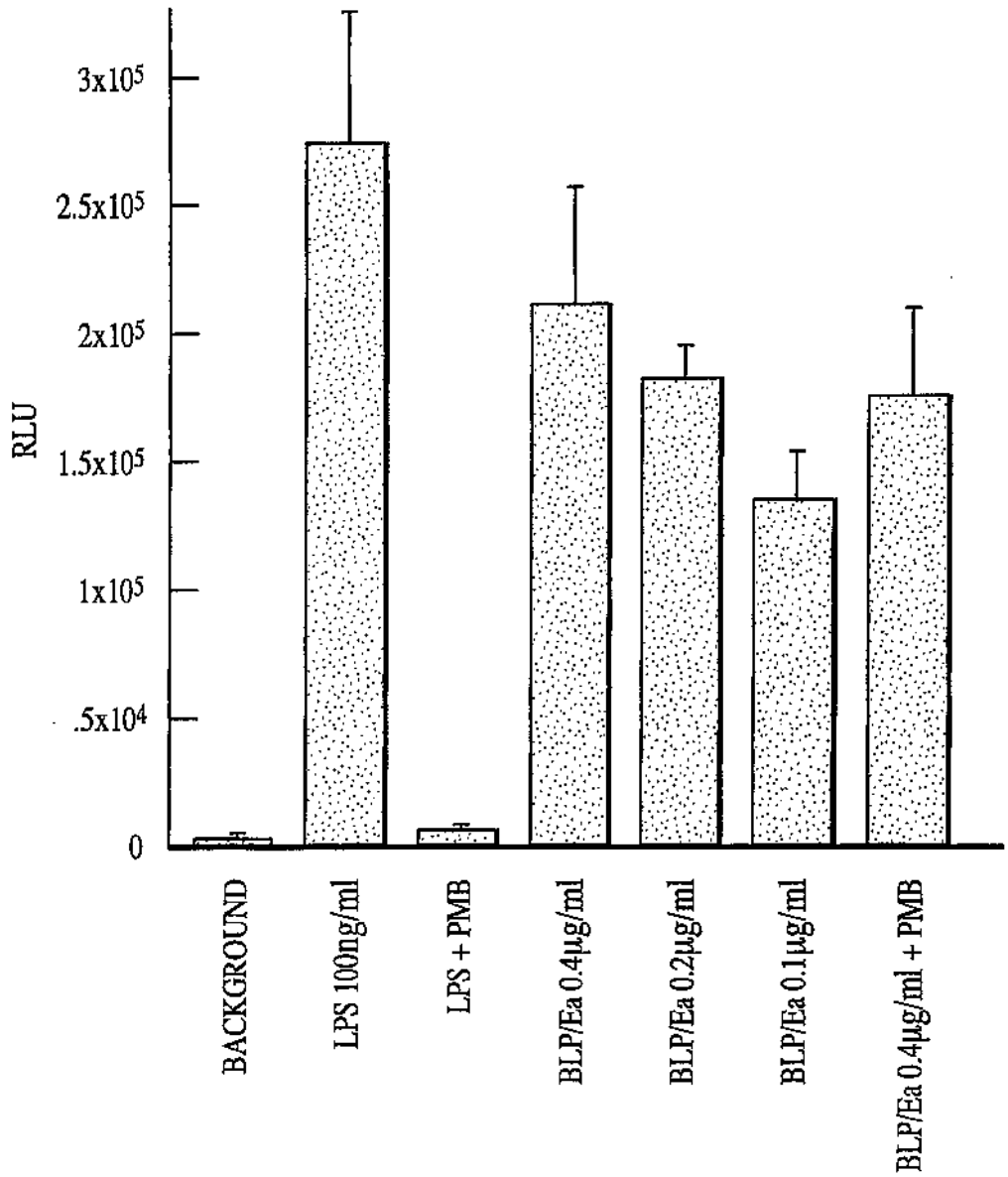

RLU
3x10^5
2.5x10^5
2x10^5
1.5x10^5
1x10^5
.5x10^4
0
BACKGROUND
LPS 100ng/ml
LPS + PMB
BLP/Ea 0.4µg/ml
BLP/Ea 0.2µg/ml
BLP/Ea 0.1µg/ml
BLP/Ea 0.4µg/ml + PMB

FIG. 4

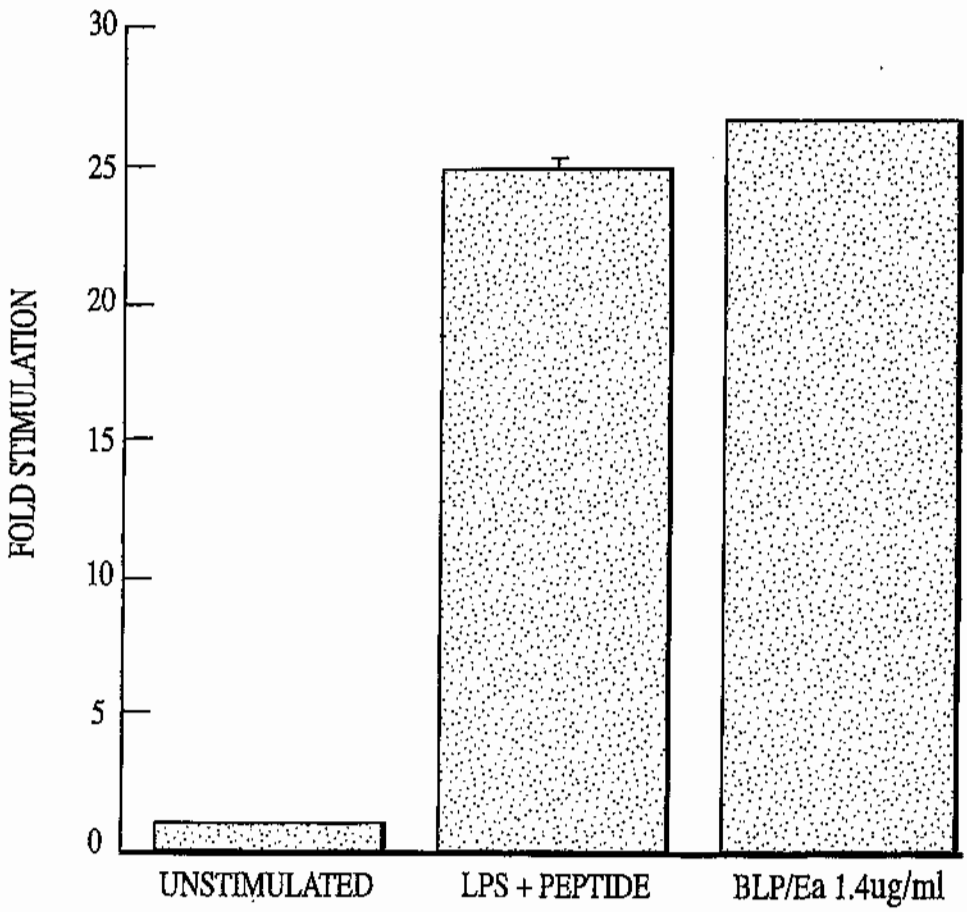
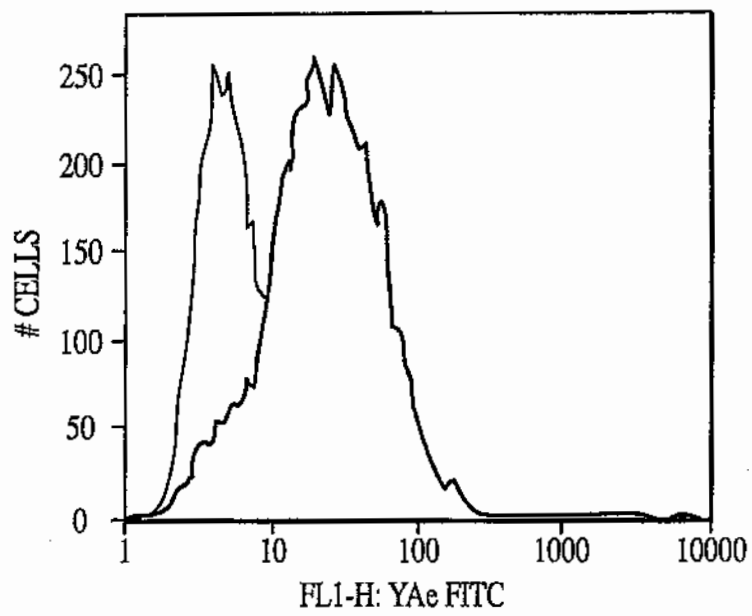

