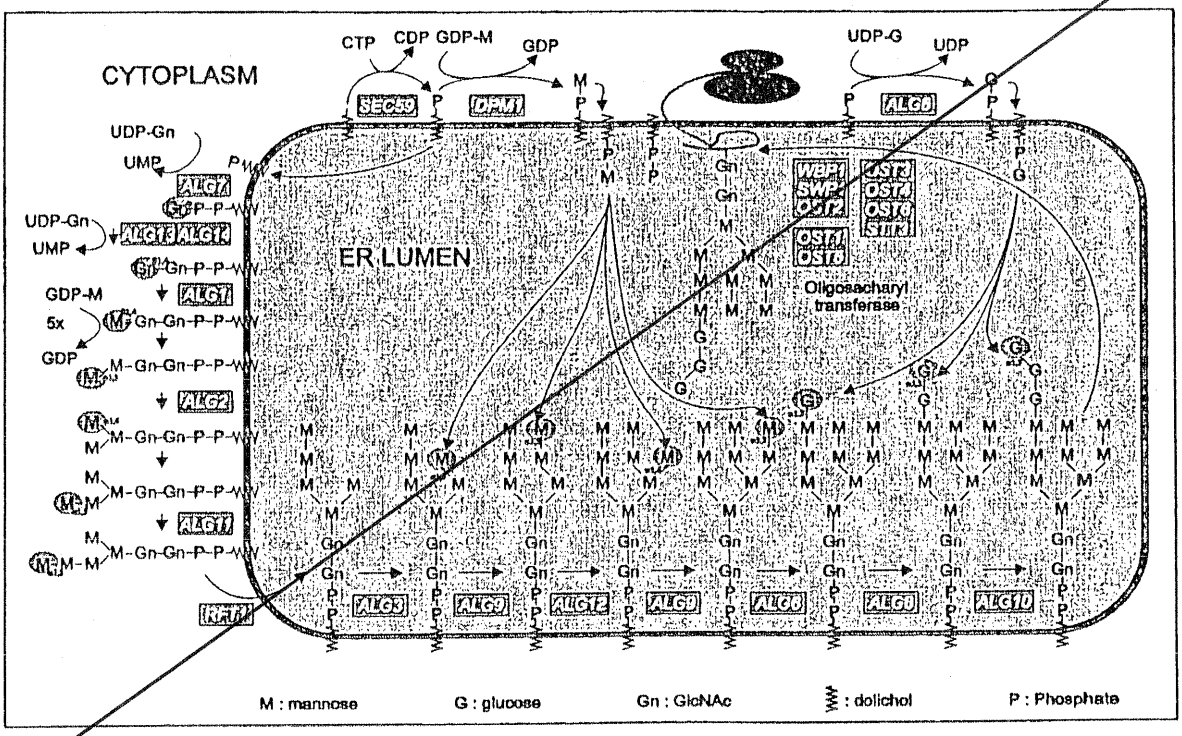

CYTOPLASM
ER LUMEN
CTP
CDP
GDP-M
GDP
UDP-G
UDP
UDP-Gn
UMP
GDP-M
5x
GDP
Oligosaccharyl transferase
M : mannose
G : glucose
Gn : GlcNAc
: dolichol
P : Phosphate

FIG. 1

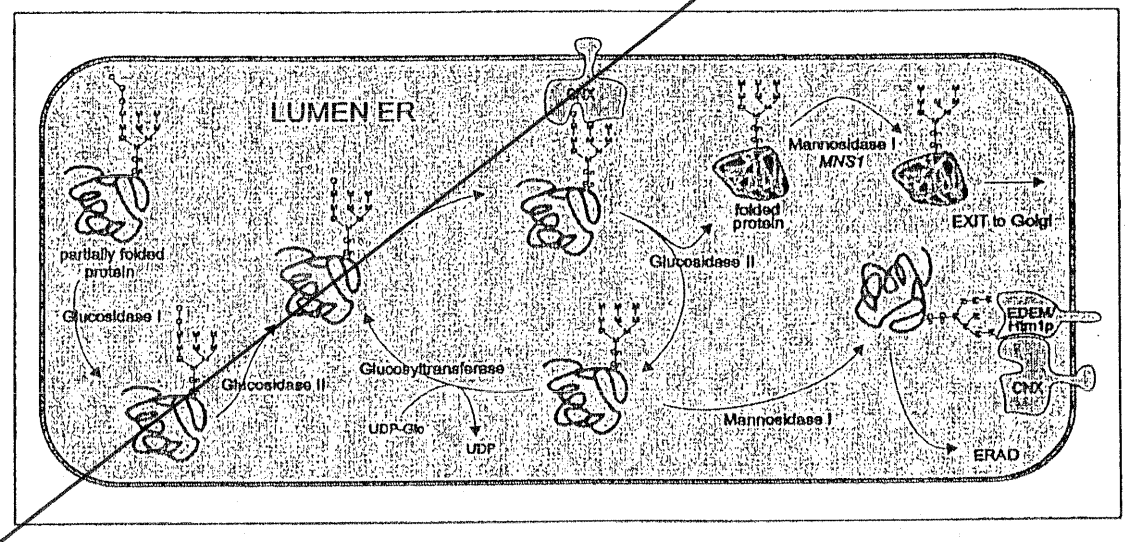

LUMEN ER
CRX
partially folded protein
Glucosidase I
Glucosidase II
Glucosyltransferase
UDP-Glc
UDP
folded protein
Mannosidase I
MNS1
EXIT to Golgi
Glucosidase II
EDEM/ Htm1p
CNX
Mannosidase I
ERAD

FIG. 2

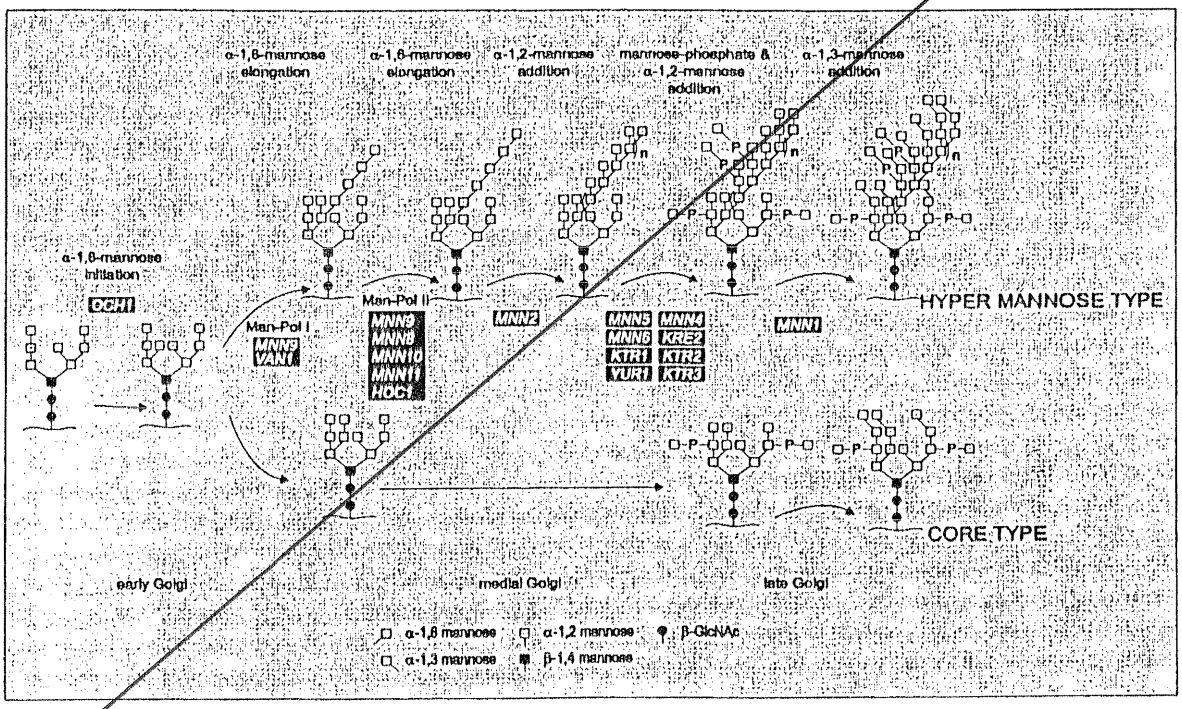

α-1,6-mannose elongation
α-1,6-mannose elongation
α-1,2-mannose addition
mannose-phosphate & α-1,2-mannose addition
α-1,3-mannose addition
α-1,6-mannose initiation
OCH1
Man-Pol I
MNN9
VAN1
Man-Pol II
MNN9
MNN8
MNN10
MNN11
HOC1
MNN2
MNN5
MNN6
KTR1
YUR1
MNN4
KRE2
KTR2
KTR3
MNN1
HYPER MANNOSE TYPE
CORE TYPE
early Golgi
medial Golgi
late Golgi
α-1,6 mannose
α-1,2 mannose
β-GlcNAc
α-1,3 mannose
β-1,4 mannose

FIG. 3

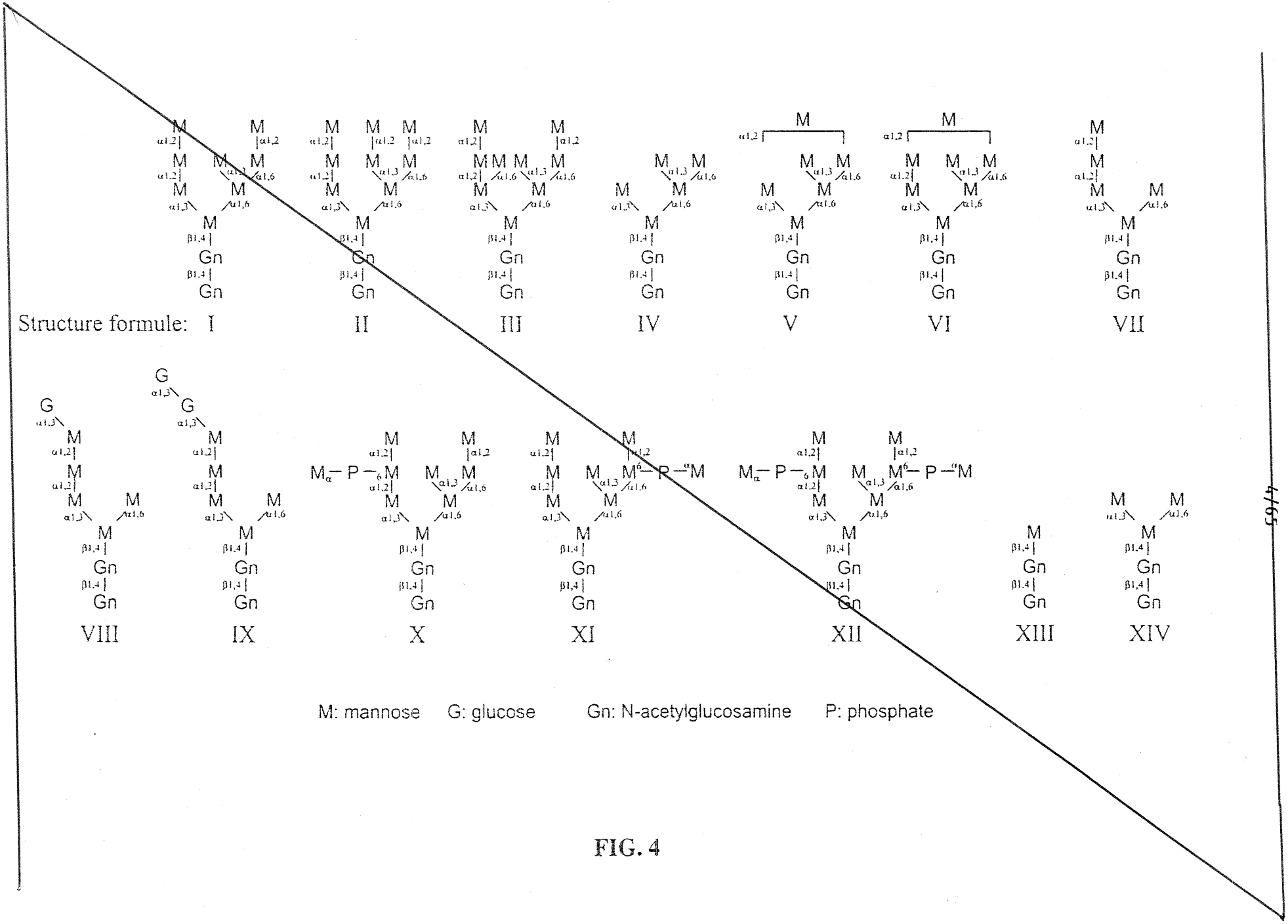

Structure formule: I II III IV V VI VII
VIII IX X XI XII XIII XIV
M: mannose G: glucose Gn: N-acetylglucosamine P: phosphate

FIG. 4

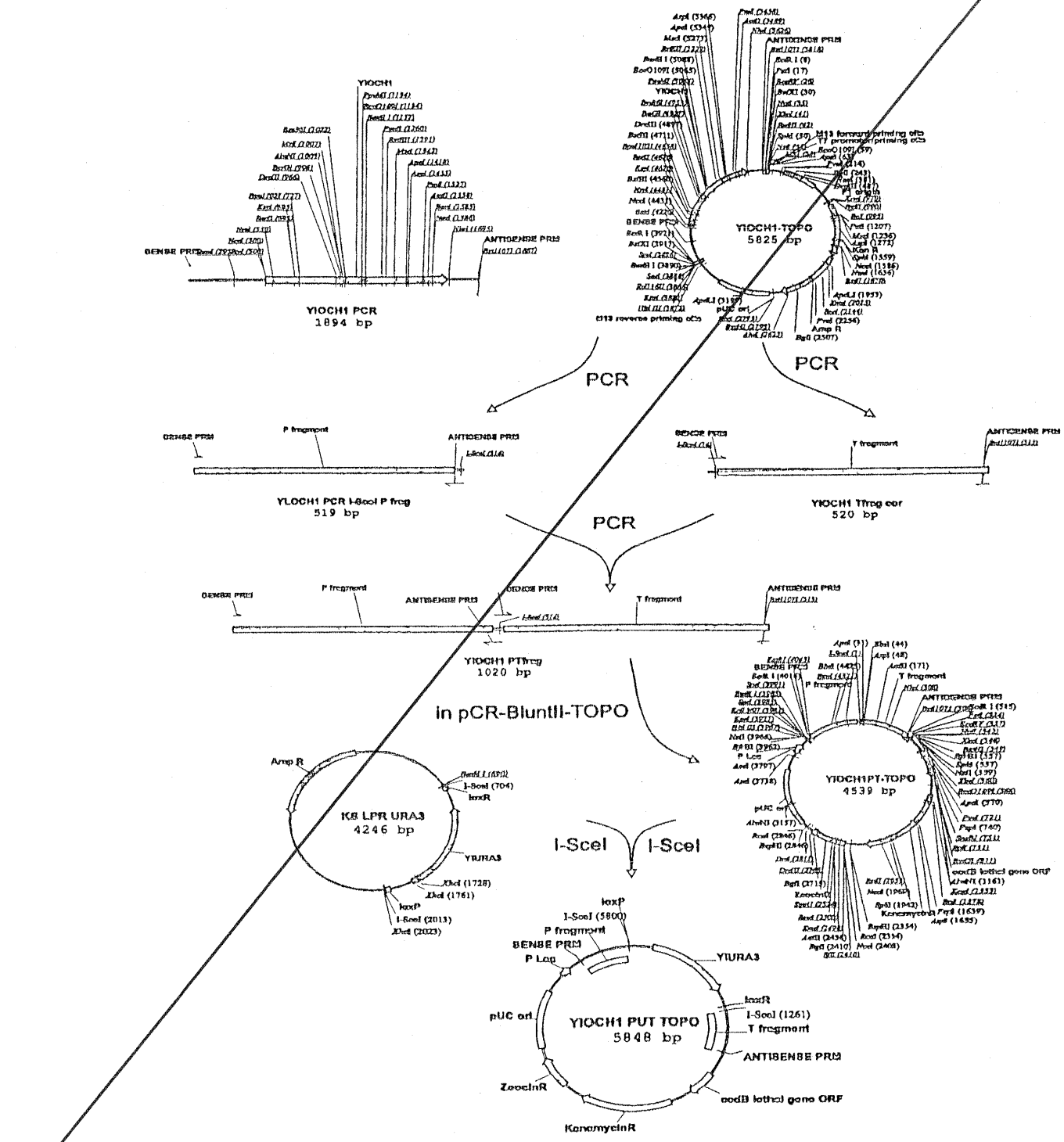

YIOCH1
YIOCH1 PCR
1894 bp
YIOCH1-TOPO
5825 bp
PCR
PCR
YLOCH1 PCR I-SceI P frag
519 bp
YIOCH1 Tfrag cor
520 bp
PCR
YIOCH1 PTfrag
1020 bp
in pCR-BluntII-TOPO
K8 LPR URA3
4246 bp
YIOCH1PT-TOPO
4539 bp
I-SceI
I-SceI
YIOCH1 PUT TOPO
5848 bp
YIURA3
loxR
I-SceI (1261)
T fragment
ANTISENSE PRM
ZeocinR
ccdB lethal gene ORF
KanamycinR
pUC ori
BENSE PRM
P Leu
P fragment
loxP
I-SceI (5800)

FIG. 5

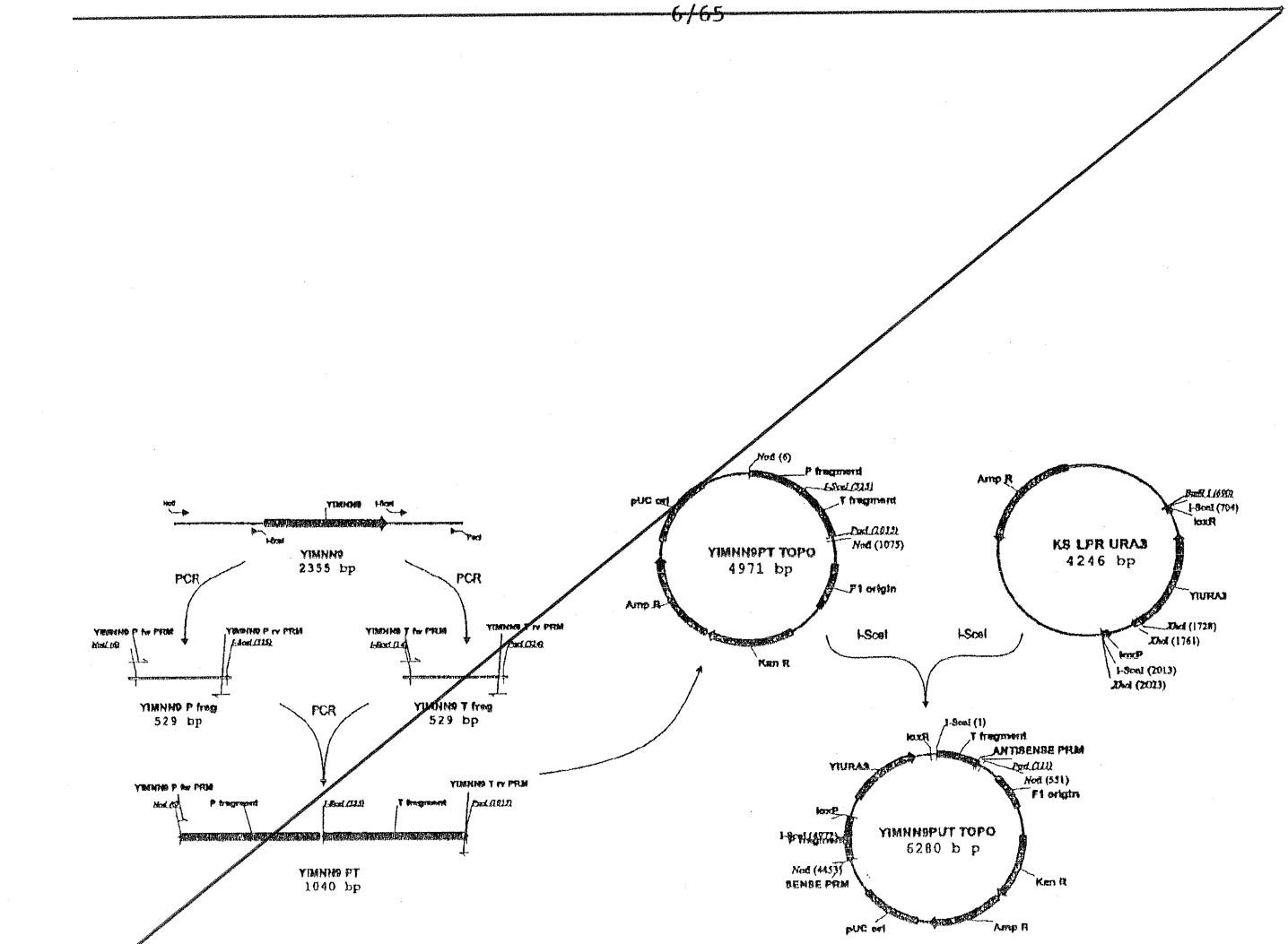

YIMNN9
2355 bp
PCR
PCR
YIMNN9 P frag
529 bp
YIMNN9 T frag
529 bp
PCR
YIMNN9 FT
1040 bp
YIMNN9PT TOPO
4971 bp
pUC ori
Amp R
Kan R
P fragment
T fragment
F1 origin
I-SceI
I-SceI
KS LFR URA3
4246 bp
YIURA3
loxR
loxP
YIMNN9PUT TOPO
6280 b p
ANTISENSE PRM
SENSE PRM
F1 origin

FIG. 6

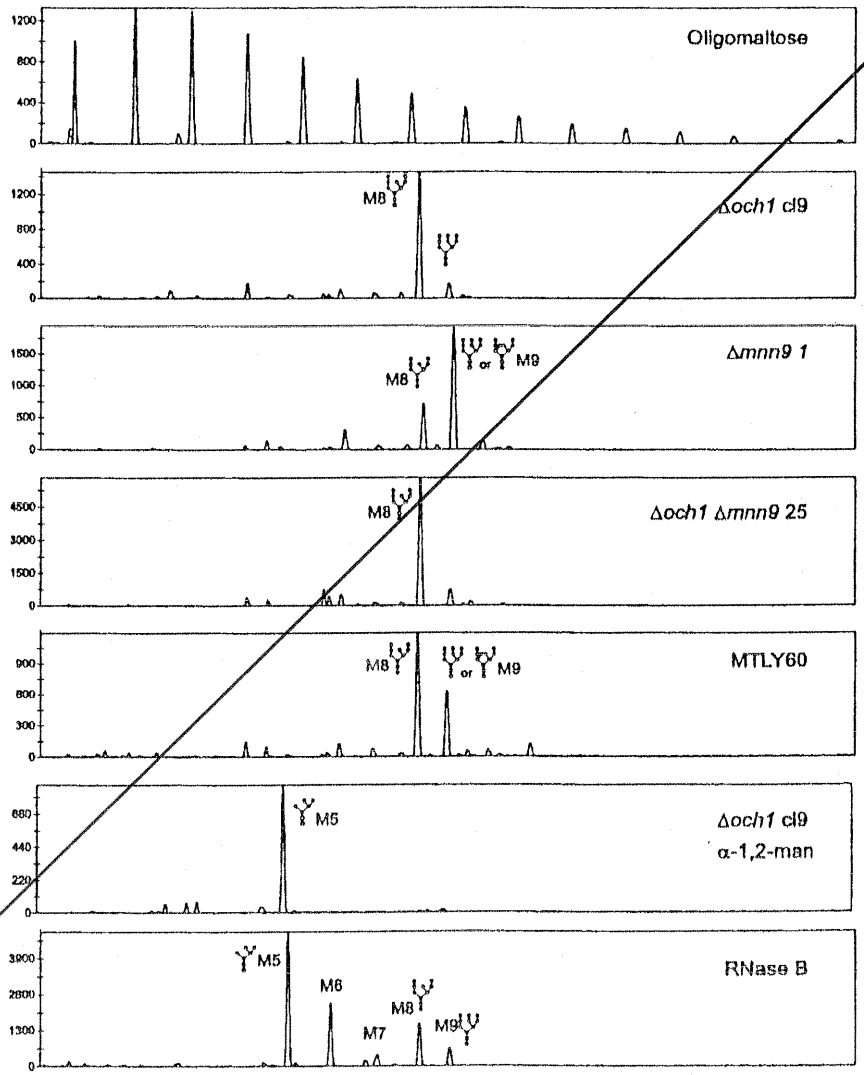

Oligomaltose
Δoch1 cl9
M8
Δmnn9 1
M8
or
M9
Δoch1 Δmnn9 25
M8
MTLY60
M8
or
M9
Δoch1 cl9
α-1,2-man
M5
RNase B
M5
M6
M8
M7
M9

FIG. 7

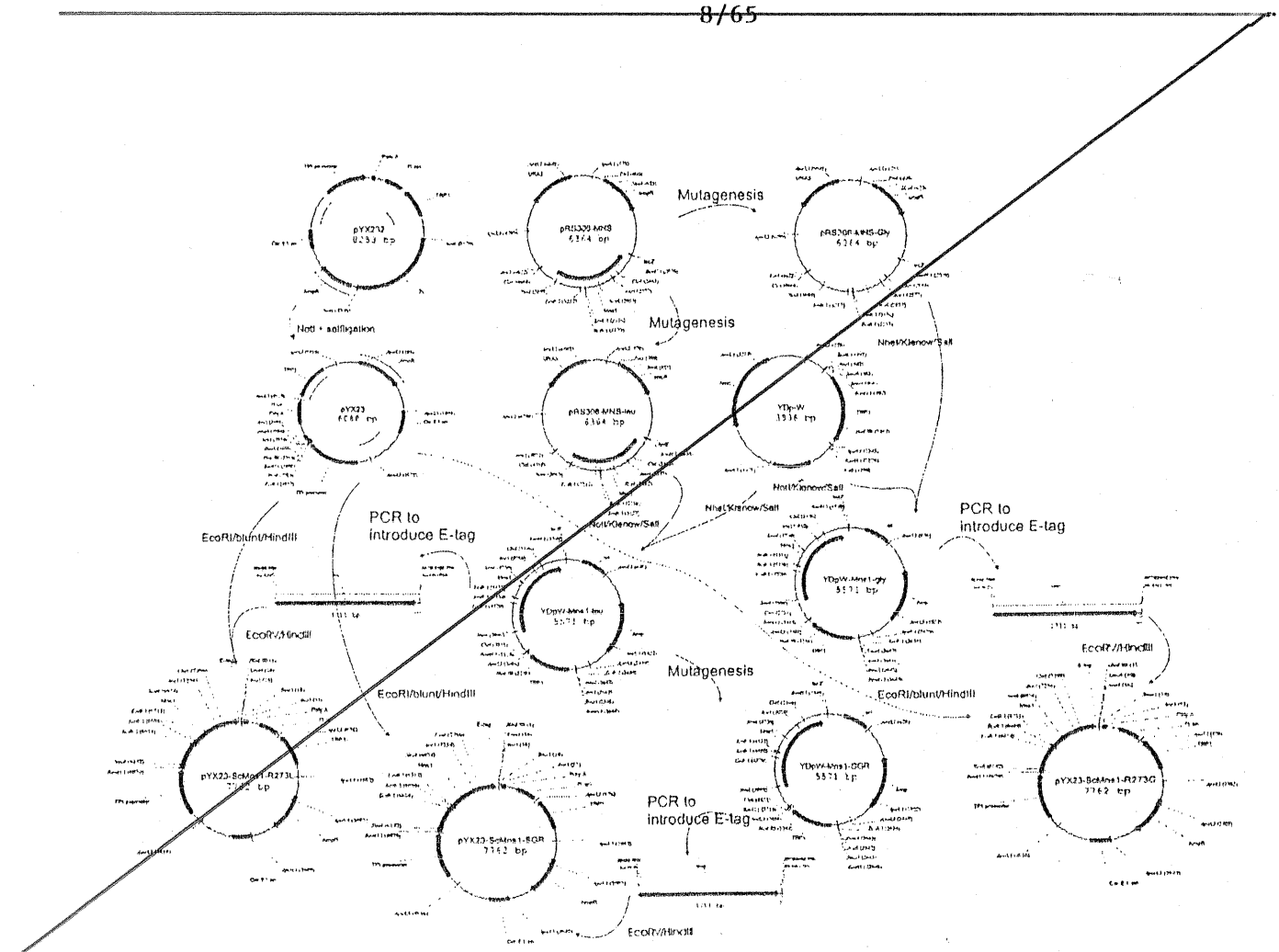

Mutagenesis
Mutagenesis
Not I + selfligation
NheI/Klenow/SalI
NheI/Klenow/SalI
NotI/Klenow/SalI
NheI/Klenow/SalI
PCR to introduce E-tag
EcoRI/blunt/HindIII
EcoRI/HindIII
EcoRI/blunt/HindIII
Mutagenesis
EcoRI/blunt/HindIII
PCR to introduce E-tag
EcoRI/HindIII
PCR to introduce E-tag
EcoRI/HindIII

FIG. 8

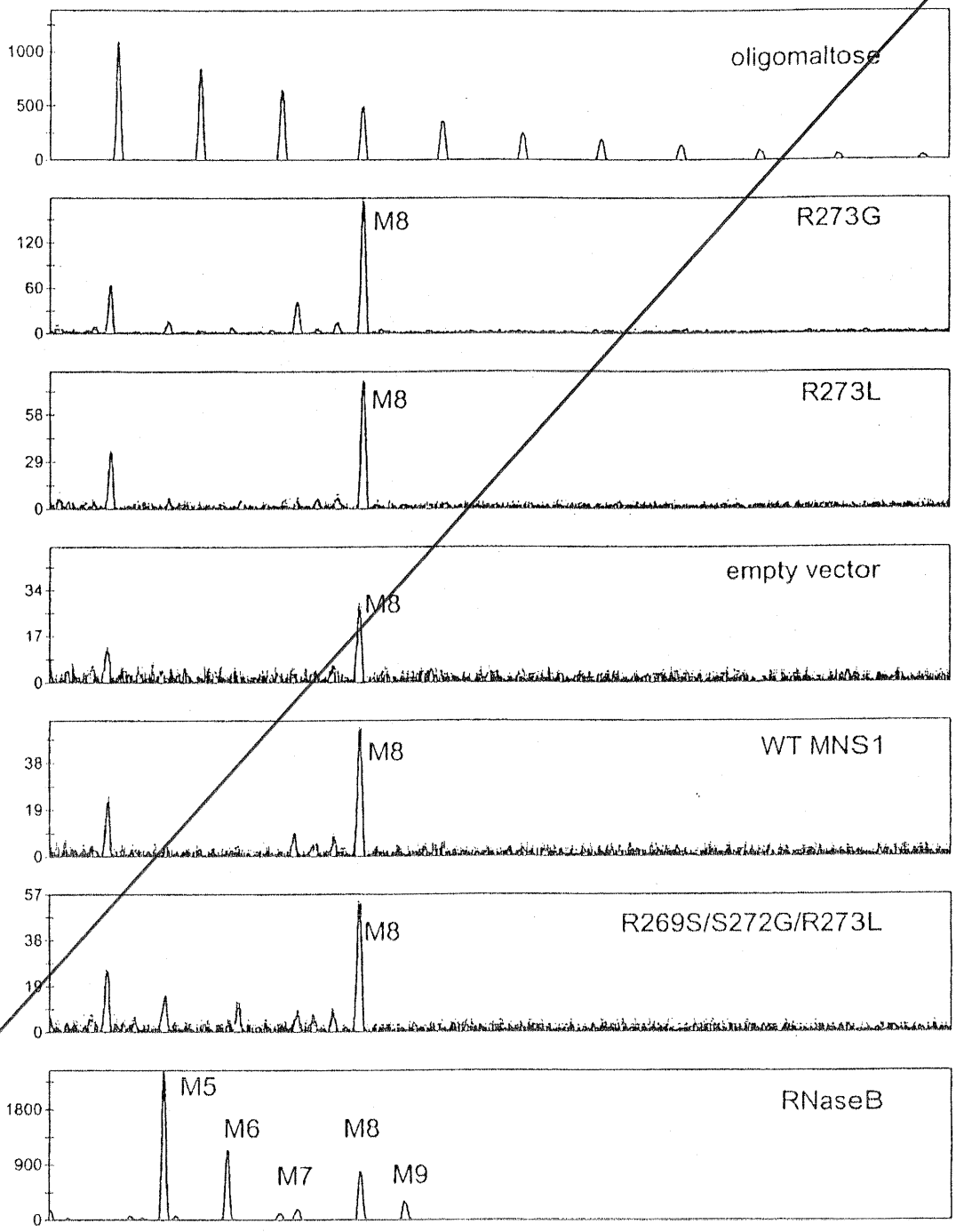

oligomaltose
1000
500
0
M8
R273G
120
60
0
M8
R273L
58
29
0
empty vector
M8
34
17
0
M8
WT MNS1
38
19
0
M8
R269S/S272G/R273L
57
38
19
0
M5
M6
M7
M8
M9
RNaseB
1800
900
0

FIG. 9

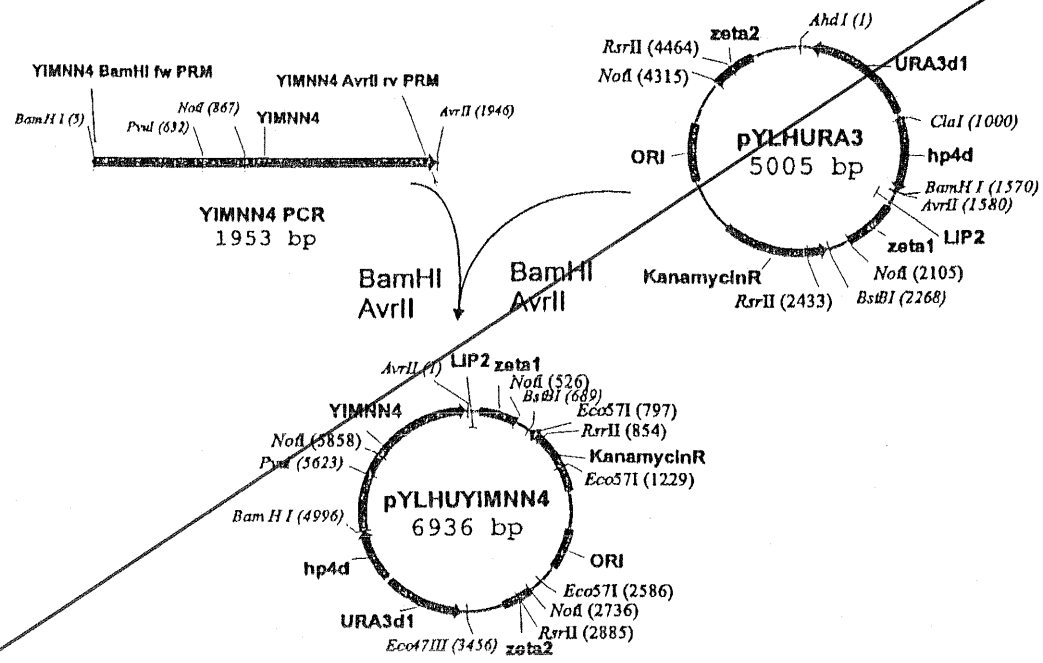

YIMNN4 BamHI fw PRM
YIMNN4 AvrII rv PRM
BamH I (5)
PvuI (632)
NotI (867)
YIMNN4
AvrII (1946)
YIMNN4 PCR
1953 bp
BamHI
AvrII
BamHI
AvrII
pYLHURA3
5005 bp
RsrII (4464)
zeta2
AhdI (1)
NotI (4315)
URA3d1
ClaI (1000)
ORI
hp4d
BamH I (1570)
AvrII (1580)
zeta1
LIP2
KanamycinR
NotI (2105)
RsrII (2433)
BstBI (2268)
pYLHUYIMNN4
6936 bp
AvrII (1)
LIP2
zeta1
NotI (526)
BstBI (669)
Eco57I (797)
RsrII (854)
KanamycinR
Eco57I (1229)
YIMNN4
NotI (5858)
PvuI (5623)
Bam H I (4996)
hp4d
ORI
Eco57I (2586)
NotI (2736)
RsrII (2885)
URA3d1
Eco47III (3456)
zeta2

FIG. 10

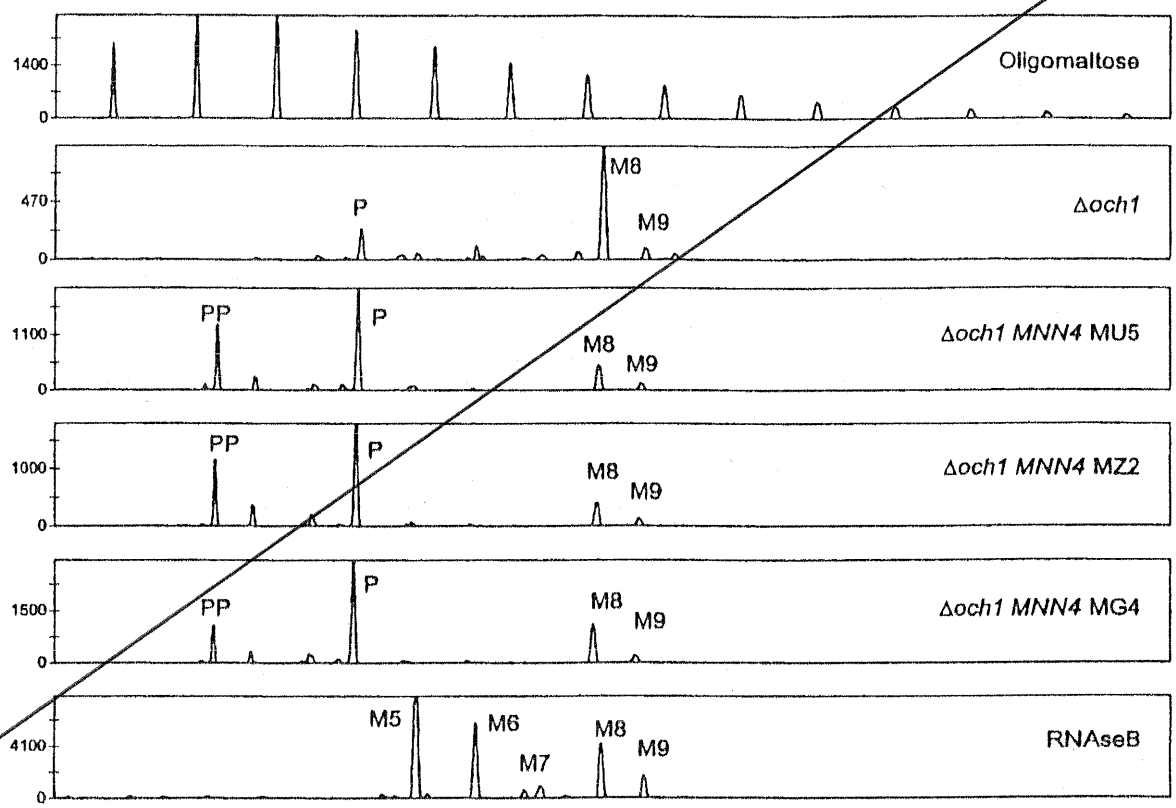

1400
0
Oligomaltose
470
0
P
M8
M9
Δoch1
1100
0
PP
P
M8
M9
Δoch1 MNN4 MU5
1000
0
PP
P
M8
M9
Δoch1 MNN4 MZ2
1500
0
PP
P
M8
M9
Δoch1 MNN4 MG4
4100
0
M5
M6
M7
M8
M9
RNAseB

FIG. 11

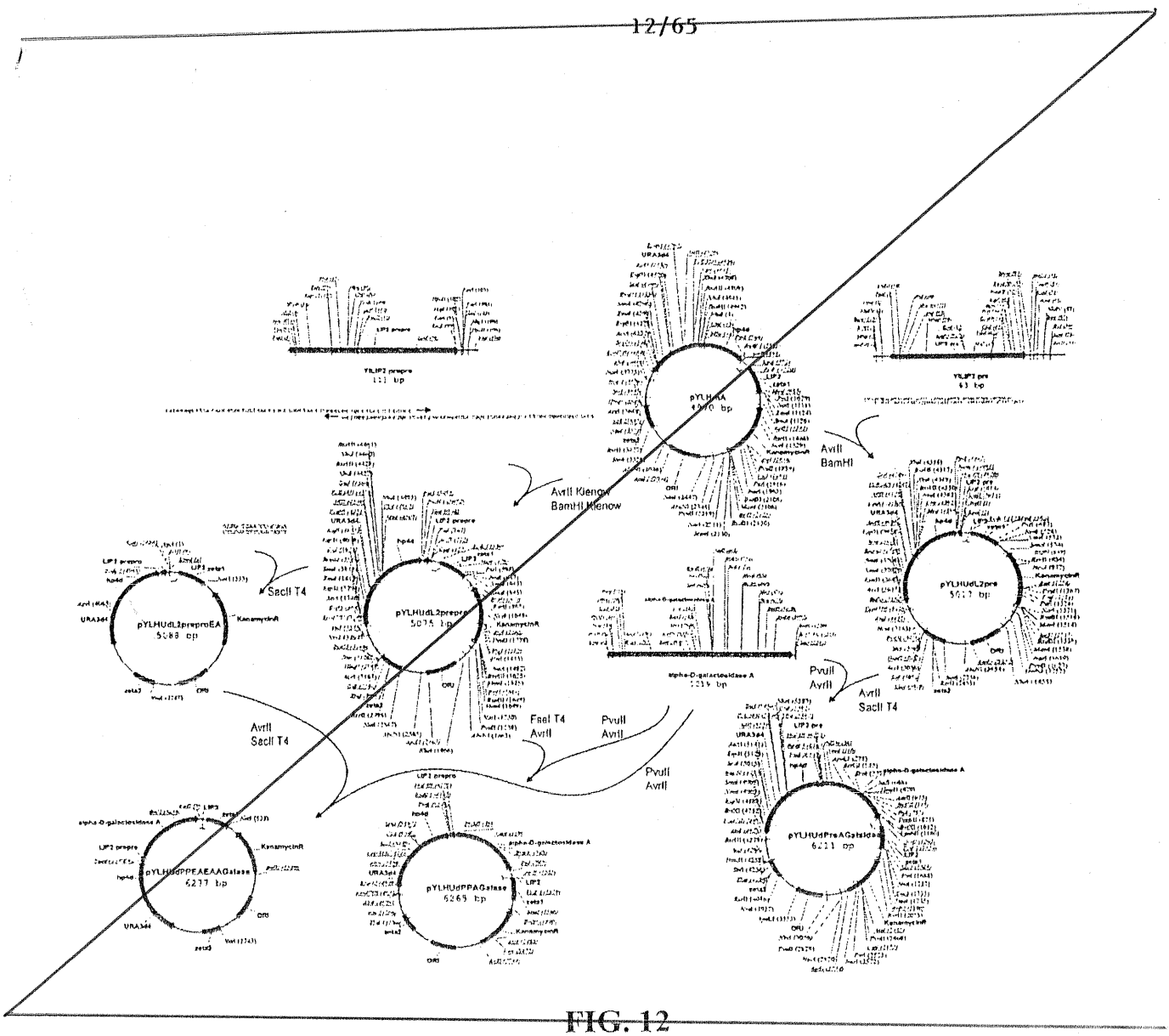

AvrII
BamHI
AvrII Klenow
BamHI Klenow
SacII T4
AvrII
SacII T4
FseI T4
AvrII
PvuII
AvrII
PvuII
AvrII
SacII T4
PvuII
AvrII

FIG. 12

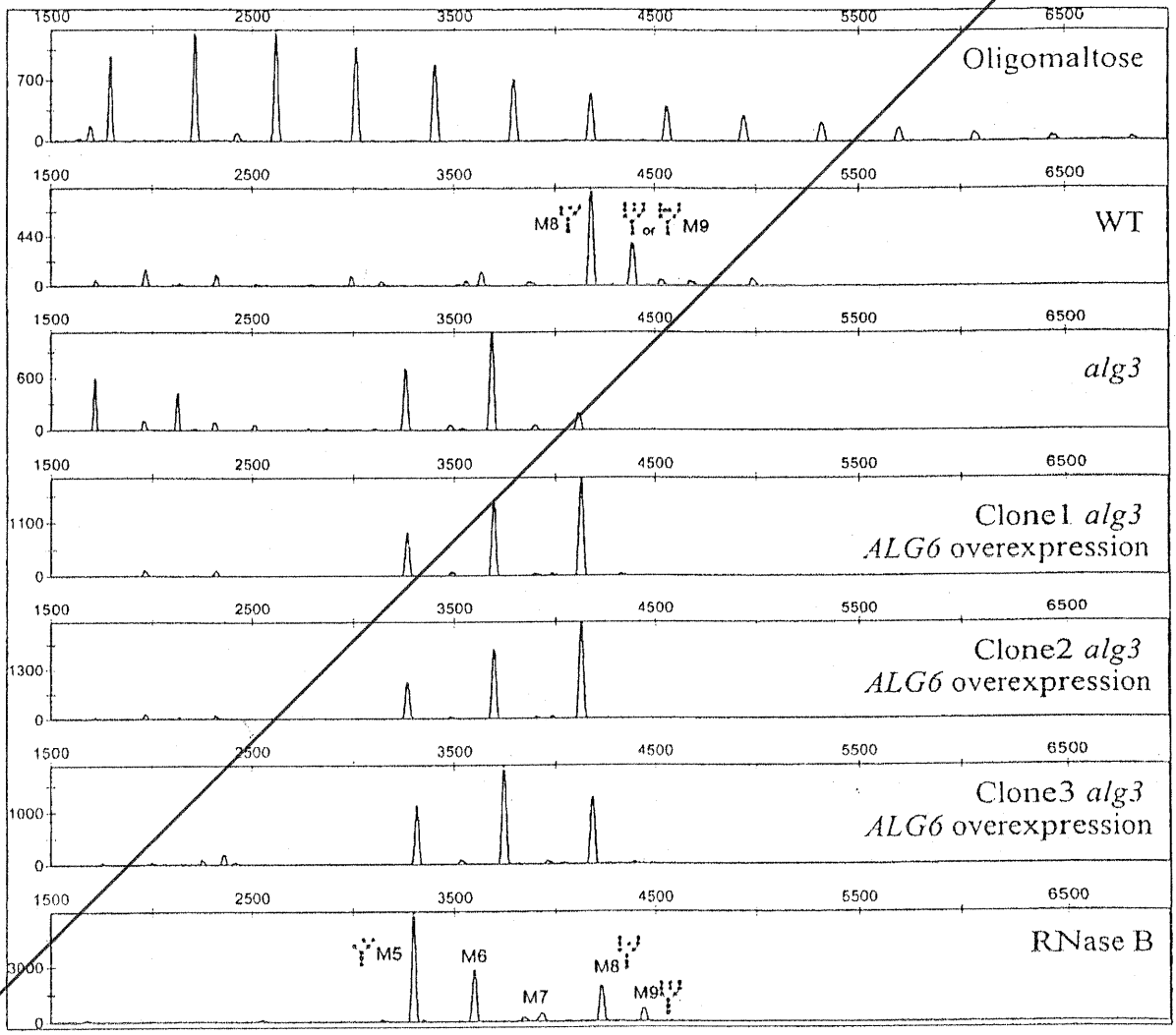

1500
2500
3500
4500
5500
6500
Oligomaltose
700
0
M8
or
M9
WT
440
alg3
600
Clone1 alg3
ALG6 overexpression
1100
Clone2 alg3
ALG6 overexpression
1300
Clone3 alg3
ALG6 overexpression
1000
M5
M6
M7
M8
M9
RNase B
3000

FIG. 13

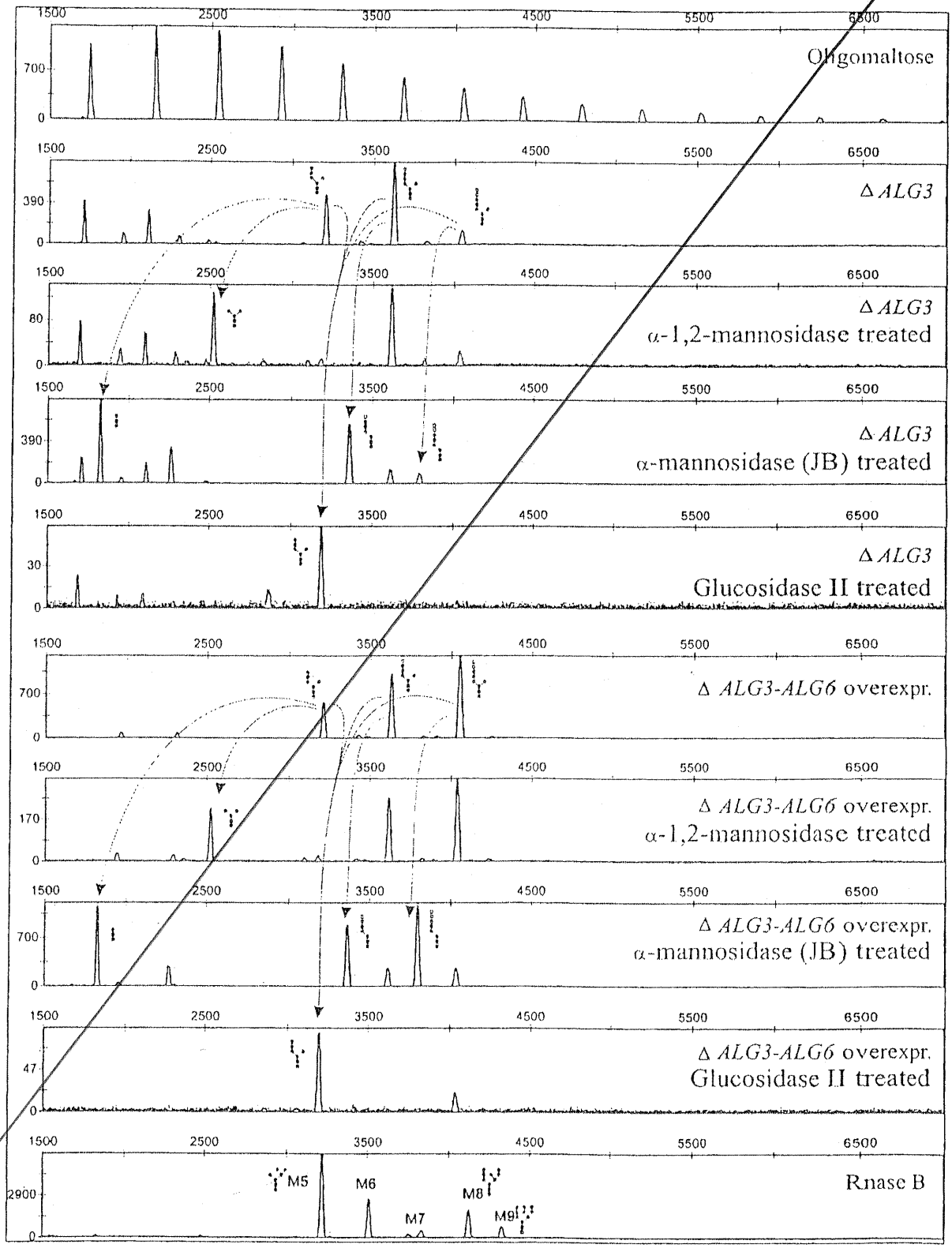

1500
2500
3500
4500
5500
6500
700
0
Oligomaltose
390
Δ ALG3
80
Δ ALG3
α-1,2-mannosidase treated
Δ ALG3
α-mannosidase (JB) treated
30
Δ ALG3
Glucosidase II treated
Δ ALG3-ALG6 overexpr.
170
Δ ALG3-ALG6 overexpr.
α-1,2-mannosidase treated
Δ ALG3-ALG6 overexpr.
α-mannosidase (JB) treated
47
Δ ALG3-ALG6 overexpr.
Glucosidase II treated
2900
M5
M6
M7
M8
M9
Rnase B

FIG. 14

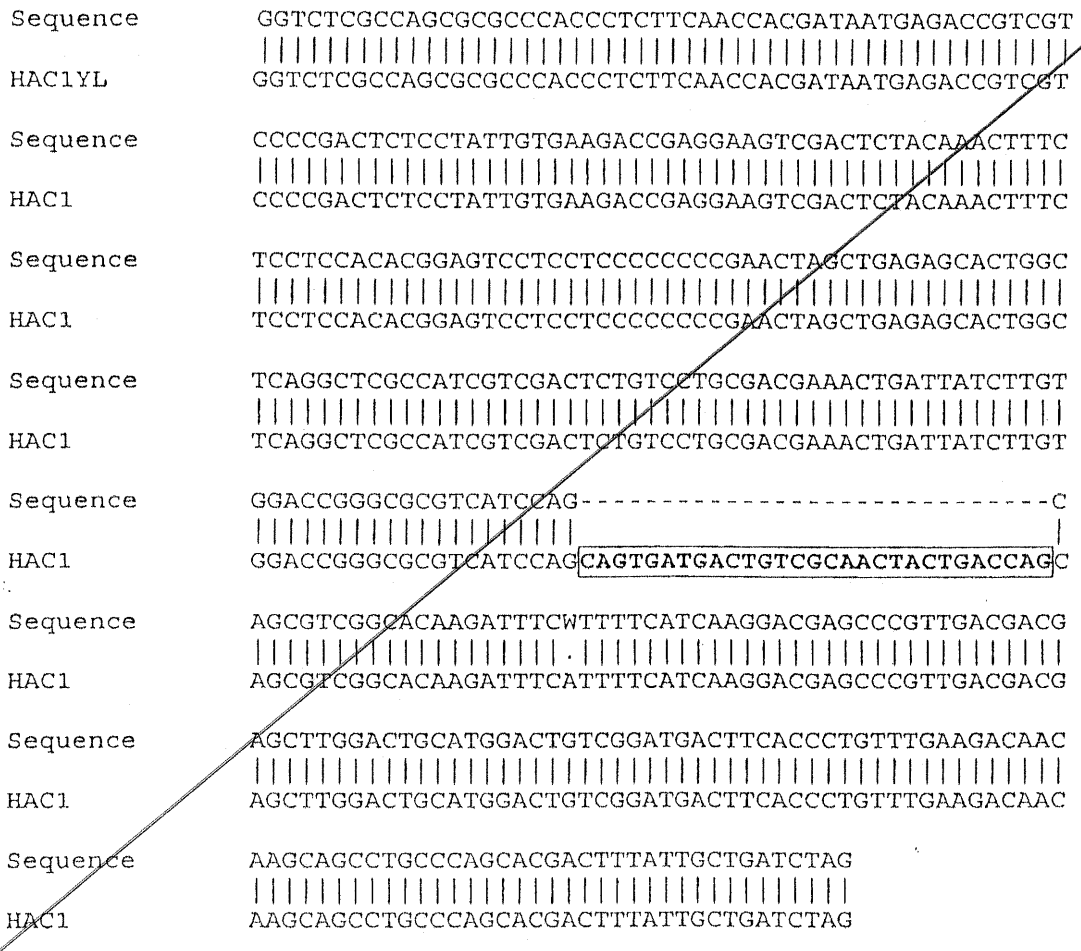

Sequence GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
HAC1YL GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
Sequence CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
HAC1 CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
Sequence TCCTCCACACGGAGTCCTCCTCCCCCCCCGAACTAGCTGAGAGCACTGGC
HAC1 TCCTCCACACGGAGTCCTCCTCCCCCCCCGAACTAGCTGAGAGCACTGGC
Sequence TCAGGCTCGCCATCGTCGACTCTGTCCTGCGACGAAACTGATTATCTTGT
HAC1 TCAGGCTCGCCATCGTCGACTCTGTCCTGCGACGAAACTGATTATCTTGT
Sequence GGACCGGGCGCGTCATCCAG----------C
HAC1 GGACCGGGCGCGTCATCCAGCAGTGATGACTGTCGCAACTACTGACCAGC
Sequence AGCGTCGGCACAAGATTTCWTTTTCATCAAGGACGAGCCCGTTGACGACG
HAC1 AGCGTCGGCACAAGATTTCATTTTCATCAAGGACGAGCCCGTTGACGACG
Sequence AGCTTGGACTGCATGGACTGTCGGATGACTTCACCCTGTTTGAAGACAAC
HAC1 AGCTTGGACTGCATGGACTGTCGGATGACTTCACCCTGTTTGAAGACAAC
Sequence AAGCAGCCTGCCCAGCACGACTTTATTGCTGATCTAG
HAC1 AAGCAGCCTGCCCAGCACGACTTTATTGCTGATCTAG

FIG. 15

5′ splice site

P. pastoris atccagcagtgatgacg
S. cerevisiae atccagccgtgattacg
******* ***** ***

3′ splice site

P. pastoris ggtctgcagcaccat
S. cerevisiae tgtccgaagcgcagt
*** * *** * *

FIG. 16

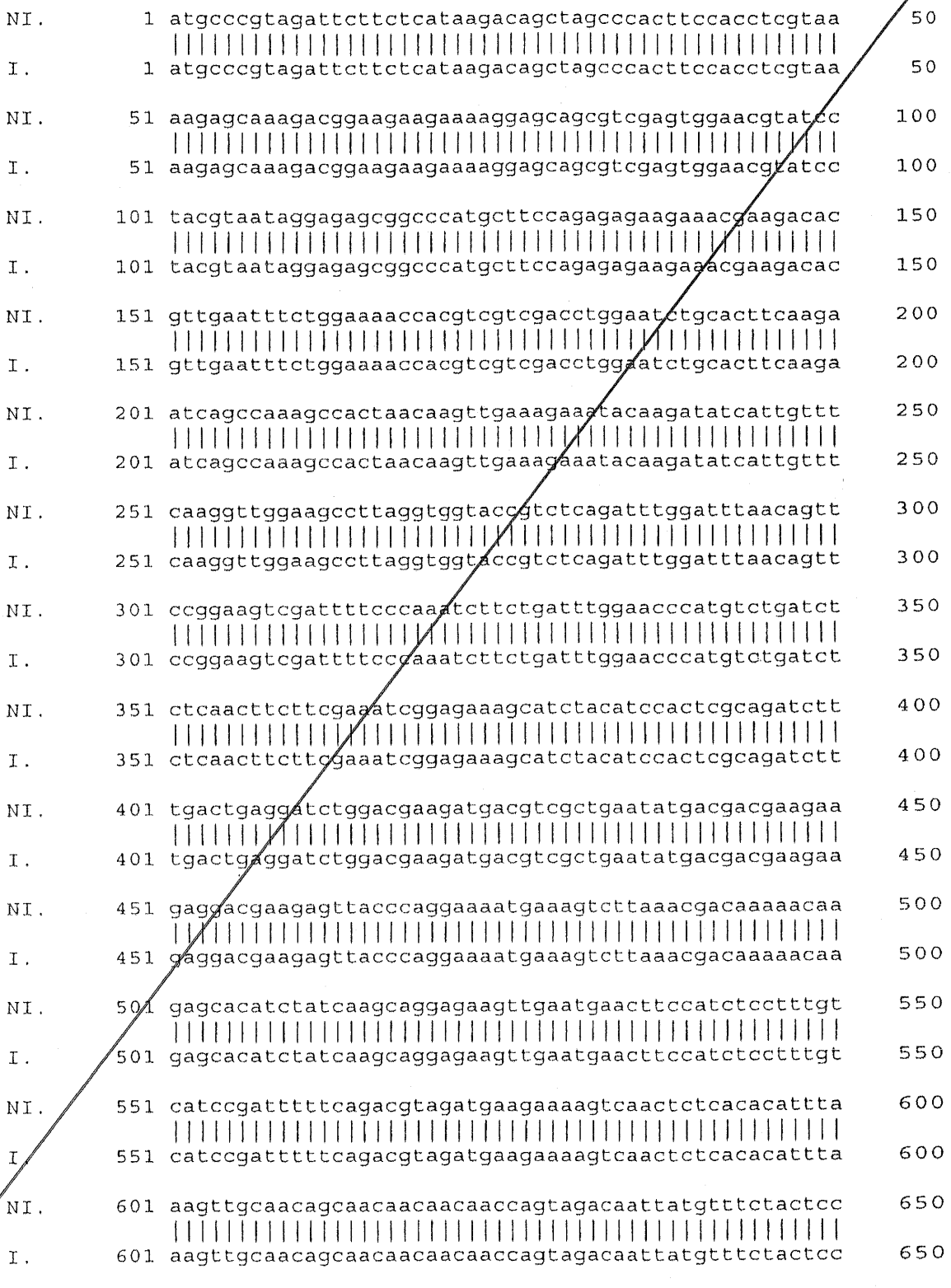

NI. 1 atgcccgtagattcttctcataagacagctagcccacttccacctcgtaa 50
I. 1 atgcccgtagattcttctcataagacagctagcccacttccacctcgtaa 50
NI. 51 aagagcaaagacggaagaagaaaaggagcagcgtcgagtggaacgtatcc 100
I. 51 aagagcaaagacggaagaagaaaaggagcagcgtcgagtggaacgtatcc 100
NI. 101 tacgtaataggagagcggcccatgcttccagagagaagaaacgaagacac 150
I. 101 tacgtaataggagagcggcccatgcttccagagagaagaaacgaagacac 150
NI. 151 gttgaatttctggaaaaccacgtcgtcgacctggaatctgcacttcaaga 200
I. 151 gttgaatttctggaaaaccacgtcgtcgacctggaatctgcacttcaaga 200
NI. 201 atcagccaaagccactaacaagttgaaagaaatacaagatatcattgttt 250
I. 201 atcagccaaagccactaacaagttgaaagaaatacaagatatcattgttt 250
NI. 251 caaggttggaagccttaggtggtaccgtctcagatttggatttaacagtt 300
I. 251 caaggttggaagccttaggtggtaccgtctcagatttggatttaacagtt 300
NI. 301 ccggaagtcgattttcccaaatcttctgatttggaacccatgtctgatct 350
I. 301 ccggaagtcgattttcccaaatcttctgatttggaacccatgtctgatct 350
NI. 351 ctcaacttcttcgaaatcggagaaagcatctacatccactcgcagatctt 400
I. 351 ctcaacttcttcgaaatcggagaaagcatctacatccactcgcagatctt 400
NI. 401 tgactgaggatctggacgaagatgacgtcgctgaatatgacgacgaagaa 450
I. 401 tgactgaggatctggacgaagatgacgtcgctgaatatgacgacgaagaa 450
NI. 451 gaggacgaagagttacccaggaaaatgaaagtcttaaacgacaaaaacaa 500
I. 451 gaggacgaagagttacccaggaaaatgaaagtcttaaacgacaaaaacaa 500
NI. 501 gagcacatctatcaagcaggagaagttgaatgaacttccatctcctttgt 550
I. 501 gagcacatctatcaagcaggagaagttgaatgaacttccatctcctttgt 550
NI. 551 catccgatttttcagacgtagatgaagaaaagtcaactctcacacattta 600
I. 551 catccgatttttcagacgtagatgaagaaaagtcaactctcacacattta 600
NI. 601 aagttgcaacagcaacaacaacaaccagtagacaattatgtttctactcc 650
I. 601 aagttgcaacagcaacaacaacaaccagtagacaattatgtttctactcc 650

