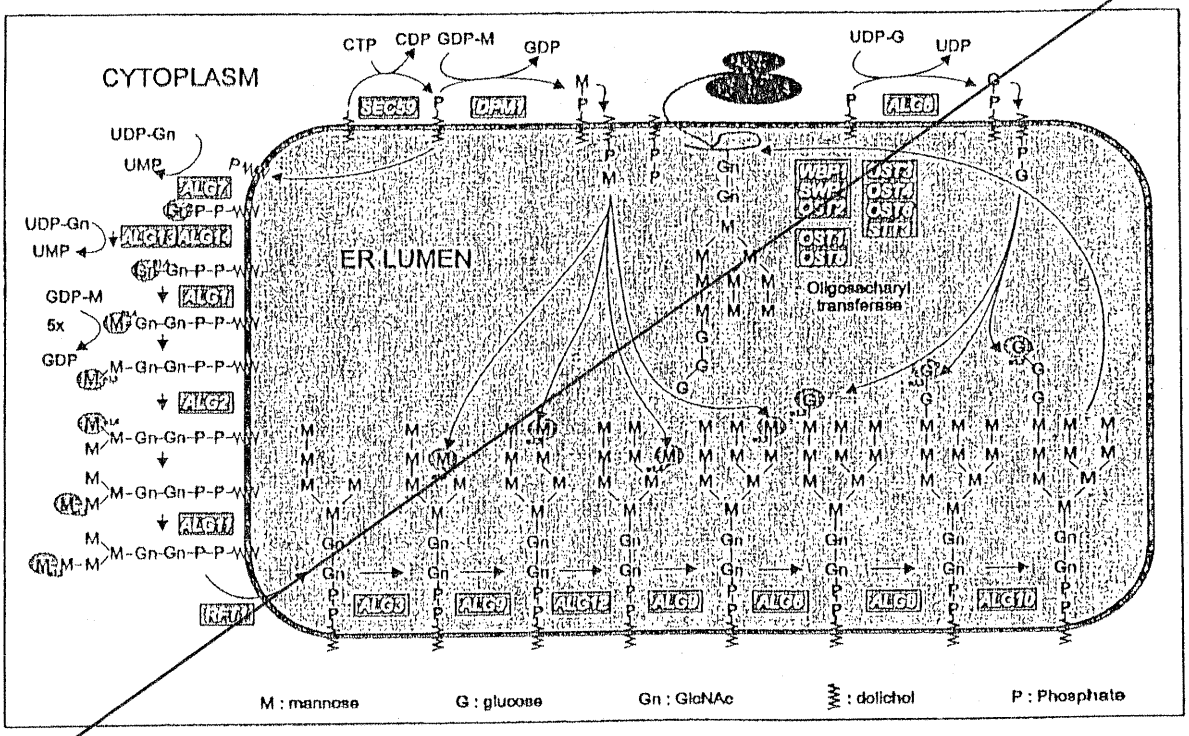
CYTOPLASM
ER LUMEN
CTP
CDP
GDP-M
GDP
UDP-G
UDP
UDP-Gn
UMP
GDP-M
5x
GDP
Oligosaccharyl transferase
M : mannose
G : glucose
Gn : GlcNAc
: dolichol
P : Phosphate

FIG. 1

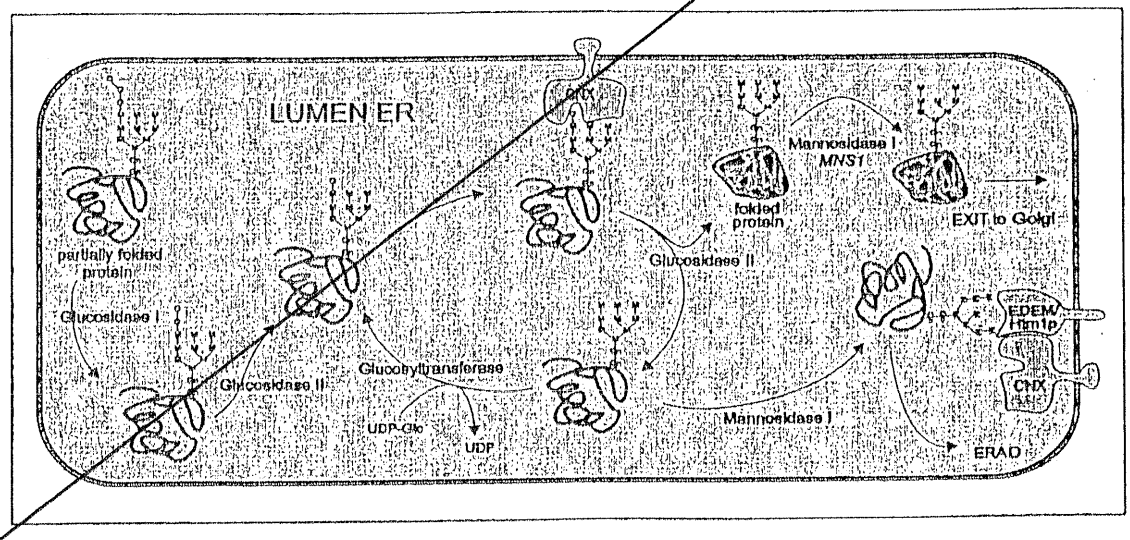
LUMEN ER
CNX
partially folded protein
Glucosidase I
Glucosidase II
Glucosyltransferase
UDP-Glc
UDP
Glucosidase II
folded protein
Mannosidase I MNS1
EXIT to Golgi
EDEM/ Htm1p
CNX
Mannosidase I
ERAD

FIG. 2

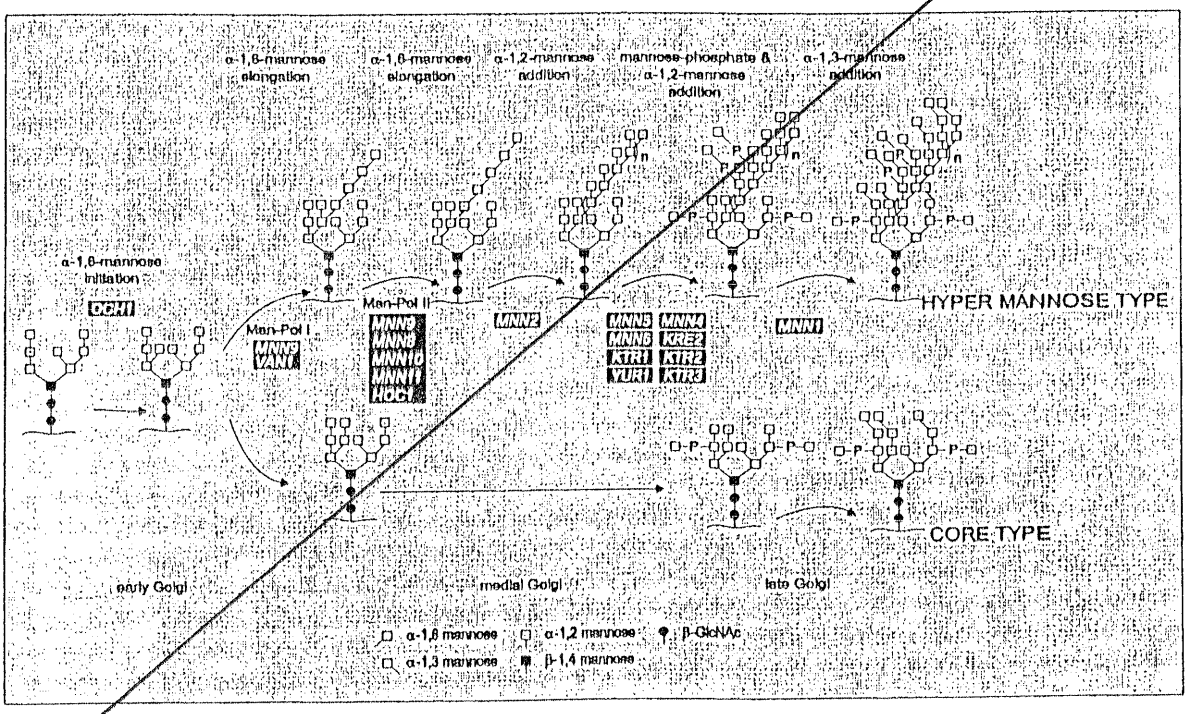
α-1,6-mannose elongation
α-1,6-mannose elongation
α-1,2-mannose addition
mannose-phosphate & α-1,2-mannose addition
α-1,3-mannose addition
α-1,6-mannose initiation
OCH1
Man-Pol I
MNN9
VAN1
Man-Pol II
MNN9
MNN1
MNN10
MNN11
HOC1
MNN2
MNN5
KTR1
YUR1
MNN4
KRE2
KTR2
KTR3
MNN1
HYPER MANNOSE TYPE
CORE TYPE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

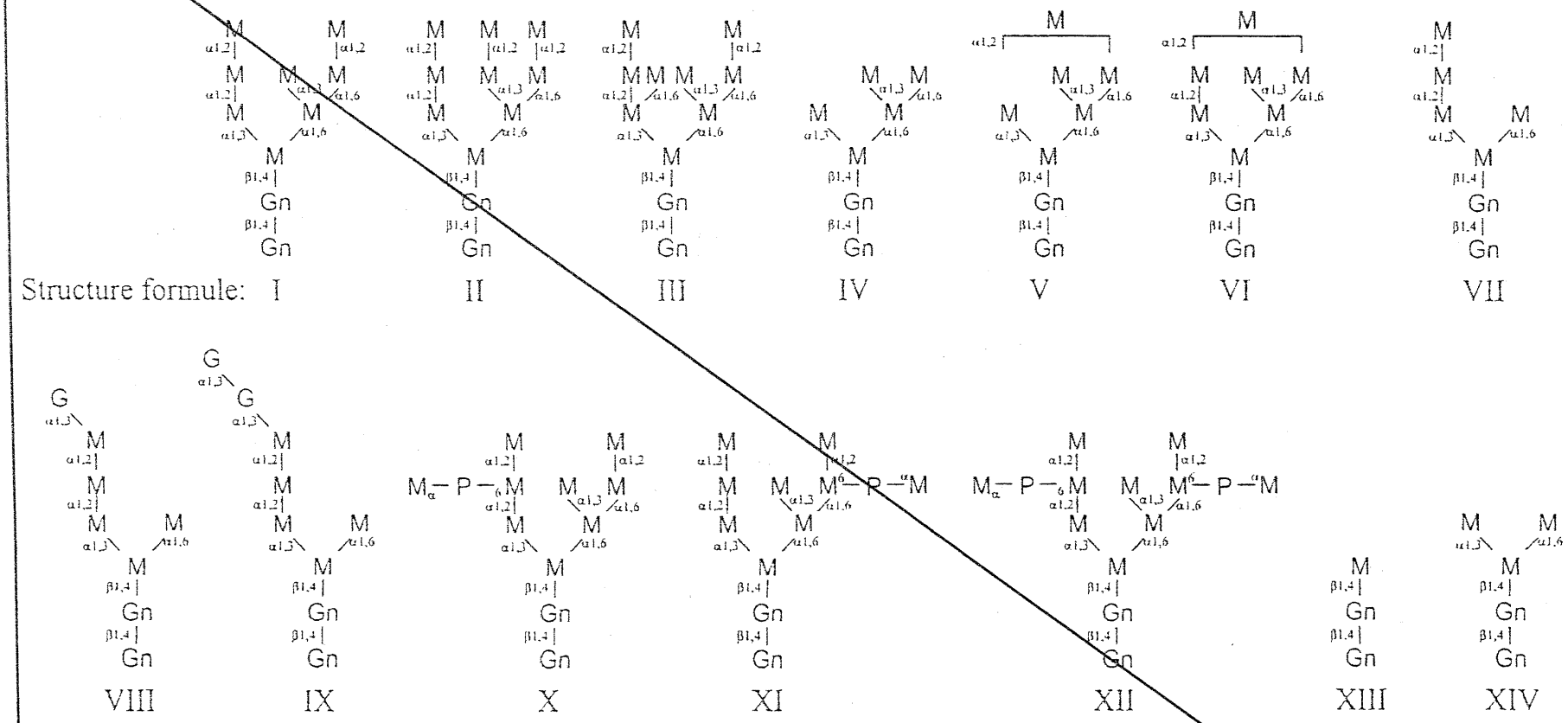
Structure formule: I II III IV V VI VII
VIII IX X XI XII XIII XIV
M: mannose G: glucose Gn: N-acetylglucosamine P: phosphate

FIG. 4

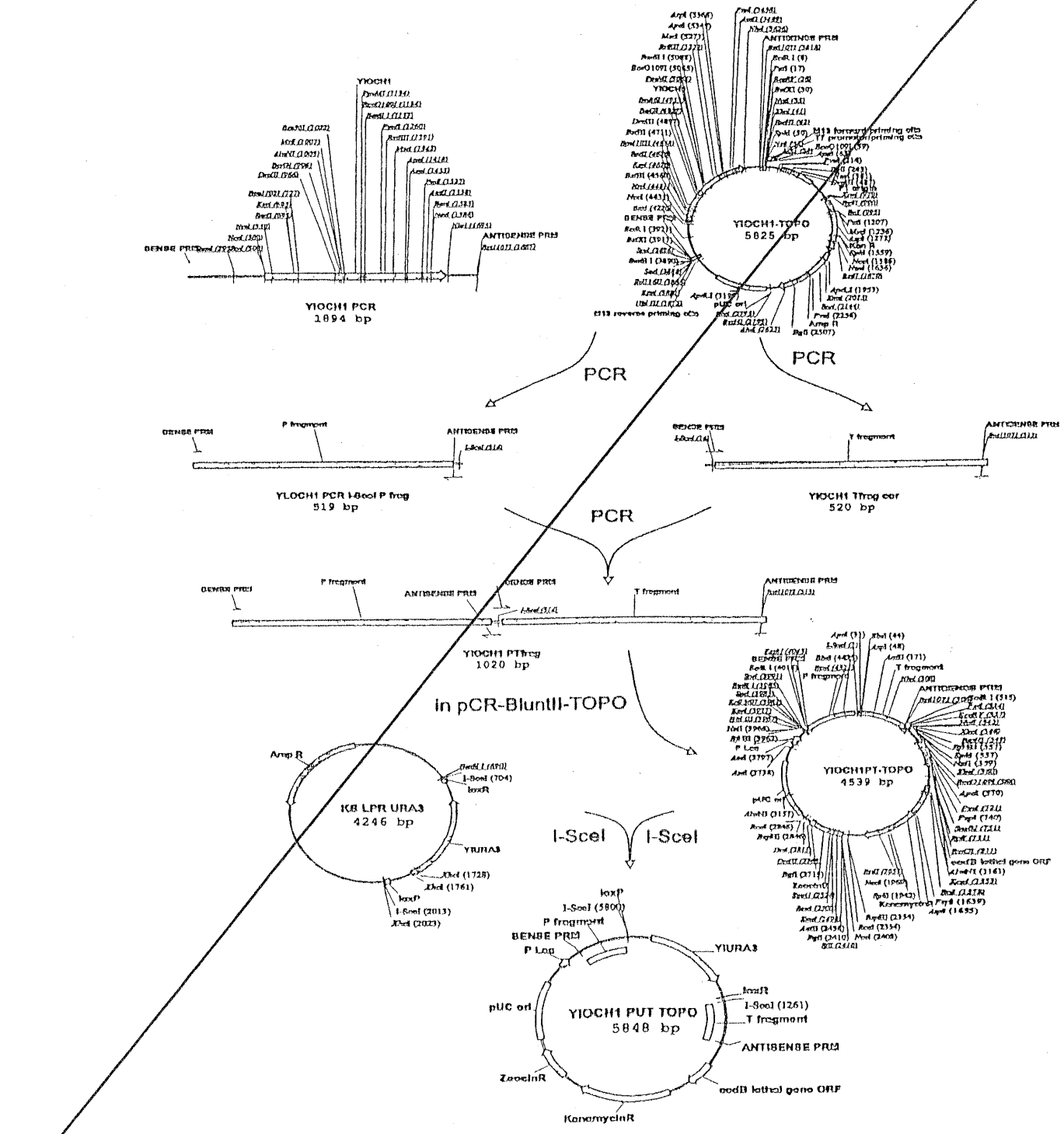
YIOCH1
YIOCH1 PCR
1894 bp
YIOCH1-TOPO
5825 bp
PCR
PCR
YLOCH1 PCR I-SceI P frag
519 bp
YIOCH1 TFrag cor
520 bp
PCR
YIOCH1 PTfrag
1020 bp
in pCR-BluntII-TOPO
YIOCH1PT-TOPO
4539 bp
KB LPR URA3
4246 bp
I-SceI
I-SceI
YIOCH1 PUT TOPO
5848 bp

FIG. 5

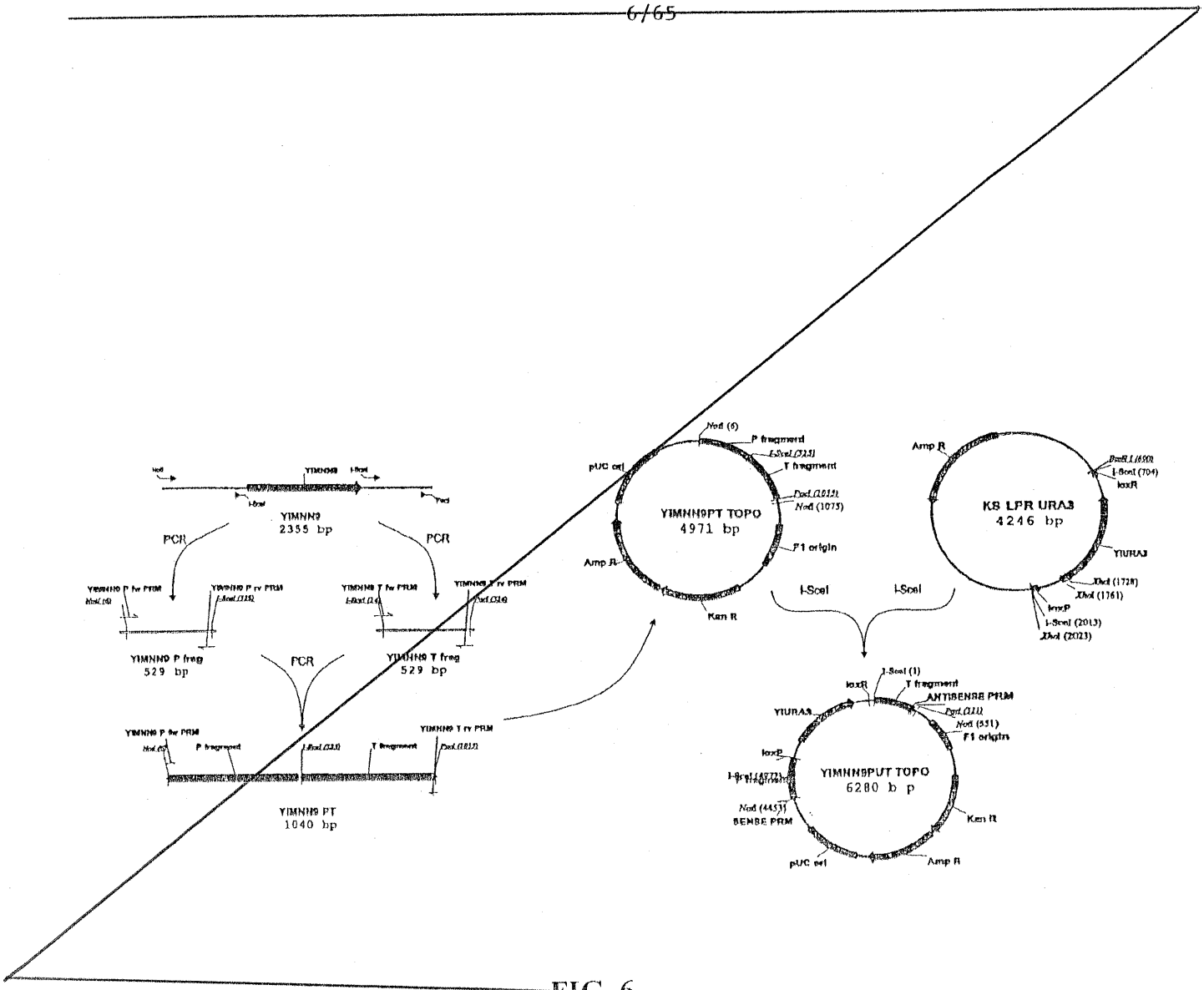
YIMNN9
2355 bp
PCR
PCR
YIMNN9 P frag
529 bp
YIMNN9 T frag
529 bp
PCR
YIMNN9 PT
1040 bp
YIMNN9PT TOPO
4971 bp
pUC ori
Amp R
Kan R
F1 origin
P fragment
T fragment
I-SceI
I-SceI
KS LPR URA3
4246 bp
Amp R
loxR
YIURA3
loxP
I-SceI (2013)
YIMNN9PUT TOPO
6280 b p
I-SceI (1)
loxR
T fragment
YIURA3
F1 origin
loxP
Kan R
pUC ori
Amp R

FIG. 6

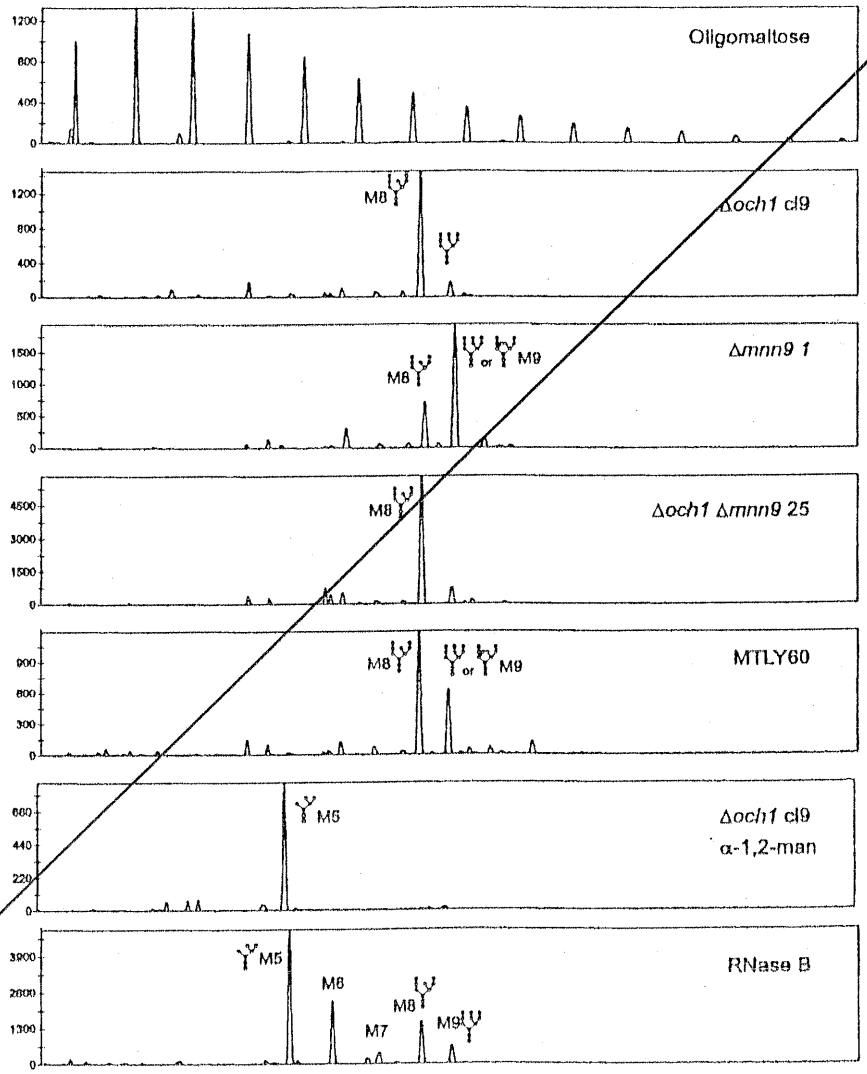
Oligomaltose
M8
Δoch1 cl9
M8
M9
Δmnn9 1
M8
MTLY60
M5
Δoch1 cl9
α-1,2-man
M5
M6
M8
M7
M9
RNase B

FIG. 7

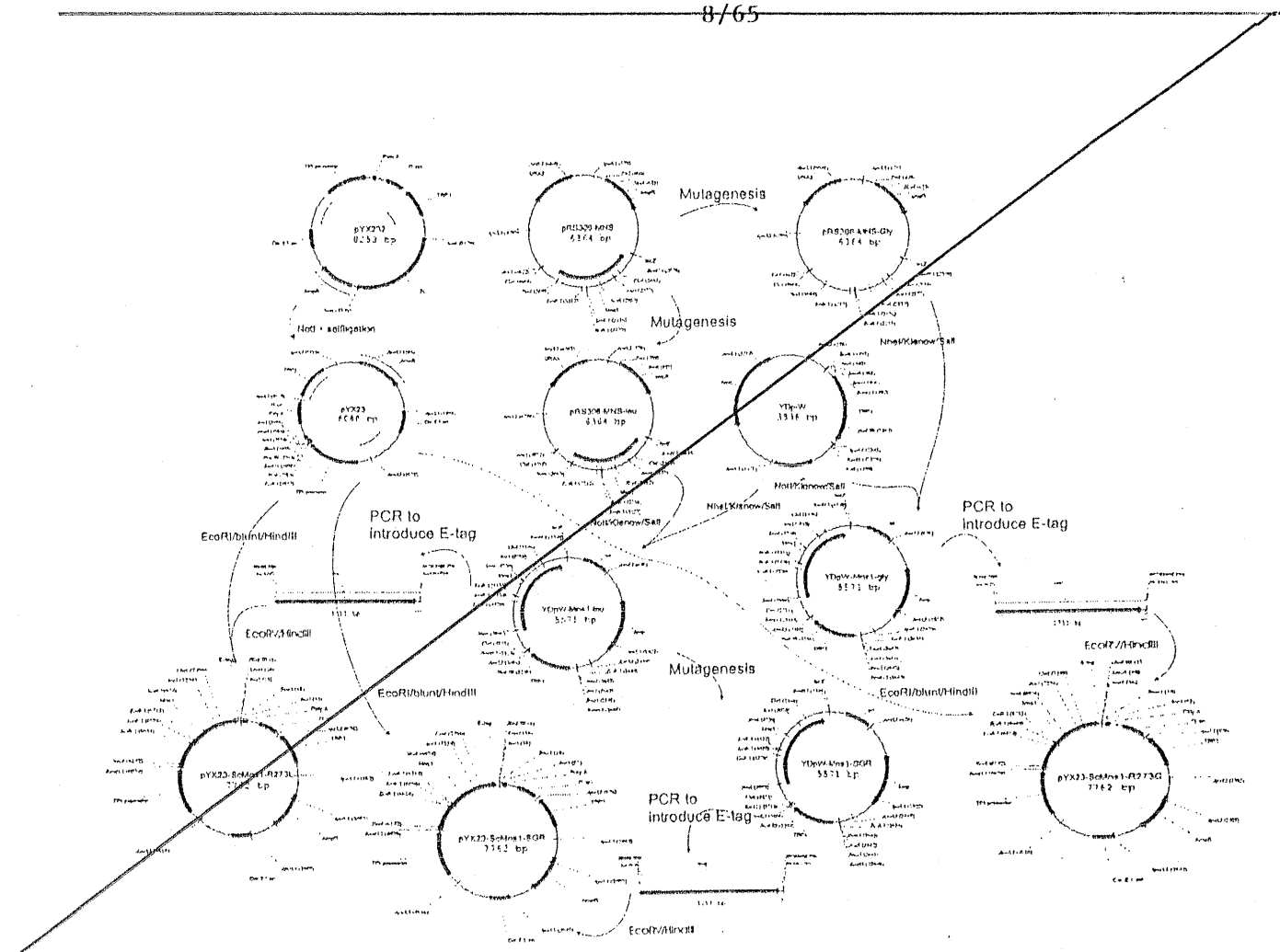
Mutagenesis
Mutagenesis
Mutagenesis
PCR to introduce E-tag
PCR to introduce E-tag
PCR to introduce E-tag
EcoRI/blunt/HindIII
EcoRI/blunt/HindIII
EcoRI/blunt/HindIII
EcoRI/HindIII
EcoRI/HindIII
EcoRI/HindIII
NotI/Klenow/SalI
NotI/Klenow/SalI
NotI/Klenow/SalI
NheI/Klenow/SalI
NotI + selfligation

FIG. 8

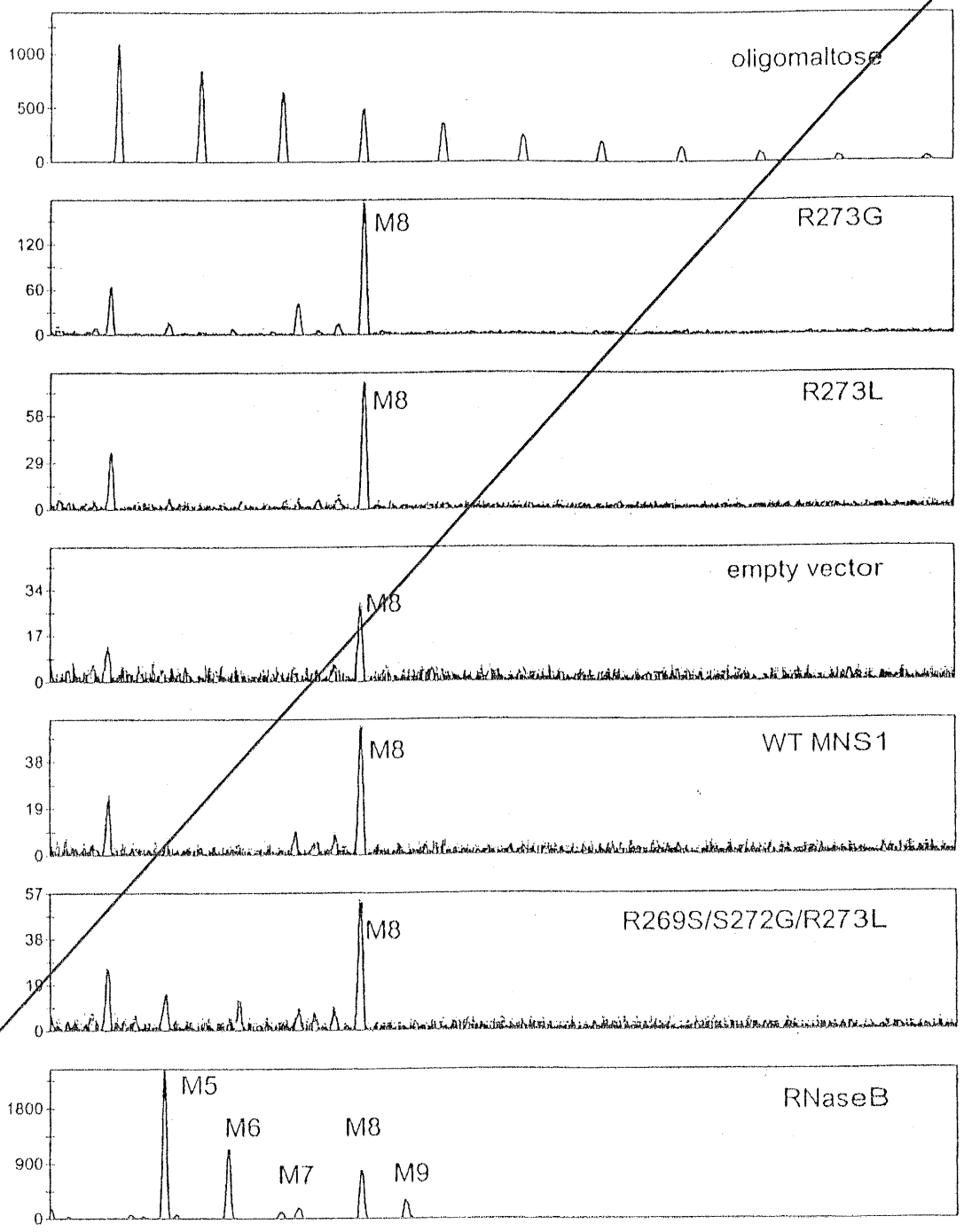
1000
500
0
oligomaltose
M8
R273G
120
60
0
M8
R273L
58
29
0
empty vector
34
17
0
M8
WT MNS1
38
19
0
M8
R269S/S272G/R273L
57
38
19
0
M5
M6
M7
M8
M9
RNaseB
1800
900
0

FIG. 9

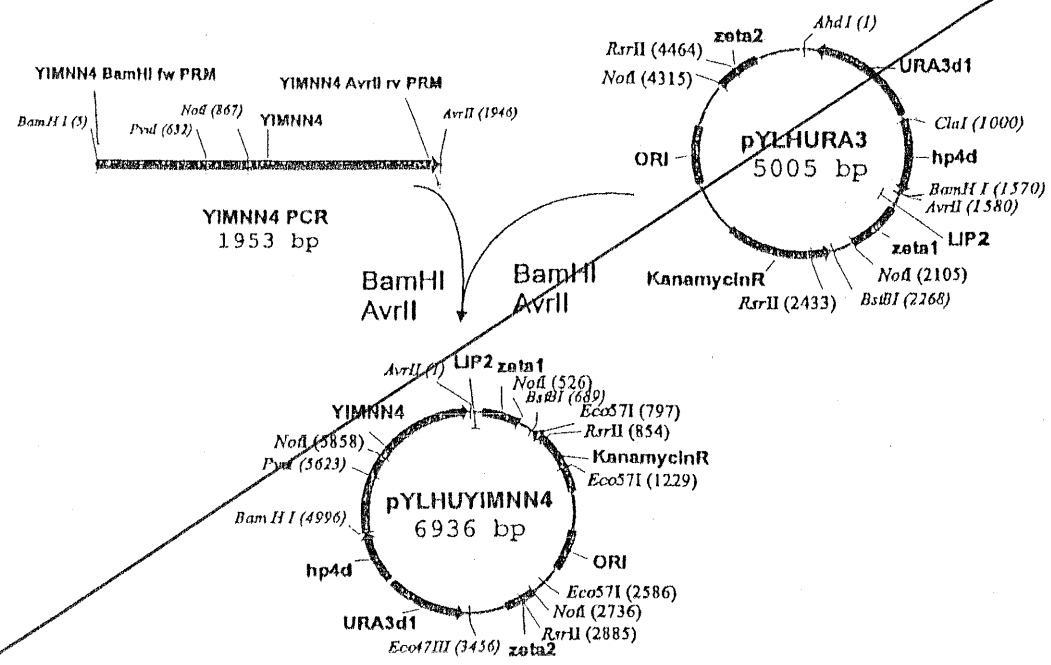
YIMNN4 BamHI fw PRM
YIMNN4 AvrII rv PRM
BamH I (5)
NotI (867)
PvuI (632)
YIMNN4
AvrII (1946)
YIMNN4 PCR
1953 bp
BamHI
AvrII
BamHI
AvrII
AhdI (1)
RsrII (4464)
zeta2
NotI (4315)
URA3d1
pYLHURA3
5005 bp
ClaI (1000)
ORI
hp4d
BamH I (1570)
AvrII (1580)
zeta1
LIP2
KanamycinR
NotI (2105)
RsrII (2433)
BstBI (2268)
AvrII (1)
LIP2
zeta1
NotI (526)
BstBI (680)
Eco57I (797)
YIMNN4
RsrII (854)
NotI (5858)
KanamycinR
PvuI (5623)
Eco57I (1229)
pYLHUYIMNN4
6936 bp
BamH I (4996)
ORI
hp4d
Eco57I (2586)
NotI (2736)
URA3d1
RsrII (2885)
Eco47III (3456)
zeta2

FIG. 10

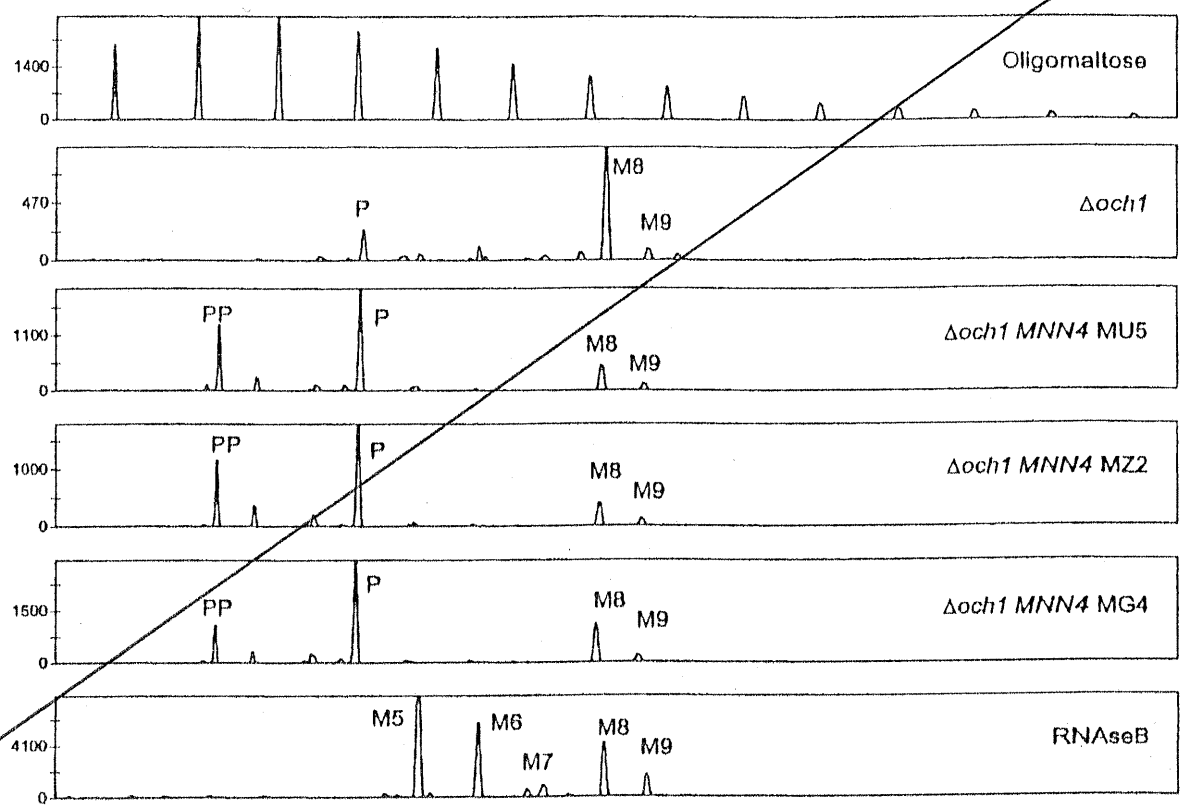
1400
0
Oligomaltose
470
0
P
M8
M9
Δoch1
1100
0
PP
P
M8
M9
Δoch1 MNN4 MU5
1000
0
PP
P
M8
M9
Δoch1 MNN4 MZ2
1500
0
PP
P
M8
M9
Δoch1 MNN4 MG4
4100
0
M5
M6
M7
M8
M9
RNAseB

FIG. 11

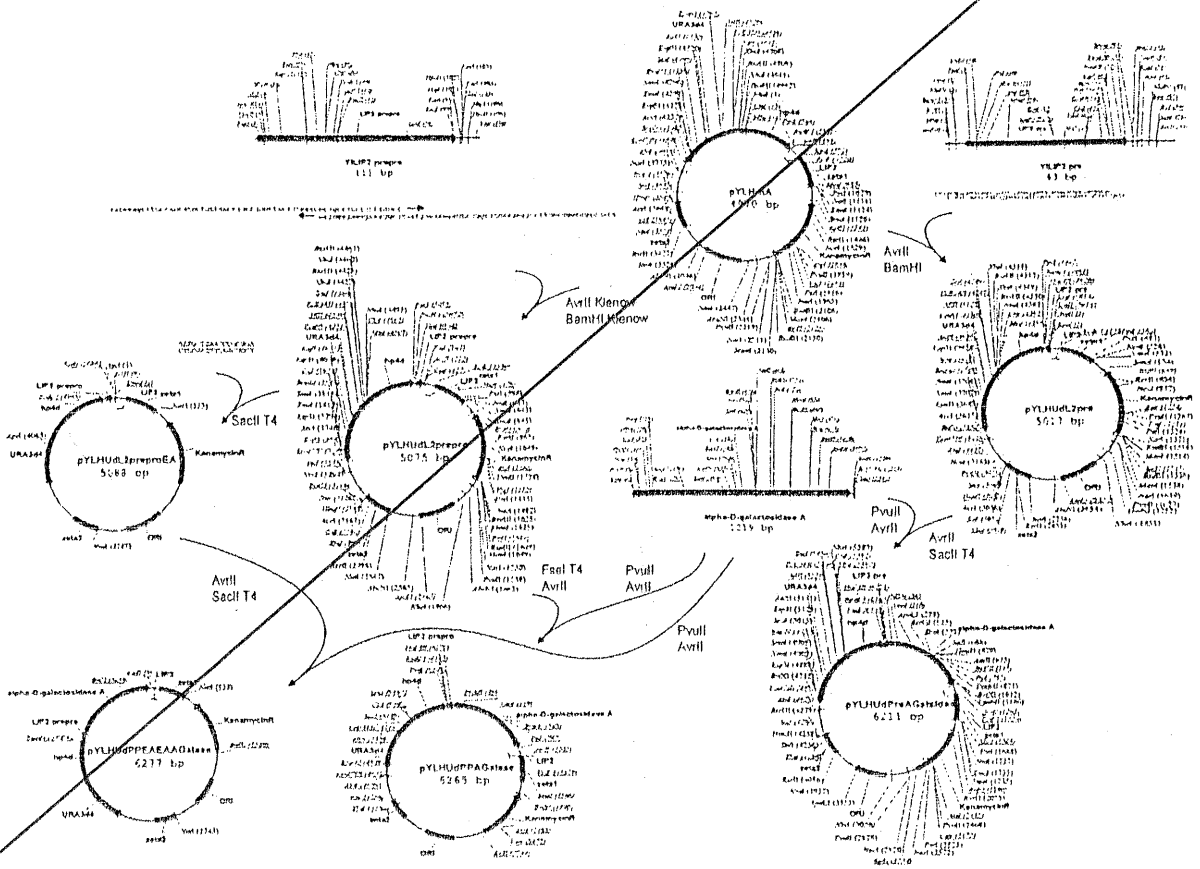
AvrII KlenowBamHI Klenow
AvrII BamHI
SacII T4
AvrII SacII T4
FseI T4 AvrII
PvuII AvrII
PvuII AvrII
AvrII SacII T4
PvuII AvrII