30
25
20
15
10
5
0
FOLD STIMULATION
UNSTIMULATED
LPS + PEPTIDE
BLP/Ea 1.4ug/ml

FIG. 5


250
200
150
100
50
0
CELLS
1
10
100
1000
10000
FL1-H: YAe FITC

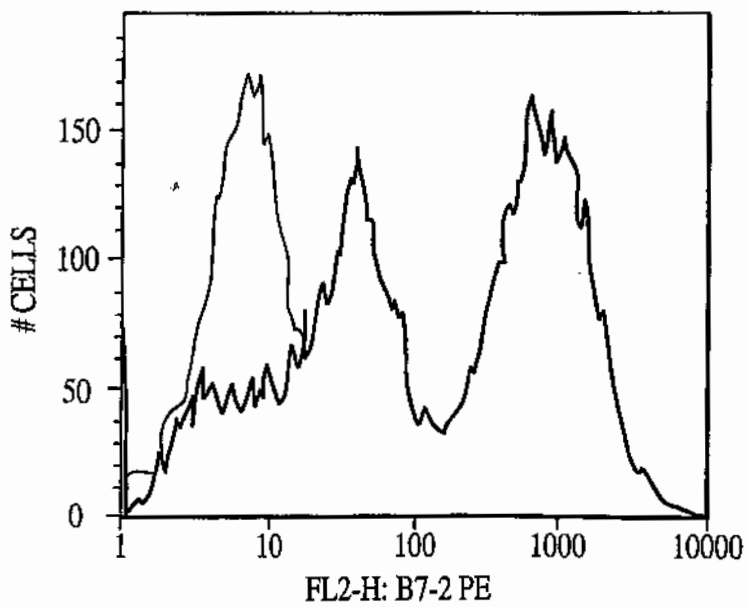

150
100
50
0
CELLS
1
10
100
1000
10000
FL2-H: B7-2 PE

FIG. 6

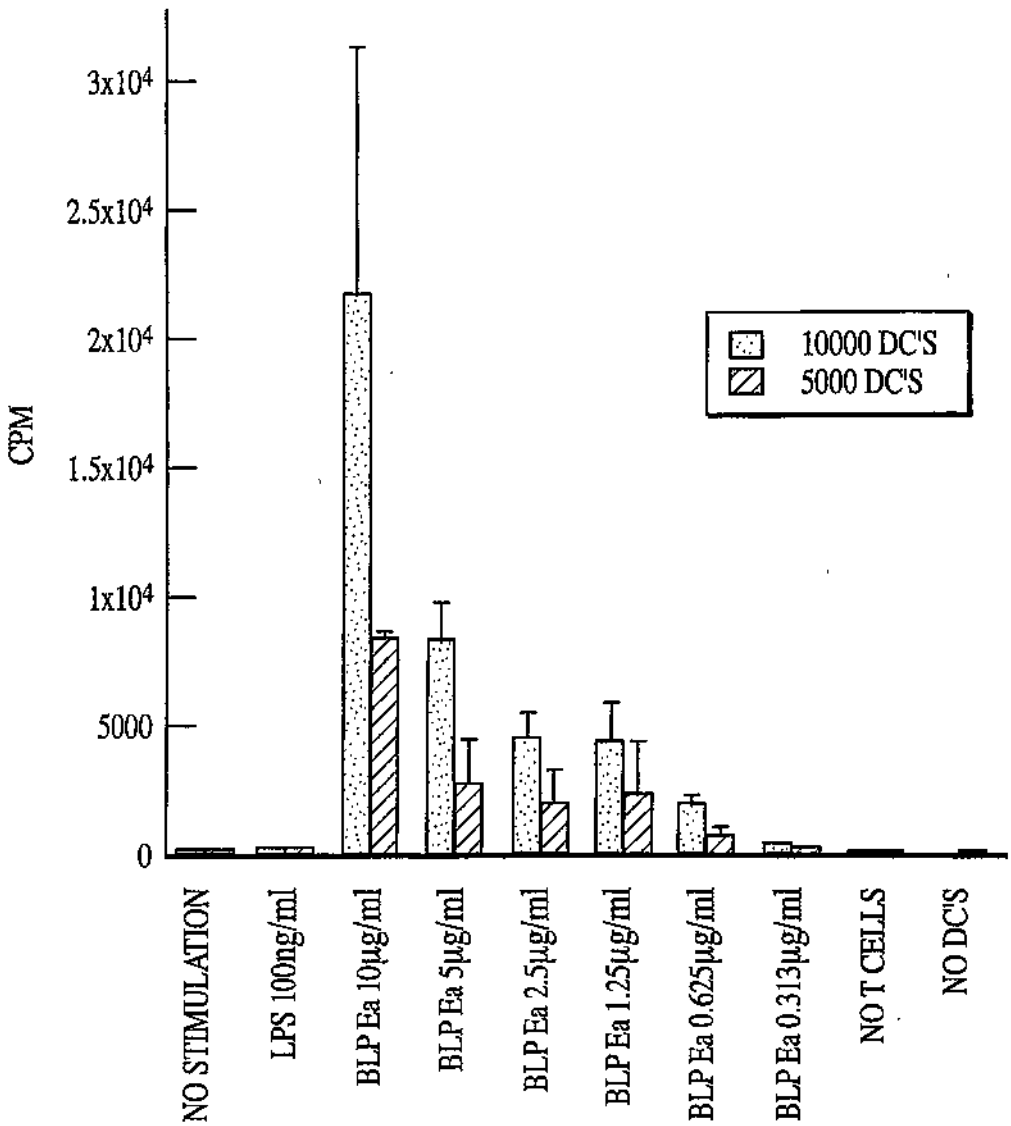

CPM
3x10⁴
2.5x10⁴
2x10⁴
1.5x10⁴
1x10⁴
5000
0
10000 DC'S
5000 DC'S
NO STIMULATION
LPS 100ng/ml
BLP Ea 10µg/ml
BLP Ea 5µg/ml
BLP Ea 2.5µg/ml
BLP Ea 1.25µg/ml
BLP Ea 0.625µg/ml
BLP Ea 0.313µg/ml
NO T CELLS
NO DC'S