FIG. 17A

```
NI.      651 tttgagtcttccggaggattcagttgattttattaacccaggtaacttaa    700
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       651 tttgagtcttccggaggattcagttgattttattaacccaggtaacttaa    700

NI.      701 aaatagagtccgatgagaacttcttgttgagttcaaatactttacaaata    750
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       701 aaatagagtccgatgagaacttcttgttgagttcaaatactttacaaata    750

NI.      751 aaacacgaaaatgacaccgactacattactacagctccatcaggttccat    800
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       751 aaacacgaaaatgacaccgactacattactacagctccatcaggttccat    800

NI.      801 caatgatttttttaattcttatgacattagcgagtcgaatcggttgcatc    850
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       801 caatgatttttttaattcttatgacattagcgagtcgaatcggttgcatc    850

NI.      851 atccagcagtgatgacggattcatctttacacattacagcaggctccatc    900
             ||||||
I.       851 atccag----------------------------------------------  855

NI.      901 ggctttttctctttgattgggggggggaaagttctgtagcagggaggcg    950

I.       856 ----------------------------------------------------  855

NI.      951 cagttcagttggcacatatcagttgacatgcatagcgatcaggtgattgc   1000

I.       856 ----------------------------------------------------  855

NI.     1001 tcttgttaactggtatgccaagtcaaccgttttcggcgtacctactactc   1050

I.       856 ----------------------------------------------------  855

NI.     1051 tactttaacttcttgaattgagatgaaacaatcgcccttgttcgagacgcg   1100

I.       856 ----------------------------------------------------  855

NI.     1101 tagttaatttaagatgaatcaatttgcaacaaaagaacaaacgaaaaaga   1150

I.       856 ----------------------------------------------------  855

NI.     1151 taacagcaaacttaagtttcgtggtctgcagcaccatttaccgctaatgc   1200
                                          ||||||||||||||||||||||
I.       856 ----------------------------cagcaccatttaccgctaatgc    878

NI.     1201 atttgatttaaatgactttgtattcttccaggaatag    1237
             ||||||||||||||||||||||||||||||||||||
I.       879 atttgatttaaatgactttgtattcttccaggaatag     915
```

FIG. 17B

```
P.pastoris          1  APFTANAFDLNDFVFFQE      18
                       |....|:|:||||....
S.cerevisiae        1  EAQSGLNSFELNDFFITS      18
```

FIG. 18

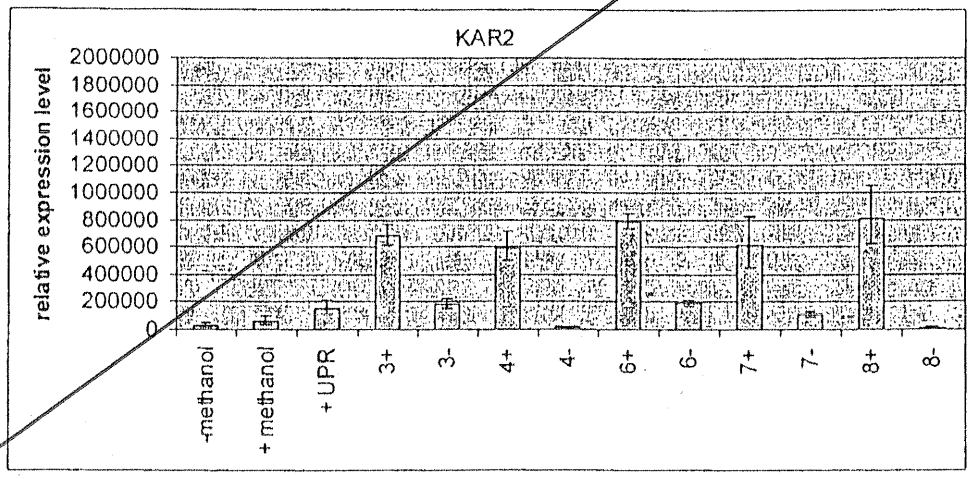

KAR2
relative expression level
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-methanol
+ methanol
+ UPR
3+
3-
4+
4-
6+
6-
7+
7-
8+
8-

FIG. 19

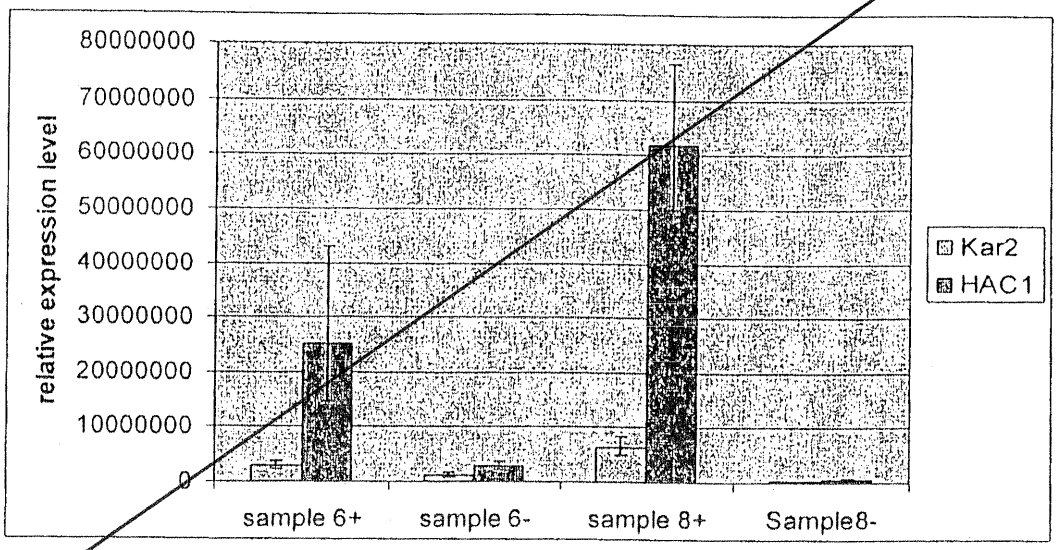

80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
relative expression level
sample 6+
sample 6-
sample 8+
Sample8-
Kar2
HAC1

FIG. 20

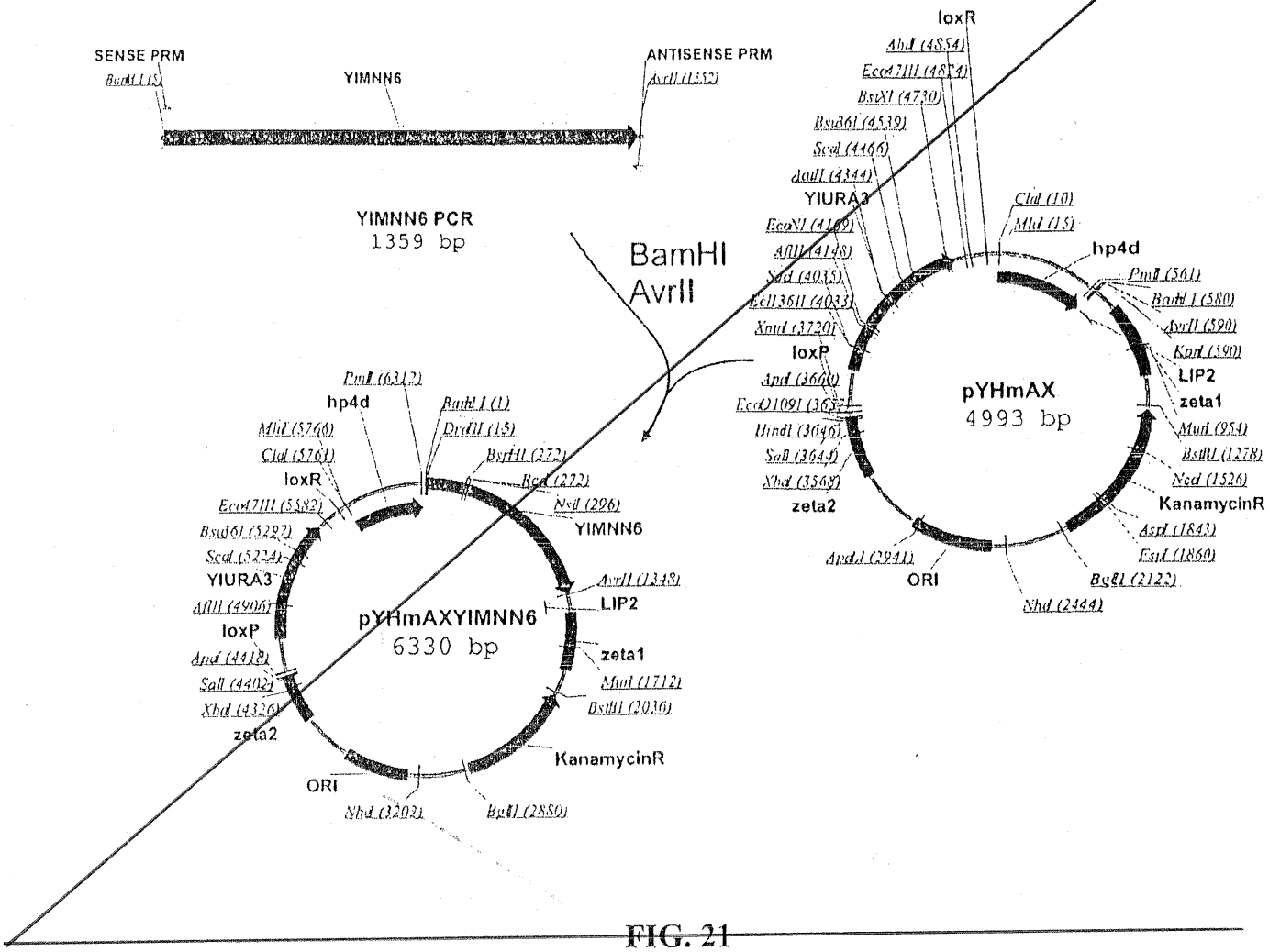

SENSE PRM
BamH I (1)
YIMNN6
ANTISENSE PRM
AvrII (1352)
YIMNN6 PCR
1359 bp
BamHI
AvrII
loxR
AhdI (4854)
Eco47III (4874)
BsrXI (4730)
Bsu36I (4539)
ScaI (4466)
dalII (4344)
YIURA3
EcoNI (4169)
AflII (4148)
SacI (4035)
Ecl136II (4033)
XmnI (3720)
loxP
ApaI (3660)
Eco0109I (3637)
HindI (3646)
SalI (3645)
XhoI (3568)
zeta2
ClaI (10)
MluI (15)
hp4d
PmlI (561)
BamH I (580)
AvrII (590)
KpnI (590)
LIP2
zeta1
pYHmAX
4993 bp
MunI (954)
BstBI (1278)
NcoI (1526)
KanamycinR
AspI (1843)
EsaI (1860)
ORI
BglI (2122)
NhdI (2444)
ApaLI (2941)
PmlI (6312)
hp4d
BamH I (1)
DraIII (15)
MluI (5746)
ClaI (5761)
loxR
BsrFHI (272)
BglI (222)
NsiI (296)
YIMNN6
Eco47III (5582)
Bsu36I (5292)
ScaI (5224)
YIURA3
AvrII (1348)
AflII (4906)
LIP2
loxP
pYHmAXYIMNN6
6330 bp
zeta1
ApaI (4418)
SalI (4402)
MunI (1712)
XhoI (4326)
BstBI (2036)
zeta2
KanamycinR
ORI
NhdI (3202)
BglI (2880)

FIG. 21

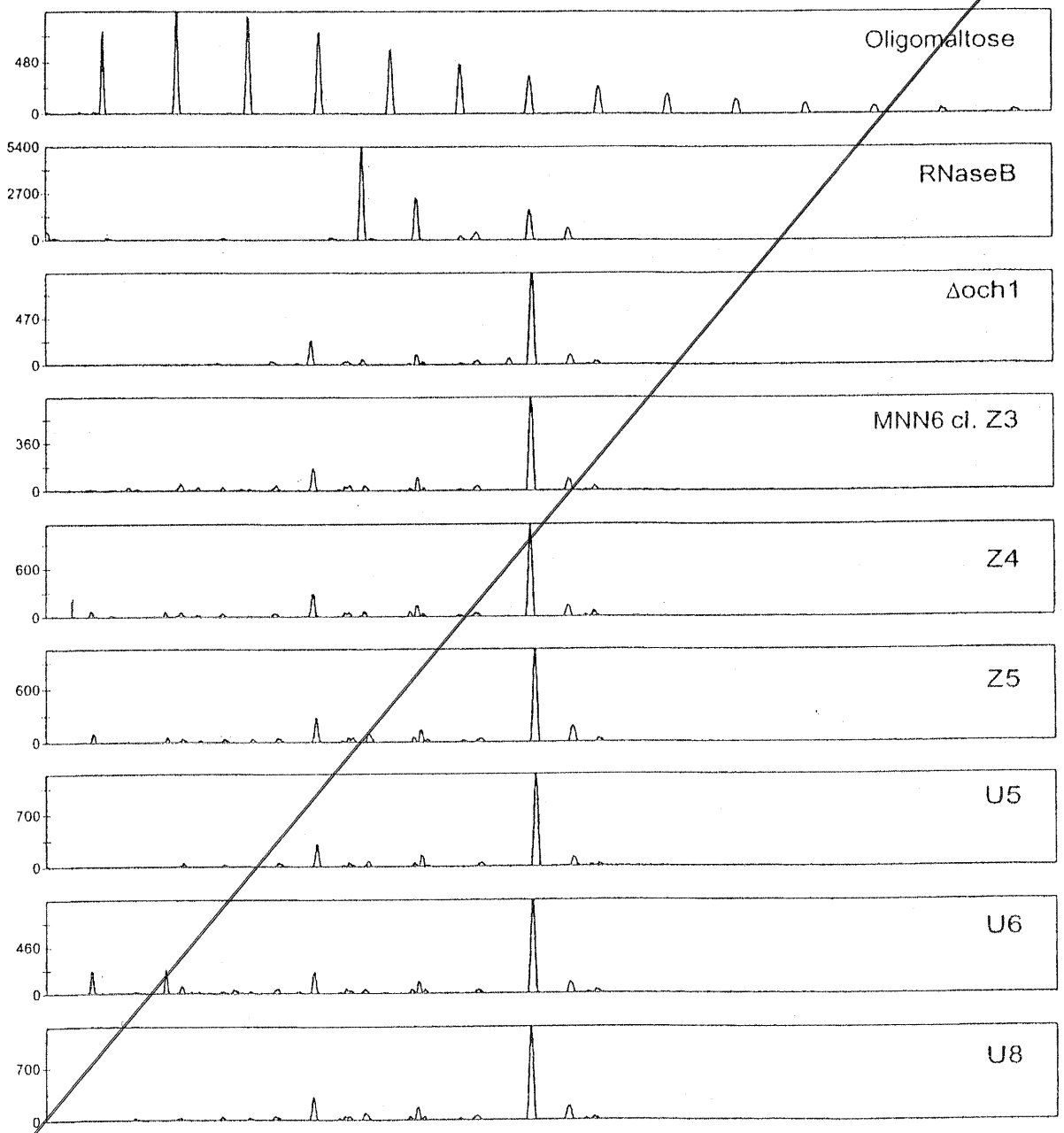

Oligomaltose
480
0
RNaseB
5400
2700
0
Δoch1
470
0
MNN6 cl. Z3
360
0
Z4
600
0
Z5
600
0
U5
700
0
U6
460
0
U8
700
0

FIG. 22

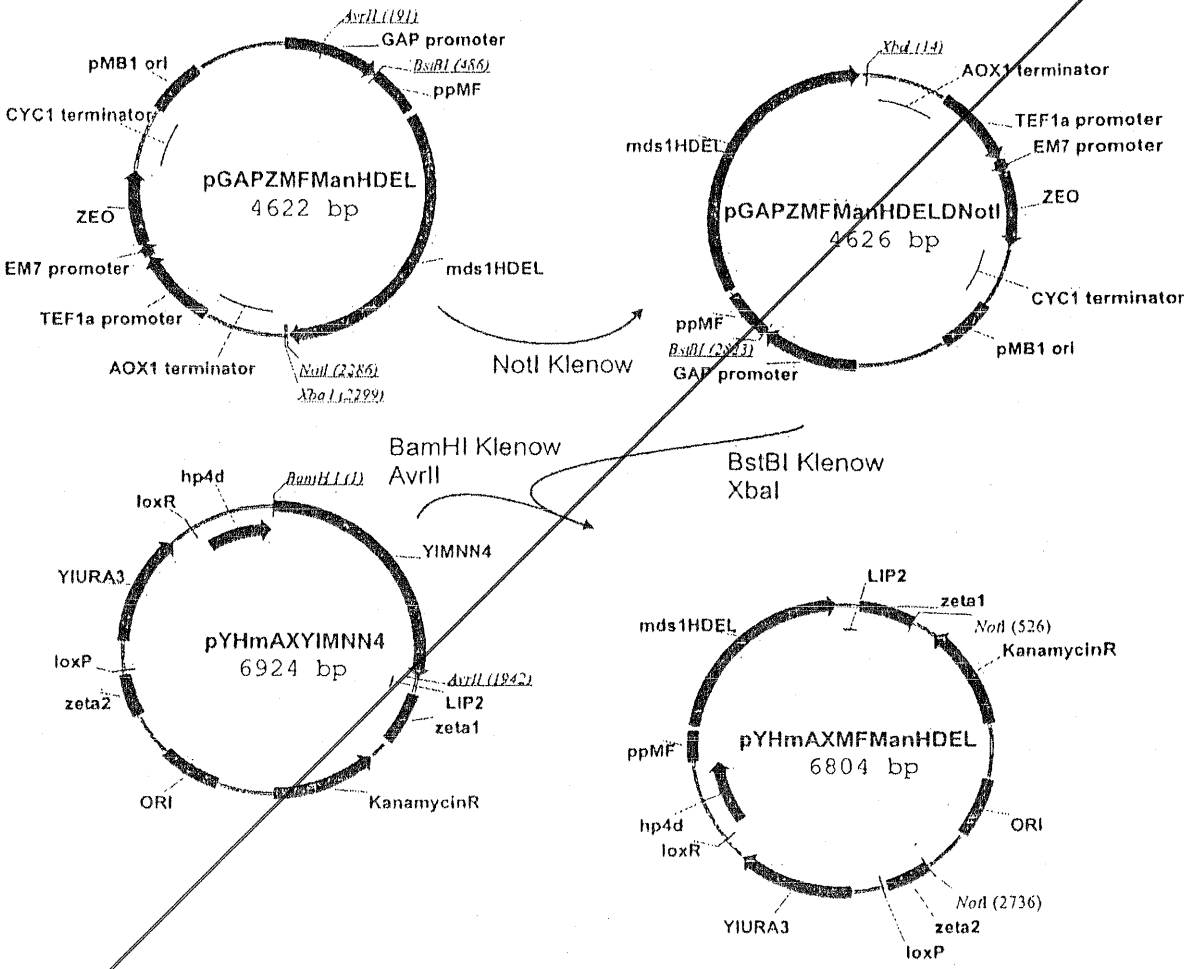

AvrII (191)
GAP promoter
BstBI (456)
ppMF
pMB1 ori
CYC1 terminator
pGAPZMFManHDEL
4622 bp
ZEO
EM7 promoter
mds1HDEL
TEF1a promoter
AOX1 terminator
NotI (2286)
XbaI (2299)
NotI Klenow
XbaI (14)
AOX1 terminator
TEF1a promoter
EM7 promoter
mds1HDEL
pGAPZMFManHDELDNotI
4626 bp
ZEO
CYC1 terminator
ppMF
BstBI (2943)
GAP promoter
pMB1 ori
BamHI Klenow
AvrII
BstBI Klenow
XbaI
hp4d
BamHI (1)
loxR
YIMNN4
YIURA3
pYHmAXYIMNN4
6924 bp
loxP
zeta2
AvrII (1942)
LIP2
zeta1
ORI
KanamycinR
LIP2
zeta1
mds1HDEL
NotI (526)
KanamycinR
pYHmAXMFManHDEL
6804 bp
ppMF
ORI
hp4d
loxR
NotI (2736)
YIURA3
zeta2
loxP

FIG. 23

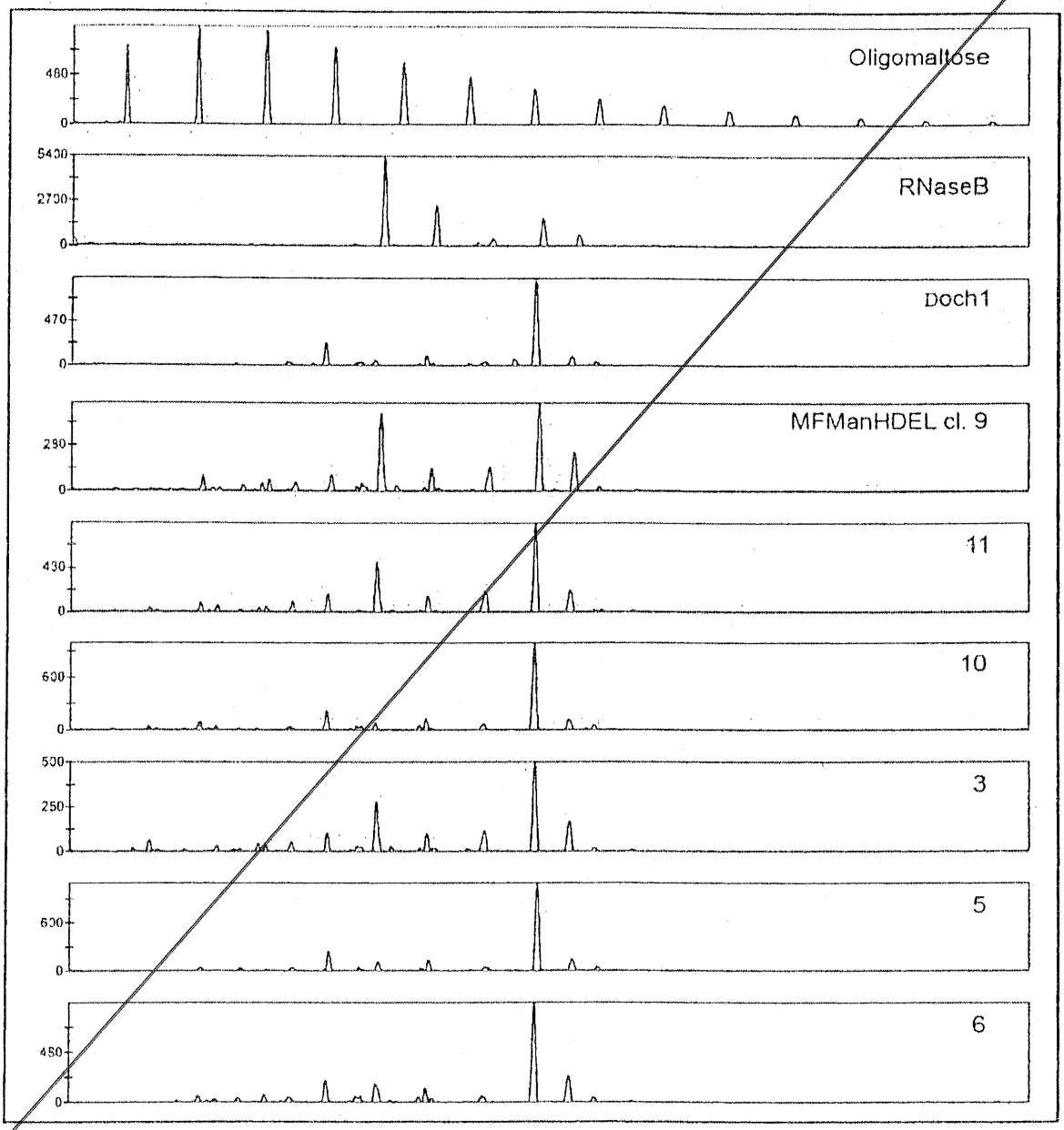

Oligomaltose
460
0
5430
2700
0
RNaseB
Doch1
470
0
MFManHDEL cl. 9
290
0
11
430
0
10
600
0
3
500
250
0
5
600
0
6
450
0

FIG. 24

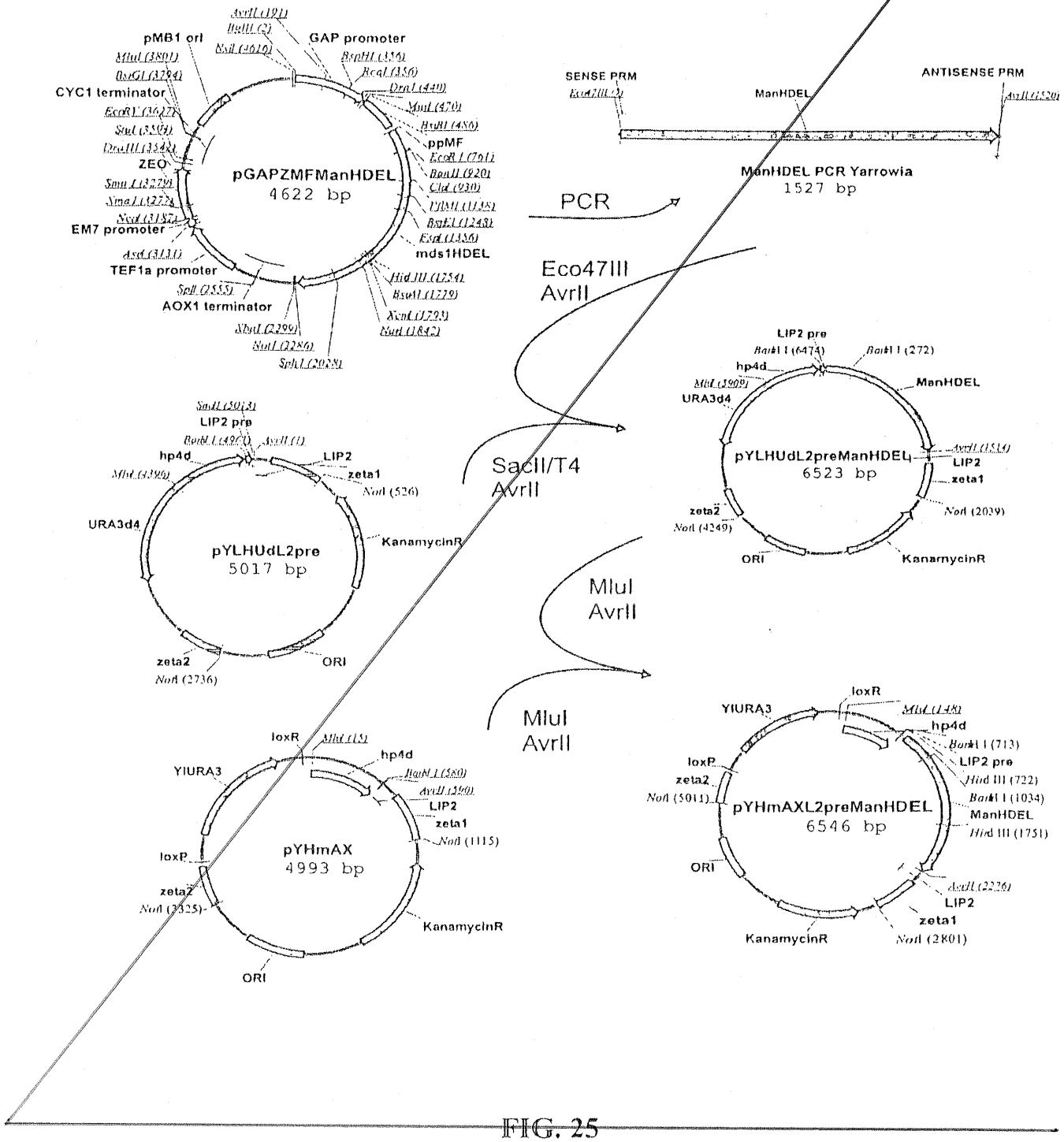

pGAPZMFManHDEL
4622 bp
pMB1 ori
GAP promoter
CYC1 terminator
ZEO
EM7 promoter
TEF1a promoter
AOX1 terminator
ppMF
mds1HDEL
SENSE PRM
ManHDEL
ANTISENSE PRM
ManHDEL PCR Yarrowia
1527 bp
PCR
Eco47III
AvrII
SacII/T4
AvrII
MluI
AvrII
MluI
AvrII
pYLHUdL2pre
5017 bp
LIP2 pre
hp4d
LIP2
zeta1
URA3d4
KanamycinR
zeta2
ORI
pYLHUdL2preManHDEL
6523 bp
ManHDEL
pYHmAX
4993 bp
loxR
YlURA3
loxP
pYHmAXL2preManHDEL
6546 bp

FIG. 25

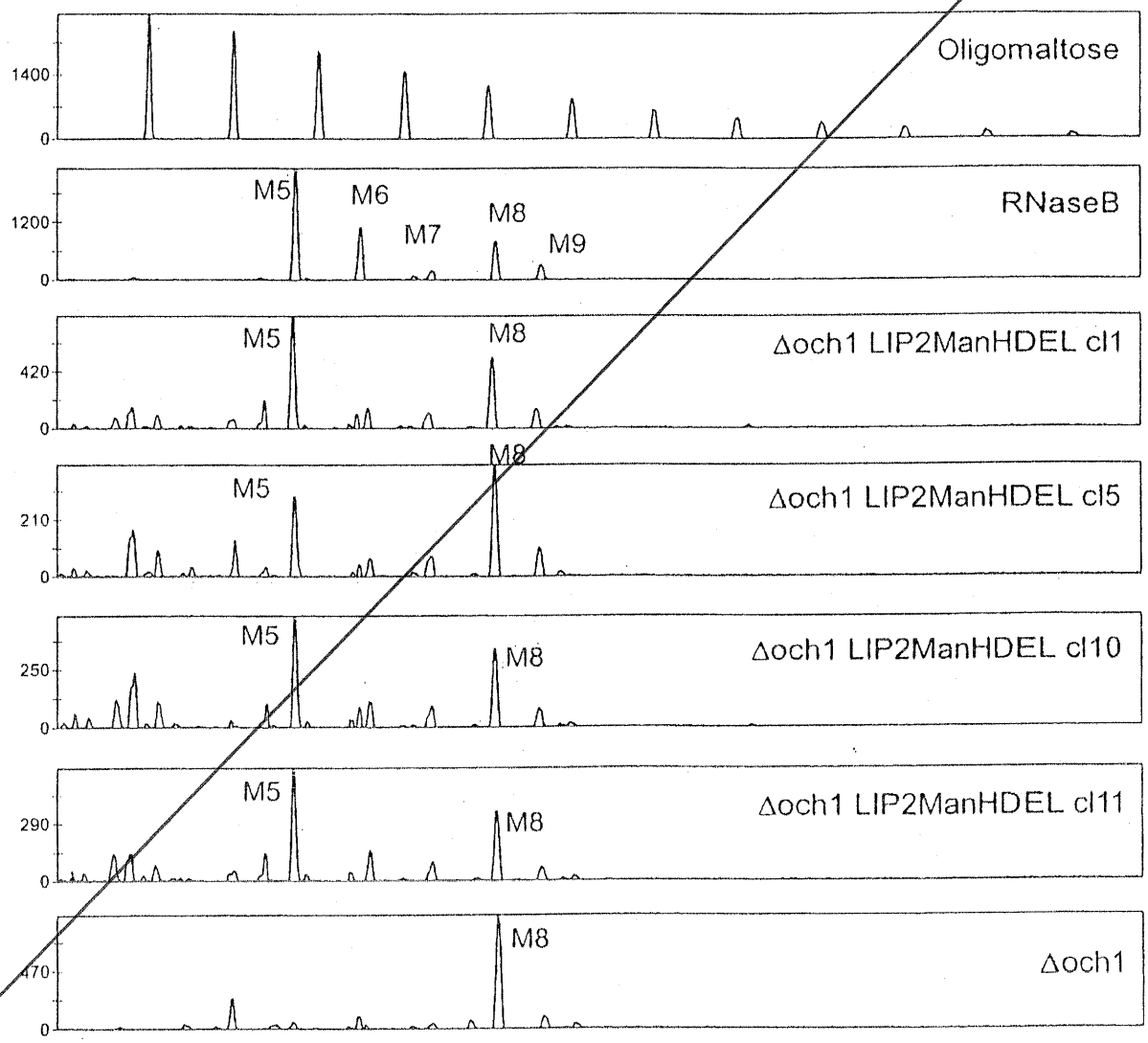

Oligomaltose
1400
0
M5
M6
M7
M8
M9
RNaseB
1200
0
M5
M8
Δoch1 LIP2ManHDEL cl1
420
0
M8
M5
Δoch1 LIP2ManHDEL cl5
210
0
M5
M8
Δoch1 LIP2ManHDEL cl10
250
0
M5
M8
Δoch1 LIP2ManHDEL cl11
290
0
M8
Δoch1
470
0

FIG. 26

MSIKREESFTPTPEDLGSPLTADSPGSPESGDKRKKDLTLP
LPAGALPPRKRAKTENEKEQRRIERIMRNRQAAHASREKKRRH
LEDLEKKCSELSSENNDLHHQVTESKKTNMHLMEQHYSLVAK
LQQLSSLVNMAKSSGALAGVDVPDMSDVSMAPKLEMPTAAPS
QPMGLASAPTLFNHDNETVVPDSPIVKTEEVDSTNFLLHTESSS
PPELAESTGSGSPSSTLSCDETDYLVDRARHPAASAQDFIFIKD
EPVDDELGLHGLSDDFTLFEDNKQPAQHDFIADLAHYESSVSN
LFGGLE

FIG. 27A

MPVDSSHKTASPLPPRKRAKTEEEKEQRRVERILRNRRAA
HASREKKRRHVEFLENHVVDLESALQESAKATNKLKEIQDIIV
SRLEALGGTVSDLDLTVPEVDFPKSSDLEPMSDLSTSSKSEKAS
TSTRRSLTEDLDEDDVAEYDDEEEDEELPRKMKVLNDKNKSTS
IKQEKLNELPSPLSSDFSDVDEEKSTLTHLKLQQQQQQPVDNY
VSTPLSLPEDSVDFINPGNLKIESDENFLLSSNTLQIKHENDTD
YITTAPSGSINDFFNSYDISESNRLHHPAAPFTANAFDLNDFVF
FQE

FIG. 27B

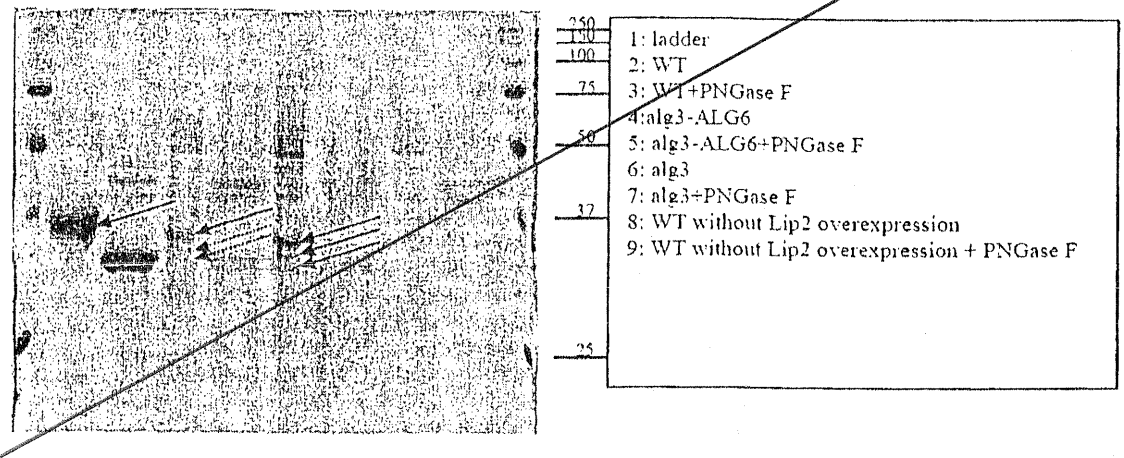

250
130
100
75
50
37
25
1: ladder
2: WT
3: WT+PNGase F
4:alg3-ALG6
5: alg3-ALG6+PNGase F
6: alg3
7: alg3+PNGase F
8: WT without Lip2 overexpression
9: WT without Lip2 overexpression + PNGase F

FIG. 28

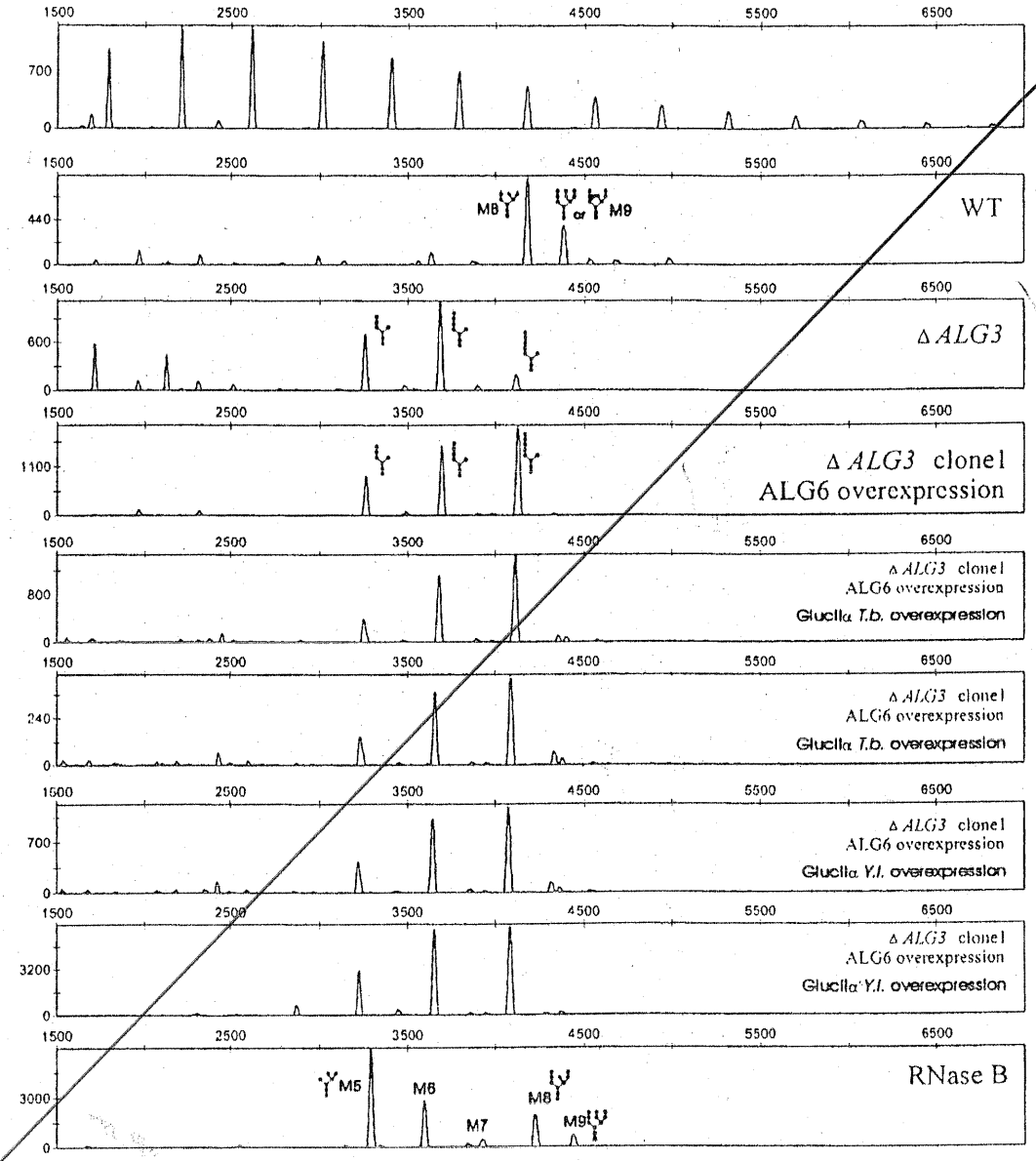

1500
2500
3500
4500
5500
6500
700
0
1500
2500
3500
4500
5500
6500
M8
or
M9
440
0
WT
1500
2500
3500
4500
5500
6500
600
0
Δ ALG3
1500
2500
3500
4500
5500
6500
1100
0
Δ ALG3 clone1
ALG6 overexpression
1500
2500
3500
4500
5500
6500
800
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα T.b. overexpression
1500
2500
3500
4500
5500
6500
240
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα T.b. overexpression
1500
2500
3500
4500
5500
6500
700
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα Y.l. overexpression
1500
2500
3500
4500
5500
6500
3200
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα Y.l. overexpression
1500
2500
3500
4500
5500
6500
M5
M6
M7
M8
M9
3000
0
RNase B

FIG. 29

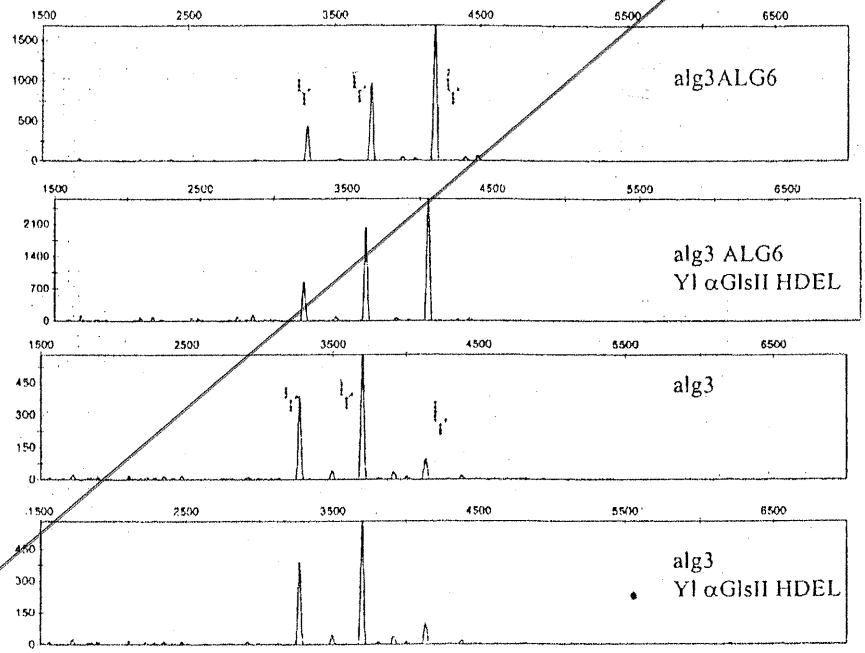

1500
2500
3500
4500
5500
6500
alg3ALG6
alg3 ALG6
Yl αGlsII HDEL
alg3
alg3
Yl αGlsII HDEL

FIG. 30

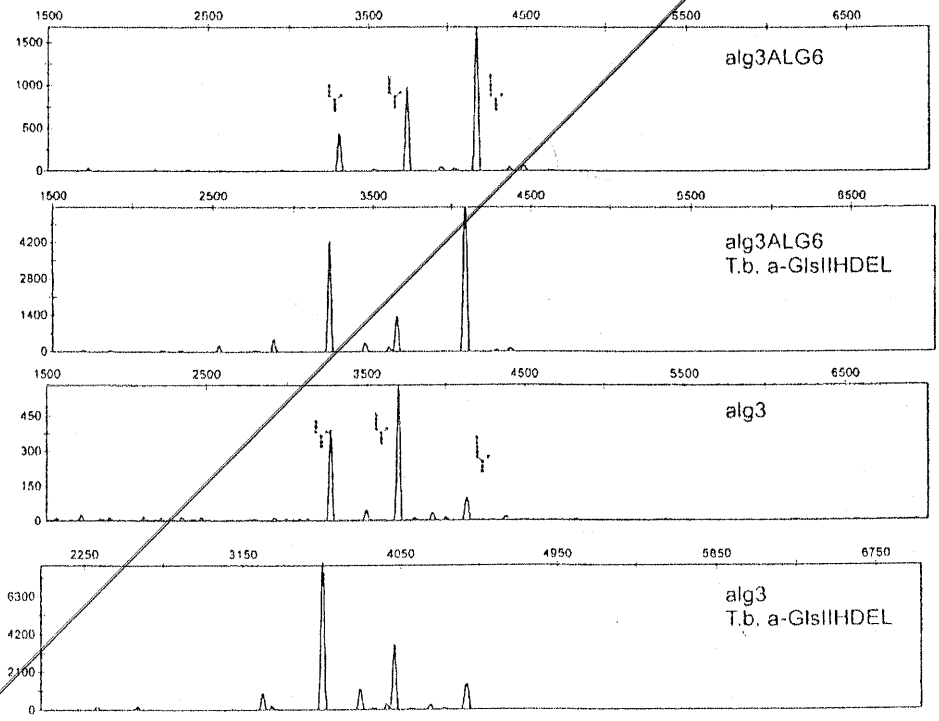

1500
2500
3500
4500
5500
6500
1500
1000
500
0
alg3ALG6
1500
2500
3500
4500
5500
6500
4200
2800
1400
0
alg3ALG6
T.b. a-GlsIIHDEL
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
2250
3150
4050
4950
5850
6750
6300
4200
2100
0
alg3
T.b. a-GlsIIHDEL

FIG. 31

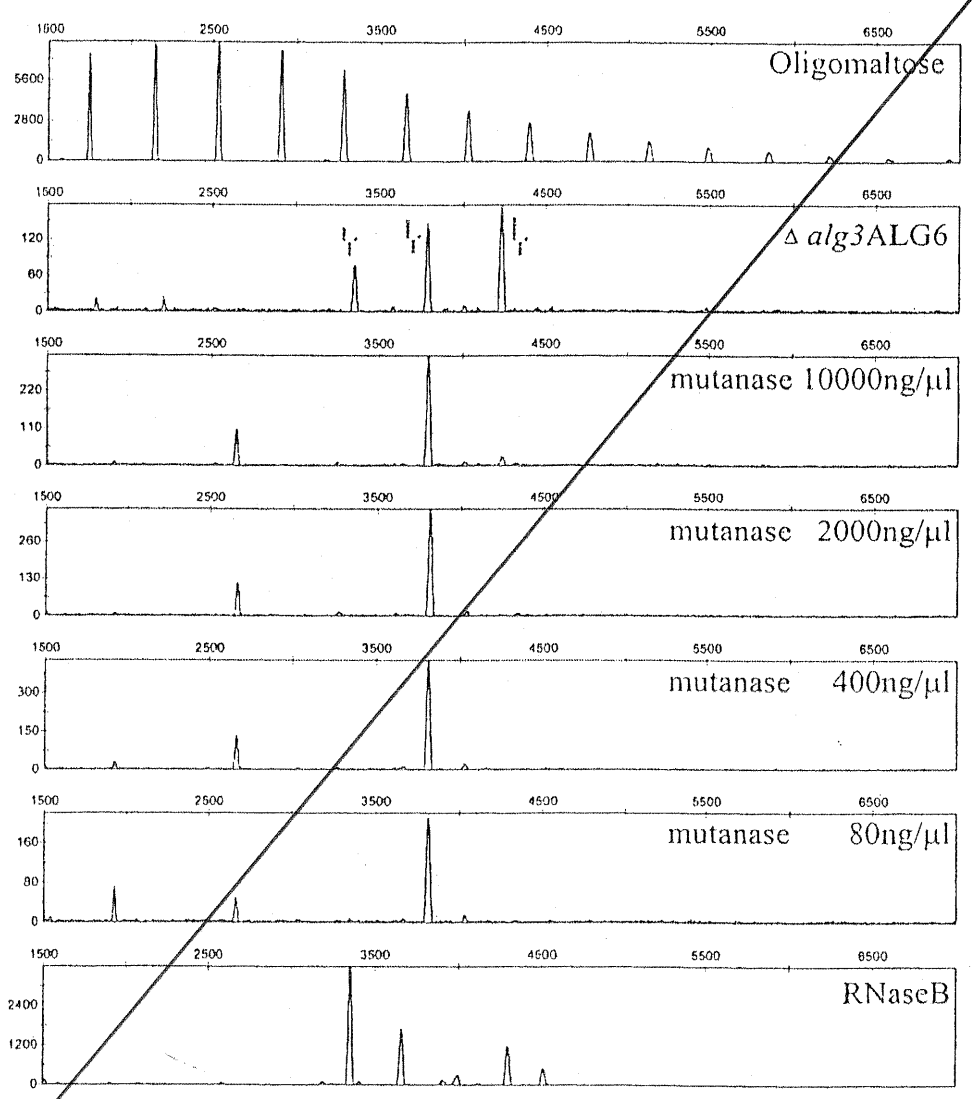

1500
2500
3500
4500
5500
6500
Oligomaltose
5600
2800
0
Δ alg3ALG6
120
60
mutanase 10000ng/μl
220
110
mutanase 2000ng/μl
260
130
mutanase 400ng/μl
300
150
mutanase 80ng/μl
160
80
RNaseB
2400
1200

FIG. 32

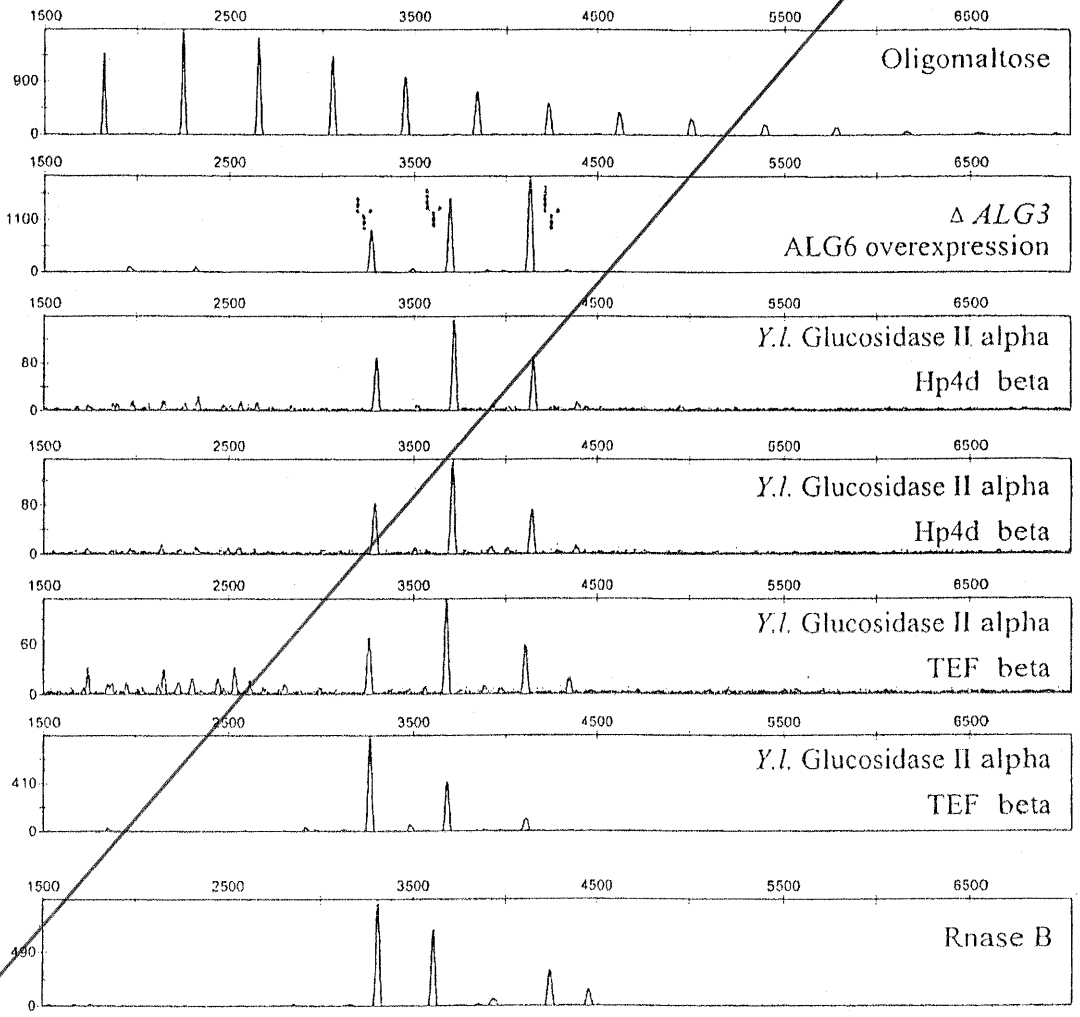

1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
1100
Δ *ALG3*
ALG6 overexpression
80
Y.l. Glucosidase II alpha
Hp4d beta
60
TEF beta
410
490
Rnase B

FIG. 33

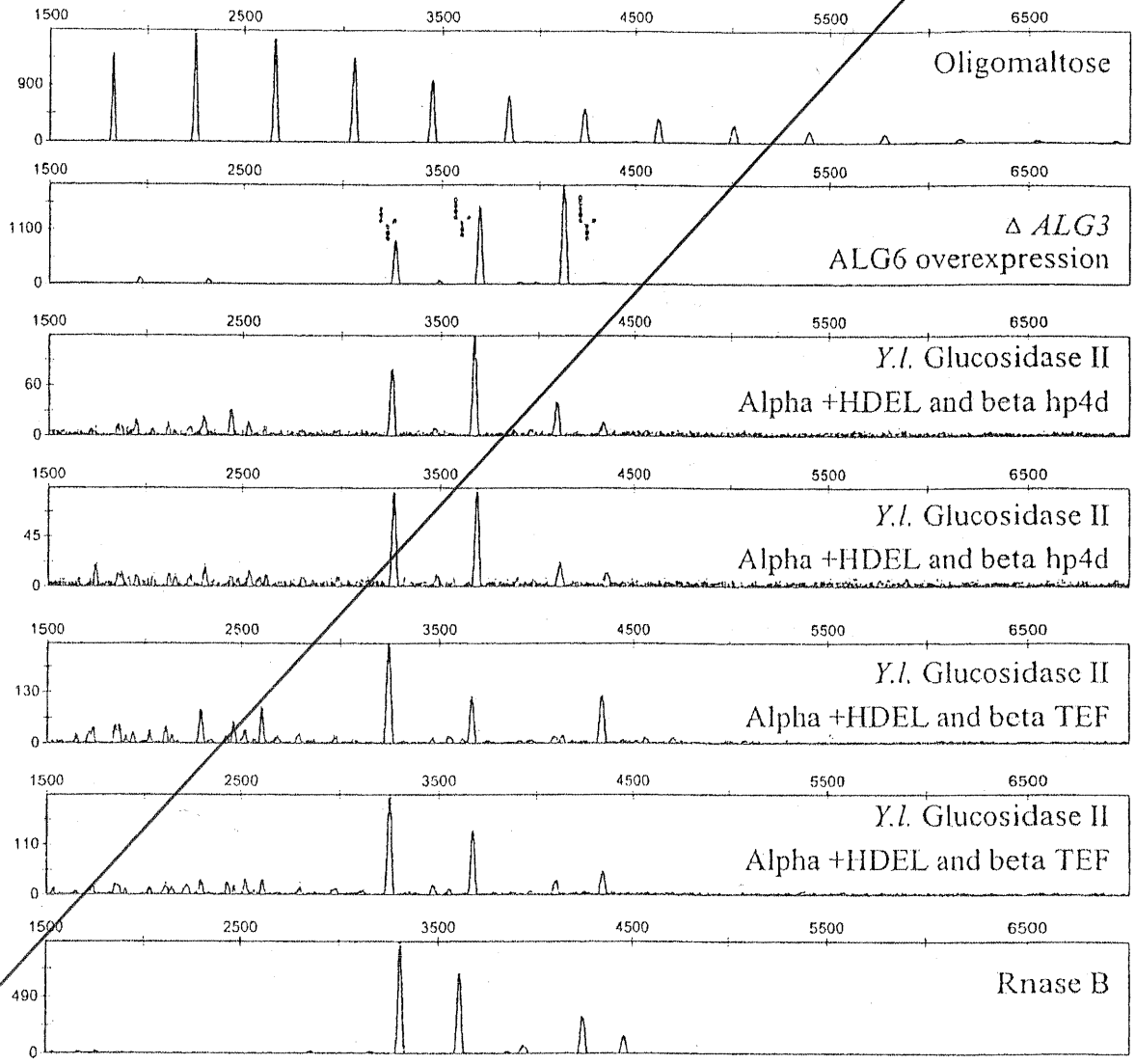

1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
1100
Δ ALG3
ALG6 overexpression
60
Y.l. Glucosidase II
Alpha +HDEL and beta hp4d
45
Y.l. Glucosidase II
Alpha +HDEL and beta hp4d
130
Y.l. Glucosidase II
Alpha +HDEL and beta TEF
110
Y.l. Glucosidase II
Alpha +HDEL and beta TEF
490
Rnase B

FIG. 34

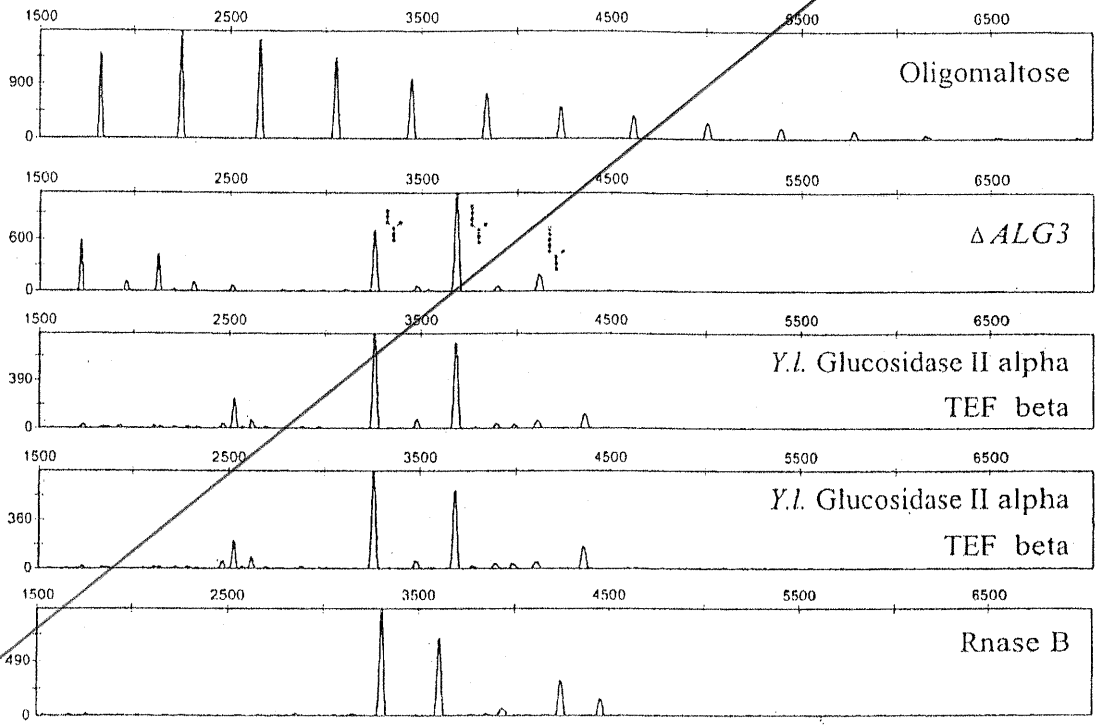

1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
600
Δ*ALG3*
390
Y.l. Glucosidase II alpha
TEF beta
360
Y.l. Glucosidase II alpha
TEF beta
490
Rnase B