FIG. 12

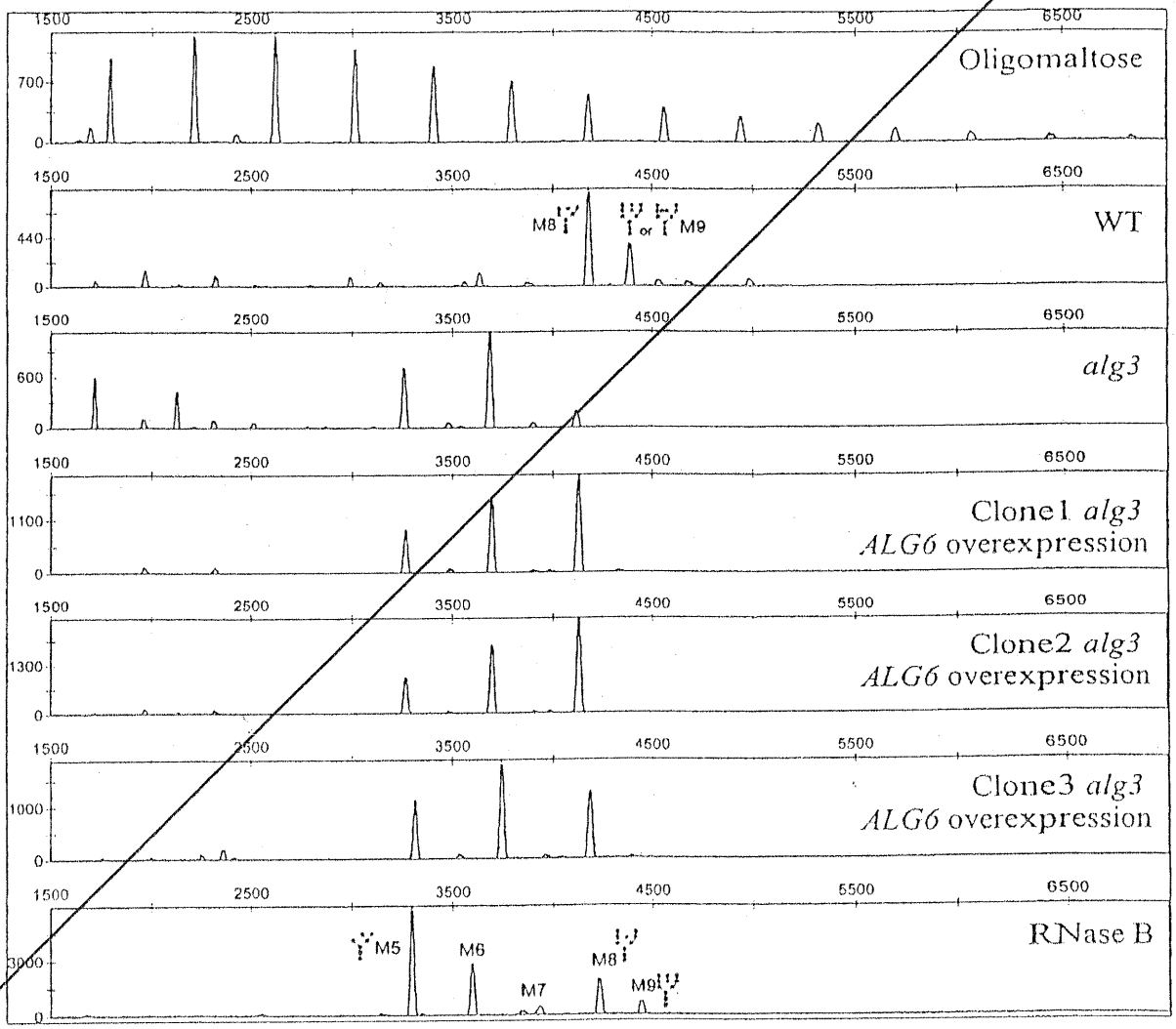
1500
2500
3500
4500
5500
6500
Oligomaltose
700
0
WT
M8
M9
or
440
alg3
600
Clone1 alg3
ALG6 overexpression
1100
Clone2 alg3
ALG6 overexpression
1300
Clone3 alg3
ALG6 overexpression
1000
RNase B
M5
M6
M7
M8
M9
3000

FIG. 13

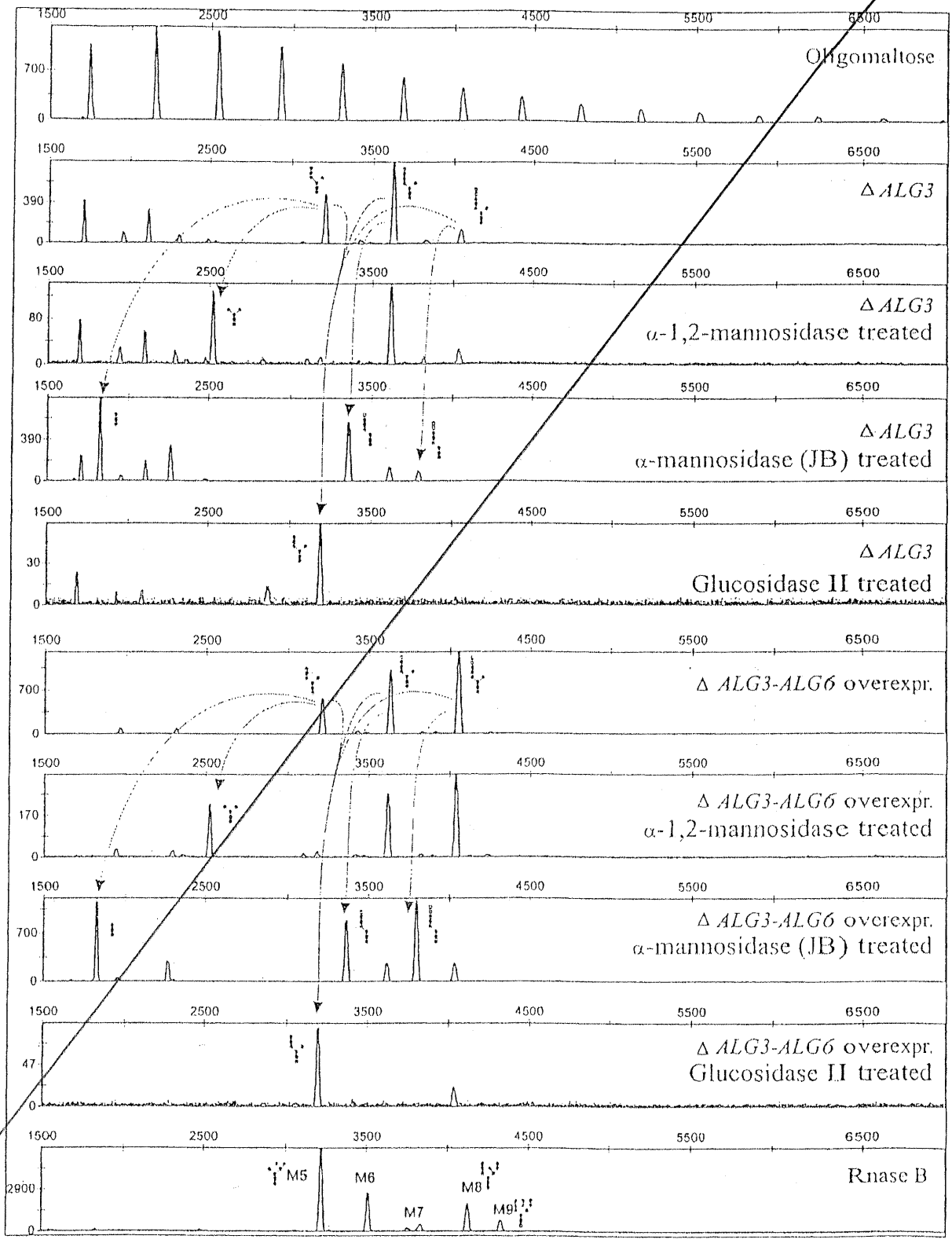
1500
2500
3500
4500
5500
6500
Oligomaltose
Δ ALG3
Δ ALG3
α-1,2-mannosidase treated
Δ ALG3
α-mannosidase (JB) treated
Δ ALG3
Glucosidase II treated
Δ ALG3-ALG6 overexpr.
Δ ALG3-ALG6 overexpr.
α-1,2-mannosidase treated
Δ ALG3-ALG6 overexpr.
α-mannosidase (JB) treated
Δ ALG3-ALG6 overexpr.
Glucosidase II treated
M5
M6
M7
M8
M9
Rnase B

FIG. 14

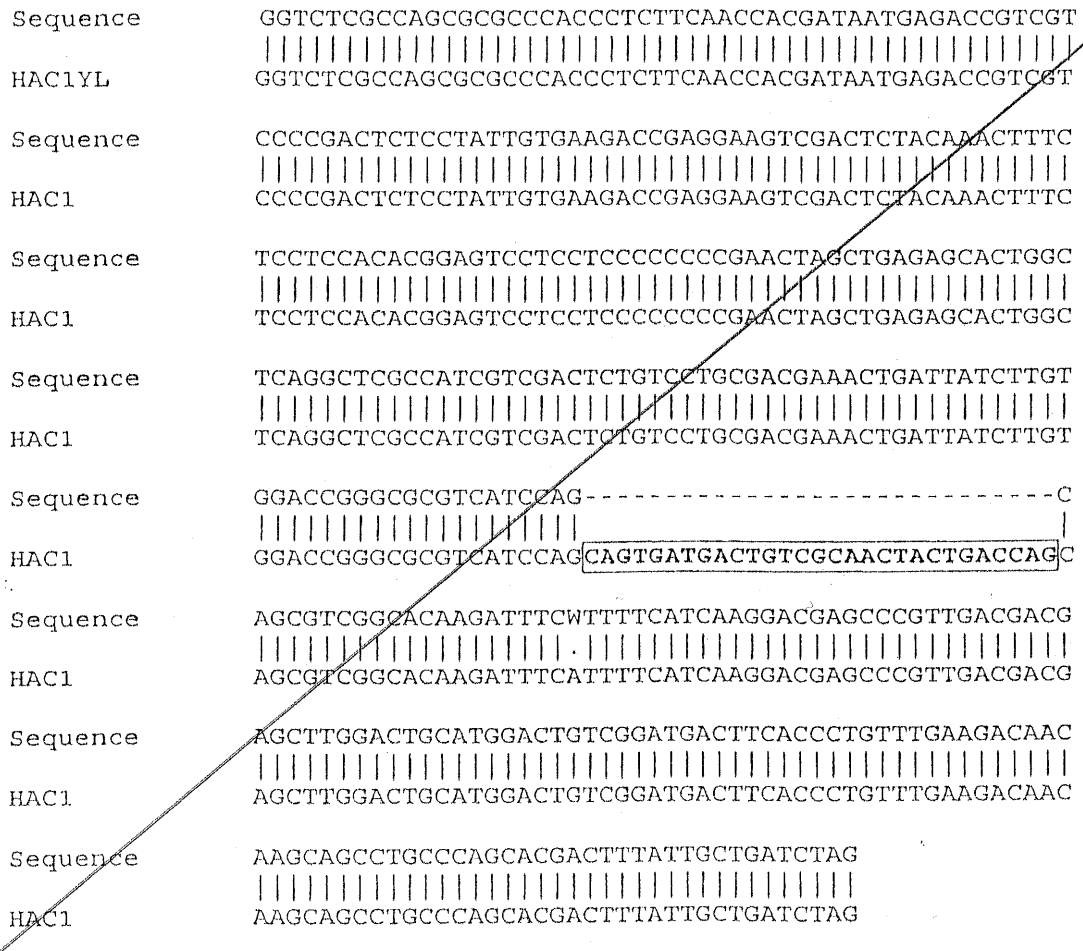
Sequence GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
HAC1YL GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
Sequence CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
HAC1 CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
Sequence TCCTCCACACGGAGTCCTCCTCCCCCCCCGAACTAGCTGAGAGCACTGGC
HAC1 TCCTCCACACGGAGTCCTCCTCCCCCCCCGAACTAGCTGAGAGCACTGGC
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

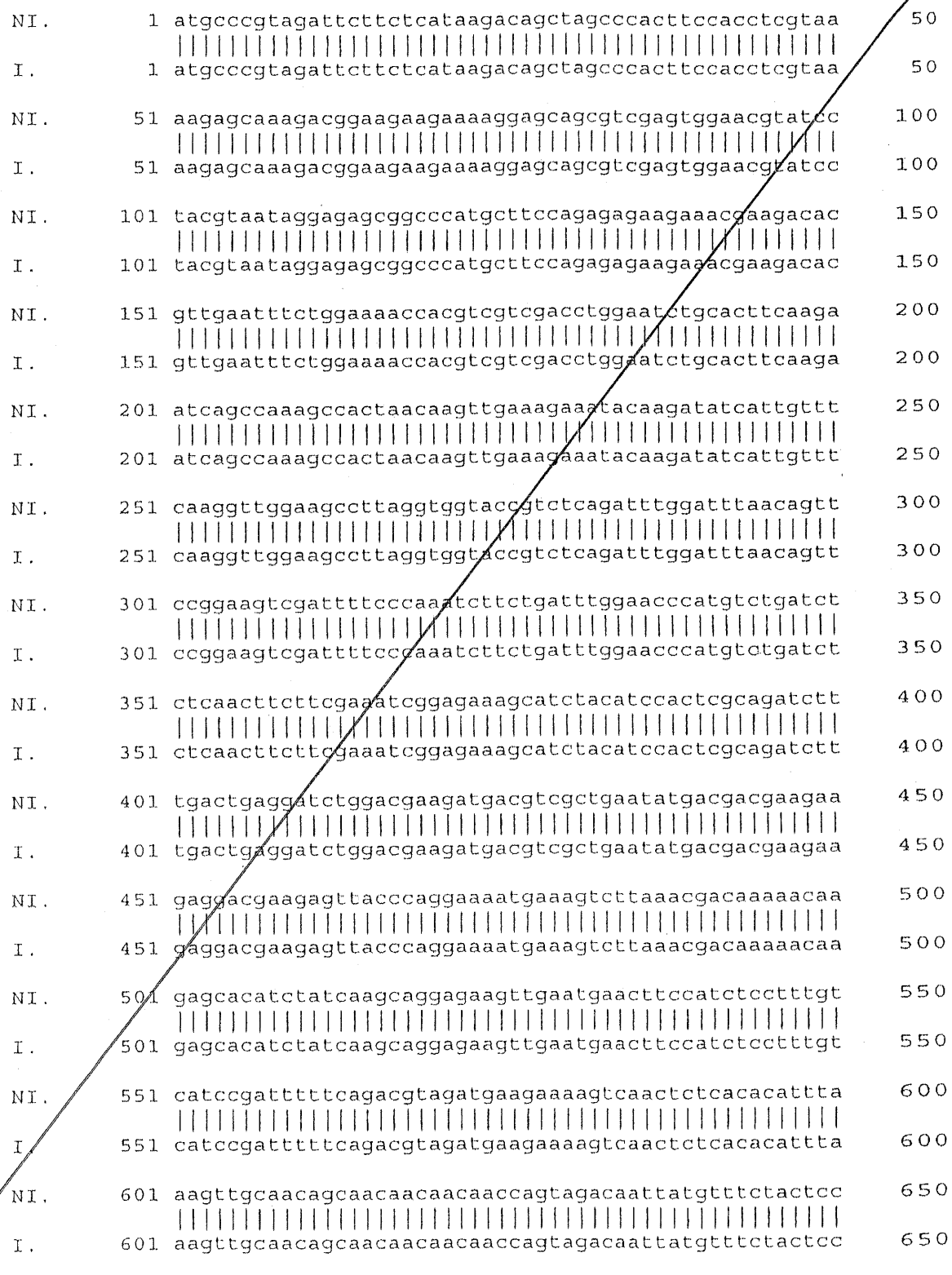
NI. 1 atgcccgtagattcttctcataagacagctagcccacttccacctcgtaa 50
I. 1 atgcccgtagattcttctcataagacagctagcccacttccacctcgtaa 50
NI. 51 aagagcaaagacggaagaagaaaaggagcagcgtcgagtggaacgtatcc 100
I. 51 aagagcaaagacggaagaagaaaaggagcagcgtcgagtggaacgtatcc 100
NI. 101 tacgtaataggagagcggcccatgcttccagagagaagaaacgaagacac 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

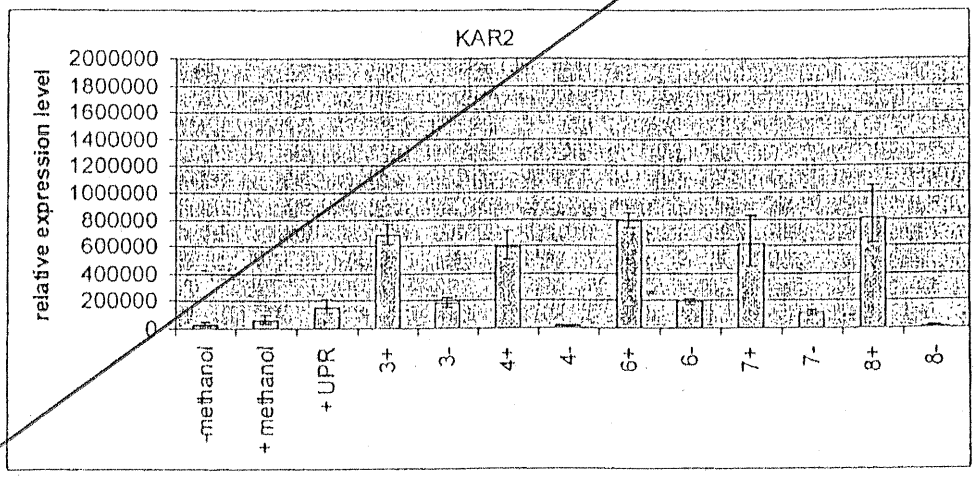
KAR2
relative expression level
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-methanol
+ methanol
+ UPR
3+
3-
4+
4-
6+
6-
7+
7-
8+
8-

FIG. 19

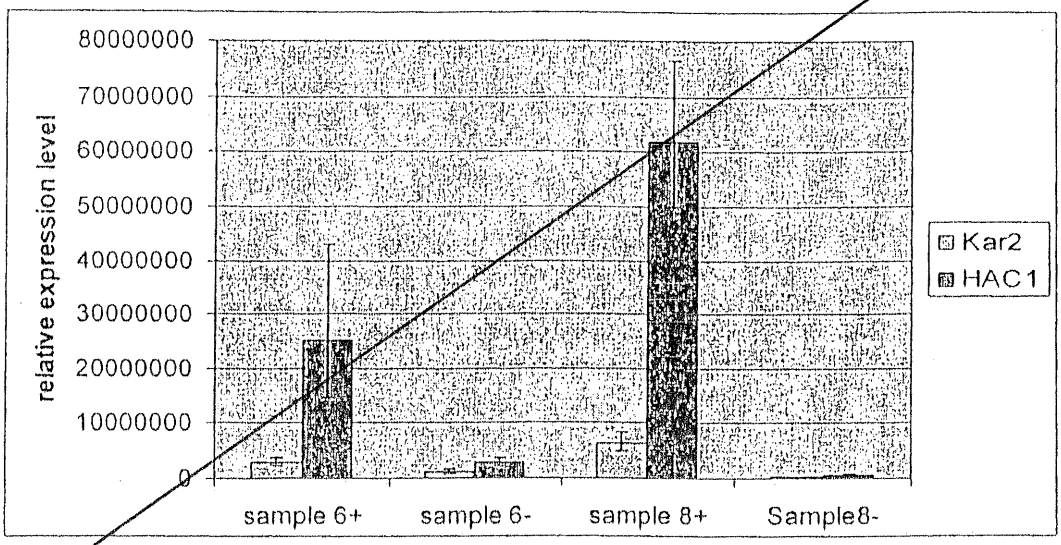
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
relative expression level
sample 6+
sample 6-
sample 8+
Sample8-
Kar2
HAC1

FIG. 20

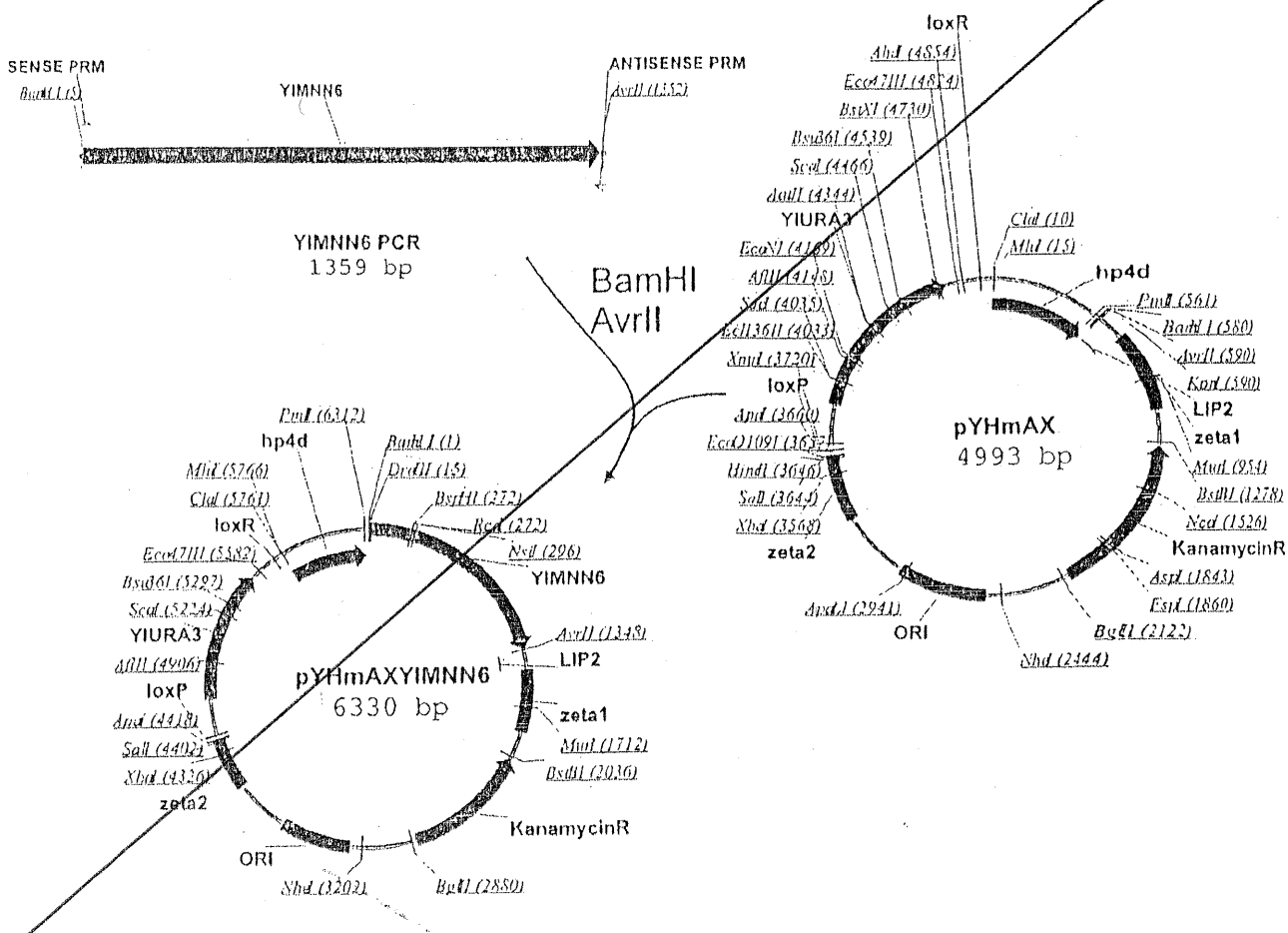
SENSE PRM
YIMNN6
ANTISENSE PRM
YIMNN6 PCR
1359 bp
BamHI
AvrII
pYHmAX
4993 bp
pYHmAXYIMNN6
6330 bp
loxR
hp4d
YIURA3
loxP
zeta1
zeta2
LIP2
KanamycinR
ORI

FIG. 21

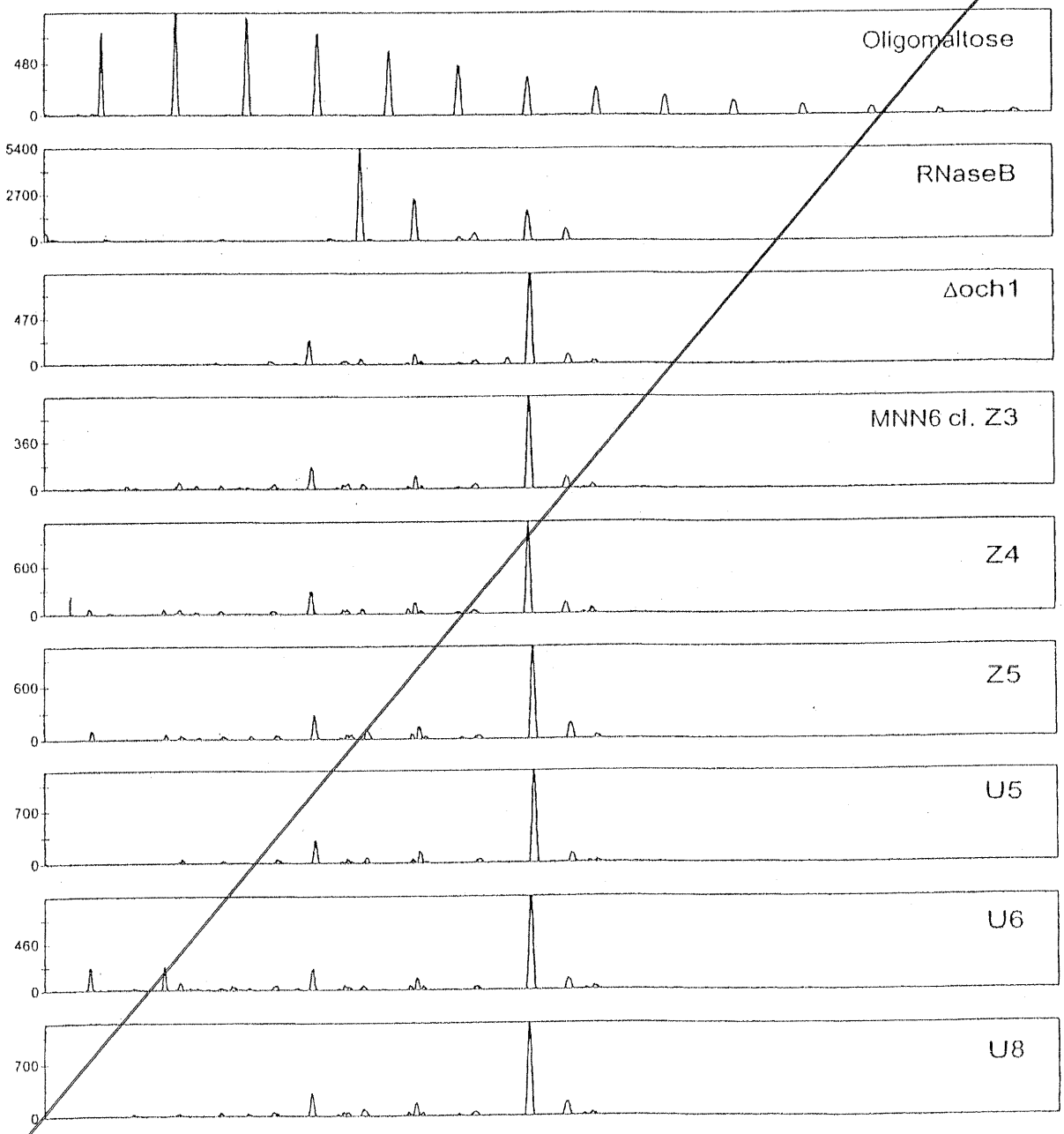
Oligomaltose
480
0
RNaseB
5400
2700
0
Δoch1
470
0
MNN6 cl. Z3
360
0
Z4
600
0
Z5
600
0
U5
700
0
U6
460
0
U8
700
0

FIG. 22

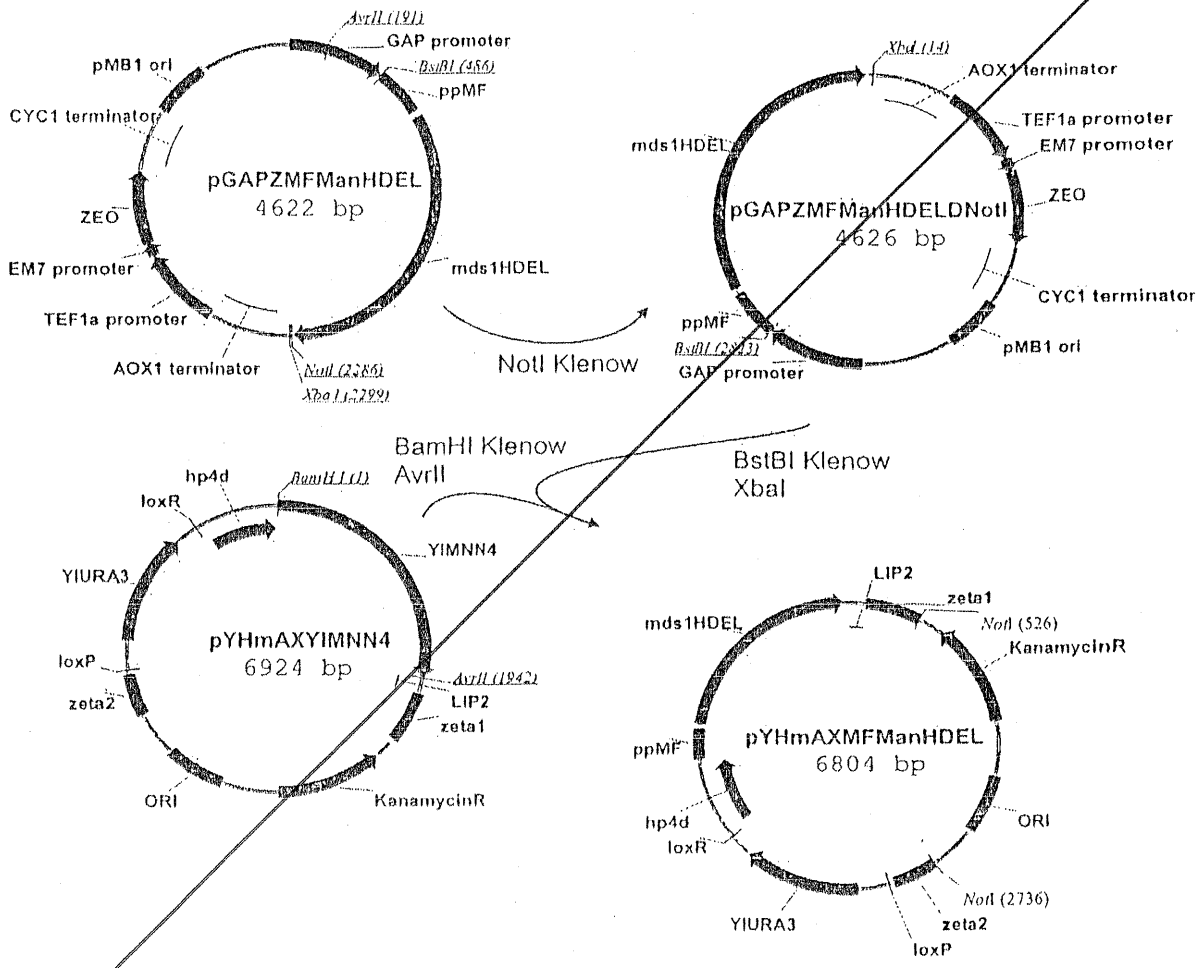
AvrII (191)
GAP promoter
pMB1 ori
BstBI (456)
ppMF
CYC1 terminator
pGAPZMFManHDEL
4622 bp
ZEO
EM7 promoter
mds1HDEL
TEF1a promoter
AOX1 terminator
NotI (2286)
XbaI (2299)
NotI Klenow
XbaI (14)
AOX1 terminator
TEF1a promoter
mds1HDEL
EM7 promoter
pGAPZMFManHDELDNotI
4626 bp
ZEO
CYC1 terminator
ppMF
BstBI (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
ppMF
pYHmAXMFManHDEL
6804 bp
ORI
hp4d
loxR
NotI (2736)
YIURA3
zeta2
loxP

FIG. 23

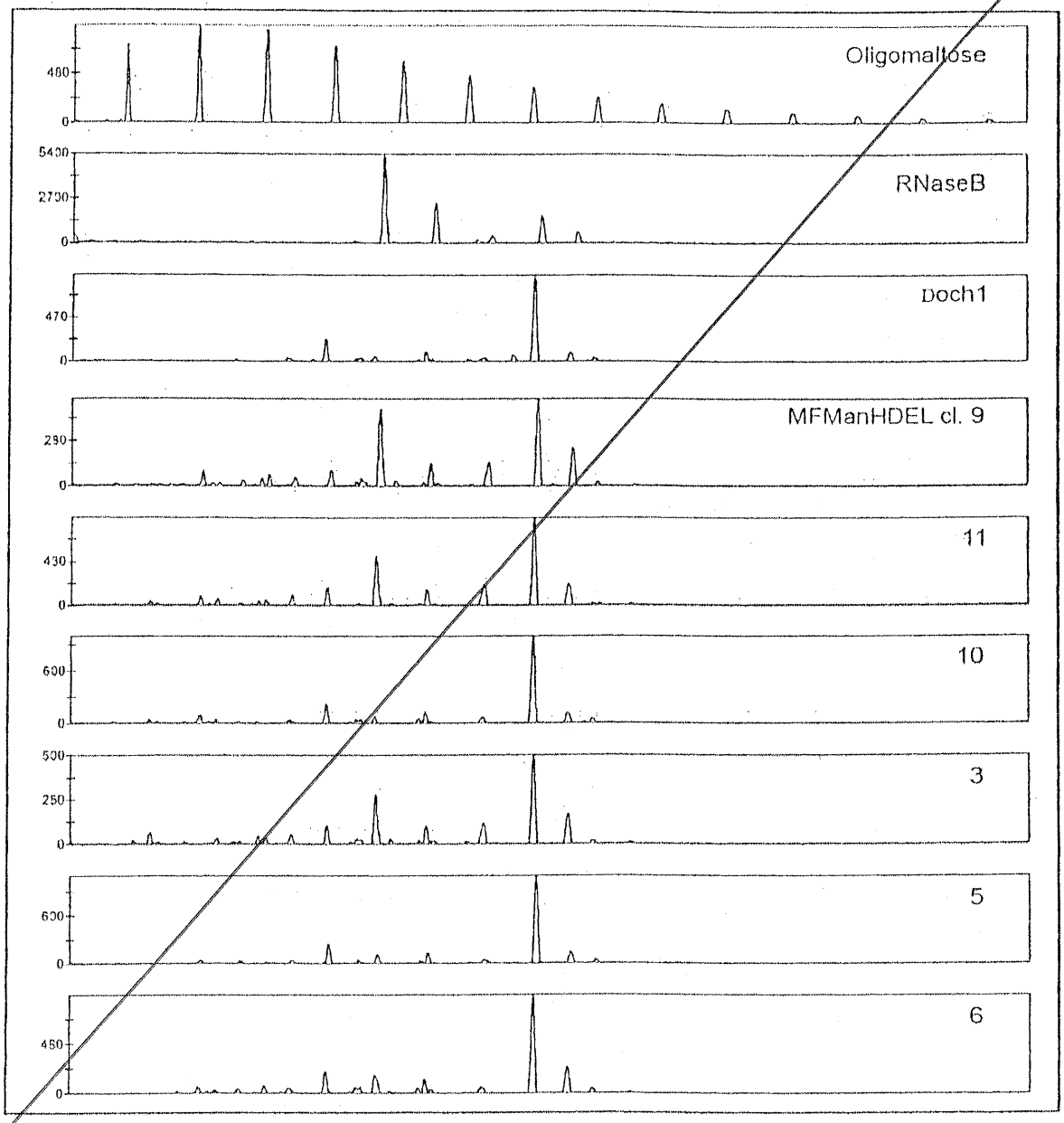
Oligomaltose
460
0
RNaseB
5400
2700
0
Doch1
470
0
MFManHDEL cl. 9
280
0
11
430
0
10
600
0
3
500
250
0
5
600
0
6
450
0

FIG. 24

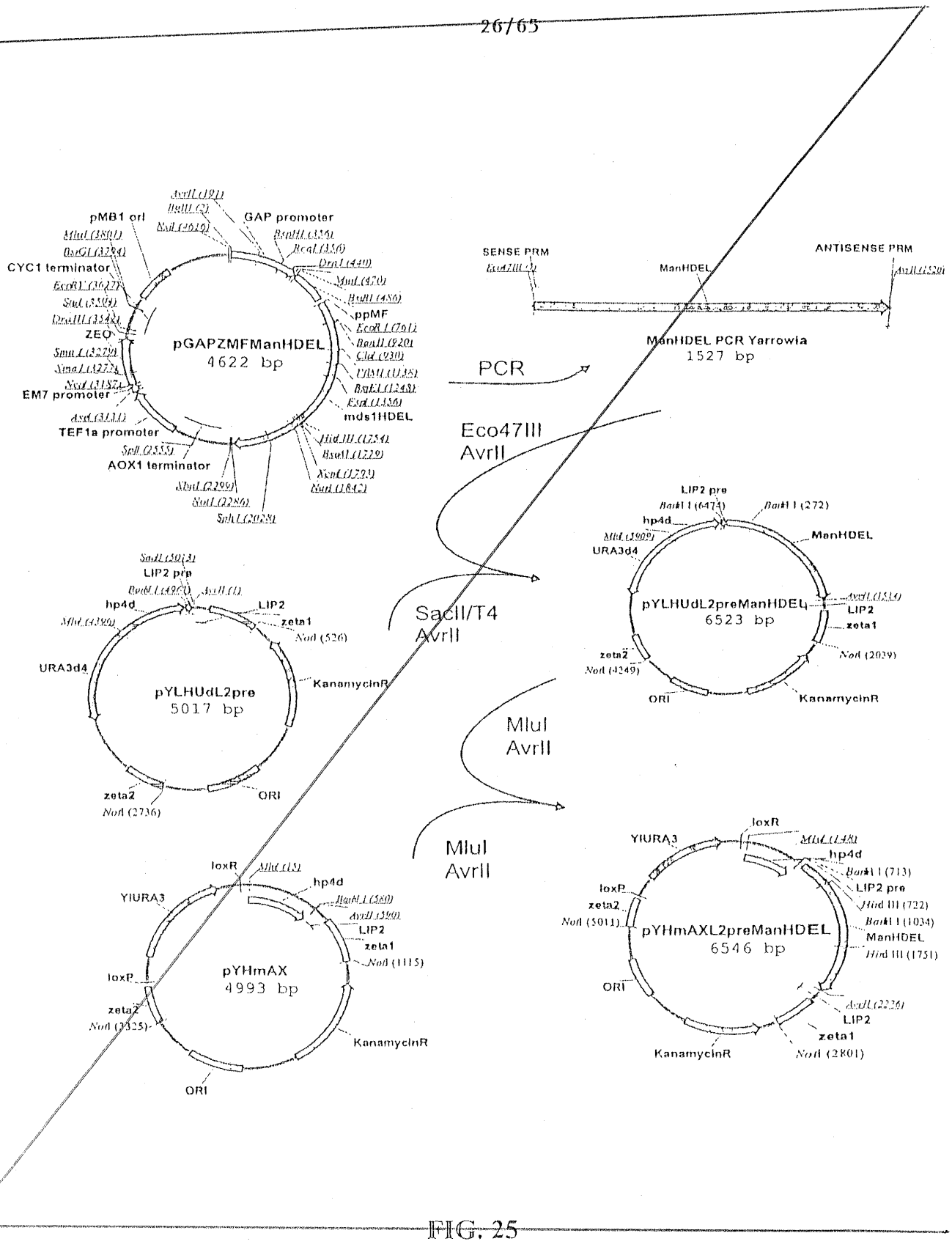
pGAPZMFManHDEL
4622 bp
pMB1 ori
GAP promoter
CYC1 terminator
ZEO
EM7 promoter
TEF1a promoter
AOX1 terminator
ppMF
mds1HDEL
SENSE PRM
ANTISENSE PRM
ManHDEL
ManHDEL PCR Yarrowia
1527 bp
PCR
Eco47III
AvrII
SacII/T4
AvrII
MluI
AvrII
MluI
AvrII
pYLHUdL2pre
5017 bp
LIP2 pre
hp4d
LIP2
zeta1
zeta2
URA3d4
KanamycinR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