FIG. 7

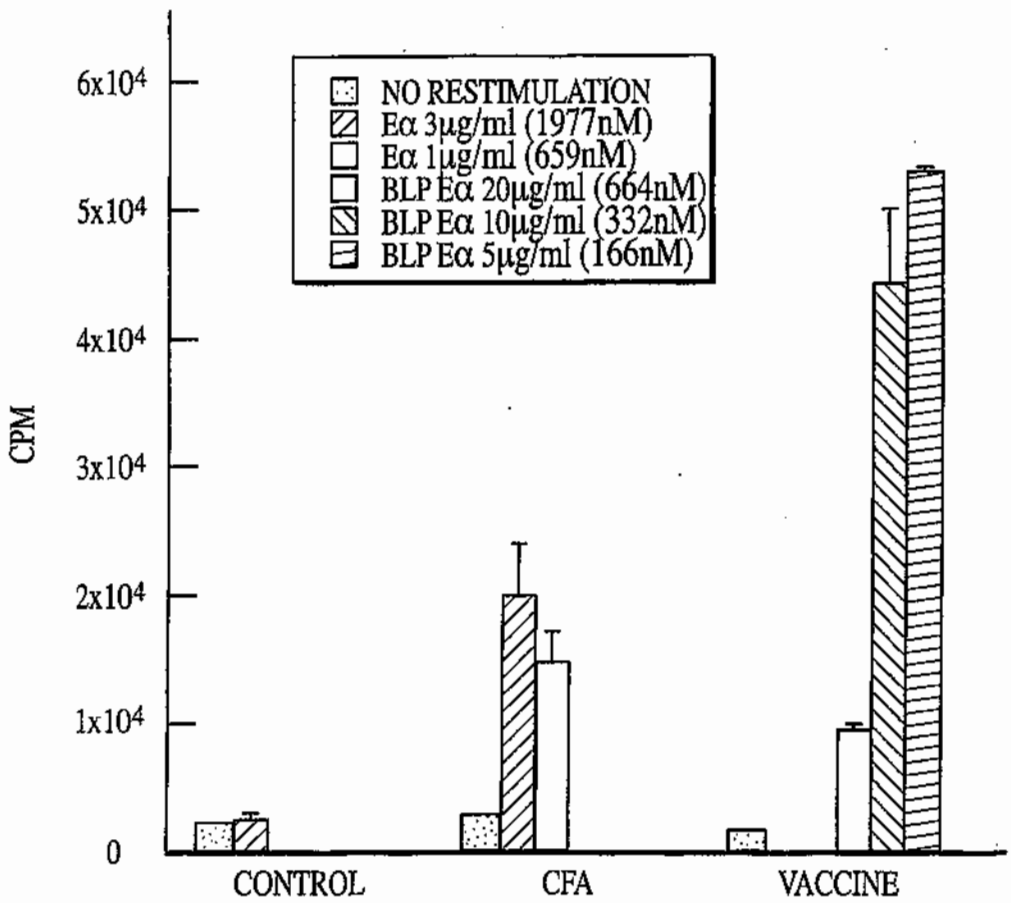

NO RESTIMULATION
Eα 3μg/ml (1977nM)
Eα 1μg/ml (659nM)
BLP Eα 20μg/ml (664nM)
BLP Eα 10μg/ml (332nM)
BLP Eα 5μg/ml (166nM)
CPM
6x10⁴
5x10⁴
4x10⁴
3x10⁴
2x10⁴
1x10⁴
0
CONTROL
CFA
VACCINE

FIG. 8

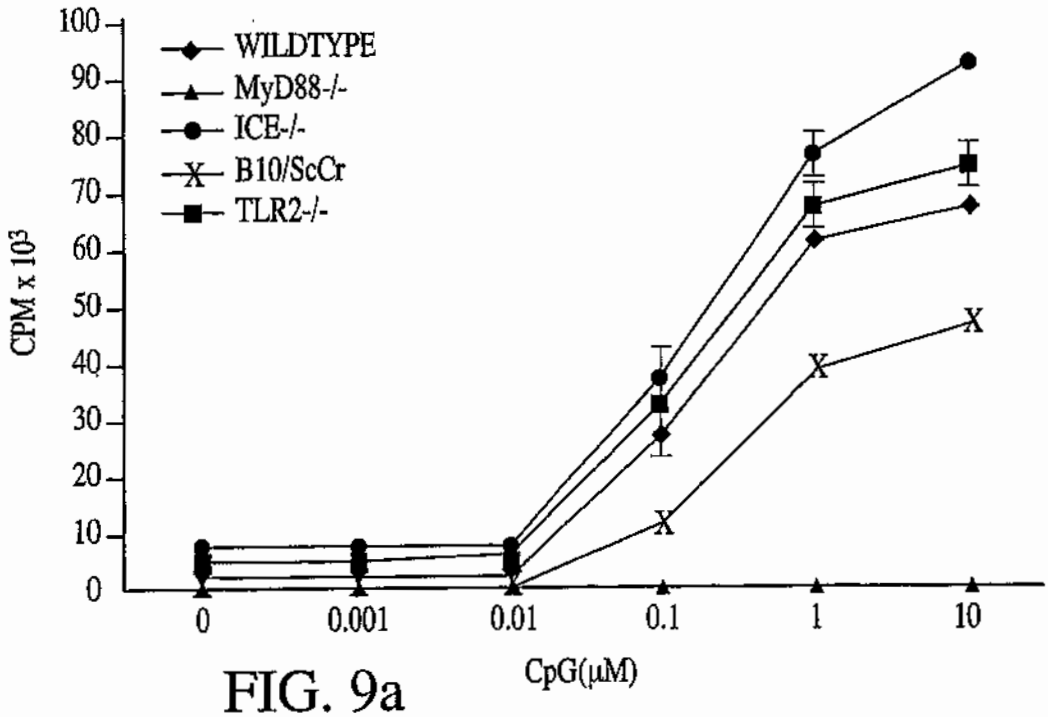

WILDTYPE
MyD88-/-
ICE-/-
B10/ScCr
TLR2-/-
CPM x 10³
100
90
80
70
60
50
40
30
20
10
0
0
0.001
0.01
0.1
1
10
CpG(μM)