FIG. 35

gtaaagcacgagaacttcaagacctgttctcagtctggcttctgtaagcgaaaccgagccttcgccgacgacgccgctgcccagggctcttc
ttgggcctctccctacgagctggactcttcttctatccagttcaaggacggccagctgcacggcaccatcctgaagtctgtgtctcccaacga
gaaggtgaagctgcccctggtggtgtctttcctggagtctggcgccgctcgagtggtggtggacgaggagaagccgaatgaacggcgacat
ccagctgcgacacgactctaaggcccgaaaggagcgatacaacgaggccgagaagtgggtgctggtcggcggcctggagctgtctaag
accgccaccctgcgacccgagaccgagtctggcttcacccgagtgctgtacggccccgacaaccagttcgaggccgtgatccgacacgc
ccccttctctgccgacttcaagcgagatggccagacccacgtgcagctgaacaacaagggctacctgaacatggagcactggcgaccca
aggtggaggtggagggcgagggtgagcagcagacccaggaggacgagtctacctggtgggacgagtctttcggcggcaacaccgaca
ccaagccccgaggccccgagtctgtgggcctggacatcaccttccccggctacaagcacgtgttcggcatccccgagcacgccgactcc
ctgtctctgaaggagacccgaggcggcgagggcaaccacgaggagccctaccgaatgtacaacgccgacgtgttcgagtacgagctgtc
ctctcccatgaccctgtacggcgccatccccttcatgcaggcccaccgaaaggactctaccgtgggcgtgttctggctgaacgccgccgag
acctgggtggacatcgtgaagtctacctcttctcccaacccccctggccctgggcgtgggagccaccaccgacacccagtctcactggttctc
tgagtctggccagctggacgtgttcgtgttcctgggccccacccccccaggagatttctaagacctacggcgagctgaccggctacacccag
ctgccccagcacttcgccattgcctaccaccagtgtcgatggaactacatcaccgacgaggacgtgaaggaggtggaccgaaacttcgac
aagtaccagatcccctacgacgtgatctggctggacatcgagtacaccgacgaccgaaagtacttcacctgggacccccctgtctttccccga
ccccatctctatggaggagcagctggacgagtctgagcgaaagctggtcgtgatcatcgaccccccacatcaagaaccaggacaagtactc
tatcgtgcaggagatgaagtctaaggacctggccaccaagaacaaggacggcgagatttacgacggctggtgttggcccggctcttctcac
tggatcgacaccttcaaccccgctgccatcaagtggtgggtgtctctgttcaagttcgacaagttcaagggcaccctgtctaacgtgttcatct
ggaacgacatgaacgagccctctgtgttcaacggccccgagaccaccatgcccaaggacaacctgcaccacggcaactgggagcaccg
agacatccacaacgtgcacggcatcaccctggtgaacgccacctacgacgccctgctggagcgaaagaagggcgagatccgacgaccc
ttcatcctgacccgatcttactacgccggtgcccagcgaatgtctgccatgtggaccggcgacaaccaggccacctgggagcacctggcc
gcctctatccccatggtgctgaacaacggaatcgccggcttccccttcgccggtgccgacgtgggcggcttcttccagaaccccctctaagg
agctgctgacccgatggtatcaggccggcatctggtatcctttcttccgagcccacgcccacatcgacacccgacgacgagagccctacct
gatcgccgagccccaccgatctatcatctctcaggccatccgactgcgataccagctgctgcccgcctggtacaccgccttccacgaggcc
tctgtgaacggcatgcccatcgtgcgaccccagtactacgcccacccctgggacgaggccggcttcgccatcgacgaccagctgtacctg
ggctctaccggcctgctggccaagcccgtggtgtctgaggaggccaccaccgccgacatctacctggctgacgacgagaagtactacga

FIG. 36A

ctacttcgactacaccgtgtaccagggcgctggcaagcgacacaccgtgcccgctcccatggagaccgtgcccctgctgatgcagggcg
gccacgtgatccccccgaaaggaccgaccccgacgatcttctgccctgatgcgatgggaccctacaccctggtggtggtgctggacaaga
acggccaggccgacggctctctgtacgtggacgacggcgagaccttcgactacgagcgaggcgcctacatccatcgacgattccgattcc
aggagtctgccctggtctccgaggacgtgggcaccaagggccccaagaccgccgagtacctcaagaccatggccaacgtgcgagtgga
gcgagtggtggtcgtggacccccccaaggagtggcagggcaagacctctgtgaccgtgatcgaggacggcgcctctgccgcctctaccg
cctccatgcagtaccactctcagcccgacggcaaggccgcctacgccgtggtgaagaaccccaacgtgggcatcggcaagacctggcg
aatcgagttctaatagcctag

FIG. 36B

agcgctggcgacgcctcttctcgaccccgaggcgtgggccccgagttcgccaagttctacaaggacaccaccaccttcacctgtatctctc
accccgccatccagattcccttctctgccgtgaacgacgactactgtgactgtcccgacggctctgacgagcccggcacctctgcctgtgcc
tttctgtctcgaaactctgccctgacccccggtgagcgacccggatctgacgacctggagctgacctctgccctgccccggcttctactgtaag
aacaagggccacaagcccggctacgtgcccttccagcgagttaacgatggcatctgtgactacgagctgtgttgtgacggatcggatgagt
gggctcgacccggcggaaccaagtgtgaggacaagtgtaaggagatcggcaaggagtggcgaaagaaggaggagaagcgacagaa
gtctatgaccgccgctctgaagaagaagaaggacctgctggtcgaggccggacgacagcagaaggaggtcgaggacaacatcaagcg
actggaggtggagatccaggcccaggagctgaaggtcaacgacctgcaggccgagctggaggaggtggagcagcaggaggcctctaa
ggtcgtcaagggcaagaccgccggcaaggtgaacgtgctggctggcctcgccaagtctcgagtggaggagctgcgaaacgccctgatg
gacgtgcgaaaggagcgagatgacacccgagcccgagttaaggaactcgaagagatcctgtctaagttcaaggtggagtacaaccccaa
cttcaacgacgagggcgtgaagcgagccgtgcgatcttgggaggactacgccgccaagggcaccctggagggcgccgtgaacaacgc
ccaggaccgagacctggacgagatcgccaagcccgacgacgagaaggccggcatcaactgggagcagtgggagaacgaggaggac
ggctgtgaggctggcctggtgtaccagctggccgcctacctgccccctctctggtggagttcatcgagggcaaggtgctgttcgtgcgag
gcctgctggaggacaacggcatcctgcccaaggccgccgagacctctacctctgagtctaaggtggtgtctgaggcccgagaggccgtg
aagtctgccgagaaggagctgggcgacaagcagaagcagctgaaggaccacaagtctgacctggagaccgactacggcgtgggctcta
tcttccgagccctgaagggcgtgtgtatctctaaggactctggcgagtacacctacgagcactgttttctggaccagaccaagcagatcccc
aagaagggcggaggctctacccgaatgggcaagtacaccggcatcggctctgtgtctgtggacgtgctgaacgaggccggcgagatcgt
gcctgaggatcgagtgaccctgcagtacgccaacggccagggctgttggaacggacccgcccgatctaccaccgtgatcctgacctgtg
gcgaggaggacgccatcctgaaggtggccgaggacgagaagtgtgtgtactctatgcacgtgacctctcccgccgtgtgtcccggcggtg
acgagggcgccaccgccccaaccgaaaggacgagctgtaatagccta

FIG. 37

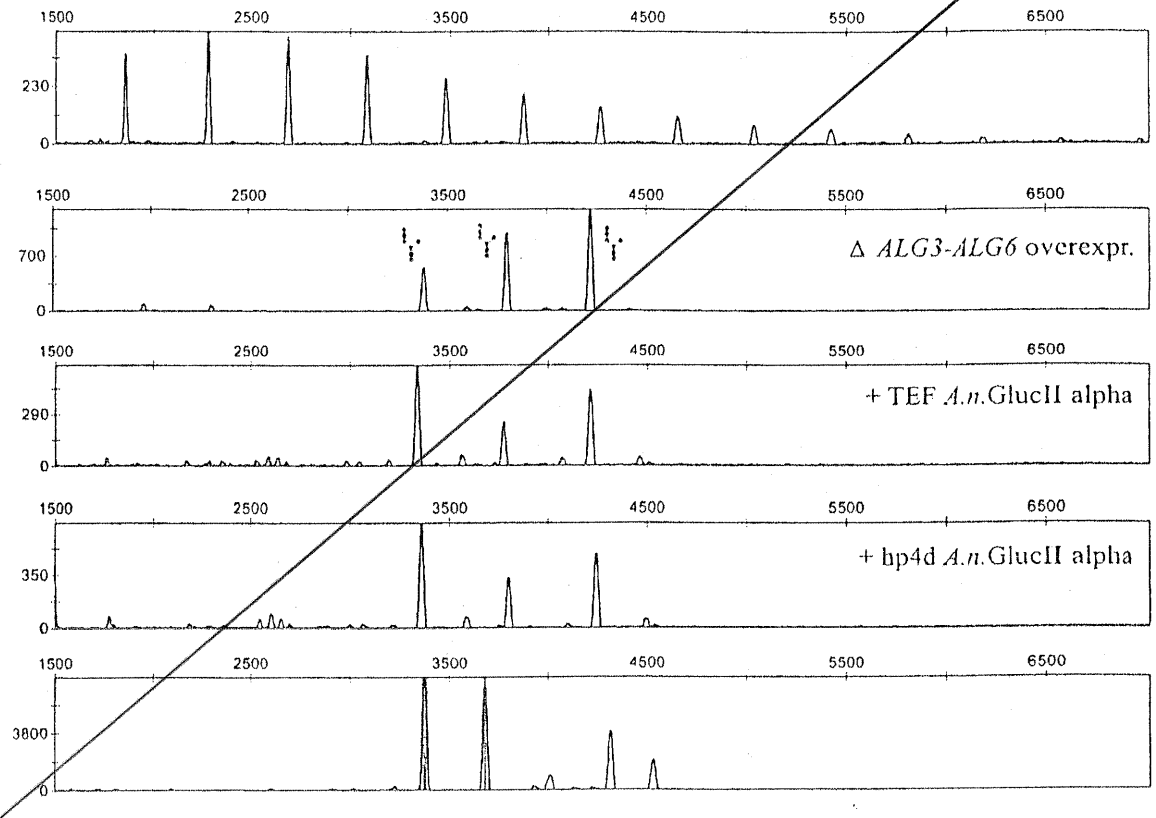

1500
2500
3500
4500
5500
6500
230
0
700
Δ ALG3-ALG6 overexpr.
290
+ TEF A.n.GlucII alpha
350
+ hp4d A.n.GlucII alpha
3800

FIG. 38

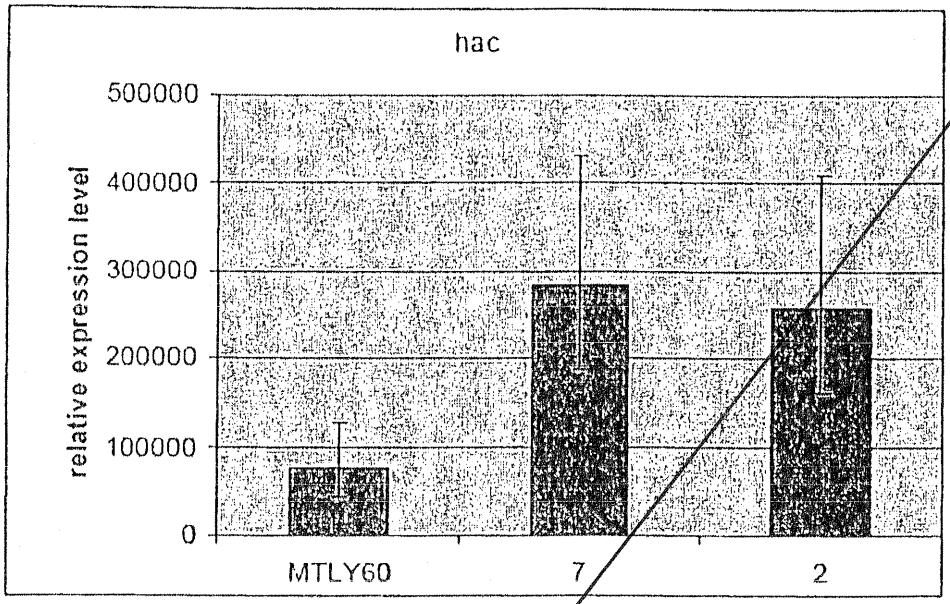


FIG. 39A

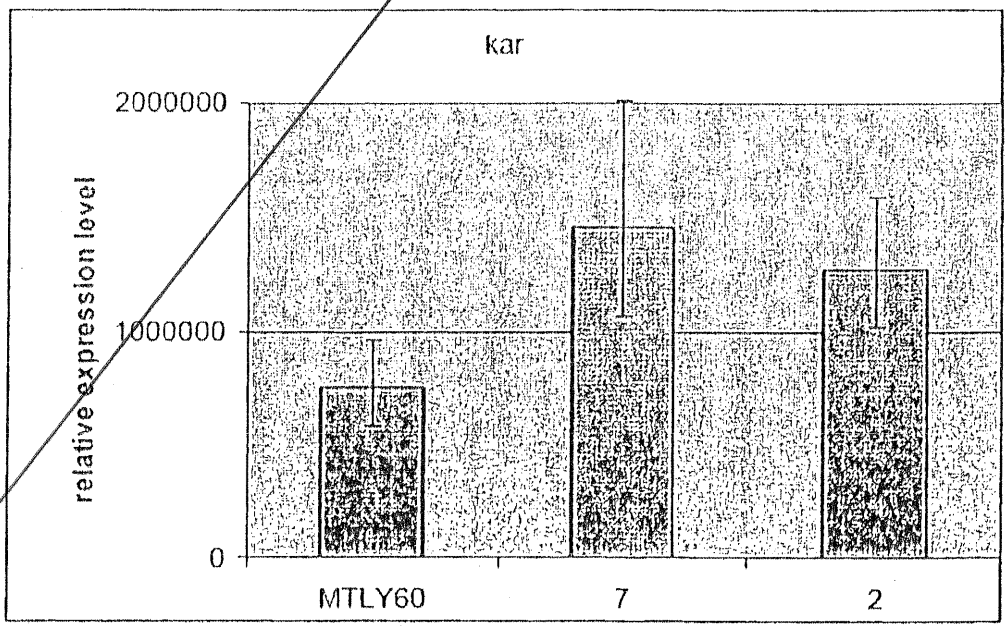


FIG. 39B

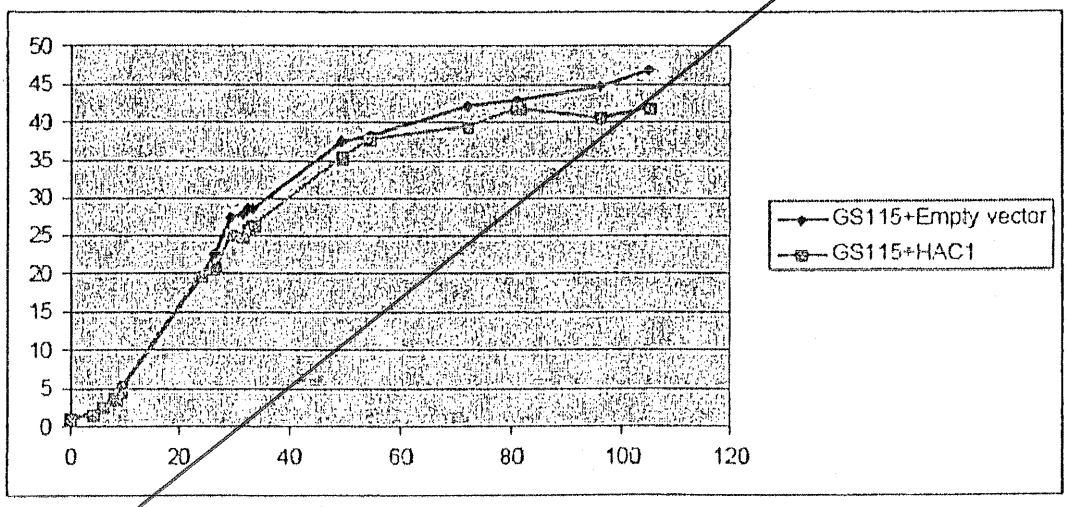

50
45
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
GS115+Empty vector
GS115+HAC1

FIG. 40

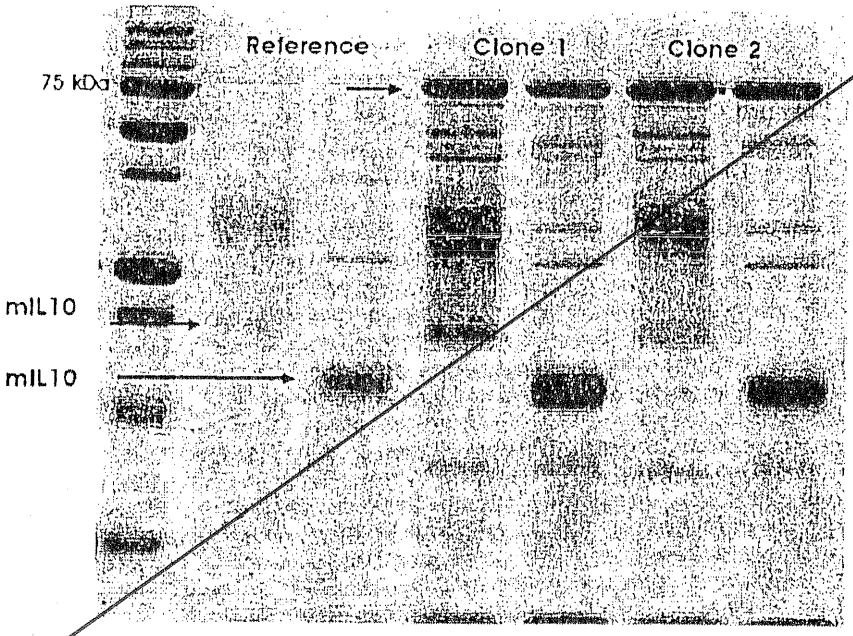


FIG. 41

gagctcagcgctaccaagcgaggctctcccaaccccacccgagccgccgctgtgaaggccgccttccagacctcttggaacgcctacca
ccacttcgccttcccccacgacgacctgcaccccgtgtctaactcgttcgacgacgagcgaaacggctgggggctcttctgccatcgacggc
ctggacaccgccatcctgatgggcgacgccgacatcgtgaacaccatcctgcagtacgtgccccagatcaacttcaccaccacgccgtg
gccaaccagggcatctctgtgttcgagaccaacatccgatacctgggcggcctgctgtctgcctacgacctgctgcgaggcccccttctcttct
ctggccaccaaccagaccctggtgaactctctgctgcgacaggcccagaccctggccaacggcctgaaggtggcttttaccaccccctctg
gcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatctggcgcctcttctaacaacgtggccgagatcggctctctggtgctg
gagtggacccgactgtctgacctgaccggcaacccccagtacgcccagctggcccagaagggcgagtcttacctgctgaaccccaaggg
ctctcccgaggcctggcccggactgatcggcaccttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgat
ggactctttctacgagtacctgatcaagatgtacctgtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggccgccgact
ctaccatcgcccacctggcctctcacccctctacccgaaaggacctgaccttcctgtcctcttacaacggccagtctacctctcccaactctgg
acacctggcttccttcgccggtggcaacttcatcctgggcggcatcctgctgaacgagcagaagtacatcgacttcggcatcaagctggcct
cttcctacttcgccacctacaaccagaccgcctctggcatcggccccgagggcttcgcctgggtggactctgtgaccggcgctggcggctc
tccccccctcttctcagtctggcttctactcttctgccggcttctgggtgaccgcccccctactacatcctgcgacccgagaccctggagtctctgt
actacgcctaccgagtgaccggcgactctaagtggcaggacctggcctgggaggccttctctgccatcgaggacgcctgtcgagccggct
ctgcctactcttctatcaacgacgtgacccaggccaacggtggcggagcctctgacgacatggagtctttctggttcgccgaggccctgaag
tacgcctacctgatcttcgccgaggaatctgacgtgcaggtgcaggccaacggcggcaacaagttcgtgttcaacaccgaggcccaccccc
ttctctatccgatcttcttctcgacgaggcggccacctggcccatgacgagctgtaatagcctaggtacc

FIG. 42

cggcggactgcgtccgaaccagctccagcagcgtttttccgggccattgagccgactgcgaccccgccaacgtgtcttggcccacgcact
catgtcatgttggtgttgggaggccacttttaagtagcacaaggcacctagctcgcagcaaggtgtccgaaccaaagaagcggctgcagt
ggtgcaaacggggcggaaacggcgggaaaaagccacgggggcacgaattgaggcacgccctcgaatttgagacgagtcacggcccc
attcgcccgcgcaatggctcgccaacgcccggtcttttgcaccacatcaggttaccccaagccaaacctttgtgttaaaaagcttaacatatta
taccgaacgtaggtttgggcgggcttgctccgtctgtccaaggcaacatttatataagggtctgcatcgccggctcaattgaatcttttttcttctt
ctcttctctatattcattcttgaattaaacacacatcaaca

FIG. 43

cttatctggggcagtgaagtatatgttatggtaatagttacgagttagttgaacttatagatagactggactatacggctatcggtccaaattaga
aagaacgtcaatggctctctgggcggaattcgtataacttcgtatagcaggagttatccgaagcgataattacccctgttatccctagcttatcga
tacgcgtgcatgctgaggtgtctcacaagtgccgtgcagtcccgccccccacttgcttctctttgtgtgtagtgtacgtacattatcgagaccgtt
gttcccgcccacctcgatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgccccccacttgcttctctttgtgtgtagtgtacgtac
attatcgagaccgttgttcccgcccacctcgatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgccccccacttgcttctctttgt
gtgtagtgtacgtacattatcgagaccgttgttcccgcccacctcgatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgccccc
acttgcttctctttgtgtgtagtgtacgtacattatcgagaccgttgttcccgcccacctcgatccggcatgcactgatcacgggcaaaagtgcg
tatatatacaagagcgtttgccagccacagattttcactccacacaccacatcacacatacaaccacacacatccacgtgggaacccgaaac
taaggatccatgaagctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctctcccaaccccacccgagccg
ccgctgtgaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgacctgcaccccgtgtctaactcgttc
gacgacgagcgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgatgggcgacgccgacatcgtgaacaccat
cctgcagtacgtgccccagatcaacttcaccaccaccgccgtggccaaccagggcatctctgtgttcgagaccaacatccgatacctgggc
ggcctgctgtctgcctacgacctgctgcgaggcccctttctcttctctggccaccaaccagaccctggtgaactctctgctgcgacaggccca
gaccctggccaacggcctgaaggtggctttaccaccccctctggcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatct
ggcgcctcttctaacaacgtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaacccccagtacgcc
cagctggcccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggcccggactgatcggcaccttcgtgtctacc
tctaacggcaccttccaggactcttccggctcttggtctggcctgatggactctttctacgagtacctgatcaagatgtacctgtacgacccgt
ggccttcgcccactacaaggaccgatgggtgctggccgccgactctaccatcgcccacctggcctctcacccctctacccgaaaggacct
gaccttcctgtcctcttacaacggccagtctacctctcccaactctggacacctggcttccttcgccggtggcaacttcatcctgggcggcatc
ctgctgaacgagcagaagtacatcgacttcggcatcaagctggcctcttcctacttcgccacctacaaccagaccgcctctggcatcggccc
cgagggcttcgcctgggtggactctgtgaccggcgctggcggctctcccccctcttctcagtctggcttctactcttctgccggcttctgggtg
accgcccccctactacatcctgcgacccgagaccctggagtctctgtactacgcctaccgagtgaccggcgactctaagtggcaggacctg
gcctgggaggccttctctgccatcgaggacgcctgtcgagccggctctgcctactcttctatcaacgacgtgacccaggccaacggtggcg
gagcctctgacgacatggagtctttctggttcgccgaggccctgaagtacgcctacctgatcttcgccgaggaatctgacgtgcaggtgcag
gccaacggcggcaacaagttcgtgttcaacaccgaggcccacccccttctctatccgatcttcttctcgacgaggcggccacctggcccatg
acgagctgtaatagcctagggtgtctgtggtatctaagctatttatcactctttacaacttctacctcaactatctactttaataaatgaatatcgttt

FIG. 44A

attctctatgattactgtatatgcgttcctctaagacaaatcgaattccatgtgtaacactcgctctggagagttagtcatccgacagggtaactct
aatctcccaacaccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaatttttcctgctcatcatctgctggattgttgtctat
cgtctggctctaatacatttattgtttattgcccaaacaactttcattgcacgtaagtgaattgttttataacagcgttcgccaattgctgcgccatc
gtcgtccggctgtcctaccgttagggtagtgtgtctcacactaccgaggttactagagttgggaaagcgatactgcctcggacacaccacct
ggtcttacgactgcagagagaatcggcgttacctcctcacaaagccctcagtgcggccgcccggggtgggcgaagaactccagcatgag
atccccgcgctggaggatcatccagccggcgtcccggaaaacgattccgaagcccaacctttcatagaaggcggcggtggaatcgaaat
ctcgtgatggcaggttgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggc
gatgcgctgcgaatcgggagcggcgataccgtaaagcacgaggaagcggtcagcccatttcgccgccaagctcttcagcaatatcacggg
tagccaacgctatgtcctgatagcggtccgccacacccagccggccacagtcgatgaatccagaaaagcggccatttttccaccatgatattc
ggcaagcaggcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgccttgagcctggcgaacagttcggctggcgcga
gcccctgatgctcttcgtccagatcatcctgatcgacaagaccggcttccatccgagtacgtgctcgctcgatgcgatgtttcgcttggtggtc
gaatgggcaggtagccggatcaagcgtatgcagccgccgcattgcatcagccatgatggatactttctcggcaggagcaaggtgagatga
caggagatcctgccccggcacttcgcccaatagcagccagtcccttcccgcttcagtgacaacgtcgagcacagctgcgcaaggaacgc
ccgtcgtggccagccacgatagccgcgctgcctcgtcctgcagttcattcagggcaccggacaggtcggtcttgacaaaaagaaccgggc
gcccctgcgctgacagccggaacacggcggcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctctccacccaag
cggccggagaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatcttgatcccctgcgccatca
gatccttggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagctggcaattccggttcgcttg
ctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaagctacctgctttctctttgcgcttgcgttttcccttgtccagatag
cccagtagctgacattcatccggggtcagcaccgtttctgcggactggctttctacgtgttccgcttcctttagcagcccttgcgccctgagtg
cttgcggcagcgtgaagctagcttatgcggtgtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctcttccgcttcctc
gctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggg
gataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgttttttccataggct
ccgccccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttcccc
ctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcat
agctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgc
cttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcga
ggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagc

FIG. 44B

cagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggcggttttttgtttgcaagcagcagattac
gcgcagaaaaaaaggatctcaagaagatcctttgatcttttcttactgaacggtgatccccaccggaattgcggccgctgtcgggaaccgcg
ttcaggtggaacaggacacctcccttgcacttcttggtatatcagtataggctgatgtattcatagtgggggttttcataataaatttactaacggc
aggcaacattcactcggcttaaacgcaaaacggaccgtcttgatatcttctgacgcattgaccaccgagaaatagtgttagttaccgggtgag
ttattgttcttctacacaggcgacgcccatcgtctagagttgatgtactaactcagatttcactacctaccctatccctggtacgcacaaagcact
ttgctagatagagtcgacaaaggcgggccccccctcgagattaccctgttatccctacataacttcgtatagcatacattatacgaagttattct
gaattccgagaaacacaacaacatgccccattggacagaccatgcggattcacaggttgtgcagtaccatacatactcgatcagacaggtc
gtctgaccatcatacaagctgaacagcgctccatacttgcacgctctctatatacacagttaaattacatatccatagtctaacctctaacagtta
atcttctggtaagcctcccagccagccttctggtatcgcttggcctcctcaataggatctcggttctggccgtacagacctcggccgacaatta
tgatatccgttccggtagacatgacatcctcaacagttcggtactgctgtccgagagcgtctcccttgtcgtcaagacccacccccgggggtca
gaataagccagtcctcagagtcgcccttaggtcggttctgggcaatgaagccaaccacaaactcggggtcggatcgggcaagctcaatgg
tctgcttggagtactcgccagtggccagagagcccttgcaagacagctcggccagcatgagcagacctctggccagcttctcgttgggaga
ggggactaggaactccttgtactgggagttctcgtagtcagagacgtcctccttcttctgttcagagacagtttcctcggcaccagctcgcag
gccagcaatgattccggttccgggtacaccgtgggcgttggtgatatcggaccactcggcgattcggtgacaccggtactggtgcttgaca
gtgttgccaatatctgcgaactttctgtcctcgaacaggaagaaaccgtgcttaagagcaagttccttgagggggagcacagtgccggcgta
ggtgaagtcgtcaatgatgtcgatatgggtcttgatcatgcacacataaggtccgaccttatcggcaagctcaatgagctccttggtggtggta
acatccagagaagcacacaggttggttttcttggctgccacgagcttgagcactcgagcggcaaaggcggacttgtggacgttagctcgag
cttcgtaggagggcattttggtggtgaagaggagactgaaataaatttagtctgcagaacttttatcggaac

FIG. 44C

tagcaggagttatccgaagcgataattaccctgttatccctagcttatcgatacggcggactgcgtccgaaccagctccagcagcgtttttcc
gggccattgagccgactgcgacccccgccaacgtgtcttggcccacgcactcatgtcatgttggtgttgggaggccacttttaagtagcaca
aggcacctagctcgcagcaaggtgtccgaaccaaagaagcggctgcagtggtgcaaacggggcggaaacggcgggaaaaagccacg
ggggcacgaattgaggcacgccctcgaatttgagacgagtcacggccccattcgcccgcgcaatggctcgccaacgcccggtcttttgca
ccacatcaggttaccccaagccaaaccttgtgttaaaaagcttaacatattataccgaacgtaggtttgggcgggcttgctccgtctgtccaa
ggcaacatttatataagggtctgcatcgccggctcaattgaatcttttttcttcttctcttctctatattcattcttgaattaaacacacatcaacagga
tccatgaagctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctctcccaaccccacccgagccgccgctgt
gaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgacctgcaccccgtgtctaactcgttcgacgac
gagcgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgatgggcgacgccgacatcgtgaacaccatcctgca
gtacgtgccccagatcaacttcaccaccaccgccgtggccaaccagggcatctctgtgttcgagaccaacatccgatacctgggcggcctg
ctgtctgcctacgacctgctgcgaggcccctttctcttctctggccaccaaccagaccctggtgaactctctgctgcgacaggcccagaccct
ggccaacggcctgaaggtggcttttaccaccccctctggcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatctggcgcc
tcttctaacaacgtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaaccccccagtacgcccagctgg
cccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggcccggactgatcggcaccttcgtgtctacctctaacgg
caccttccaggactcttccggctcttggtctggcctgatggactctttctacgagtacctgatcaagatgtacctgtacgaccccgtggccttcg
cccactacaaggaccgatgggtgctggccgccgactctaccatcgcccacctggcctctcacccctctacccgaaaggacctgaccttcct
gtcctcttacaacggccagtctacctctcccaactctggacacctggcttccttcgccggtggcaacttcatcctgggcggcatcctgctgaa
cgagcagaagtacatcgacttcggcatcaagctggcctcttcctacttcgccacctacaaccagaccgcctctggcatcggccccgagggc
ttcgcctgggtggactctgtgaccggcgctggcggctctcccccctcttctcagtctggcttctactcttctgccggcttctgggtgaccgccc
cctactacatcctgcgacccgagaccctggagtctctgtactacgcctaccgagtgaccggcgactctaagtggcaggacctggcctggga
ggccttctctgccatcgatggacgcctgtcgagccggctctgcctactcttctatcaacgacgtgacccaggccaacggtggcggagcctct
gacgacatggagtctttctggttcgccgaggccctgaagtacgcctacctgatcttcgccgaggaatctgacgtgcaggtgcaggccaacg
gcggcaacaagttcgtgttcaacaccgaggcccacccctttctctatccgatcttcttctcgacgaggcggccacctggcccatgacgagctg
taatagcctagggtgtctgtggtatctaagctatttatcactctttacaacttctacctcaactatctactttaataaatgaatatcgtttattctctatg
attactgtatatgcgttcctctaagacaaatcgaattccatgtgtaacactcgctctggagagttagtcatccgacagggtaactctaatctccca
acaccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaattttcctgctcatcatctgctggattgttgtctatcgtctggct

FIG. 45A

ctaatacatttattgtttattgcccaaacaactttcattgcacgtaagtgaattgttttataacagcgttcgccaattgctgcgccatcgtcgtccgg
ctgtcctaccgttagggtagtgtgtctcacactaccgaggttactagagttgggaaagcgatactgcctcggacacaccacctggtcttacga
ctgcagagagaatcggcgttacctcctcacaaagccctcagtgcggccgcccggggtgggcgaagaactccagcatgagatccccgcg
ctggaggatcatccagccggcgtcccggaaaacgattccgaagcccaacctttcatagaaggcggcggtggaatcgaaatctcgtgatgg
caggttgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggcgatgcgctgc
gaatcgggagcggcgataccgtaaagcacgaggaagcggtcagcccattcgccgccaagctcttcagcaatatcacgggtagccaacgc
tatgtcctgatagcggtccgccacacccagccggccacagtcgatgaatccagaaaagcggccattttccaccatgatattcggcaagcag
gcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgccttgagcctggcgaacagttcggctggcgcgagcccctgatg
ctcttcgtccagatcatcctgatcgacaagaccggcttccatccgagtacgtgctcgctcgatgcgatgtttcgcttggtggtcgaatgggca
ggtagccggatcaagcgtatgcagccgccgcattgcatcagccatgatggatactttctcggcaggagcaaggtgagatgacaggagatc
ctgccccggcacttcgcccaatagcagccagtcccttcccgcttcagtgacaacgtcgagcacagctgcgcaaggaacgcccgtcgtggc
cagccacgatagccgcgctgcctcgtcctgcagttcattcagggcaccggacaggtcggtcttgacaaaaagaaccgggcgcccctgcg
ctgacagccggaacacggcggcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctctccacccaagcggccgga
gaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatcttgatcccctgcgccatcagatccttggc
ggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagctggcaattccggttcgcttgctgtccataa
aaccgcccagtctagctatcgccatgtaagcccactgcaagctacctgctttctctttgcgcttgcgttttcccttgtccagatagcccagtagct
gacattcatccggggtcagcaccgtttctgcggactggctttctacgtgttccgcttcctttagcagcccttgcgccctgagtgcttgcggcag
cgtgaagctagcttatgcggtgtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctcttccgcttcctcgctcactgac
tcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgca
ggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgttttccataggctccgcccccc
tgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttccccctggaagctc
cctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagctcacgct
gtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgccttatccggta
actatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgtagg
cggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagccagttaccttc
ggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggcggttttttgtttgcaagcagcagattacgcgcagaaaa

FIG. 45B

aaaggatctcaagaagatcctttgatcttttcttactgaacggtgatccccaccggaattgcggccgctgtcgggaaccgcgttcaggtggaa
caggacacctcccttgcacttcttggtatatcagtataggctgatgtattcatagtggggtttttcataataaatttactaacggcaggcaacattc
actcggcttaaacgcaaaacggaccgtcttgatatcttctgacgcattgaccaccgagaaatagtgttagttaccgggtgagttattgttcttcta
cacaggcgacgcccatcgtctagagttgatgtactaactcagatttcactacctaccctatcccctggtacgcacaaagcactttgctagataga
gtcgacaaaggcgggccccccctcgagattaccctgttatccctacataacttcgtatagcatacattatacgaagttattctgaattccgccca
gagagccattgacgttctttctaatttggaccgatagccgtatagtccagtctatctataagttcaactaactcgtaactattaccataacatatact
tcactgccccagataaggttccgataaaaagttctgcagactaaatttatttcagtctcctcttcaccaccaaaatgccctcctacgaagctcga
gctaacgtccacaagtccgcctttgccgctcgagtgctcaagctcgtggcagccaagaaaaccaacctgtgtgcttctctggatgttaccacc
accaaggagctcattgagcttgccgataaggtcggaccttatgtgtgcatgatcaagacccatatcgacatcattgacgacttcacctacgcc
ggcactgtgctccccctcaaggaacttgctcttaagcacggttcttcctgttcgaggacagaaagttcgcagatattggcaacactgtcaagc
accagtaccggtgtcaccgaatcgccgagtggtccgatatcaccaacgcccacggtgtacccggaaccggaatcattgctggcctgcgag
ctggtgccgaggaaactgtctctgaacagaagaaggaggacgtctctgactacgagaactcccagtacaaggagttcctagtcccctctcc
caacgagaagctggccagaggtctgctcatgctggccgagctgtcttgcaagggctctctggccactggcgagtactccaagcagaccatt
gagcttgcccgatccgaccccgagtttgtggttggcttcattgcccagaaccgacctaagggcgactctgaggactggcttattctgacccc
cggggtgggtcttgacgacaagggagacgctctcggacagcagtaccgaactgttgaggatgtcatgtctaccggaacggatatcataatt
gtcggccgaggtctgtacggccagaaccgagatcctattgaggaggccaagcgataccagaaggctggctgggaggcttaccagaagat
taactgttagaggttagactatggatatgtaatttaactgtgtatatagagagcgtgcaagtatggagcgctgttcagcttgtatgatggtcagac
gacctgtctgatcgagtatgtatggtactgcacaacctgtgtatccgcatggtctgtccaatggggcatgttgttgtgtttctcggaattcgtata
acttcgta

FIG. 45C

caaggggtaggcgaatggtacgattccgccaagtgagactggcgatcgggagaagggttggtggtcatgggggatagaatttgtacaagt
ggaaaaaccactacgagtagcggatttgataccacaagtagcagagatatacagcaatggtgggagtgcaagtatcggaatgtactgtacc
tcctgtactcgtactcgtacggcactcgtagaaacggggcaatacgggggagaagcgatcgcccgtctgttcaatcgccacaagtccgagt
aatgctcgagtatcgaagtcttgtacctccctgtcaatcatggcaccactggtcttgacttgtctattcatactggacaagcgccagagttagct
agcgaatttcgccctcggacatcaccccatacgacggacacacatgcccgacaaacagcctctcttattgtagctgaaagtatattgaatgtg
aacgtgtacaatatcaggtaccagcgggaggttacggccaaggtgataccggaataaccctggcttggagatggtcggtccattgtactga
agtgtccgtgtcgtttccgtcactgccccaattggacatgtttgtttttccgatctttcgggcgccctctccttgtctccttgtctgtctcctggactg
ttgctaccccatttctttggcctccattggttcctccccgtctttcacgtcgtctatggttgcatggtttcccttatacttttccccacagtcacatgtt
atggaggggtctagatggacatggtgcaaggcccgcagggttgattcgacgctttccgcgaaaaaaacaagtccaaataccccccgtttatt
ctccctcggctctcggtatttcacatgaaaactataacctagactacacgggcaaccttaaccccagagtatacttatataccaaagggatgg
gtcctcaaaaatcacacaagcaacggatccatgaagctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctc
tcccaaccccaccccgagccgccgctgtgaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgacct
gcaccccgtgtctaactcgttcgacgacgagcgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgatgggcga
cgccgacatcgtgaacaccatcctgcagtacgtgccccagatcaacttcaccaccaccgccgtggccaaccagggcatctctgtgttcgag
accaacatccgatacctgggcggcctgctgtctgcctacgacctgctgcgaggcccctctcttctctggccaccaaccagaccctggtgaa
ctctctgctgcgacaggcccagaccctggccaacggcctgaaggtggcttttaccaccccctctggcgtgccccgaccccaccgtgttcttca
accccaccgtgcgacgatctggcgcctcttctaacaacgtggccgagatcggctctctggtgctggagtggaccccgactgtctgacctgac
cggcaacccccagtacgcccagctggcccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggccccggactg
atcggcaccttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgatggactctttctacgagtacctgatcaa
gatgtacctgtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggccgccgactctaccatcgcccacctggcctctcac
ccctctacccgaaaggacctgaccttcctgtcctcttacaacggccagtctacctctcccaactctggacacctggcttccttcgccggtggc
aacttcatcctgggcggcatcctgctgaacgagcagaagtacatcgacttcggcatcaagctggcctcttcctacttcgccacctacaacca
gaccgcctctggcatcggccccgagggcttcgcctgggtggactctgtgaccggcgctggcggctctccccctcttctcagtctggcttct
actcttctgccggcttctgggtgaccgcccctactacatcctgcgacccgagaccctggagtctctgtactacgcctaccgagtgaccggc
gactctaagtggcaggacctggcctgggaggccttctctgccatcgaggacgcctgtcgagccggctctgcctactcttctatcaacgacgt
gacccaggccaacggtggcggagcctctgacgacatggagtctttctggttcgccgaggccctgaagtacgcctacctgatcttcgccgag

FIG. 46A

gaatctgacgtgcaggtgcaggccaacggcggcaacaagttcgtgttcaacaccgaggcccacccccttctctatccgatcttcttctcgacg
aggcggccacctggcccatgacgagctgtaatagcctagggtgtctgtggtatctaagctatttatcactctttacaacttctacctcaactatct
actttaataaatgaatatcgtttattctctatgattactgtatatgcgttcctctaagacaaatcgaattccatgtgtaacactcgctctggagagtta
gtcatccgacagggtaactctaatctcccaacaccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaatttttcctgctca
tcatctgctggattgttgtctatcgtctggctctaatacatttattgtttattgcccaaacaactttcattgcacgtaagtgaattgttttataacagcgt
tcgccaattgctgcgccatcgtcgtccggctgtcctaccgttagggtagtgtgtctcacactaccgaggttactagagttgggaaagcgatac
tgcctcggacacaccacctggtcttacgactgcagagagaatcggcgttacctcctcacaaagccctcagtgcggccgcccggggtgggc
gaagaactccagcatgagatccccgcgctggaggatcatccagccggcgtccccggaaaacgattccgaagcccaacctttcatagaagg
cggcggtggaatcgaaatctcgtgatggcaggttgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtca
agaaggcgatagaaggcgatgcgctgcgaatcgggagcggcgataccgtaaagcacgaggaagcggtcagcccattcgccgccaagc
tcttcagcaatatcacgggtagccaacgctatgtcctgatagcggtccgccacacccagccggccacagtcgatgaatccagaaaagcgg
ccatttttccaccatgatattcggcaagcaggcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgccttgagcctggcga
acagttcggctggcgcgagcccctgatgctcttcgtccagatcatcctgatcgacaagaccggcttccatccgagtacgtgctcgctcgatg
cgatgtttcgcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgccgcattgcatcagccatgatggatactttctcggc
aggagcaaggtgagatgacaggagatcctgccccggcacttcgcccaatagcagccagtcccttcccgcttcagtgacaacgtcgagcac
agctgcgcaaggaacgcccgtcgtggccagccacgatagccgcgctgcctcgtcctgcagttcattcagggcaccggacaggtcggtctt
gacaaaaagaaccgggcgcccctgcgctgacagccggaacacggcggcatcagagcagccgattgtctgttgtgcccagtcatagccg
aatagcctctccacccaagcggccggagaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatc
ttgatccccctgcgccatcagatccttggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagct
ggcaattccggttcgcttgctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaagctacctgctttctctttgcgcttgc
gttttcccttgtccagatagcccagtagctgacattcatccggggtcagcaccgtttctgcggactggctttctacgtgttccgcttcctttagca
gcccttgcgccctgagtgcttgcggcagcgtgaagctagcttatgcggtgtgaaataccgcacagatgcgtaaggagaaaataccgcatca
ggcgctcttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacgg
ttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgc
tggcgttttccataggctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaag

FIG. 46B

ataccaggcgtttcccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcggga
agcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttc
agcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaa
caggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggta
tctgcgctctgctgaagccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggcggttttttgtt
tgcaagcagcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttcttactgaacggtgatccccaccggaattgcggcc
gctgtcgggaaccgcgttcaggtggaacaggacacctcccttgcacttcttggtatatcagtatataggctgatgtattcatagtgggggttttcat
aataaatttactaacggcaggcaacattcactcggcttaaacgcaaaacggaccgtcttgatatcttctgacgcattgaccaccgagaaatag
tgttagttaccgggtgagttattgttcttctacacaggcgacgcccatcgtctagagttgatgtactaactcagatttcactacctaccctatccct
ggtacgcacaaagcactttgctagatagagtcgacaaaggcgggcccccctctcgagattaccctgttatccctacataacttcgtatagcata
cattatacgaagttattctgaattccgcccagagagccattgacgttcttctaatttggaccgatagccgtatagtccagtctatctataagttca
actaactcgtaactattaccataacatatacttcactgccccagataaggttccgataaaaagttctgcagactaaatttatttcagtctcctcttca
ccaccaaaatgccctcctacgaagctcgagctaacgtccacaagtccgccttgccgctcgagtgctcaagctcgtggcagccaagaaaac
caacctgtgtgcttctctggatgttaccaccaccaaggagctcattgagcttgccgataaggtcggaccttatgtgtgcatgatcaagacccat
atcgacatcattgacgacttcacctacgccggcactgtgctccccctcaaggaacttgctcttaagcacggtttcttcctgttcgaggacagaa
agttcgcagatattggcaacactgtcaagcaccagtaccggtgtcaccgaatcgccgagtggtccgatatcaccaacgcccacggtgtacc
cggaaccggaatcattgctggcctgcgagctggtgccgaggaaactgtctctgaacagaagaaggaggacgtctctgactacgagaactc
ccagtacaaggagttcctagtcccctctcccaacgagaagctggccagaggtctgctcatgctggccgagctgtcttgcaagggctctctgg
ccactggcgagtactccaagcagaccattgagcttgcccgatccgaccccgagtttgtggttggcttcattgcccagaaccgacctaaggg
cgactctgaggactggcttattctgacccccgggggtgggtcttgacgacaagggagacgctctcggacagcagtaccgaactgttgaggat
gtcatgtctaccggaacggatatcataattgtcggccgaggtctgtacggccagaaccgagatcctattgaggaggccaagcgataccaga
aggctggctgggaggcttaccagaagattaactgttagaggttagactatggatatgtaatttaactgtgtatatagagagcgtgcaagtatgg
agcgctgttcagcttgtatgatggtcagacgacctgtctgatcgagtatgtatggtactgcacaacctgtgtatccgcatggtctgtccaatgg
ggcatgttgttgtgtttctcggaattcgtataacttcgtatagcaggagttatccgaagcgataattaccctgttatccctagcttatcgattcccac
aagacgaacaagtgataggccgagagccgaggacgaggtggagtgca

FIG. 46C

taattaccctgttatccctagcttatcgatagagaccgggttggcggcgtatttgtgtcccaaaaaacagccccaattgccccaattgacccca
aattgacccagtagcgggcccaaccccggcgagagcccccttcaccccacatatcaaacctcccccggttcccacacttgccgttaaggg
cgtagggtactgcagtctggaatctacgcttgttcagactttgtactagtttctttgtctggccatccgggtaacccatgccggacgcaaaatag
actactgaaaatttttttgctttgtggttgggactttagccaagggtataaaagaccaccgtcccccgaattacctttcctcttctttttctctctcctt
gtcaactcacacccgaaggatccatgaagctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctctcccaac
cccacccgagccgccgctgtgaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgacctgcacccc
gtgtctaactcgttcgacgacgagcgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgatgggcgacgccgac
atcgtgaacaccatcctgcagtacgtgccccagatcaacttcaccaccaccgccgtggccaaccagggcatctctgtgttcgagaccaaca
tccgatacctgggcggcctgctgtctgcctacgacctgctgcgaggcccctctcttctctggccaccaaccagaccctggtgaactctctgc
tgcgacaggcccagaccctggccaacggcctgaaggtggcttttaccacccctctggcgtgcccgaccccaccgtgttcttcaaccccac
cgtgcgacgatctggcgcctcttctaacaacgtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaac
ccccagtacgcccagctggcccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggcccggactgatcggcac
cttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgatggactctttctacgagtacctgatcaagatgtacct
gtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggccgccgactctaccatcgcccacctggcctctcaccccctctacc
cgaaaggacctgaccttcctgtcctcttacaacggccagtctacctctcccaactctggacacctggcttccttcgccggtggcaacttcatcc
tgggcggcatcctgctgaacgagcagaagtacatcgacttcggcatcaagctggcctcttcctacttcgccacctacaaccagaccgcctct
ggcatcggccccgagggcttcgcctgggtggactctgtgaccggcgctggcggctctccccccttcttctcagtctggcttctactcttctgcc
ggcttctgggtgaccgcccccctactacatcctgcgacccgagaccctggagtctctgtactacgcctaccgagtgaccggcgactctaagt
ggcaggacctggcctgggaggccttctctgccatcgaggacgcctgtcgagccggctctgcctactcttctatcaacgacgtgacccaggc
caacggtggcggagcctctgacgacatggagtctttctggttcgccgaggccctgaagtacgcctacctgatcttcgccgaggaatctgac
gtgcaggtgcaggccaacggcggcaacaagttcgtgttcaacaccgaggcccacccctttctctatccgatcttcttctcgacgaggcggcc
acctggcccatgacgagctgtaatagcctagggtgtctgtggtatctaagctatttatcactctttacaacttctacctcaactatctacttaataa
atgaatatcgtttattctctatgattactgtatatgcgttcctctaagacaaatcgaattccatgtgtaacactcgctctggagagttagtcatccga
cagggtaactctaatctcccaacaccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaatttcctgctcatcatctgctg
gattgttgtctatcgtctggctctaatacatttattgtttattgcccaaacaacttttcattgcacgtaagtgaattgttttataacagcgttcgccaatt
gctgcgccatcgtcgtccggctgtcctaccgttagggtagtgtgtctcacactaccgaggttactagagttgggaaagcgatactgcctcgg

FIG. 47A

acacaccacctggtcttacgactgcagagagaatcggcgttacctcctcacaaagccctcagtgcggccgcccggggtgggcgaagaac
tccagcatgagatccccgcgctggaggatcatccagccggcgtcccggaaaacgattccgaagcccaacctttcatagaaggcggcggt
ggaatcgaaatctcgtgatggcaggttgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtcaagaaggc
gatagaaggcgatgcgctgcgaatcgggagcggcgataccgtaaagcacgaggaagcggtcagcccatttcgccgccaagctcttcagc
aatatcacgggtagccaacgctatgtcctgatagcggtccgccacacccagccggccacagtcgatgaatccagaaaagcggccattttcc
accatgatattcggcaagcaggcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgccttgagcctggcgaacagttcg
gctggcgcgagcccctgatgctcttcgtccagatcatcctgatcgacaagaccggcttccatccgagtacgtgctcgctcgatgcgatgtttc
gcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgccgcattgcatcagccatgatggatactttctcggcaggagcaa
ggtgagatgacaggagatcctgccccggcacttcgcccaatagcagccagtcccttcccgcttcagtgacaacgtcgagcacagctgcgc
aaggaacgcccgtcgtggccagccacgatagccgcgctgcctcgtcctgcagttcattcagggcaccggacaggtcggtcttgacaaaaa
gaaccgggcgcccctgcgctgacagccggaacacggcggcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctct
ccacccaagcggccggagaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatcttgatcccct
gcgccatcagatccttggcggcaagaaagccatccagtttacttgcagggcttcccaaccttaccagagggcgccccagctggcaattcc
ggttcgcttgctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaagctacctgctttctctttgcgcttgcgttttcccttg
tccagatagcccagtagctgacattcatccggggtcagcaccgtttctgcggactggctttctacgtgttccgcttcctttagcagcccttgcgc
cctgagtgcttgcggcagcgtgaagctagcttatgcggtgtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctcttc
cgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacag
aatcaggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaggccgcgttgctggcgttttc
cataggctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggc
gtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaagcgtggcg
ctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgacc
gctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagc
agagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctg
ctgaagccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggcggttttttgtttgcaagcagc
agattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttcttactgaacggtgatccccaccggaattgcggccgctgtcggga
accgcgttcaggtggaacaggacacctccttgcacttcttggtatatcagtataggctgatgtattcatagtggggttttttcataataaatttact

FIG. 47B

aacggcaggcaacattcactcggcttaaacgcaaaacggaccgtcttgatatcttctgacgcattgaccaccgagaaatagtgttagttaccg
ggtgagttattgttcttctacacaggcgacgcccatcgtctagagttgatgtactaactcagatttcactacctaccctatccctggtacgcacaa
agcactttgctagatagagtcgacaaaggcgggccccccctcgagattaccctgttatccctacataacttcgtatagcatacattatacgaag
ttattctgaattccgagaaacacaacaacatgccccattggacagaccatgcggatacacaggttgtgcagtaccatacatactcgatcagac
aggtcgtctgaccatcatacaagctgaacagcgctccatacttgcacgctctctatatacacagttaaattacatatccatagtctaacctctaac
agttaatcttctggtaagcctcccagccagccttctggtatcgcttggcctcctcaataggatctcggttctggccgtacagacctcggccgac
aattatgatatccgttccggtagacatgacatcctcaacagttcggtactgctgtccgagagcgtctcccttgtcgtcaagacccacccccggg
ggtcagaataagccagtcctcagagtcgcccttaggtcggttctgggcaatgaagccaaccacaaactcggggtcggatcgggcaagctc
aatggtctgcttggagtactcgccagtggccagagagcccttgcaagacagctcggccagcatgagcagacctctggccagcttctcgttg
ggagaggggactaggaactccttgtactgggagttctcgtagtcagagacgtcctccttcttctgttcagagacagtttcctcggcaccagct
cgcaggccagcaatgattccggttccgggtacaccgtgggcgttggtgatatcggaccactcggcgattcggtgacaccggtactggtgct
tgacagtgttgccaatatctgcgaactttctgtcctcgaacaggaagaaaccgtgcttaagagcaagttccttgagggggagcacagtgccg
gcgtaggtgaagtcgtcaatgatgtcgatatgggtcttgatcatgcacacataaggtccgaccttatcggcaagctcaatgagctccttggtg
gtggtaacatccagagaagcacacaggttggttttcttggctgccacgagcttgagcactcgagcggcaaaggcggacttgtggacgttag
ctcgagcttcgtaggagggcattttggtggtgaagaggagactgaaataaatttagtctgcagaacttttatcggaaccttatctggggcagt
gaagtatatgttatggtaatagttacgagttagttgaacttatagatagactggactatacggctatcggtccaaattagaaagaacgtcaatgg
ctctctgggcggaattcgtataacttcgtatagcaggagttatccgaagcga

FIG. 47C

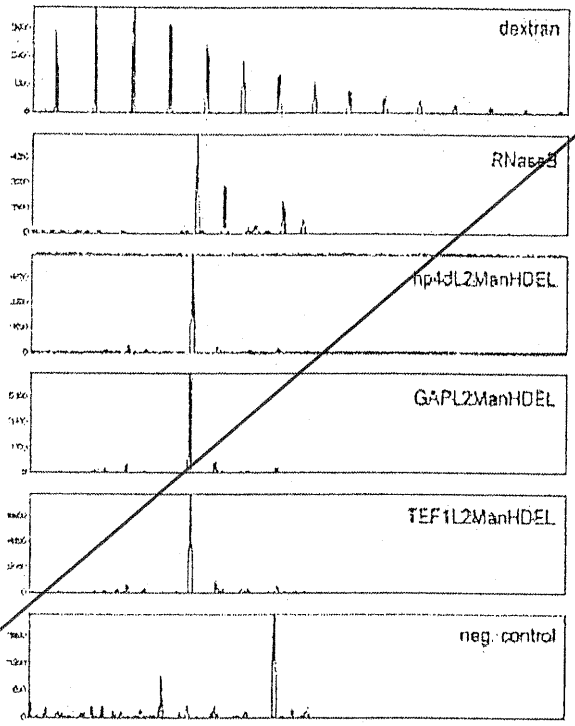

dextran
RNaseB
hp4dL2ManHDEL
GAPL2ManHDEL
TEF1L2ManHDEL
neg. control

FIG. 48

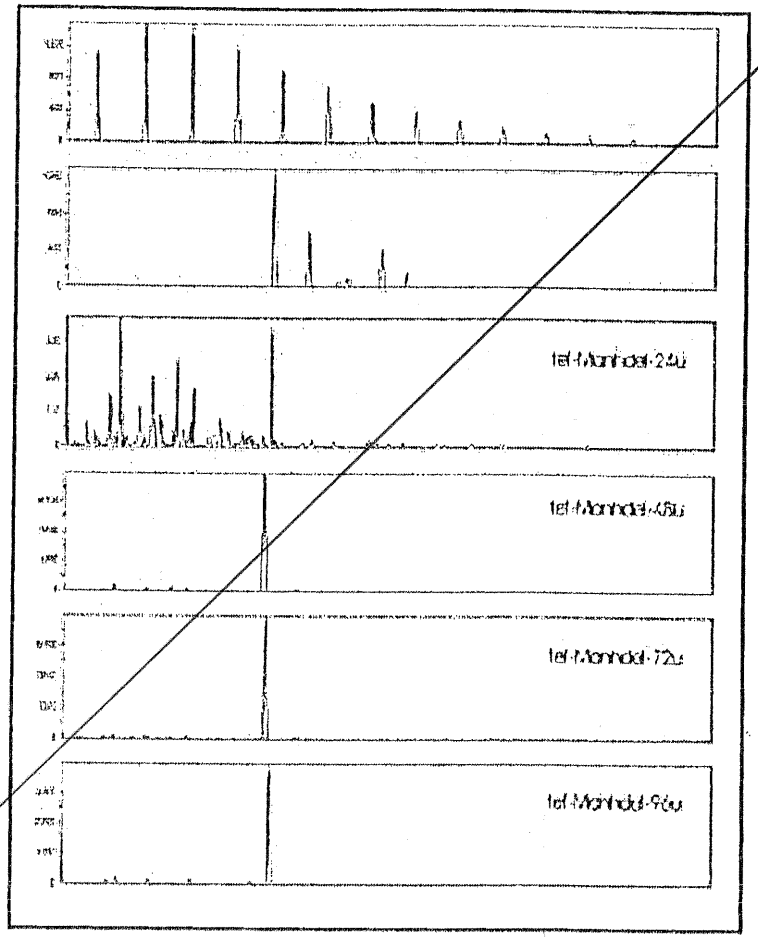

Ref-Monodisperse-24h
Ref-Monodisperse-48h
Ref-Monodisperse-72h
Ref-Monodisperse-96h

FIG. 49

gagctcagcgctcgaccctgtatccccaagtctttcggctactcttctgtggtgtgtgtgtgtaacgccacctactgtgactcgttcgaccccccc
caccttccccgccctgggcaccttctctcgatacgagtctacccgatctggccgacgaatggagctgtctatgggccccatccaggccaac
cacaccggcaccggcctgctgctgaccctgcagcccgagcagaagttccagaaggtcaagggcttcggcggagccatgaccgacgccg
ctgccctgaacatcctggccctgtctccccccgctcagaacctgctcctgaagtcttacttctctgaggagggcatcggctacaacatcatcc
gagtgcctatggcctcttgtgacttctctatccgaacctacacctacgccgacacccccgacgacttccagctgcacaacttctccctgcccg
aggaggacaccaagctgaagatcccccctgatccaccgagccctgcagctggcccagcgacccgtgtctctgctggcctctccctggacct
ctcccacctggctcaagaccaacggcgccgtgaacggcaagggctctctgaagggccagcccggcgacatctaccaccagacctgggc
ccgatacttcgtgaagttcctggacgcctacgccgagcacaagctgcagttctgggccgtgaccgccgagaacgagccctctgctggact
gctgtccggctacccctttccagtgtctgggcttcaccccccgagcaccagcgagacttcatcgcccgagacctgggacccaccctggccaa
ctctacccaccacaacgtgcgactgctgatgctggacgaccagcgactgctgctgccccactgggccaaggtggtgctgaccgaccccg
aggccgccaagtacgtccacggcatcgctgtccattggtatctggacttctggctcccgccaaggccaccctgggcgagacccaccgact
gttccccaacaccatgctgttcgcctctgaggcttgtgtgggctctaagttctgggagcagtctgtgcgactgggctcttgggaccgaggcat
gcagtactctcactctatcatcaccaacctgctgtaccacgtggtgggctggaccgactggaacctggccctgaaccccgagggcggacc
caactgggtgcgaaacttcgtggactctcccatcatcgtggacatcaccaaggacaccttctacaagcagcccatgttctaccacctgggcc
acttctctaagttcatccccgagggctctcagcgagtgggcctggtggcctctcagaagaacgacctggacgccgtggccctgatgcaccc
cgacggctctgccgtggtggtggtcctgaaccgatcttctaaggacgtgcccctgaccatcaaggaccccgccgtgggcttcctggagacc
atctctcccggctactctatccacacctacctgtggcgacgacagtaatagcctaggggtacc

FIG. 50

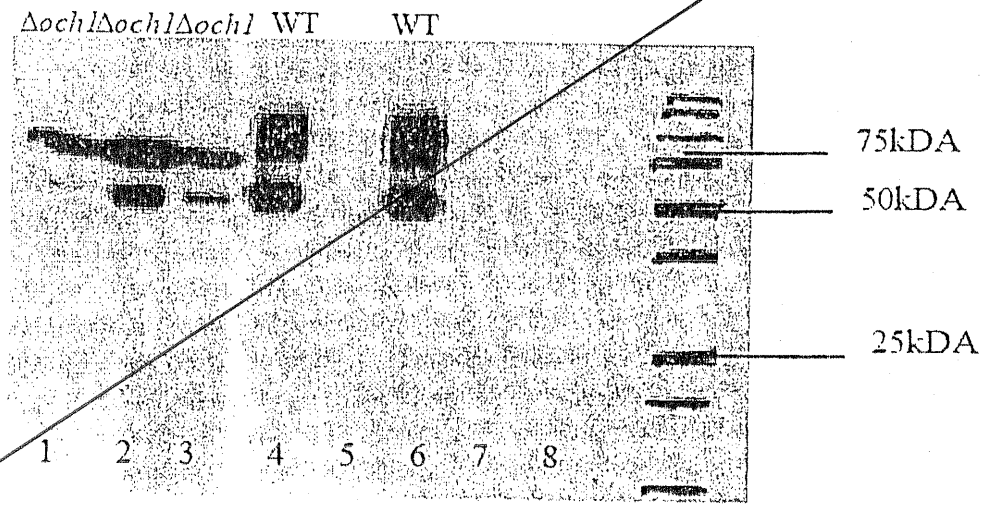


FIG. 51

gctccccccgactgatctgtgactctcgagtgctggaacgatacctgctggaagccaaggaagccgagaacatcaccaccggctgtgcc
gagcactgttctctgaacgagaacattaccgtgcccgacaccaaggtgaacttctacgcctggaagcgaatggaggtgggccagcaggcc
gtggaggtgtggcagggactggctctgctgtctgaggccgtgctgcgaggacaggctctcctggtgaactcttctcagccctgggagcccc
tgcagctgcacgtggacaaggccgtgtctggcctgcgatctctgaccaccctgctgcgagccctcggtgctcagaaggaagccatctctcc
ccccgacgccgcctctgctgccccccctgcgaaccatcaccgccgacaccttccgaaagctgttccgagtgtactctaacttcctgcgaggc
aagctgaagctgtacaccggcgaggcttgtcgaaccggcgaccga