FIG. 25

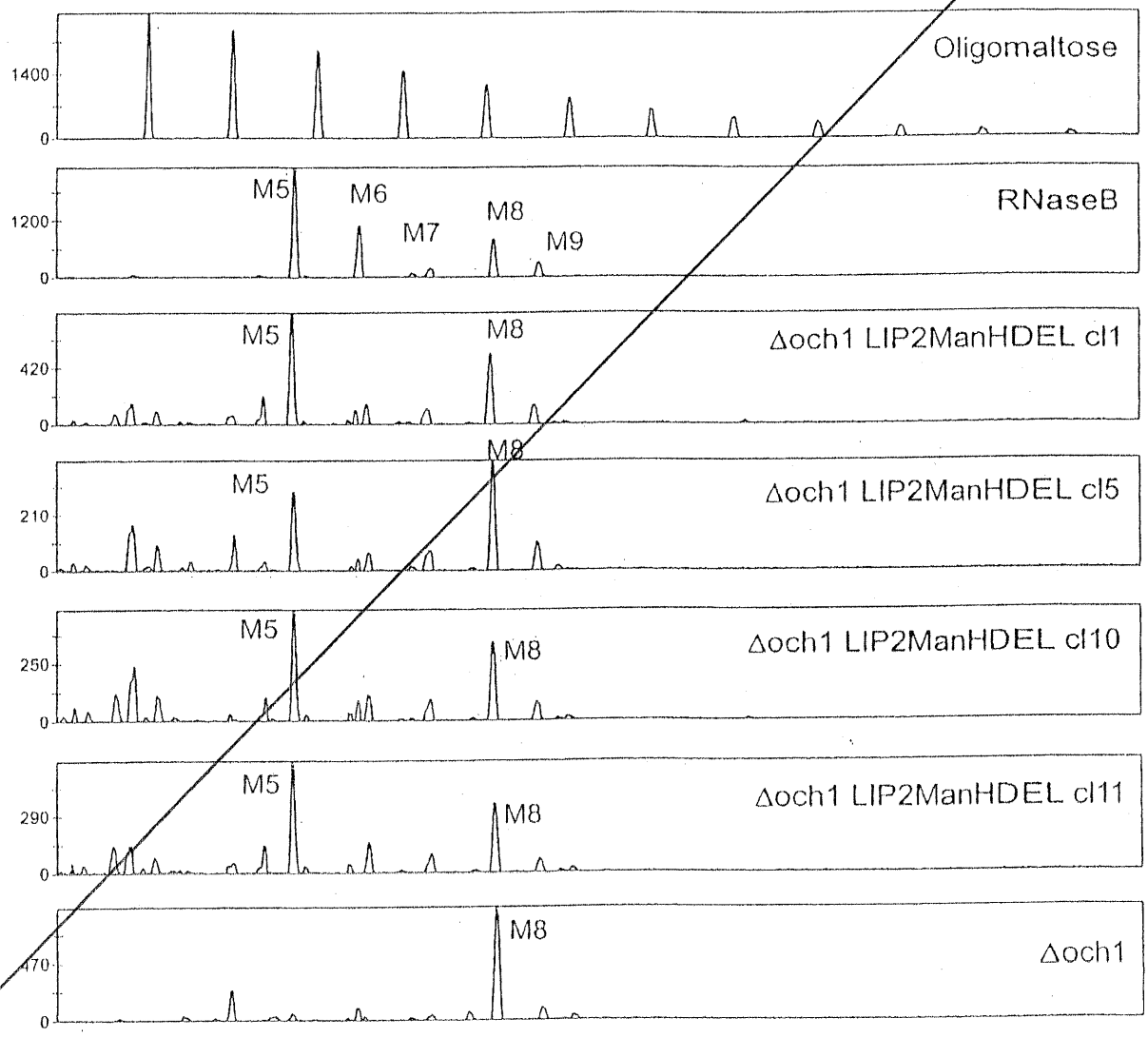
Oligomaltose
1400
0
M5
M6
M7
M8
M9
RNaseB
1200
0
M5
M8
Δoch1 LIP2ManHDEL cl1
420
0
M8
M5
Δoch1 LIP2ManHDEL cl5
210
0
M5
M8
Δoch1 LIP2ManHDEL cl10
250
0
M5
M8
Δoch1 LIP2ManHDEL cl11
290
0
M8
Δoch1
470
0

FIG. 26

MSIKREESFTPTPEDLGSPLTADSPGSPESGDKRKKDLTLP
LPAGALPPRKRAKTENEKEQRRIERIMRNRQAAHASREKKRRH
LEDLEKKCSELSSENNDLHHQVTESKKTNMHLMEQHYSLVAK
LQQLSSLVNMAKSSGALAGVDVPDMSDVSMAPKLEMPTAAPS
QPMGLASAPTLFNHDNETVVPDSPIVKTEEVDSTNFLLHTESSS
PPELAESTGSGSPSSTLSCDETDYLVDRARHPAASAQDFIFIKD
EPVDDELGLHGLSDDFTLFEDNKQPAQHDFIADLAHYESSVSN
LFGGLE

FIG. 27A

MPVDSSHKTASPLPPRKRAKTEEEKEQRRVERILRNRRAA
HASREKKRRHVEFLENHVVDLESALQESAKATNKLKEIQDIIV
SRLEALGGTVSDLDLTVPEVDFPKSSDLEPMSDLSTSSKSEKAS
TSTRRSLTEDLDEDDVAEYDDEEEDEELPRKMKVLNDKNKSTS
IKQEKLNELPSPLSSDFSDVDEEKSTLTHLKLQQQQQQPVDNY
VSTPLSLPEDSVDFINPGNLKIESDENFLLSSNTLQIKHENDTD
YITTAPSGSINDFFNSYDISESNRLHHPAAPFTANAFDLNDFVF
FQE

FIG. 27B

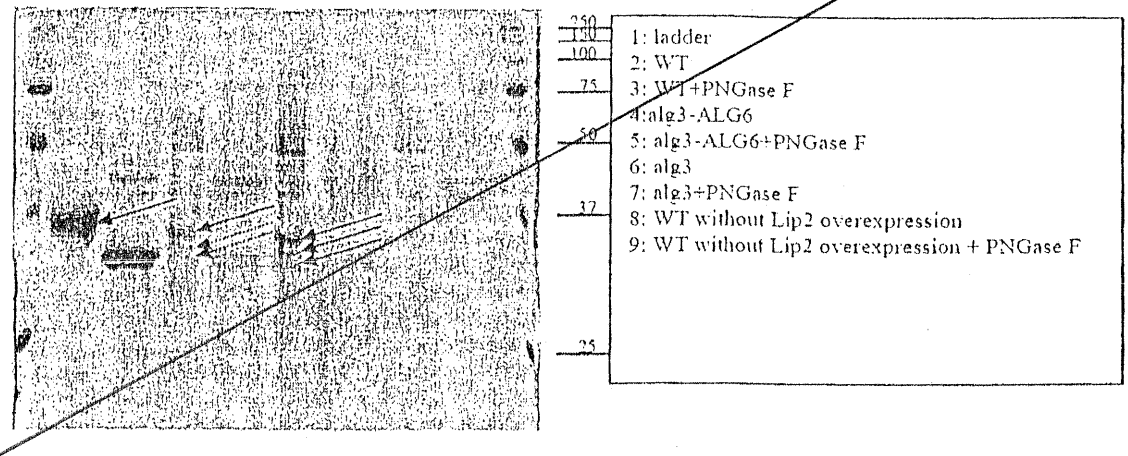
250
150
100
75
50
37
25
1: ladder
2: WT
3: WT+PNGase F
4:alg3-ALG6
5: alg3-ALG6+PNGase F
6: alg3
7: alg3+PNGase F
8: WT without Lip2 overexpression
9: WT without Lip2 overexpression + PNGase F

FIG. 28

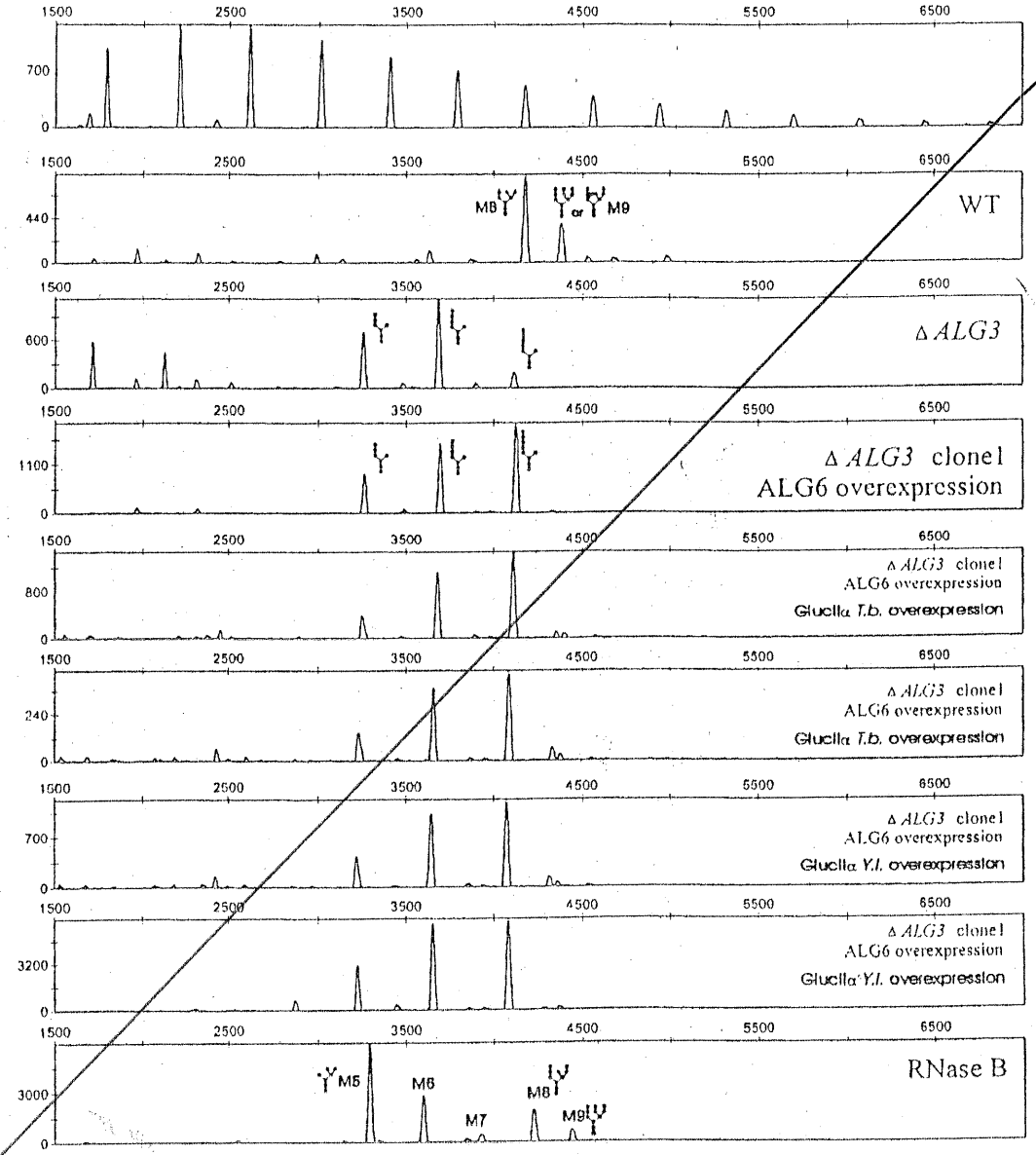
1500
2500
3500
4500
5500
6500
700
0
M8
M9
or
WT
440
Δ ALG3
600
Δ ALG3 clone1
ALG6 overexpression
1100
Δ ALG3 clone1
ALG6 overexpression
GlucIIα T.b. overexpression
800
Δ ALG3 clone1
ALG6 overexpression
GlucIIα T.b. overexpression
240
Δ ALG3 clone1
ALG6 overexpression
GlucIIα Y.l. overexpression
700
Δ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

FIG. 29

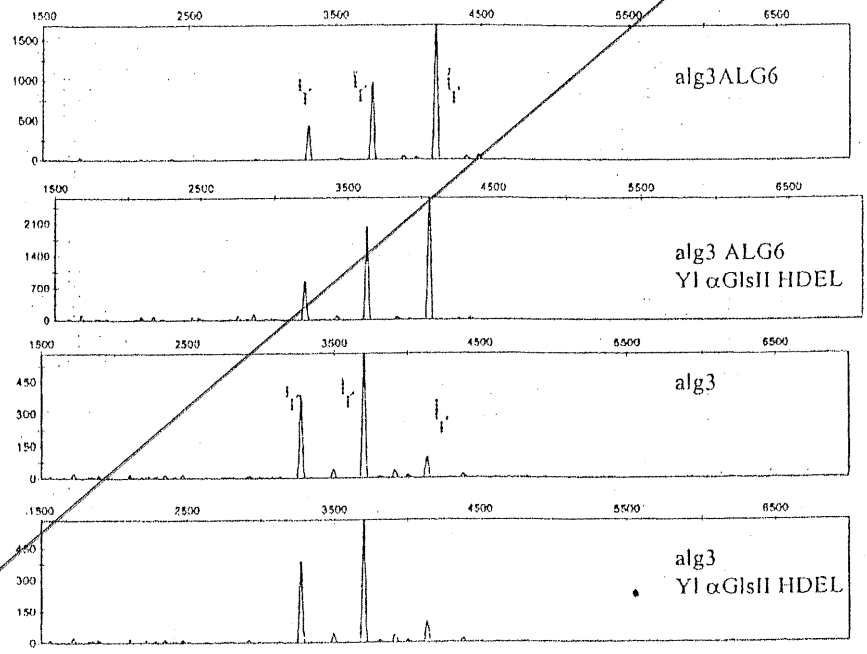
1500
2500
3500
4500
5500
6500
alg3ALG6
alg3 ALG6
Yl αGlsII HDEL
alg3
alg3
Yl αGlsII HDEL

FIG. 30

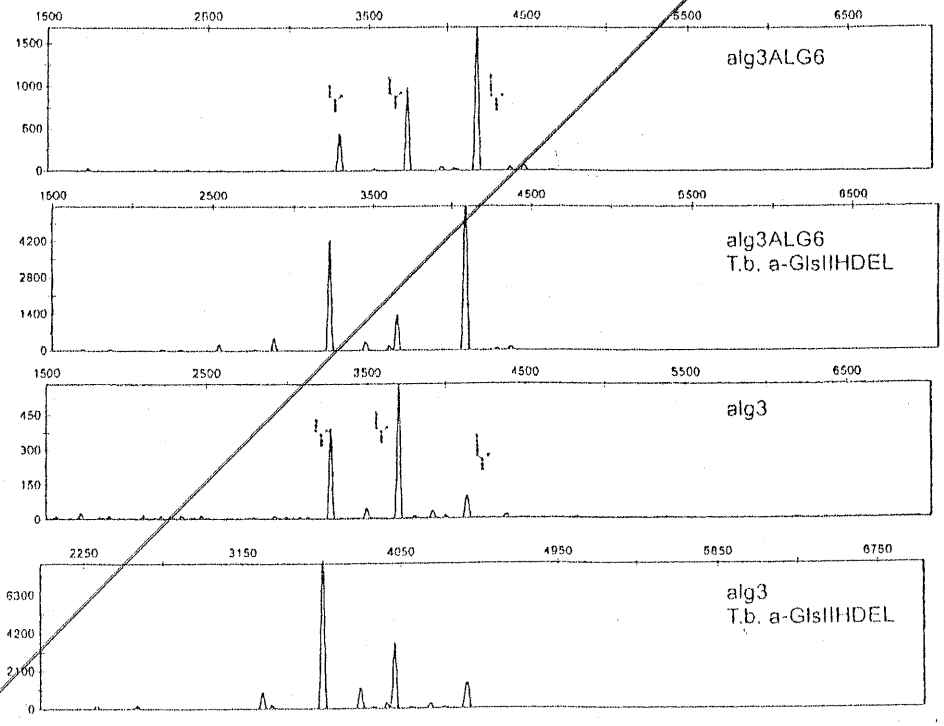
1500
2500
3500
4500
5500
6500
1500
1000
600
0
alg3ALG6
1500
2500
3500
4500
5500
6500
4200
2800
1400
0
alg3ALG6
T.b. a-GlsIIHDEL
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
2250
3150
4050
4950
5850
6750
6300
4200
2100
0
alg3
T.b. a-GlsIIHDEL

FIG. 31

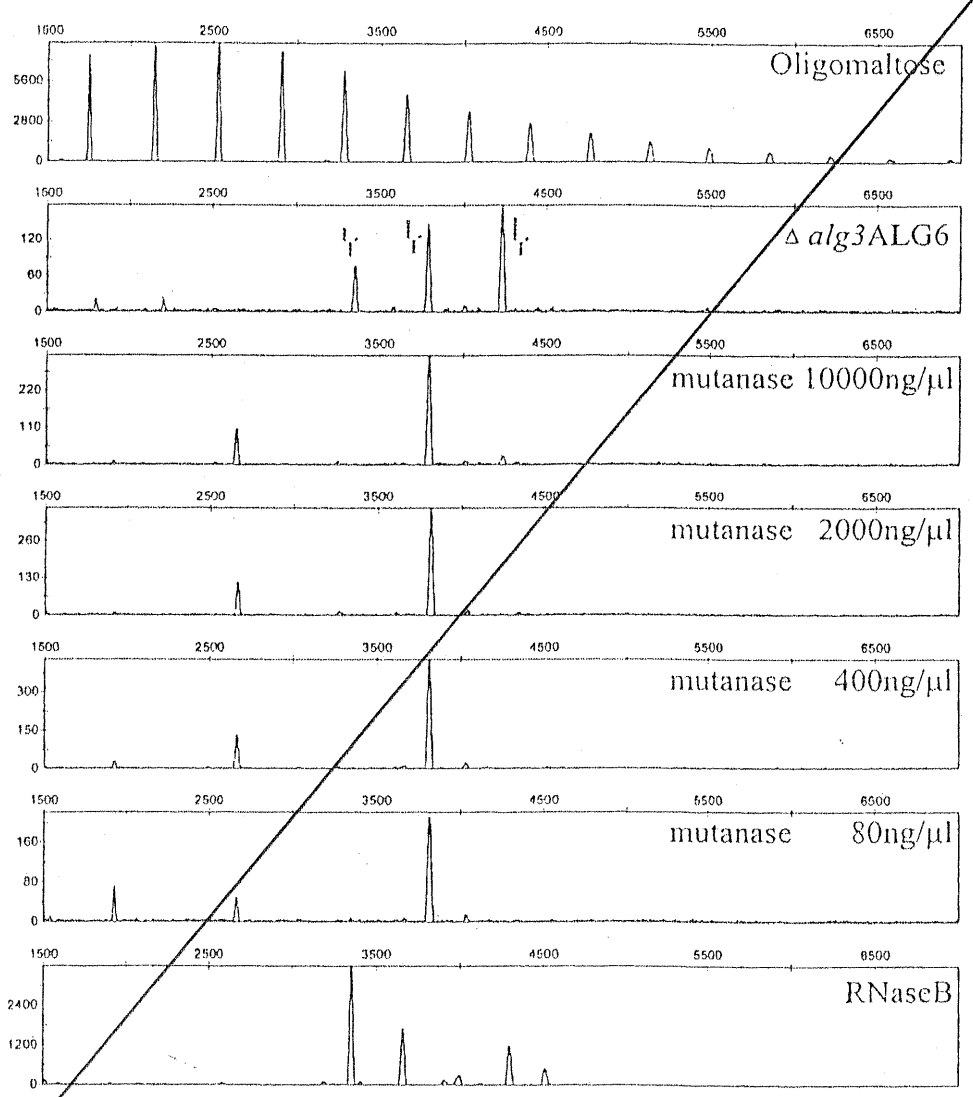
1500
2500
3500
4500
5500
6500
Oligomaltose
5600
2800
0
Δ alg3ALG6
120
60
mutanase 10000ng/μl
220
110
mutanase 2000ng/μl
260
130
mutanase 400ng/μl
300
150
mutanase 80ng/μl
160
80
RNaseB
2400
1200

FIG. 32

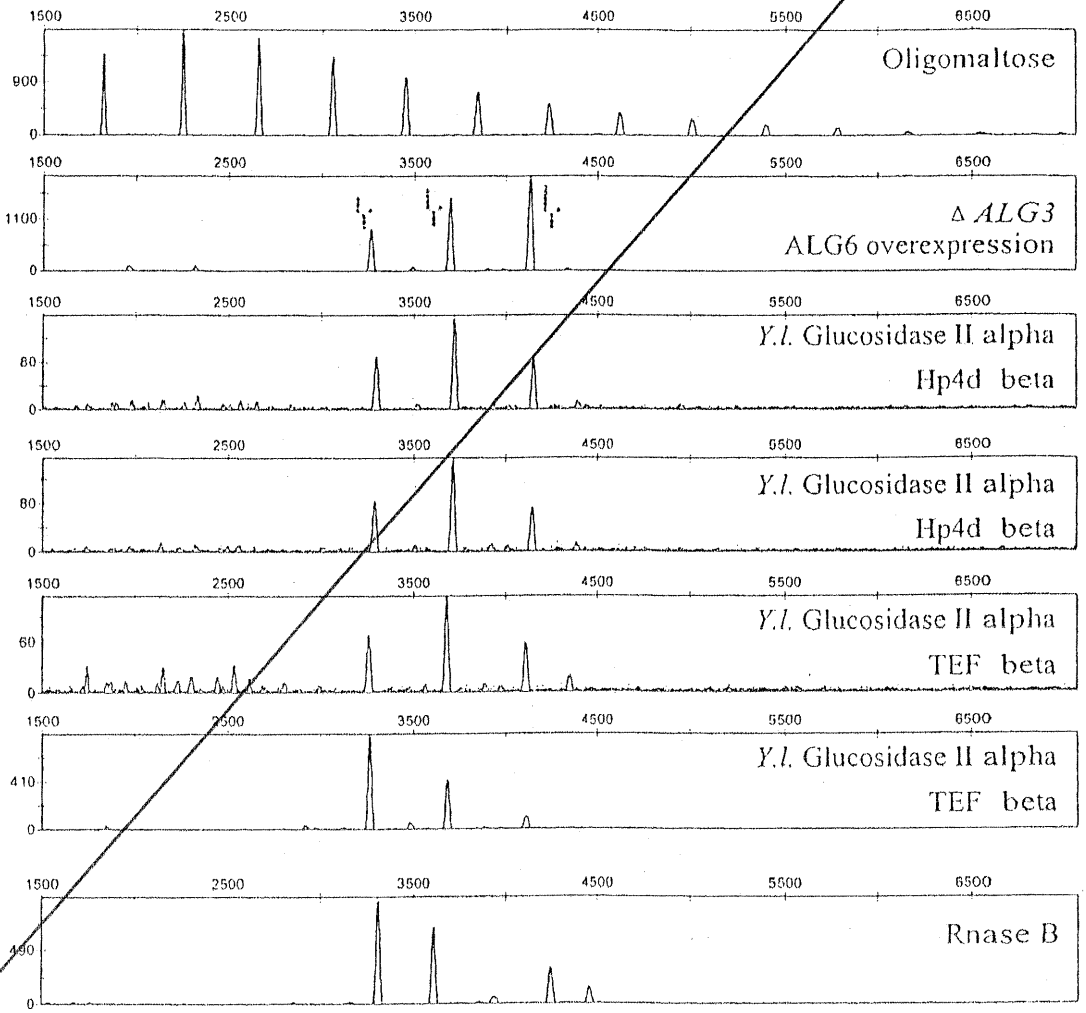
1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
1100
Δ ALG3
ALG6 overexpression
80
Y.l. Glucosidase II alpha
Hp4d beta
60
TEF beta
410
490
Rnase B

FIG. 33

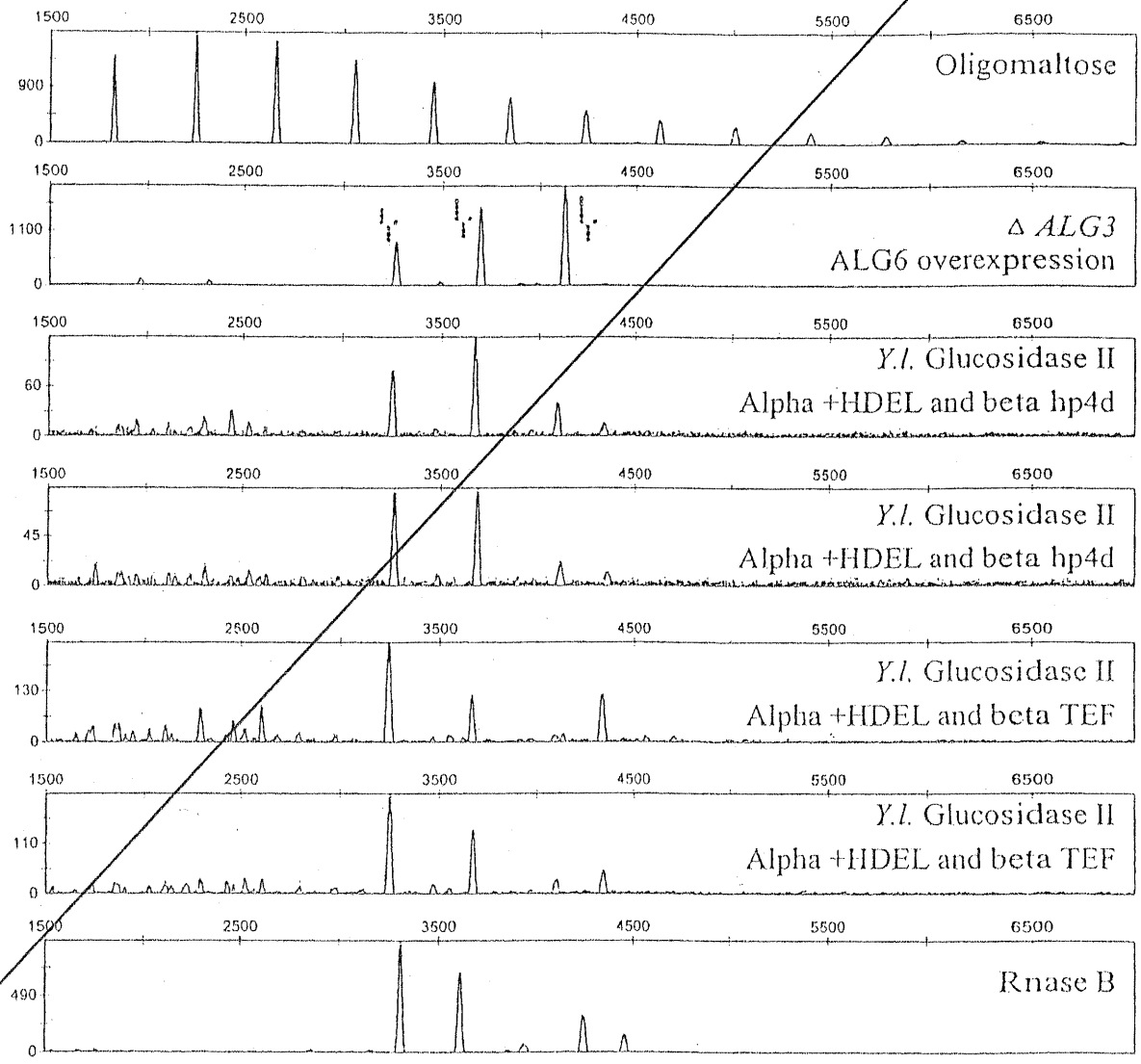
1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
1100
Δ ALG3
ALG6 overexpression
60
Y.l. Glucosidase II
Alpha +HDEL and beta hp4d
45
130
Y.l. Glucosidase II
Alpha +HDEL and beta TEF
110
490
Rnase B

FIG. 34

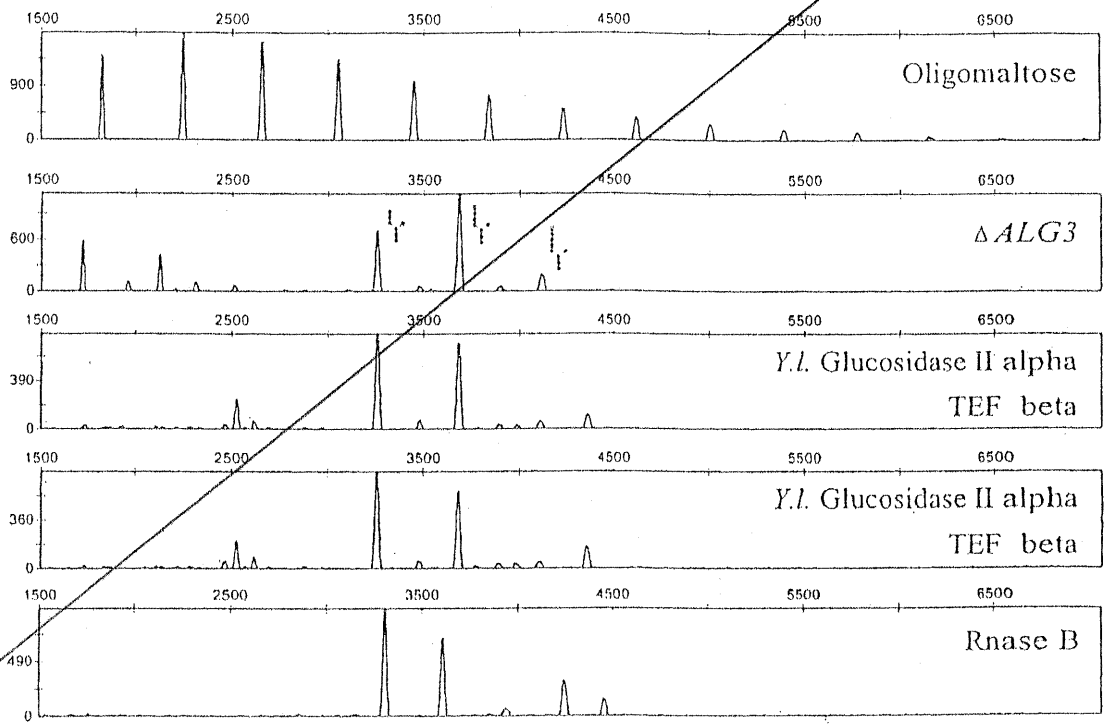
1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
600
ΔALG3
390
Y.l. Glucosidase II alpha
TEF beta
360
Y.l. Glucosidase II alpha
TEF beta
490
Rnase B

FIG. 35

gtaaagcacgagaacttcaagacctgttctcagtctggcttctgtaagcgaaaccgagccttcgccgacgacgccgctgcccagggctcttc
ttgggcctctccctacgagctggactcttcttctatccagttcaaggacggccagctgcacggcaccatcctgaagtctgtgtctcccaacga
gaaggtgaagctgcccctggtggtgtctttcctggagtctggcgccgctcgagtggtggtggacgaggagaagcgaatgaacggcgacat
ccagctgcgacacgactctaaggcccgaaaggagcgatacaacgaggccgagaagtgggtgctggtcggcggcctggagctgtctaag
accgccaccctgcgacccgagaccgagtctggcttcacccgagtgctgtacggccccgacaaccagttcgaggccgtgatccgacacgc
ccccttctctgccgacttcaagcgagatggccagacccacgtgcagctgaacaacaagggctacctgaacatggagcactggcgaccca
aggtggaggtggagggcgagggtgagcagcagacccaggaggacgagtctacctggtgggacgagtctttcggcggcaacaccgaca
ccaagccccgaggccccgagtctgtgggcctggacatcaccttccccggctacaagcacgtgttcggcatccccgagcacgccgactcc
ctgtctctgaaggagaccccgaggcggcgagggcaaccacgaggagccctaccgaatgtacaacgccgacgtgttcgagtacgagctgtc
ctctcccatgaccctgtacggcgccatccccttcatgcaggcccaccgaaaggactctaccgtgggcgtgttctggctgaacgccgccgag
acctgggtggacatcgtgaagtctacctcttctcccaacccccctggccctgggcgtgggagccaccaccgacacccagtctcactggttctc
tgagtctggccagctggacgtgttcgtgttcctgggccccaccccccaggagatttctaagacctacggcgagctgaccggctacacccag
ctgccccagcacttcgccattgcctaccaccagtgtcgatggaactacatcaccgacgaggacgtgaaggaggtggaccgaaacttcgac
aagtaccagatcccctacgacgtgatctggctggacatcgagtacaccgacgaccgaaagtacttcacctgggacccccctgtctttccccga
ccccatctctatggaggagcagctggacgagtctgagcgaaagctggtcgtgatcatcgacccccacatcaagaaccaggacaagtactc
tatcgtgcaggagatgaagtctaaggacctggccaccaagaacaaggacggcgagatttacgacggctggtgttggcccggctcttctcac
tggatcgacaccttcaaccccgctgccatcaagtggtgggtgtctctgttcaagttcgacaagttcaagggcaccctgtctaacgtgttcatct
ggaacgacatgaacgagccctctgtgttcaacggccccgagaccaccatgcccaaggacaacctgcaccacggcaactgggagcaccg
agacatccacaacgtgcacggcatcaccctggtgaacgccacctacgacgccctgctggagcgaaagaagggcgagatccgacgaccc
ttcatcctgacccgatcttactacgccggtgcccagcgaatgtctgccatgtggaccggcgacaaccaggccacctgggagcacctggcc
gcctctatccccatggtgctgaacaacggaatcgccggcttccccttcgccggtgccgacgtgggcggcttcttccagaaccccctctaagg
agctgctgacccgatggtatcaggccggcatctggtatcctttcttccgagcccacgcccacatcgacacccgacgacgagagccctacct
gatcgccgagccccaccgatctatcatctctcaggccatccgactgcgataccagctgctgcccgcctggtacaccgccttccacgaggcc
tctgtgaacggcatgcccatcgtgcgaccccagtactacgcccacccctgggacgaggccggcttcgccatcgacgaccagctgtacctg
ggctctaccggcctgctggccaagcccgtggtgtctgaggaggccaccaccgccgacatctacctggctgacgacgagaagtactacga

FIG. 36A

ctacttcgactacaccgtgtaccagggcgctggcaagcgacacaccgtgcccgctcccatggagaccgtgcccctgctgatgcagggcg
gccacgtgatcccccgaaaggaccgaccccgacgatcttctgccctgatgcgatgggaccctacaccctggtggtggtgctggacaaga
acggccaggccgacggctctctgtacgtggacgacggcgagaccttcgactacgagcgaggcgcctacatccatcgacgattccgattcc
aggagtctgccctggtctccgaggacgtgggcaccaagggccccaagaccgccgagtacctcaagaccatggccaacgtgcgagtgga
gcgagtggtggtcgtggacccccccaaggagtggcagggcaagacctctgtgaccgtgatcgaggacggcgcctctgccgcctctaccg
cctccatgcagtaccactctcagcccgacggcaaggccgcctacgccgtggtgaagaaccccaacgtgggcatcggcaagacctggcg
aatcgagttctaatagcctag

FIG. 36B

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

FIG. 37

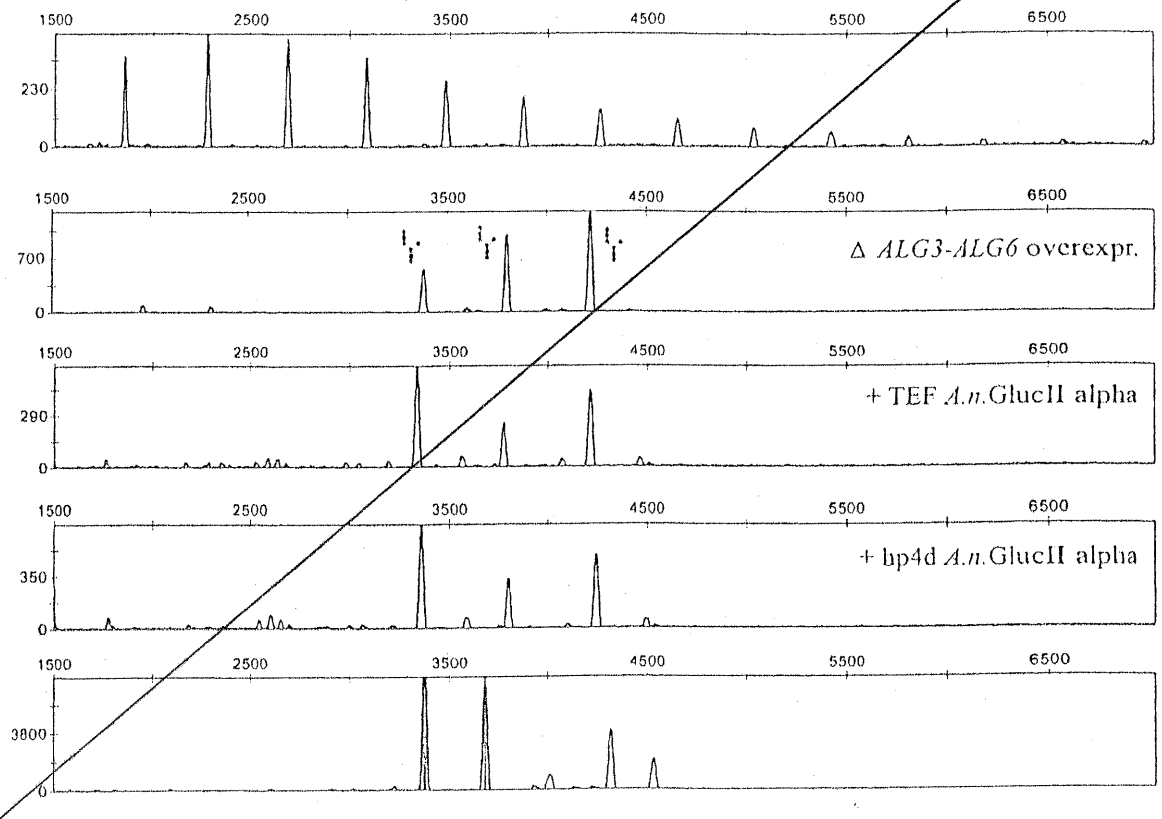
1500
2500
3500
4500
5500
6500
230
0
700
Δ ALG3-ALG6 overexpr.
290
+ TEF A.n.GlucII alpha
350
+ hp4d A.n.GlucII alpha
3800

FIG. 38

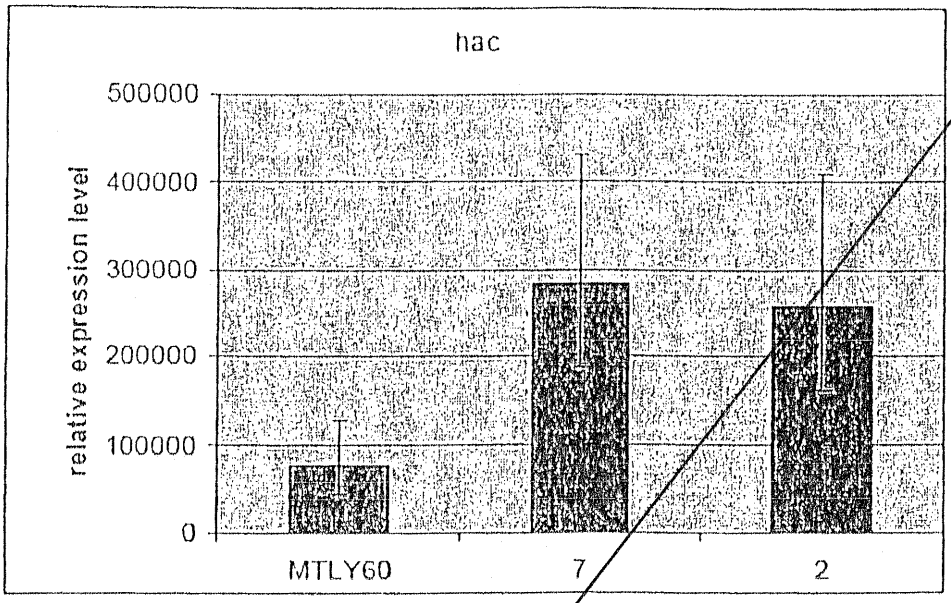

FIG. 39A

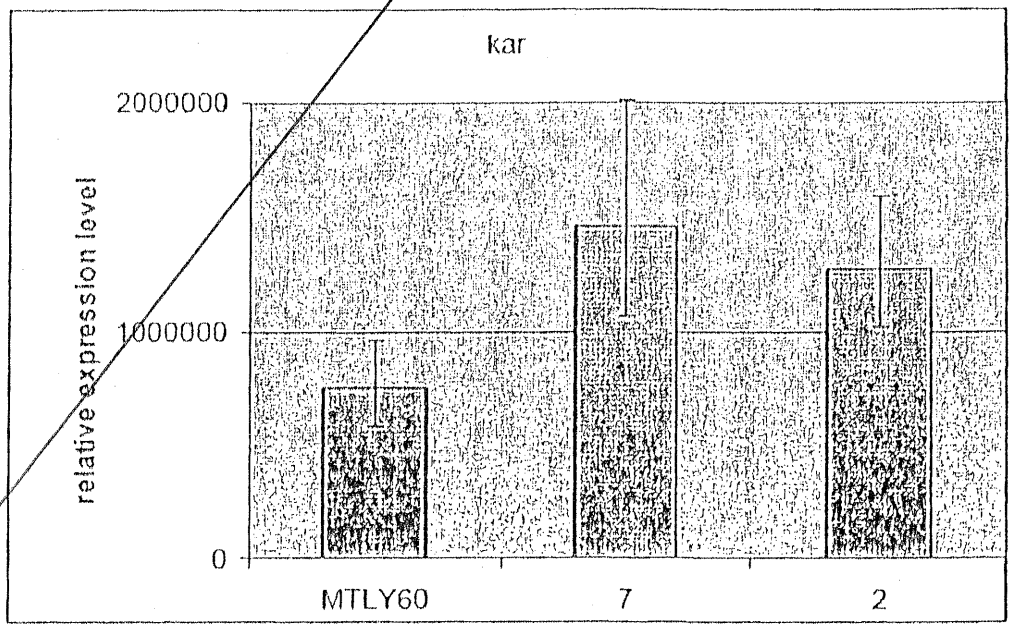

FIG. 39B

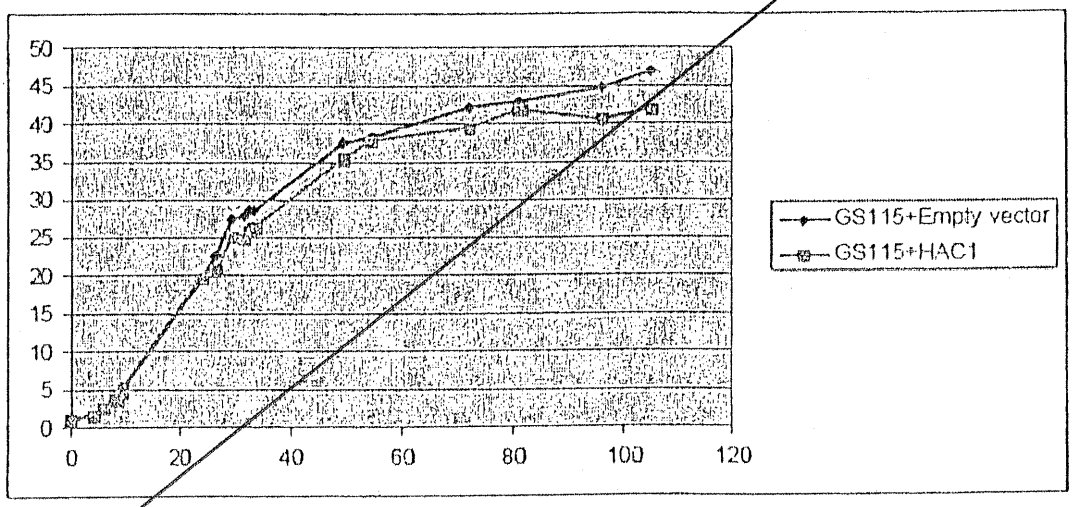
50
45
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
GS115+Empty vector
GS115+HAC1