FIG. 9a

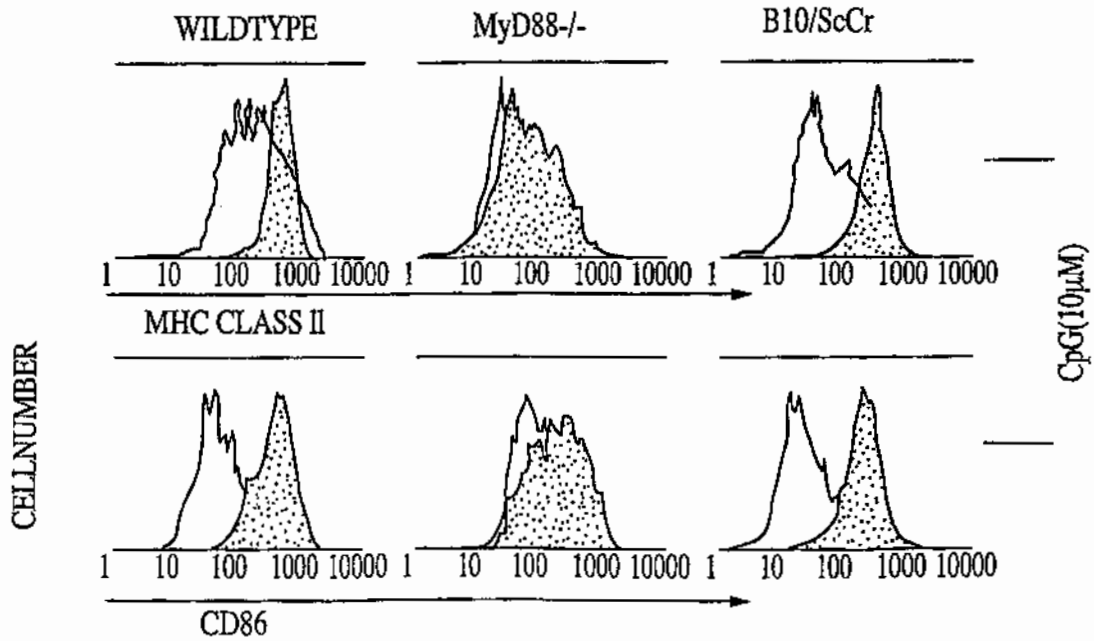

WILDTYPE
MyD88-/-
B10/ScCr
1 10 100 1000 10000
MHC CLASS II
CELLNUMBER
CD86
CpG(10μM)