FIG. 52

ctggacaacggcctggcccgaacccccaccatgggctggctgcactgggagcgattcatgtgtaacctggactgtcaggaagagcccga
ctcttgtatctctgagaagctgttcatggaaatggccgagctgatggtgtctgagggctggaaggacgccggctacgagtacctgtgtatcg
acgactgttggatggcccccccagcgagactctgagggccgactccaggccgacccccagcgattcccccacggcatccgacagctcgc
caactacgtgcactctaagggcctgaagctgggcatctacgccgacgtgggcaacaagacctgtgccggcttccccggctctttcggctac
tacgacatcgacgcccagaccttcgccgactggggcgtggacctgctgaagttcgacggctgttactgtgactctctcgagaacctggccg
acggctacaagcacatgtctctggccctgaaccgaaccggccgatctatcgtgtactcttgtgagtggcccctgtacatgtggcccttccaga
agcccaactacaccgagatccgacagtactgtaaccactggcgaaacttcgccgacatcgacgactcgtggaagtctatcaagtctattctg
gactggacctctttcaaccaggagcgaatcgtcgacgtcgccggacccggcggatggaacgaccccgacatgctggtgatcggcaacttc
ggcctgtcttggaaccagcaggtgacccagatggccctgtgggctatcatggctgcccccctgttcatgtctaacgacctgcgacacatctct
ccccaggccaaggccctgctccaggacaaggacgtgatcgccatcaaccaggacccccctgggcaagcagggctaccagctccgacag
ggcgacaacttcgaggtgtgggagcgaccccctgtctggcctggcctgggccgtggccatgatcaaccgacaggagatcggcggacccc
gatcttacaccatcgccgtggcctccctgggaaagggcgtggcctgtaaccccgcctgtttcatcacccagctcctgcccgtgaagcgaaa
gctgggattctacgagtggacctctcgactgcgatctcacatcaaccccaccggcaccgtgctgctccagctcgagaacaccatgcagatg
tctctgaaggacctgctg

FIG. 53

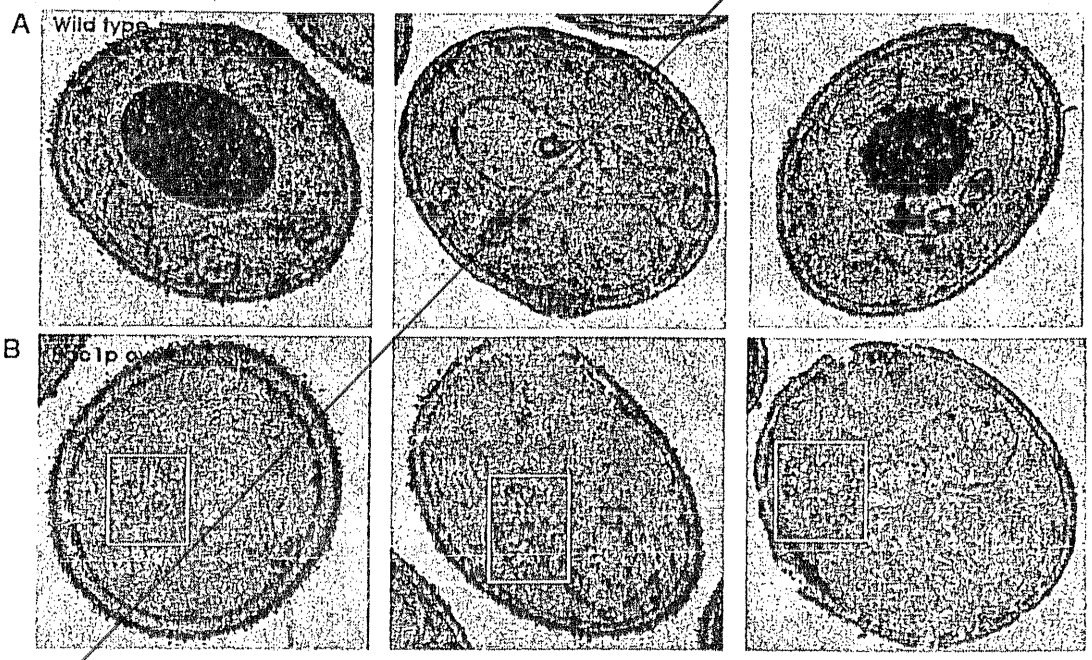


FIG. 54

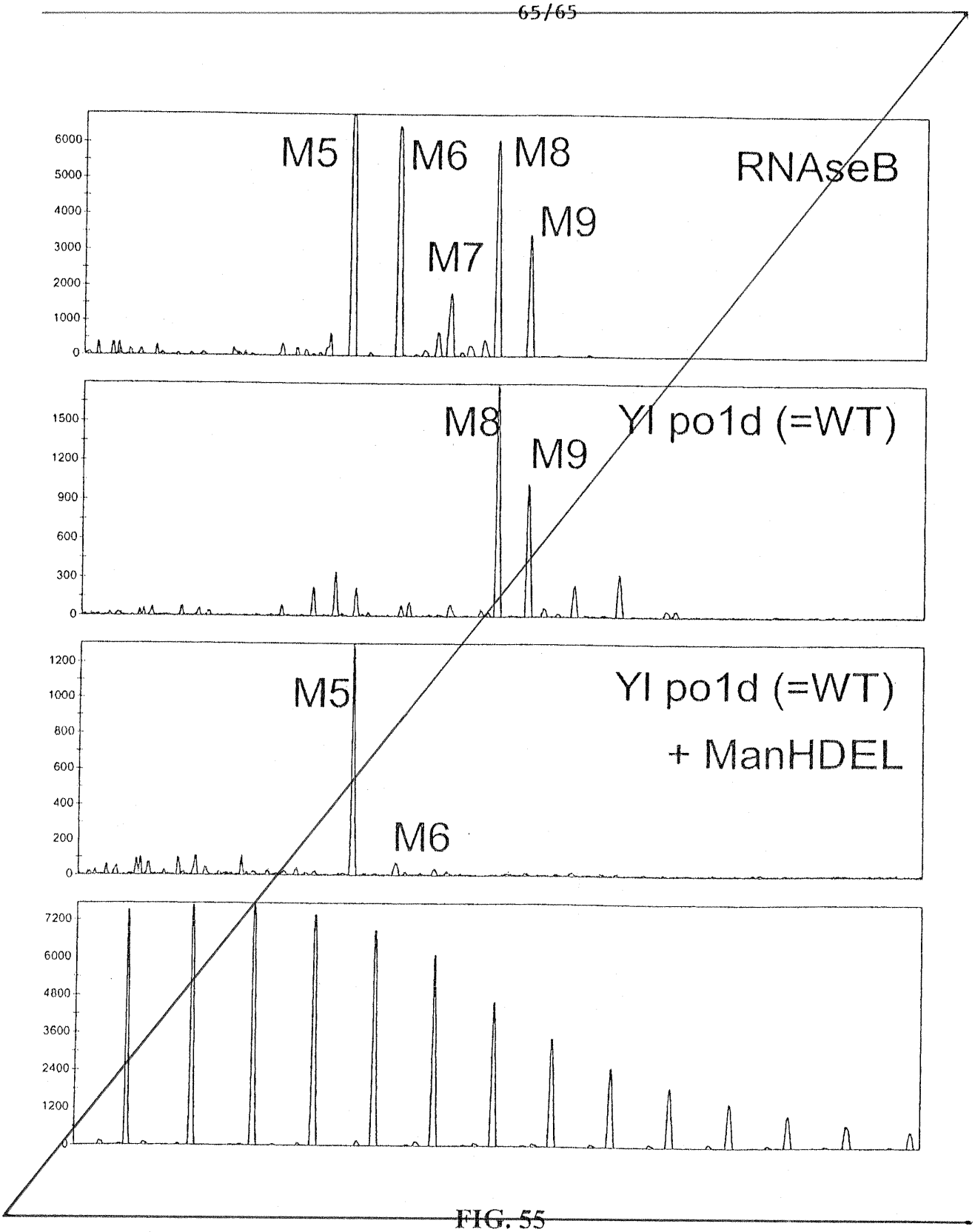


FIG. 55

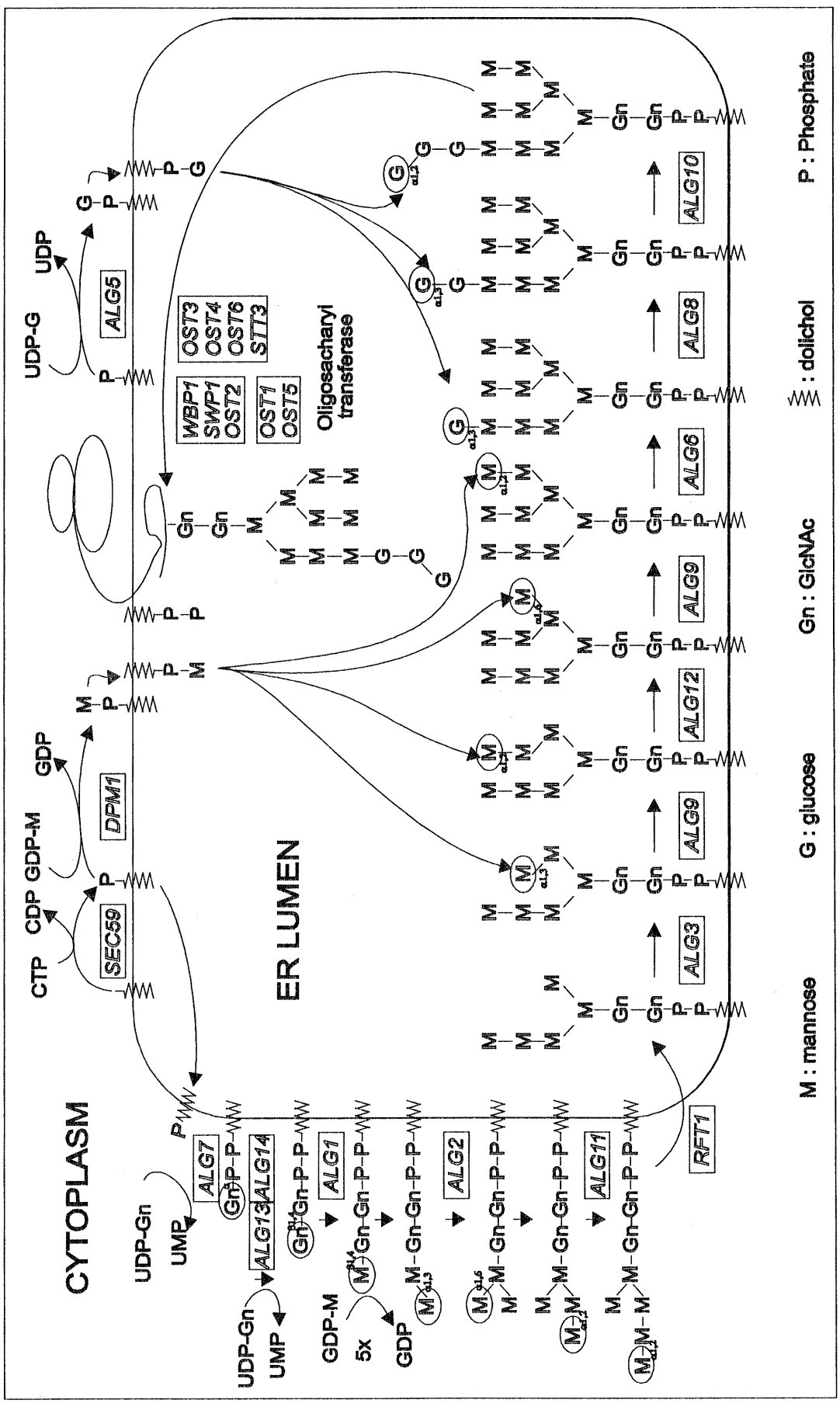

CYTOPLASM
ER LUMEN
UDP-Gn
UMP
CTP
CDP
GDP-M
GDP
UDP-G
UDP
ALG7
ALG13
ALG14
ALG1
ALG2
ALG11
RFT1
SEC59
DPM1
ALG5
ALG3
ALG9
ALG12
ALG6
ALG8
ALG10
WBP1
SWP1
OST2
OST3
OST4
OST6
STT3
OST1
OST5
Oligosaccharyl transferase
5x
M : mannose
G : glucose
Gn : GlcNAc
: dolichol
P : Phosphate

FIG. 1

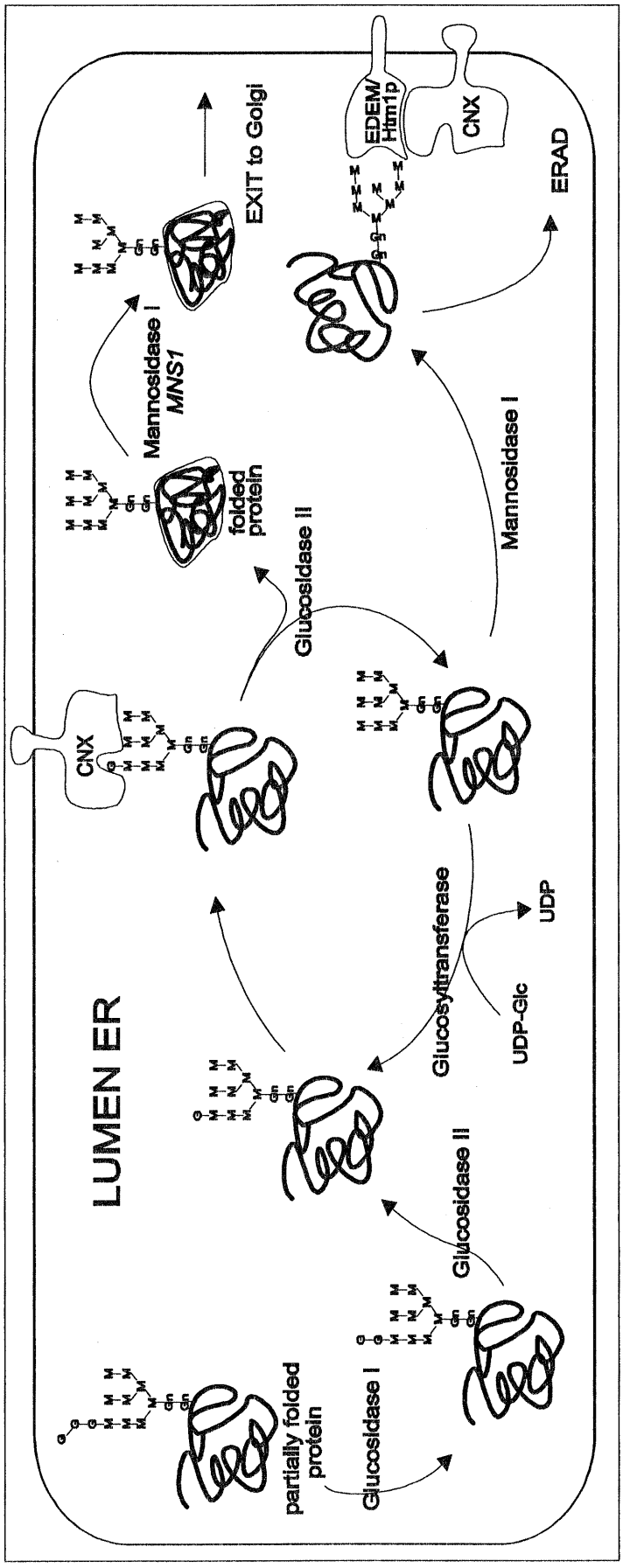

LUMEN ER
partially folded protein
Glucosidase I
Glucosidase II
Glucosyltransferase
UDP-Glc
UDP
folded protein
Mannosidase I
MNS1
EXIT to Golgi
Glucosidase II
CNX
EDEM/ Htm1p
CNX
ERAD
Mannosidase I

FIG. 2

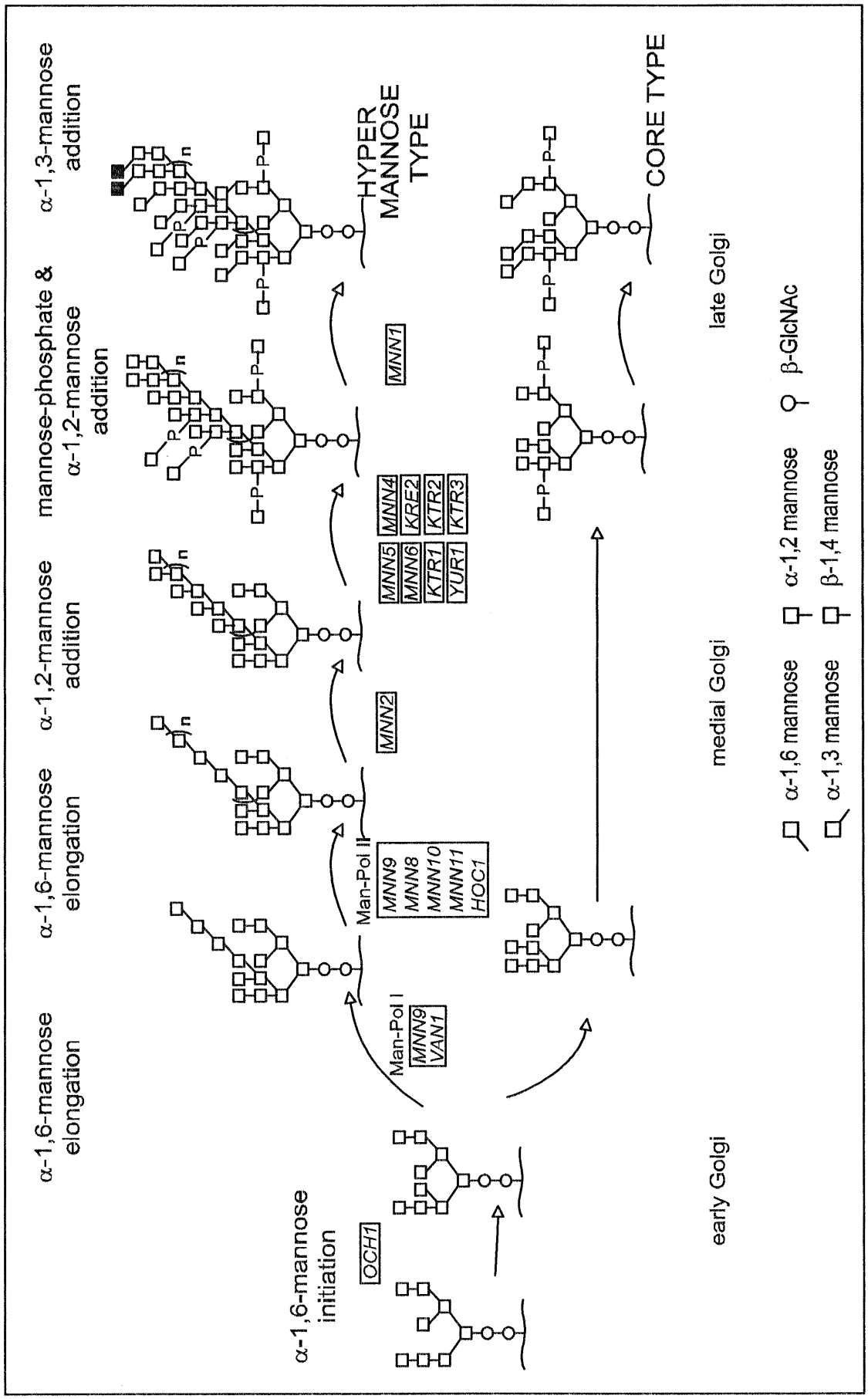

α-1,6-mannose elongation
α-1,6-mannose elongation
mannose-phosphate & α-1,2-mannose addition
α-1,3-mannose addition
α-1,6-mannose initiation
α-1,2-mannose addition
HYPER MANNOSE TYPE
CORE TYPE
OCH1
Man-Pol I
MNN9
VAN1
Man-Pol II
MNN9
MNN8
MNN10
MNN11
HOC1
MNN2
MNN5
MNN6
KTR1
YUR1
MNN4
KRE2
KTR2
KTR3
MNN1
early Golgi
medial Golgi
late Golgi
α-1,6 mannose
α-1,3 mannose
α-1,2 mannose
β-1,4 mannose
β-GlcNAc

FIG. 3

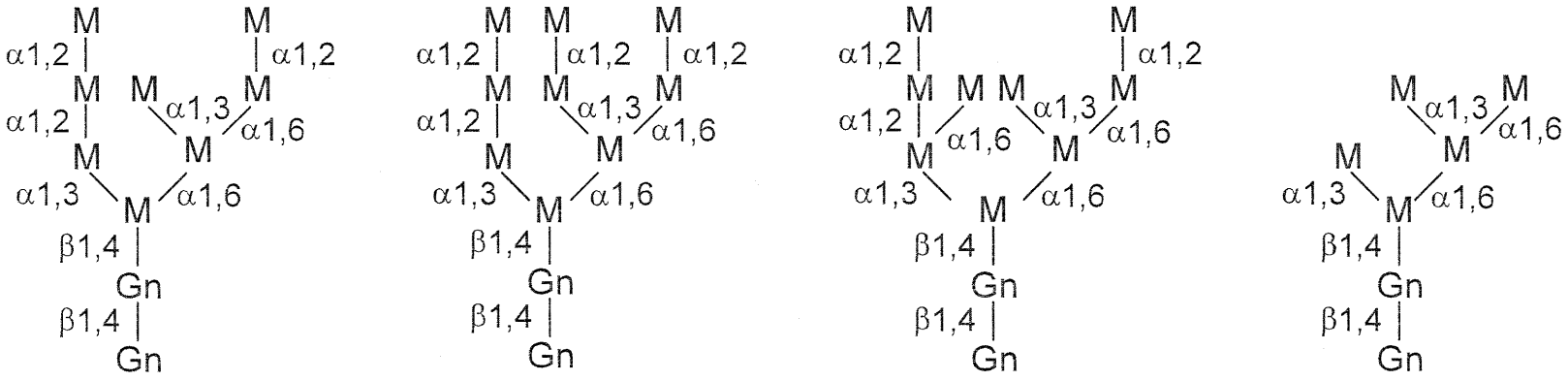
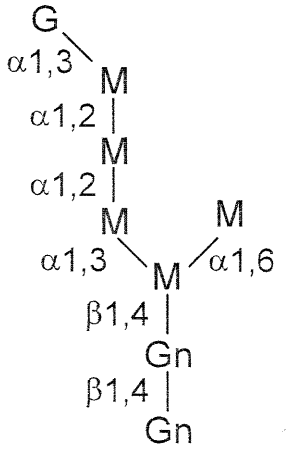
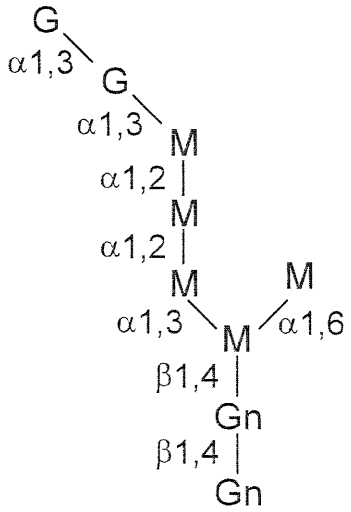


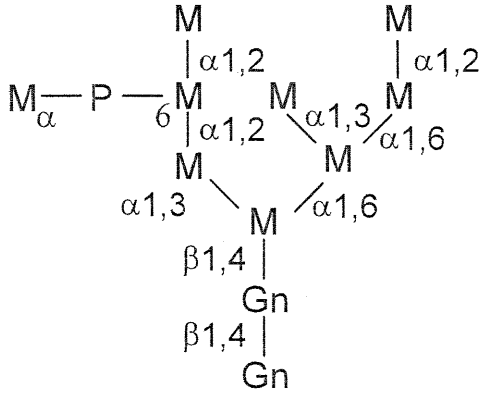
FIG. 4A



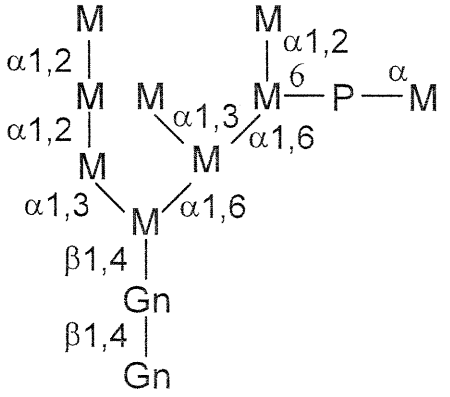
VIII



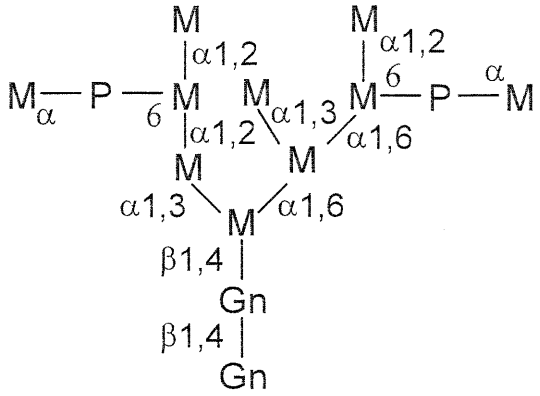
IX



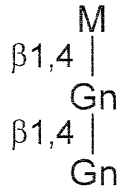
X



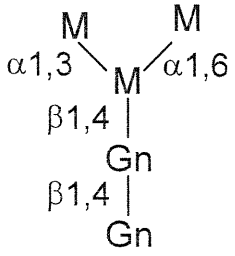
XI



XII



XIII



XIV

M: Mannose G: Glucose Gn: N-acetylglucosamine P: Phosphate

FIG. 4B

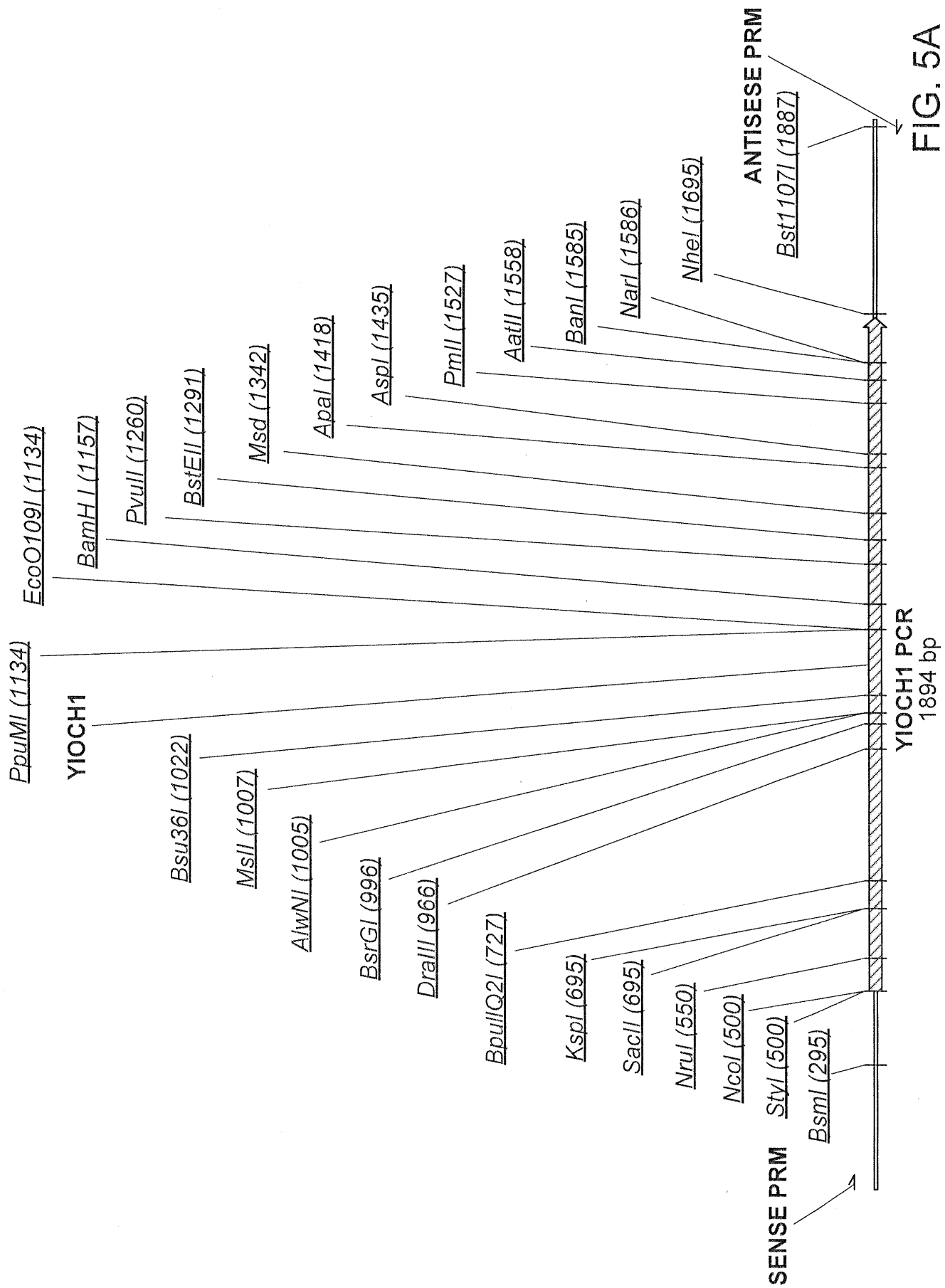

PpuMI (1134)
YIOCH1
Bsu36I (1022)
MslI (1007)
AlwNI (1005)
BsrGI (996)
DraIII (966)
BpuIIQ2I (727)
KspI (695)
SacII (695)
NruI (550)
NcoI (500)
StyI (500)
BsmI (295)
SENSE PRM
EcoO109I (1134)
BamH I (1157)
PvuII (1260)
BstEII (1291)
Msd (1342)
ApaI (1418)
AspI (1435)
PmlI (1527)
AatII (1558)
BanI (1585)
NarI (1586)
NheI (1695)
Bst1107I (1887)
ANTISESE PRM
YIOCH1 PCR
1894 bp

FIG. 5A

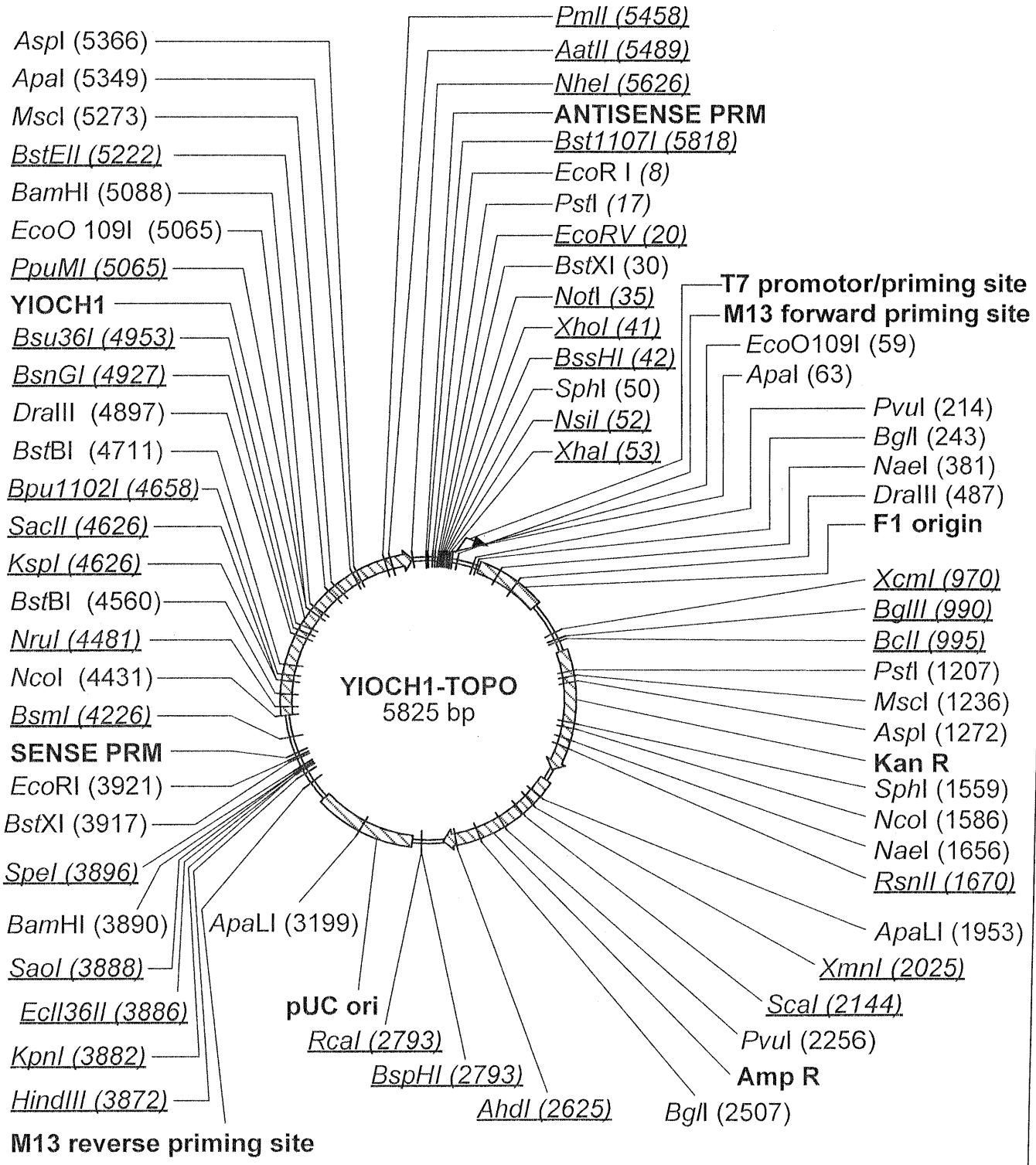

YIOCH1-TOPO
5825 bp
PmlI (5458)
AatII (5489)
NheI (5626)
ANTISENSE PRM
Bst1107I (5818)
EcoR I (8)
PstI (17)
EcoRV (20)
BstXI (30)
NotI (35)
XhoI (41)
BssHI (42)
SphI (50)
NsiI (52)
XhaI (53)
T7 promotor/priming site
M13 forward priming site
EcoO109I (59)
ApaI (63)
PvuI (214)
BglI (243)
NaeI (381)
DraIII (487)
F1 origin
XcmI (970)
BglII (990)
BclI (995)
PstI (1207)
MscI (1236)
AspI (1272)
Kan R
SphI (1559)
NcoI (1586)
NaeI (1656)
RsnII (1670)
ApaLI (1953)
XmnI (2025)
ScaI (2144)
PvuI (2256)
Amp R
BglI (2507)
AhdI (2625)
BspHI (2793)
RcaI (2793)
pUC ori
ApaLI (3199)
BamHI (3890)
SaoI (3888)
EcII36II (3886)
KpnI (3882)
HindIII (3872)
M13 reverse priming site
SpeI (3896)
BstXI (3917)
EcoRI (3921)
SENSE PRM
BsmI (4226)
NcoI (4431)
NruI (4481)
BstBI (4560)
KspI (4626)
SacII (4626)
Bpu1102I (4658)
BstBI (4711)
DraIII (4897)
BsnGI (4927)
Bsu36I (4953)
YIOCH1
PpuMI (5065)
EcoO 109I (5065)
BamHI (5088)
BstEII (5222)
MscI (5273)
ApaI (5349)
AspI (5366)
PCR
A
PCR
B

FIG. 5B

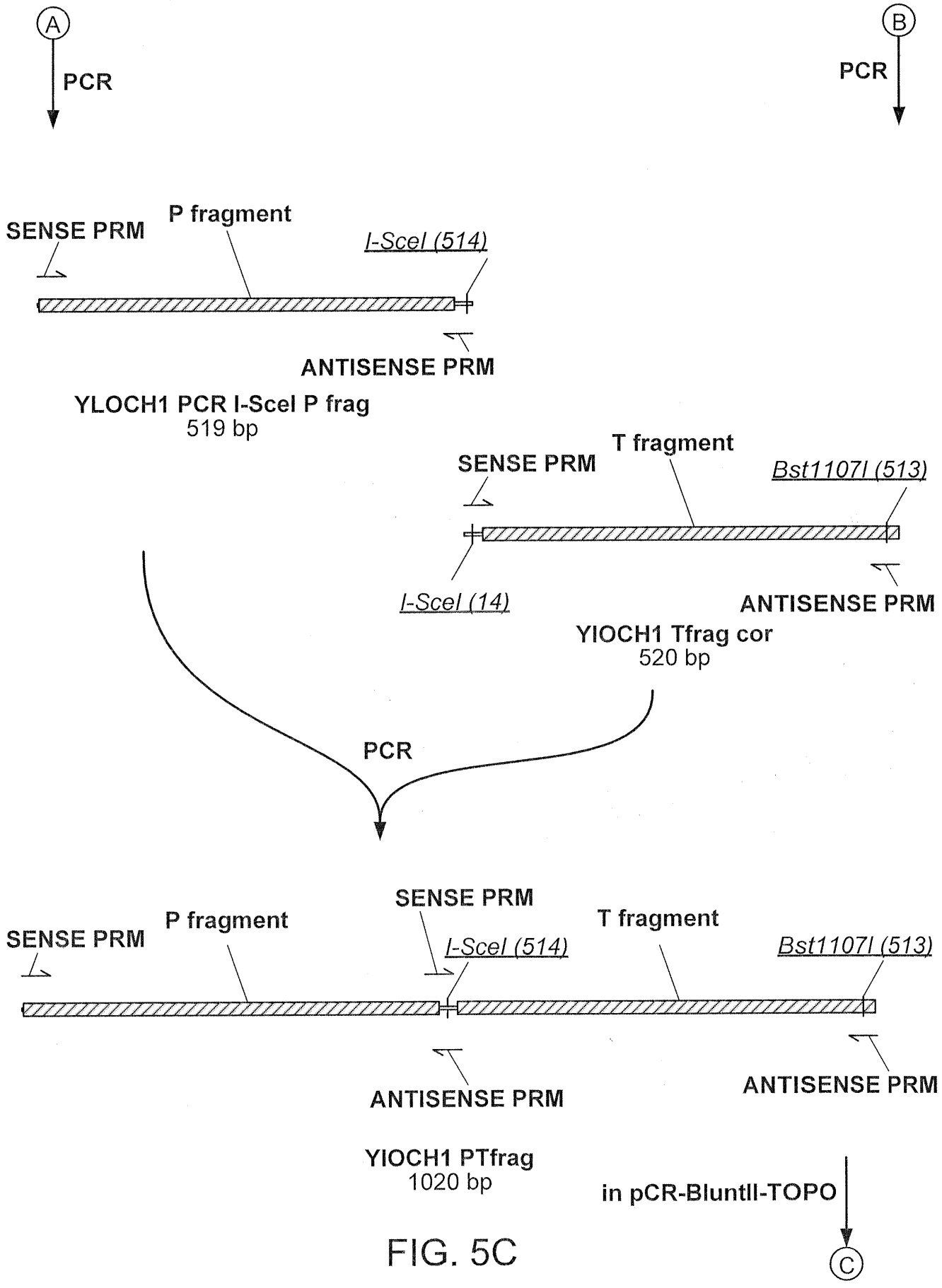

A
PCR
B
PCR
SENSE PRM
P fragment
I-SceI (514)
ANTISENSE PRM
YLOCH1 PCR I-SceI P frag
519 bp
T fragment
SENSE PRM
Bst1107I (513)
I-SceI (14)
ANTISENSE PRM
YIOCH1 Tfrag cor
520 bp
PCR
SENSE PRM
P fragment
SENSE PRM
T fragment
I-SceI (514)
Bst1107I (513)
ANTISENSE PRM
ANTISENSE PRM
YIOCH1 PTfrag
1020 bp
in pCR-BluntII-TOPO
C

FIG. 5C

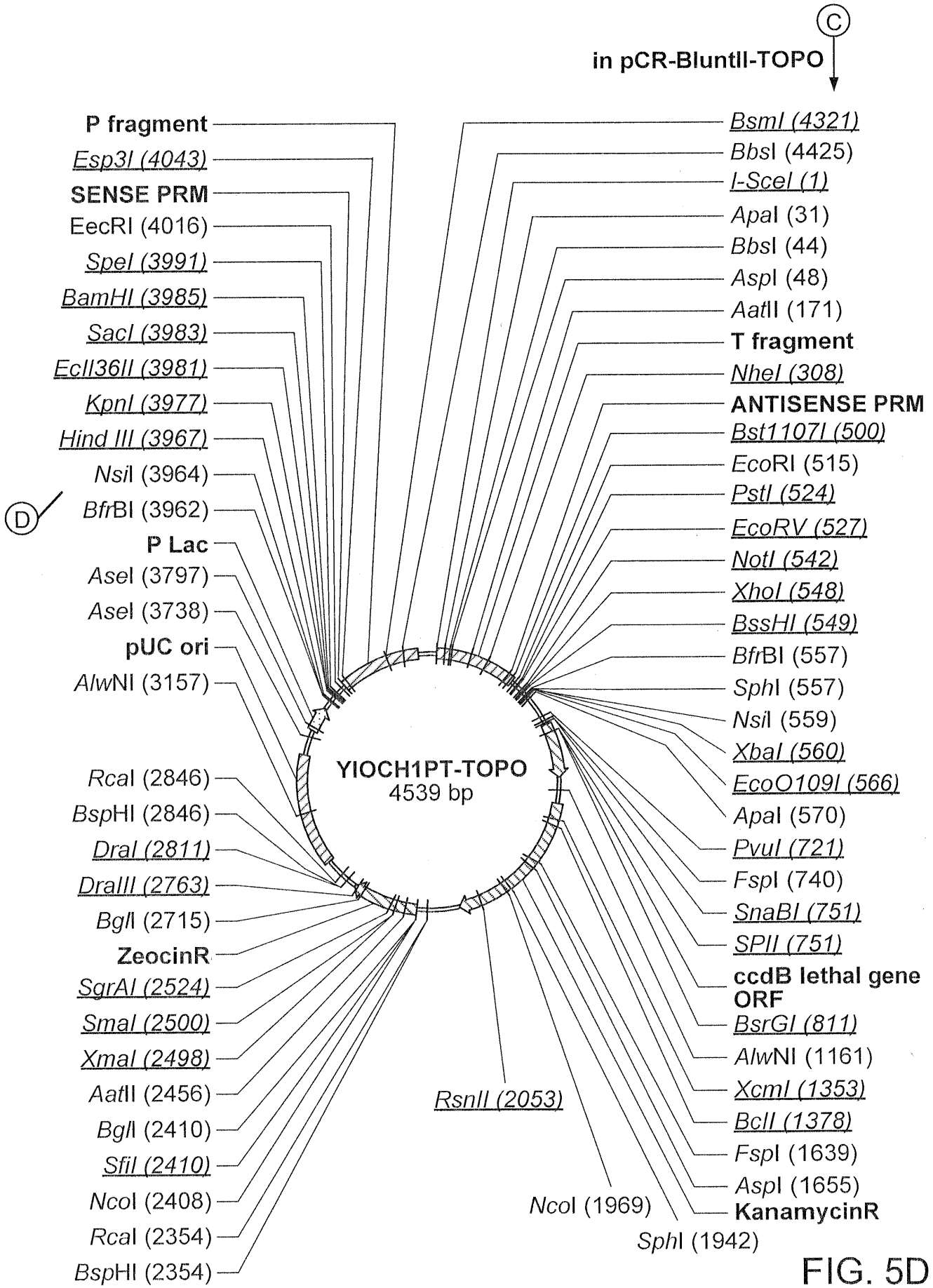

C
in pCR-BluntII-TOPO
P fragment
Esp3I (4043)
SENSE PRM
EecRI (4016)
SpeI (3991)
BamHI (3985)
SacI (3983)
EcII36II (3981)
KpnI (3977)
Hind III (3967)
NsiI (3964)
D
BfrBI (3962)
P Lac
AseI (3797)
AseI (3738)
pUC ori
AlwNI (3157)
RcaI (2846)
BspHI (2846)
DraI (2811)
DraIII (2763)
BglI (2715)
ZeocinR
SgrAI (2524)
SmaI (2500)
XmaI (2498)
AatII (2456)
BglI (2410)
SfiI (2410)
NcoI (2408)
RcaI (2354)
BspHI (2354)
YIOCH1PT-TOPO
4539 bp
RsnII (2053)
NcoI (1969)
SphI (1942)
BsmI (4321)
BbsI (4425)
I-SceI (1)
ApaI (31)
BbsI (44)
AspI (48)
AatII (171)
T fragment
NheI (308)
ANTISENSE PRM
Bst1107I (500)
EcoRI (515)
PstI (524)
EcoRV (527)
NotI (542)
XhoI (548)
BssHI (549)
BfrBI (557)
SphI (557)
NsiI (559)
XbaI (560)
EcoO109I (566)
ApaI (570)
PvuI (721)
FspI (740)
SnaBI (751)
SPII (751)
ccdB lethal gene ORF
BsrGI (811)
AlwNI (1161)
XcmI (1353)
BclI (1378)
FspI (1639)
AspI (1655)
KanamycinR

FIG. 5D

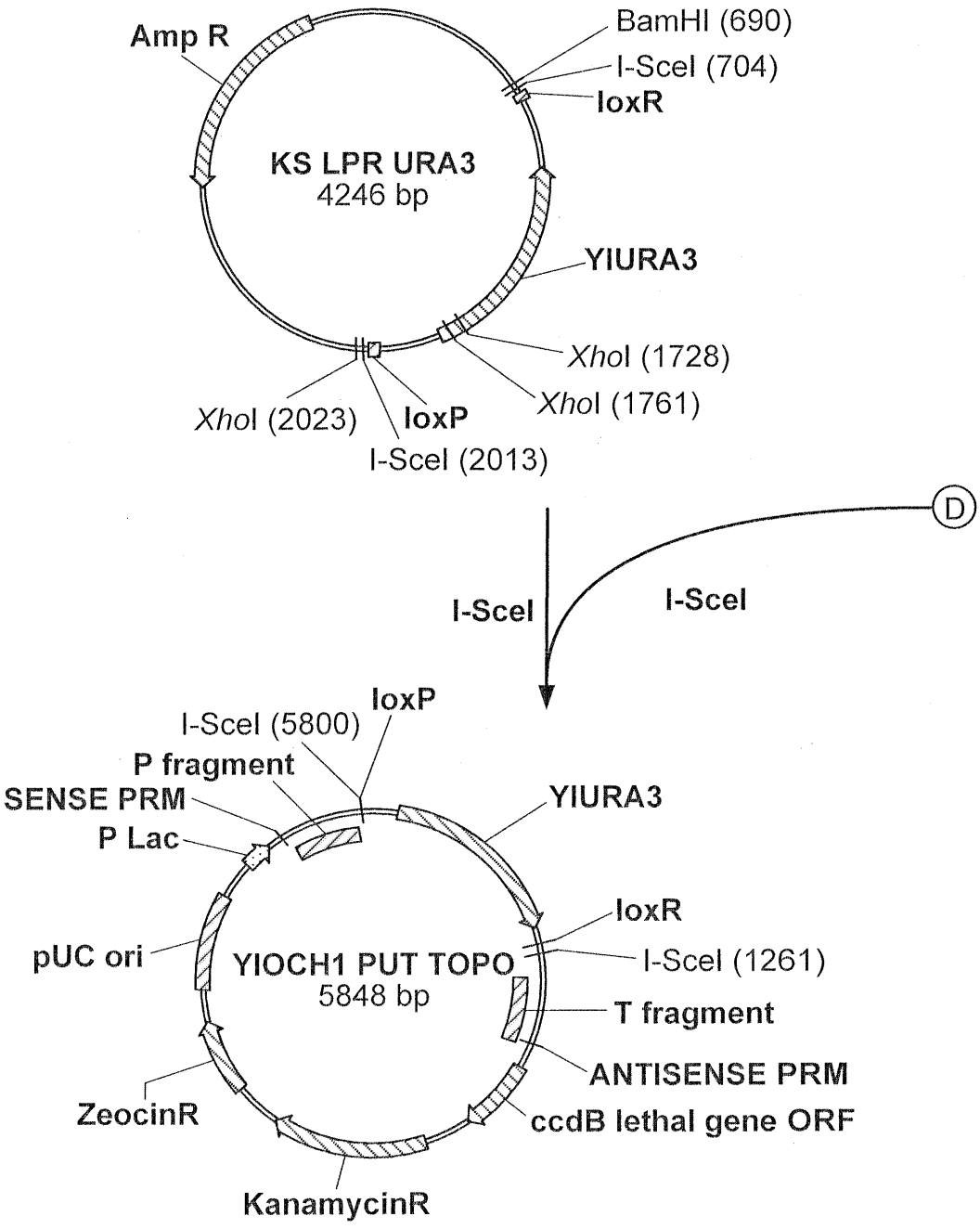

Amp R
BamHI (690)
I-SceI (704)
loxR
KS LPR URA3
4246 bp
YIURA3
XhoI (1728)
XhoI (1761)
XhoI (2023)
loxP
I-SceI (2013)
D
I-SceI
I-SceI
loxP
I-SceI (5800)
P fragment
SENSE PRM
P Lac
YIURA3
loxR
pUC ori
YIOCH1 PUT TOPO
5848 bp
I-SceI (1261)
T fragment
ANTISENSE PRM
ZeocinR
ccdB lethal gene ORF
KanamycinR

FIG. 5E

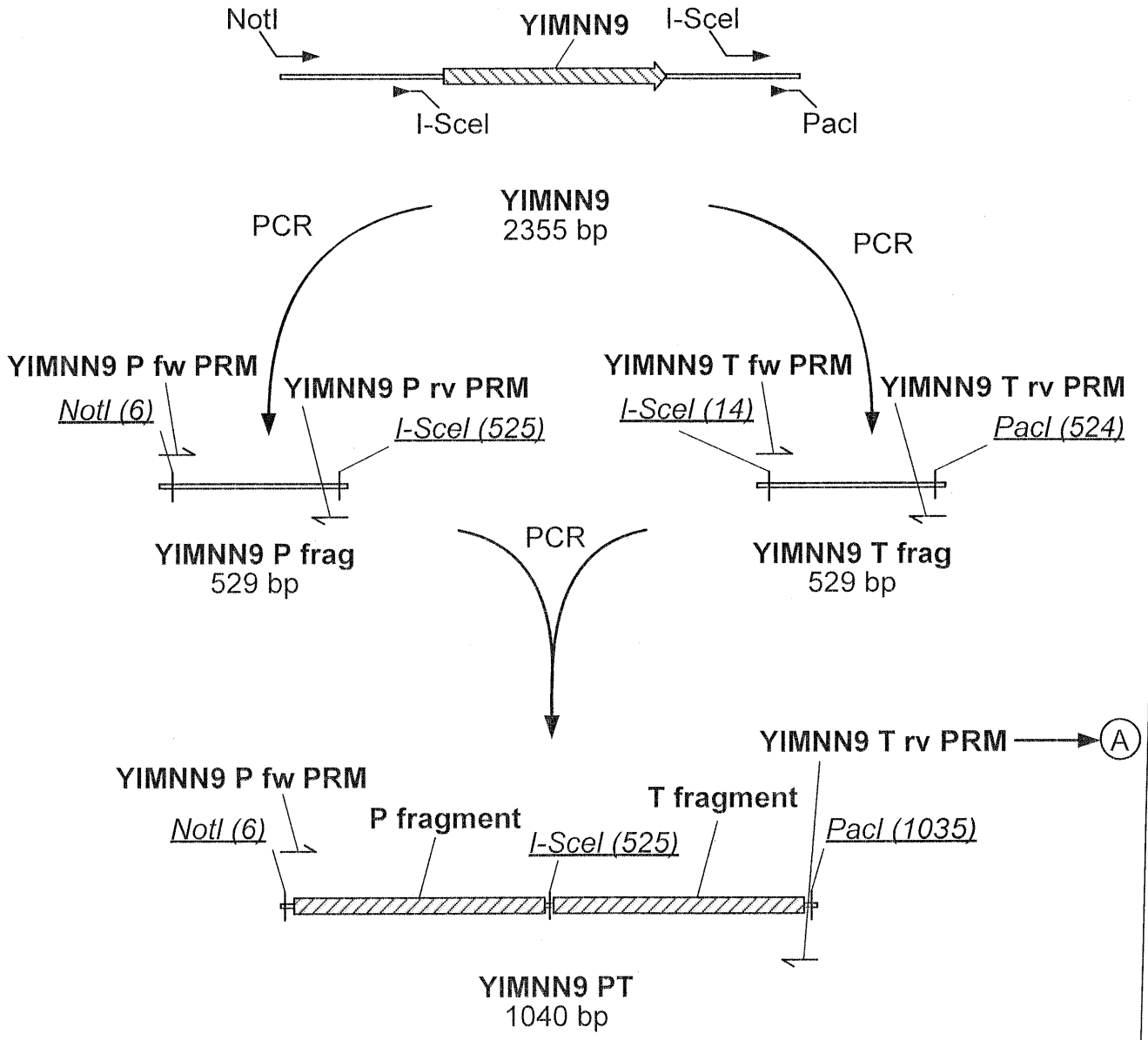

NotI
YIMNN9
I-SceI
I-SceI
PacI
YIMNN9
2355 bp
PCR
PCR
YIMNN9 P fw PRM
NotI (6)
YIMNN9 P rv PRM
I-SceI (525)
YIMNN9 T fw PRM
I-SceI (14)
YIMNN9 T rv PRM
PacI (524)
YIMNN9 P frag
529 bp
PCR
YIMNN9 T frag
529 bp
YIMNN9 T rv PRM
A
YIMNN9 P fw PRM
NotI (6)
P fragment
I-SceI (525)
T fragment
PacI (1035)
YIMNN9 PT
1040 bp

FIG. 6A

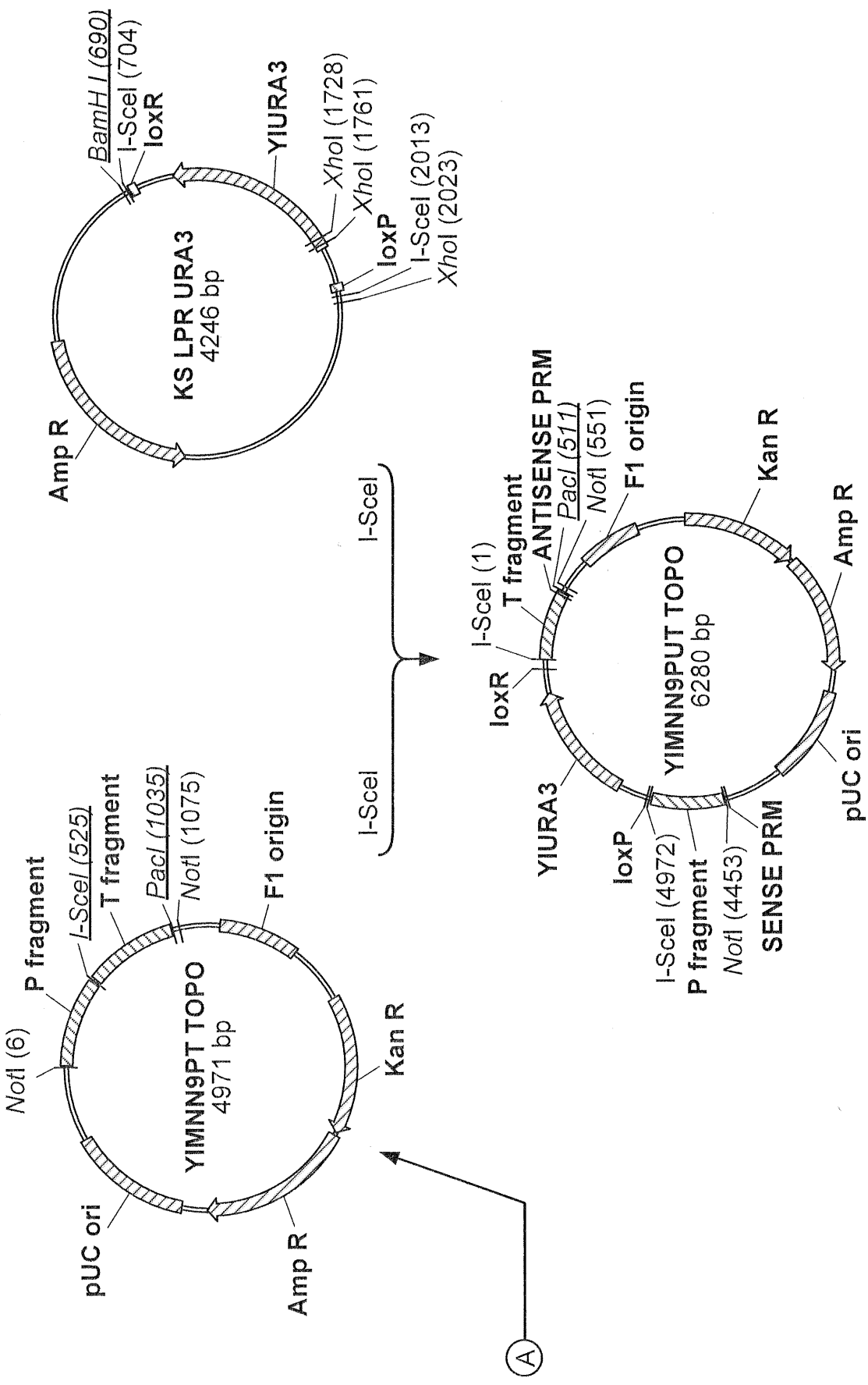

BamH I (690)
I-SceI (704)
loxR
YIURA3
XhoI (1728)
XhoI (1761)
loxP
I-SceI (2013)
XhoI (2023)
KS LPR URA3
4246 bp
Amp R
I-SceI
I-SceI
I-SceI (1)
T fragment
ANTISENSE PRM
PacI (511)
NotI (551)
F1 origin
Kan R
Amp R
loxR
YIURA3
YIMNN9PUT TOPO
6280 bp
loxP
I-SceI (4972)
P fragment
NotI (4453)
SENSE PRM
pUC ori
NotI (6)
P fragment
I-SceI (525)
T fragment
PacI (1035)
NotI (1075)
F1 origin
Kan R
YIMNN9PT TOPO
4971 bp
pUC ori
Amp R
A

FIG. 6B

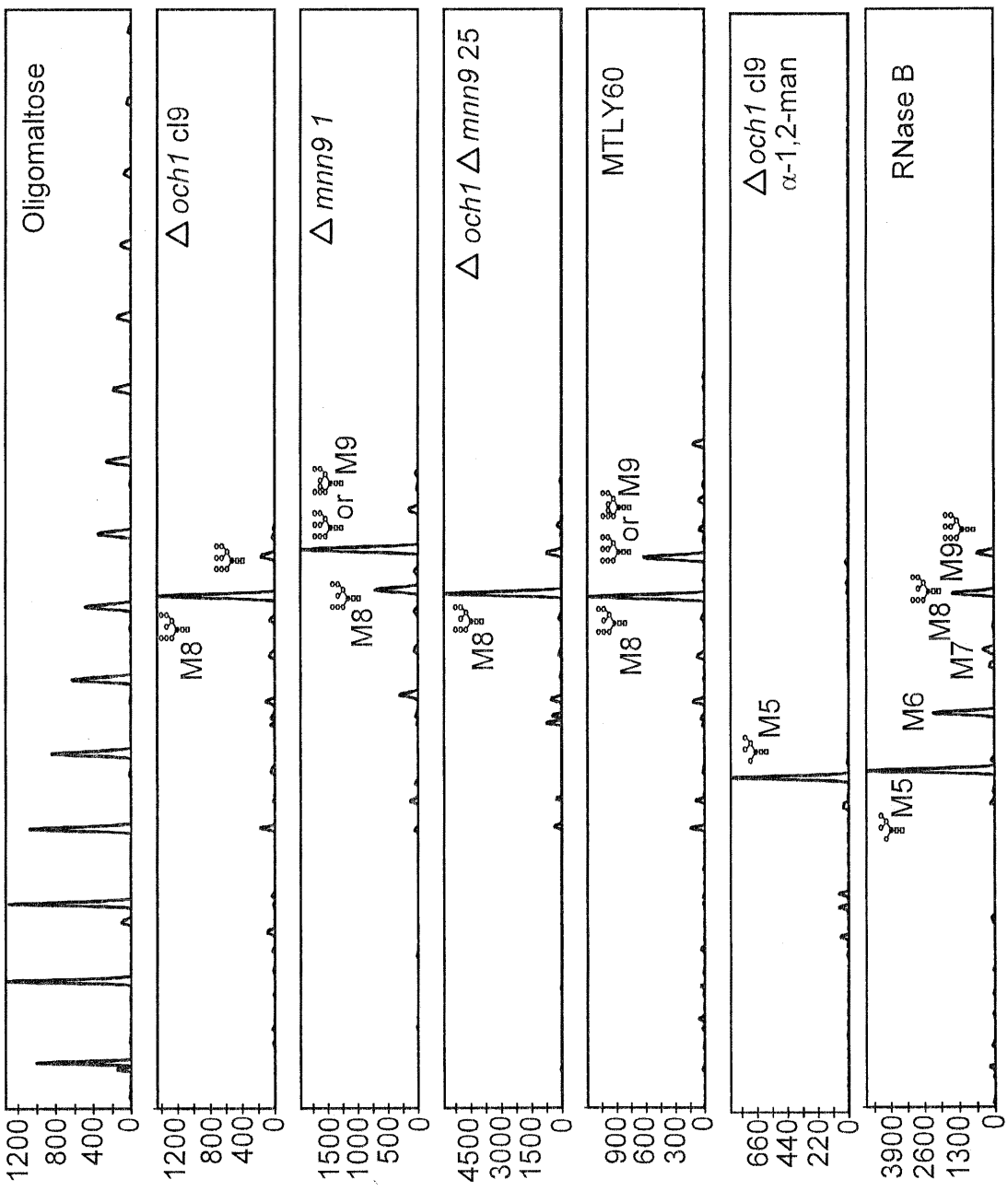

Oligomaltose
Δ och1 cl9
M8
Δ mnn9 1
M8
or M9
Δ och1 Δ mnn9 25
M8
MTLY60
M8
or M9
Δ och1 cl9
α-1,2-man
M5
RNase B
M5
M6
M7
M8
M9
1200
800
400
0
1200
800
400
0
1500
1000
500
0
4500
3000
1500
0
900
600
300
0
660
440
220
0
3900
2600
1300
0