FIG. 40

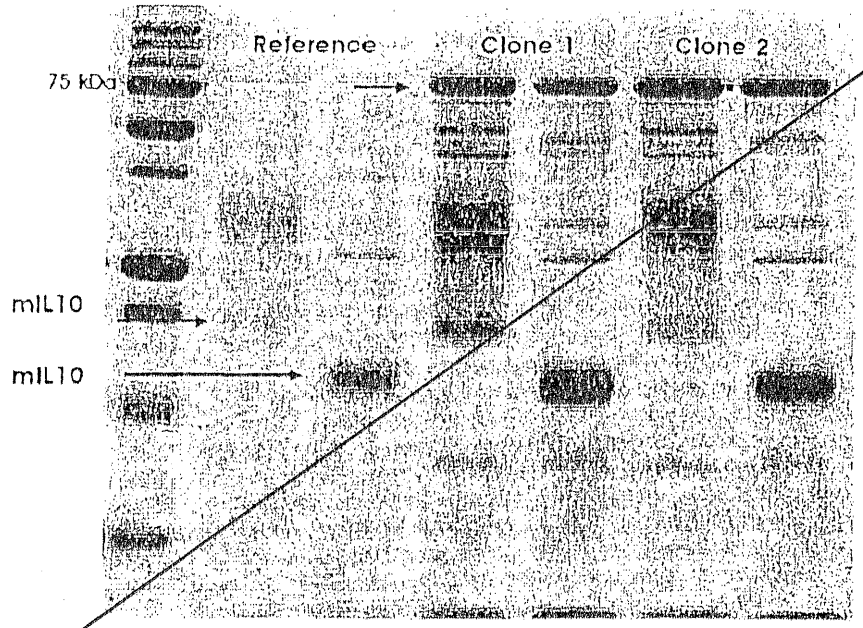

FIG. 41

gagctcagcgctaccaagcgaggctctcccaaccccaccgagccgccgctgtgaaggccgccttccagacctcttggaacgcctacca
ccacttcgccttcccccacgacgacctgcaccccgtgtctaactcgttcgacgacgagcgaaacggctgggggctcttctgccatcgacggc
ctggacaccgccatcctgatgggcgacgccgacatcgtgaacaccatcctgcagtacgtgccccagatcaacttcaccaccaccgccgtg
gccaaccagggcatctctgtgttcgagaccaacatccgatacctgggcggcctgctgtctgcctacgacctgctgcgaggcccctctcttct
ctggccaccaaccagaccctggtgaactctctgctgcgacaggcccagaccctggccaacggcctgaaggtggcttttaccaccccctctg
gcgtgccccgaccccaccgtgttcttcaaccccaccgtgcgacgatctggcgcctcttctaacaacgtggccgagatcggctctctgggtgctg
gagtggaccccgactgtctgacctgaccggcaacccccagtacgcccagctggcccagaagggcgagtcttacctgctgaaccccaagggc
ctctccccgaggcctggccccggactgatcggcaccttcgtgtctacctctaacggcaccttccaggactcttccggctcttggtctggcctgat
ggactctttctacgagtacctgatcaagatgtacctgtacgaccccgtggccttcgcccactacaaggaccgatgggtgctggccgccgact
ctaccatcgcccacctggcctctcaccctctaccccgaaaggacctgaccttcctgtcctcttacaacggccagtctacctctccccaactctgg
acacctggcttccttcgccggtggcaacttcatcctgggcggcatcctgctgaacgagcagaagtacatcgacttcggcatcaagctggcct
cttcctacttcgccacctacaaccagaccgcctctggcatcggccccgagggcttcgcctgggtggactctgtgaccggcgctggcggctc
tccccccctcttctcagtctggcttctactcttctgccggcttctgggtgaccgcccccctactacatcctgcgacccgagaccctggagtctctgt
actacgcctaccgagtgaccggcgactctaagtggcaggacctggcctgggaggccttctctgccatcgaggacgcctgtcgagccggct
ctgcctactcttctatcaacgacgtgacccaggccaacggtggcggagcctctgacgacatggagtctttctggttcgccgaggccctgaag
tacgcctacctgatcttcgccgaggaatctgacgtgcaggtgcaggccaacggcggcaacaagttcgtgttcaacaccgaggcccacccc
ttctctatccgatcttcttctcgacgaggcggccacctggcccatgacgagctgtaatagcctaggtacc

FIG. 42

cggcggactgcgtccgaaccagctccagcagcgtttttccgggccattgagccgactgcgaccccgccaacgtgtcttggcccacgcact
catgtcatgttggtgttgggaggccacttttaagtagcacaaggcacctagctcgcagcaaggtgtccgaaccaaagaagcggctgcagt
ggtgcaaacggggcggaaacggcgggaaaaagccacggggcacgaattgaggcacgccctcgaatttgagacgagtcacggcccc
attcgcccgcgcaatggctcgccaacgcccggtcttttgcaccacatcaggttaccccaagccaaacctttgtgttaaaaagcttaacatatta
taccgaacgtaggtttgggcgggcttgctccgtctgtccaaggcaacatttatataagggtctgcatcgccggctcaattgaatcttttttcttctt
ctcttctctatattcattcttgaattaaacacacatcaaca

FIG. 43

cttatctggggcagtgaagtatatgttatggtaatagttacgagttagttgaacttatagatagactggactatacggctatcggtccaaattaga
aagaacgtcaatggctctctgggcggaattcgtataacttcgtatagcaggagttatccgaagcgataattacccctgttatcccctagcttatcga
tacgcgtgcatgctgaggtgtctcacaagtgccgtgcagtcccgccccccacttgcttctctttgtgtgtagtgtacgtacattatcgagaccgtt
gttcccgcccacctcgatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgccccccacttgcttctctttgtgtgtagtgtacgtac
attatcgagaccgttgttcccgcccacctcgatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgccccccacttgcttctctttgt
gtgtagtgtacgtacattatcgagaccgttgttcccgcccacctcgatccggcatgctgaggtgtctcacaagtgccgtgcagtcccgccccc
acttgcttctctttgtgtgtagtgtacgtacattatcgagaccgttgttcccgcccacctcgatccggcatgcactgatcacgggcaaaagtgcg
tatatatacaagagcgtttgccagccacagattttcactccacacaccacatcacacatacaaccacacacatccacgtgggaacccgaaac
taaggatccatgaagctttccaccatcctcttcacagcctgcgctaccctggccgctaccaagcgaggctctcccaaccccacccgagccg
ccgctgtgaaggccgccttccagacctcttggaacgcctaccaccacttcgccttcccccacgacgacctgcaccccgtgtctaactcgttc
gacgacgagcgaaacggctggggctcttctgccatcgacggcctggacaccgccatcctgatgggcgacgccgacatcgtgaacaccat
cctgcagtacgtgccccagatcaacttcaccaccaccgccgtggccaaccagggcatctctgtgttcgagaccaacatccgatacctgggc
ggcctgctgtctgcctacgacctgctgcgaggcccctttctttctctggccaccaaccagaccctggtgaactctctgctgcgacaggccca
gaccctggccaacggcctgaaggtggctttaccacccctctggcgtgcccgaccccaccgtgttcttcaaccccaccgtgcgacgatct
ggcgcctcttctaacaacgtggccgagatcggctctctggtgctggagtggacccgactgtctgacctgaccggcaaccccccagtacgcc
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FIG. 44A

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FIG. 44B

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FIG. 44C

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FIG. 45A

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FIG. 45B

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FIG. 45C

caaggggtaggcgaatggtacgattccgccaagtgagactggcgatcgggagaagggttggtggtcatgggggatagaatttgtacaagt
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FIG. 46A

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FIG. 46B

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FIG. 46C

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FIG. 47A

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FIG. 47B

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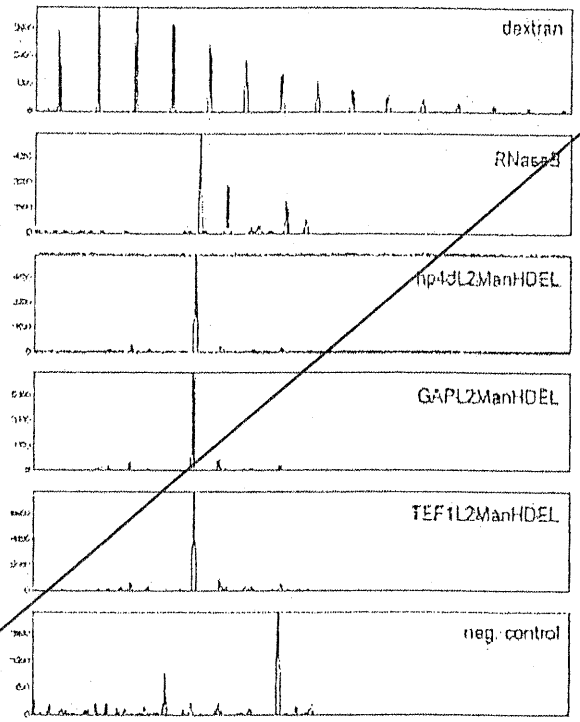
FIG. 47C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RNaseB
hp4dL2ManHDEL
GAPL2ManHDEL
TEF1L2ManHDEL
neg. control

FIG. 48

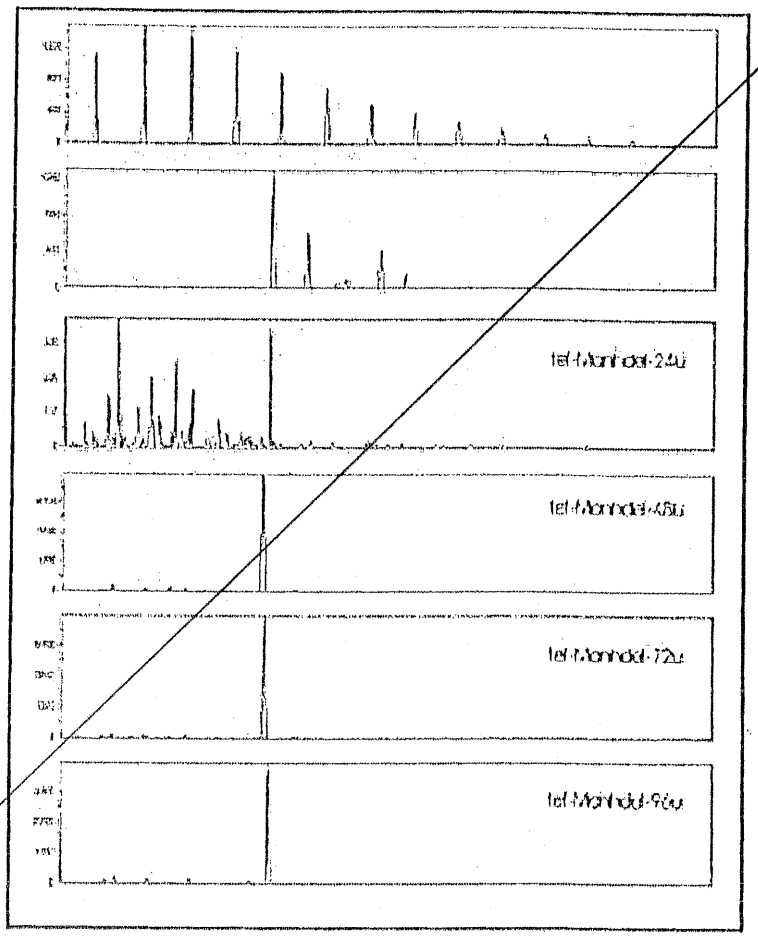

FIG. 49

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FIG. 50

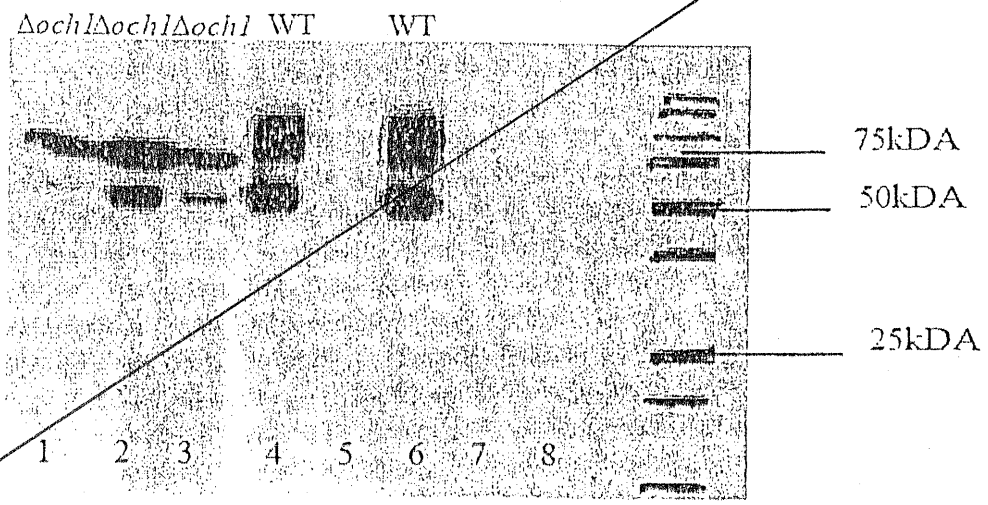

FIG. 51

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FIG. 52

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FIG. 53

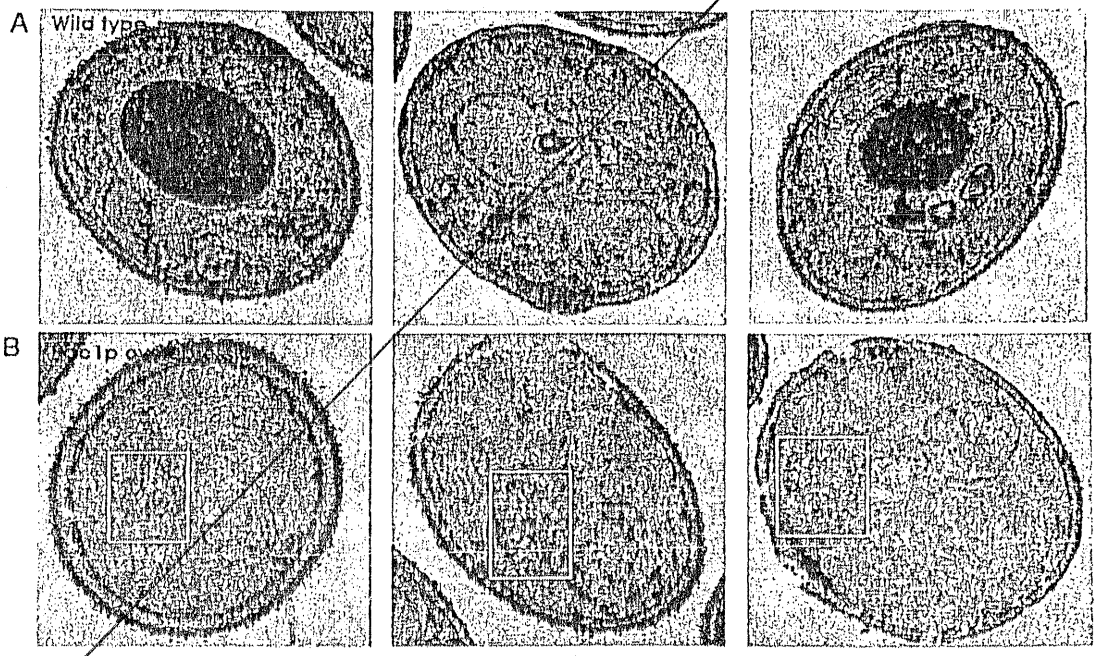


FIG. 54

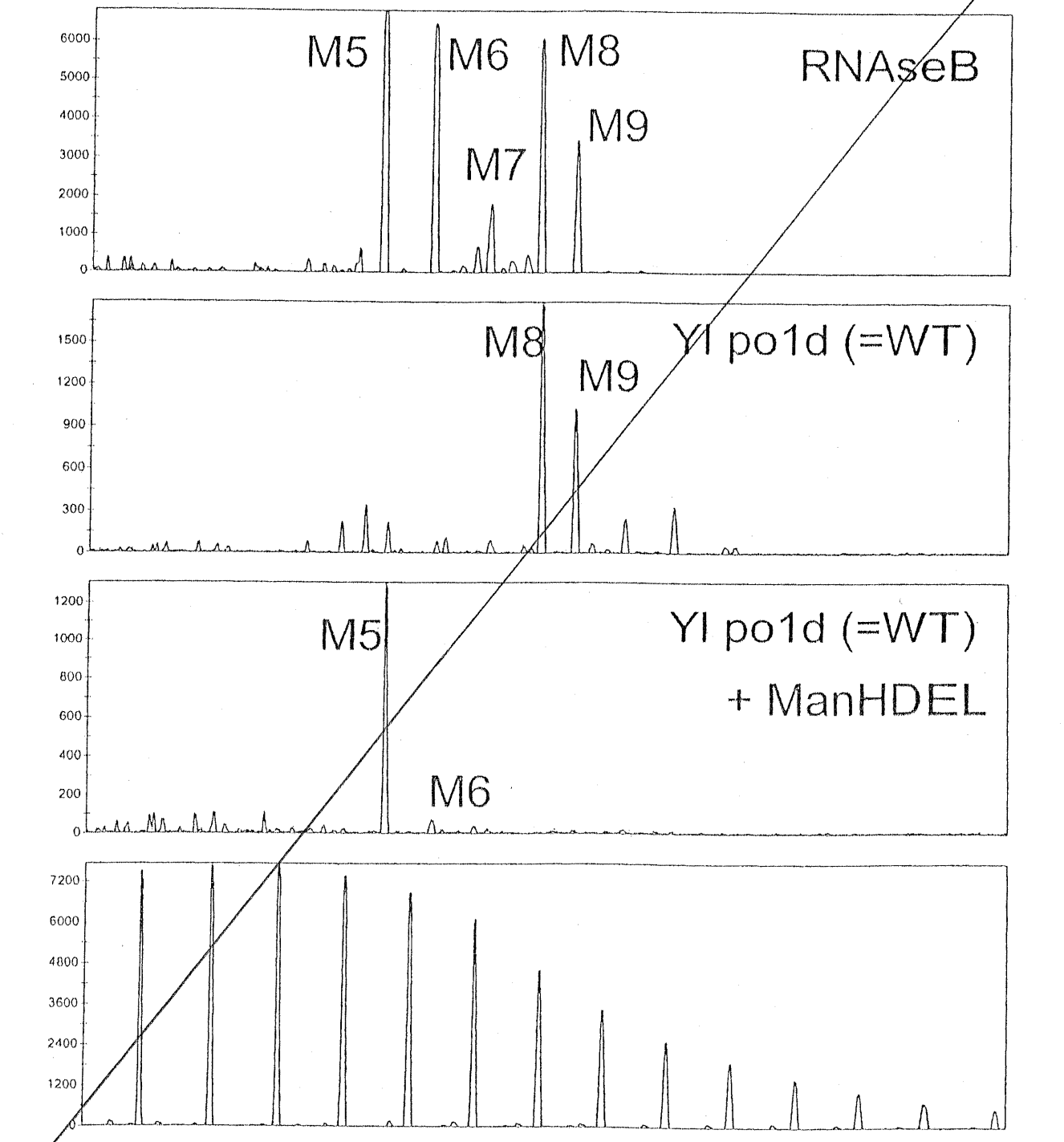
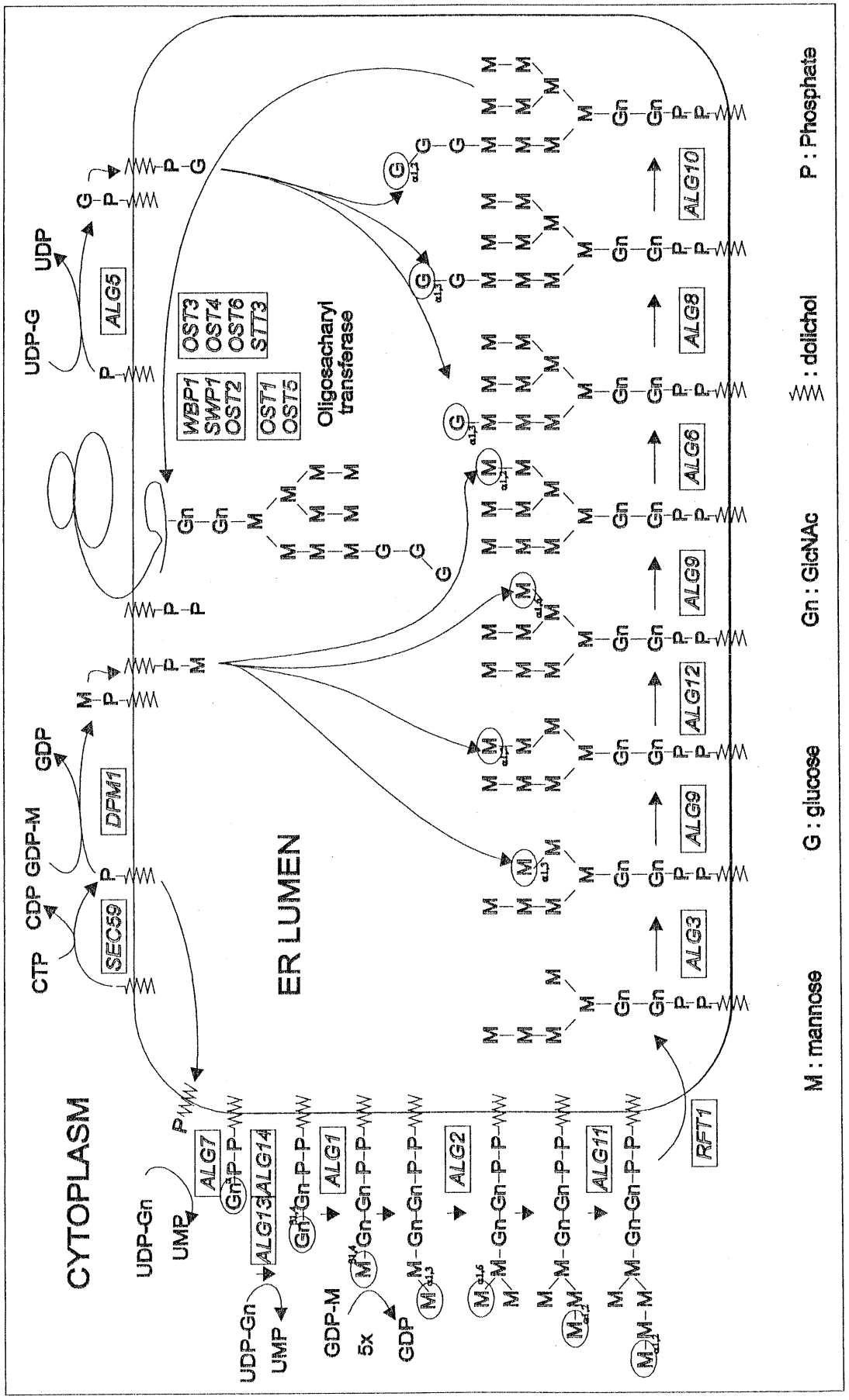


FIG. 55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ER LUMEN
UDP-Gn
UMP
GDP-M
5x
GDP
CTP
CDP
GDP-M
GDP
UDP-G
UDP
ALG7
ALG13/ALG14
ALG1
ALG2
ALG11
RFT1
SEC59
DPM1
ALG5
ALG3
ALG9
ALG12
ALG6
ALG8
ALG10
WBP1
SWP1
OST2
OST3
OST4
OST6
STT3
OST1
OST5
Oligosaccharyl transferase
M : mannose
G : glucose
Gn : GlcNAc
: dolichol
P : Phosphate

FIG. 1

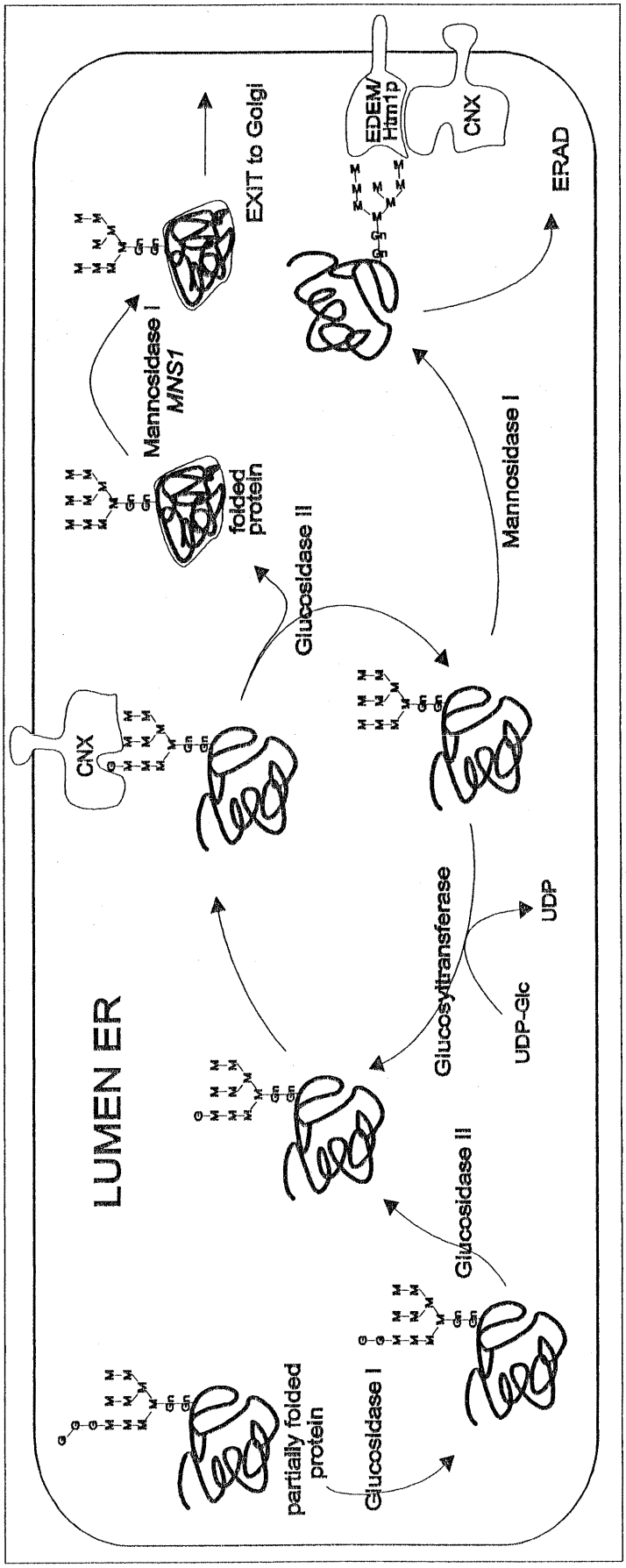

LUMEN ER
partially folded protein
Glucosidase I
Glucosidase II
Glucosyltransferase
UDP-Glc
UDP
folded protein
Mannosidase I
MNS1
EXIT to Golgi
Glucosidase II
CNX
EDEM/ Htm1p
CNX
Mannosidase I
ERAD

FIG. 2

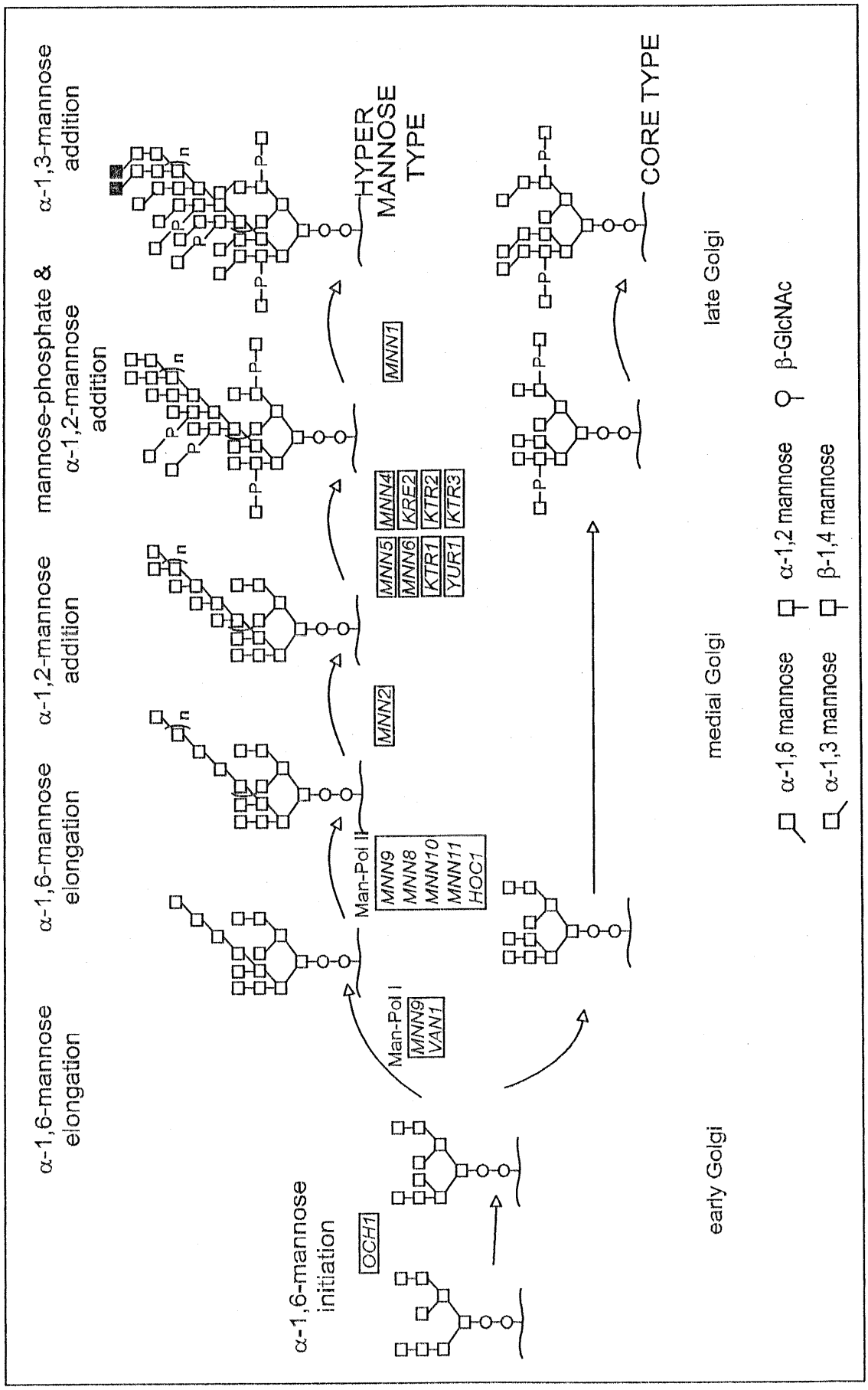
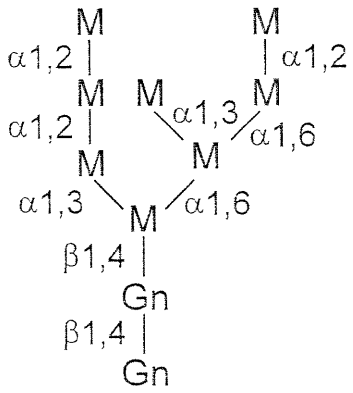
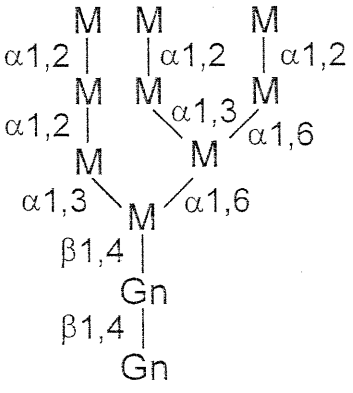

α-1,6-mannose elongation
α-1,6-mannose elongation
α-1,2-mannose addition
mannose-phosphate & α-1,2-mannose addition
α-1,3-mannose addition
α-1,6-mannose initiation
OCH1
Man-Pol I
MNN9
VAN1
Man-Pol II
MNN9
MNN8
MNN10
MNN11
HOC1
MNN2
MNN5
MNN6
KTR1
YUR1
MNN4
KRE2
KTR2
KTR3
MNN1
HYPER MANNOSE TYPE
CORE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

FIG. 3

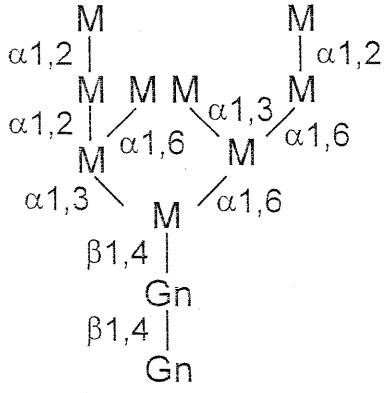


Structure Formule:

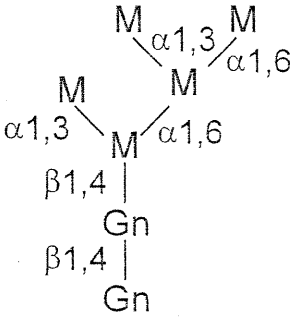
I



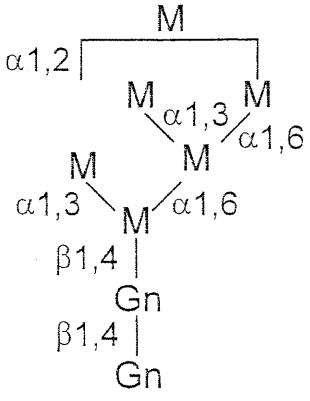
II



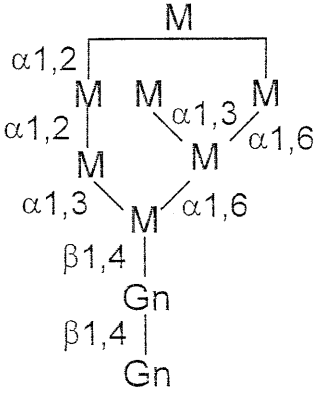
III



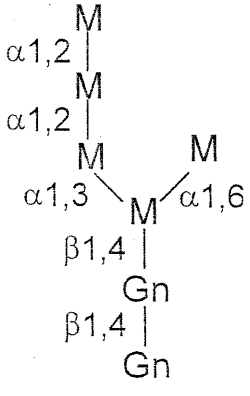
IV



V

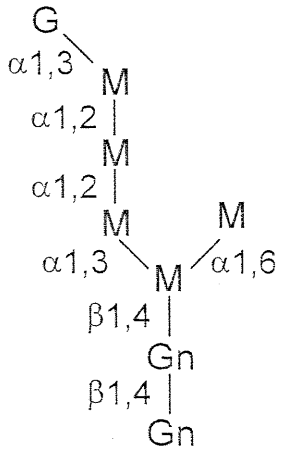


VI

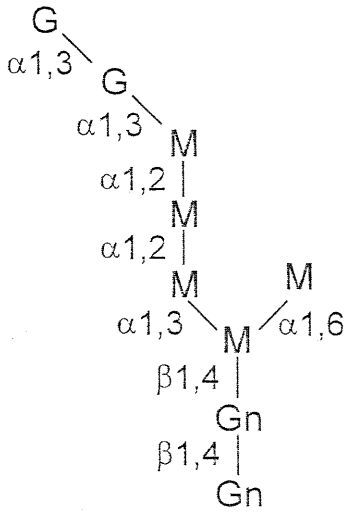


VII

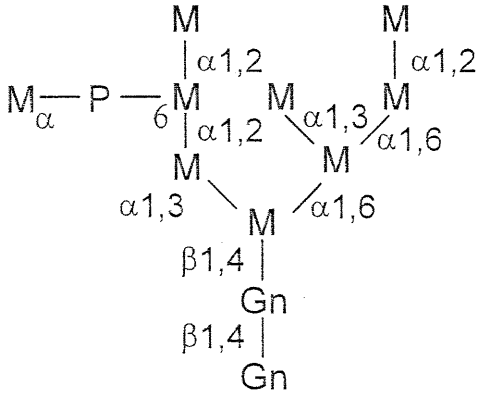
FIG.4



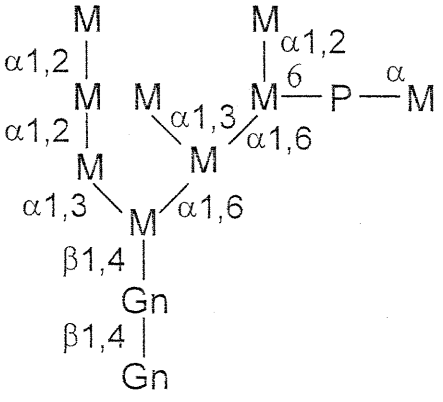
VIII



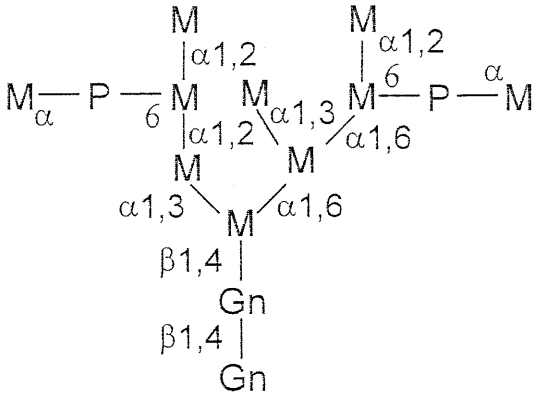
IX



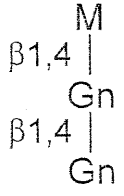
X



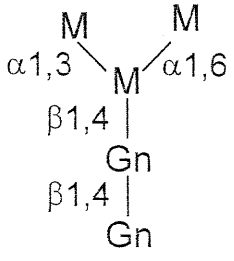
XI



XII



XIII



XIV

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

FIG.4(Continuous)

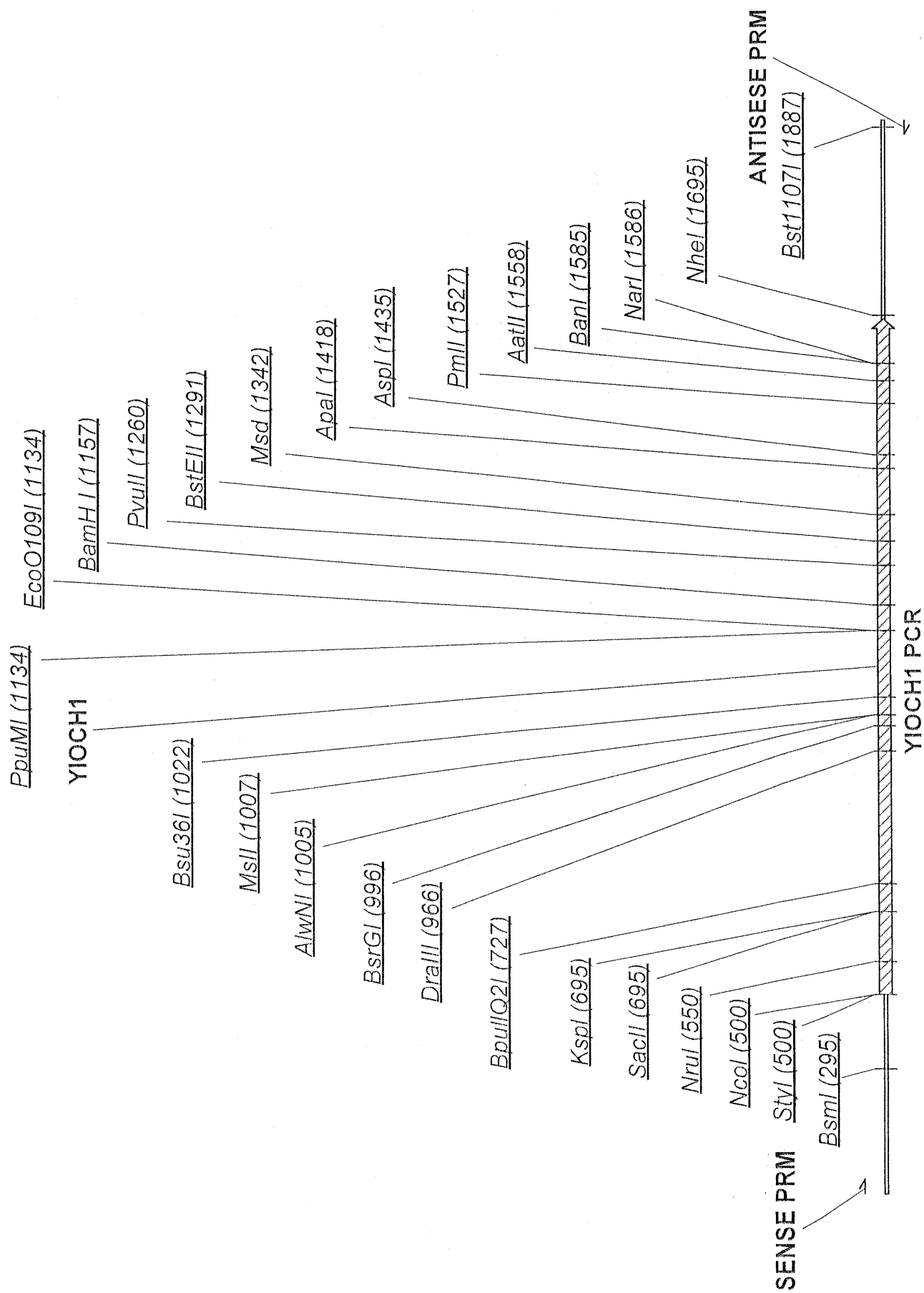

PpuMI (1134)
EcoO109I (1134)
BamH I (1157)
PvuII (1260)
BstEII (1291)
Msd (1342)
ApaI (1418)
AspI (1435)
PmlI (1527)
AatII (1558)
BanI (1585)
NarI (1586)
NheI (1695)
Bst1107I (1887)
ANTISESE PRM
YIOCH1
Bsu36I (1022)
MslI (1007)
AlwNI (1005)
BsrGI (996)
DraIII (966)
BpuIIQ2I (727)
KspI (695)
SacII (695)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