FIG. 9b

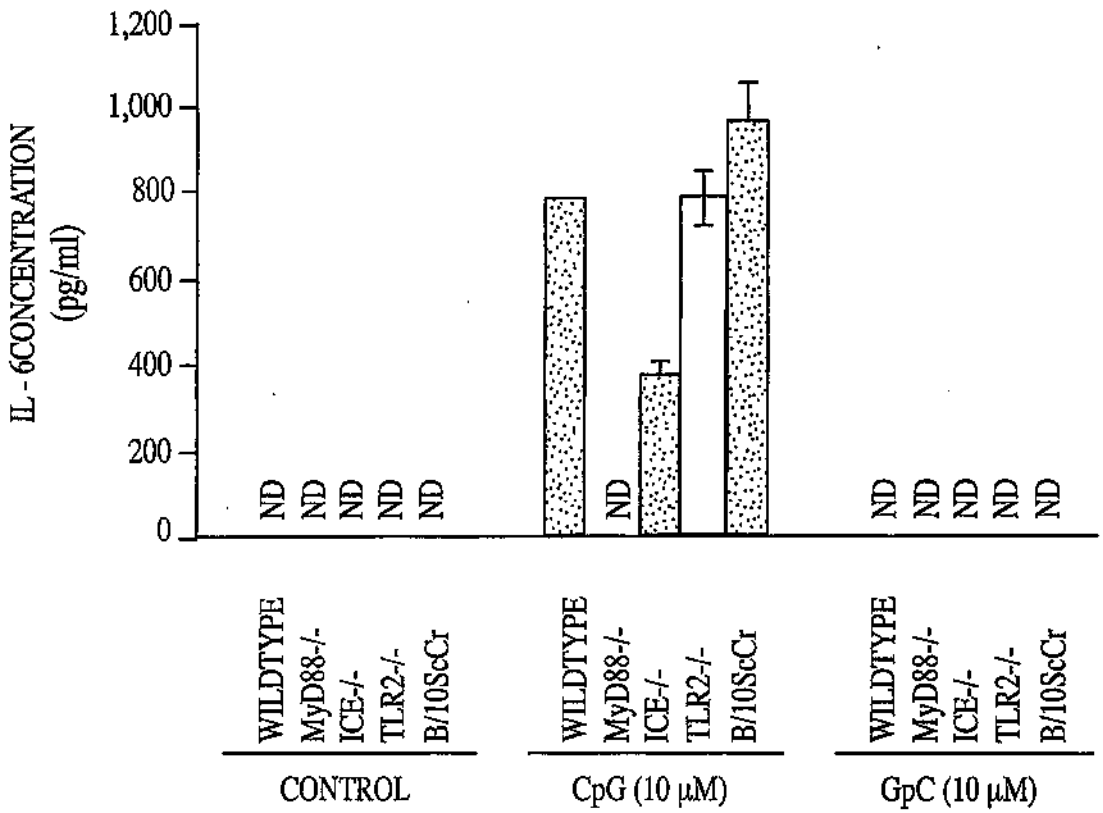


FIG. 10a

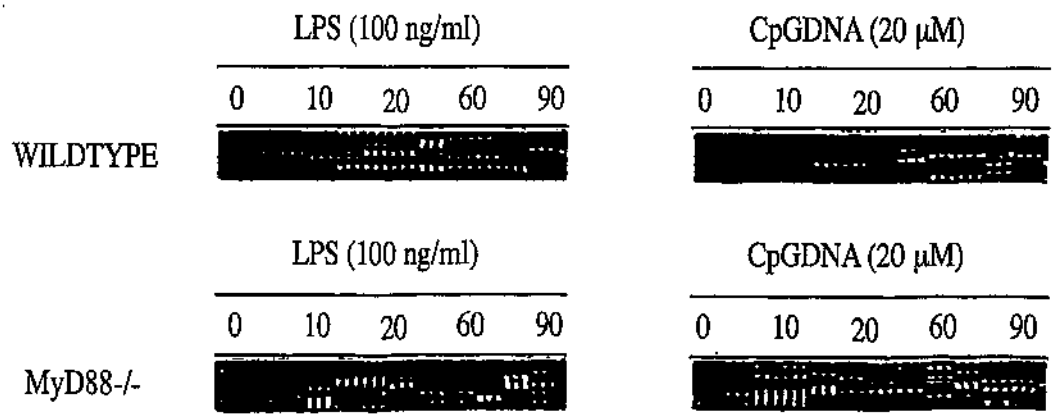


FIG. 10b

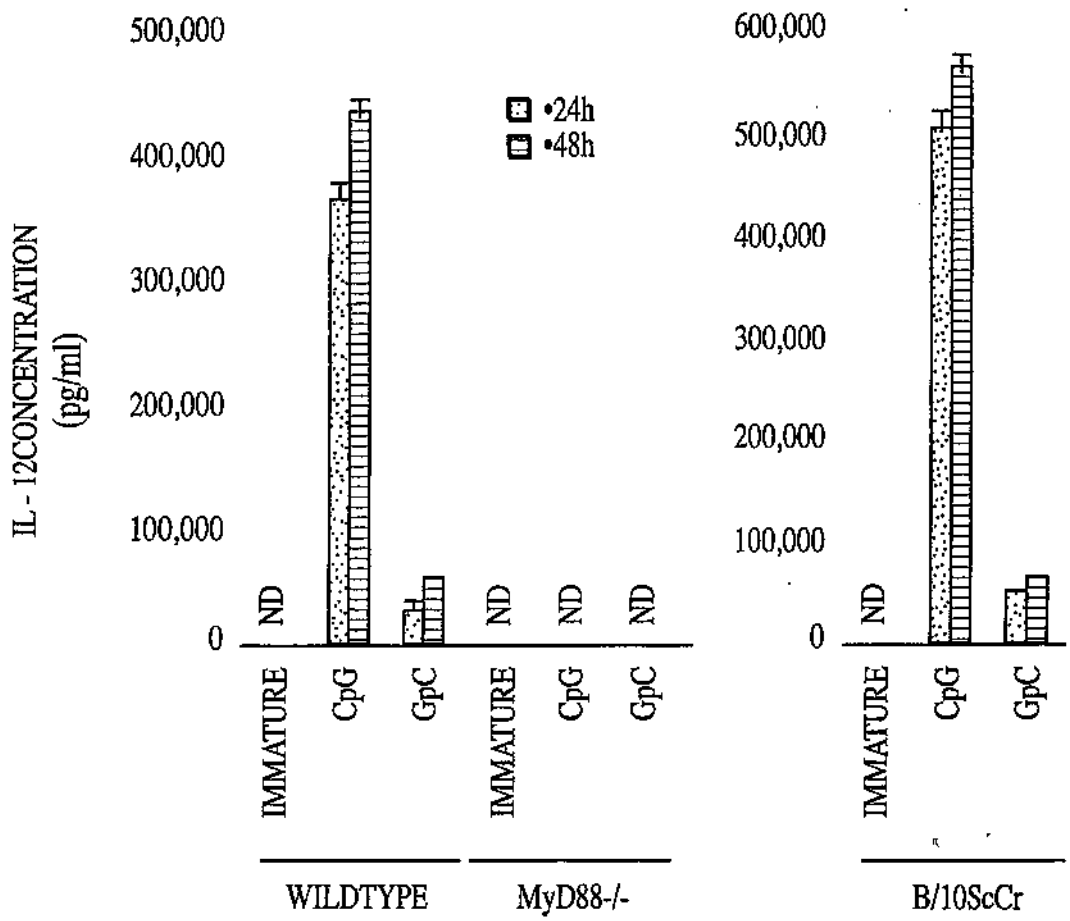

IL - 12CONCENTRATION (pg/ml)
500,000
400,000
300,000
200,000
100,000
0
•24h
•48h
ND
IMMATURE
CpG
GpC
WILDTYPE
MyD88-/-
600,000
B/10ScCr