FIG. 7

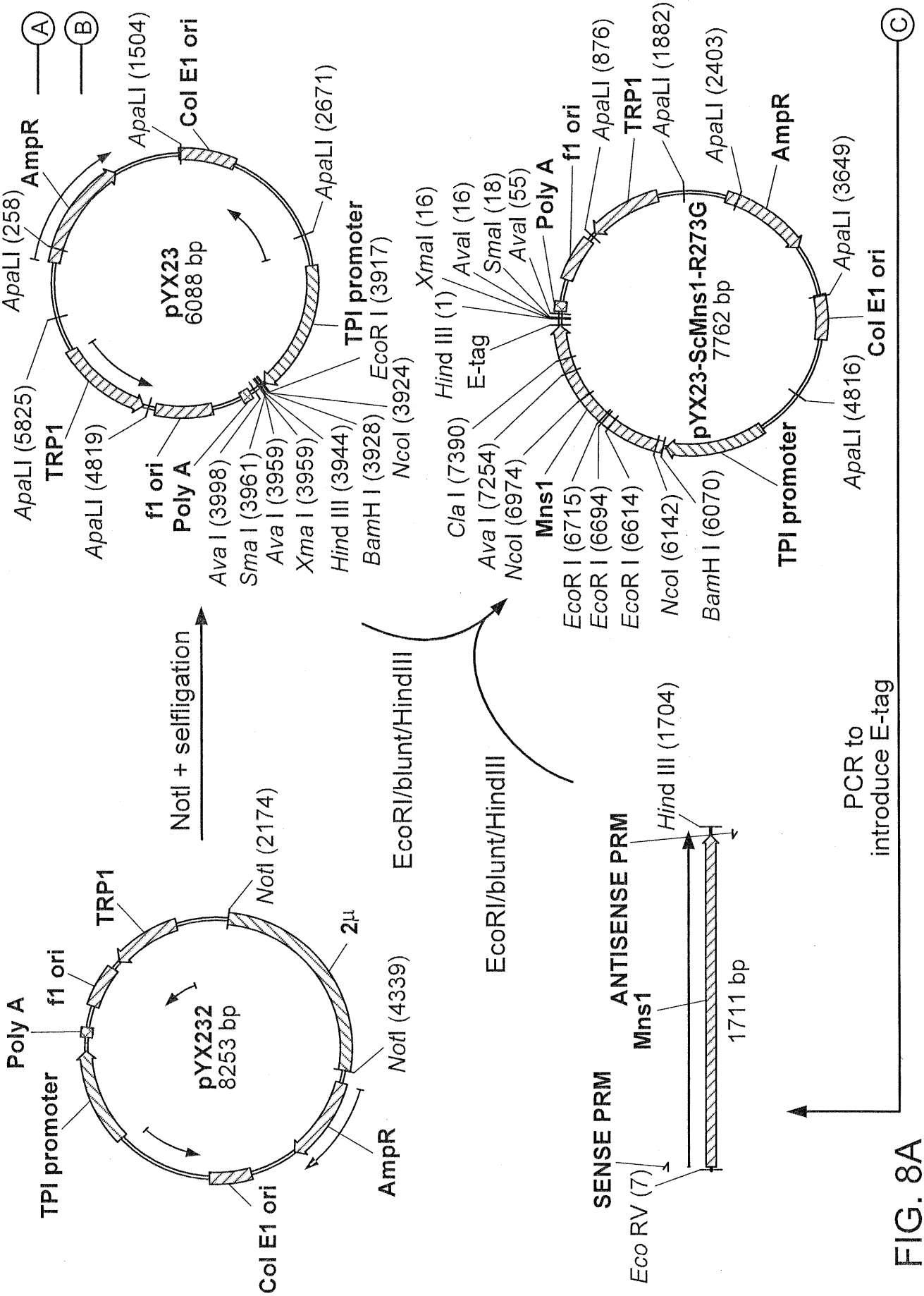

pYX23
6088 bp
AmpR
ApaLI (258)
ApaLI (1504)
Col E1 ori
ApaLI (2671)
TPI promoter
EcoR I (3917)
NcoI (3924)
BamH I (3928)
Hind III (3944)
Xma I (3959)
Ava I (3959)
Sma I (3961)
Ava I (3998)
f1 ori
Poly A
ApaLI (4819)
TRP1
ApaLI (5825)
A
B
NotI + selfligation
pYX232
8253 bp
Poly A
f1 ori
TRP1
NotI (2174)
2μ
NotI (4339)
AmpR
Col E1 ori
TPI promoter
EcoRI/blunt/HindIII
EcoRI/blunt/HindIII
pYX23-ScMns1-R273G
7762 bp
Xma I (16)
Ava I (16)
Sma I (18)
Ava I (55)
Poly A
f1 ori
ApaLI (876)
TRP1
ApaLI (1882)
ApaLI (2403)
AmpR
ApaLI (3649)
Col E1 ori
ApaLI (4816)
TPI promoter
BamH I (6070)
NcoI (6142)
EcoR I (6614)
EcoR I (6694)
EcoR I (6715)
Mns1
NcoI (6974)
Ava I (7254)
Cla I (7390)
E-tag
Hind III (1)
SENSE PRM
ANTISENSE PRM
Mns1
Hind III (1704)
Eco RV (7)
1711 bp
PCR to
introduce E-tag
C

FIG. 8A

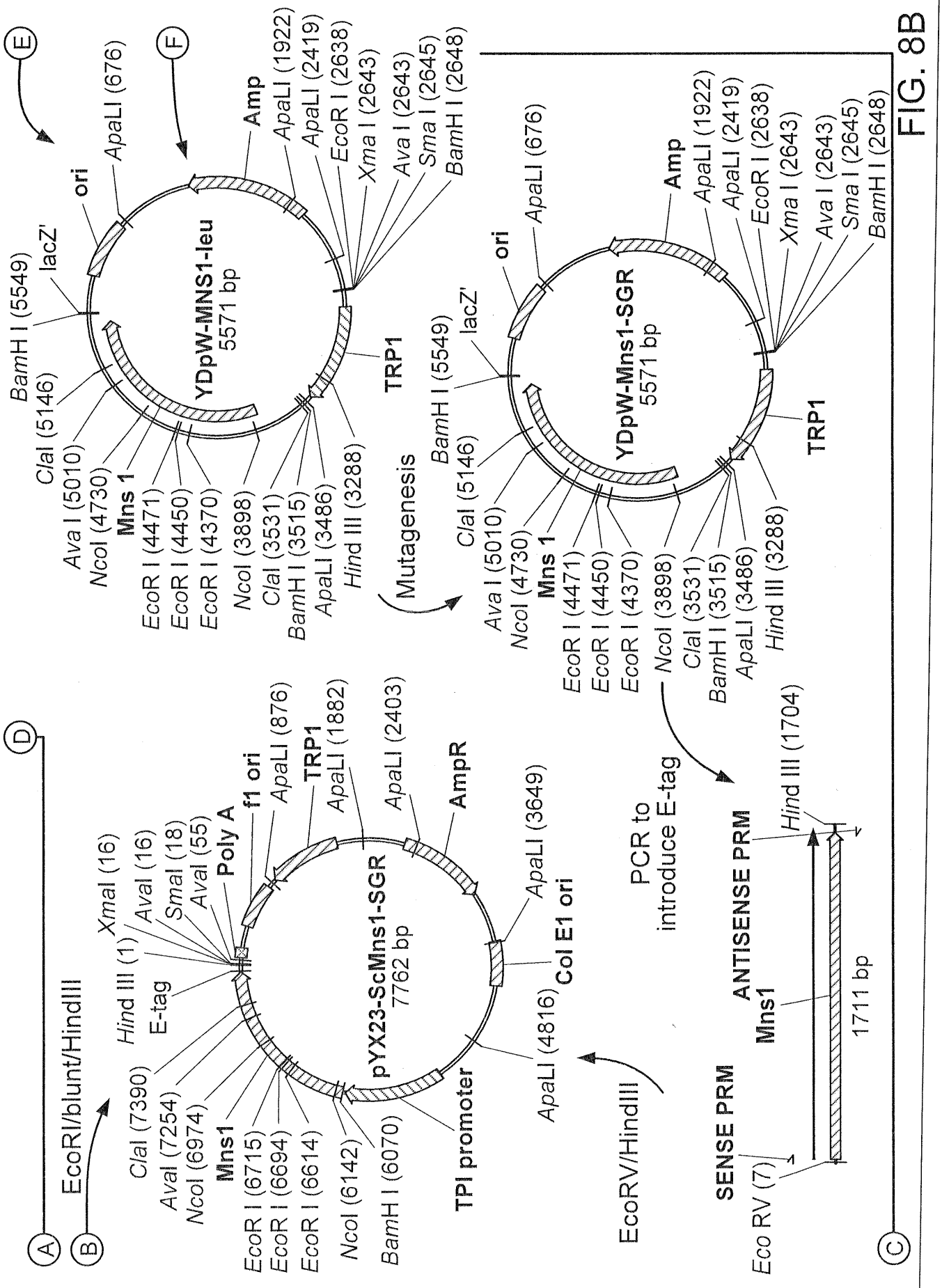

A
B
C
D
E
F
EcoRI/blunt/HindIII
pYX23-ScMns1-SGR
7762 bp
Hind III (1)
Xmal (16)
Aval (16)
Smal (18)
Aval (55)
Poly A
f1 ori
ApaLI (876)
TRP1
ApaLI (1882)
ApaLI (2403)
AmpR
ApaLI (3649)
Col E1 ori
ApaLI (4816)
TPI promoter
BamH I (6070)
Ncol (6142)
EcoR I (6614)
EcoR I (6694)
EcoR I (6715)
Mns1
Ncol (6974)
Aval (7254)
ClaI (7390)
E-tag
EcoRV/HindIII
PCR to
introduce E-tag
SENSE PRM
Eco RV (7)
ANTISENSE PRM
Mns1
Hind III (1704)
1711 bp
YDpW-MNS1-leu
5571 bp
BamH I (5549)
lacZ'
ori
ApaLI (676)
Amp
ApaLI (1922)
ApaLI (2419)
EcoR I (2638)
Xma I (2643)
Ava I (2643)
Sma I (2645)
BamH I (2648)
ClaI (5146)
Ava I (5010)
Ncol (4730)
Mns 1
EcoR I (4471)
EcoR I (4450)
EcoR I (4370)
Ncol (3898)
ClaI (3531)
BamH I (3515)
ApaLI (3486)
Hind III (3288)
TRP1
Mutagenesis
YDpW-Mns1-SGR
5571 bp

FIG. 8B

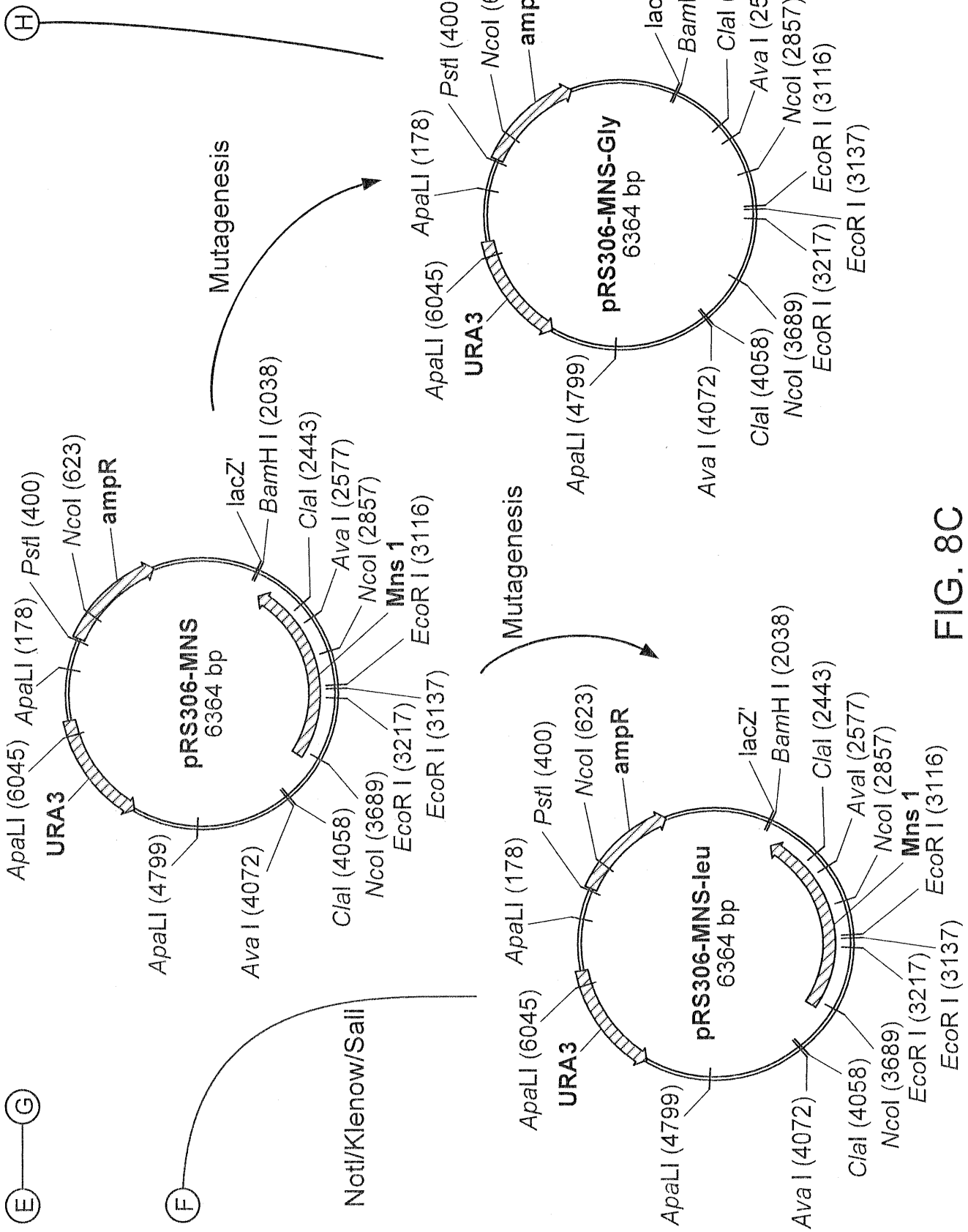

E
G
F
H
NotI/Klenow/SalI
pRS306-MNS
6364 bp
ApaLI (6045)
URA3
ApaLI (178)
PstI (400)
NcoI (623)
ampR
ApaLI (4799)
AvaI (4072)
ClaI (4058)
NcoI (3689)
EcoR I (3217)
EcoR I (3137)
lacZ'
BamH I (2038)
ClaI (2443)
AvaI (2577)
NcoI (2857)
Mns 1
EcoR I (3116)
Mutagenesis
pRS306-MNS-Gly
6364 bp
pRS306-MNS-leu
6364 bp
AvaI (2577)

FIG. 8C

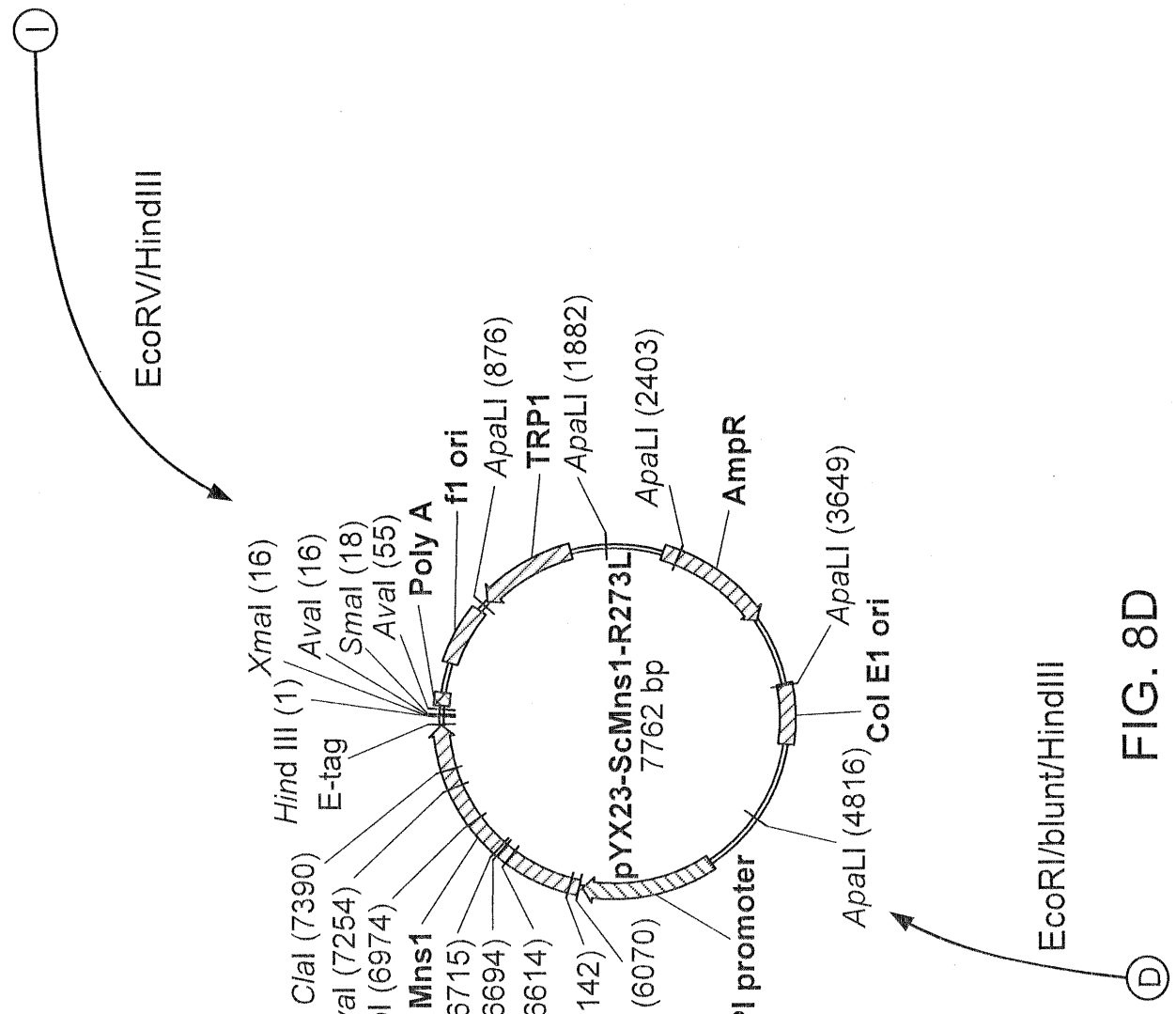

1
EcoRV/HindIII
XmaI (16)
AvaI (16)
SmaI (18)
AvaI (55)
Poly A
f1 ori
ApaLI (876)
TRP1
ApaLI (1882)
ApaLI (2403)
AmpR
ApaLI (3649)
Col E1 ori
ApaLI (4816)
EcoRI/blunt/HindIII
D
Hind III (1)
E-tag
ClaI (7390)
AvaI (7254)
NcoI (6974)
Mns1
EcoR I (6715)
EcoR I (6694)
EcoR I (6614)
NcoI (6142)
BamH I (6070)
TPI promoter
pYX23-ScMns1-R273L
7762 bp

FIG. 8D

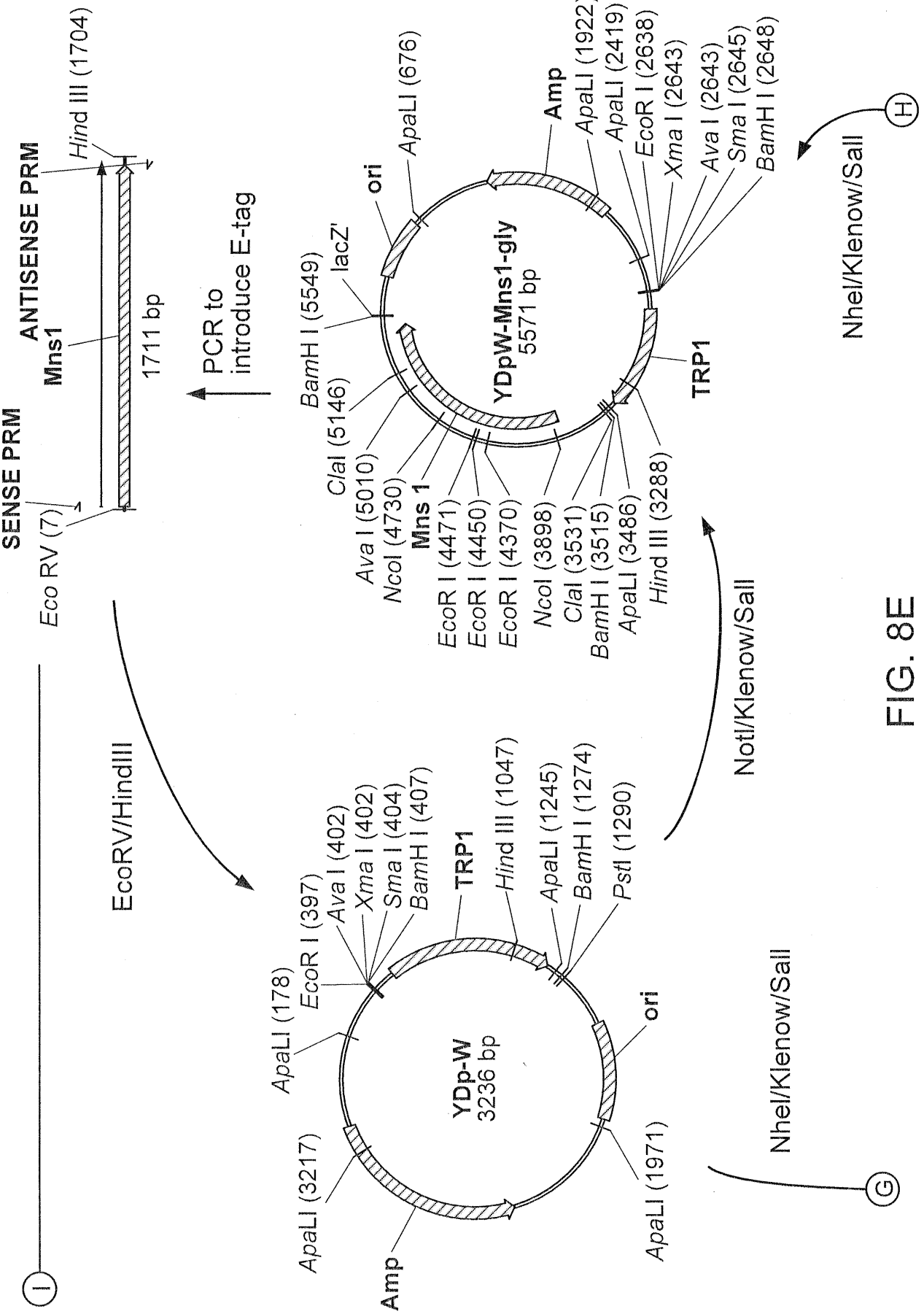

SENSE PRM
ANTISENSE PRM
Mns1
Eco RV (7)
Hind III (1704)
1711 bp
PCR to introduce E-tag
EcoRV/HindIII
YDpW-Mns1-gly
5571 bp
ori
ApaLI (676)
Amp
ApaLI (1922)
ApaLI (2419)
EcoR I (2638)
Xma I (2643)
Ava I (2643)
Sma I (2645)
BamH I (2648)
TRP1
Hind III (3288)
ApaLI (3486)
BamH I (3515)
ClaI (3531)
NcoI (3898)
EcoR I (4370)
EcoR I (4450)
EcoR I (4471)
Mns 1
NcoI (4730)
AvaI (5010)
ClaI (5146)
BamH I (5549)
lacZ'
NheI/Klenow/SalI
NotI/Klenow/SalI
YDp-W
3236 bp
TRP1
Hind III (1047)
ApaLI (1245)
BamH I (1274)
PstI (1290)
ori
ApaLI (1971)
Amp
ApaLI (3217)
ApaLI (178)
EcoR I (397)
Ava I (402)
Xma I (402)
Sma I (404)
BamH I (407)
NheI/Klenow/SalI
I
G
H

FIG. 8E

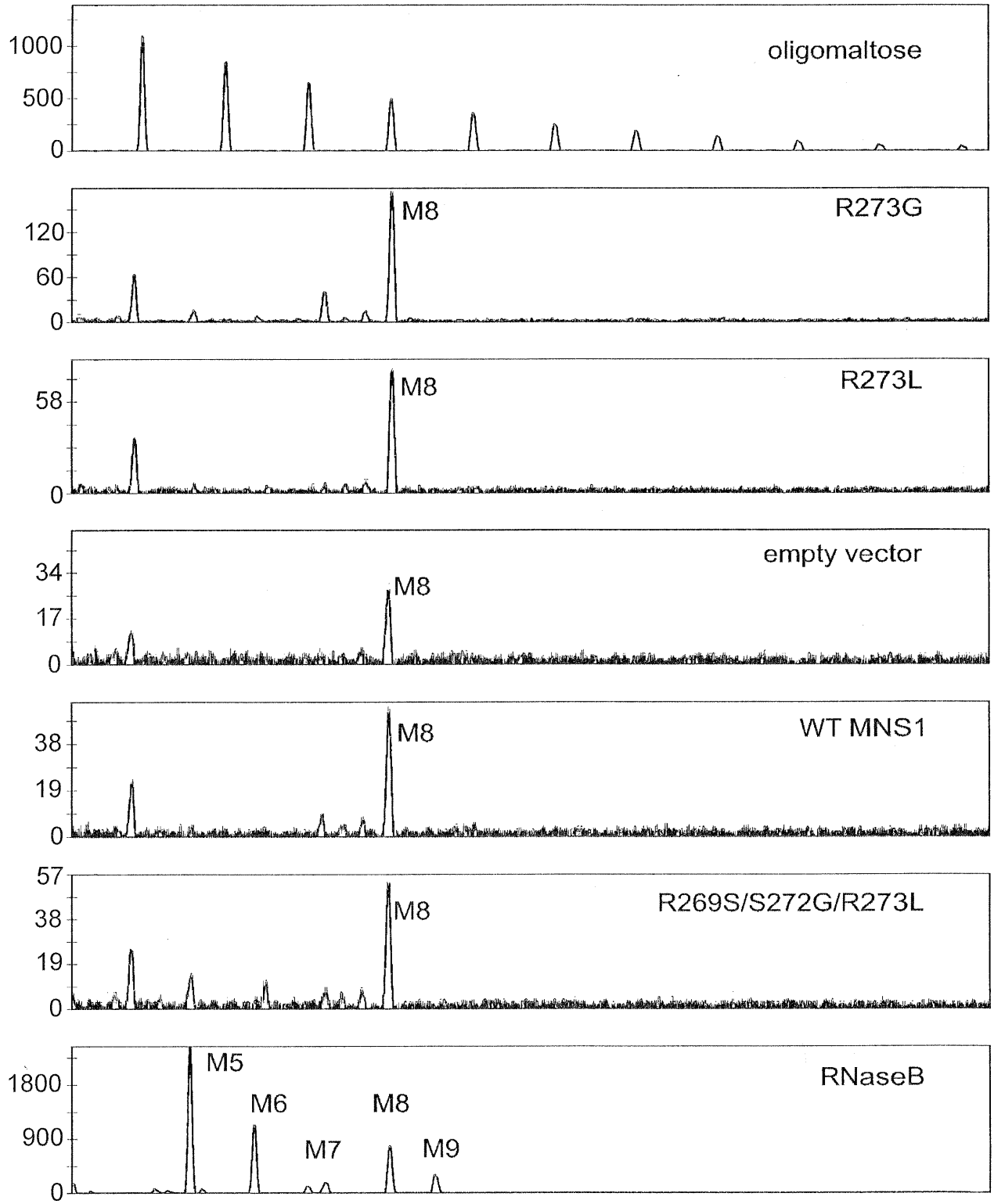

1000
500
0
oligomaltose
120
60
0
M8
R273G
58
0
M8
R273L
34
17
0
empty vector
M8
38
19
0
M8
WT MNS1
57
38
19
0
M8
R269S/S272G/R273L
1800
900
0
M5
M6
M7
M8
M9
RNaseB

FIG. 9

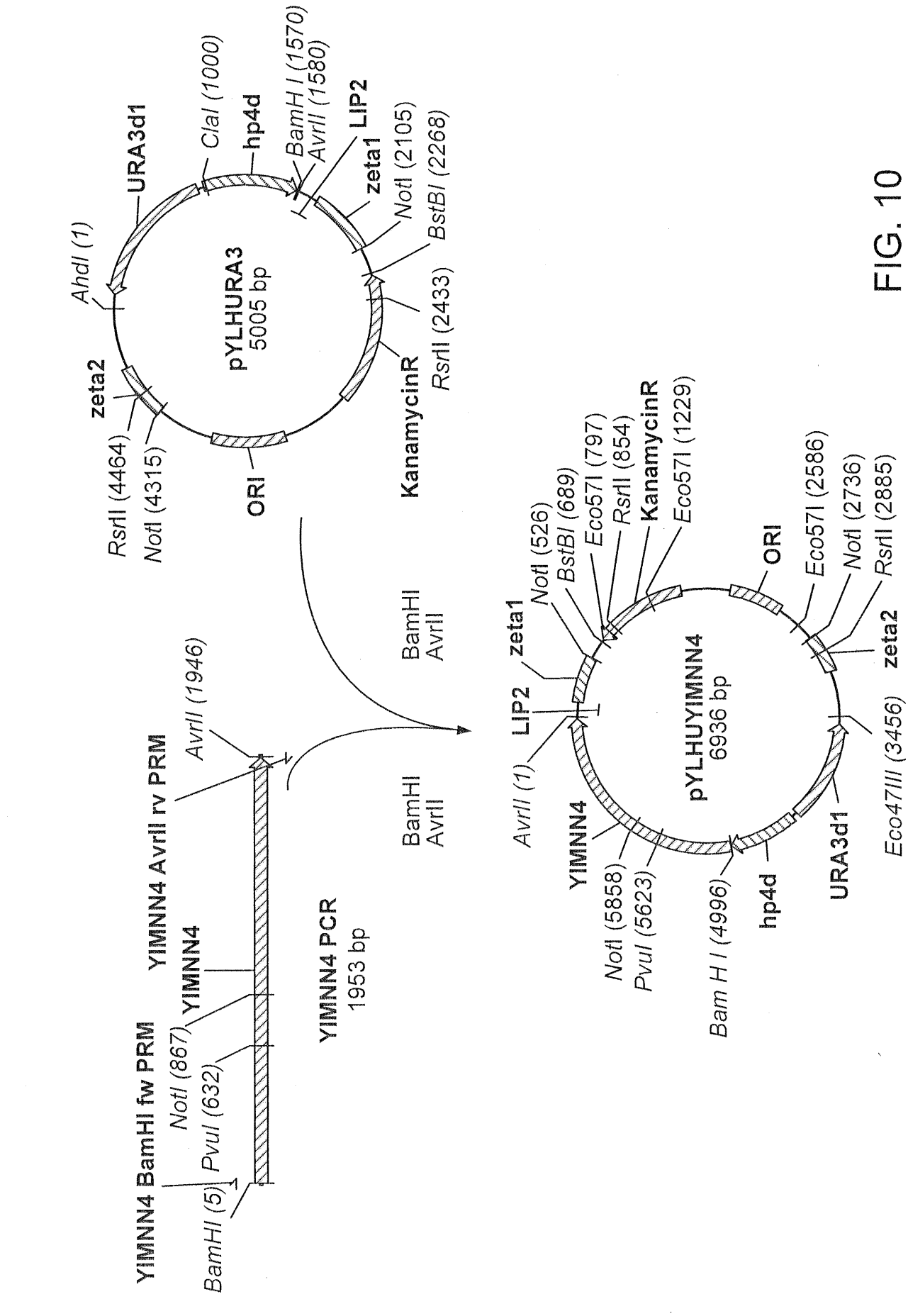

pYLHURA3
5005 bp
URA3d1
AhdI (1)
ClaI (1000)
hp4d
BamH I (1570)
AvrII (1580)
LIP2
zeta1
NotI (2105)
BstBI (2268)
RsrII (2433)
KanamycinR
ORI
NotI (4315)
RsrII (4464)
zeta2
YIMNN4 BamHI fw PRM
YIMNN4
YIMNN4 AvrII rv PRM
AvrII (1946)
NotI (867)
PvuI (632)
BamHI (5)
YIMNN4 PCR
1953 bp
BamHI
AvrII
BamHI
AvrII
pYLHUYIMNN4
6936 bp
AvrII (1)
LIP2
zeta1
NotI (526)
BstBI (689)
Eco57I (797)
RsrII (854)
KanamycinR
Eco57I (1229)
ORI
Eco57I (2586)
NotI (2736)
RsrII (2885)
zeta2
Eco47III (3456)
URA3d1
hp4d
Bam H I (4996)
PvuI (5623)
NotI (5858)
YIMNN4

FIG. 10

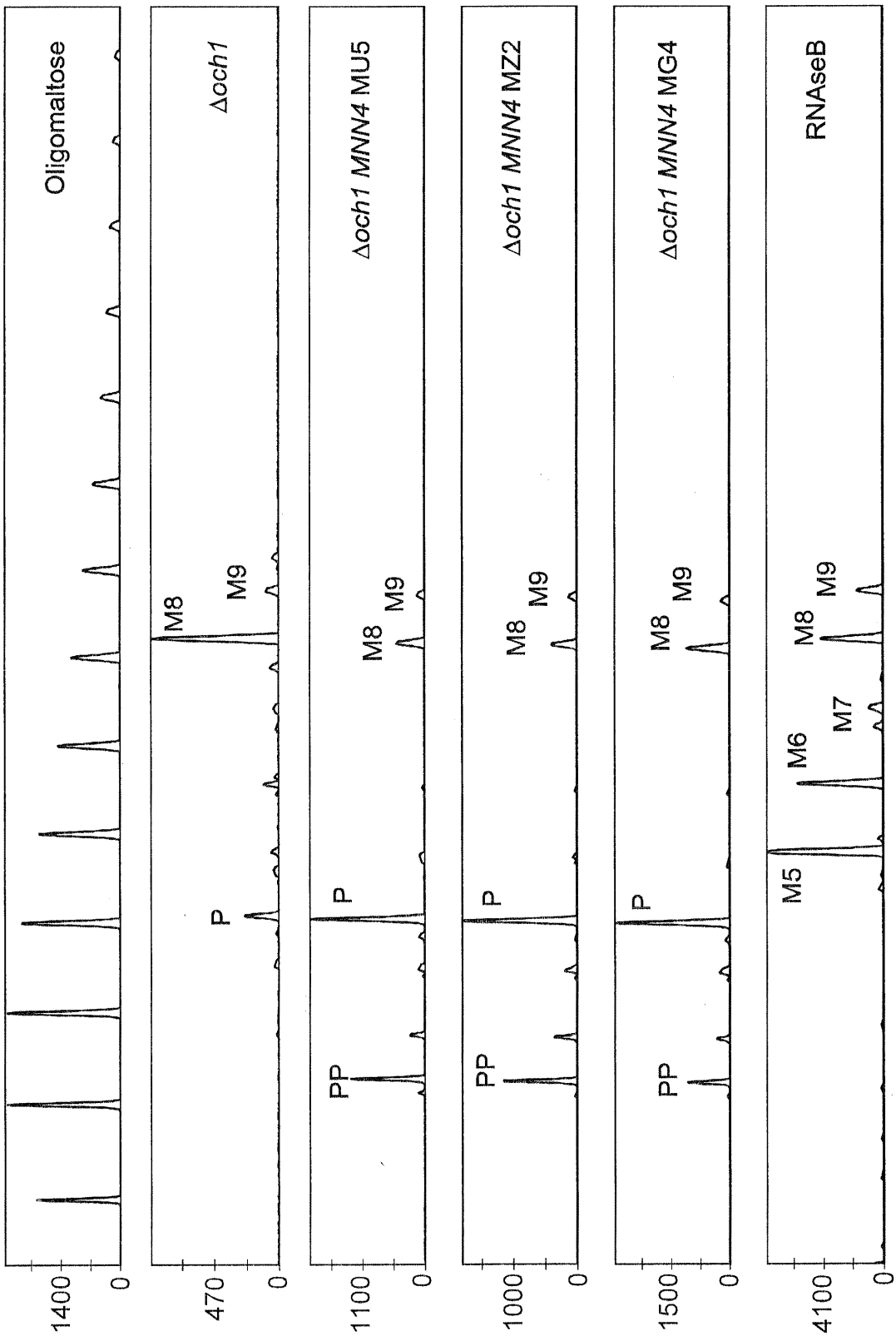
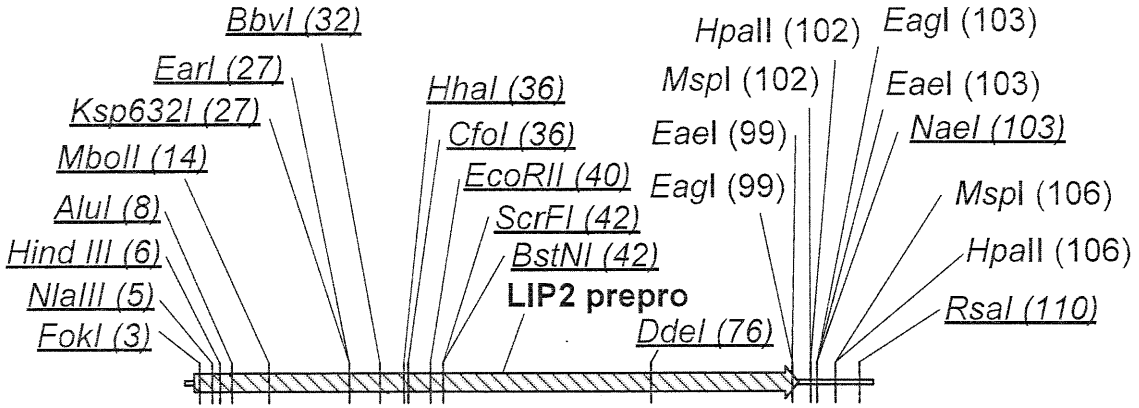

Oligomaltose
1400
0
Δoch1
M8
M9
P
470
0
Δoch1 MNN4 MU5
M8
M9
P
PP
1100
0
Δoch1 MNN4 MZ2
M8
M9
P
PP
1000
0
Δoch1 MNN4 MG4
M8
M9
P
PP
1500
0
RNAseB
M5
M6
M7
M8
M9
4100
0

FIG. 11



catgaactttccaccatcctcttcacagcctgcgctaccctggctgccgccctcccttccccc →

← cggcgggagggaaggggtagtgaggaagactccggcgtcaagaggtcttcgccggccggccatg

FIG. 12A

Ⓐ

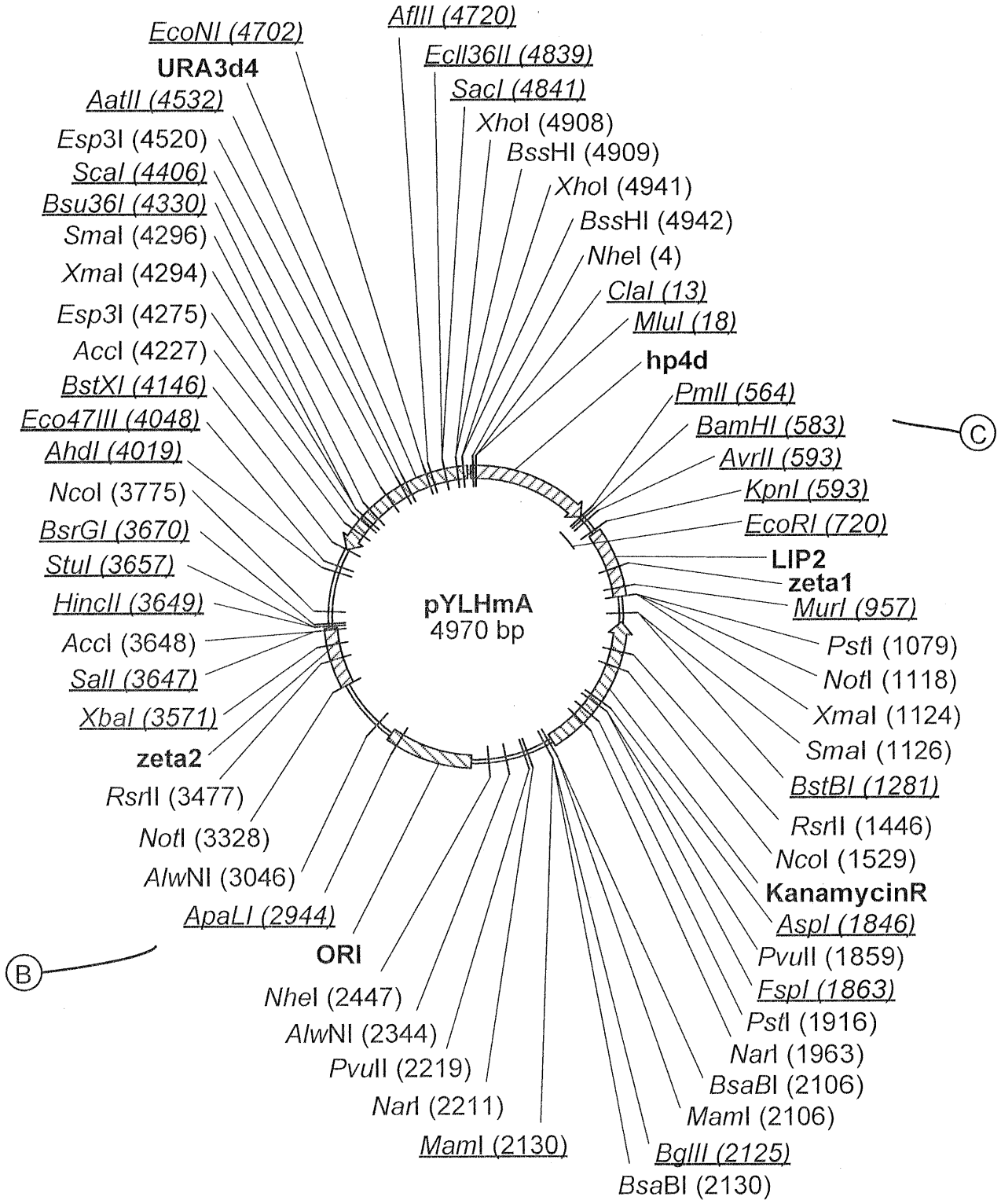

pYLHmA
4970 bp
EcoNI (4702)
URA3d4
AatII (4532)
Esp3I (4520)
ScaI (4406)
Bsu36I (4330)
SmaI (4296)
XmaI (4294)
Esp3I (4275)
AccI (4227)
BstXI (4146)
Eco47III (4048)
AhdI (4019)
NcoI (3775)
BsrGI (3670)
StuI (3657)
HincII (3649)
AccI (3648)
SalI (3647)
XbaI (3571)
zeta2
RsrII (3477)
NotI (3328)
AlwNI (3046)
ApaLI (2944)
ORI
NheI (2447)
AlwNI (2344)
PvuII (2219)
NarI (2211)
MamI (2130)
BsaBI (2130)
BglII (2125)
MamI (2106)
BsaBI (2106)
NarI (1963)
PstI (1916)
FspI (1863)
PvuII (1859)
AspI (1846)
KanamycinR
NcoI (1529)
RsrII (1446)
BstBI (1281)
SmaI (1126)
XmaI (1124)
NotI (1118)
PstI (1079)
MurI (957)
zeta1
LIP2
EcoRI (720)
KpnI (593)
AvrII (593)
BamHI (583)
PmlI (564)
hp4d
MluI (18)
ClaI (13)
NheI (4)
BssHI (4942)
XhoI (4941)
BssHI (4909)
XhoI (4908)
SacI (4841)
EcoICI36II (4839)
AflIII (4720)
B
C

FIG. 12B

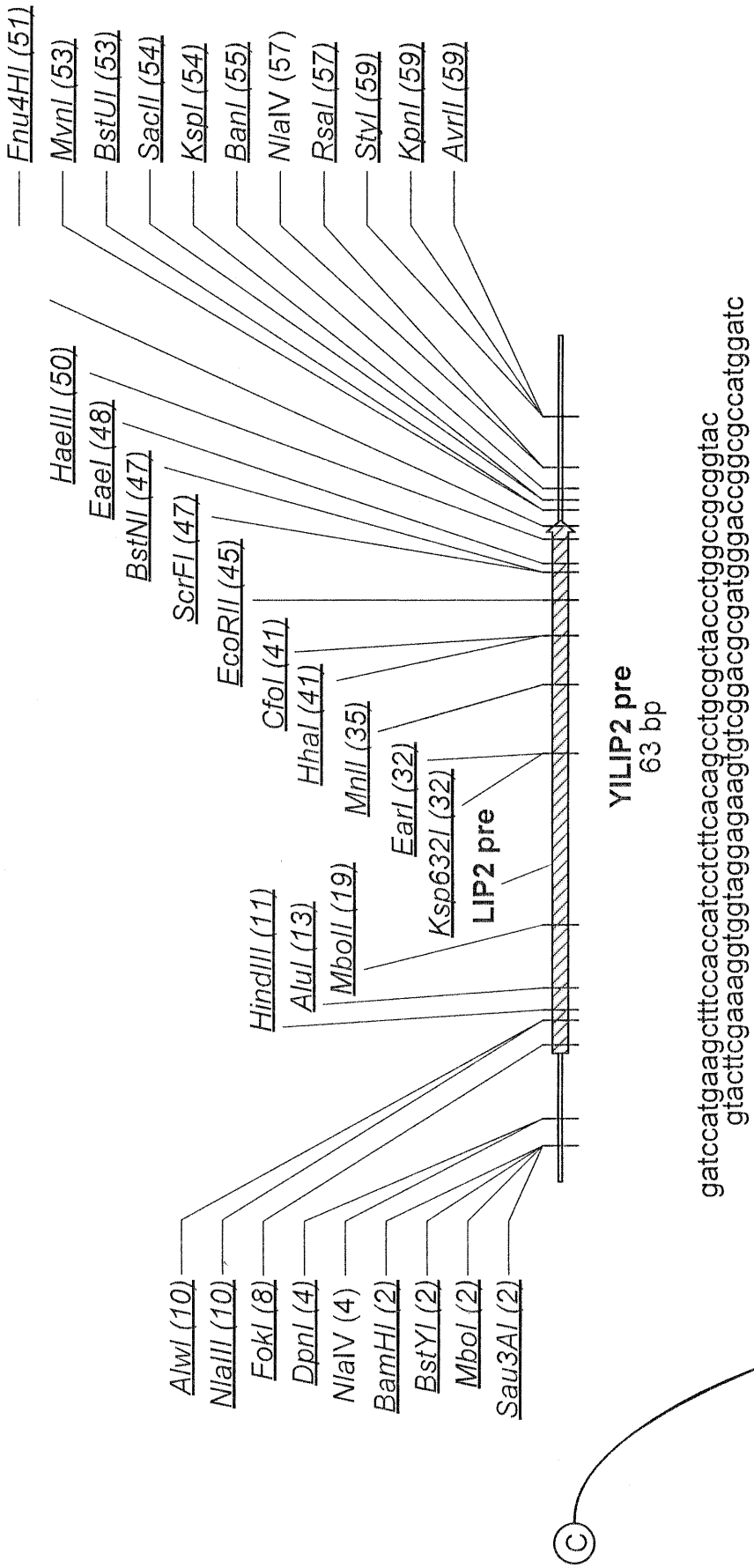

Fnu4HI (51)
MvnI (53)
BstUI (53)
SacII (54)
KspI (54)
BanI (55)
NlaIV (57)
RsaI (57)
StyI (59)
KpnI (59)
AvrII (59)
HaeIII (50)
EaeI (48)
BstNI (47)
ScrFI (47)
EcoRII (45)
CfoI (41)
HhaI (41)
MnlI (35)
EarI (32)
Ksp632I (32)
LIP2 pre
HindIII (11)
AluI (13)
MboII (19)
AlwI (10)
NlaIII (10)
FokI (8)
DpnI (4)
NlaIV (4)
BamHI (2)
BstYI (2)
MboI (2)
Sau3AI (2)
YlLIP2 pre
63 bp
gatccatgaagctttccaccatcctcttcacagcctgcgctacctggccgcggtac
gtacttcgaaaggtggtaggagaagtgtcggacgcgatgggaccggcgccatggatc
C
D
AvrII
BamHI

FIG. 12C

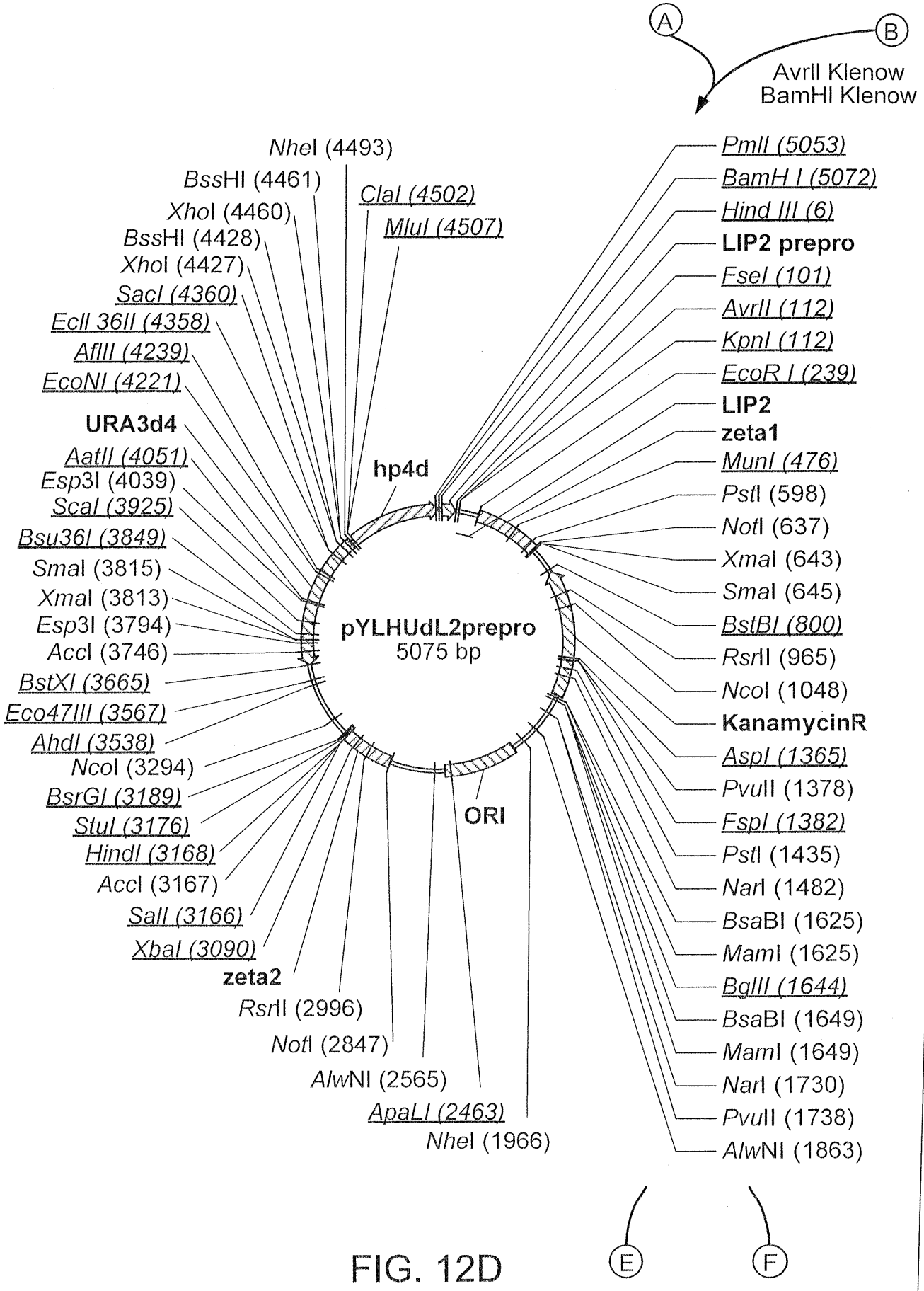

A
B
AvrII Klenow
BamHI Klenow
NheI (4493)
BssHI (4461)
ClaI (4502)
XhoI (4460)
MluI (4507)
BssHI (4428)
XhoI (4427)
SacI (4360)
EcII 36II (4358)
AflII (4239)
EcoNI (4221)
URA3d4
AatII (4051)
Esp3I (4039)
ScaI (3925)
Bsu36I (3849)
SmaI (3815)
XmaI (3813)
Esp3I (3794)
AccI (3746)
BstXI (3665)
Eco47III (3567)
AhdI (3538)
NcoI (3294)
BsrGI (3189)
StuI (3176)
HindI (3168)
AccI (3167)
SalI (3166)
XbaI (3090)
zeta2
RsrII (2996)
NotI (2847)
AlwNI (2565)
ApaLI (2463)
NheI (1966)
hp4d
pYLHUdL2prepro
5075 bp
ORI
PmlI (5053)
BamH I (5072)
Hind III (6)
LIP2 prepro
FseI (101)
AvrII (112)
KpnI (112)
EcoR I (239)
LIP2
zeta1
MunI (476)
PstI (598)
NotI (637)
XmaI (643)
SmaI (645)
BstBI (800)
RsrII (965)
NcoI (1048)
KanamycinR
AspI (1365)
PvuII (1378)
FspI (1382)
PstI (1435)
NarI (1482)
BsaBI (1625)
MamI (1625)
BglII (1644)
BsaBI (1649)
MamI (1649)
NarI (1730)
PvuII (1738)
AlwNI (1863)
E
F

FIG. 12D

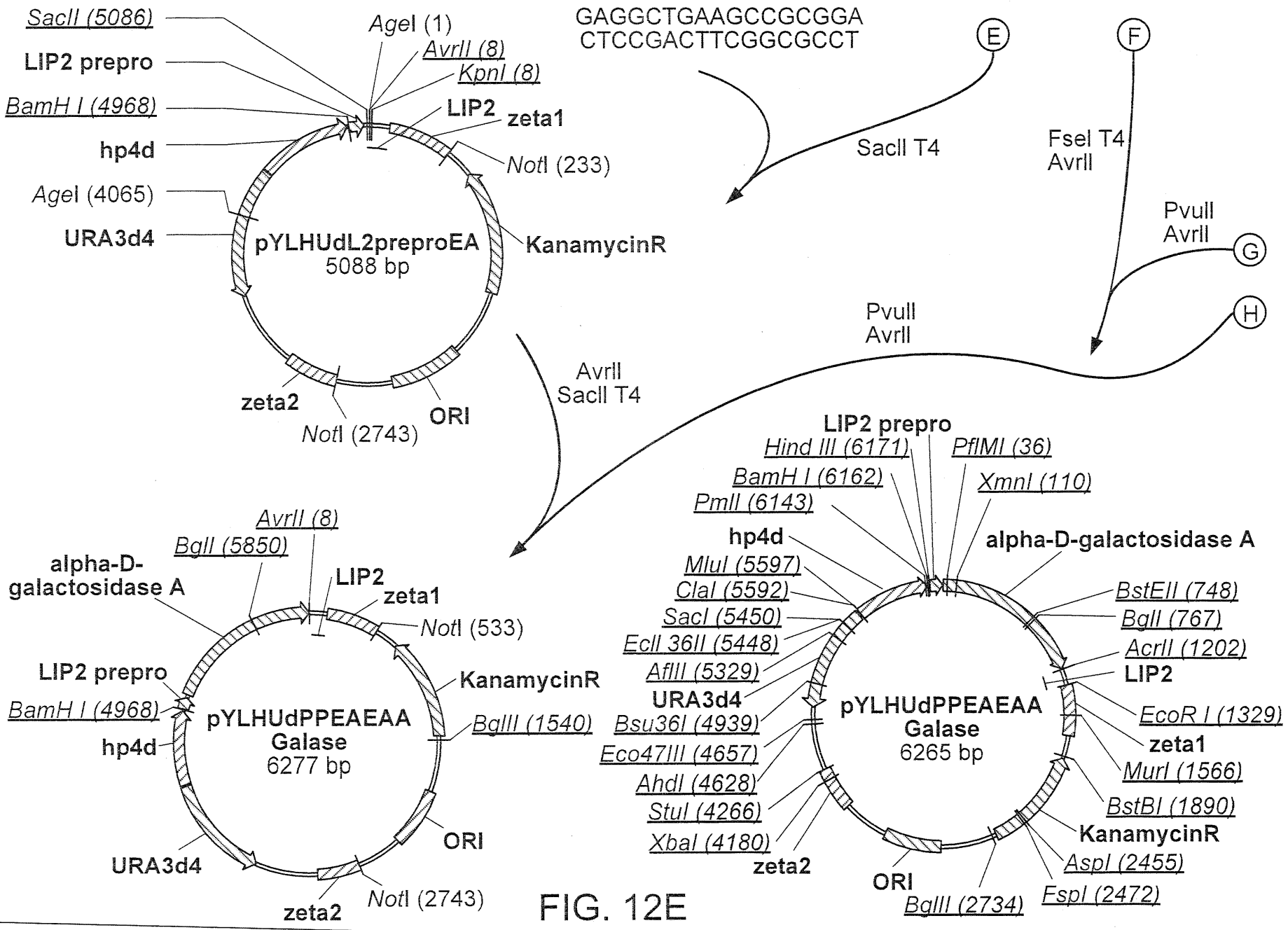
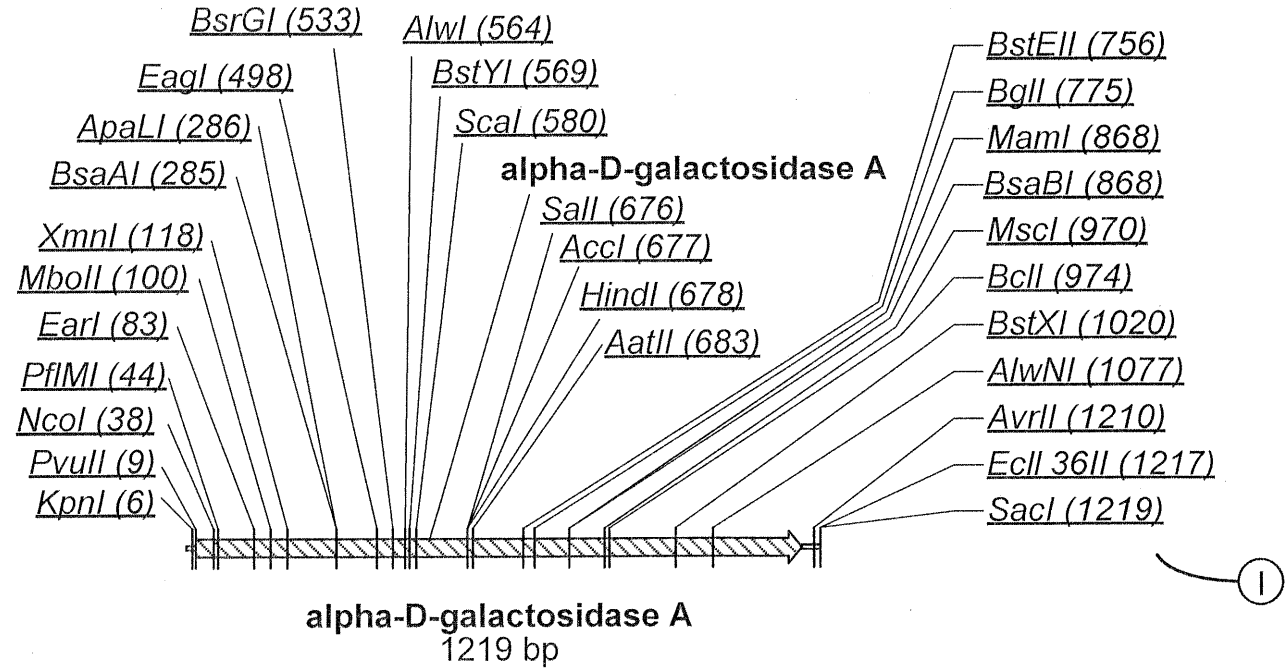