FIG.5

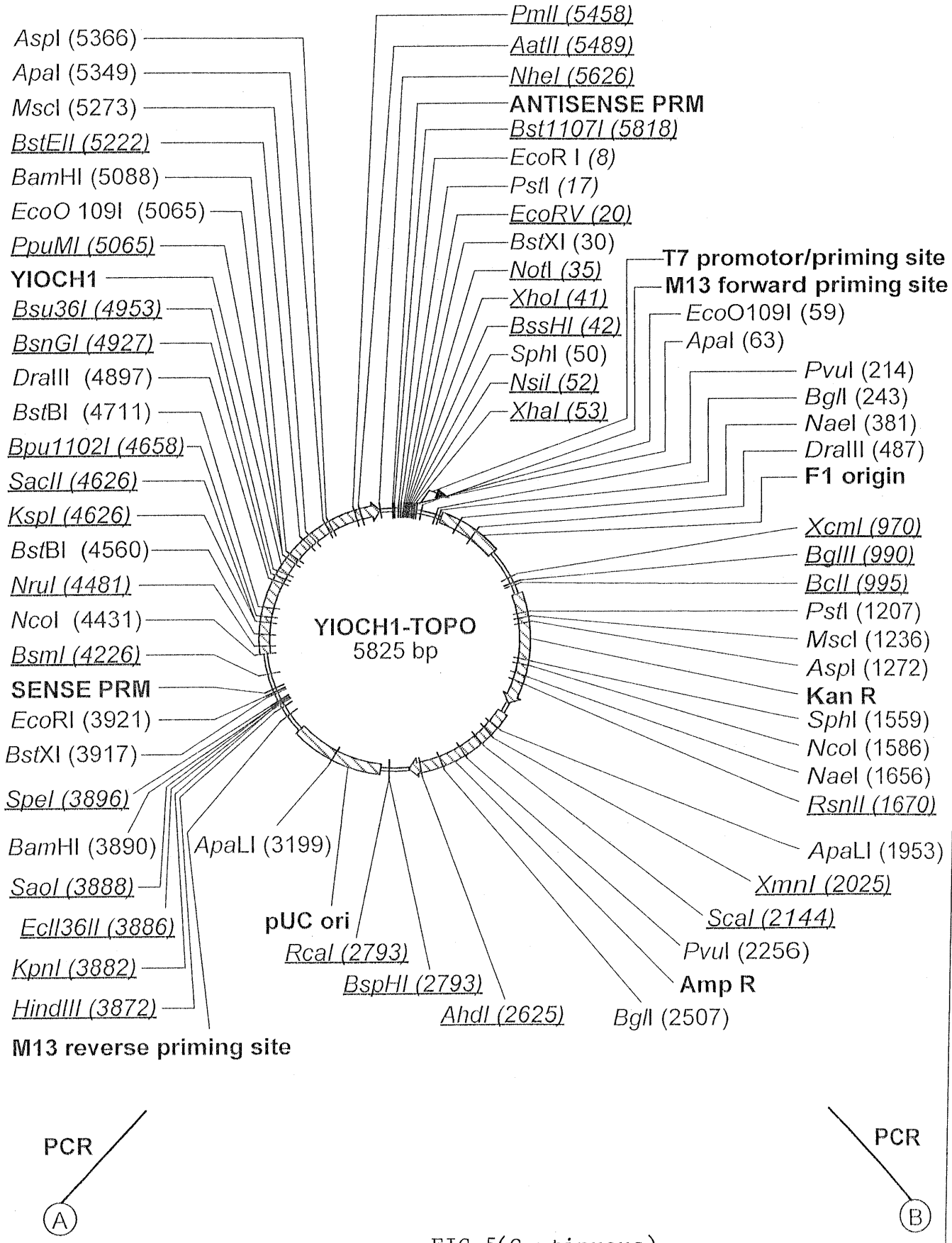


FIG.5(Continuous)

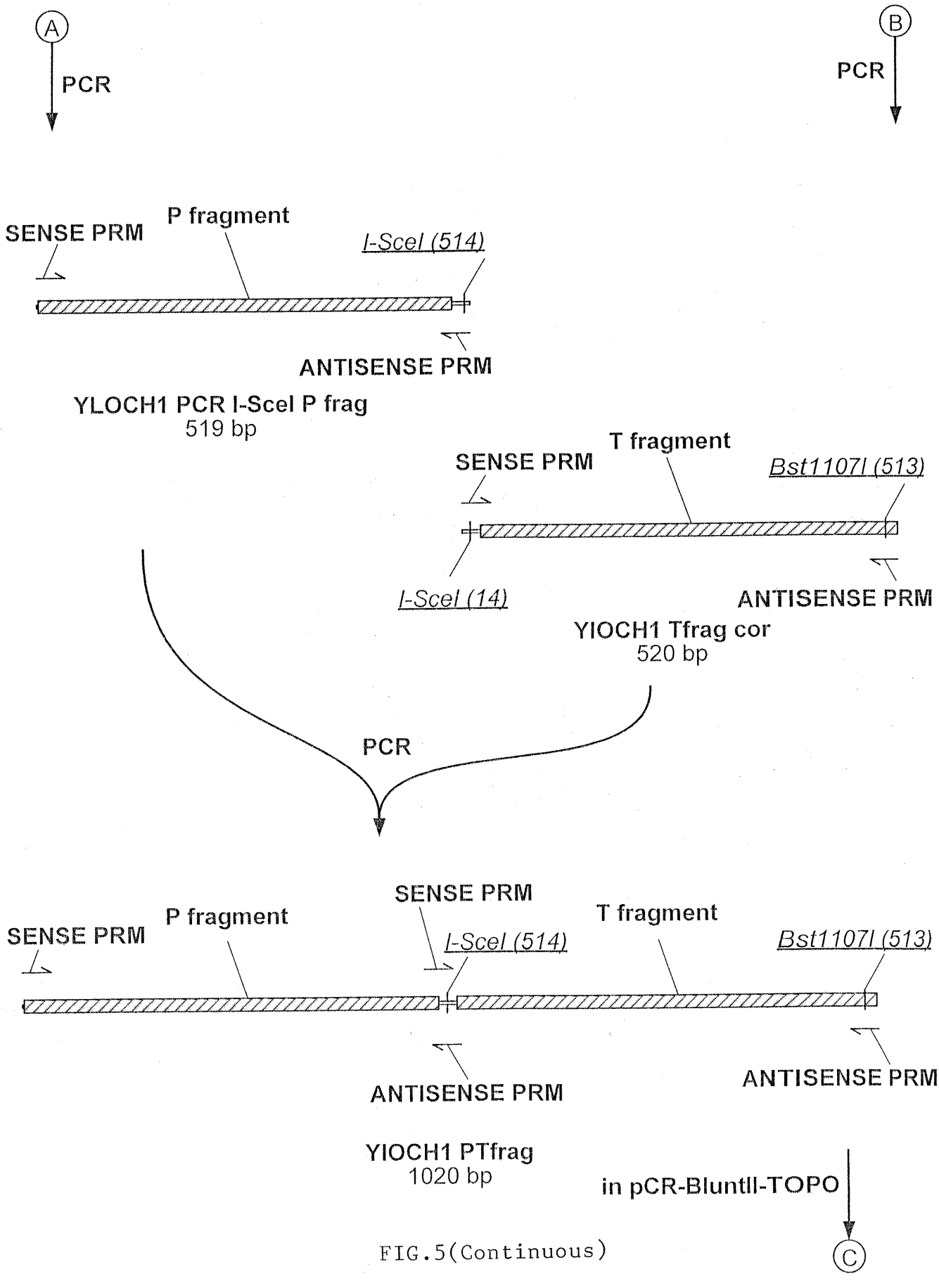


FIG.5(Continuous)

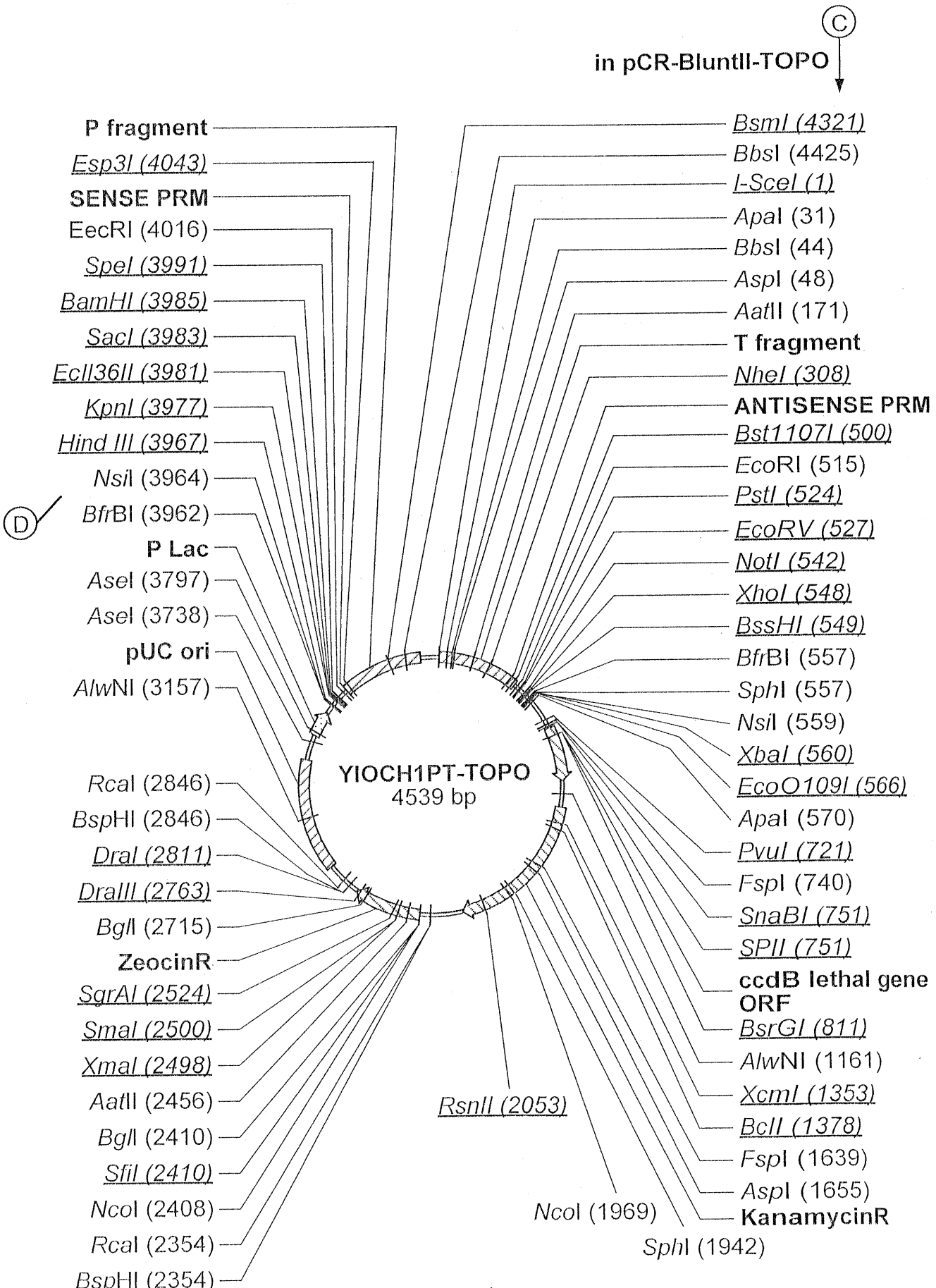


FIG.5(Continuous)

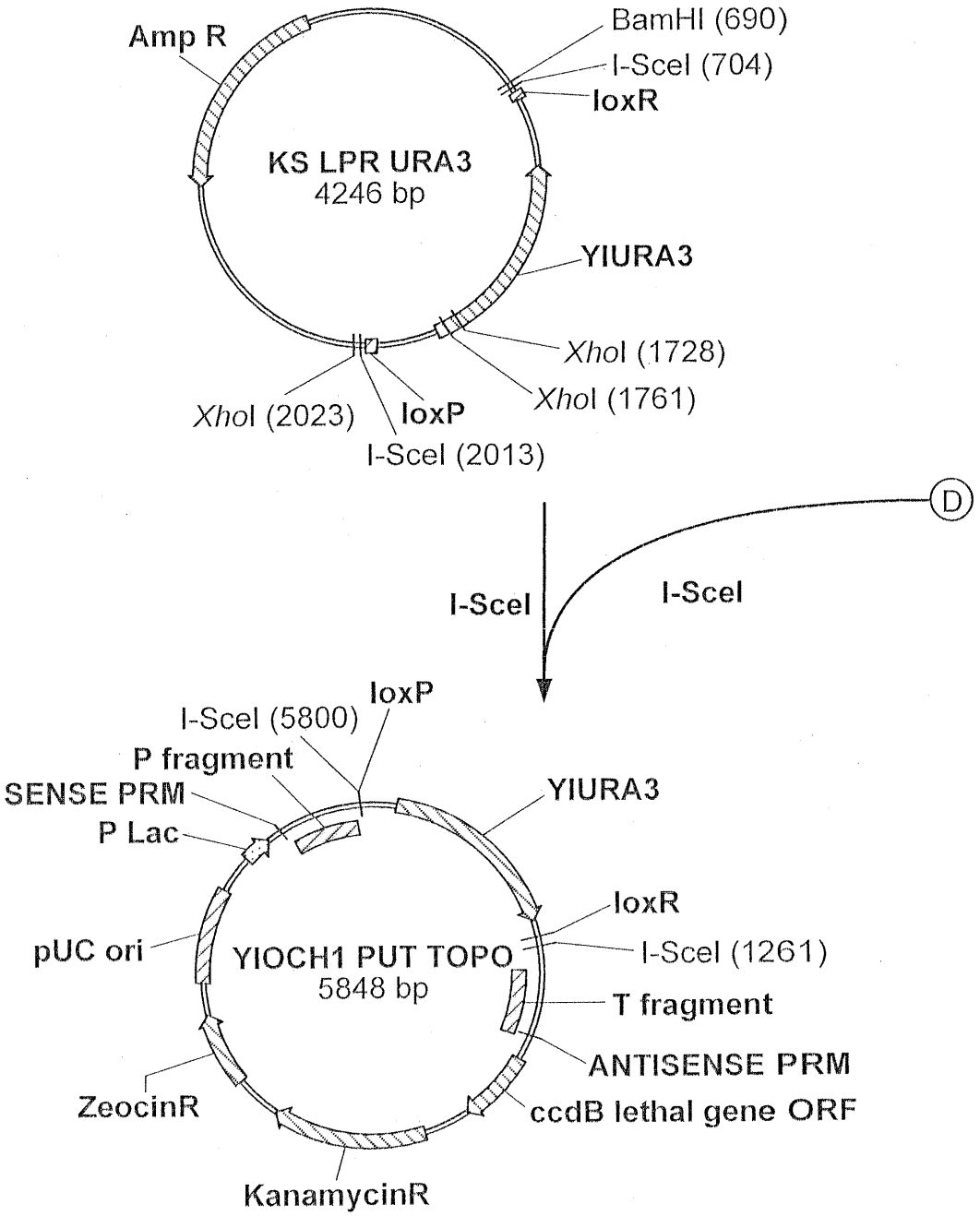


FIG.5(Continuous)

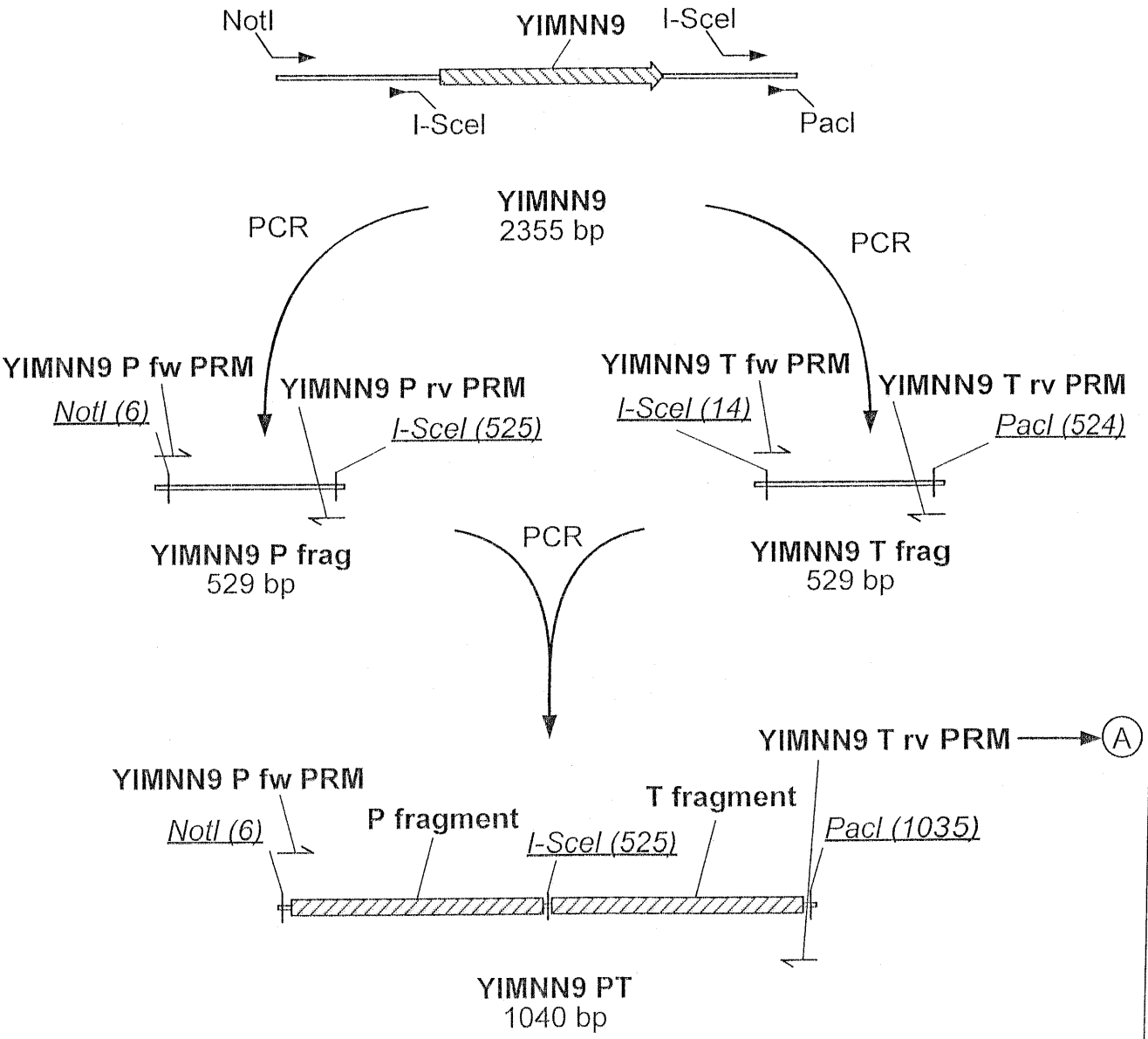

NotI
YIMNN9
I-SceI
I-SceI
PacI
YIMNN9
2355 bp
PCR
PCR
YIMNN9 P fw PRM
NotI (6)
YIMNN9 P rv PRM
I-SceI (525)
YIMNN9 T fw PRM
I-SceI (14)
YIMNN9 T rv PRM
PacI (524)
YIMNN9 P frag
529 bp
PCR
YIMNN9 T frag
529 bp
YIMNN9 T rv PRM
A
YIMNN9 P fw PRM
NotI (6)
P fragment
I-SceI (525)
T fragment
PacI (1035)
YIMNN9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

FIG.6

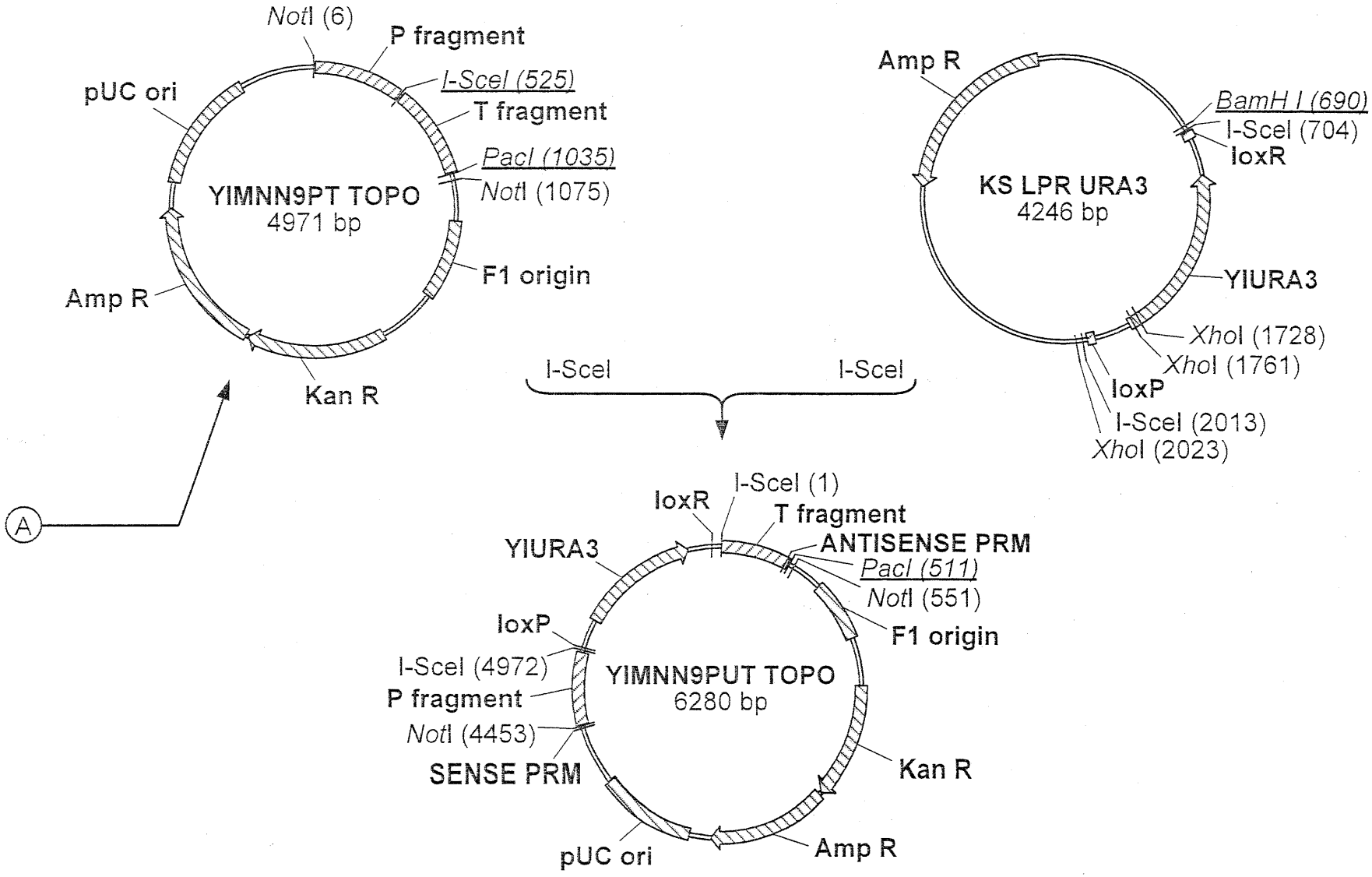


FIG.6(Continuous)

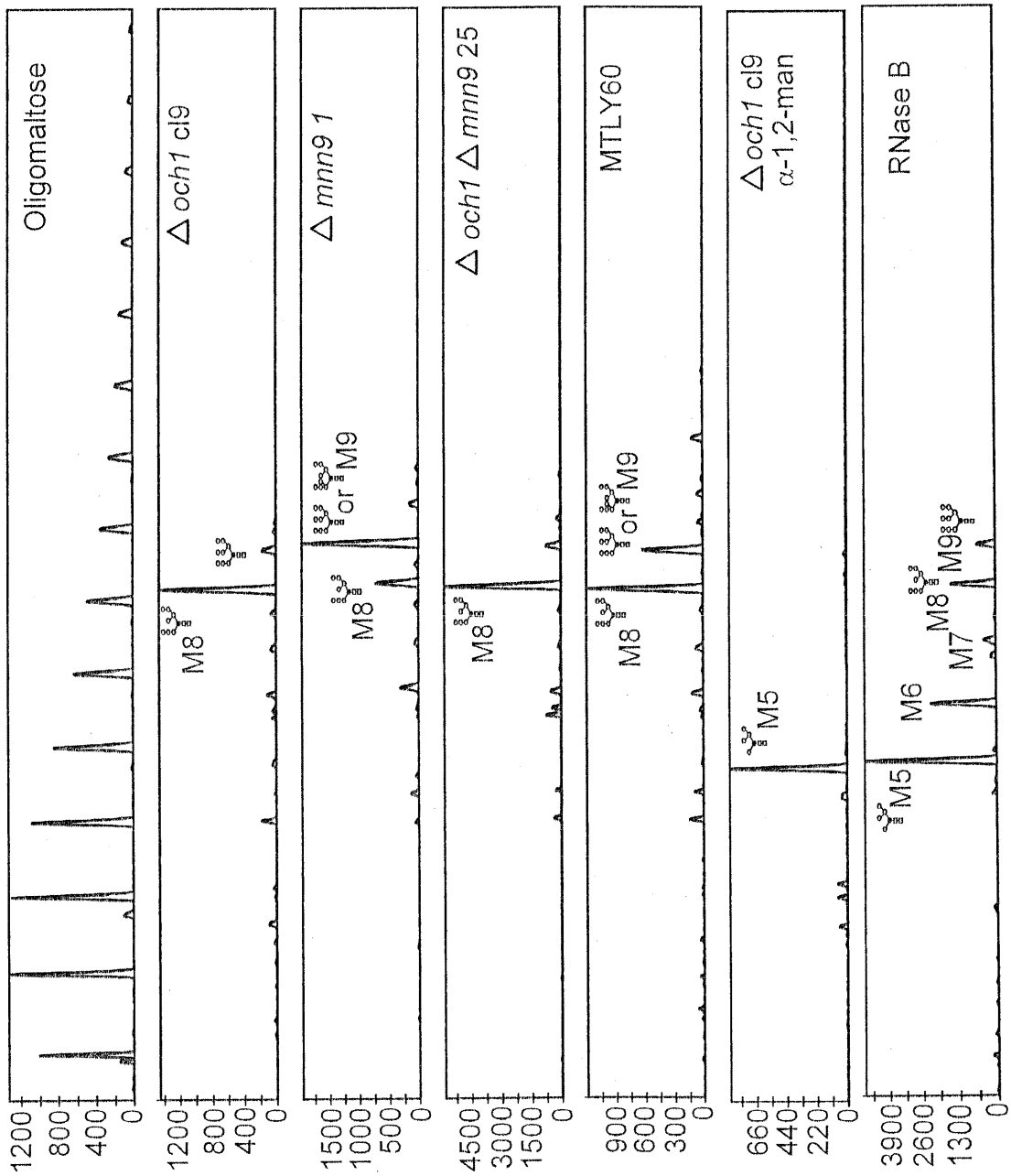

Oligomaltose
1200
800
400
0
Δ och1 cl9
M8
1200
800
400
0
Δ mnn9 1
or M9
M8
1500
1000
500
0
Δ och1 Δ mnn9 25
M8
4500
3000
1500
0
MTLY60
or M9
M8
900
600
300
0
Δ och1 cl9
α-1,2-man
M5
660
440
220
0
RNase B
M5
M6
M7
M8
M9
3900
2600
1300
0

FIG. 7

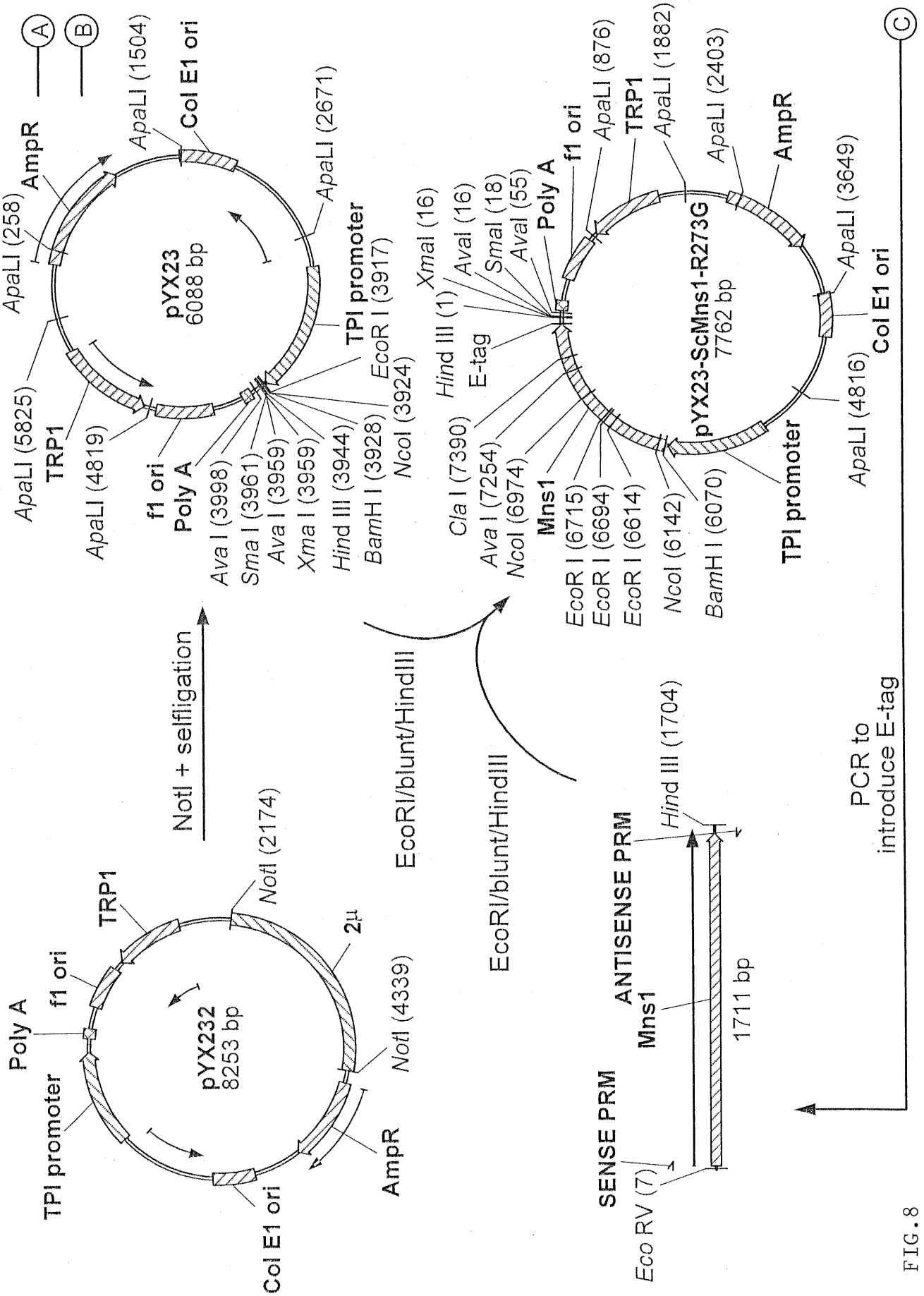

pYX23
6088 bp
AmpR
Col E1 ori
TRP1
f1 ori
Poly A
TPI promoter
ApaLI (258)
ApaLI (1504)
ApaLI (2671)
ApaLI (5825)
ApaLI (4819)
Ava I (3998)
Sma I (3961)
Ava I (3959)
Xma I (3959)
Hind III (3944)
BamH I (3928)
EcoR I (3917)
NcoI (3924)
XmaI (16)
AvaI (16)
SmaI (18)
AvaI (55)
Hind III (1)
E-tag
Cla I (7390)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

FIG. 8

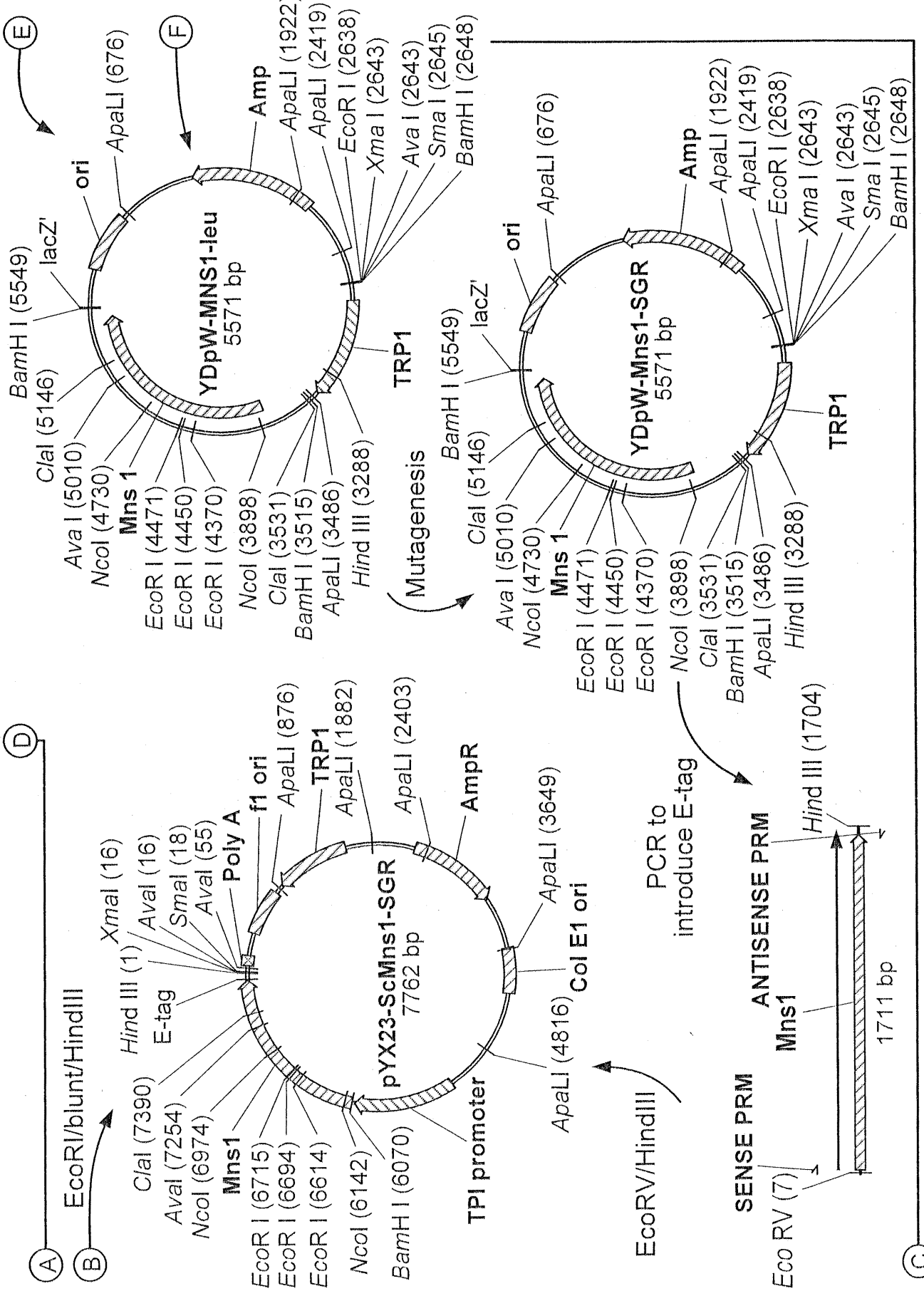


FIG.8(Continuous)

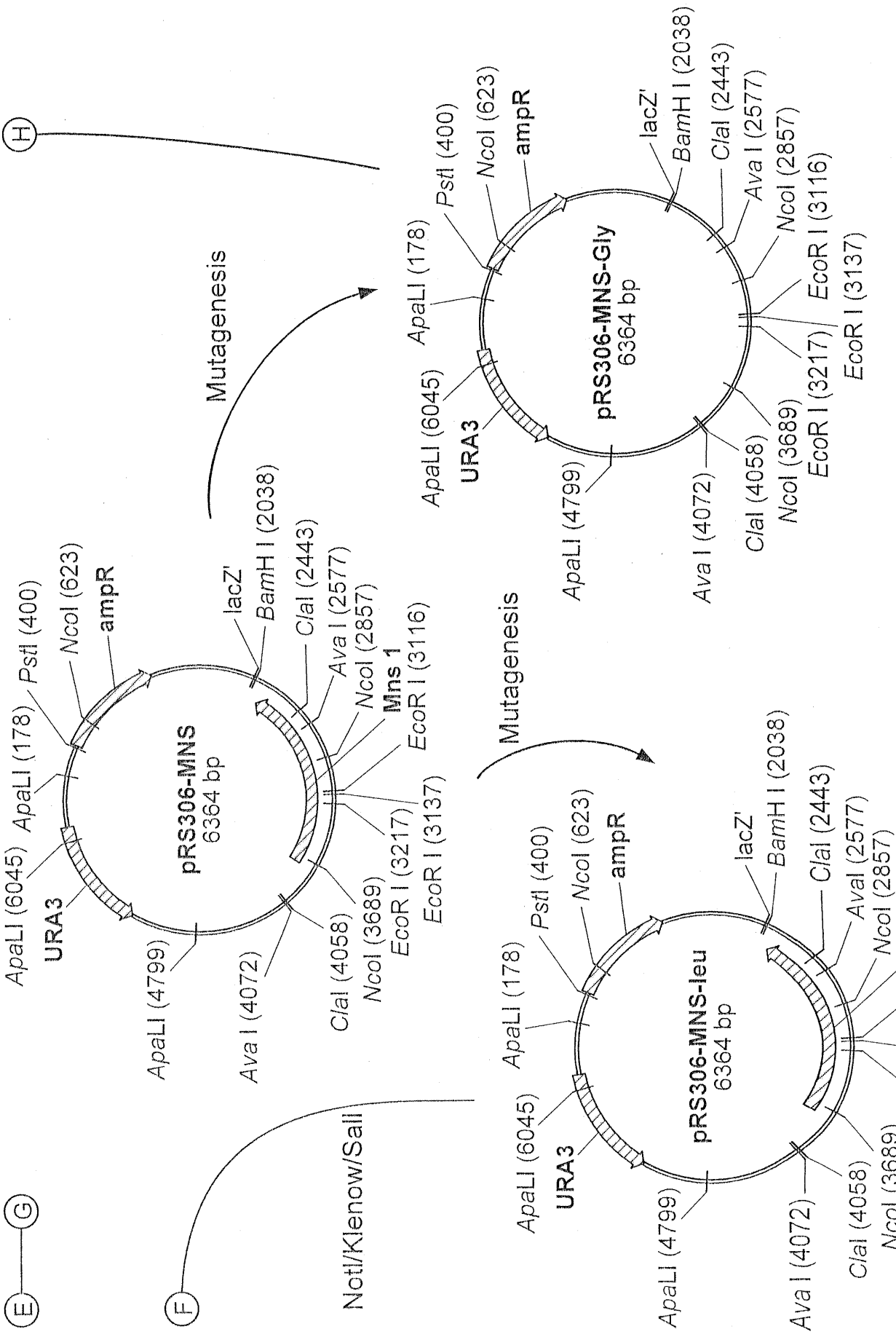


FIG.8(Continuous)