FIG. 11

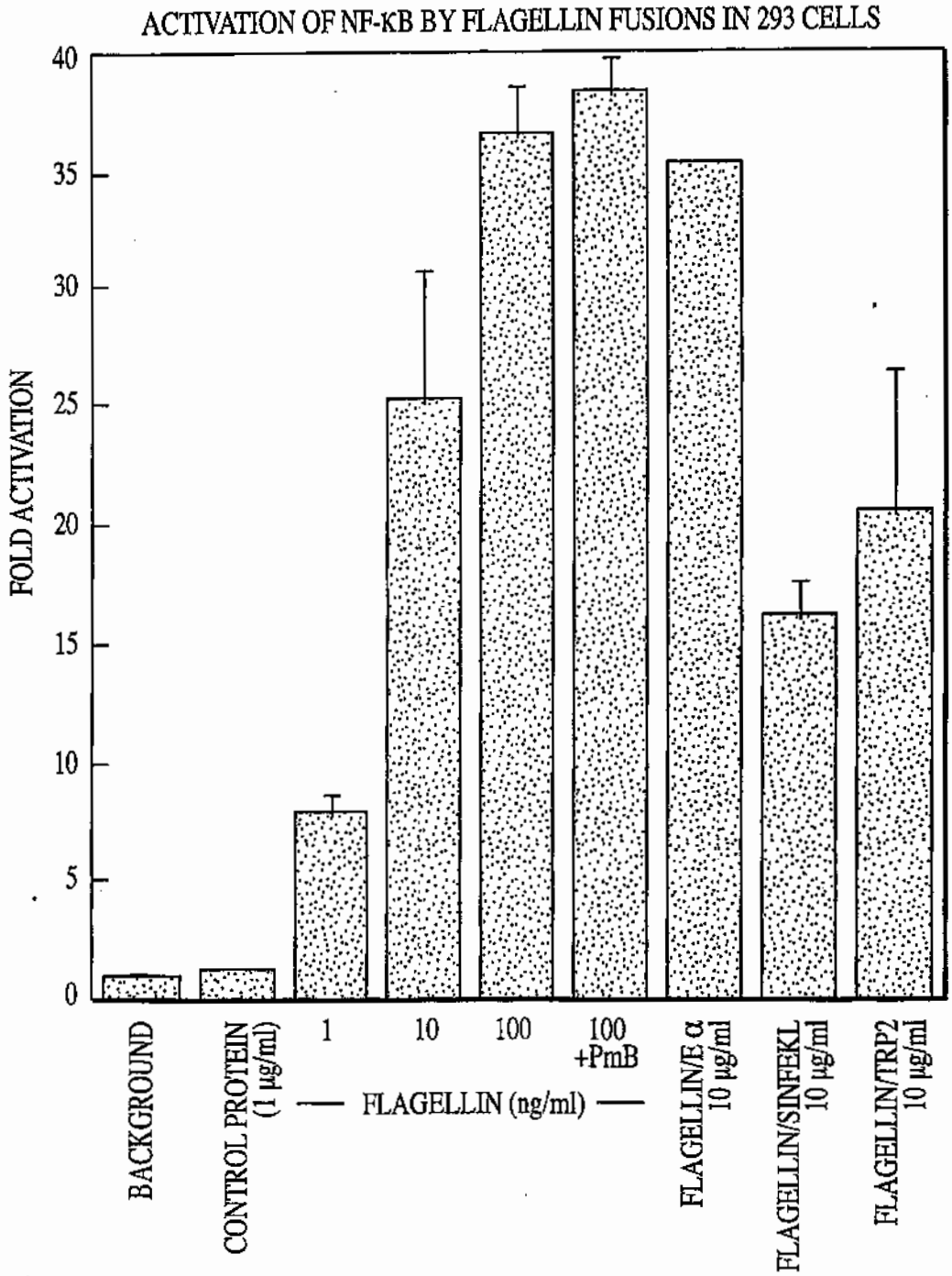

ACTIVATION OF NF-κB BY FLAGELLIN FUSIONS IN 293 CELLS
FOLD ACTIVATION
0
5
10
15
20
25
30
35
40
BACKGROUND
CONTROL PROTEIN (1 μg/ml)
1
10
100
100 +PmB
FLAGELLIN (ng/ml)
FLAGELLIN/E α 10 μg/ml
FLAGELLIN/SINFEKL 10 μg/ml
FLAGELLIN/TRP2 10 μg/ml

FIG. 12

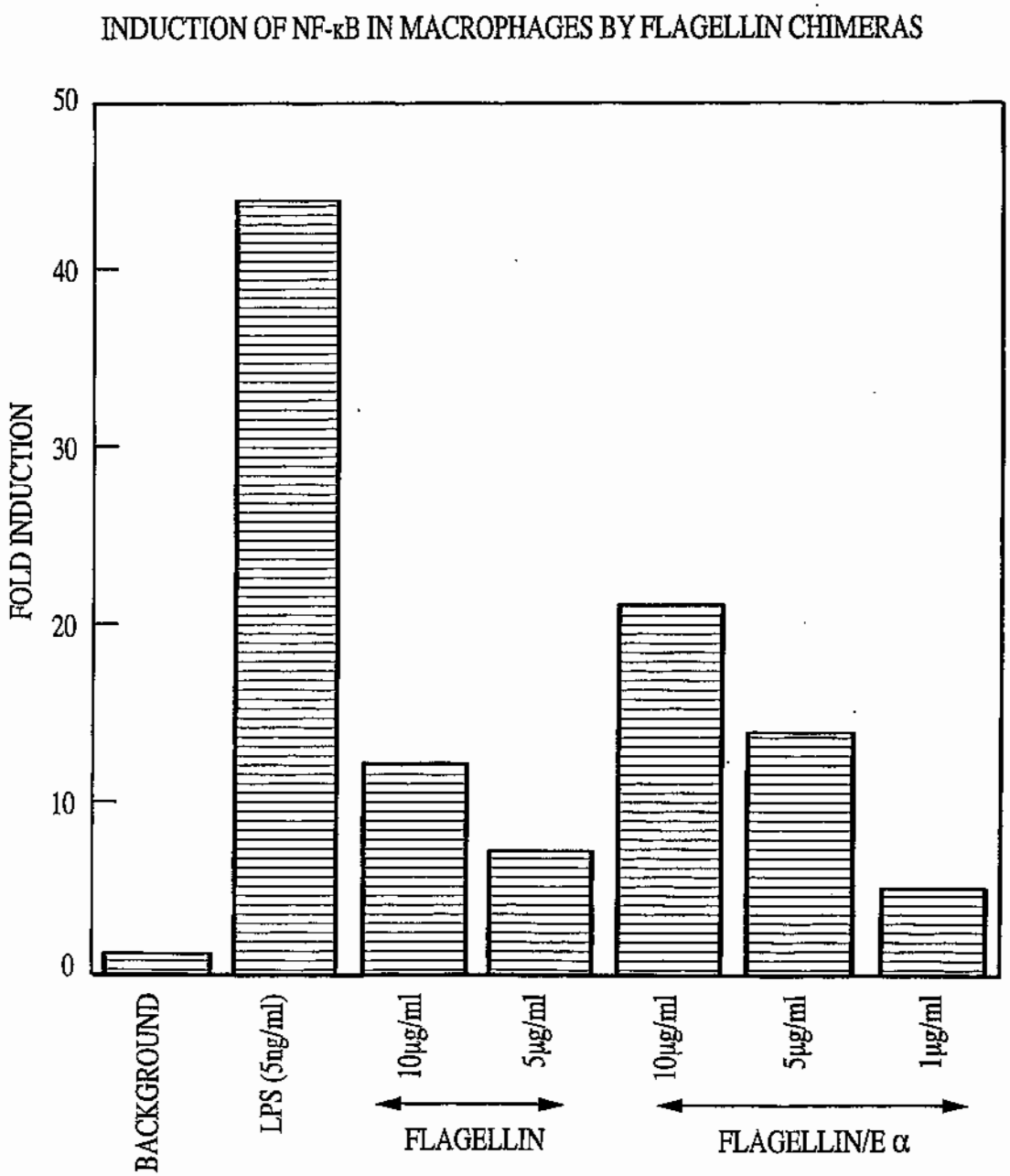

INDUCTION OF NF-κB IN MACROPHAGES BY FLAGELLIN CHIMERAS
FOLD INDUCTION
50
40
30
20
10
0
BACKGROUND
LPS (5ng/ml)
10μg/ml
5μg/ml
FLAGELLIN
10μg/ml
5μg/ml
1μg/ml
FLAGELLIN/E α