SacII (5086)
AgeI (1)
AvrII (8)
KpnI (8)
LIP2 prepro
BamH I (4968)
LIP2
zeta1
hp4d
NotI (233)
AgeI (4065)
URA3d4
pYLHUdL2preproEA
5088 bp
KanamycinR
zeta2
NotI (2743)
ORI
GAGGCTGAAGCCGCGGA
CTCCGACTTCGGCGCCT
E
SacII T4
F
FseI T4
AvrII
PvuII
AvrII
G
PvuII
AvrII
H
AvrII
SacII T4
AvrII (8)
BglI (5850)
alpha-D-
galactosidase A
LIP2
zeta1
NotI (533)
LIP2 prepro
KanamycinR
BamH I (4968)
pYLHUdPPEAEAA
Galase
6277 bp
BglII (1540)
hp4d
ORI
URA3d4
zeta2
NotI (2743)
LIP2 prepro
Hind III (6171)
PflMI (36)
BamH I (6162)
XmnI (110)
PmlI (6143)
hp4d
alpha-D-galactosidase A
MluI (5597)
ClaI (5592)
BstEII (748)
SacI (5450)
BglI (767)
EclI 36II (5448)
AcrII (1202)
AflII (5329)
LIP2
URA3d4
pYLHUdPPEAEAA
Galase
6265 bp
EcoR I (1329)
Bsu36I (4939)
zeta1
Eco47III (4657)
MurI (1566)
AhdI (4628)
BstBI (1890)
StuI (4266)
KanamycinR
XbaI (4180)
AspI (2455)
zeta2
ORI
BglII (2734)
FspI (2472)

FIG. 12E


BsrGI (533)
AlwI (564)
EagI (498)
BstYI (569)
ApaLI (286)
ScaI (580)
BsaAI (285)
alpha-D-galactosidase A
SalI (676)
XmnI (118)
AccI (677)
MboII (100)
HindI (678)
EarI (83)
AatII (683)
PflMI (44)
NcoI (38)
PvuII (9)
KpnI (6)
BstEII (756)
BglII (775)
MamI (868)
BsaBI (868)
MscI (970)
BclI (974)
BstXI (1020)
AlwNI (1077)
AvrII (1210)
EclI 36II (1217)
SacI (1219)
I
alpha-D-galactosidase A
1219 bp

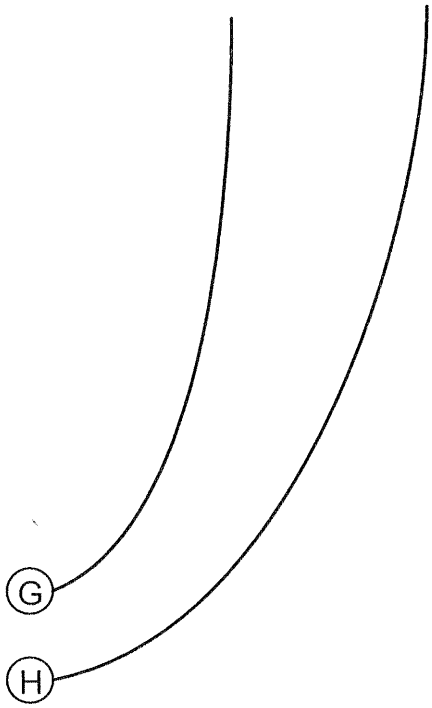

G
H

FIG. 12F

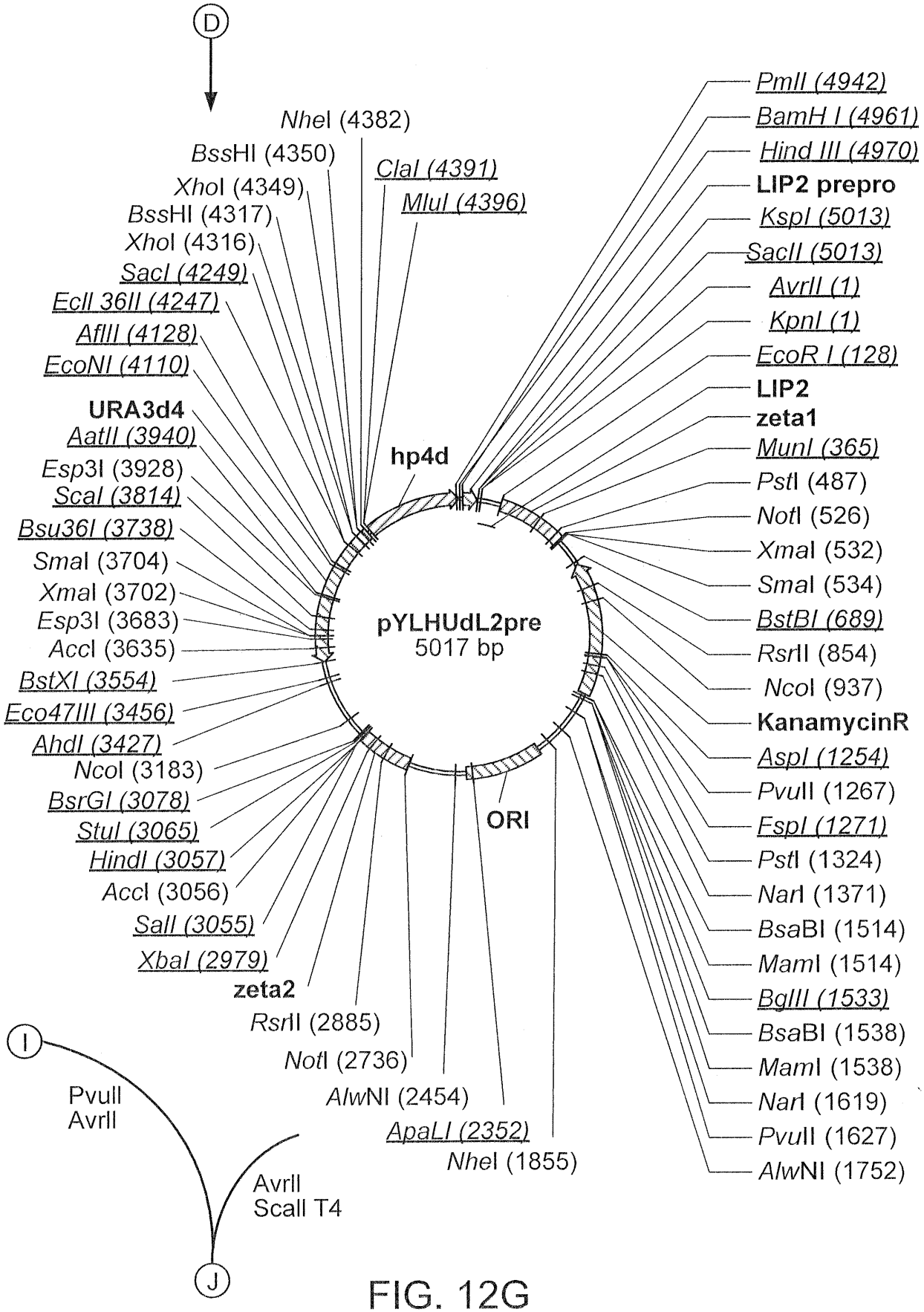

D
NheI (4382)
BssHII (4350)
ClaI (4391)
XhoI (4349)
MluI (4396)
BssHII (4317)
XhoI (4316)
SacI (4249)
EcII 36II (4247)
AflII (4128)
EcoNI (4110)
URA3d4
AatII (3940)
hp4d
Esp3I (3928)
ScaI (3814)
Bsu36I (3738)
SmaI (3704)
XmaI (3702)
pYLHUdL2pre
5017 bp
Esp3I (3683)
AccI (3635)
BstXI (3554)
Eco47III (3456)
AhdI (3427)
NcoI (3183)
BsrGI (3078)
ORI
StuI (3065)
HindI (3057)
AccI (3056)
SalI (3055)
XbaI (2979)
zeta2
RsrII (2885)
NotI (2736)
AlwNI (2454)
ApaLI (2352)
NheI (1855)
PmlI (4942)
BamH I (4961)
Hind III (4970)
LIP2 prepro
KspI (5013)
SacII (5013)
AvrII (1)
KpnI (1)
EcoR I (128)
LIP2
zeta1
MunI (365)
PstI (487)
NotI (526)
XmaI (532)
SmaI (534)
BstBI (689)
RsrII (854)
NcoI (937)
KanamycinR
AspI (1254)
PvuII (1267)
FspI (1271)
PstI (1324)
NarI (1371)
BsaBI (1514)
MamI (1514)
BglII (1533)
BsaBI (1538)
MamI (1538)
NarI (1619)
PvuII (1627)
AlwNI (1752)
I
PvuII
AvrII
AvrII
ScaII T4
J

FIG. 12G

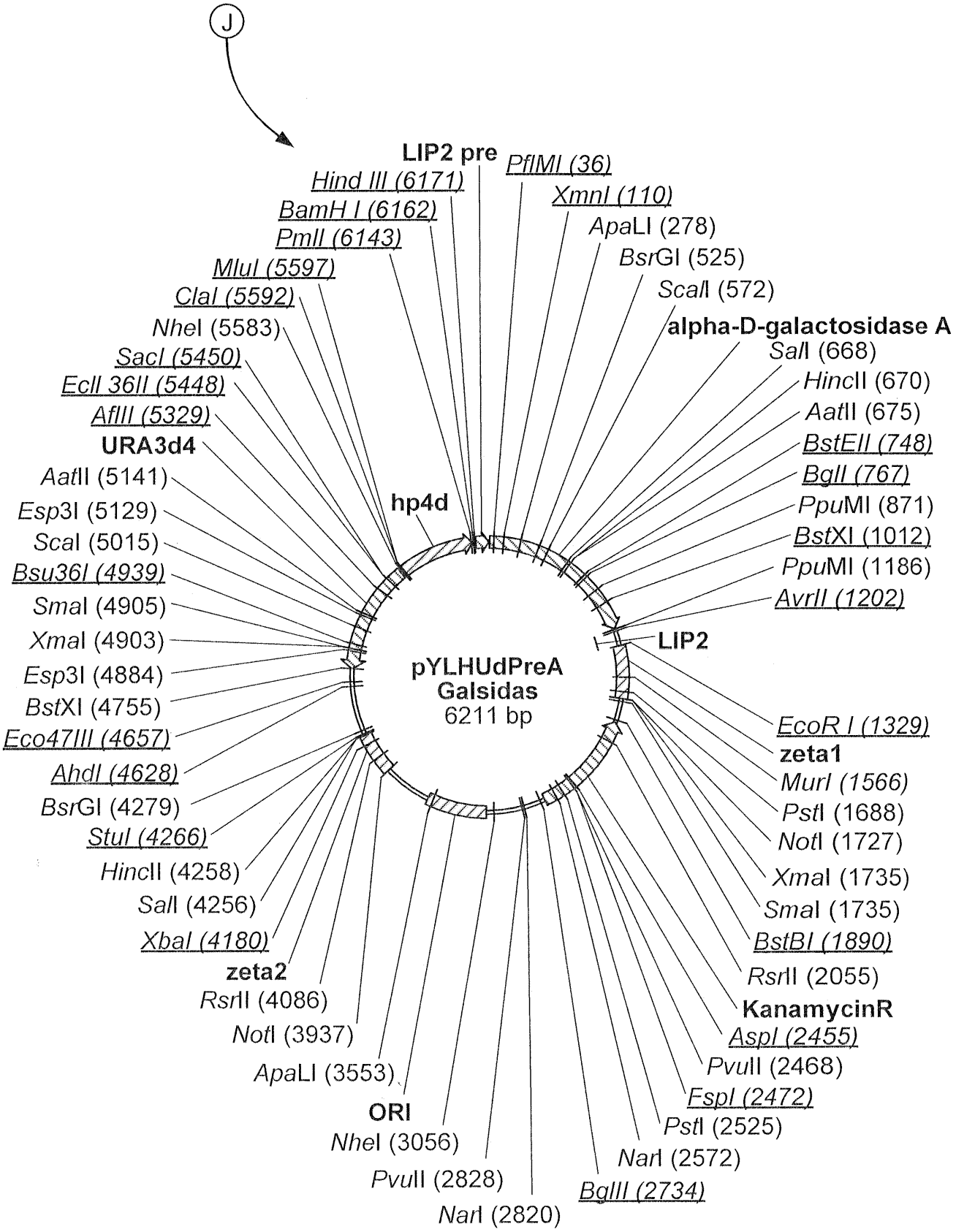

J
LIP2 pre
PflMI (36)
Hind III (6171)
XmnI (110)
BamH I (6162)
ApaLI (278)
PmlI (6143)
BsrGI (525)
MluI (5597)
ScaII (572)
ClaI (5592)
alpha-D-galactosidase A
NheI (5583)
SalI (668)
SacI (5450)
HincII (670)
EcII 36II (5448)
AatII (675)
AflII (5329)
BstEII (748)
URA3d4
BglI (767)
AatII (5141)
hp4d
PpuMI (871)
Esp3I (5129)
BstXI (1012)
ScaI (5015)
PpuMI (1186)
Bsu36I (4939)
AvrII (1202)
SmaI (4905)
LIP2
XmaI (4903)
pYLHUdPreA
Galsidas
6211 bp
Esp3I (4884)
EcoR I (1329)
BstXI (4755)
zeta1
Eco47III (4657)
MurI (1566)
AhdI (4628)
PstI (1688)
BsrGI (4279)
NotI (1727)
StuI (4266)
XmaI (1735)
HincII (4258)
SmaI (1735)
SalI (4256)
BstBI (1890)
XbaI (4180)
RsrII (2055)
zeta2
KanamycinR
RsrII (4086)
AspI (2455)
NotI (3937)
PvuII (2468)
ApaLI (3553)
FspI (2472)
ORI
PstI (2525)
NheI (3056)
NarI (2572)
PvuII (2828)
BglII (2734)
NarI (2820)

FIG. 12H

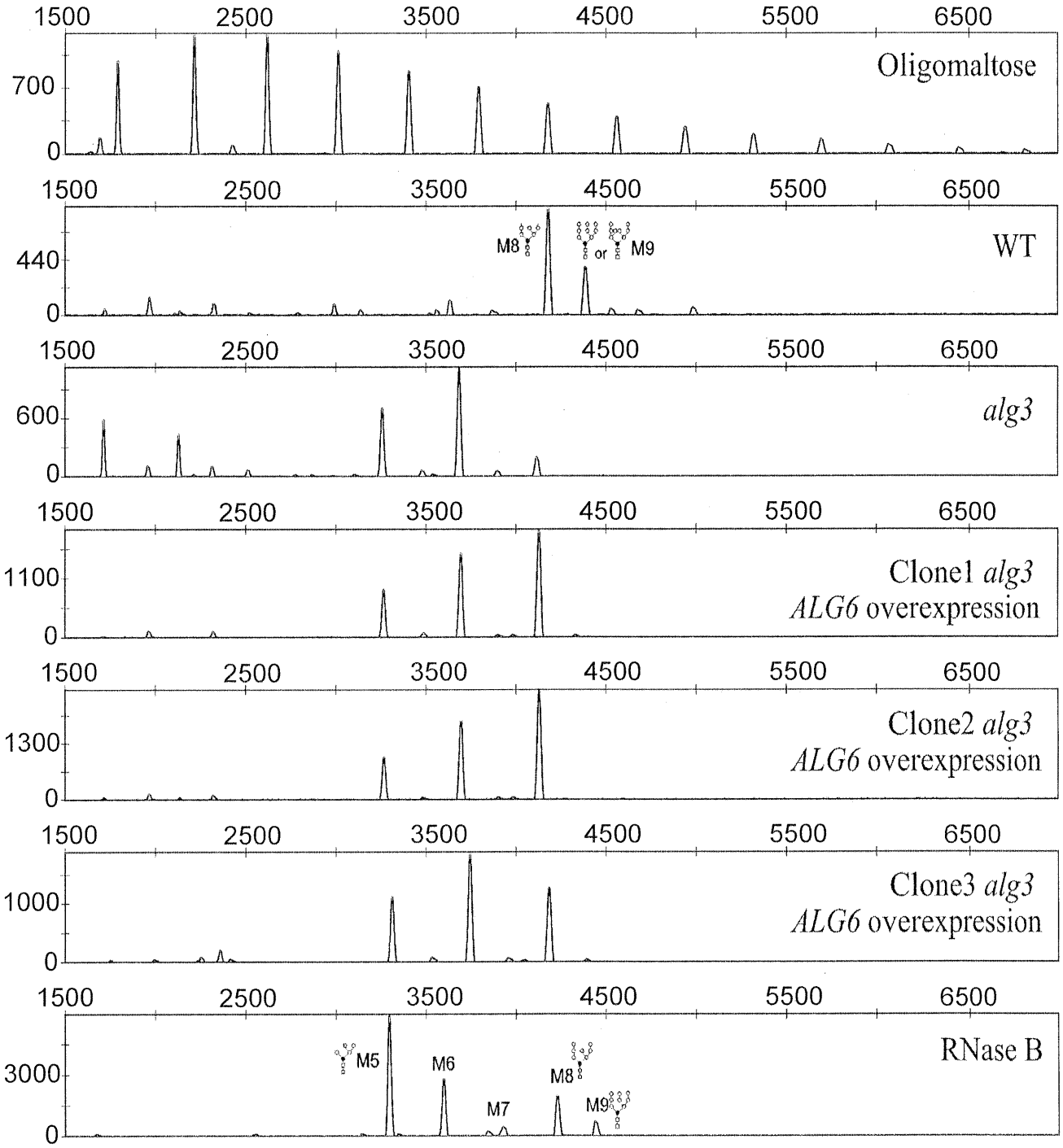

1500
2500
3500
4500
5500
6500
700
0
Oligomaltose
440
M8
or
M9
WT
600
alg3
1100
Clone1 alg3
ALG6 overexpression
1300
Clone2 alg3
ALG6 overexpression
1000
Clone3 alg3
ALG6 overexpression
3000
M5
M6
M7
M8
M9
RNase B

FIG. 13

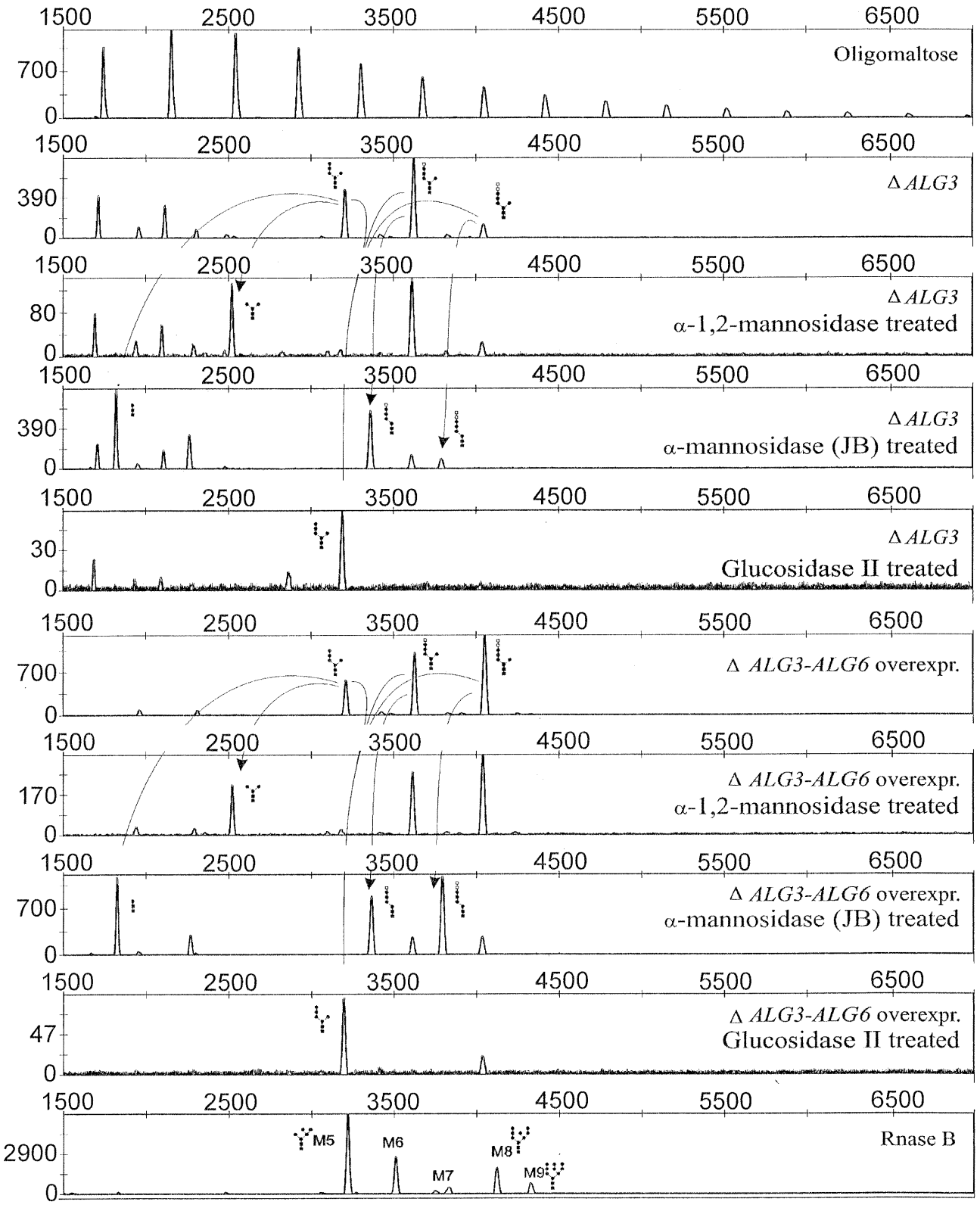

1500
2500
3500
4500
5500
6500
700
0
Oligomaltose
390
Δ ALG3
80
Δ ALG3
α-1,2-mannosidase treated
Δ ALG3
α-mannosidase (JB) treated
30
Δ ALG3
Glucosidase II treated
Δ ALG3-ALG6 overexpr.
170
Δ ALG3-ALG6 overexpr.
α-1,2-mannosidase treated
Δ ALG3-ALG6 overexpr.
α-mannosidase (JB) treated
47
Δ ALG3-ALG6 overexpr.
Glucosidase II treated
2900
M5
M6
M7
M8
M9
Rnase B

FIG. 14

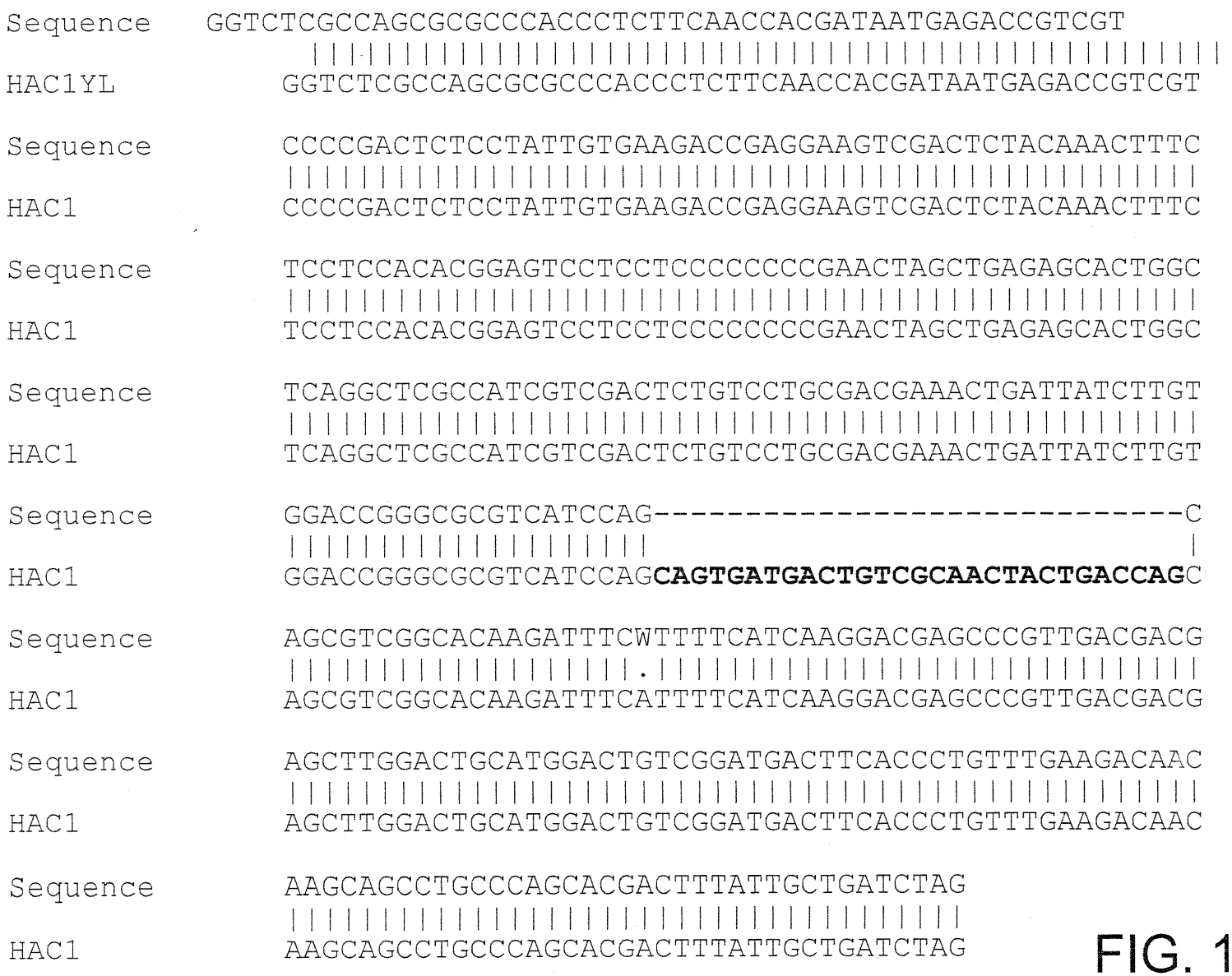

Sequence GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
HAC1YL GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
Sequence CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
HAC1 CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
Sequence TCCTCCACACGGAGTCCTCCTCCCCCCCCCGAACTAGCTGAGAGCACTGGC
HAC1 TCCTCCACACGGAGTCCTCCTCCCCCCCCCGAACTAGCTGAGAGCACTGGC
Sequence TCAGGCTCGCCATCGTCGACTCTGTCCTGCGACGAAACTGATTATCTTGT
HAC1 TCAGGCTCGCCATCGTCGACTCTGTCCTGCGACGAAACTGATTATCTTGT
Sequence GGACCGGGCGCGTCATCCAG----------C
HAC1 GGACCGGGCGCGTCATCCAGCAGTGATGACTGTCGCAACTACTGACCAGC
Sequence AGCGTCGGCACAAGATTTCWTTTTCATCAAGGACGAGCCCGTTGACGACG
HAC1 AGCGTCGGCACAAGATTTCATTTTCATCAAGGACGAGCCCGTTGACGACG
Sequence AGCTTGGACTGCATGGACTGTCGGATGACTTCACCCTGTTTGAAGACAAC
HAC1 AGCTTGGACTGCATGGACTGTCGGATGACTTCACCCTGTTTGAAGACAAC
Sequence AAGCAGCCTGCCCAGCACGACTTTATTGCTGATCTAG
HAC1 AAGCAGCCTGCCCAGCACGACTTTATTGCTGATCTAG

FIG. 15

5′ splice site

P. pastoris atc**cagcagt**gatgacg
S. cerevisiae atc**cagccgt**gattacg
******* ***** ***

3′ splice site

P. pastoris ggt**ctgcagc**accat
S. cerevisiae tgt**ccgaagc**gcagt
*** * *** * *

FIG. 16

```
NI.       1 atgcccgtagattcttctcataagacagctagcccacttccacctcgtaa   50
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.        1 atgcccgtagattcttctcataagacagctagcccacttccacctcgtaa   50

NI.      51 aagagcaaagacggaagaagaaaaggagcagcgtcgagtggaacgtatcc  100
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       51 aagagcaaagacggaagaagaaaaggagcagcgtcgagtggaacgtatcc  100

NI.     101 tacgtaataggagagcggcccatgcttccagagagaagaaacgaagacac  150
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      101 tacgtaataggagagcggcccatgcttccagagagaagaaacgaagacac  150

NI.     151 gttgaatttctggaaaaccacgtcgtcgacctggaatctgcacttcaaga  200
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      151 gttgaatttctggaaaaccacgtcgtcgacctggaatctgcacttcaaga  200

NI.     201 atcagccaaagccactaacaagttgaaagaaatacaagatatcattgttt  250
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      201 atcagccaaagccactaacaagttgaaagaaatacaagatatcattgttt  250

NI.     251 caaggttggaagccttaggtggtaccgtctcagatttggatttaacagtt  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      251 caaggttggaagccttaggtggtaccgtctcagatttggatttaacagtt  300

NI.     301 ccggaagtcgattttcccaaatcttctgatttggaacccatgtctgatct  350
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      301 ccggaagtcgattttcccaaatcttctgatttggaacccatgtctgatct  350

NI.     351 ctcaacttcttcgaaatcggagaaagcatctacatccactcgcagatctt  400
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      351 ctcaacttcttcgaaatcggagaaagcatctacatccactcgcagatctt  400

NI.     401 tgactgaggatctggacgaagatgacgtcgctgaatatgacgacgaagaa  450
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      401 tgactgaggatctggacgaagatgacgtcgctgaatatgacgacgaagaa  450

NI.     451 gaggacgaagagttacccaggaaaatgaaagtcttaaacgacaaaaacaa  500
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      451 gaggacgaagagttacccaggaaaatgaaagtcttaaacgacaaaaacaa  500

NI.     501 gagcacatctatcaagcaggagaagttgaatgaacttccatctcctttgt  550
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      501 gagcacatctatcaagcaggagaagttgaatgaacttccatctcctttgt  550

NI.     551 catccgatttttcagacgtagatgaagaaaagtcaactctcacacattta  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      551 catccgatttttcagacgtagatgaagaaaagtcaactctcacacattta  600

NI.     601 aagttgcaacagcaacaacaacaaccagtagacaattatgtttctactcc  650
            ||||||||||||||||||||||||||||||||||||||||||||||||||
I.      601 aagttgcaacagcaacaacaacaaccagtagacaattatgtttctactcc  650
```

FIG. 17A

```
NI.      651 tttgagtcttccggaggattcagttgattttattaacccaggtaacttaa  700
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       651 tttgagtcttccggaggattcagttgattttattaacccaggtaacttaa  700

NI.      701 aaatagagtccgatgagaacttcttgttgagttcaaatactttacaaata  750
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       701 aaatagagtccgatgagaacttcttgttgagttcaaatactttacaaata  750

NI.      751 aaacacgaaaatgacaccgactacattactacagctccatcaggttccat  800
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       751 aaacacgaaaatgacaccgactacattactacagctccatcaggttccat  800

NI.      801 caatgatttttttaattcttatgacattagcgagtcgaatcggttgcatc  850
             ||||||||||||||||||||||||||||||||||||||||||||||||||
I.       801 caatgatttttttaattcttatgacattagcgagtcgaatcggttgcatc  850

NI.      851 atccagcagtgatgacggattcatctttacacattacagcaggctccatc  900
             ||||||
I.       851 atccag----------------------------------------------  855

NI.      901 ggctttttctctttgattgggggggggaaagttctgtagcagggaggcg  950

I.       856 ----------------------------------------------------  855

NI.      951 cagttcagttggcacatatcagttgacatgcatagcgatcaggtgattgc 1000

I.       856 ----------------------------------------------------  855

NI.     1001 tcttgttaactggtatgccaagtcaaccgttttcggcgtacctactactc 1050

I.       856 ----------------------------------------------------  855

NI.     1051 tactttaactcttgaattgagatgaaacaatcgcccttgttcgagacgcg 1100

I.       856 ----------------------------------------------------  855

NI.     1101 tagttaatttaagatgaatcaatttgcaacaaaagaacaaacgaaaaaga 1150

I.       856 ----------------------------------------------------  855

NI.     1151 taacagcaaacttaagtttcgtggtctgcagcaccatttaccgctaatgc 1200
                                          ||||||||||||||||||||||
I.       856 ----------------------------cagcaccatttaccgctaatgc  878

NI.     1201 atttgatttaaatgactttgtattcttccaggaatag   1237
             ||||||||||||||||||||||||||||||||||
I.       879 atttgatttaaatgactttgtattcttccaggaatag    915
```

FIG. 17B

```
P.pastoris          1  APFTANAFDLNDFVFFQE
18
                       |....|:|:||||....
S.cerevisiae        1 EAQSGLNSFELNDFFITS
18
```

FIG. 18

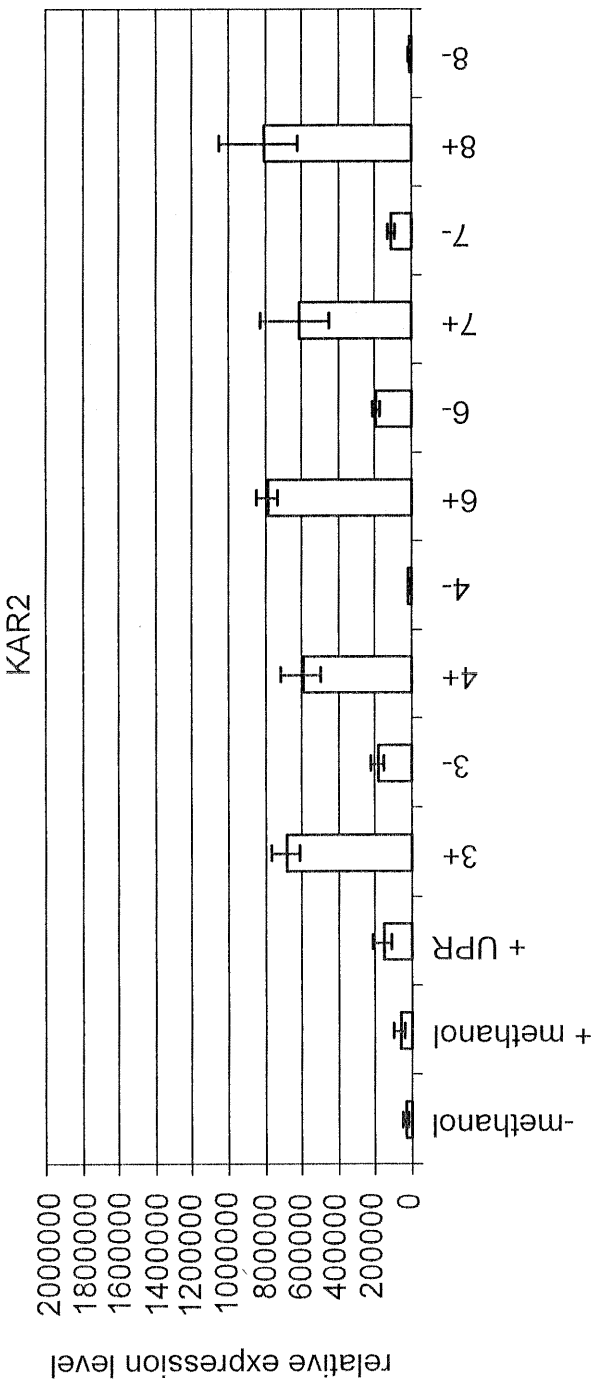

KAR2
relative expression level
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-methanol
+ methanol
+ UPR
3+
3-
4+
4-
6+
6-
7+
7-
8+
8-

FIG. 19

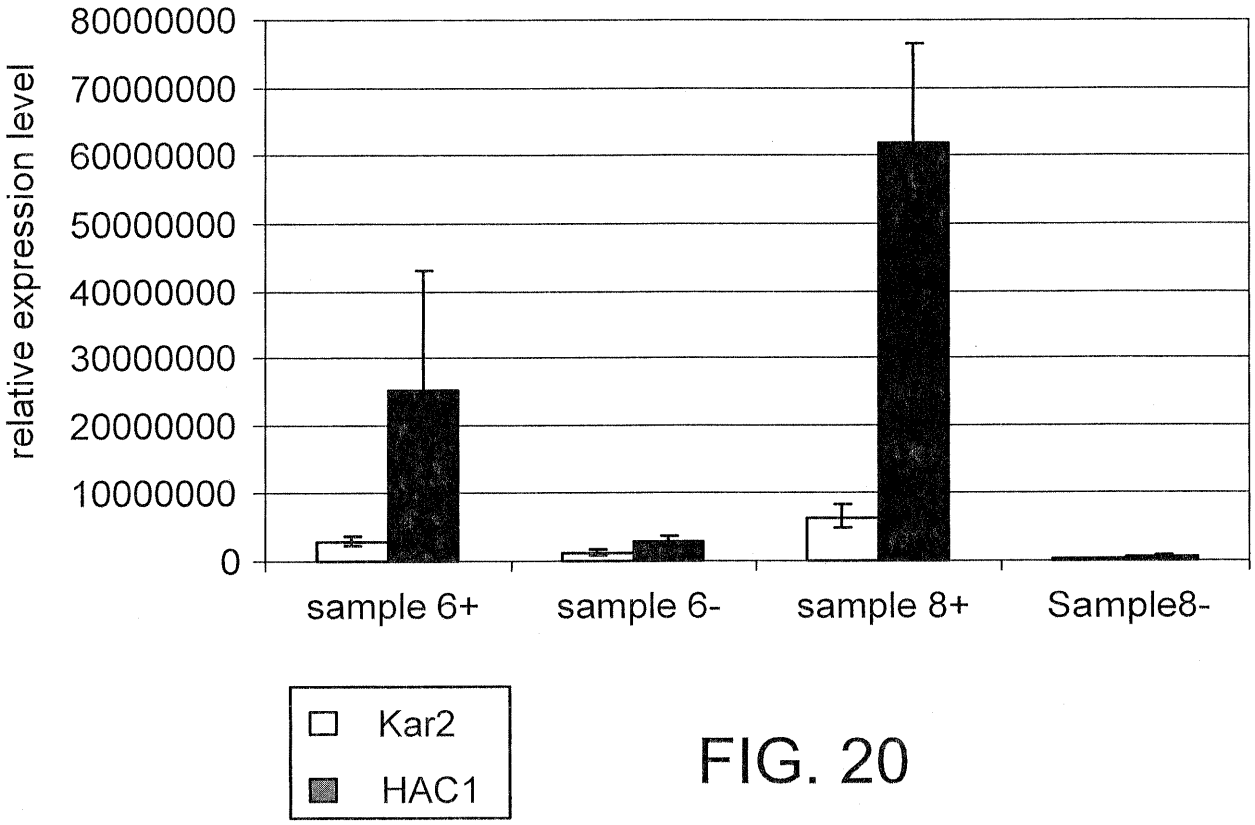

relative expression level
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
sample 6+
sample 6-
sample 8+
Sample8-
Kar2
HAC1

FIG. 20

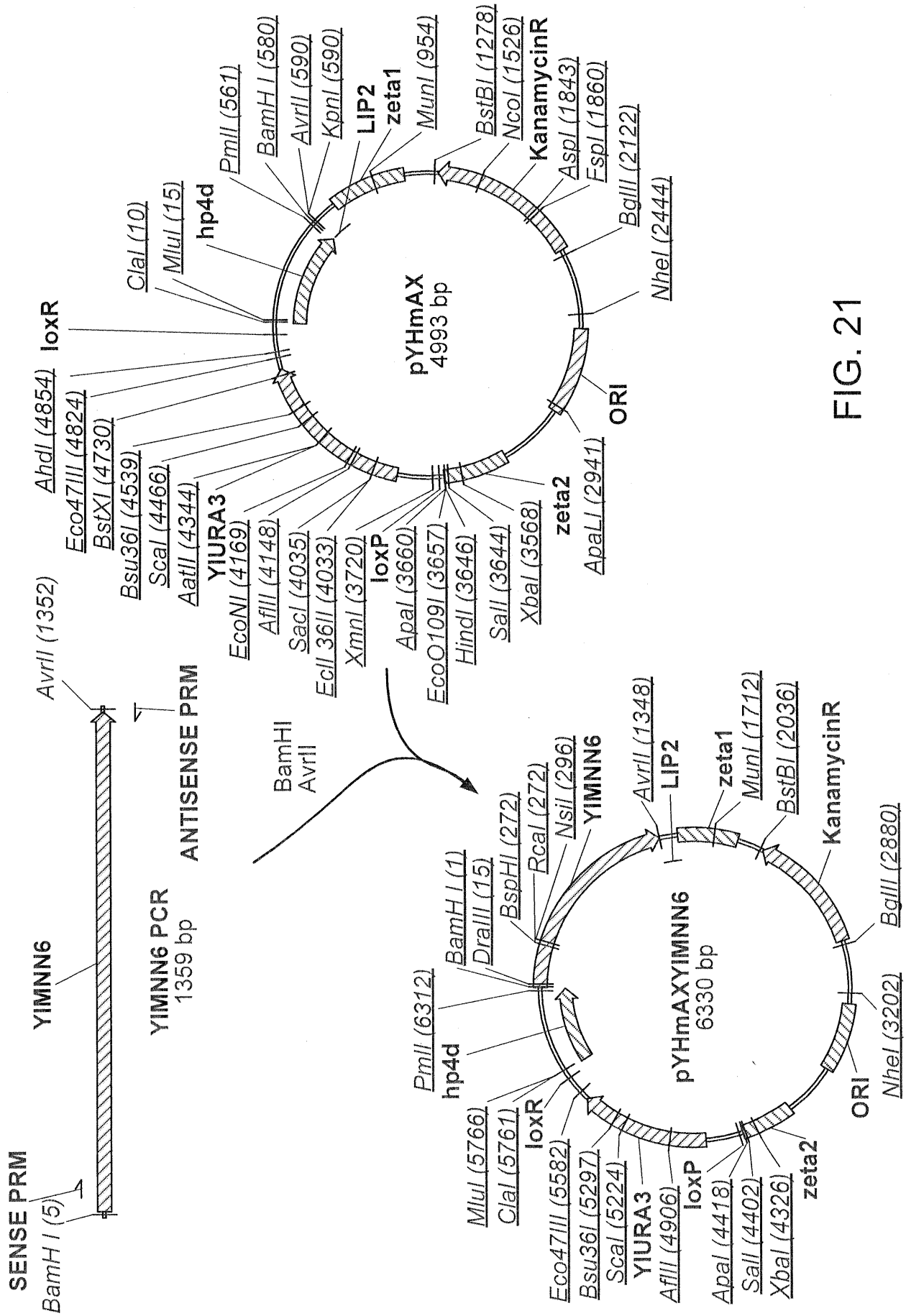

SENSE PRM
BamH I (5)
YIMNN6
YIMNN6 PCR
1359 bp
AvrII (1352)
ANTISENSE PRM
BamHI
AvrII
pYHmAX
4993 bp
ClaI (10)
MluI (15)
hp4d
PmlI (561)
BamH I (580)
AvrII (590)
KpnI (590)
LIP2
zeta1
MunI (954)
BstBI (1278)
NcoI (1526)
KanamycinR
AspI (1843)
FspI (1860)
BglII (2122)
NheI (2444)
ORI
ApaLI (2941)
zeta2
XbaI (3568)
SalI (3644)
HindI (3646)
EcoO109I (3657)
ApaI (3660)
loxP
XmnI (3720)
EclI 36II (4033)
SacI (4035)
AflII (4148)
EcoNI (4169)
YIURA3
AatII (4344)
ScaI (4466)
Bsu36I (4539)
BstXI (4730)
Eco47III (4824)
AhdI (4854)
loxR
pYHmAXYIMNN6
6330 bp
BamH I (1)
DraIII (15)
BspHI (272)
RcaI (272)
NsiI (296)
YIMNN6
AvrII (1348)
LIP2
zeta1
MunI (1712)
BstBI (2036)
KanamycinR
BglII (2880)
NheI (3202)
ORI
zeta2
XbaI (4326)
SalI (4402)
ApaI (4418)
loxP
AflII (4906)
YIURA3
ScaI (5224)
Bsu36I (5297)
Eco47III (5582)
loxR
ClaI (5761)
MluI (5766)
hp4d
PmlI (6312)

FIG. 21

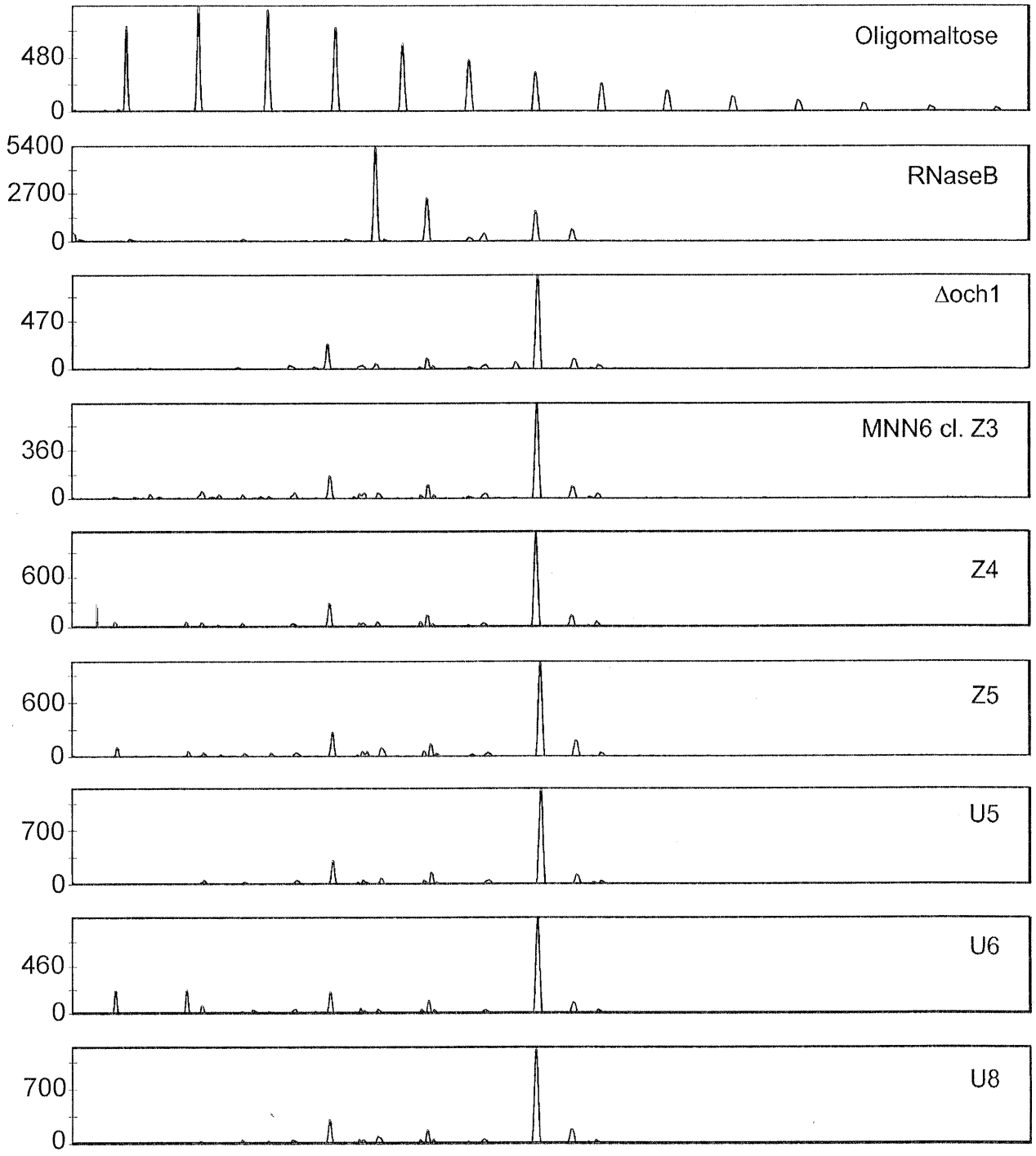

Oligomaltose
480
0
RNaseB
5400
2700
0
Δoch1
470
0
MNN6 cl. Z3
360
0
Z4
600
0
Z5
600
0
U5
700
0
U6
460
0
U8
700
0

FIG. 22

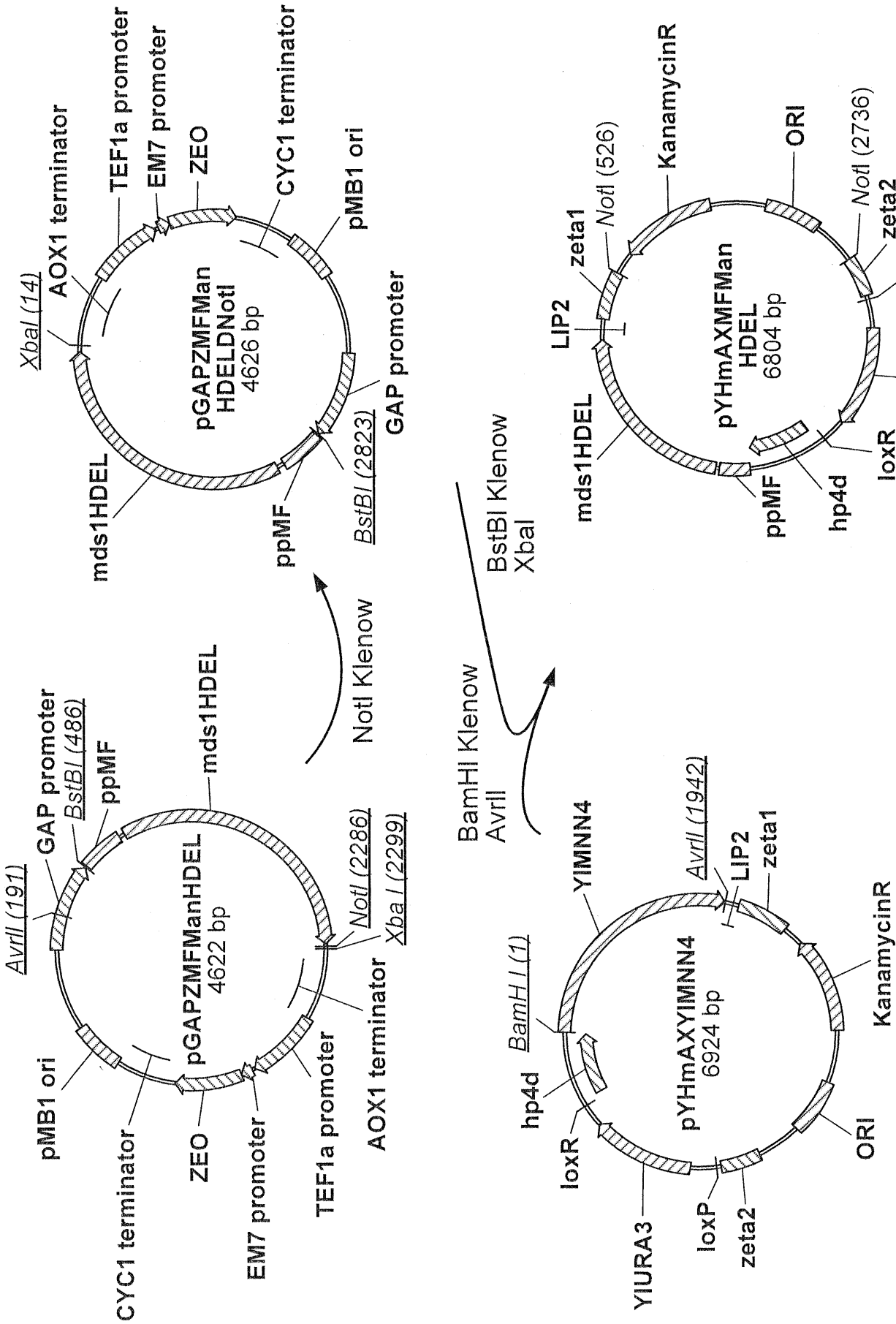

pGAPZMFMan
HDELDNotI
4626 bp
XbaI (14)
AOX1 terminator
TEF1a promoter
EM7 promoter
ZEO
CYC1 terminator
pMB1 ori
GAP promoter
BstBI (2823)
ppMF
mds1HDEL
NotI Klenow
BstBI Klenow
XbaI
pGAPZMFManHDEL
4622 bp
AvrII (191)
GAP promoter
BstBI (486)
ppMF
mds1HDEL
NotI (2286)
Xba I (2299)
AOX1 terminator
TEF1a promoter
EM7 promoter
ZEO
CYC1 terminator
pMB1 ori
BamHI Klenow
AvrII
pYHmAXMFMan
HDEL
6804 bp
LIP2
zeta1
NotI (526)
KanamycinR
ORI
NotI (2736)
zeta2
loxP
YIURA3
loxR
hp4d
ppMF
mds1HDEL
pYHmAXYIMNN4
6924 bp
BamH I (1)
YIMNN4
AvrII (1942)
LIP2
zeta1
KanamycinR
ORI
YIURA3
loxP
zeta2
loxR
hp4d

FIG. 23

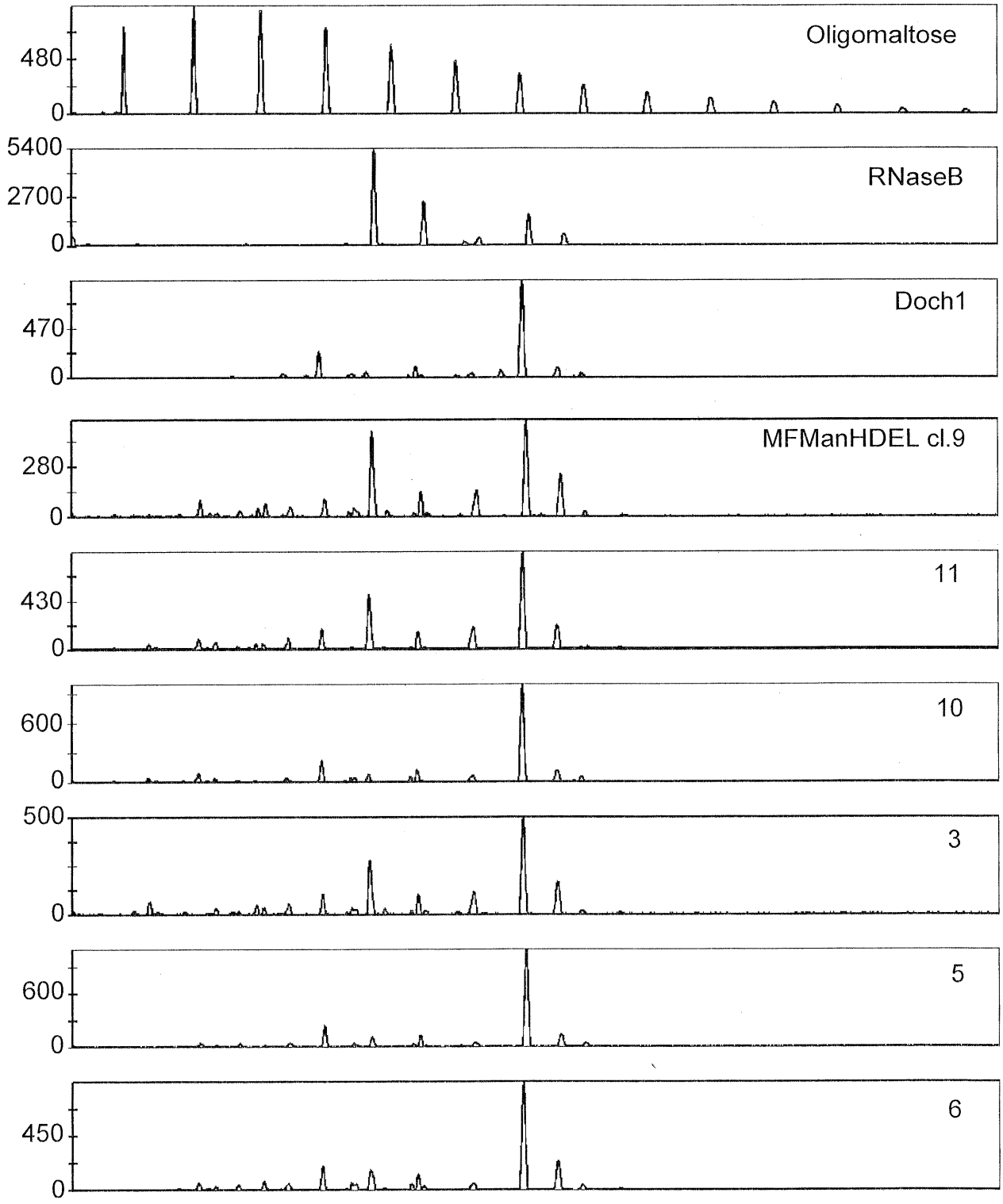

Oligomaltose
480
0
5400
2700
0
RNaseB
Doch1
470
0
MFManHDEL cl.9
280
0
11
430
0
10
600
0
3
500
0
5
600
0
6
450
0

FIG. 24

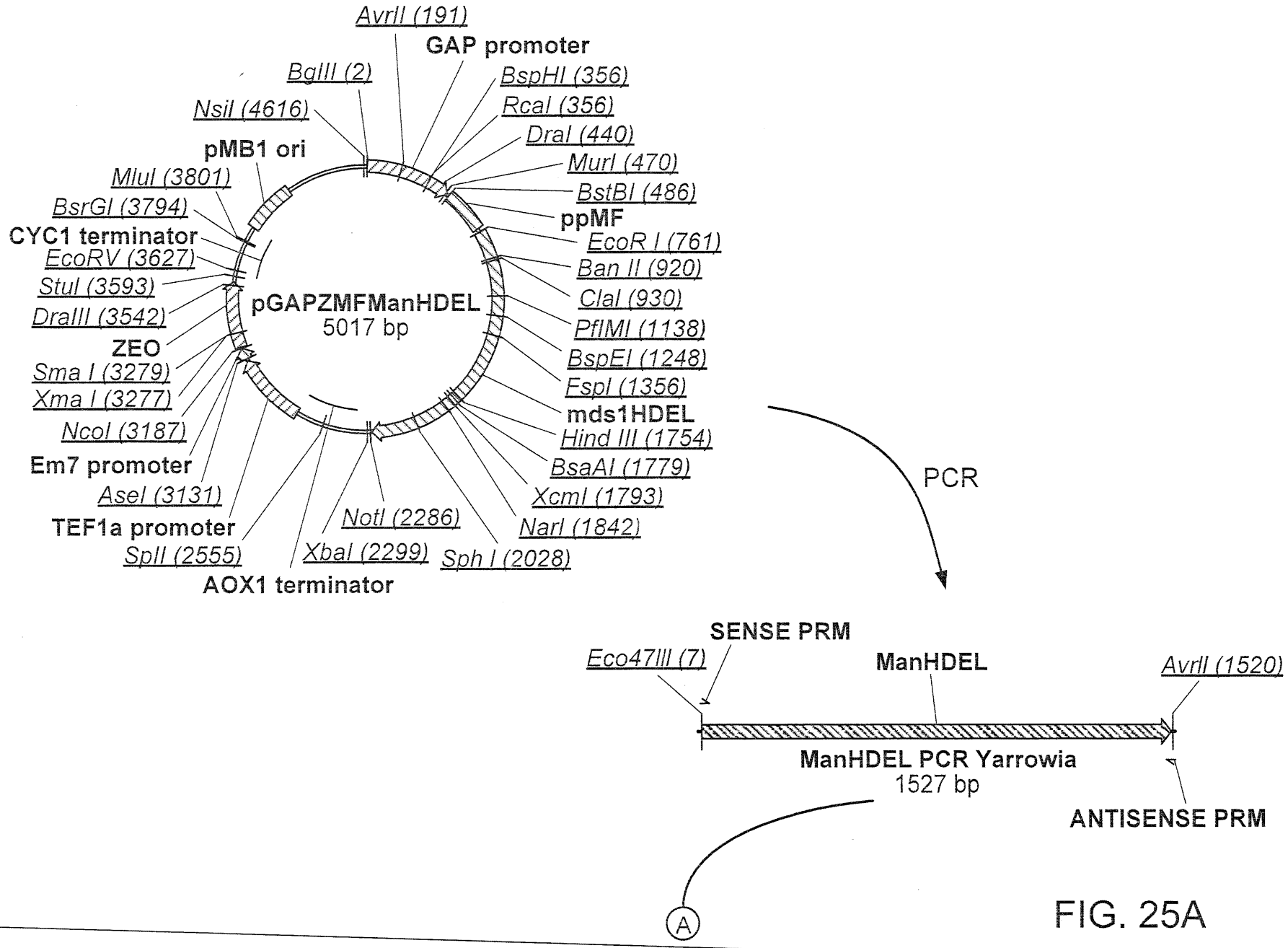

AvrII (191)
GAP promoter
BglII (2)
BspHI (356)
NsiI (4616)
RcaI (356)
pMB1 ori
DraI (440)
MluI (3801)
MurI (470)
BsrGI (3794)
BstBI (486)
CYC1 terminator
ppMF
EcoRV (3627)
EcoR I (761)
StuI (3593)
Ban II (920)
pGAPZMFManHDEL
5017 bp
DraIII (3542)
ClaI (930)
ZEO
PflMI (1138)
Sma I (3279)
BspEI (1248)
Xma I (3277)
FspI (1356)
NcoI (3187)
mds1HDEL
Hind III (1754)
Em7 promoter
BsaAI (1779)
AseI (3131)
XcmI (1793)
TEF1a promoter
NotI (2286)
NarI (1842)
SpII (2555)
XbaI (2299)
Sph I (2028)
AOX1 terminator
PCR
SENSE PRM
Eco47III (7)
ManHDEL
AvrII (1520)
ManHDEL PCR Yarrowia
1527 bp
ANTISENSE PRM
A