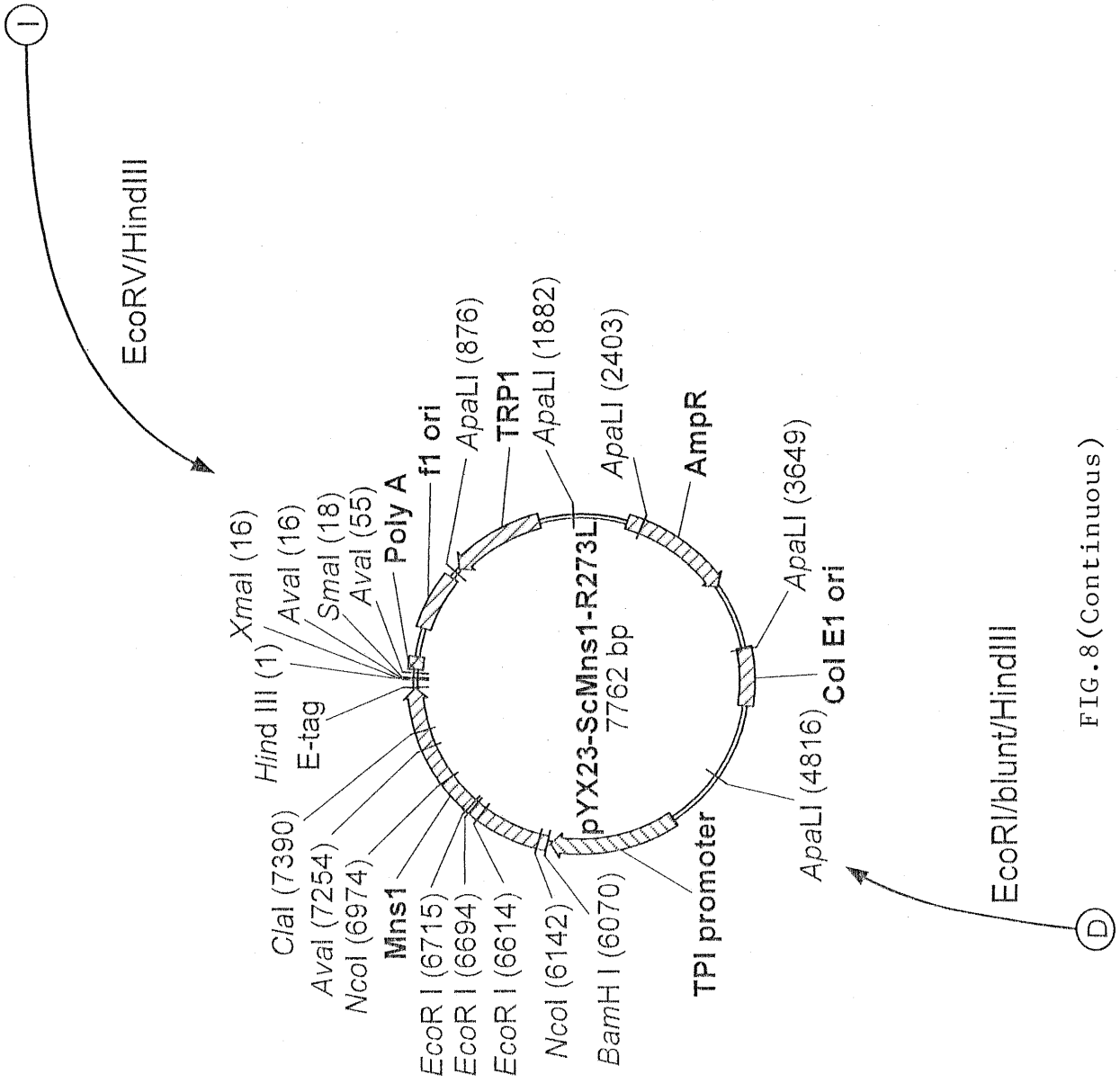


FIG.8(Continuous)

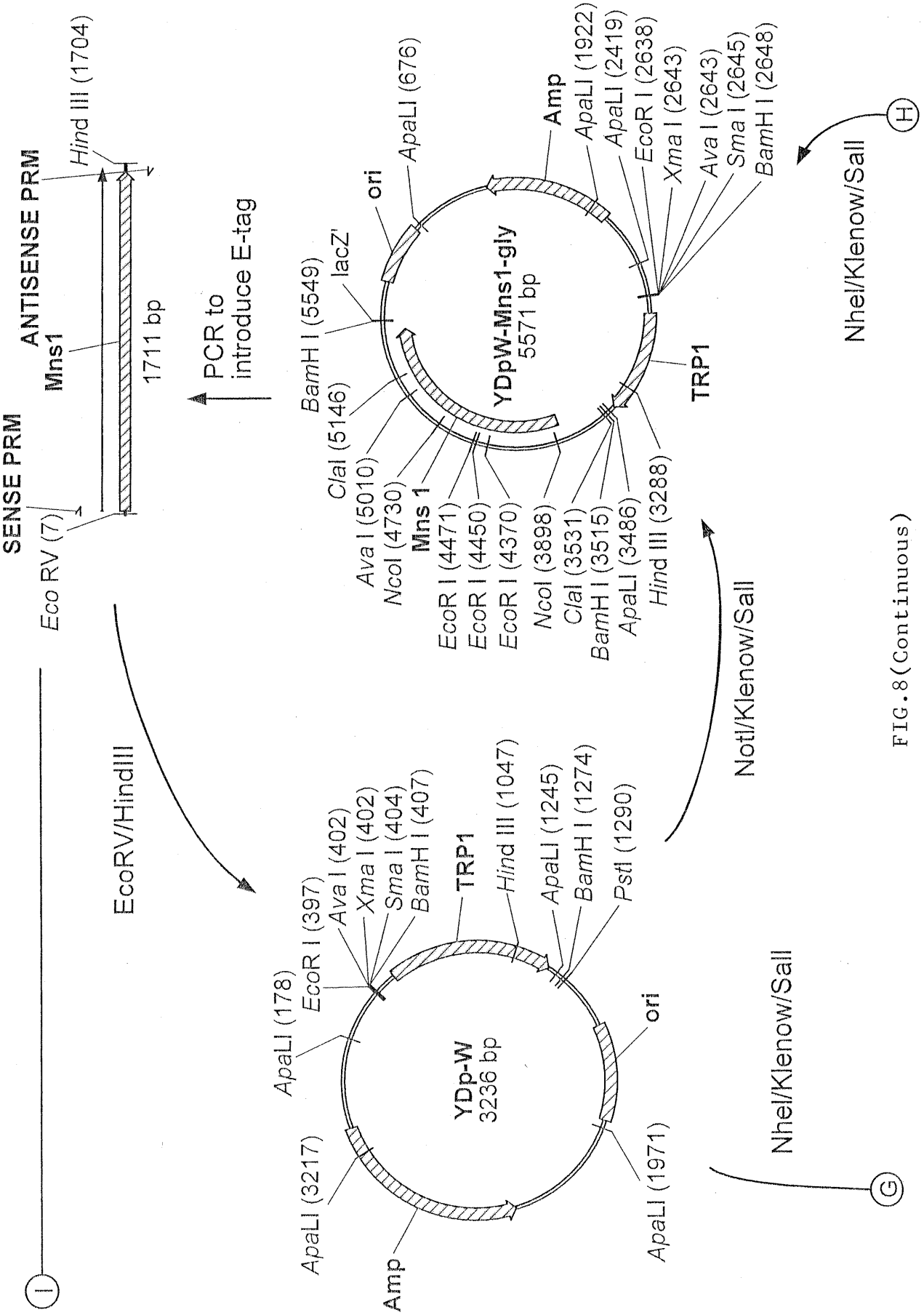


FIG.8(Continuous)

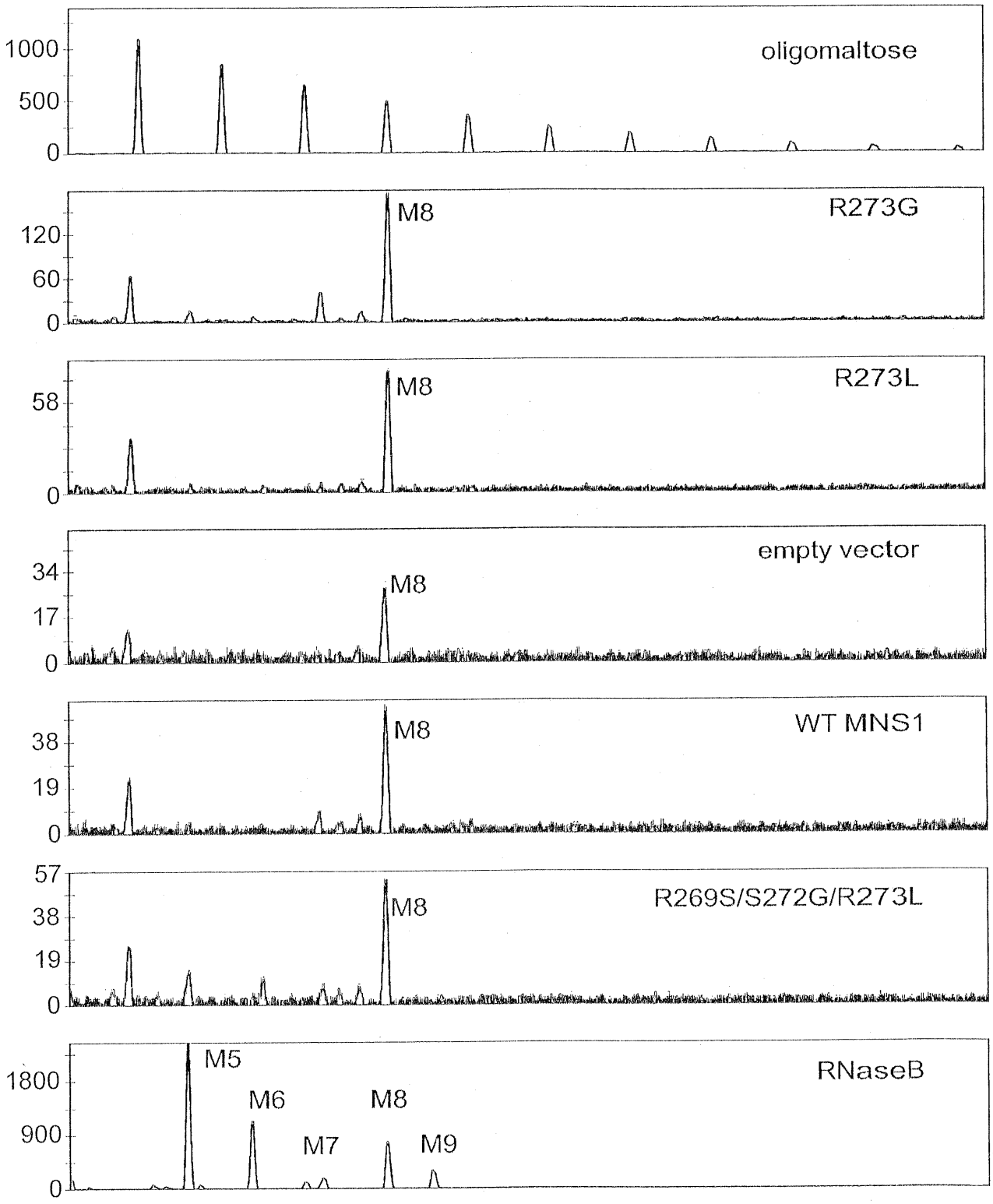

1000
500
0
oligomaltose
120
60
0
M8
R273G
58
0
M8
R273L
34
17
0
M8
empty vector
38
19
0
M8
WT MNS1
57
38
19
0
M8
R269S/S272G/R273L
1800
900
0
M5
M6
M7
M8
M9
RNaseB

FIG. 9

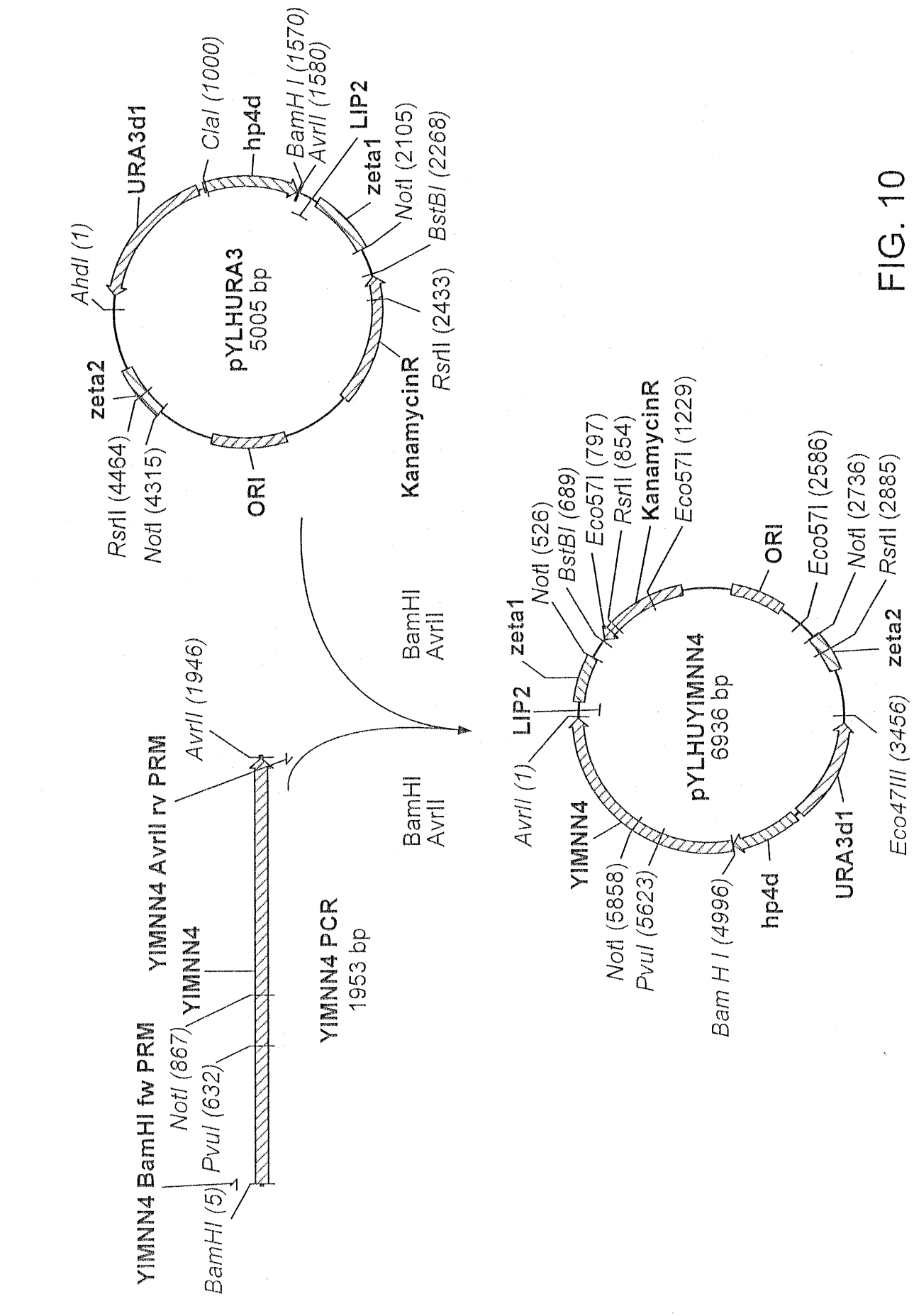

pYLHURA3
5005 bp
URA3d1
AhdI (1)
ClaI (1000)
hp4d
BamH I (1570)
AvrII (1580)
LIP2
zeta1
NotI (2105)
BstBI (2268)
RsrII (2433)
KanamycinR
ORI
zeta2
RsrII (4464)
NotI (4315)
YIMNN4 BamHI fw PRM
YIMNN4
NotI (867)
PvuI (632)
BamHI (5)
YIMNN4 AvrII rv PRM
AvrII (1946)
YIMNN4 PCR
1953 bp
BamHI
AvrII
BamHI
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

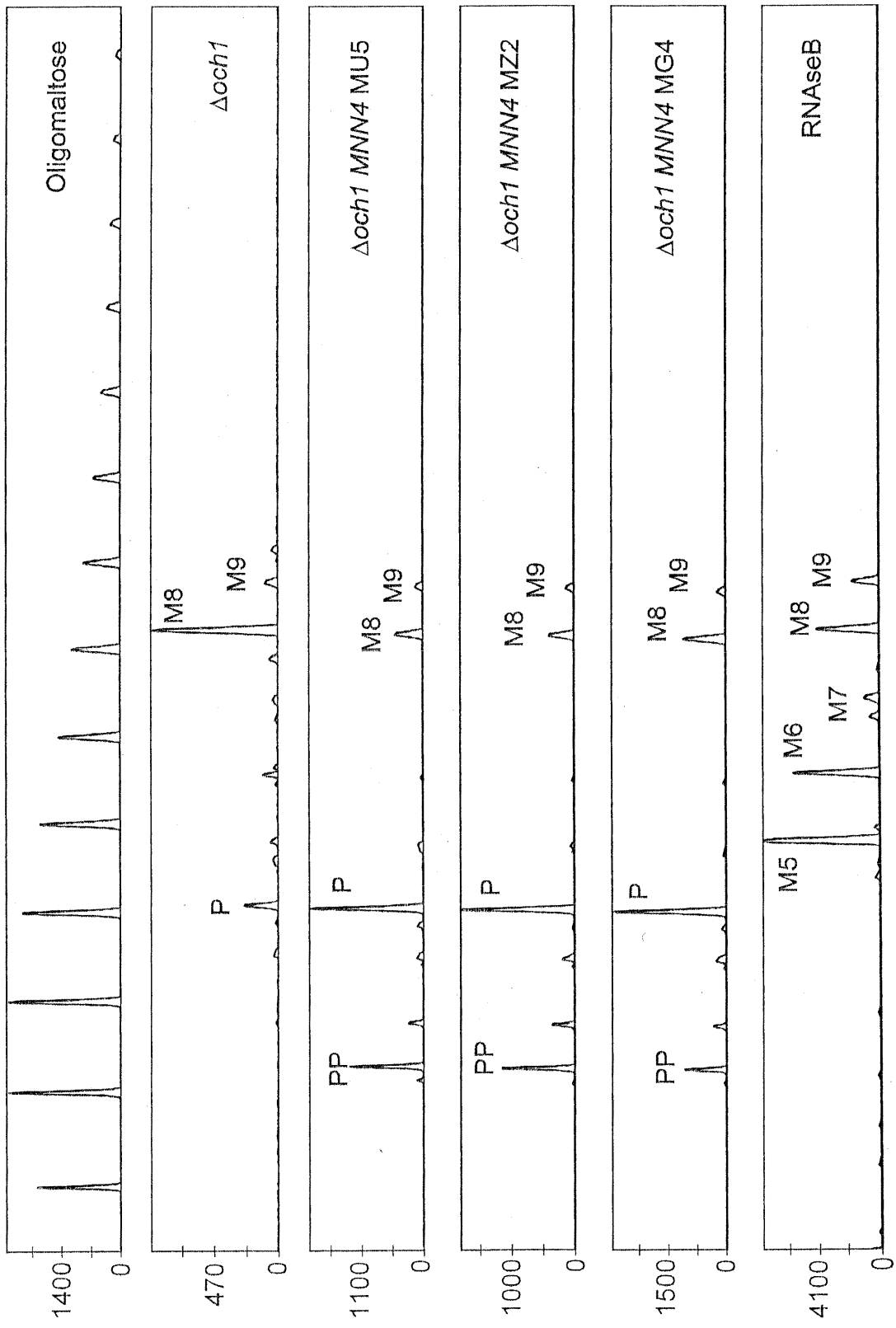
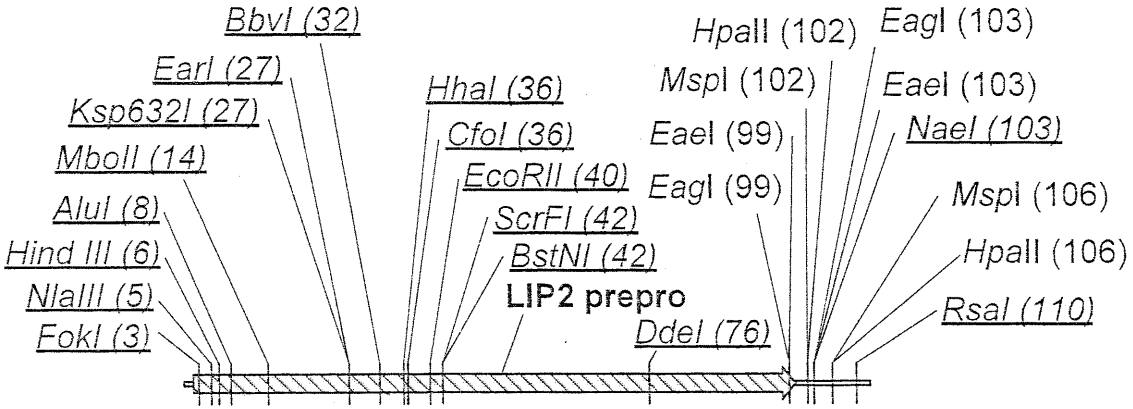

Oligomaltose
1400
0
Δoch1
M8
M9
P
470
0
Δoch1 MNN4 MU5
M8
M9
P
PP
1100
0
Δoch1 MNN4 MZ2
M8
M9
P
PP
1000
0
Δoch1 MNN4 MG4
M8
M9
P
PP
1500
0
RNAseB
M5
M6
M7
M8
M9
4100
0

FIG. 11



YlLIP2 prepro
111 bp

catgaactttccaccatcctcttcacagcctgcgctaccctggctgccgccctcccttccccc ⟶
⟵ cggcgggagggaaggggtagtgaggaagactccggcgtcaagaggtcttcgccggccggccatg

FIG.12

A

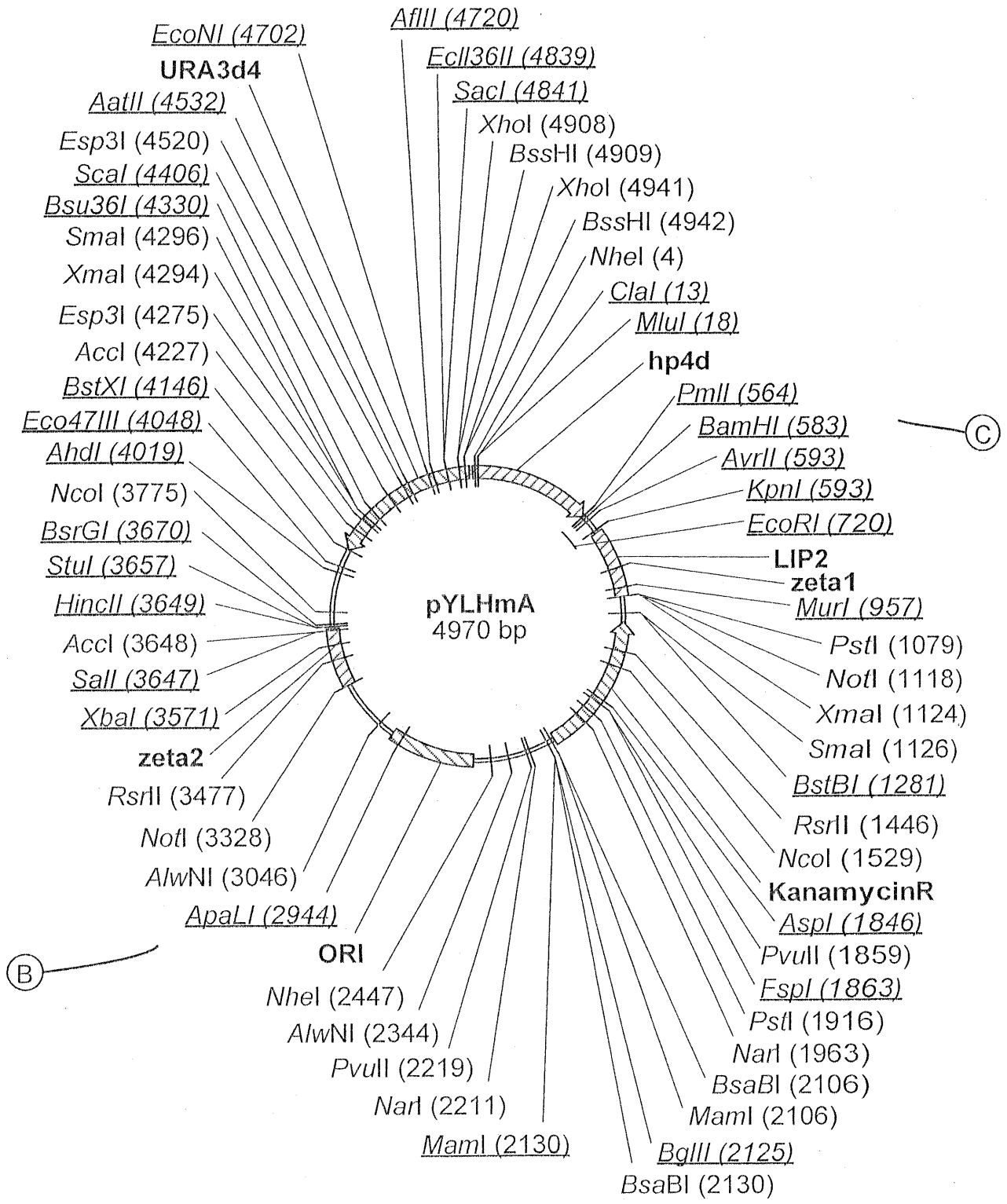


FIG.12(Continuous)

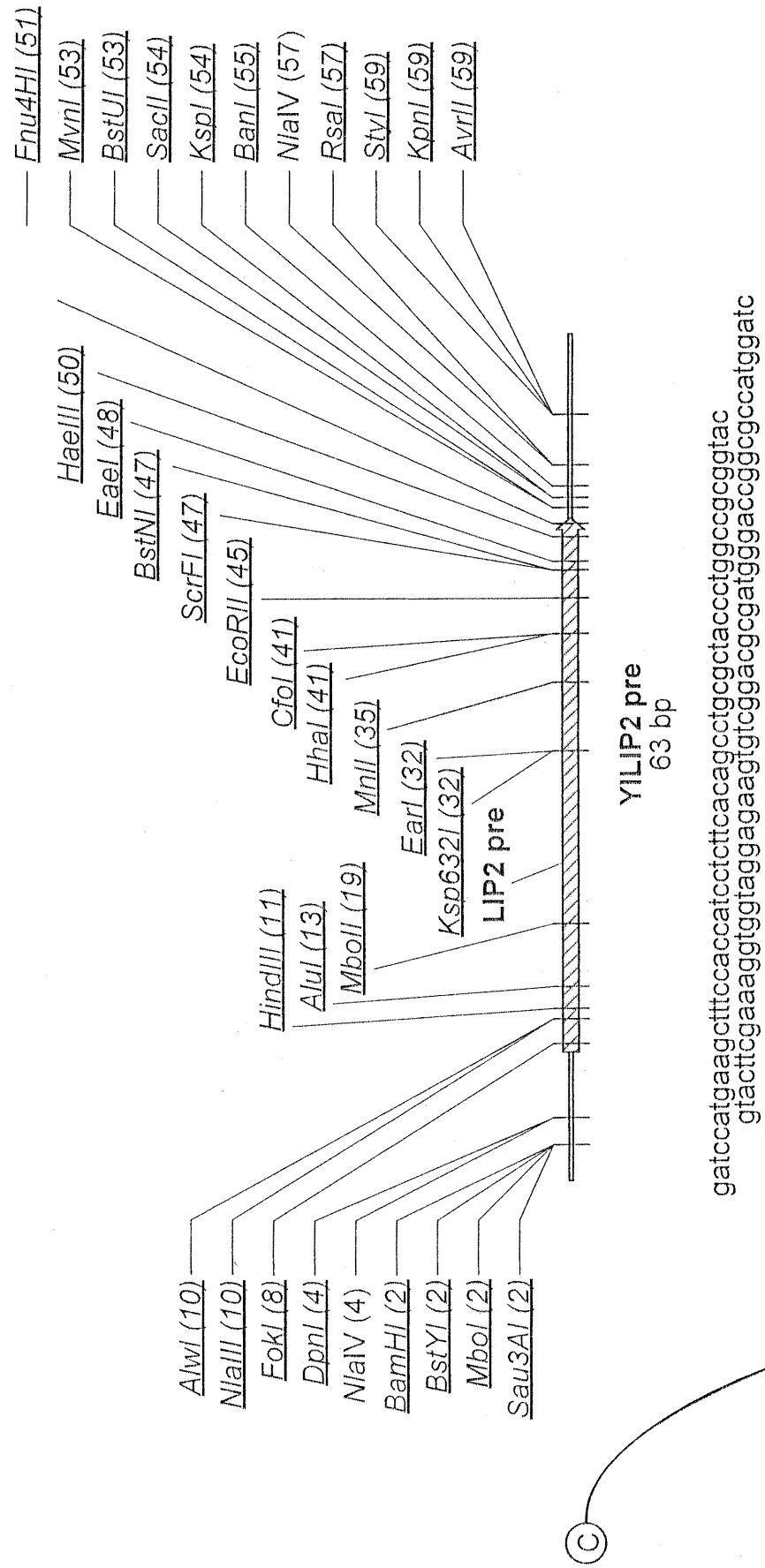


FIG.12(Continuous)

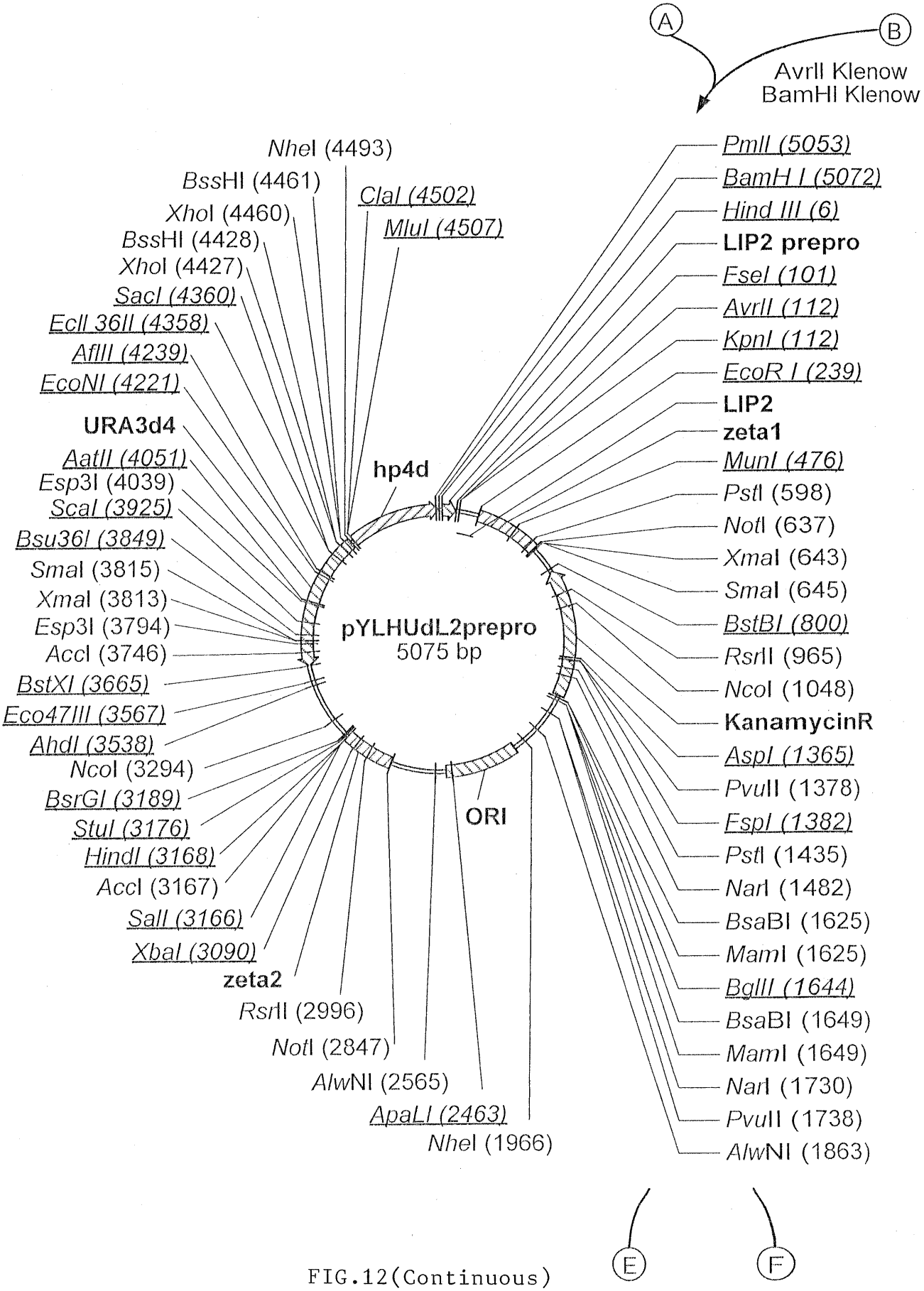


FIG.12(Continuous)

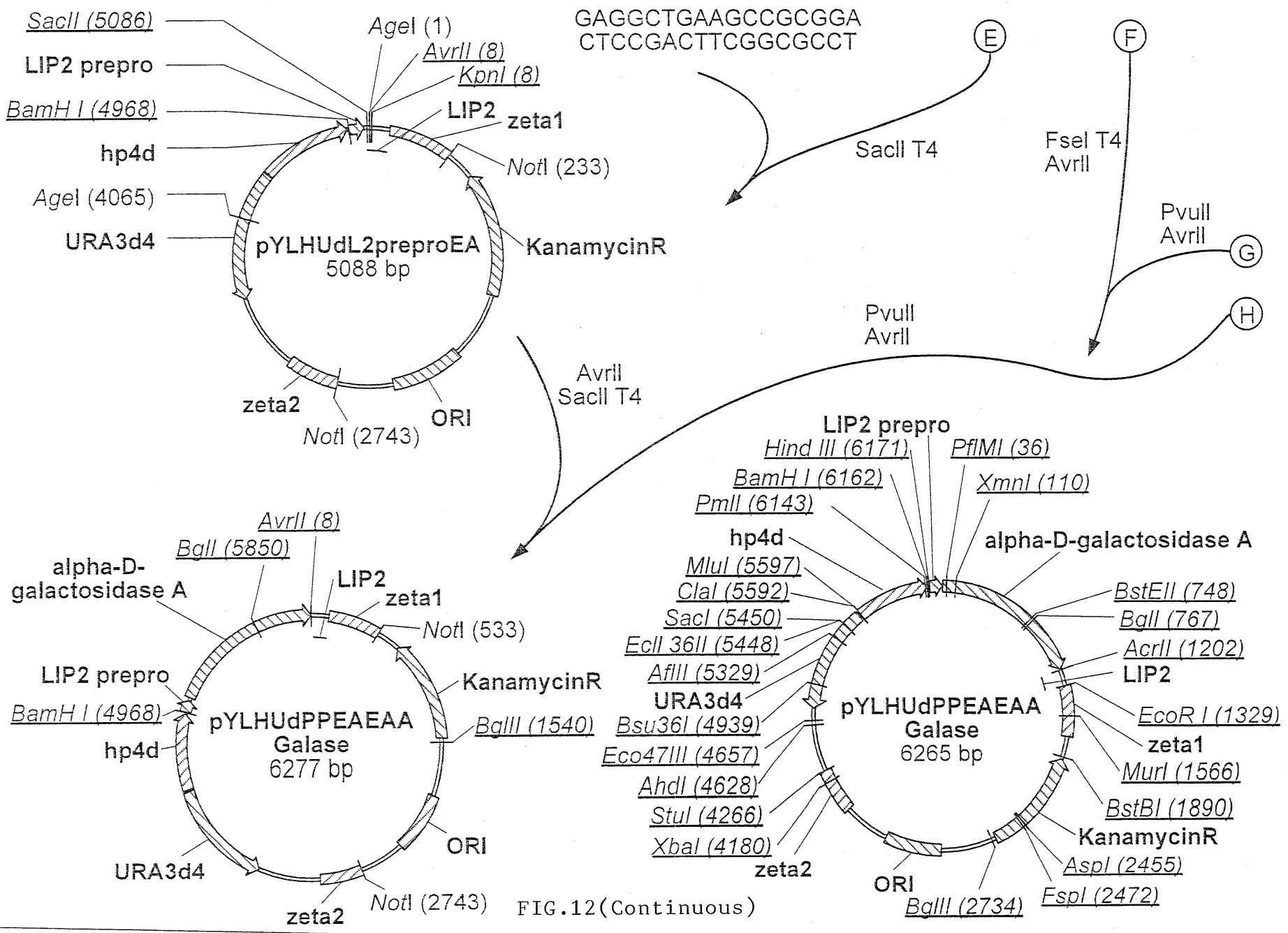
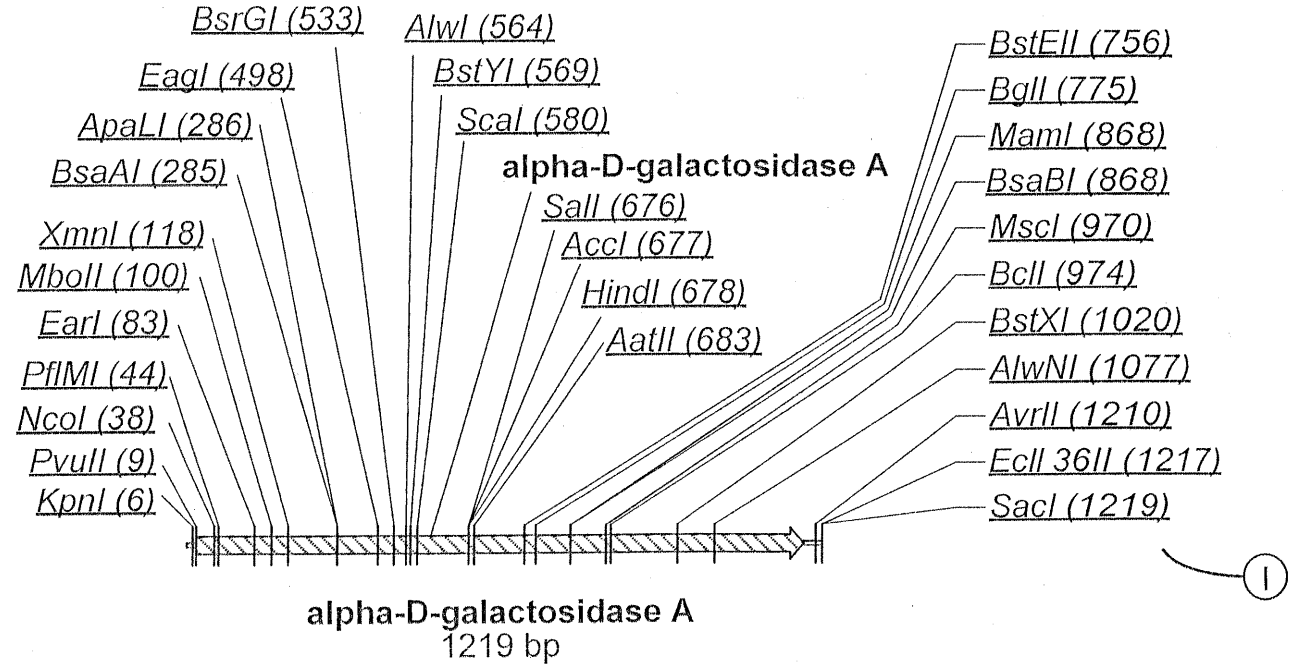


FIG.12(Continuous)



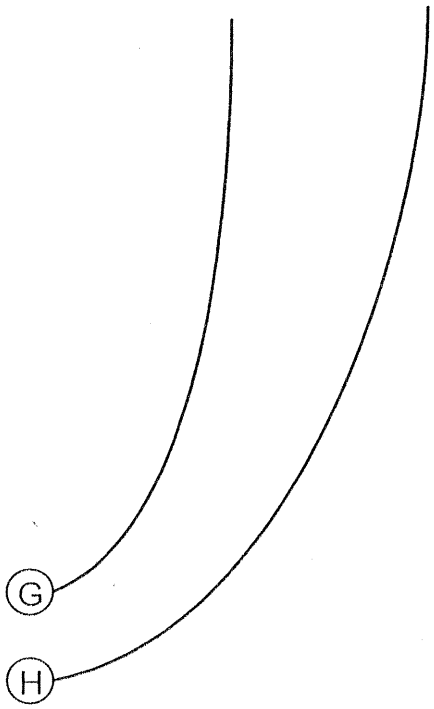


FIG.12(Continuous)

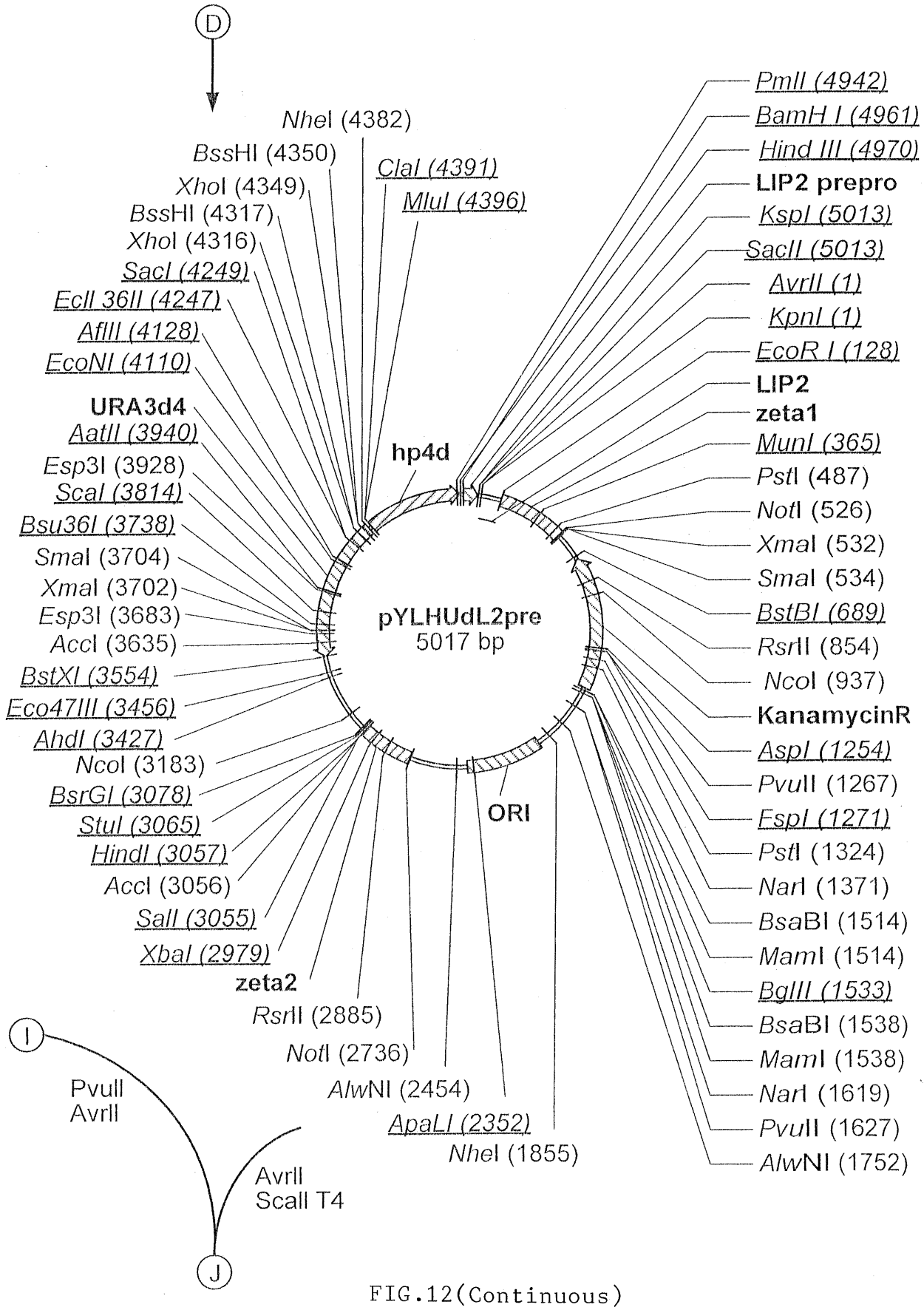


FIG.12(Continuous)