FIG. 13

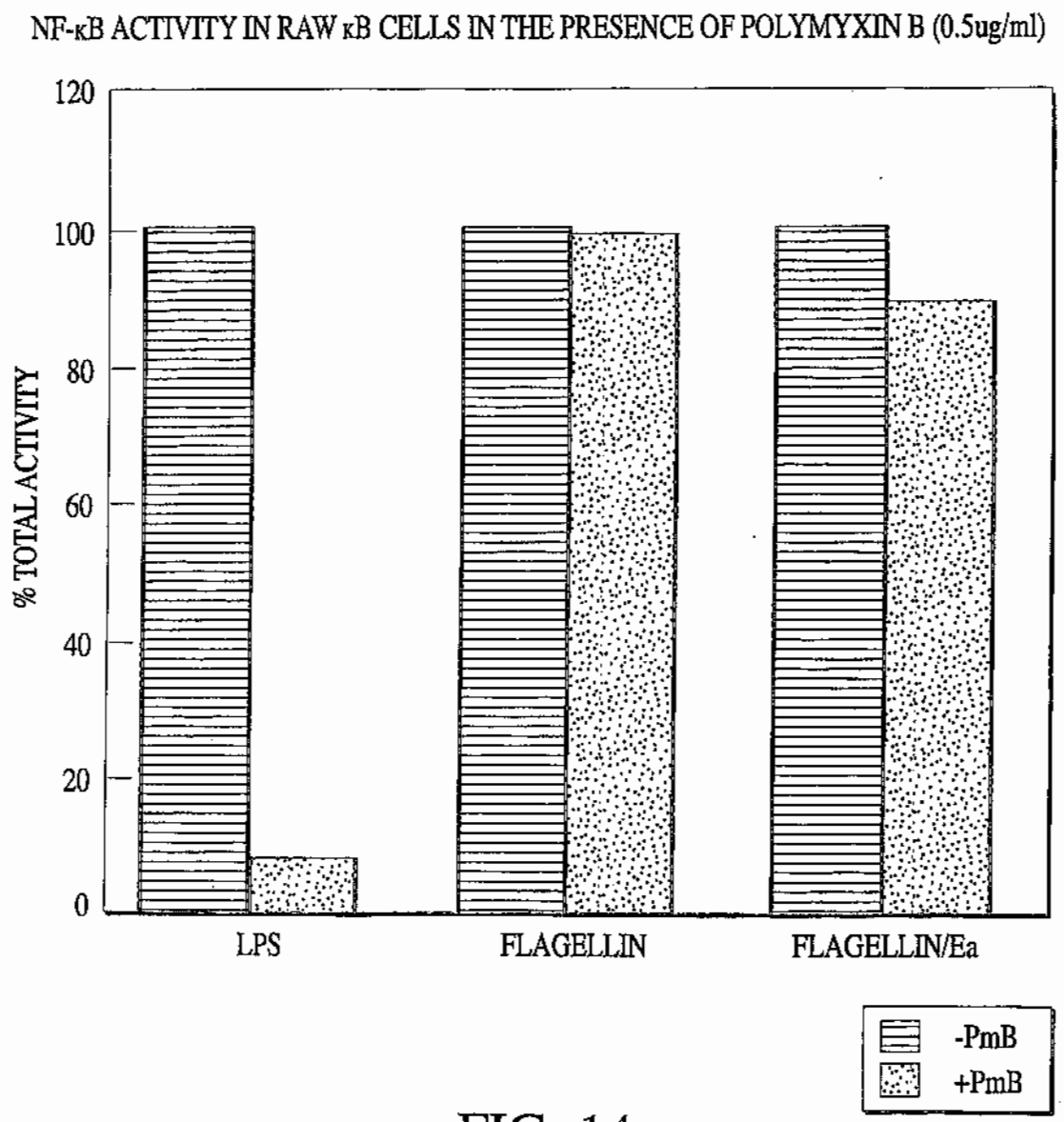

NF-κB ACTIVITY IN RAW κB CELLS IN THE PRESENCE OF POLYMYXIN B (0.5ug/ml)
% TOTAL ACTIVITY
120
100
80
60
40
20
0
LPS
FLAGELLIN
FLAGELLIN/Ea
-PmB
+PmB

FIG. 14

FIG. 15

```
MET LYS ALA THR LYS LEU VAL LEU GLY ALA
1               5                   10

VAL ILE LEU GLY SER THR LEU LEU ALA GLY
                15                  20
```

FIG. 16

```
MET ASN ARG THR LYS LEU VAL LEU GLY ALA
1               5                   10

VAL ILE LEU GLY SER THR LEU LEU ALA GLY
                15                  20
```

FIG. 17

MET ASN ARG THR LYS LEU VAL LEU GLY ALA
1 5 10

VAL ILE LEU GLY SER HIS SER ALA GLY
15

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FIG. 18

```
MET LYS ALA LYS ILE VAL LEU GLY ALA VAL
1               5                   10

ILE LEU ALA SER GLY LEU LEU ALA GLY
                15
```

FIG. 19

```
MET LYS LYS TYR LEU LEU GLY ILE GLY LEU
1               5                   10
ILE LEU ALA LEU ILE ALA
                15
```

FIG. 20A

GCAGCATAAT AAGCAGTATG GCTGGAGCAC TCTGTAAATT 40
AACTCAATTA GACAGAGCCT GATTTAACAA GGAAGACTGG 80
CGAGAAGCTC CCCTCATTAA ACCTGATGTT AGAGGAGCTT 120
CGGATGAAAT TAAATCAGTG TTAGTTGTTT GAGTCACATA 160
AAATTGCATG AGCGTGTACA CATGTGCACA CGTGTAGGCT 200
CTGTGATTTA GGTGGGAATT TTGAGAGGAG AGGAAAGGGC 240
TAGAACTAAA CCCAAAGAAA AGGAAAGAAG AGAAGAGGAA 280
AGGAAAGAAA AAAGAAAAGG CAATTTGAGT GAGTAAAGGT 320
TCCAGAACTC AGGAGTGGAA GACAAGGAGT AAAGTCAGAC 360
AGAAACCAGG TGGGACGCCG GCCAGGCCTC CCAATTAAGA 400
AGGCATGGGC CTTGTGGGAT GGGGGCTTCT GCTGGGTTGT 440
CTGGGCTGCG GAATTCTGCT CAGAGCTCGG GCTCAGTTTC 480
CCCGAGTCTG CATGACCTTG GATGGCGTGC TGAACAAGGA 520
ATGCTGCCCG CCTCTGGGTC CCGAGGCAAC CAACATCTGT 560
GGATTTCTAG AGGGCAGGGG GCAGTGCGCA GAGGTGCAAA 600
CAGACACCAG ACCCTGGAGT GGCCCTTATA TCCTTCGAAA 640
CCAGGATGAC CGTGAGCAAT GGCCGAGAAA ATTCTTCAAC 680
CGGACATGCA AATGCACAGG AAACTTTGCT GGTTATAATT 720
GTGGAGGCTG CAAGTTCGGC TGGACCGGCC CCGACTGTAA 760
TCGGAAGAAG CCGGCCATCC TAAGACGGAA TATCCATTCC 800
CTGACTGCCC AGGAGAGGGA GCAGTTCTTG GGCGCCTTAG 840
ACCTGGCCAA GAAGAGTATC CATCCAGACT ACGTGATCAC 880
CACGCAACAC TGGCTGGGGC TGCTCGGACC CAACGGGACC 920
CAGCCCCAGA TCGCCAACTG CAGCGTGTAT GACTTTTTTG 960
TGTGGCTCCA TTATTATTCT GTTCGAGACA CATTATTAGG 1000
TCCAGGACGC CCCTATAAGG CCATTGATTT CTCTCACCAA 1040
GGGCCTGCCT TTGTCACGTG GCACAGGTAC CATCTGTTGT 1080
GGCTGGAAAG AGAACTCCAG AGACTCACTG GCAATGAGTC 1120
CTTTGCGTTG CCCTACTGGA ACTTTGCAAC CGGGAAGAAC 1160
GAGTGTGACG TGTGCACAGA CGACTGGCTT GGAGCAGCAA 1200
GACAAGATGA CCCAACGCTG ATTAGTCGGA ACTCGAGATT 1240
CTCTACCTGG GAGATTGTGT GCGACAGCTT GGATGACTAC 1280
AACCGCCGGG TCACACTGTG TAATGGAACC TATGAAGGTT 1320
TGCTGAGAAG AAACAAAGTA GGCAGAAATA ATGAGAAACT 1360
GCCAACCTTA AAAAATGTGC AAGATTGCCT GTCTCTCCAG 1400
AAGTTTGACA GCCCTCCCTT CTTCCAGAAC TCTACCTTCA 1440
GCTTCAGGAA TGCACTGGAA GGGTTTGATA AAGCAGACGG 1480
AACACTGGAC TCTCAAGTCA TGAACCTTCA TAACTTGGCT 1520
CACTCCTTCC TGAATGGGAC CAATGCCTTG CCACACTCAG 1560