FIG. 25A

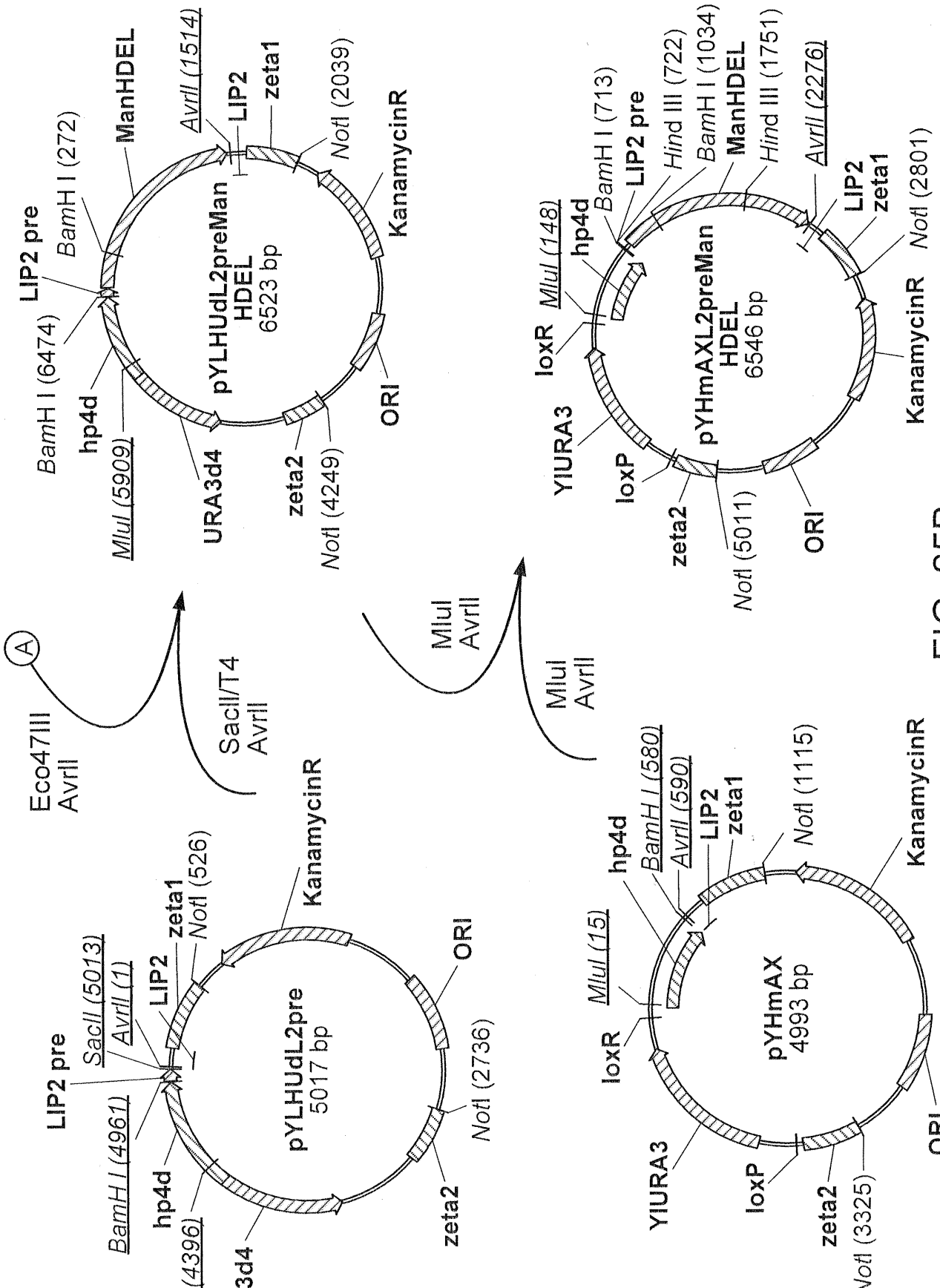

pYLHUdL2preMan
HDEL
6523 bp
LIP2 pre
BamH I (6474)
BamH I (272)
ManHDEL
AvrII (1514)
LIP2
zeta1
NotI (2039)
KanamycinR
ORI
NotI (4249)
zeta2
URA3d4
MluI (5909)
hp4d
pYLHUdL2pre
5017 bp
LIP2 pre
SacII (5013)
AvrII (1)
BamH I (4961)
hp4d
MluI (4396)
URA3d4
LIP2
zeta1
NotI (526)
KanamycinR
ORI
NotI (2736)
zeta2
Eco47III
AvrII
A
SacII/T4
AvrII
MluI
AvrII
MluI
AvrII
pYHmAXL2preMan
HDEL
6546 bp
loxR
MluI (148)
hp4d
BamH I (713)
LIP2 pre
Hind III (722)
BamH I (1034)
ManHDEL
Hind III (1751)
AvrII (2276)
LIP2
zeta1
NotI (2801)
KanamycinR
YlURA3
loxP
zeta2
NotI (5011)
ORI
pYHmAX
4993 bp
loxR
MluI (15)
hp4d
BamH I (580)
AvrII (590)
LIP2
zeta1
NotI (1115)
KanamycinR
YlURA3
loxP
zeta2
NotI (3325)
ORI

FIG. 25B

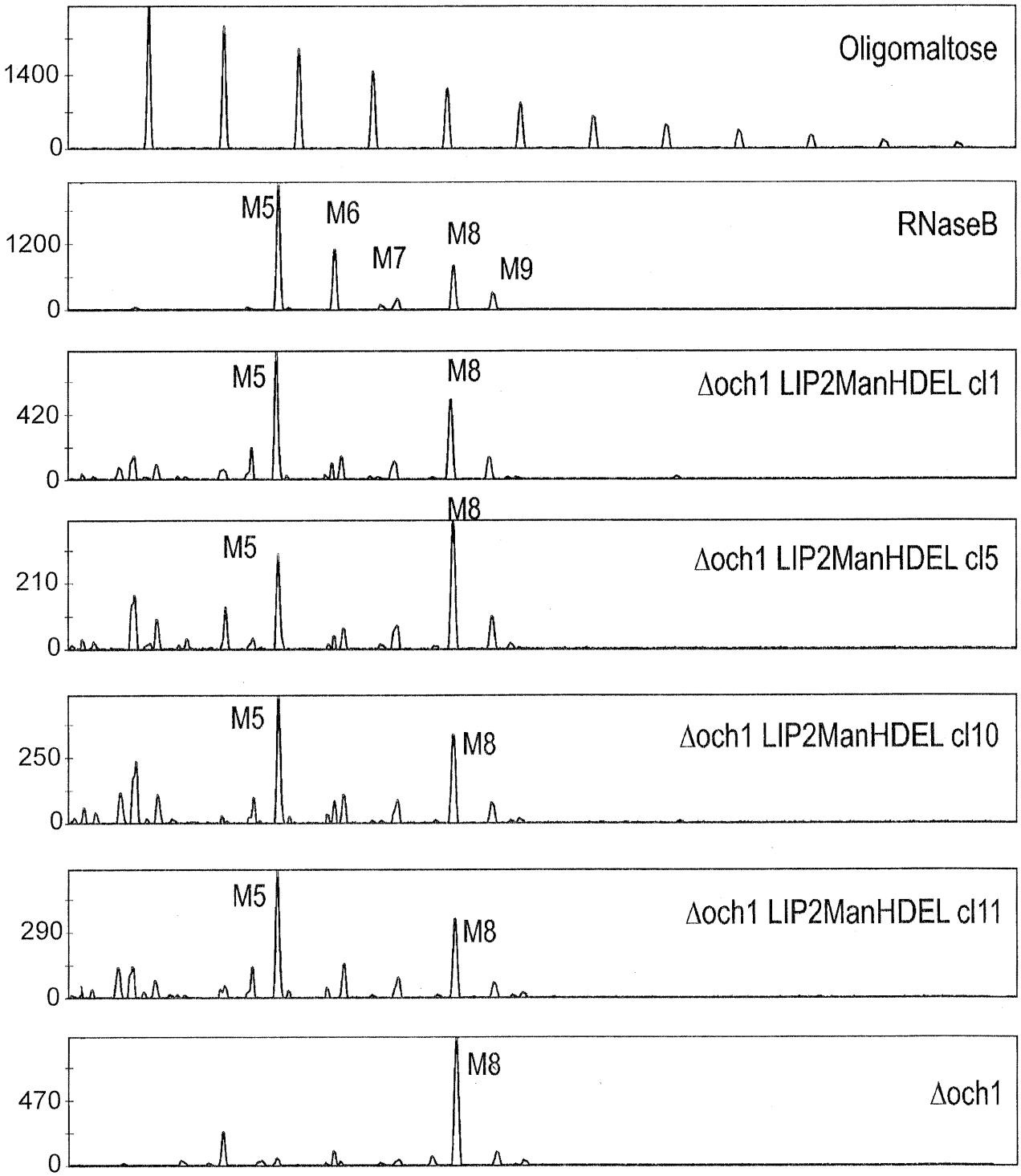

Oligomaltose
1400
0
M5
M6
M7
M8
M9
RNaseB
1200
0
M5
M8
Δoch1 LIP2ManHDEL cl1
420
0
M8
M5
Δoch1 LIP2ManHDEL cl5
210
0
M5
M8
Δoch1 LIP2ManHDEL cl10
250
0
M5
M8
Δoch1 LIP2ManHDEL cl11
290
0
M8
Δoch1
470
0

FIG. 26

MSIKREESFTPTPEDLGSPLTADSPGSPESGDKRKKDLTLP
LPAGALPPRKRAKTENEKEQRRIERIMRNRQAAHASREKKRRH
LEDLEKKCSELSSENNDLHHQVTESKKTNMHLMEQHYSLVAK
LQQLSSLVNMAKSSGALAGVDVPDMSDVSMAPKLEMPTAAPS
QPMGLASAPTLFNHDNETVVPDSPIVKTEEVDSTNFLLHTESSS
PPELAESTGSGSPSSTLSCDETDYLVDRARHPAASAQDFIFIKD
EPVDDELGLHGLSDDFTLFEDNKQPAQHDFIADLAHYESSVSN
LFGGLE

FIG. 27A

MPVDSSHKTASPLPPRKRAKTEEEKEQRRVERILRNRRAA
HASREKKRRHVEFLENHVVDLESALQESAKATNKLKEIQDIIV
SRLEALGGTVSDLDLTVPEVDFPKSSDLEPMSDLSTSSKSEKAS
TSTRRSLTEDLDEDDVAEYDDEEEDEELPRKMKVLNDKNKSTS
IKQEKLNELPSPLSSDFSDVDEEKSTLTHLKLQQQQQQPVDNY
VSTPLSLPEDSVDFINPGNLKIESDENFLLSSNTLQIKHENDTD
YITTAPSGSINDFFNSYDISESNRLHHPAAPFTANAFDLNDFVF
FQE

FIG. 27B

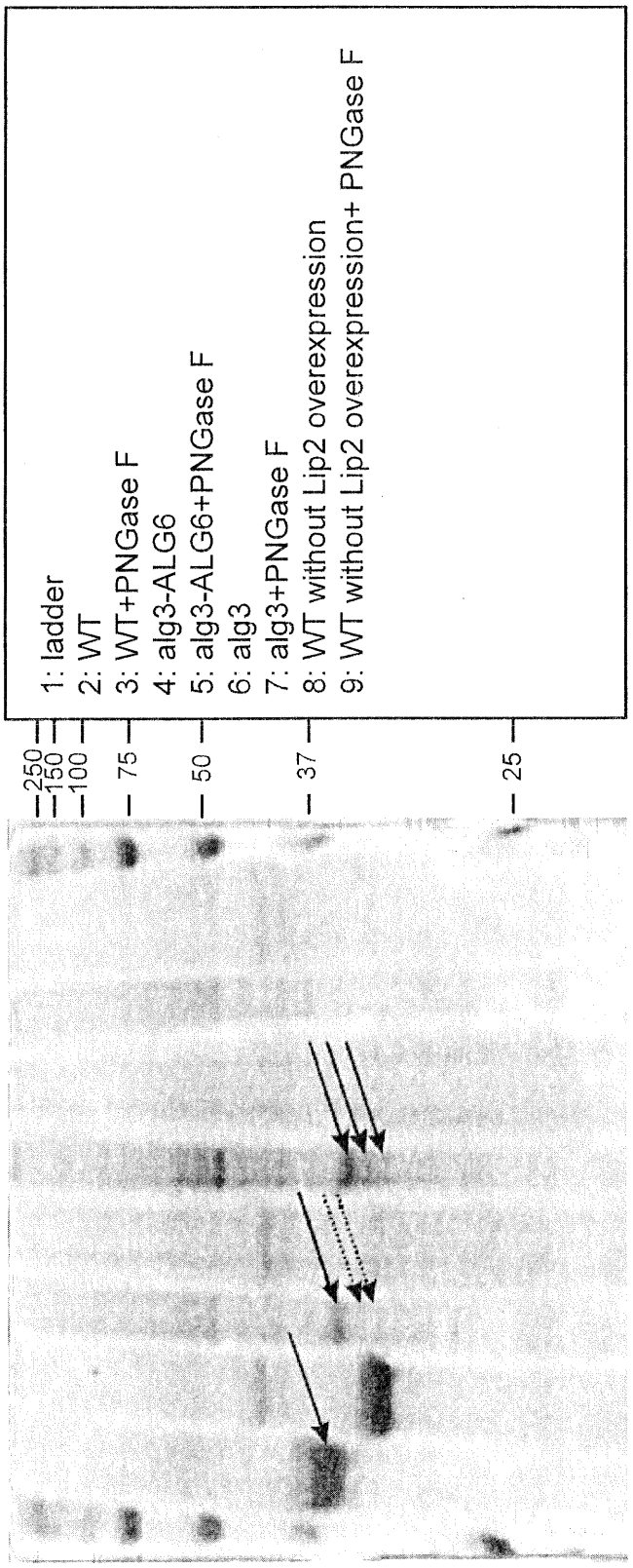

1: ladder
2: WT
3: WT+PNGase F
4: alg3-ALG6
5: alg3-ALG6+PNGase F
6: alg3
7: alg3+PNGase F
8: WT without Lip2 overexpression
9: WT without Lip2 overexpression+ PNGase F
250
150
100
75
50
37
25

FIG. 28

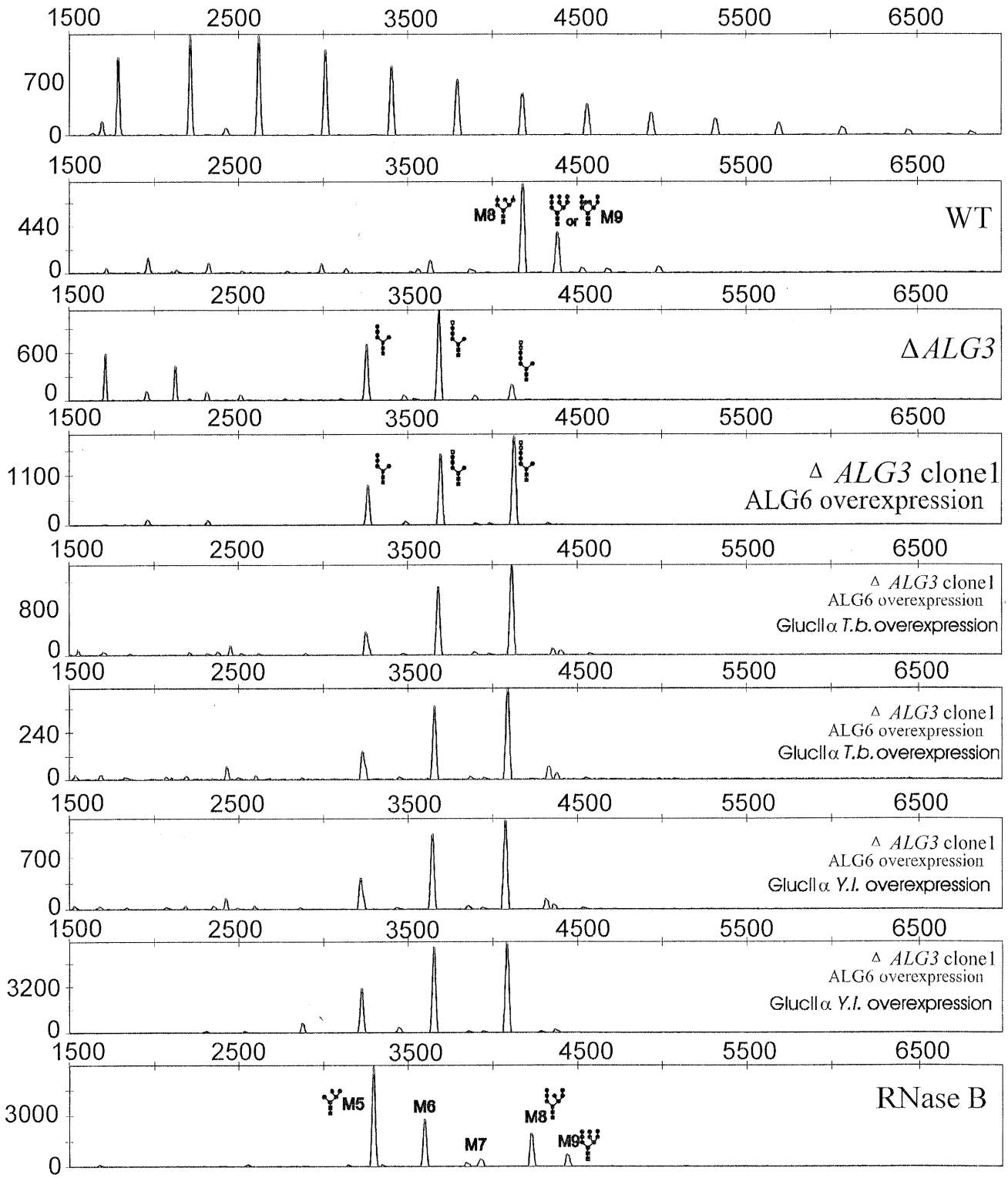

1500
2500
3500
4500
5500
6500
700
0
1500
2500
3500
4500
5500
6500
440
0
M8
or
M9
WT
1500
2500
3500
4500
5500
6500
600
0
ΔALG3
1500
2500
3500
4500
5500
6500
1100
0
Δ ALG3 clone1
ALG6 overexpression
1500
2500
3500
4500
5500
6500
800
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα T.b. overexpression
1500
2500
3500
4500
5500
6500
240
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα T.b. overexpression
1500
2500
3500
4500
5500
6500
700
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα Y.l. overexpression
1500
2500
3500
4500
5500
6500
3200
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα Y.l. overexpression
1500
2500
3500
4500
5500
6500
3000
0
M5
M6
M7
M8
M9
RNase B

FIG. 29

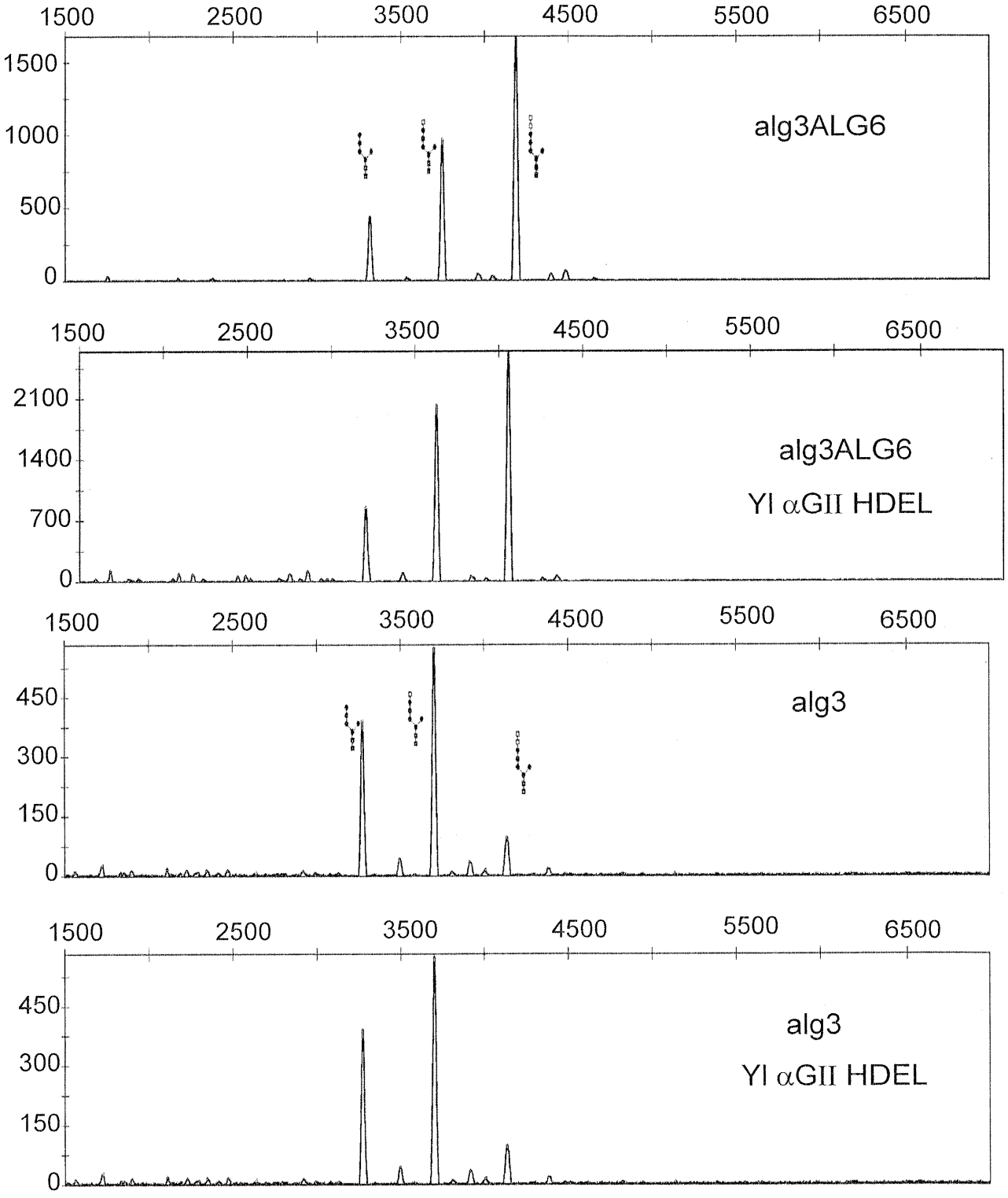

1500
2500
3500
4500
5500
6500
1500
1000
500
0
alg3ALG6
1500
2500
3500
4500
5500
6500
2100
1400
700
0
alg3ALG6
Yl αGII HDEL
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
Yl αGII HDEL

FIG. 30

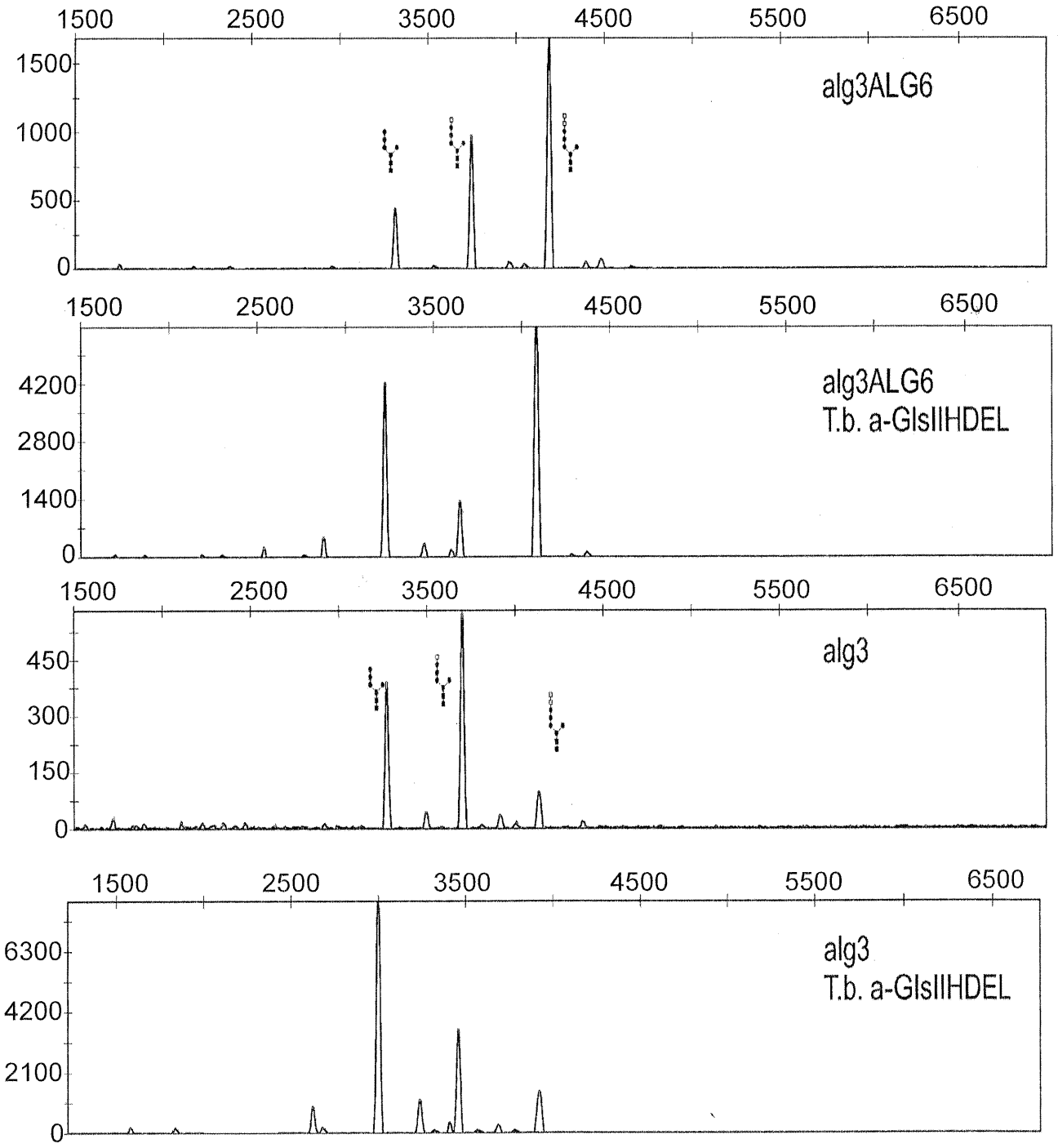

1500
2500
3500
4500
5500
6500
1500
1000
500
0
alg3ALG6
1500
2500
3500
4500
5500
6500
4200
2800
1400
0
alg3ALG6
T.b. a-GlsIIHDEL
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
1500
2500
3500
4500
5500
6500
6300
4200
2100
0
alg3
T.b. a-GlsIIHDEL

FIG. 31

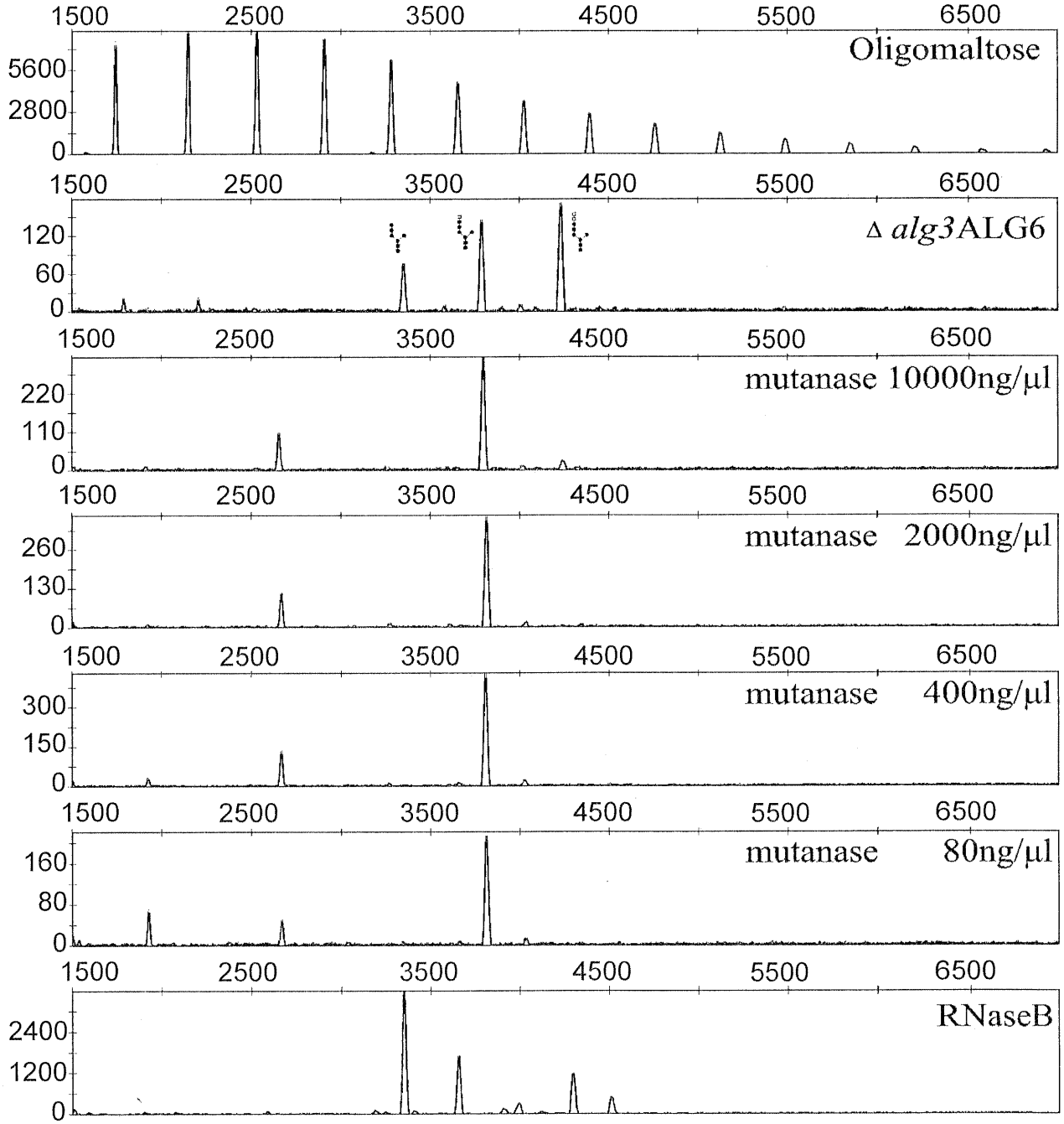

1500
2500
3500
4500
5500
6500
Oligomaltose
5600
2800
0
Δ alg3ALG6
120
60
mutanase 10000ng/μl
220
110
mutanase 2000ng/μl
260
130
mutanase 400ng/μl
300
150
mutanase 80ng/μl
160
80
RNaseB
2400
1200

FIG. 32

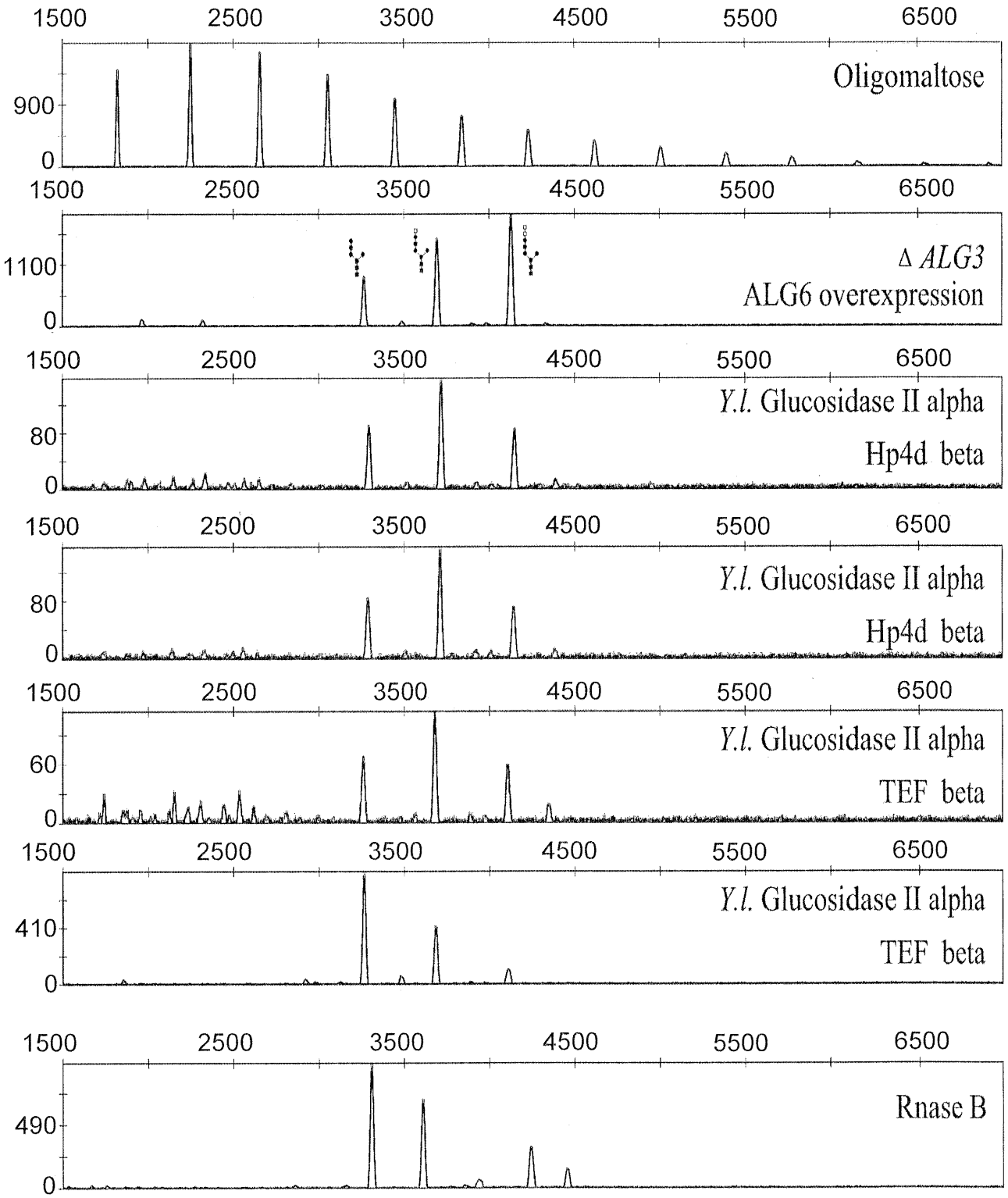

1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
1100
Δ ALG3
ALG6 overexpression
80
Y.l. Glucosidase II alpha
Hp4d beta
60
TEF beta
410
490
Rnase B

FIG. 33

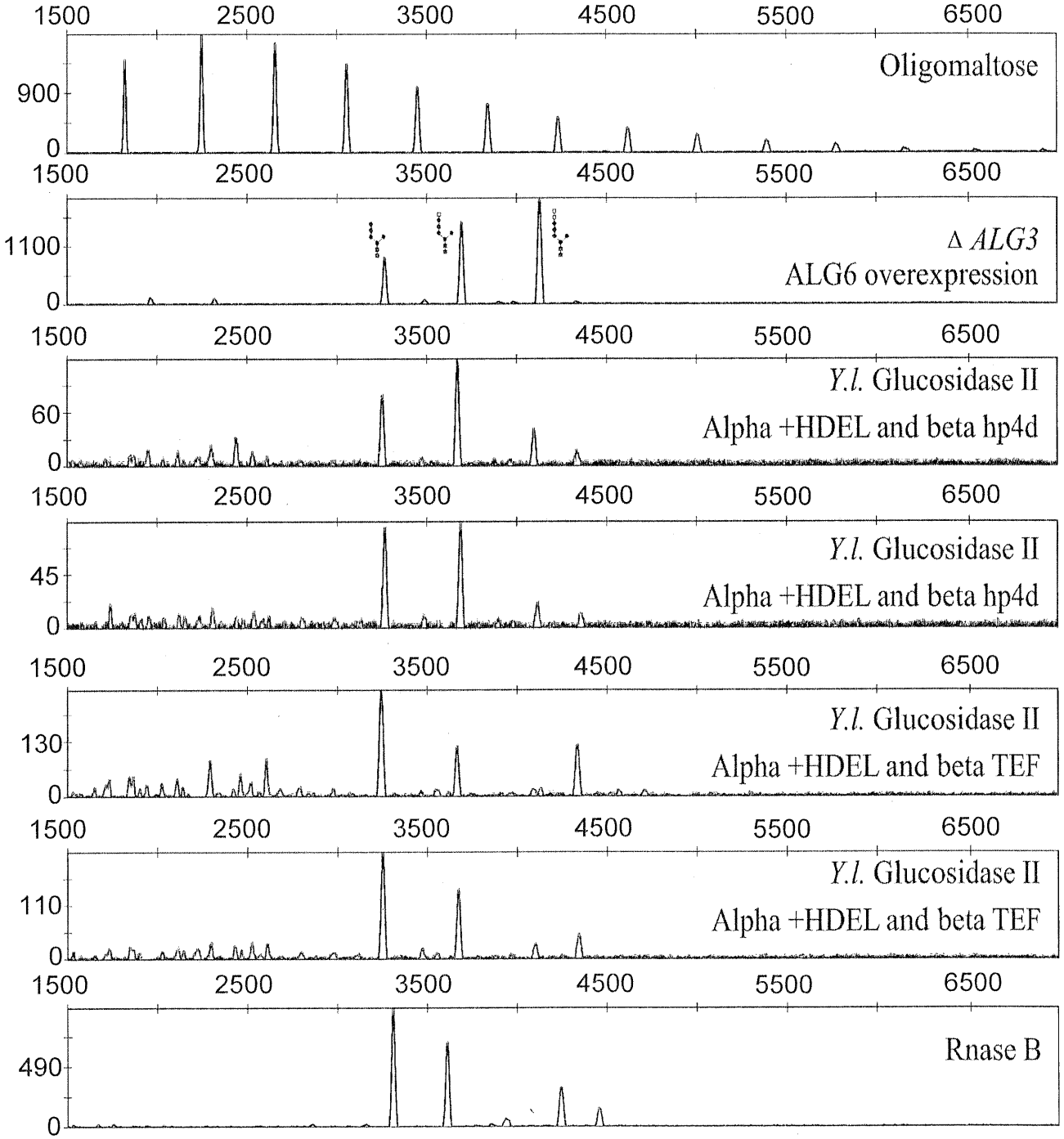

1500
2500
3500
4500
5500
6500
Oligomaltose
900
0
Δ ALG3
ALG6 overexpression
1100
0
Y.l. Glucosidase II
Alpha +HDEL and beta hp4d
60
0
Y.l. Glucosidase II
Alpha +HDEL and beta hp4d
45
0
Y.l. Glucosidase II
Alpha +HDEL and beta TEF
130
0
Y.l. Glucosidase II
Alpha +HDEL and beta TEF
110
0
Rnase B
490
0

FIG. 34

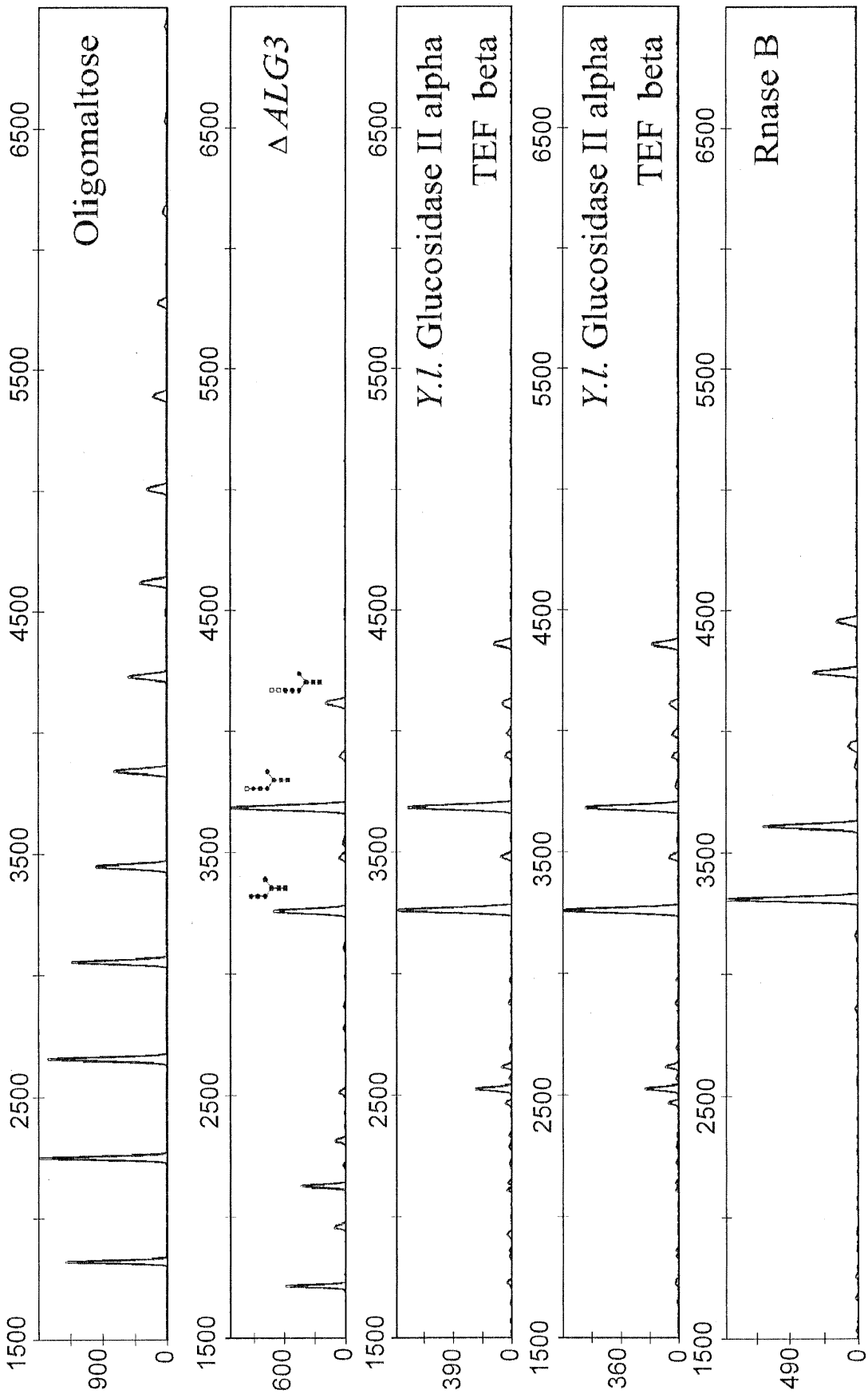

Oligomaltose
ΔALG3
Y.l. Glucosidase II alpha
TEF beta
Y.l. Glucosidase II alpha
TEF beta
Rnase B
1500
2500
3500
4500
5500
6500
900
600
390
360
490
0

FIG. 35

gtaaagcacgagaacttcaagacctgttctcagtctggcttctgtaagcgaaaccgagccttcgccgacgacgccgctg
cccagggctcttcttgggcctctccctacgagctggactcttcttctatccagttcaaggacggccagctgcacggcacc
atcctgaagtctgtgtctcccaacgagaaggtgaagctgcccctggtggtgtctttcctggagtctggcgccgctcgagt
ggtggtggacgaggagaagcgaatgaacggcgacatccagctgcgacacgactctaaggcccgaaaggagcgata
caacgaggccgagaagtgggtgctggtcggcggcctggagctgtctaagaccgccaccctgcgacccgagaccga
gtctggcttcacccgagtgctgtacggccccgacaaccagttcgaggccgtgatccgacacgccccttctctgccgac
ttcaagcgagatggccagacccacgtgcagctgaacaacaagggctacctgaacatggagcactggcgacccaagg
tggaggtggagggcgagggtgagcagcagacccaggaggacgagtctacctggtgggacgagtctttcggcggca
acaccgacaccaagccccgaggccccgagtctgtgggcctggacatcaccttccccggctacaagcacgtgttcggc
atccccgagcacgccgactccctgtctctgaaggagacccgaggcggcgagggcaaccacgaggagccctaccga
atgtacaacgccgacgtgttcgagtacgagctgtcctctcccatgaccctgtacggcgccatccccttcatgcaggccca
ccgaaaggactctaccgtgggcgtgttctggctgaacgccgccgagacctgggtggacatcgtgaagtctacctcttct
cccaaccccctggccctgggcgtgggagccaccaccgacacccagtctcactggttctctgagtctggccagctggac
gtgttcgtgttcctgggccccacccccccaggagatttctaagacctacggcgagctgaccggctacacccagctgcccc
agcacttcgccattgcctaccaccagtgtcgatggaactacatcaccgacgaggacgtgaaggaggtggaccgaaact
tcgacaagtaccagatcccctacgacgtgatctggctggacatcgagtacaccgacgaccgaaagtacttcacctggg
acccccctgtctttccccgaccccatctctatggaggagcagctggacgagtctgagcgaaagctggtcgtgatcatcga
cccccacatcaagaaccaggacaagtactctatcgtgcaggagatgaagtctaaggacctggccaccaagaacaagg
acggcgagatttacgacggctggtgttggcccggctcttctcactggatcgacaccttcaaccccgctgccatcaagtg
gtgggtgtctctgttcaagttcgacaagttcaagggcaccctgtctaacgtgttcatctggaacgacatgaacgagccctc
tgtgttcaacggccccgagaccaccatgcccaaggacaacctgcaccacggcaactgggagcaccgagacatccac
aacgtgcacggcatcaccctggtgaacgccacctacgacgccctgctggagcgaaagaagggcgagatccgacgac
ccttcatcctgacccgatcttactacgccggtgcccagcgaatgtctgccatgtggaccggcgacaaccaggccacctg
ggagcacctggccgcctctatccccatggtgctgaacaacggaatcgccggcttccccttcgccggtgccgacgtggg
cggcttcttccagaaccccctctaaggagctgctgacccgatggtatcaggccggcatctggtatcctttcttccgagccca
cgcccacatcgacacccgacgacgagagccctacctgatcgccgagccccaccgatctatcatctctcaggccatccg
actgcgataccagctgctgcccgcctggtacaccgccttccacgaggcctctgtgaacggcatgcccatcgtgcgacc
ccagtactacgcccacccctgggacgaggccggcttcgccatcgacgaccagctgtacctgggctctaccggcctgct
ggccaagcccgtggtgtctgaggaggccaccaccgccgacatctacctggctgacgacgagaagtactacga

FIG. 36A

ctacttcgactacaccgtgtaccagggcgctggcaagcgacacaccgtgcccgctcccatggagaccgtgcccctg
ctgatgcagggcggccacgtgatccccccgaaaggaccgaccccgacgatcttctgccctgatgcgatgggacccct
acaccctggtggtggtgctggacaagaacggccaggccgacggctctctgtacgtggacgacggcgagaccttcga
ctacgagcgaggcgcctacatccatcgacgattccgattccaggagtctgccctggtctccgaggacgtgggcacca
agggccccaagaccgccgagtacctcaagaccatggccaacgtgcgagtggagcgagtggtggtcgtggacccccc
ccaaggagtggcagggcaagacctctgtgaccgtgatcgaggacggcgcctctgccgcctctaccgcctccatgca
gtaccactctcagcccgacggcaaggccgcctacgccgtggtgaagaaccccaacgtgggcatcggcaagacctg
gcgaatcgagttctaatagcctag

FIG. 36B

agcgctggcgacgcctcttctcgaccccgaggcgtgggccccgagttcgccaagttctacaaggacaccaccacc
ttcacctgtatctctcaccccgccatccagattcccttctctgccgtgaacgacgactactgtgactgtcccgacggct
ctgacgagcccggcacctctgcctgtgcctttctgtctcgaaactctgccctgacccccggtgagcgacccggatct
gacgacctggagctgacctctgccctgcccggcttctactgtaagaacaagggccacaagcccggctacgtgccc
ttccagcgagttaacgatggcatctgtgactacgagctgtgttgtgacggatcggatgagtgggctcgacccggcg
gaaccaagtgtgaggacaagtgtaaggagatcggcaaggagtggcgaaagaaggaggagaagcgacagaagt
ctatgaccgccgctctgaagaagaagaaggacctgctggtcgaggccggacgacagcagaaggaggtcgagga
caacatcaagcgactggaggtggagatccaggcccaggagctgaaggtcaacgacctgcaggccgagctggag
gaggtggagcagcaggaggcctctaaggtcgtcaagggcaagaccgccggcaaggtgaacgtgctggctggcc
tcgccaagtctcgagtggaggagctgcgaaacgccctgatggacgtgcgaaaggagcgagatgacacccgagc
ccgagttaaggaactcgaagagatcctgtctaagttcaaggtggagtacaaccccaacttcaacgacgagggcgtg
aagcgagccgtgcgatcttgggaggactacgccgccaagggcaccctggagggcgccgtgaacaacgcccag
gaccgagacctggacgagatcgccaagcccgacgacgagaaggccggcatcaactgggagcagtgggagaac
gaggaggacggctgtgaggctggcctggtgtaccagctggccgcctacctgcccccctctctggtggagttcatcg
agggcaaggtgctgttcgtgcgaggcctgctggaggacaacggcatcctgcccaaggccgccgagacctctacc
tctgagtctaaggtggtgtctgaggcccgagaggccgtgaagtctgccgagaaggagctgggcgacaagcagaa
gcagctgaaggaccacaagtctgacctggagaccgactacggcgtgggctctatcttccgagccctgaagggcgt
gtgtatctctaaggactctggcgagtacacctacgagcactgttttctggaccagaccaagcagatccccaagaagg
gcggaggctctacccgaatgggcaagtacaccggcatcggctctgtgtctgtggacgtgctgaacgaggccggc
gagatcgtgcctgaggatcgagtgaccctgcagtacgccaacggccagggctgttggaacggacccgcccgatc
taccaccgtgatcctgacctgtggcgaggaggacgccatcctgaaggtggccgaggacgagaagtgtgtgtactc
tatgcacgtgacctctcccgccgtgtgtcccggcggtgacgagggcgccaccgcccccaaccgaaaggacgagc
tgtaatagccta

FIG. 37

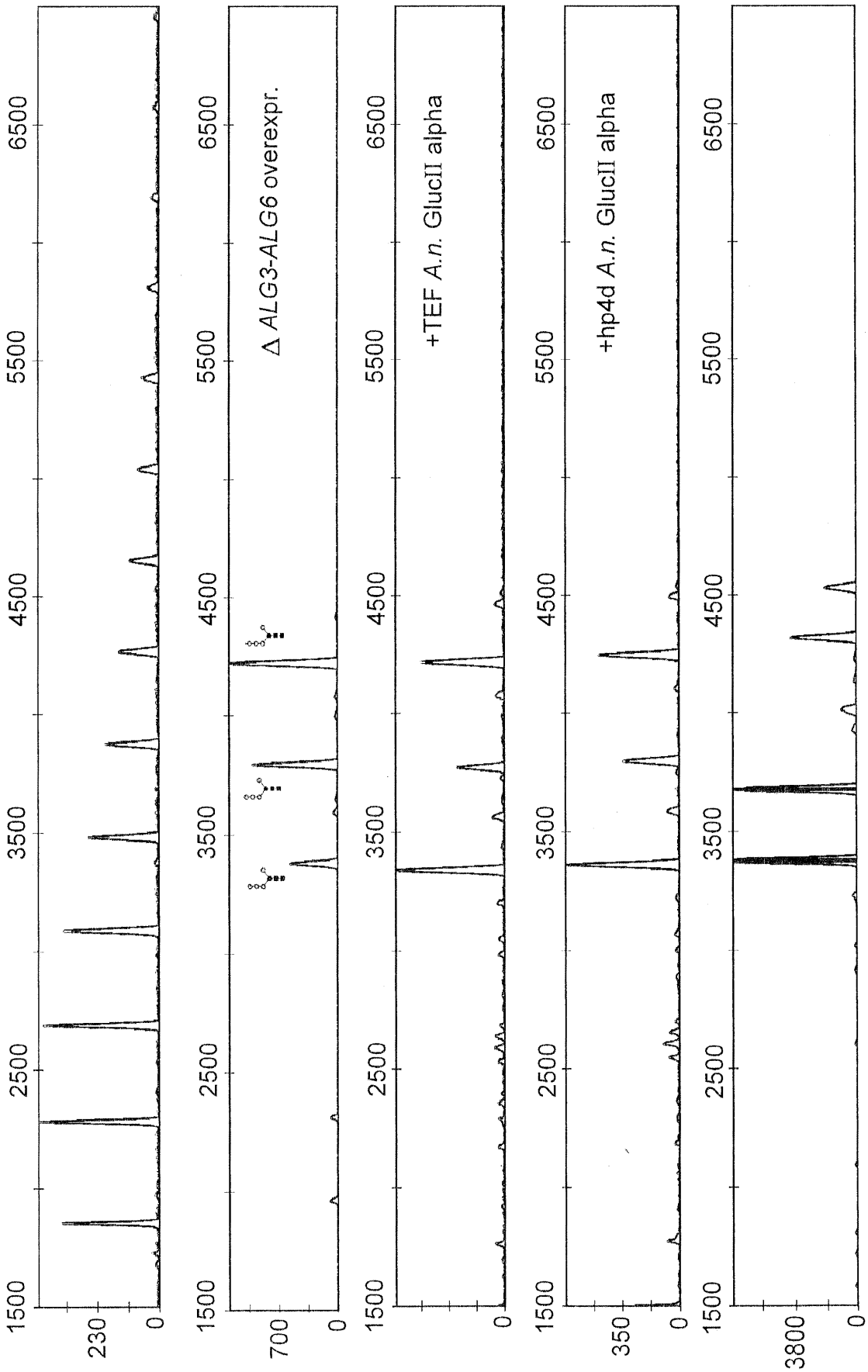

1500
2500
3500
4500
5500
6500
230
0
Δ ALG3-ALG6 overexpr.
700
+TEF A.n. GlucII alpha
+hp4d A.n. GlucII alpha
350
3800

FIG. 38

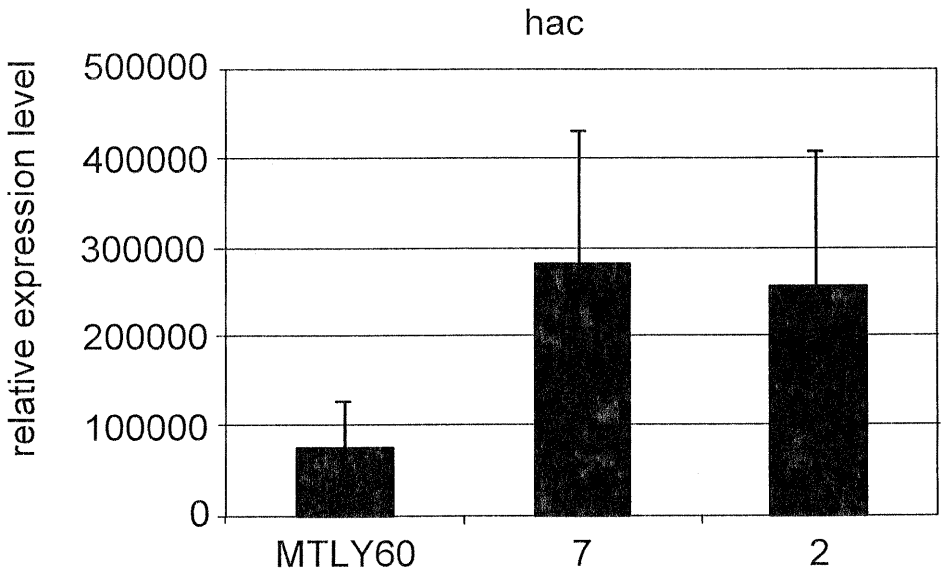


FIG. 39A

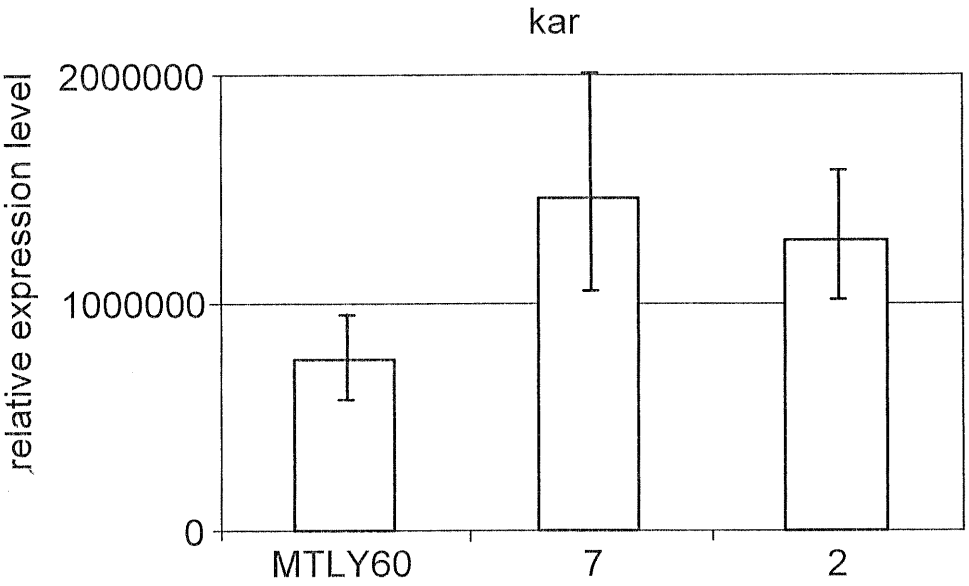


FIG. 39B

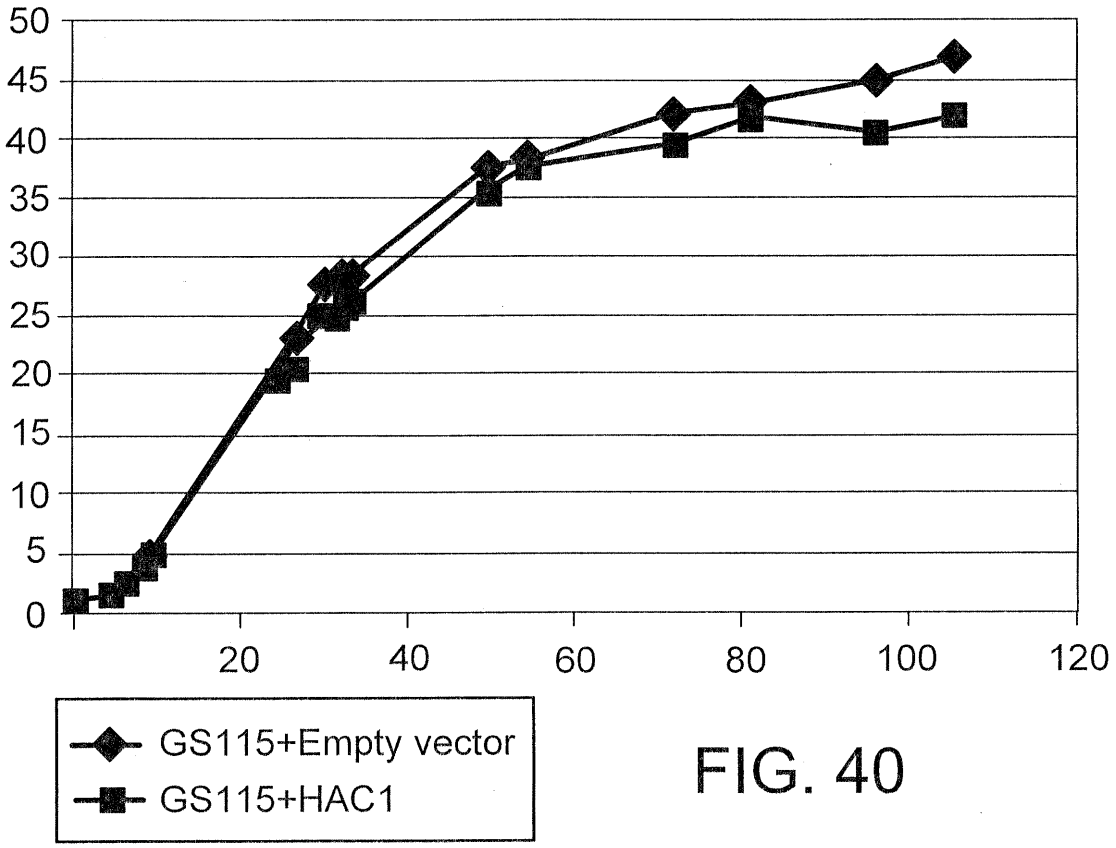

50
45
40
35
30
25
20
15
10
5
0
20
40
60
80
100
120
GS115+Empty vector
GS115+HAC1

FIG. 40

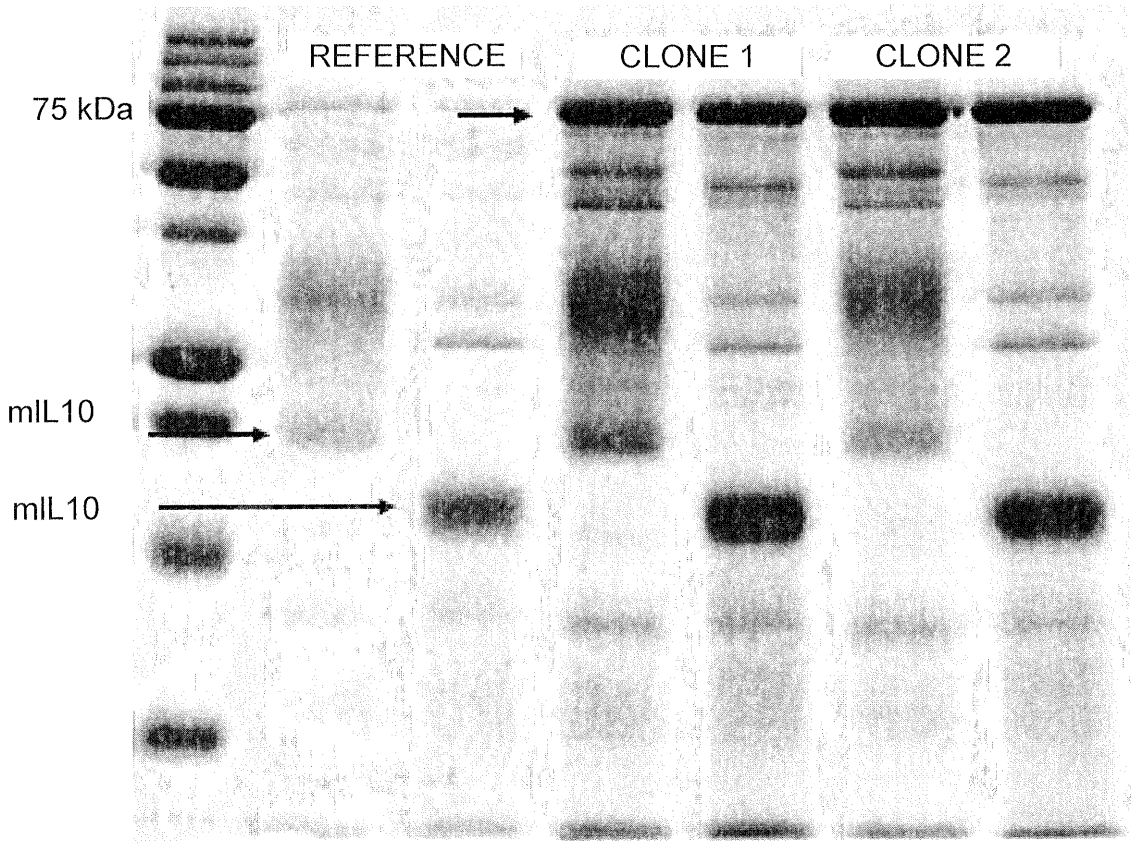


FIG. 41

gagctcagcgctaccaagcgaggctctcccaaccccacccgagccgccgctgtgaaggccgccttccagacc
tcttggaacgcctaccaccacttcgccttcccccacgacgacctgcaccccgtgtctaactcgttcgacgacgag
cgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgatgggcgacgccgacatcgtga
acaccatcctgcagtacgtgccccagatcaacttcaccaccaccgccgtggccaaccagggcatctctgtgttcg
agaccaacatccgatacctgggcggcctgctgtctgcctacgacctgctgcgaggcccttctcttctctggcca
ccaaccagaccctggtgaactctctgctgcgacaggcccagaccctggccaacggcctgaaggtggcttttacc
acccccctctggcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatctggcgcctcttctaacaac
gtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaacccccagtacgccc
agctggcccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggcccggactgatcg
gcaccttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgatggactctttctacg
agtacctgatcaagatgtacctgtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggccgcc
gactctaccatcgcccacctggcctctcacccctctacccgaaaggacctgaccttcctgtcctcttacaacggcc
agtctacctctcccaactctggacacctggcttccttcgccggtggcaacttcatcctgggcggcatcctgctgaa
cgagcagaagtacatcgacttcggcatcaagctggcctcttcctacttcgccacctacaaccagaccgcctctgg
catcggccccgagggcttcgcctgggtggactctgtgaccggcgctggcggctctcccccctcttctcagtctgg
cttctactcttctgccggcttctgggtgaccgcccccctactacatcctgcgacccgagaccctggagtctctgtact
acgcctaccgagtgaccggcgactctaagtggcaggacctggcctgggaggccttctctgccatcgaggacgc
ctgtcgagccggctctgcctactcttctatcaacgacgtgacccaggccaacggtggcggagcctctgacgaca
tggagtctttctggttcgccgaggccctgaagtacgcctacctgatcttcgccgaggaatctgacGtgcaggtgc
aggccaacggcggcaacaagttcgtgttcaacaccgaggcccacccctctctatccgatcttcttctcgacgag
gcggccacctggcccatgacgagctgtaatagcctaggtacc