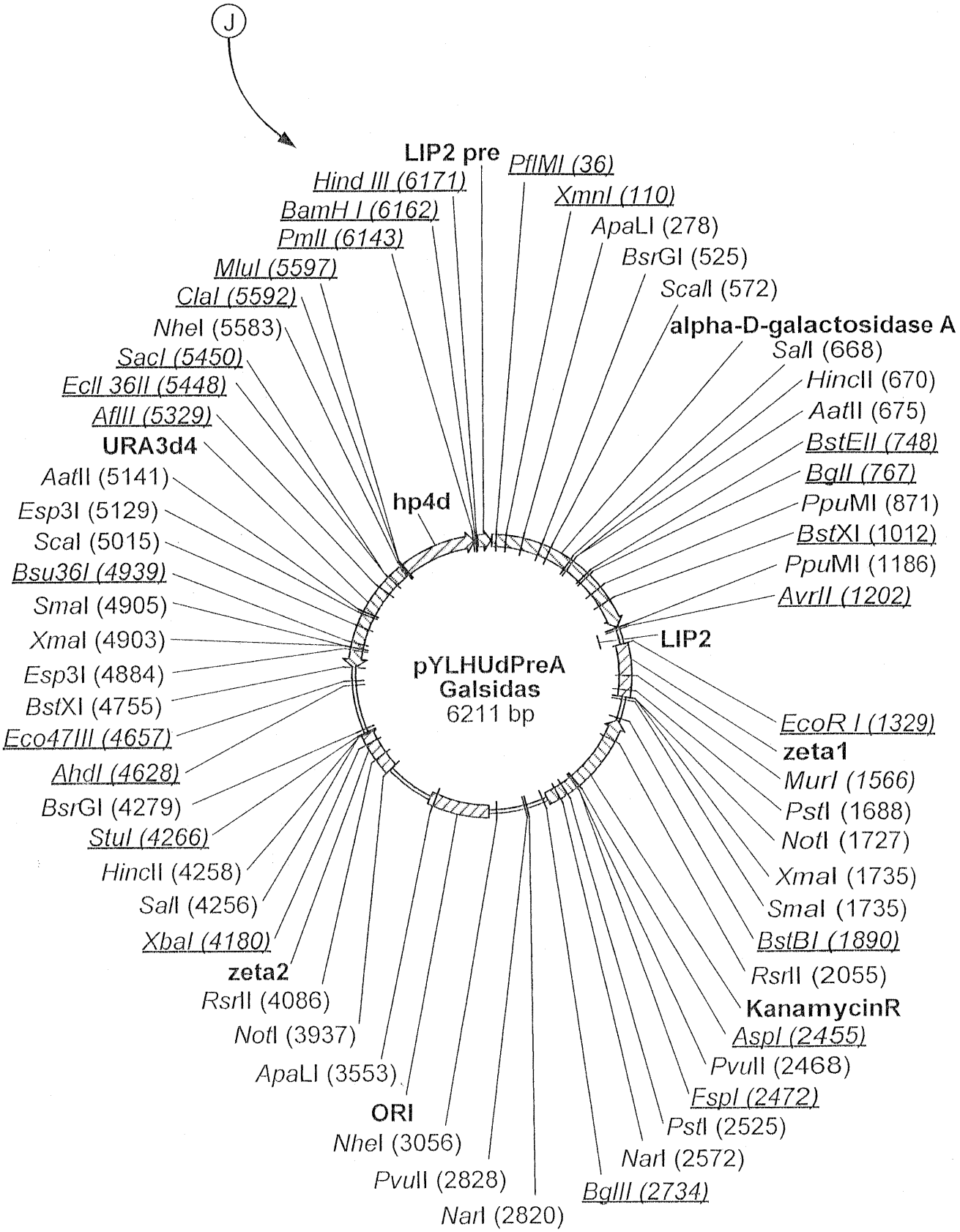


FIG.12(Continuous)

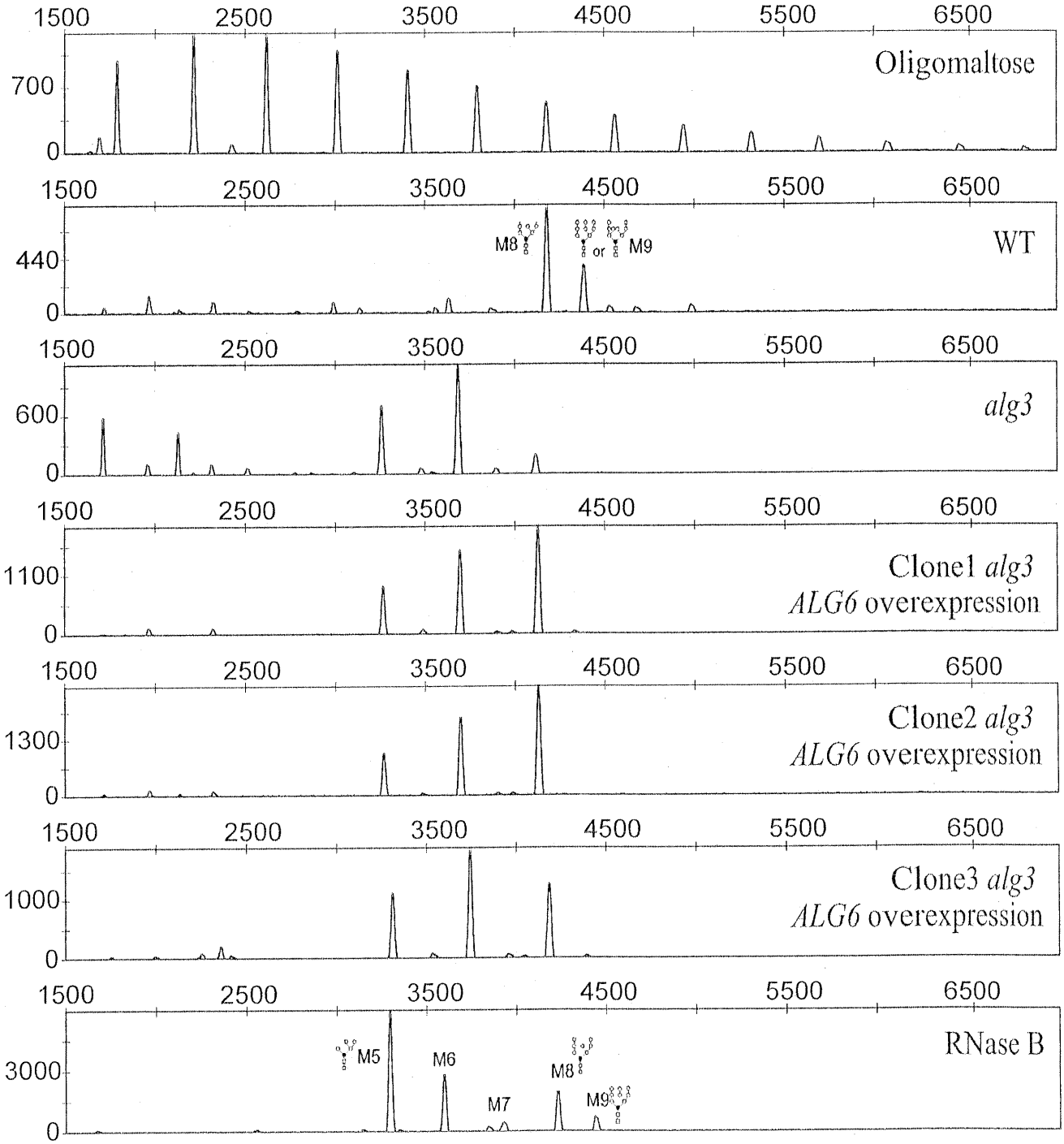

1500
2500
3500
4500
5500
6500
700
0
Oligomaltose
440
M8
or
M9
WT
600
alg3
1100
Clone1 alg3
ALG6 overexpression
1300
Clone2 alg3
ALG6 overexpression
1000
Clone3 alg3
ALG6 overexpression
3000
M5
M6
M7
M8
M9
RNase B

FIG. 13

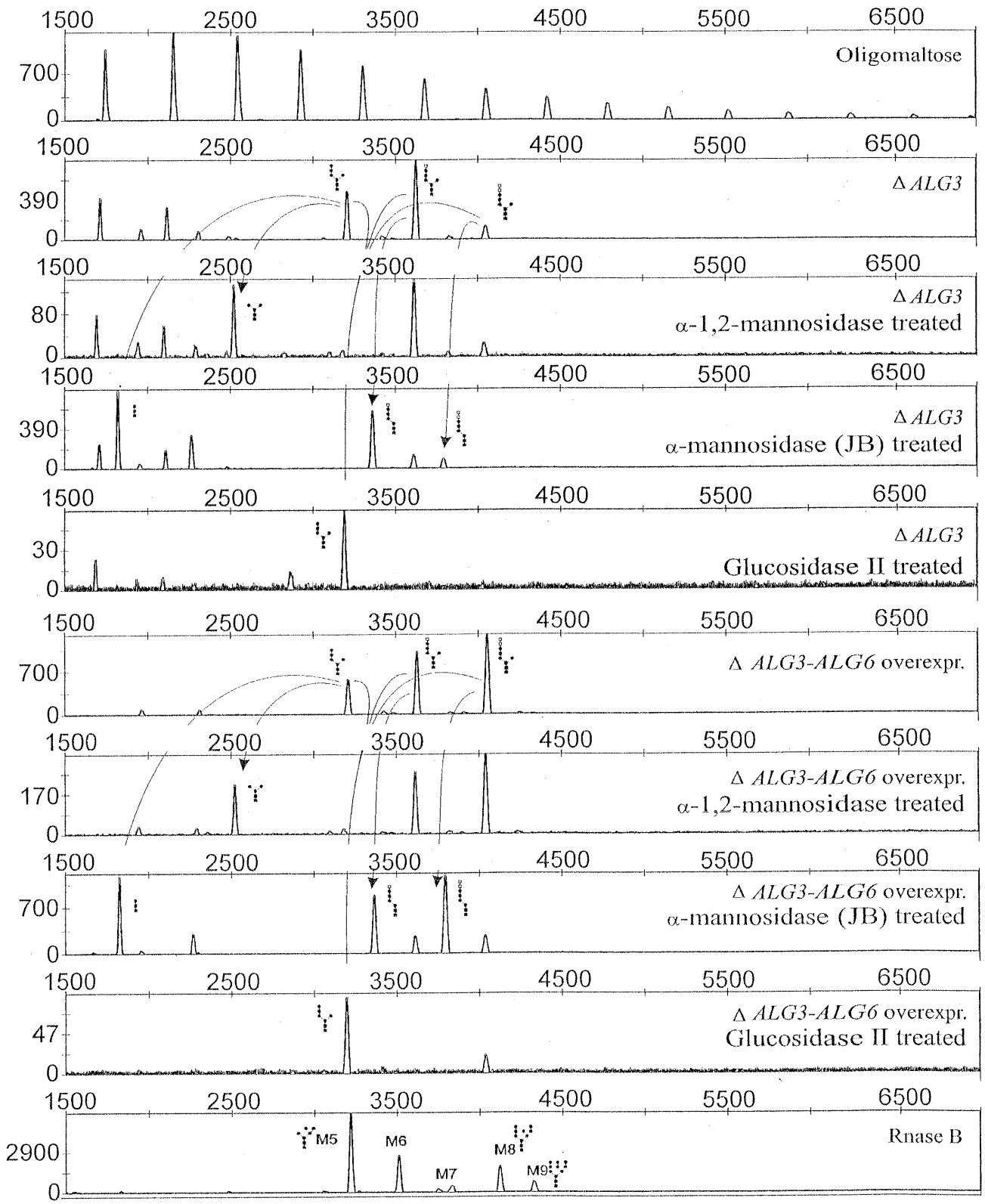

1500
2500
3500
4500
5500
6500
700
0
Oligomaltose
390
Δ ALG3
80
Δ ALG3
α-1,2-mannosidase treated
Δ ALG3
α-mannosidase (JB) treated
30
Δ ALG3
Glucosidase II treated
Δ ALG3-ALG6 overexpr.
170
Δ ALG3-ALG6 overexpr.
α-1,2-mannosidase treated
Δ ALG3-ALG6 overexpr.
α-mannosidase (JB) treated
47
Δ ALG3-ALG6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

FIG. 14

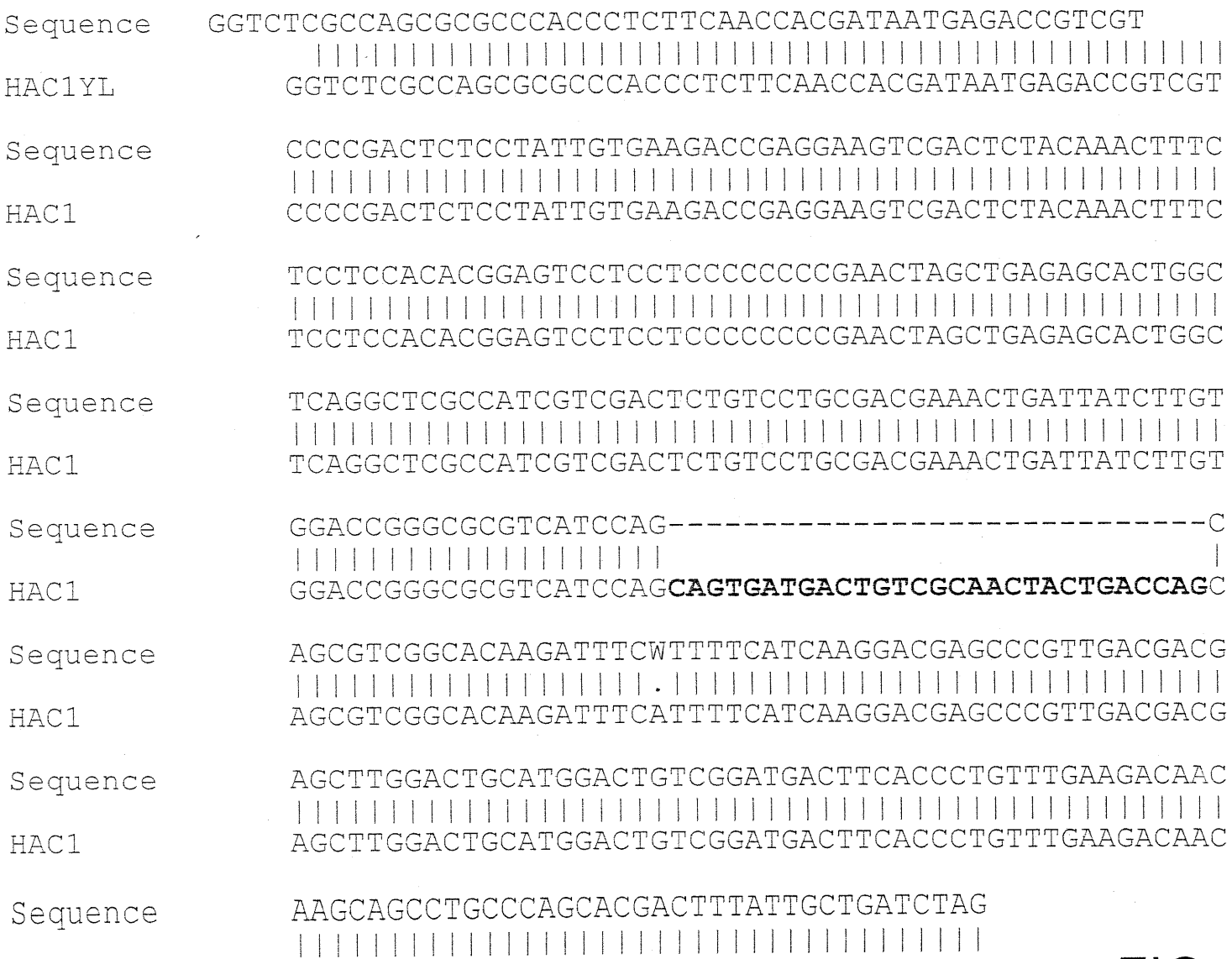

Sequence GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
HAC1YL GGTCTCGCCAGCGCGCCCACCCTCTTCAACCACGATAATGAGACCGTCGT
Sequence CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
HAC1 CCCCGACTCTCCTATTGTGAAGACCGAGGAAGTCGACTCTACAAACTTTC
Sequence TCCTCCACACGGAGTCCTCCTCCCCCCCCGAACTAGCTGAGAGCACTGGC
HAC1 TCCTCCACACGGAGTCCTCCTCCCCCCCCGAACTAGCTGAGAGCACTGGC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

FIG. 15

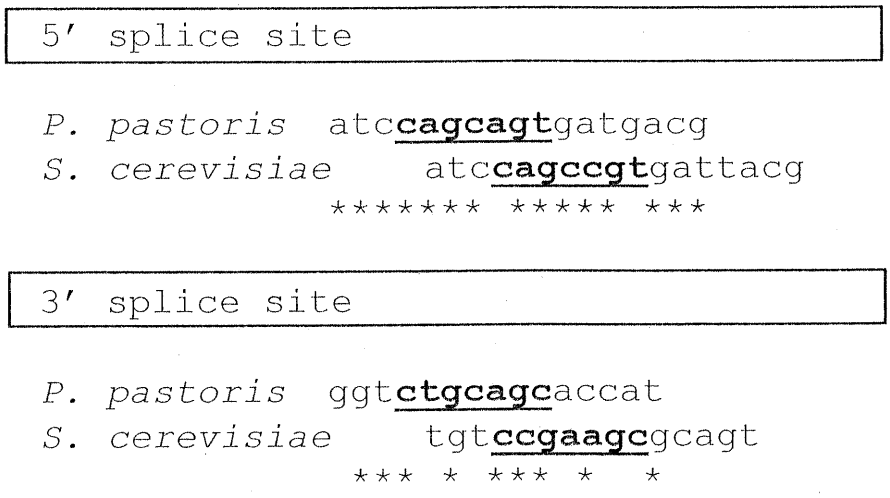

5′ splice site
P. pastoris atccagcagtgatgacg
S. cerevisiae atccagccgtgattacg
******* ***** ***
3′ splice site
P. pastoris ggtctgcagcaccat
S. cerevisiae tgtccgaagcgcagt
*** * *** * *

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.17

```
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.17(Continuous)