FIG. 20B

```
CAGCCAACGA CCCTGTGTTT GTGGTCCTCC ACTCTTTTAC 1600
AGACGCCATC TTTGATGAGT GGCTGAAGAG AAACAACCCT 1640
TCCACAGATG CCTGGCCTCA GGAACTGGCA CCCATTGGTC 1680
ACAACCGAAT GTATAACATG GTCCCCTTCT TCCCACCGGT 1720
GACTAATGAG GAGCTCTTCC TAACCGCAGA GCAACTTGGC 1760
TACAATTACG CCGTTGATCT GTCAGAGGAA GAAGCTCCAG 1800
TTTGGTCCAC AACTCTCTCA GTGGTCATTG GAATCCTGGG 1840
AGCTTTCGTC TTGCTCTTGG GGTTGCTGGC TTTTCTTCAA 1880
TACAGAAGGC TTCGCAAAGG CTATGCGCCC TTAATGGAGA 1920
CAGGTCTCAG CAGCAAGAGA TACACGGAGG AAGCCTAGCA 1960
TGCTCCTACC TGGCCTGACC TGGGTAGTAA CTAATTACAC 2000
CGTCGCTCAT CTTGAGACAG GTGGAACTCT TCAGCGTGTG 2040
CTCTTTAGTA GTGATGATGA TGATGCCTTA GCAATGACAA 2080
TTATCTCTAG TTGCTGCTTT GCTTATTGTA CACAGACAAA 2120
ATGCTTGGGT CATTCACCAC GGTCAAAGTA AGGTGTGGCT 2160
AGTATATGTG ACCTTTGATT AG                    2182
```

FIG. 21A

```
MET GLY LEU VAL GLY TRP GLY LEU LEU LEU
1               5                  10

GLY CYS LEU GLY CYS GLY ILE LEU LEU ARG
                15                 20

ALA ARG ALA GLN PHE PRO ARG VAL CYS MET
                25                 30

THR LEU ASP GLY VAL LEU ASN LYS GLU CYS
                35                 40

CYS PRO PRO LEU GLY PRO GLU ALA THR ASN
                45                 50

ILE CYS GLY PHE LEU GLU GLY ARG GLY GLN
                55                 60

CYS ALA GLU VAL GLN THR ASP THR ARG PRO
                65                 70

TRP SER GLY PRO TYR ILE LEU ARG ASN GLN
                75                 80

ASP ASP ARG GLU GLN TRP PRO ARG LYS PHE
                85                 90

PHE ASN ARG THR CYS LYS CYS THR GLY ASN
                95                 100

PHE ALA GLY TYR ASN CYS GLY GLY CYS LYS
                105                110

PHE GLY TRP THR GLY PRO ASP CYS ASN ARG
                115                120

LYS LYS PRO ALA ILE LEU ARG ARG ASN ILE
                125                130
```

FIG. 21B

```
HIS SER LEU THR ALA GLN GLU ARG GLU GLN
                135                 140

PHE LEU GLY ALA LEU ASP LEU ALA LYS LYS
                145                 150

SER ILE HIS PRO ASP TYR VAL ILE THR THR
                155                 160

GLN HIS TRP LEU GLY LEU LEU GLY PRO ASN
                165                 170

GLY THR GLN PRO GLN ILE ALA ASN CYS SER
                175                 180

VAL TYR ASP PHE PHE VAL TRP LEU HIS TYR
                185                 190

TYR SER VAL ARG ASP THR LEU LEU GLY PRO
                195                 200

GLY ARG PRO TYR LYS ALA ILE ASP PHE SER
                205                 210

HIS GLN GLY PRO ALA PHE VAL THR TRP HIS
                215                 220

ARG TYR HIS LEU LEU TRP LEU GLU ARG GLU
                225                 230

LEU GLN ARG LEU THR GLY ASN GLU SER PHE
                235                 240

ALA LEU PRO TYR TRP ASN PHE ALA THR GLY
                245                 250

LYS ASN GLU CYS ASP VAL CYS THR ASP ASP
                255                 260
```

FIG. 21C

```
TRP LEU GLY ALA ALA ARG GLN ASP ASP PRO
                265                 270

THR LEU ILE SER ARG ASN SER ARG PHE SER
                275                 280

THR TRP GLU ILE VAL CYS ASP SER LEU ASP
                285                 290

ASP TYR ASN ARG ARG VAL THR LEU CYS ASN
                295                 300

GLY THR THR GLU GLY LEU LEU ARG ARG ASN
                305                 310

LYS VAL GLY ARG ASN ASN GLU LYS LEU PRO
                315                 320

THR LEU LYS ASN VAL GLN ASP CYS LEU SER
                325                 330

LEU GLN LYS PHE ASP SER PRO PRO PHE PHE
                335                 340

GLN ASN SER THR PHE SER PHE ARG ASN ALA
                345                 350

LEU GLU GLY PHE ASP LYS ALA ASP GLY THR
                355                 360

LEU ASP SER GLN VAL MET ASN LEU HIS ASN
                365                 370

LEU ALA HIS SER PHE LEU ASN GLY THR ASN
                375                 380

ALA LEU PRO HIS SER ALA ALA ASN ASP PRO
                385                 390
```

FIG. 21D

```
VAL PHE VAL VAL LEU HIS SER PHE THR ASP
                395                 400

ALA ILE PHE ASP GLU TRP LEU LYS ARG ASN
                405                 410

ASN PRO SER THR ASP ALA TRP PRO GLN GLU
                415                 420

LEU ALA PRO ILE GLY HIS ASN ARG MET TYR
                425                 430

ASN MET VAL PRO PHE PHE PRO PRO VAL THR
                435                 440

ASN GLU GLU LEU PHE LEU THR ALA GLU GLN
                445                 450

LEU GLY TYR ASN TYR ALA VAL ASP LEU SER
                455                 460

GLU GLU GLU ALA PRO VAL TRP SER THR THR
                465                 470

LEU SER VAL VAL ILE GLY ILE LEU GLY ALA
                475                 480

PHE VAL LEU LEU LEU GLY LEU LEU ALA PHE
                485                 490

LEU GLN TYR ARG ARG LEU ARG LYS GLY TYR
                495                 500

ALA PRO LEU MET GLU THR GLY LEU SER SER
                505                 510

LYS ARG TYR THR GLU GLU ALA
                515
```