FIG. 42

cggcggactgcgtccgaaccagctccagcagcgtttttttccgggccattgagccgactgcgaccccgccaacgtgtct
tggcccacgcactcatgtcatgttggtgttgggaggccacttttaagtagcacaaggcacctagctcgcagcaaggtgt
ccgaaccaaagaagcggctgcagtggtgcaaacggggcggaaacggcgggaaaaagccacgggggcacgaatt
gaggcacgccctcgaatttgagacgagtcacggccccattcgcccgcgcaatggctcgccaacgcccggtcttttgca
ccacatcaggttaccccaagccaaacctttgtgttaaaaagcttaacatattataccgaacgtaggtttgggcgggcttgc
tccgtctgtccaaggcaacatttatataagggtctgcatcgccggctcaattgaatctttttttcttcttctcttctctatattcatt
cttgaattaaacacacatcaaca

FIG. 43

cttatctggggcagtgaagtatatgttatggtaatagttacgagttagttgaacttatagatagactggactatacggctatc
ggtccaaattagaaagaacgtcaatggctctctgggcggaattcgtataacttcgtatagcaggagttatccgaagcgat
aattaccctgttatccctagcttatcgatacgcgtgcatgctgaggtgtctcacaagtgccgtgcagtcccgcccccactt
gcttctctttgtgtgtagtgtacgtacattatcgagaccgttgttcccgcccacctcgatccggcatgctgaggtgtctcaca
agtgccgtgcagtcccgcccccacttgcttctctttgtgtgtagtgtacgtacattatcgagaccgttgttcccgcccacctc
gatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgcccccacttgcttctctttgtgtgtagtgtacgtacatt
atcgagaccgttgttcccgcccacctcgatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgcccccacttt
gcttctctttgtgtgtagtgtacgtacattatcgagaccgttgttcccgcccacctcgatccggcatgcactgatcacgggc
aaaagtgcgtatatatacaagagcgtttgccagccacagattttcactccacacaccacatcacacatacaaccacacac
atccacgtgggaacccgaaactaaggatccatgaagctttccaccatcctcttcacagcctgcgctaccctggccgctac
caagcgaggctctcccaaccccacccgagccgccgctgtgaaggccgccttccagacctcttggaacgcctaccacc
acttcgccttcccccacgacgacctgcaccccgtgtctaactcgttcgacgacgagcgaaacggctggggctcttctgc
catcgacggcctggacaccgccatcctgatgggcgacgccgacatcgtgaacaccatcctgcagtacgtgccccaga
tcaacttcaccaccaccgccgtggccaaccagggcatctctgtgttcgagaccaacatccgatacctgggcggcctgct
gtctgcctacgacctgctgcgaggcccttctcttctctggccaccaaccagaccctggtgaactctctgctgcgacagg
cccagaccctggccaacggcctgaaggtggcttttaccacccctctggcgtgcccgaccccaccgtgttcttcaaccc
caccgtgcgacgatctggcgcctcttctaacaacgtggccgagatcggctctctggtgctggagtggacccgactgtct
gacctgaccggcaaccccccagtacgcccagctggcccagaagggcgagtcttacctgctgaaccccaagggctctcc
cgaggcctggcccggactgatcggcaccttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctg
gcctgatggactctttctacgagtacctgatcaagatgtacctgtacgaccccgtggccttcgcccactacaaggaccga
tgggtgctggccgccgactctaccatcgcccacctggcctctcacccctctacccgaaaggacctgaccttcctgtcctc
ttacaacggccagtctacctctcccaactctggacacctggcttccttcgccggtggcaacttcatcctgggcggcatcct
gctgaacgagcagaagtacatcgacttcggcatcaagctggcctcttcctacttcgccacctacaaccagaccgcctct
ggcatcggccccgagggcttcgcctgggtggactctgtgaccggcgctggcggctctcccccctcttctcagtctggct
tctactcttctgccggcttctgggtgaccgcccccctactacatcctgcgacccgagaccctggagtctctgtactacgcct
accgagtgaccggcgactctaagtggcaggacctggcctgggaggccttctctgccatcgaggacgcctgtcgagcc
ggctctgcctactcttctatcaacgacgtgacccaggccaacggtggcggagcctctgacgacatggagtctttctggtt
cgccgaggccctgaagtacgcctacctgatcttcgccgaggaatctgacgtgcaggtgcaggccaacggcggcaaca
agttcgtgttcaacaccgaggcccacccctctctatccgatcttcttctcgacgaggcggccacctggcccatgacgag
ctgtaatagcctagggtgtctgtggtatctaagctatttatcactctttacaacttctacctcaactatctactttaataaatgaa
tatcgttt

FIG. 44A

attctctatgattactgtatatgcgttcctctaagacaaatcgaattccatgtgtaacactcgctctggagagttagtcatccga
cagggtaactctaatctcccaacaccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaattttcctgct
catcatctgctggattgttgtctatcgtctggctctaatacatttattgtttattgcccaaacaactttcattgcacgtaagtgaatt
gttttataacagcgttcgccaattgctgcgccatcgtcgtccggctgtcctaccgttagggtagtgtgtctcacactaccgag
gttactagagttgggaaagcgatactgcctcggacacaccacctggtcttacgactgcagagagaatcggcgttacctcct
cacaaagccctcagtgcggccgcccggggtgggcgaagaactccagcatgagatccccgcgctggaggatcatccag
ccggcgtcccggaaaacgattccgaagcccaacctttcatagaaggcggcggtggaatcgaaatctcgtgatggcaggt
tgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggcgatg
cgctgcgaatcgggagcggcgataccgtaaagcacgaggaagcggtcagcccattcgccgccaagctcttcagcaata
tcacgggtagccaacgctatgtcctgatagcggtccgccacacccagccggccacagtcgatgaatccagaaaagcgg
ccattttccaccatgatattcggcaagcaggcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgcctt
gagcctggcgaacagttcggctggcgcgagcccctgatgctcttcgtccagatcatcctgatcgacaagaccggcttcca
tccgagtacgtgctcgctcgatgcgatgtttcgcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgc
cgcattgcatcagccatgatggatactttctcggcaggagcaaggtgagatgacaggagatcctgccccggcacttcgcc
caatagcagccagtcccttcccgcttcagtgacaacgtcgagcacagctgcgcaaggaacgcccgtcgtggccagcca
cgatagccgcgctgcctcgtcctgcagttcattcagggcaccggacaggtcggtcttgacaaaaagaaccgggcgcccc
tgcgctgacagccggaacacggcggcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctctcca
cccaagcggccggagaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatctt
gatcccctgcgccatcagatccttggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggc
gccccagctggcaattccggttcgcttgctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaagct
acctgctttctctttgcgcttgcgttttcccttgtccagatagcccagtagctgacattcatccggggtcagcaccgtttctgcg
gactggctttctacgtgttccgcttcctttagcagcccttgcgccctgagtgcttgcggcagcgtgaagctagcttatgcggt
gtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctcttccgcttcctcgctcactgactcgctgcgct
cggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgc
aggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccatag
gctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagata
ccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcc
cttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgt
gtgcacgaaccccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacga
cttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagt
ggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagc

FIG. 44B

cagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggcggttttt
tgtttgcaagcagcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttcttactgaacg
gtgatccccaccggaattgcggccgctgtcgggaaccgcgttcaggtggaacaggacacctcccttgcac
ttcttggtatatcagtataggctgatgtattcatagtggggtttttcataataaatttactaacggcaggcaacatt
cactcggcttaaacgcaaaacggaccgtcttgatatcttctgacgcattgaccaccgagaaatagtgttagtt
accgggtgagttattgttcttctacacaggcgacgcccatcgtctagagttgatgtactaactcagatttcact
acctaccctatccctggtacgcacaaagcactttgctagatagagtcgacaaaggcgggccccccctcga
gattaccctgttatccctacataacttcgtatagcatacattatacgaagttattctgaattccgagaaacacaa
caacatgccccattggacagaccatgcggatacacaggttgtgcagtaccatacatactcgatcagacagg
tcgtctgaccatcatacaagctgaacagcgctccatacttgcacgctctctatatacacagttaaattacatat
ccatagtctaacctctaacagttaatcttctggtaagcctcccagccagccttctggtatcgcttggcctcctc
aataggatctcggttctggccgtacagacctcggccgacaattatgatatccgttccggtagacatgacatc
ctcaacagttcggtactgctgtccgagagcgtctcccttgtcgtcaagacccaccccgggggtcagaataa
gccagtcctcagagtcgcccttaggtcggttctgggcaatgaagccaaccacaaactcggggtcggatcg
ggcaagctcaatggtctgcttggagtactcgccagtggccagagagcccttgcaagacagctcggccag
catgagcagacctctggccagcttctcgttgggagaggggactaggaactccttgtactgggagttctcgta
gtcagagacgtcctccttcttctgttcagagacagtttcctcggcaccagctcgcaggccagcaatgattcc
ggttccgggtacaccgtgggcgttggtgatatcggaccactcggcgattcggtgacaccggtactggtgct
tgacagtgttgccaatatctgcgaactttctgtcctcgaacaggaagaaaccgtgcttaagagcaagttcctt
gaggggggagcacagtgccggcgtaggtgaagtcgtcaatgatgtcgatatgggtcttgatcatgcacacat
aaggtccgaccttatcggcaagctcaatgagctccttggtggtggtaacatccagagaagcacacaggttg
gttttcttggctgccacgagcttgagcactcgagcggcaaaggcggacttgtggacgttagctcgagcttc
gtaggagggcattttggtggtgaagaggagactgaaataaatttagtctgcagaacttttatcggaac

FIG. 44C

tagcaggagttatccgaagcgataattaccctgttatccctagcttatcgatacggcggactgcgtccgaaccagctcca
gcagcgtttttttccgggccattgagccgactgcgaccccgccaacgtgtcttggcccacgcactcatgtcatgttggtgtt
gggaggccacttttaagtagcacaaggcacctagctcgcagcaaggtgtccgaaccaaagaagcggctgcagtggt
gcaaacggggcggaaacggcgggaaaaagccacgggggcacgaattgaggcacgccctcgaatttgagacgagt
cacggccccattcgcccgcgcaatggctcgccaacgcccggtcttttgcaccacatcaggttaccccaagccaaacctt
tgtgttaaaaagcttaacatattataccgaacgtaggtttgggcgggcttgctccgtctgtccaaggcaacatttatataag
ggtctgcatcgccggctcaattgaatctttttttcttcttctcttctctatattcattcttgaattaaacacacatcaacaggatcc
atgaagctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctctcccaaccccacccgag
ccgccgctgtgaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgacctgcacc
ccgtgtctaactcgttcgacgacgagcgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgat
gggcgacgccgacatcgtgaacaccatcctgcagtacgtgccccagatcaacttcaccaccaccgccgtggccaacc
agggcatctctgtgttcgagaccaacatccgatacctgggcggcctgctgtctgcctacgacctgctgcgaggcccctt
ctcttctctggccaccaaccagaccctggtgaactctctgctgcgacaggcccagaccctggccaacggcctgaaggt
ggcttttaccacccctctggcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatctggcgcctcttcta
acaacgtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaacccccagtacgcc
cagctggcccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggcccggactgatcggca
ccttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgatggactctttctacgagtacctg
atcaagatgtacctgtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggccgccgactctaccatc
gcccacctggcctctcacccctctacccgaaaggacctgaccttcctgtcctcttacaacggccagtctacctctcccaa
ctctggacacctggcttccttcgccggtggcaacttcatcctgggcggcatcctgctgaacgagcagaagtacatcgac
ttcggcatcaagctggcctcttcctacttcgccacctacaaccagaccgcctctggcatcggccccgagggcttcgcct
gggtggactctgtgaccggcgctggcggctctccccctcttctcagtctggcttctactcttctgccggcttctgggtga
ccgcccccctactacatcctgcgacccgagaccctggagtctctgtactacgcctaccgagtgaccggcgactctaagtg
gcaggacctggcctgggaggccttctctgccatcgaggacgcctgtcgagccggctctgcctactcttctatcaacgac
gtgacccaggccaacggtggcggagcctctgacgacatggagtctttctggttcgccgaggccctgaagtacgcctac
ctgatcttcgccgaggaatctgacgtgcaggtgcaggccaacggcggcaacaagttcgtgttcaacaccgaggccca
ccccttctctatccgatcttcttctcgacgaggcggccacctggcccatgacgagctgtaatagcctagggtgtctgtggt
atctaagctatttatcactctttacaacttctacctcaactatctactttaataaatgaatatcgtttattctctatgattactgtata
tgcgttcctctaagacaaatcgaattccatgtgtaacactcgctctggagagttagtcatccgacagggtaactctaatctc
ccaacaccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaattttcctgctcatcatctgctggattg
ttgtctatcgtctggct

FIG. 45A

ctaatacatttattgtttattgcccaaacaactttcattgcacgtaagtgaattgttttataacagcgttcgccaattgctgcgc
catcgtcgtccggctgtcctaccgttagggtagtgtgtctcacactaccgaggttactagagttgggaaagcgatactgc
ctcggacacaccacctggtcttacgactgcagagagaatcggcgttacctcctcacaaagccctcagtgcggccgcc
cggggtgggcgaagaactccagcatgagatccccgcgctggaggatcatccagccggcgtcccggaaaacgattc
cgaagcccaacctttcatagaaggcggcggtggaatcgaaatctcgtgatggcaggttgggcgtcgcttggtcggtca
tttcgaaccccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggcgatgcgctgcgaatcgggagcg
gcgataccgtaaagcacgaggaagcggtcagcccattcgccgccaagctcttcagcaatatcacgggtagccaacg
ctatgtcctgatagcggtccgccacacccagccggccacagtcgatgaatccagaaaagcggccattttccaccatga
tattcggcaagcaggcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgccttgagcctggcgaac
agttcggctggcgcgagcccctgatgctcttcgtccagatcatcctgatcgacaagaccggcttccatccgagtacgtg
ctcgctcgatgcgatgtttcgcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgccgcattgcatc
agccatgatggatactttctcggcaggagcaaggtgagatgacaggagatcctgccccggcacttcgcccaatagca
gccagtcccttcccgcttcagtgacaacgtcgagcacagctgcgcaaggaacgcccgtcgtggccagccacgatag
ccgcgctgcctcgtcctgcagttcattcagggcaccggacaggtcggtcttgacaaaaagaaccgggcgcccctgcg
ctgacagccggaacacggcggcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctctccaccc
aagcggccggagaacctgcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatcttg
atcccctgcgccatcagatccttggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagaggg
cgccccagctggcaattccggttcgcttgctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaa
gctacctgctttctctttgcgcttgcgttttcccttgtccagatagcccagtagctgacattcatccggggtcagcaccgttt
ctgcggactggctttctacgtgttccgcttcctttagcagcccttgcgccctgagtgcttgcggcagcgtgaagctagctt
atgcggtgtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctcttccgcttcctcgctcactgact
cgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcag
gggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgctg
gcgtttttccataggctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgac
aggactataaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggat
acctgtccgcctttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgt
tcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgag
tccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgtagg
cggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctga
agccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggcggttttttgttt
gcaagcagcagattacgcgcagaaaa

FIG. 45B

aaaggatctcaagaagatcctttgatcttttcttactgaacggtgatccccaccggaattgcggccgctgtcgg
gaaccgcgttcaggtggaacaggacacctcccttgcacttcttggtatatcagtataggctgatgtattcatag
tggggtttttcataataaatttactaacggcaggcaacattcactcggcttaaacgcaaaacggaccgtcttga
tatcttctgacgcattgaccaccgagaaatagtgttagttaccgggtgagttattgttcttctacacaggcgacg
cccatcgtctagagttgatgtactaactcagatttcactacctaccctatccctggtacgcacaaagcactttgc
tagatagagtcgacaaaggcgggccccccctcgagattaccctgttatccctacataacttcgtatagcatac
attatacgaagttattctgaattccgcccagagagccattgacgttctttctaatttggaccgatagccgtatagt
ccagtctatctataagttcaactaactcgtaactattaccataacatatacttcactgccccagataaggttccga
taaaaagttctgcagactaaatttatttcagtctcctcttcaccaccaaaatgccctcctacgaagctcgagcta
acgtccacaagtccgcctttgccgctcgagtgctcaagctcgtggcagccaagaaaaccaacctgtgtgctt
ctctggatgttaccaccaccaaggagctcattgagcttgccgataaggtcggaccttatgtgtgcatgatcaa
gacccatatcgacatcattgacgacttcacctacgccggcactgtgctccccctcaaggaacttgctcttaag
cacggtttcttcctgttcgaggacagaaagttcgcagatattggcaacactgtcaagcaccagtaccggtgtc
accgaatcgccgagtggtccgatatcaccaacgcccacggtgtacccggaaccggaatcattgctggcct
gcgagctggtgccgaggaaactgtctctgaacagaagaaggaggacgtctctgactacgagaactcccag
tacaaggagttcctagtcccctctcccaacgagaagctggccagaggtctgctcatgctggccgagctgtct
tgcaagggctctctggccactggcgagtactccaagcagaccattgagcttgcccgatccgaccccgagttt
gtggttggcttcattgcccagaaccgacctaagggcgactctgaggactggcttattctgacccccggggtg
ggtcttgacgacaagggagacgctctcggacagcagtaccgaactgttgaggatgtcatgtctaccggaac
ggatatcataattgtcggccgaggtctgtacggccagaaccgagatcctattgaggaggccaagcgatacc
agaaggctggctgggaggcttaccagaagattaactgttagaggttagactatggatatgtaatttaactgtgt
atatagagagcgtgcaagtatggagcgctgttcagcttgtatgatggtcagacgacctgtctgatcgagtatg
tatggtactgcacaacctgtgtatccgcatggtctgtccaatggggcatgttgttgtgtttctcggaattcgtata
acttcgta

FIG. 45C

caaggggtaggcgaatggtacgattccgccaagtgagactggcgatcgggagaagggttggtggtcatgggggata
gaatttgtacaagtggaaaaaccactacgagtagcggatttgataccacaagtagcagagatatacagcaatggtggg
agtgcaagtatcggaatgtactgtacctcctgtactcgtactcgtacggcactcgtagaaacggggcaatacgggggga
gaagcgatcgcccgtctgttcaatcgccacaagtccgagtaatgctcgagtatcgaagtcttgtacctccctgtcaatca
tggcaccactggtcttgacttgtctattcatactggacaagcgccagagttagctagcgaatttcgccctcggacatcac
cccatacgacggacacacatgcccgacaaacagcctctcttattgtagctgaaagtatattgaatgtgaacgtgtacaat
atcaggtaccagcgggaggttacggccaaggtgataccggaataaccctggcttggagatggtcggtccattgtactg
aagtgtccgtgtcgtttccgtcactgccccaattggacatgtttgtttttccgatctttcgggcgccctctccttgtctccttgt
ctgtctcctggactgttgctaccccatttctttggcctccattggttcctccccgtctttcacgtcgtctatggttgcatggttt
cccttatacttttccccacagtcacatgttatggaggggtctagatggacatggtgcaaggcccgcagggttgattcgac
gcttttccgcgaaaaaaacaagtccaaataccccccgtttattctccctcggctctcggtatttcacatgaaaactataacct
agactacacgggcaaccttaaccccagagtatacttatataccaaagggatgggtcctcaaaaatcacacaagcaacg
gatccatgaagctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctctcccaacccca
cccgagccgccgctgtgaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgac
ctgcaccccgtgtctaactcgttcgacgacgagcgaaacggctggggctcttctgccatcgacggcctggacaccgc
catcctgatgggcgacgccgacatcgtgaacaccatcctgcagtacgtgccccagatcaacttcaccaccaccgccg
tggccaaccagggcatctctgtgttcgagaccaacatccgatacctgggcggcctgctgtctgcctacgacctgctgc
gaggcccctctcttctctggccaccaaccagaccctggtgaactctctgctgcgacaggcccagaccctggccaac
ggcctgaaggtggcttttaccacccctctggcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatct
ggcgcctcttctaacaacgtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaa
cccccagtacgcccagctggcccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggccc
ggactgatcggcaccttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgatggactct
ttctacgagtacctgatcaagatgtacctgtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggcc
gccgactctaccatcgcccacctggcctctcacccctctacccgaaaggacctgaccttcctgtcctcttacaacggcc
agtctacctctcccaactctggacacctggcttccttcgccggtggcaacttcatcctgggcggcatcctgctgaacga
gcagaagtacatcgacttcggcatcaagctggcctcttcctacttcgccacctacaaccagaccgcctctggcatcgg
ccccgagggcttcgcctgggtggactctgtgaccggcgctggcggctctcccccctcttctcagtctggcttctactctt
ctgccggcttctgggtgaccgcccccctactacatcctgcgacccgagaccctggagtctctgtactacgcctaccgag
tgaccggcgactctaagtggcaggacctggcctgggaggccttctctgccatcgaggacgcctgtcgagccggctct
gcctactcttctatcaacgacgtgacccaggccaacggtggcggagcctctgacgacatggagtctttctggttcgcc
gaggccctgaagtacgcctacctgatcttcgccgag

FIG. 46A

gaatctgacgtgcaggtgcaggccaacggcggcaacaagttcgtgttcaacaccgaggcccacccccttctctatc
cgatcttcttctcgacgaggcggccacctggcccatgacgagctgtaatagcctagggtgtctgtggtatctaagct
atttatcactctttacaacttctacctcaactatctactttaataaatgaatatcgtttattctctatgattactgtatatgcgtt
cctctaagacaaatcgaattccatgtgtaacactcgctctggagagttagtcatccgacagggtaactctaatctccc
aacaccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaattttcctgctcatcatctgctggatt
gttgtctatcgtctggctctaatacatttattgtttattgcccaaacaactttcattgcacgtaagtgaattgttttataaca
gcgttcgccaattgctgcgccatcgtcgtccggctgtcctaccgttagggtagtgtgtctcacactaccgaggttact
agagttgggaaagcgatactgcctcggacacaccacctggtcttacgactgcagagagaatcggcgttacctcct
cacaaagccctcagtgcggccgcccggggtgggcgaagaactccagcatgagatccccgcgctggaggatcat
ccagccggcgtcccggaaaacgattccgaagcccaacctttcatagaaggcggcggtggaatcgaaatctcgtg
atggcaggttgggcgtcgcttggtcggtcatttcgaaccccagagtcccgctcagaagaactcgtcaagaaggcg
atagaaggcgatgcgctgcgaatcgggagcggcgataccgtaaagcacgaggaagcggtcagcccattcgcc
gccaagctcttcagcaatatcacgggtagccaacgctatgtcctgatagcggtccgccacacccagccggccaca
gtcgatgaatccagaaaagcggccattttccaccatgatattcggcaagcaggcatcgccatgggtcacgacgag
atcctcgccgtcgggcatgcgcgccttgagcctggcgaacagttcggctggcgcgagcccctgatgctcttcgtc
cagatcatcctgatcgacaagaccggcttccatccgagtacgtgctcgctcgatgcgatgtttcgcttggtggtcga
atgggcaggtagccggatcaagcgtatgcagccgccgcattgcatcagccatgatggatactttctcggcaggag
caaggtgagatgacaggagatcctgccccggcacttcgcccaatagcagccagtcccttcccgcttcagtgacaa
cgtcgagcacagctgcgcaaggaacgcccgtcgtggccagccacgatagccgcgctgcctcgtcctgcagttca
ttcagggcaccggacaggtcggtcttgacaaaaagaaccgggcgcccctgcgctgacagccggaacacggcg
gcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctctccacccaagcggccggagaacct
gcgtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatcttgatcccctgcgccatca
gatccttggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagctgg
caattccggttcgcttgctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaagctacctgctt
tctctttgcgcttgcgttttcccttgtccagatagcccagtagctgacattcatccggggtcagcaccgtttctgcgga
ctggctttctacgtgttccgcttcctttagcagcccttgcgccctgagtgcttgcggcagcgtgaagctagcttatgcg
gtgtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctcttccgcttcctcgctcactgactcg
ctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatca
ggggataacgcaggaaagaacatgtgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttg
ctggcgtttttccataggctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaac
ccgacaggactataaag

FIG. 46B

ataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcc
tttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaag
ctgggctgtgtgcacgaaccccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccg
gtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagagcgaggtatgtaggcggtgcta
cagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagccagtt
accttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggcggttttttgtttgcaagca
gcagattacgcgcagaaaaaaaggatctcaagaagatcctttgatcttttcttactgaacggtgatccccaccggaatt
gcggccgctgtcgggaaccgcgttcaggtggaacaggacacctcccttgcacttcttggtatatcagtataggctgat
gtattcatagtggggtttttcataataaatttactaacggcaggcaacattcactcggcttaaacgcaaaacggaccgtct
tgatatcttctgacgcattgaccaccgagaaatagtgttagttaccgggtgagttattgttcttctacacaggcgacgccc
atcgtctagagttgatgtactaactcagatttcactacctaccctatccctggtacgcacaaagcactttgctagatagag
tcgacaaaggcgggccccccctcgagattaccctgttatccctacataacttcgtatagcatacattatacgaagttattc
tgaattccgcccagagagccattgacgttctttctaatttggaccgatagccgtatagtccagtctatctataagttcaact
aactcgtaactattaccataacatatacttcactgccccagataaggttccgataaaaagttctgcagactaaatttatttc
agtctcctcttcaccaccaaaatgccctcctacgaagctcgagctaacgtccacaagtccgcctttgccgctcgagtgc
tcaagctcgtggcagccaagaaaaccaacctgtgtgcttctctggatgttaccaccaccaaggagctcattgagcttgc
cgataaggtcggaccttatgtgtgcatgatcaagacccatatcgacatcattgacgacttcacctacgccggcactgtg
ctccccctcaaggaacttgctcttaagcacggtttcttcctgttcgaggacagaaagttcgcagatattggcaacactgt
caagcaccagtaccggtgtcaccgaatcgccgagtggtccgatatcaccaacgcccacggtgtacccggaaccgg
aatcattgctggcctgcgagctggtgccgaggaaactgtctctgaacagaagaaggaggacgtctctgactacgaga
actcccagtacaaggagttcctagtcccctctcccaacgagaagctggccagaggtctgctcatgctggccgagctgt
cttgcaagggctctctggccactggcgagtactccaagcagaccattgagcttgcccgatccgaccccgagtttgtgg
ttggcttcattgcccagaaccgacctaagggcgactctgaggactggcttattctgacccccggggtgggtcttgacg
acaagggagacgctctcggacagcagtaccgaactgttgaggatgtcatgtctaccggaacggatatcataattgtcg
gccgaggtctgtacggccagaaccgagatcctattgaggaggccaagcgataccagaaggctggctgggaggctt
accagaagattaactgttagaggttagactatggatatgtaatttaactgtgtatatagagagcgtgcaagtatggagcg
ctgttcagcttgtatgatggtcagacgacctgtctgatcgagtatgtatggtactgcacaacctgtgtatccgcatggtct
gtccaatggggcatgttgttgtgtttctcggaattcgtataacttcgtatagcaggagttatccgaagcgataattaccctg
ttatccctagcttatcgattcccacaagacgaacaagtgataggccgagagccgaggacgaggtggagtgca

FIG. 46C

taattaccctgttatccctagcttatcgatagagaccgggttggcggcgtatttgtgtcccaaaaaacagccccaattgcc
ccaattgaccccaaattgacccagtagcgggcccaaccccggcgagagccccccttcaccccacatatcaaacctcccc
cggttcccacacttgccgttaagggcgtagggtactgcagtctggaatctacgcttgttcagactttgtactagtttctttgt
ctggccatccgggtaacccatgccggacgcaaaatagactactgaaaatttttttgctttgtggttgggactttagccaag
ggtataaaagaccaccgtccccgaattacctttcctcttcttttctctctctccttgtcaactcacacccgaaggatccatga
agctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctctcccaaccccacccgagccg
ccgctgtgaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgacctgcaccccgt
gtctaactcgttcgacgacgagcgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgatggg
cgacgccgacatcgtgaacaccatcctgcagtacgtgccccagatcaacttcaccaccaccgccgtggccaaccagg
gcatctctgtgttcgagaccaacatccgatacctgggcggcctgctgtctgcctacgacctgctgcgaggccccttctctt
ctctggccaccaaccagaccctggtgaactctctgctgcgacaggcccagaccctggccaacggcctgaaggtggct
tttaccacccctctggcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatctggcgcctcttctaacaa
cgtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaaccccccagtacgcccagc
tggcccagaagggcgagtcttacctgctgaaccccaagggctctcccgaggcctggcccggactgatcggcaccttc
gtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgatggactctttctacgagtacctgatca
agatgtacctgtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggccgccgactctaccatcgccc
acctggcctctcacccctctacccgaaaggacctgaccttcctgtcctcttacaacggccagtctacctctcccaactctg
gacacctggcttccttcgccggtggcaacttcatcctgggcggcatcctgctgaacgagcagaagtacatcgacttcgg
catcaagctggcctcttcctacttcgccacctacaaccagaccgcctctggcatcggccccgagggcttcgcctgggtg
gactctgtgaccggcgctggcggctctcccccctcttctcagtctggcttctactcttctgccggcttctgggtgaccgcc
ccctactacatcctgcgacccgagaccctggagtctctgtactacgcctaccgagtgaccggcgactctaagtggcag
gacctggcctgggaggccttctctgccatcgaggacgcctgtcgagccggctctgcctactcttctatcaacgacgtga
cccaggccaacggtggcggagcctctgacgacatggagtctttctggttcgccgaggccctgaagtacgcctacctga
tcttcgccgaggaatctgacgtgcaggtgcaggccaacggcggcaacaagttcgtgttcaacaccgaggcccacccc
ttctctatccgatcttcttctcgacgaggcggccacctggcccatgacgagctgtaatagcctagggtgtctgtggtatcta
agctatttatcactctttacaacttctacctcaactatctactttaataaatgaatatcgtttattctctatgattactgtatatgcgt
tcctctaagacaaatcgaattccatgtgtaacactcgctctggagagttagtcatccgacagggtaactctaatctcccaa
caccttattaactctgcgtaactgtaactcttcttgccacgtcgatcttactcaattttcctgctcatcatctgctggattgttgt
ctatcgtctggctctaatacatttattgtttattgcccaaacaactttcattgcacgtaagtgaattgttttataacagcgttcgc
caattgctgcgccatcgtcgtccggctgtcctaccgttagggtagtgtgtctcacactaccgaggttactagagttgggaa
agcgatactgcctcgg

FIG. 47A

acacaccacctggtcttacgactgcagagagaatcggcgttacctcctcacaaagccctcagtgcggccgcccggggt
gggcgaagaactccagcatgagatccccgcgctggaggatcatccagccggcgtcccggaaaacgattccgaagcc
caacctttcatagaaggcggcggtggaatcgaaatctcgtgatggcaggttgggcgtcgcttggtcggtcatttcgaacc
ccagagtcccgctcagaagaactcgtcaagaaggcgatagaaggcgatgcgctgcgaatcgggagcggcgataccg
taaagcacgaggaagcggtcagcccattcgccgccaagctcttcagcaatatcacgggtagccaacgctatgtcctgat
agcggtccgccacacccagccggccacagtcgatgaatccagaaaagcggccattttccaccatgatattcggcaagc
aggcatcgccatgggtcacgacgagatcctcgccgtcgggcatgcgcgccttgagcctggcgaacagttcggctggc
gcgagcccctgatgctcttcgtccagatcatcctgatcgacaagaccggcttccatccgagtacgtgctcgctcgatgcg
atgtttcgcttggtggtcgaatgggcaggtagccggatcaagcgtatgcagccgccgcattgcatcagccatgatggata
ctttctcggcaggagcaaggtgagatgacaggagatcctgccccggcacttcgcccaatagcagccagtcccttcccgc
ttcagtgacaacgtcgagcacagctgcgcaaggaacgcccgtcgtggccagccacgatagccgcgctgcctcgtcctg
cagttcattcagggcaccggacaggtcggtcttgacaaaaagaaccgggcgcccctgcgctgacagccggaacacgg
cggcatcagagcagccgattgtctgttgtgcccagtcatagccgaatagcctctccacccaagcggccggagaacctgc
gtgcaatccatcttgttcaatcatgcgaaacgatcctcatcctgtctcttgatcagatcttgatcccctgcgccatcagatcct
tggcggcaagaaagccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagctggcaattccggtt
cgcttgctgtccataaaaccgcccagtctagctatcgccatgtaagcccactgcaagctacctgctttctctttgcgcttgcg
ttttcccttgtccagatagcccagtagctgacattcatccggggtcagcaccgtttctgcggactggctttctacgtgttccg
cttcctttagcagcccttgcgccctgagtgcttgcggcagcgtgaagctagcttatgcggtgtgaaataccgcacagatg
cgtaaggagaaaataccgcatcaggcgctcttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggc
gagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgag
caaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccccctgacg
agcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttccccctg
gaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaagcgtgg
cgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaacccc
cgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactgg
cagcagccactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaacta
cggctacactagaaggacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggtagctcttg
atccggcaaacaaaccaccgctggtagcggcggttttttgtttgcaagcagcagattacgcgcagaaaaaaaggatctc
aagaagatcctttgatcttttcttactgaacggtgatccccaccggaattgcggccgctgtcgggaaccgcgttcaggtgg
aacaggacacctcccttgcacttcttggtatatcagtataggctgatgtattcatagtggggtttttcataataaatttact

FIG. 47B

aacggcaggcaacattcactcggcttaaacgcaaaacggaccgtcttgatatcttctgacgcattgaccaccgaga
aatagtgttagttaccgggtgagttattgttcttctacacaggcgacgcccatcgtctagagttgatgtactaactcag
atttcactacctaccctatccctggtacgcacaaagcactttgctagatagagtcgacaaaggcgggcccccccctc
gagattaccctgttatccctacataacttcgtatagcatacattatacgaagttattctgaattccgagaaacacaacaa
catgccccattggacagaccatgcggatacacaggttgtgcagtaccatacatactcgatcagacaggtcgtctga
ccatcatacaagctgaacagcgctccatacttgcacgctctctatatacacagttaaattacatatccatagtctaacct
ctaacagttaatcttctggtaagcctcccagccagccttctggtatcgcttggcctcctcaataggatctcggttctgg
ccgtacagacctcggccgacaattatgatatccgttccggtagacatgacatcctcaacagttcggtactgctgtcc
gagagcgtctcccttgtcgtcaagacccaccccgggggtcagaataagccagtcctcagagtcgcccttaggtcg
gttctgggcaatgaagccaaccacaaactcggggtcggatcgggcaagctcaatggtctgcttggagtactcgcc
agtggccagagagcccttgcaagacagctcggccagcatgagcagacctctggccagcttctcgttgggagagg
ggactaggaactccttgtactgggagttctcgtagtcagagacgtcctccttcttctgttcagagacagtttcctcggc
accagctcgcaggccagcaatgattccggttccgggtacaccgtgggcgttggtgatatcggaccactcggcgat
tcggtgacaccggtactggtgcttgacagtgttgccaatatctgcgaactttctgtcctcgaacaggaagaaaccgt
gcttaagagcaagttccttgagggggagcacagtgccggcgtaggtgaagtcgtcaatgatgtcgatatgggtctt
gatcatgcacacataaggtccgaccttatcggcaagctcaatgagctccttggtggtggtaacatccagagaagca
cacaggttggttttcttggctgccacgagcttgagcactcgagcggcaaaggcggacttgtggacgttagctcgag
cttcgtaggagggcattttggtggtgaagaggagactgaaataaatttagtctgcagaacttttatcggaaccttatc
tggggcagtgaagtatatgttatggtaatagttacgagttagttgaacttatagatagactggactatacggctatcgg
tccaaattagaaagaacgtcaatggctctctgggcggaattcgtataacttcgtatagcaggagttatccgaagcga

FIG. 47C

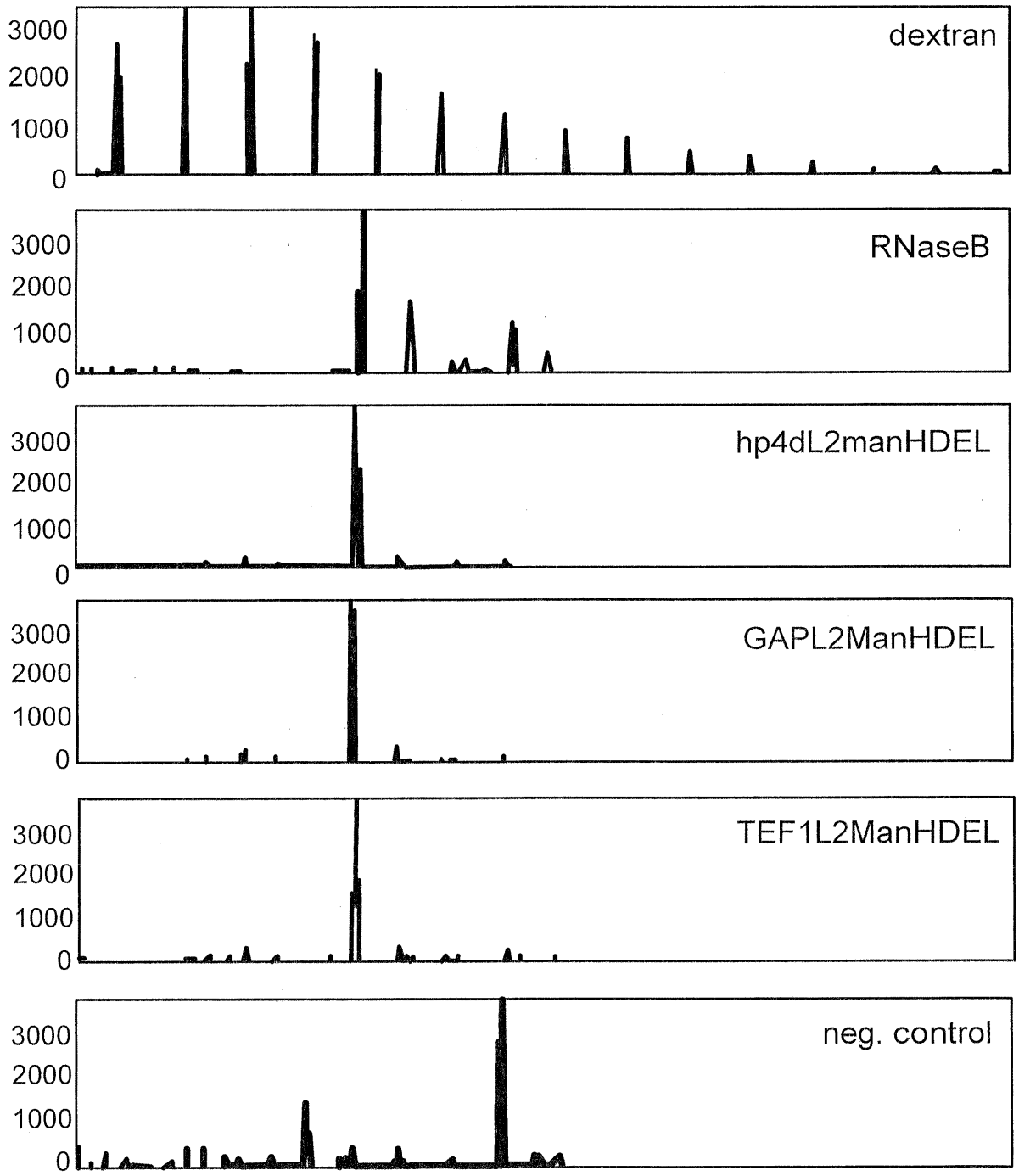

3000
2000
1000
0
dextran
RNaseB
hp4dL2manHDEL
GAPL2ManHDEL
TEF1L2ManHDEL
neg. control

FIG. 48

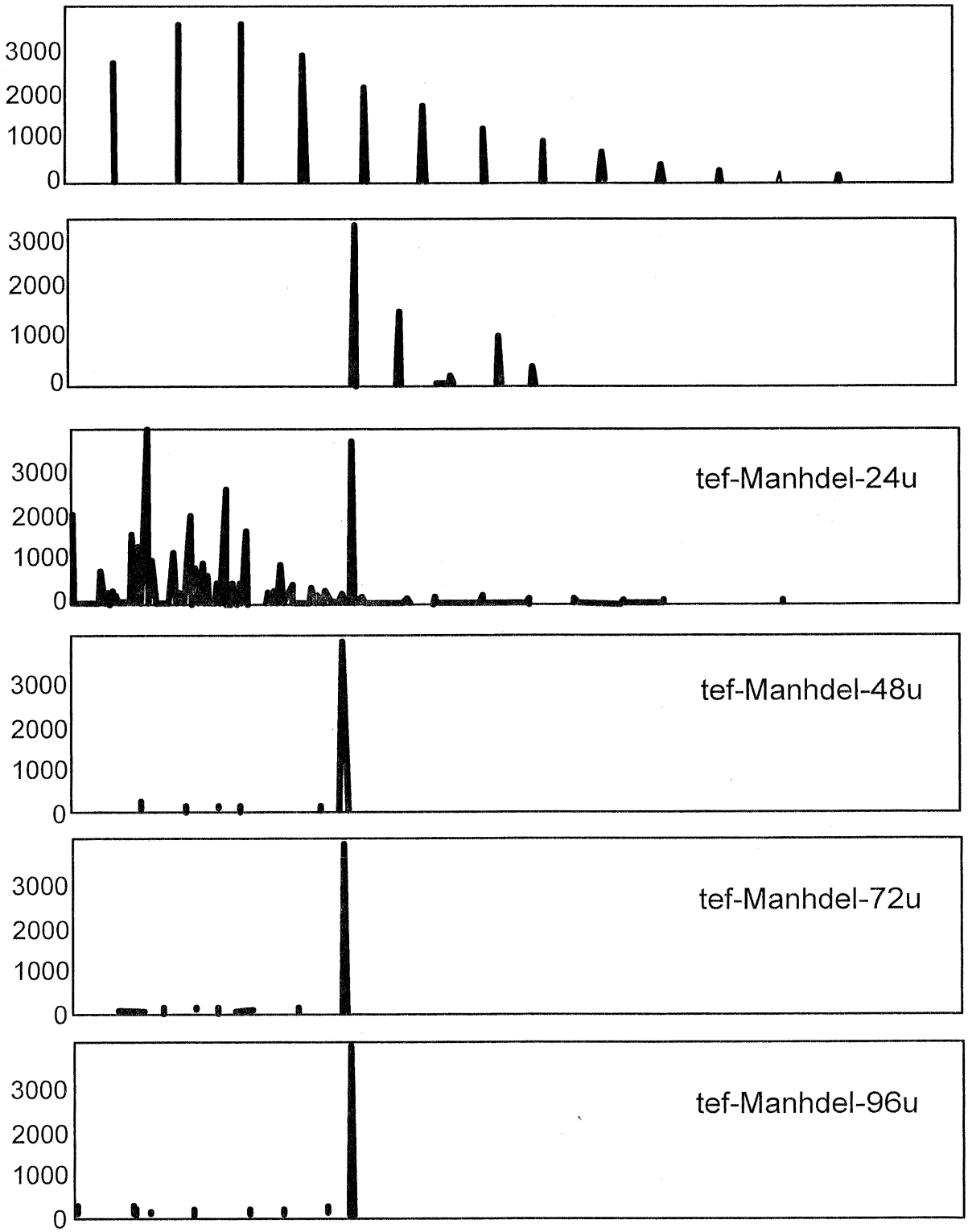

3000
2000
1000
0
3000
2000
1000
0
3000
2000
1000
0
tef-Manhdel-24u
3000
2000
1000
0
tef-Manhdel-48u
3000
2000
1000
0
tef-Manhdel-72u
3000
2000
1000
0
tef-Manhdel-96u

FIG. 49

gagctcagcgctcgaccctgtatccccaagtctttcggctactcttctgtggtgtgtgtgtgtaacgccacctactgtgact
cgttcgacccccccacctttccccgccctgggcaccttctctcgatacgagtctacccgatctggccgacgaatggagct
gtctatgggccccatccaggccaaccacaccggcaccggcctgctgctgaccctgcagcccgagcagaagttccaga
aggtcaagggcttcggcggagccatgaccgacgccgctgccctgaacatcctggccctgtctcccccccgctcagaac
ctgctcctgaagtcttacttctctgaggagggcatcggctacaacatcatccgagtgcctatggcctcttgtgacttctctat
ccgaacctacacctacgccgacacccccgacgacttccagctgcacaacttctccctgcccgaggaggacaccaagct
gaagatcccccctgatccaccgagccctgcagctggcccagcgacccgtgtctctgctggcctctccctggacctctccc
acctggctcaagaccaacggcgccgtgaacggcaagggctctctgaagggccagcccggcgacatctaccaccaga
cctgggcccgatacttcgtgaagttcctggacgcctacgccgagcacaagctgcagttctgggccgtgaccgccgaga
acgagccctctgctggactgctgtccggctacccccttccagtgtctgggcttcacccccgagcaccagcgagacttcatc
gcccgagacctgggacccaccctggccaactctacccaccacaacgtgcgactgctgatgctggacgaccagcgact
gctgctgccccactgggccaaggtggtgctgaccgaccccgaggccgccaagtacgtccacggcatcgctgtccatt
ggtatctggactttctggctcccgccaaggccaccctgggcgagacccaccgactgttccccaacaccatgctgttcgc
ctctgaggcttgtgtgggctctaagttctgggagcagtctgtgcgactgggctcttgggaccgaggcatgcagtactctc
actctatcatcaccaacctgctgtaccacgtggtgggctggaccgactggaacctggccctgaaccccgagggcggac
ccaactgggtgcgaaacttcgtggactctcccatcatcgtggacatcaccaaggacaccttctacaagcagcccatgttc
taccacctgggccacttctctaagttcatccccgagggctctcagcgagtgggcctggtggcctctcagaagaacgacc
tggacgccgtggccctgatgcaccccgacggctctgccgtggtggtggtcctgaaccgatcttctaaggacgtgccct
gaccatcaaggaccccgccgtgggcttcctggagaccatctctcccggctactctatccacacctacctgtggcgacga
cagtaatagcctaggggtacc

FIG. 50

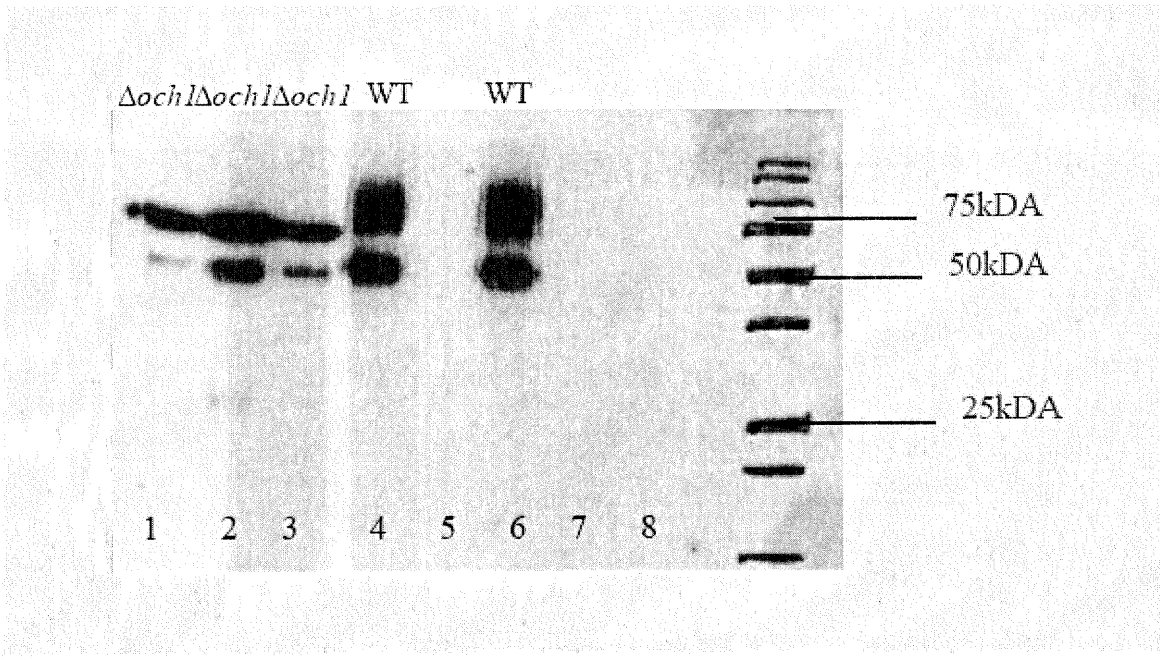


FIG. 51

gctccccccgactgatctgtgactctcgagtgctggaacgatacctgctggaagccaaggaagccgag
aacatcaccaccggctgtgccgagcactgttctctgaacgagaacattaccgtgcccgacaccaaggtg
aacttctacgcctggaagcgaatggaggtgggccagcaggccgtggaggtgtggcagggactggctct
gctgtctgaggccgtgctgcgaggacaggctctcctggtgaactcttctcagccctgggagccccctgcag
ctgcacgtggacaaggccgtgtctggcctgcgatctctgaccaccctgctgcgagccctcggtgctcaga
aggaagccatctctccccccgacgccgcctctgctgcccccctgcgaaccatcaccgccgacaccttccg
aaagctgttccgagtgtactctaacttcctgcgaggcaagctgaagctgtacaccggcgaggcttgtcg
aaccggcgaccga

FIG. 52

ctggacaacggcctggcccgaacccccaccatgggctggctgcactgggagcgattcatgtgtaacct
ggactgtcaggaagagcccgactcttgtatctctgagaagctgttcatggaaatggccgagctgatggt
gtctgagggctggaaggacgccggctacgagtacctgtgtatcgacgactgttggatggcccccccagc
gagactctgagggccgactccaggccgacccccagcgattcccccacggcatccgacagctcgccca
actacgtgcactctaagggcctgaagctgggcatctacgccgacgtgggcaacaagacctgtgccgg
cttccccggctctttcggctactacgacatcgacgcccagaccttcgccgactggggcgtggacctgct
gaagttcgacggctgttactgtgactctctcgagaacctggccgacggctacaagcacatgtctctggcc
ctgaaccgaaccggccgatctatcgtgtactcttgtgagtggcccctgtacatgtggcccttccagaagc
ccaactacaccgagatccgacagtactgtaaccactggcgaaacttcgccgacatcgacgactcgtgg
aagtctatcaagtctattctggactggacctctttcaaccaggagcgaatcgtcgacgtcgccggacccg
gcggatggaacgaccccgacatgctggtgatcggcaacttcggcctgtcttggaaccagcaggtgacc
cagatggccctgtgggctatcatggctgcccccctgttcatgtctaacgacctgcgacacatctctcccc
aggccaaggccctgctccaggacaaggacgtgatcgccatcaaccaggacccccctgggcaagcagg
gctaccagctccgacagggcgacaacttcgaggtgtgggagcgacccctgtctggcctggcctgggc
cgtggccatgatcaaccgacaggagatcggcggaccccgatcttacaccatcgccgtggcctccctgg
gaaagggcgtggcctgtaaccccgcctgtttcatcacccagctcctgcccgtgaagcgaaagctggga
ttctacgagtggacctctcgactgcgatctcacatcaaccccaccggcaccgtgctgctccagctcgag
aacaccatgcagatgtctctgaaggacctgctg

FIG. 53

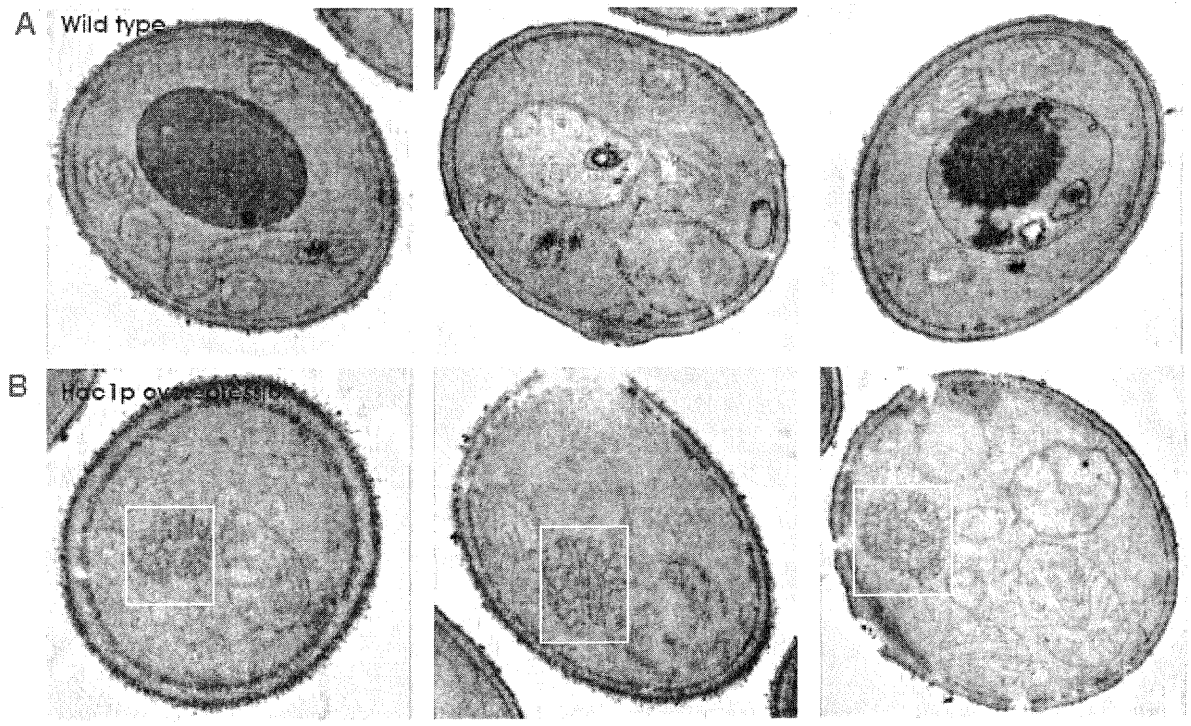


FIG. 54

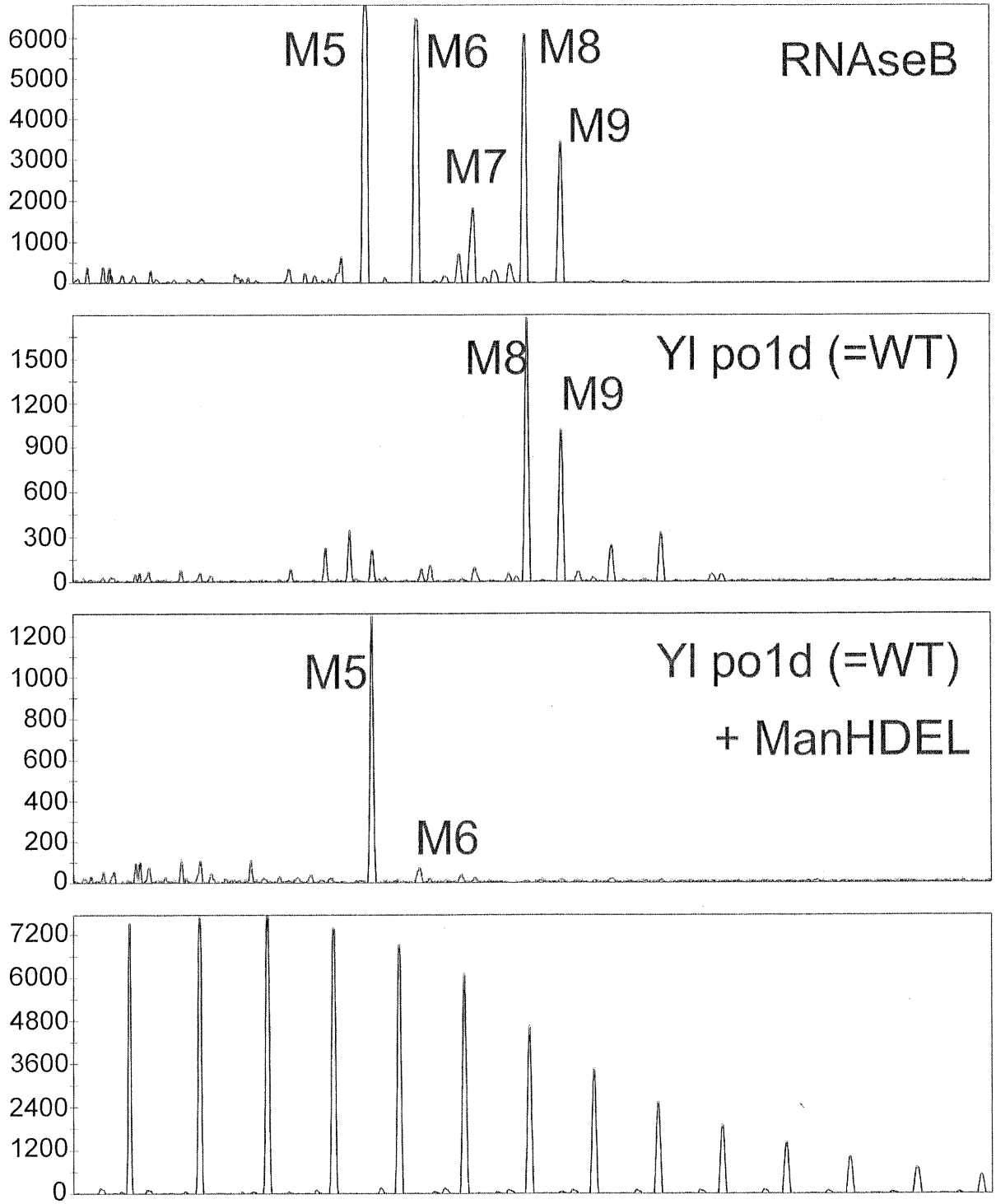

6000
5000
4000
3000
2000
1000
0
M5
M6
M8
M9
M7
RNAseB
1500
1200
900
600
300
0
M8
M9
Yl po1d (=WT)
1200
1000
800
600
400
200
0
M5
M6
Yl po1d (=WT)
+ ManHDEL
7200
6000
4800
3600
2400
1200
0

FIG. 55