```
P.pastoris          1  APFTANAFDLNDFVFFQE
18

                       |....|:|:||||....
S.cerevisiae        1 EAQSGLNSFELNDFFITS
18
```

FIG. 18

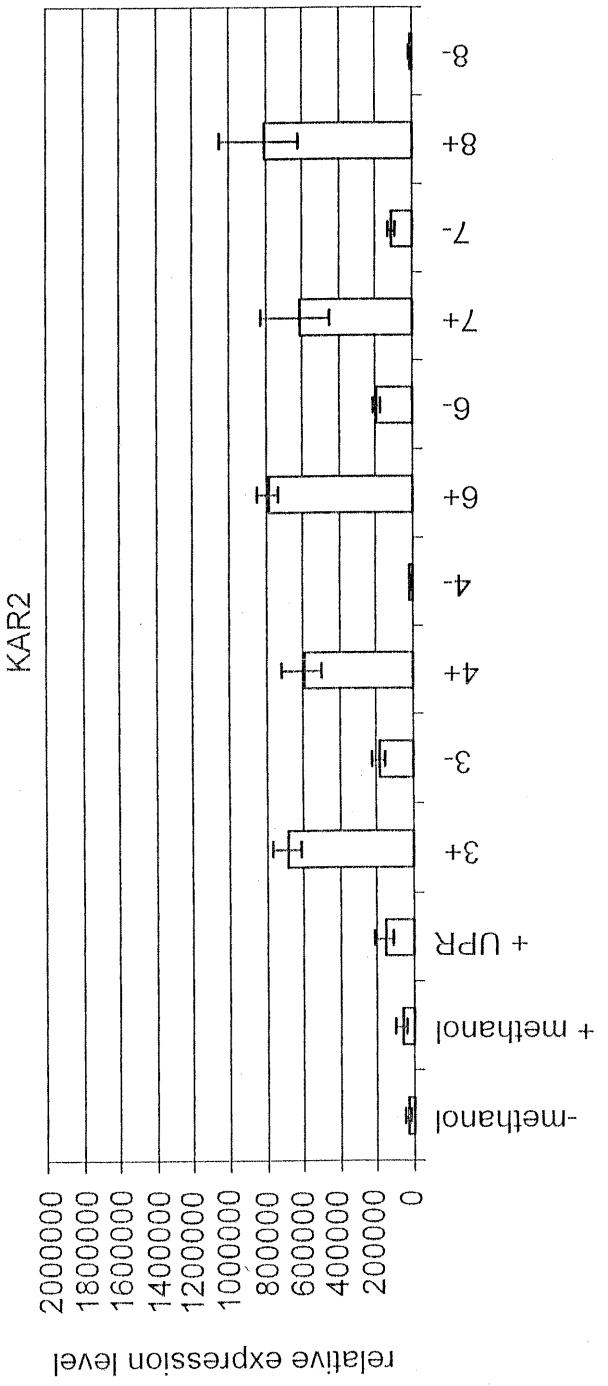

KAR2
relative expression level
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-methanol
+ methanol
+ UPR
3+
3-
4+
4-
6+
6-
7+
7-
8+
8-

FIG. 19

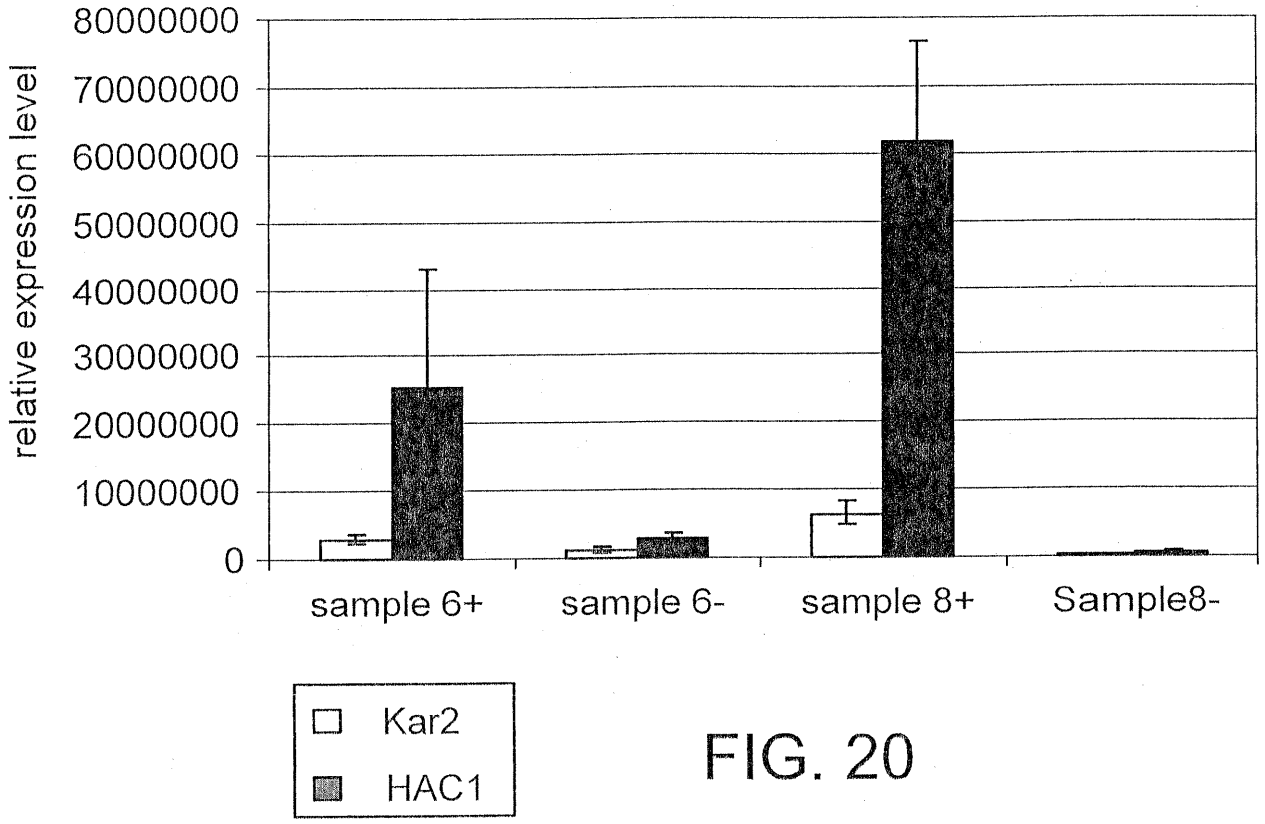

relative expression level
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
sample 6+
sample 6-
sample 8+
Sample8-
Kar2
HAC1

FIG. 20

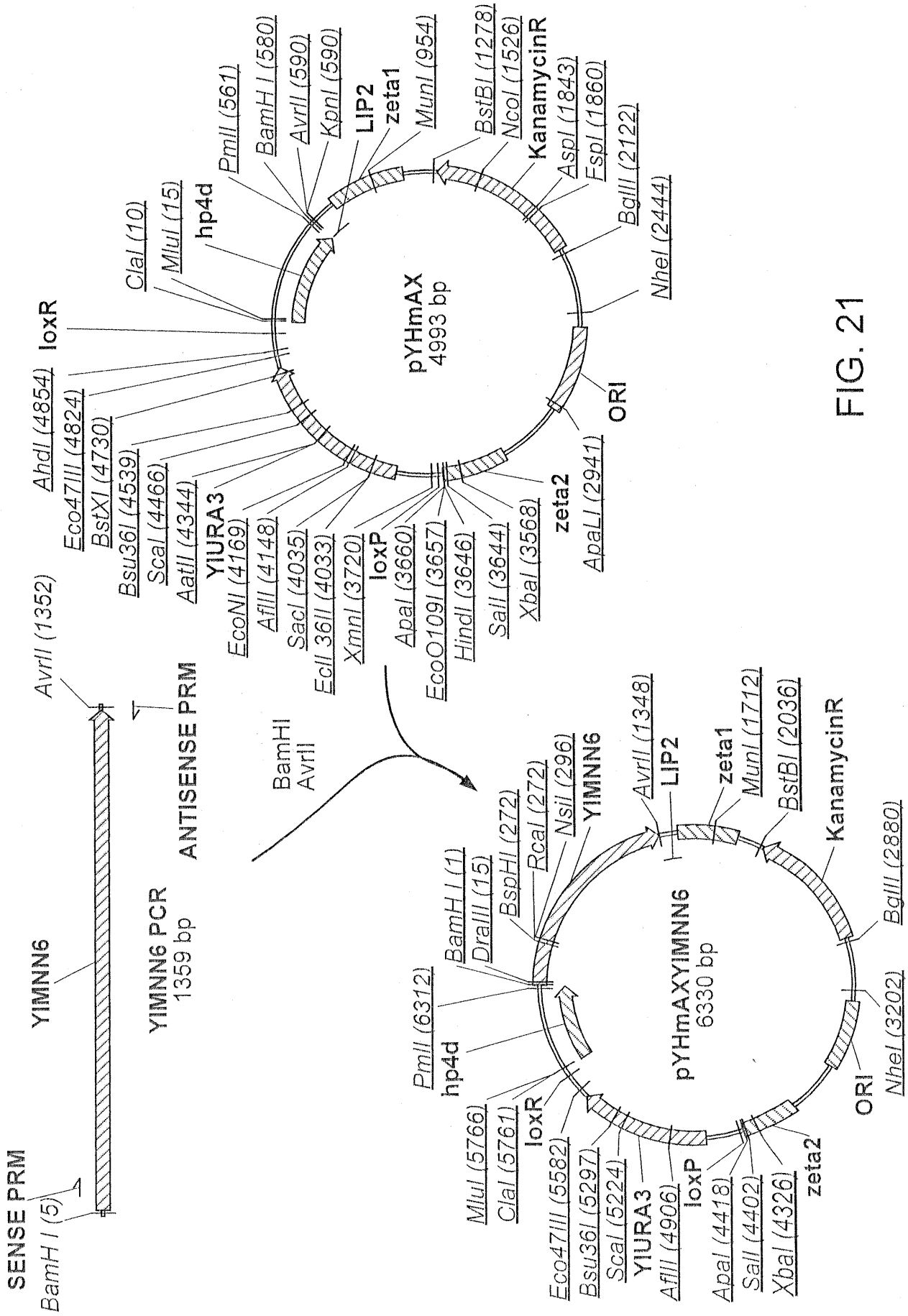

SENSE PRM
BamH I (5)
YIMNN6
AvrII (1352)
YIMNN6 PCR
1359 bp
ANTISENSE PRM
BamHI
AvrII
pYHmAX
4993 bp
AhdI (4854)
loxR
Eco47III (4824)
BstXI (4730)
Bsu36I (4539)
ScaI (4466)
AatII (4344)
YIURA3
EcoNI (4169)
AflII (4148)
SacI (4035)
EcII 36II (4033)
XmnI (3720)
loxP
ApaI (3660)
EcoO109I (3657)
HindI (3646)
SalI (3644)
XbaI (3568)
zeta2
ApaLI (2941)
ORI
NheI (2444)
BglII (2122)
AspI (1843)
FspI (1860)
BstBI (1278)
NcoI (1526)
KanamycinR
MunI (954)
LIP2
zeta1
KpnI (590)
AvrII (590)
BamH I (580)
PmlI (561)
hp4d
MluI (15)
ClaI (10)
pYHmAXYIMNN6
6330 bp
PmlI (6312)
hp4d
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)

FIG. 21

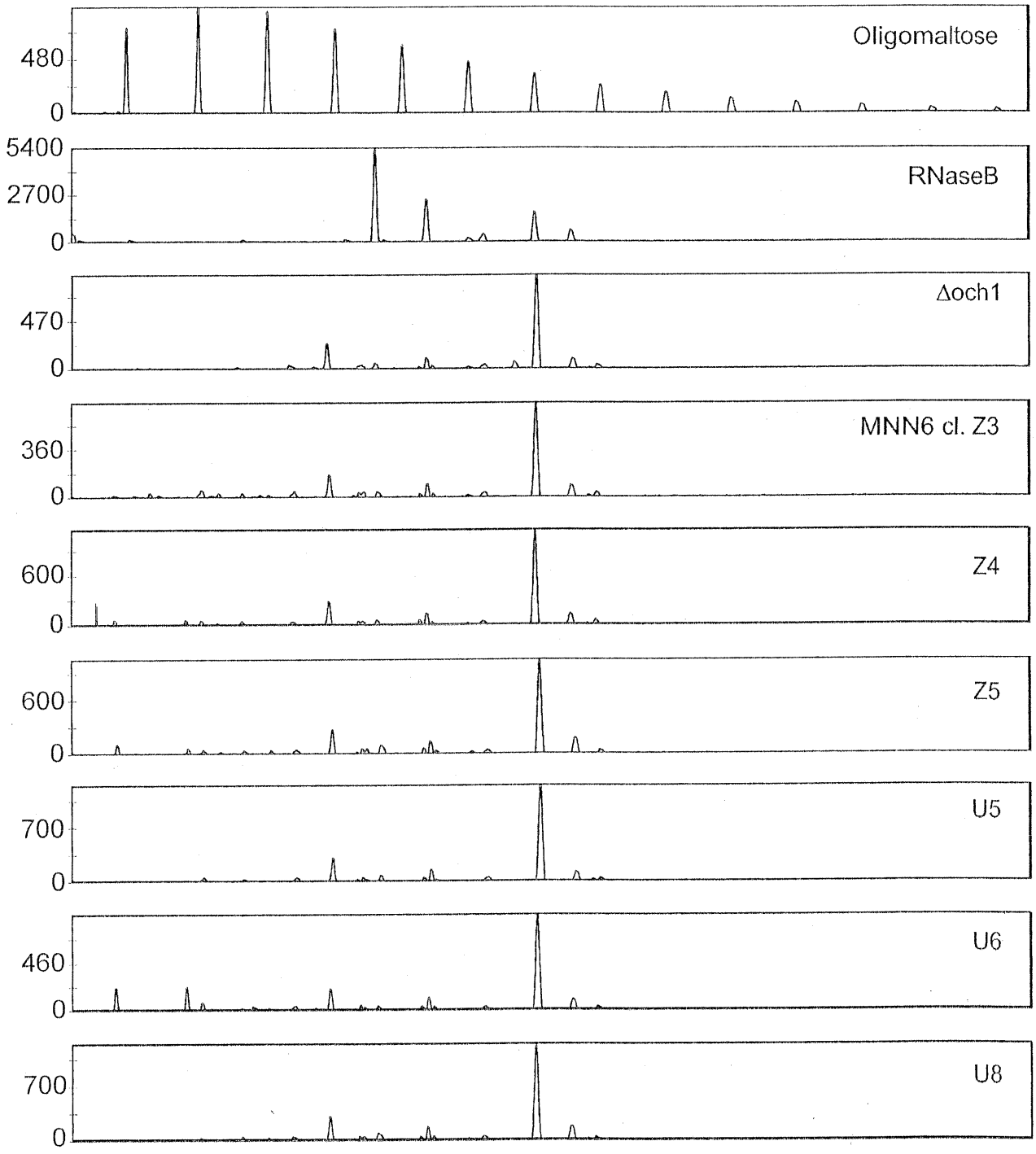

Oligomaltose
480
0
RNaseB
5400
2700
0
Δoch1
470
0
MNN6 cl. Z3
360
0
Z4
600
0
Z5
600
0
U5
700
0
U6
460
0
U8
700
0

FIG. 22

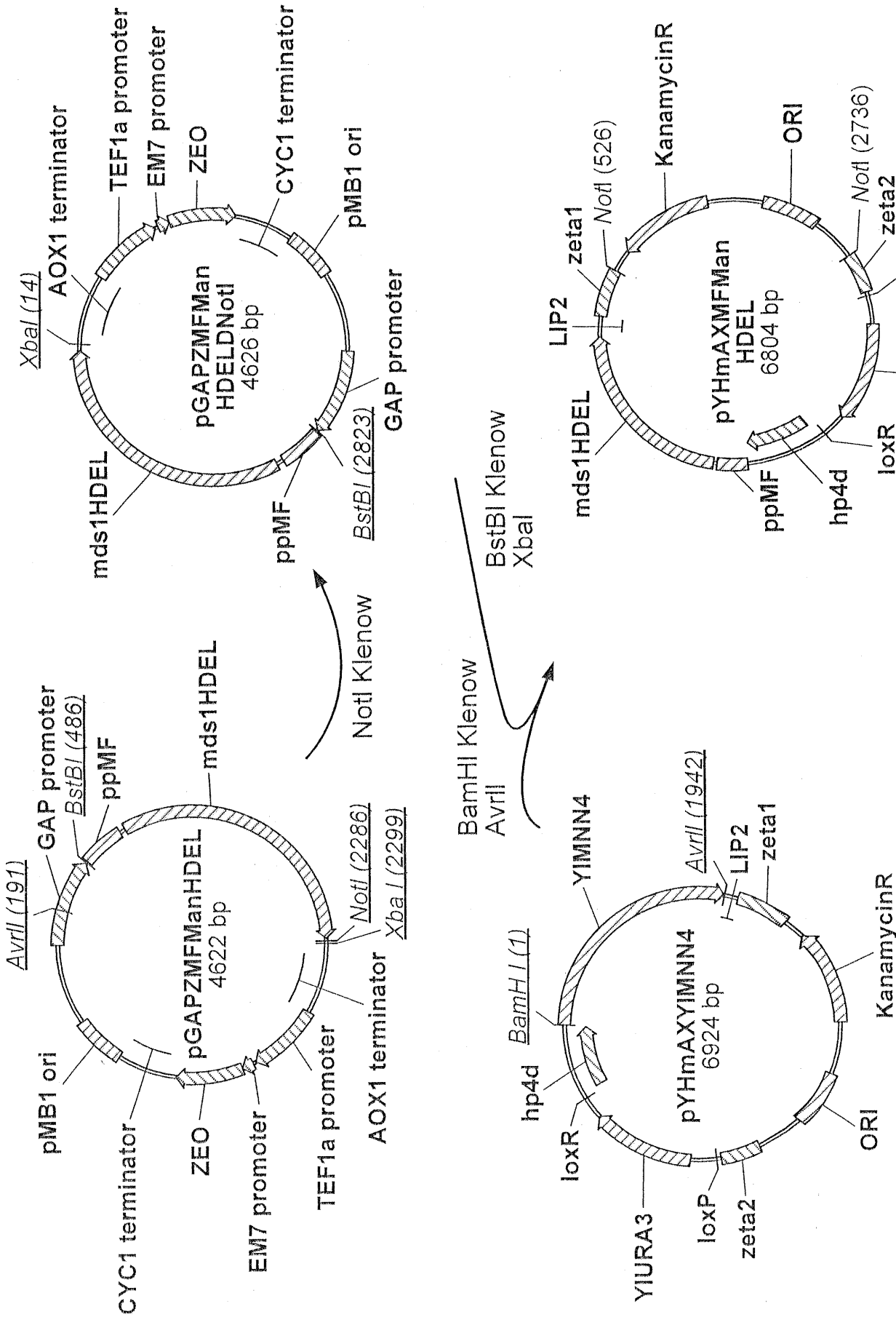

pGAPZMFMan
HDELDNotI
4626 bp
AOX1 terminator
TEF1a promoter
EM7 promoter
ZEO
CYC1 terminator
pMB1 ori
GAP promoter
mds1HDEL
ppMF
XbaI (14)
BstBI (2823)
pGAPZMFManHDEL
4622 bp
AvrII (191)
BstBI (486)
NotI (2286)
Xba I (2299)
NotI Klenow
BstBI Klenow
XbaI
BamHI Klenow
AvrII
pYHmAXMFMan
HDEL
6804 bp
LIP2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

FIG. 23

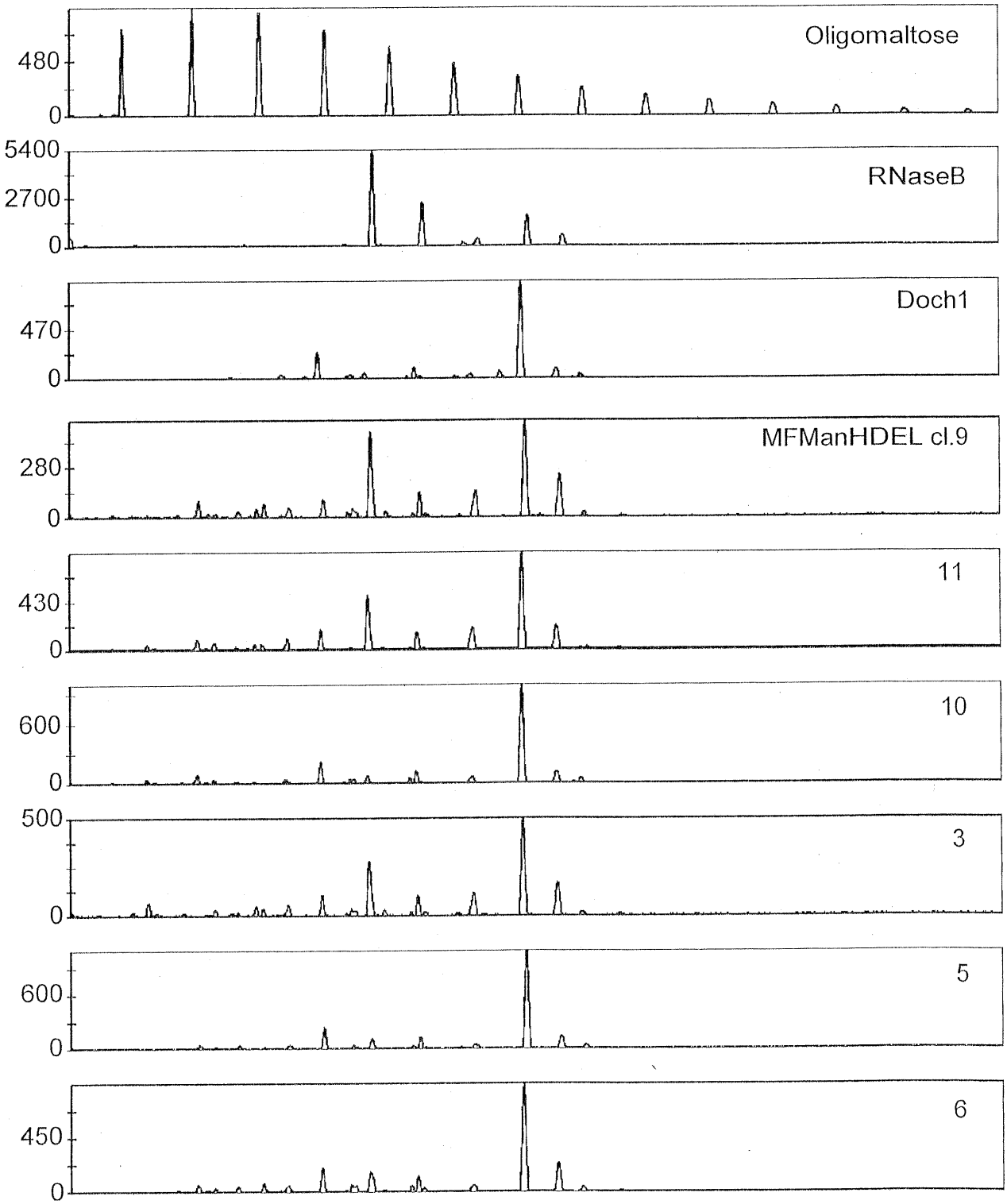

Oligomaltose
480
0
5400
2700
0
RNaseB
Doch1
470
0
MFManHDEL cl.9
280
0
11
430
0
10
600
0
3
500
0
5
600
0
6
450
0

FIG. 24

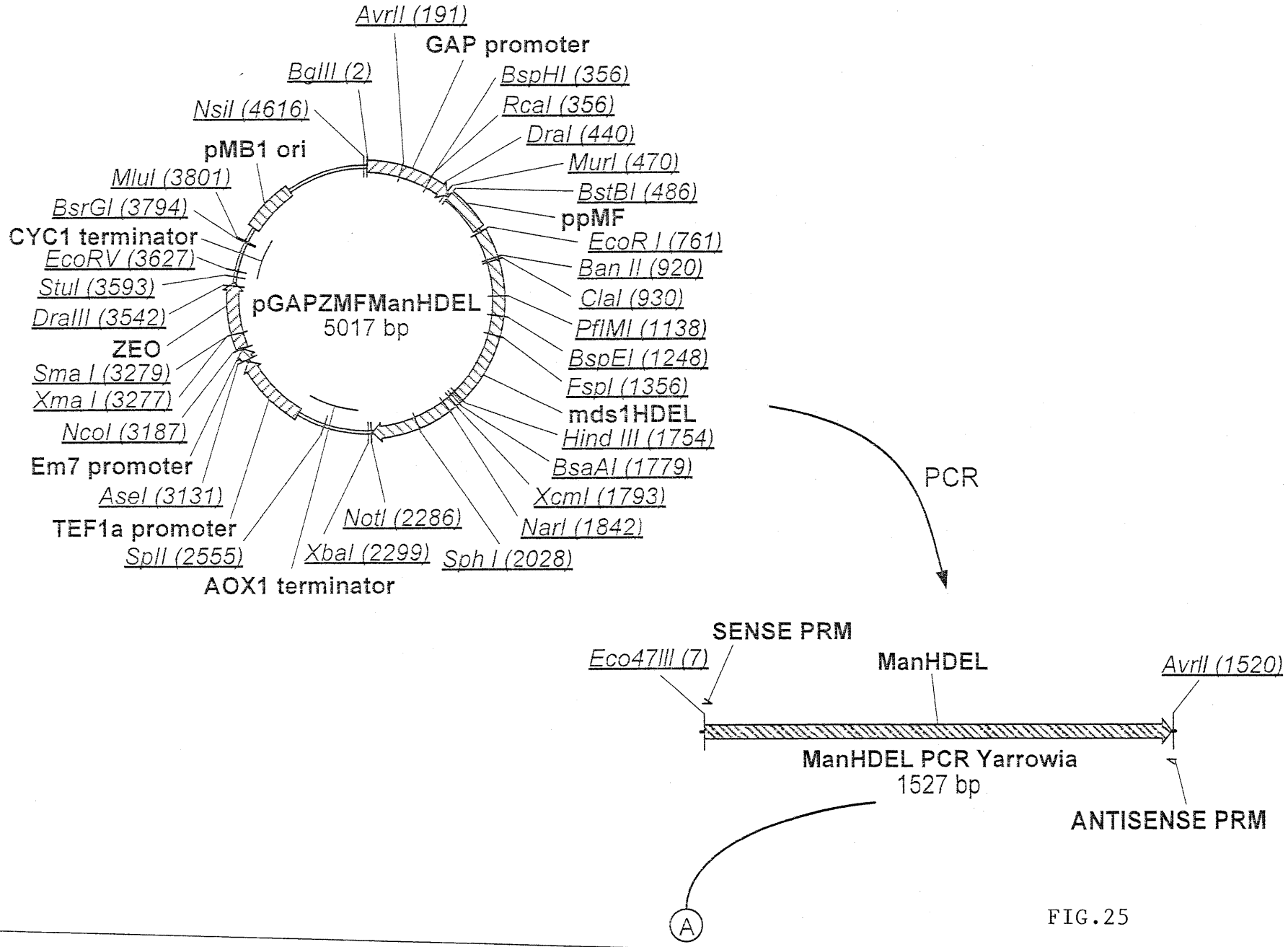

AvrII (191)
GAP promoter
BglII (2)
BspHI (356)
NsiI (4616)
RcaI (356)
pMB1 ori
DraI (440)
MluI (3801)
MurI (470)
BsrGI (3794)
BstBI (486)
CYC1 terminator
ppMF
EcoRV (3627)
EcoR I (761)
StuI (3593)
Ban II (920)
pGAPZMFManHDEL
5017 bp
DraIII (3542)
ClaI (930)
PflMI (1138)
ZEO
BspEI (1248)
Sma I (3279)
FspI (1356)
Xma I (3277)
mds1HDEL
NcoI (3187)
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.25

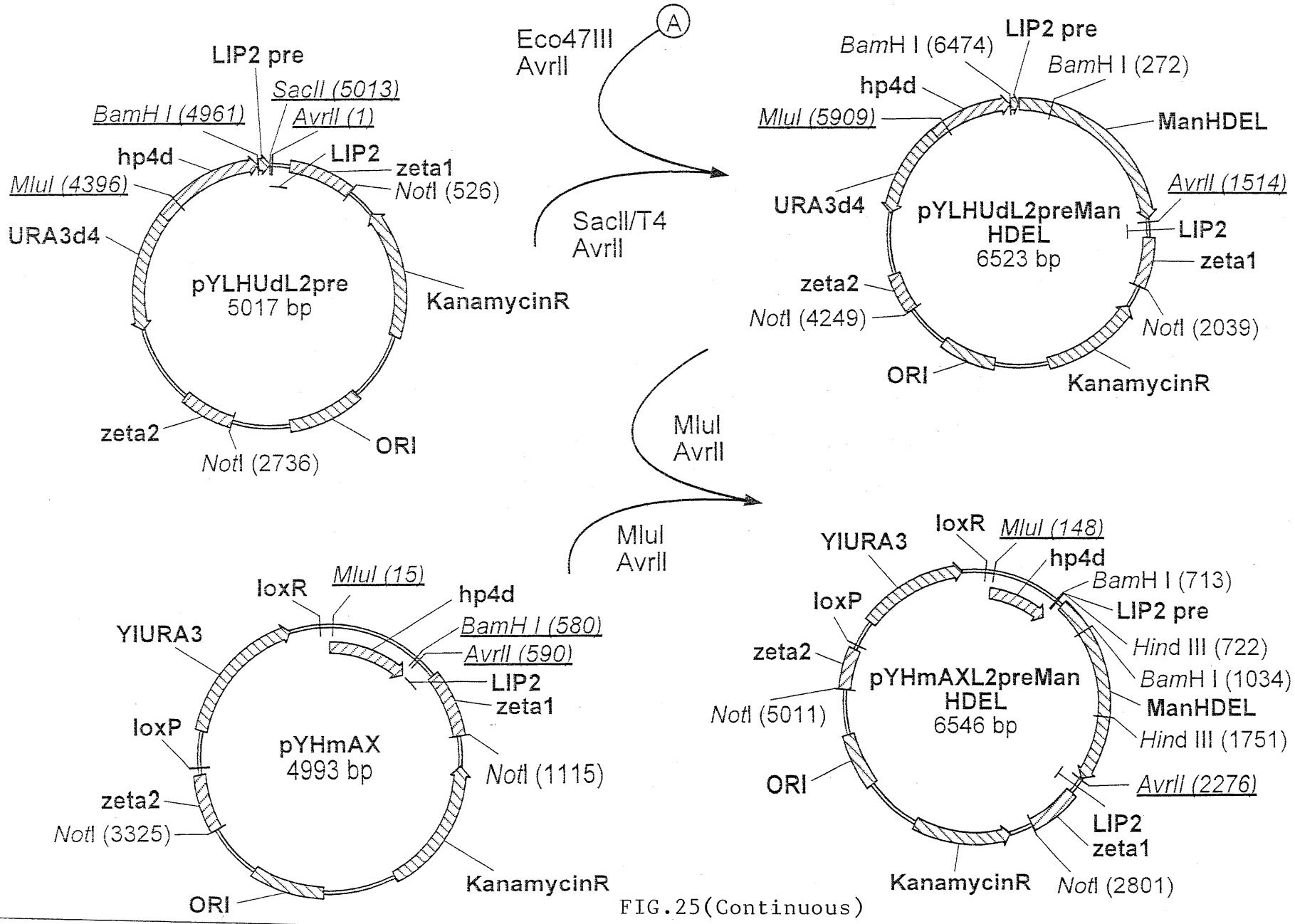


FIG.25(Continuous)

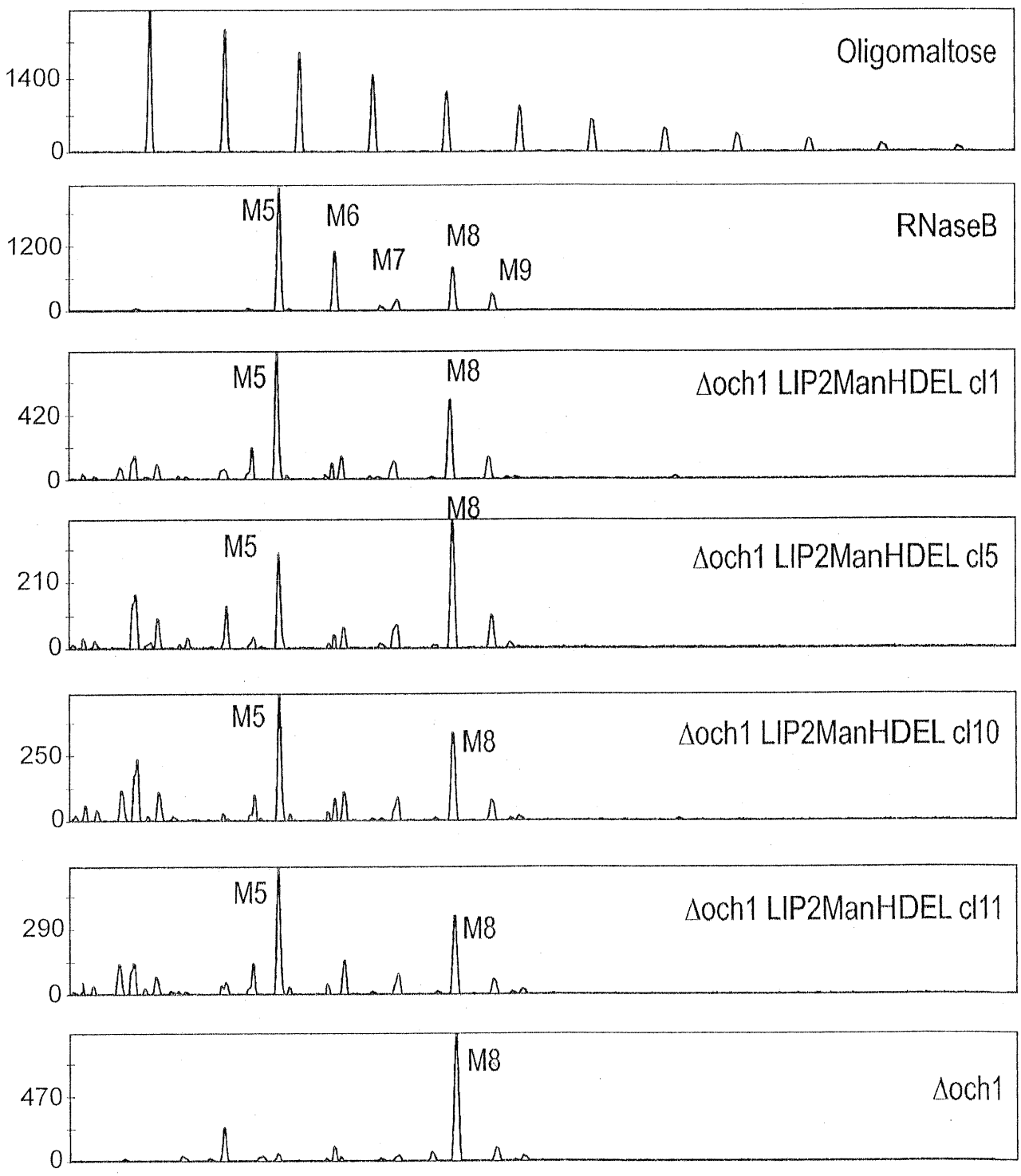

Oligomaltose
1400
0
M5
M6
M7
M8
M9
RNaseB
1200
0
M5
M8
Δoch1 LIP2ManHDEL cl1
420
0
M8
M5
Δoch1 LIP2ManHDEL cl5
210
0
M5
M8
Δoch1 LIP2ManHDEL cl10
250
0
M5
M8
Δoch1 LIP2ManHDEL cl11
290
0
M8
Δoch1
470
0

FIG. 26

MSIKREESFTPTPEDLGSPLTADSPGSPESGDKRKKDLTLP
LPAGALPPRKRAKTENEKEQRRIERIMRNRQAAHASREKKRRH
LEDLEKKCSELSSENNDLHHQVTESKKTNMHLMEQHYSLVAK
LQQLSSLVNMAKSSGALAGVDVPDMSDVSMAPKLEMPTAAPS
QPMGLASAPTLFNHDNETVVPDSPIVKTEEVDSTNFLLHTESSS
PPELAESTGSGSPSSTLSCDETDYLVDRARHPAASAQDFIFIKD
EPVDDELGLHGLSDDFTLFEDNKQPAQHDFIADLAHYESSVSN
LFGGLE

FIG. 27A

MPVDSSHKTASPLPPRKRAKTEEEKEQRRVERILRNRRAA
HASREKKRRHVEFLENHVVDLESALQESAKATNKLKEIQDIIV
SRLEALGGTVSDLDLTVPEVDFPKSSDLEPMSDLSTSSKSEKAS
TSTRRSLTEDLDEDDVAEYDDEEEEDEELPRKMKVLNDKNKSTS
IKQEKLNELPSPLSSDFSDVDEEKSTLTHLKLQQQQQQQPVDNY
VSTPLSLPEDSVDFINPGNLKIESDENFLLSSNTLQIKHENDTD
YITTAPSGSINDFFNSYDISESNRLHHPAAPFTANAFDLNDFVF
FQE

FIG. 27B

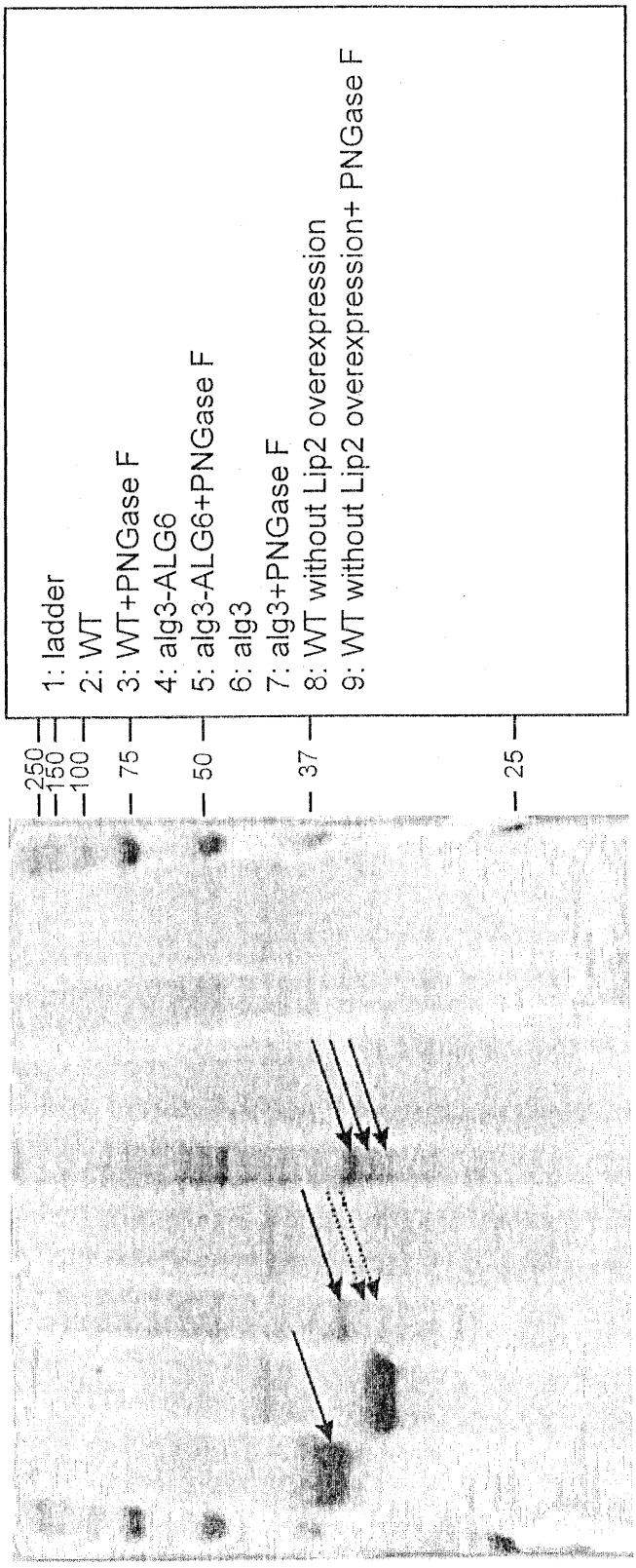

1: ladder
2: WT
3: WT+PNGase F
4: alg3-ALG6
5: alg3-ALG6+PNGase F
6: alg3
7: alg3+PNGase F
8: WT without Lip2 overexpression
9: WT without Lip2 overexpression+ PNGase F
250
150
100
75
50
37
25

FIG. 28

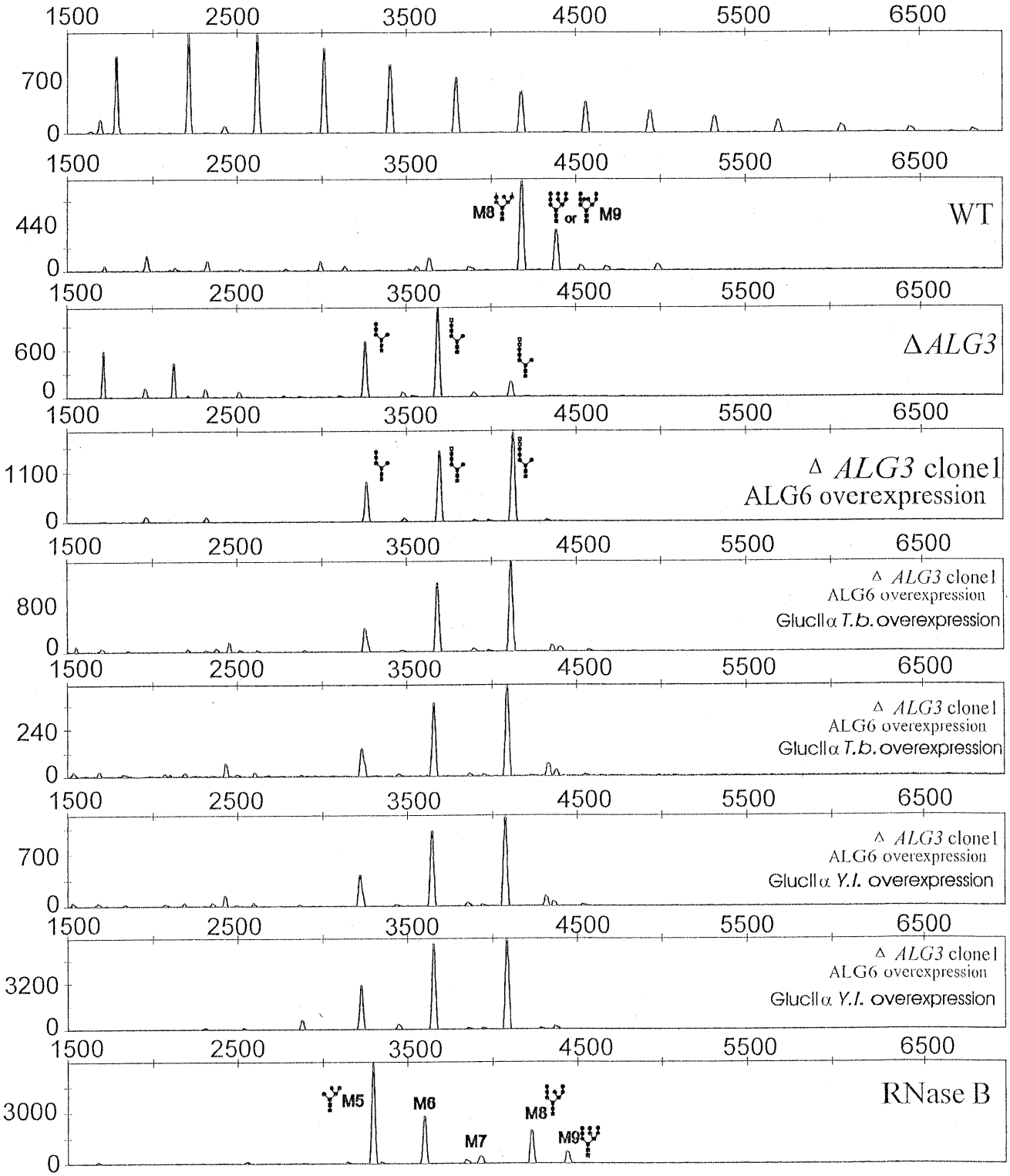

1500
2500
3500
4500
5500
6500
700
0
1500
2500
3500
4500
5500
6500
M8
or
M9
440
0
WT
1500
2500
3500
4500
5500
6500
600
0
ΔALG3
1500
2500
3500
4500
5500
6500
1100
0
Δ ALG3 clone1
ALG6 overexpression
1500
2500
3500
4500
5500
6500
800
0
Δ ALG3 clone1
ALG6 overexpression
GlucIIα T.b. overexpression
1500
2500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

FIG. 29

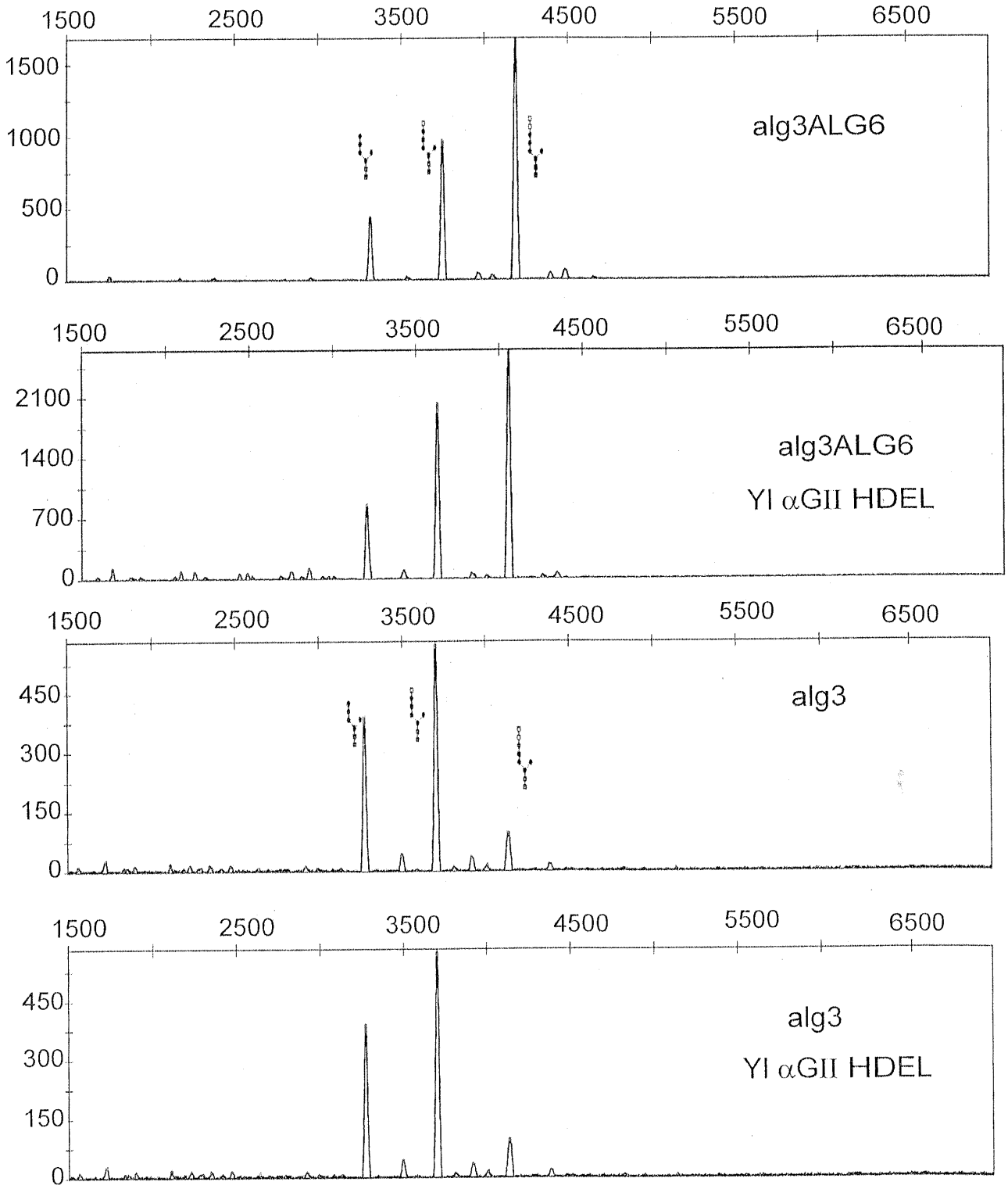

1500
2500
3500
4500
5500
6500
1500
1000
500
0
alg3ALG6
1500
2500
3500
4500
5500
6500
2100
1400
700
0
alg3ALG6
Yl αGII HDEL
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
Yl αGII HDEL

FIG. 30

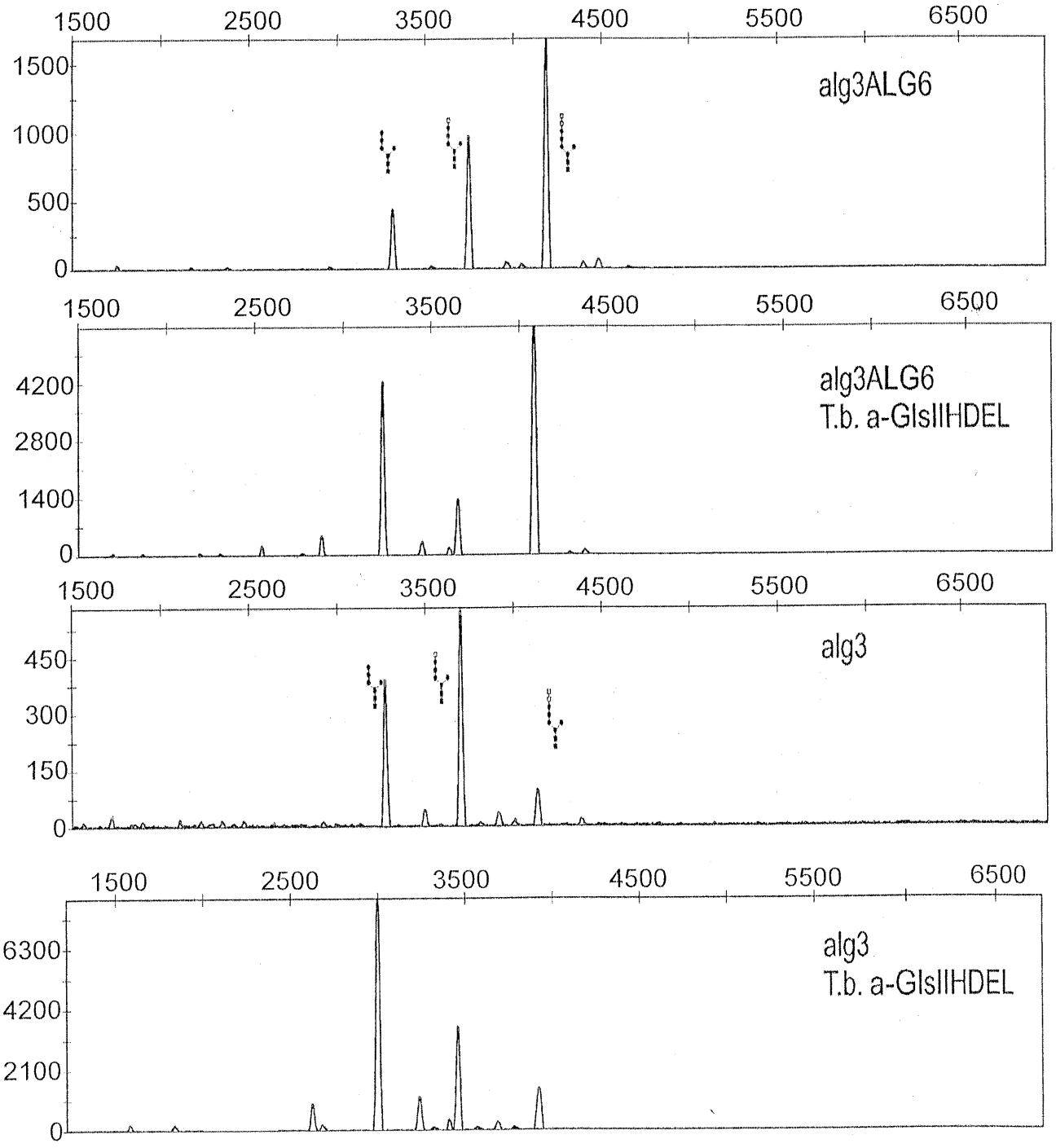

1500
2500
3500
4500
5500
6500
1500
1000
500
0
alg3ALG6
1500
2500
3500
4500
5500
6500
4200
2800
1400
0
alg3ALG6
T.b. a-GlsIIHDEL
1500
2500
3500
4500
5500
6500
450
300
150
0
alg3
1500
2500
3500
4500
5500
6500
6300
4200
2100
0
alg3
T.b. a-GlsIIHDEL

FIG. 31

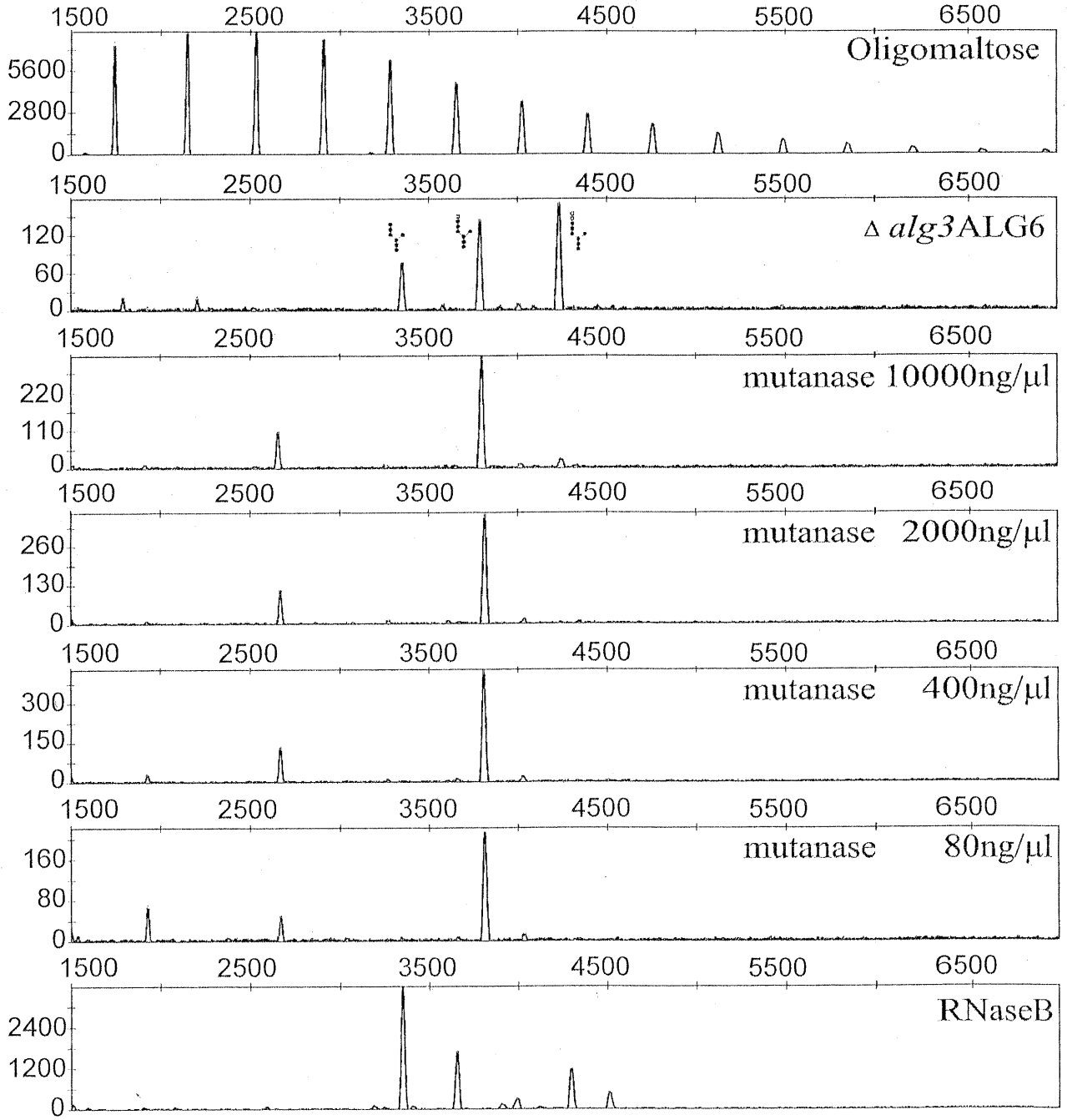

1500
2500
3500
4500
5500
6500
Oligomaltose
5600
2800
0
Δ alg3ALG6
120
60
mutanase 10000ng/μl
220
110
mutanase 2000ng/μl
260
130
mutanase 400ng/μl
300
150
mutanase 80ng/μl
160
80
RNaseB
2400
1200

FIG. 32

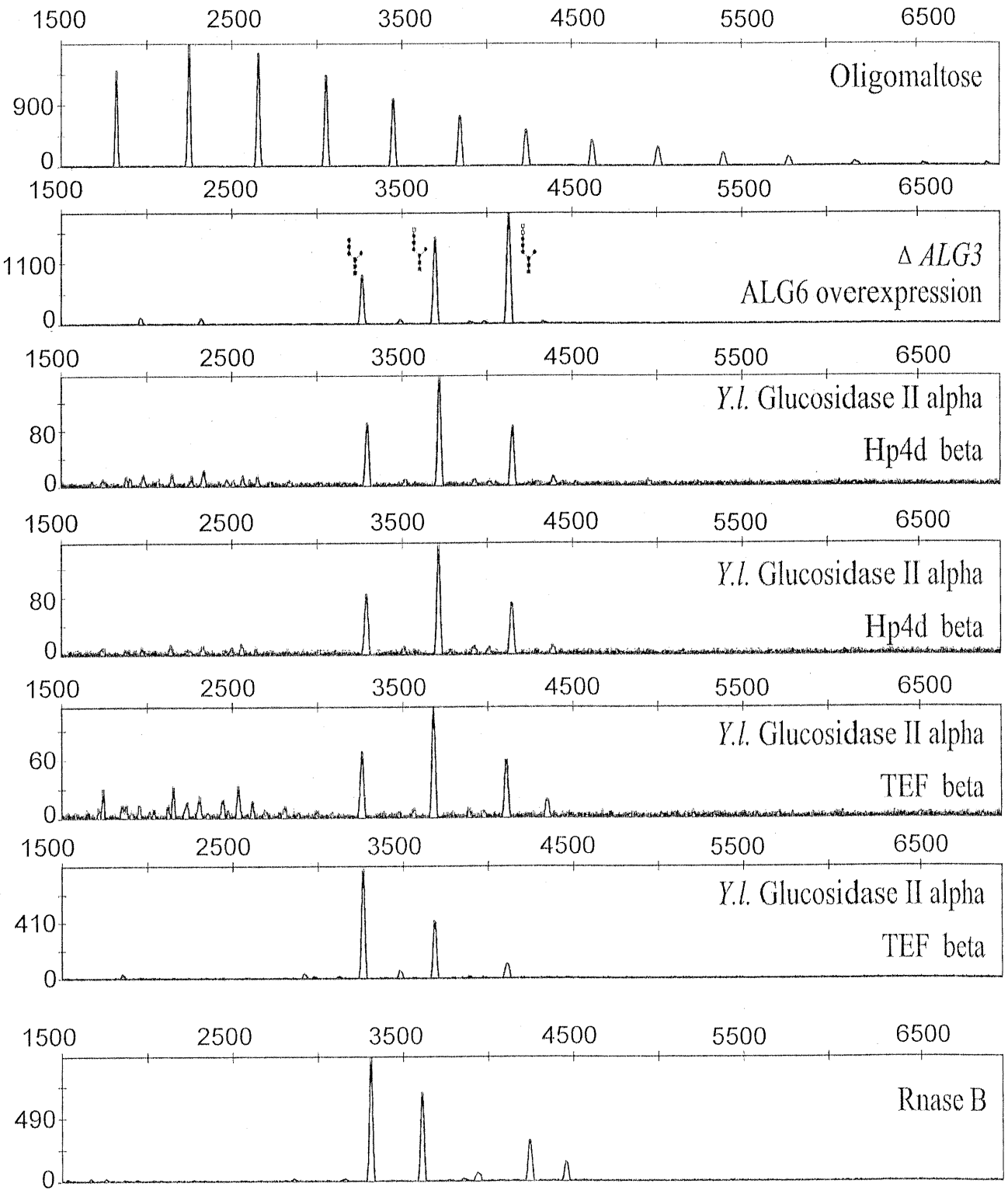

1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
1100
Δ ALG3
ALG6 overexpression
80
Y.l. Glucosidase II alpha
Hp4d beta
60
TEF beta
410
490
Rnase B

FIG. 33

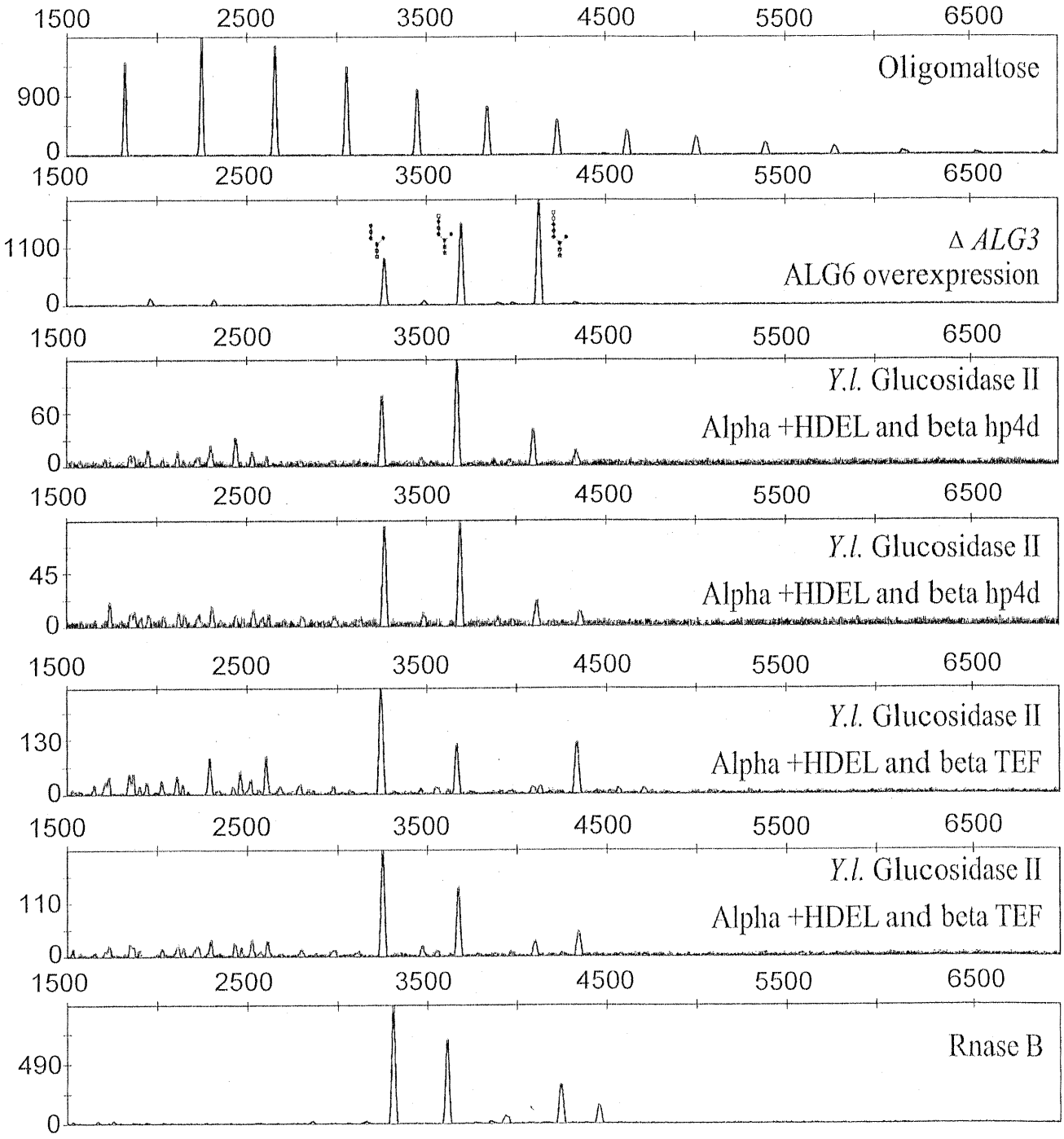

1500
2500
3500
4500
5500
6500
900
0
Oligomaltose
1100
0
Δ ALG3
ALG6 overexpression
60
0
Y.l. Glucosidase II
Alpha +HDEL and beta hp4d
45
0
Y.l. Glucosidase II
Alpha +HDEL and beta hp4d
130
0
Y.l. Glucosidase II
Alpha +HDEL and beta TEF
110
0
Y.l. Glucosidase II
Alpha +HDEL and beta TEF
490
0
Rnase B

FIG. 34

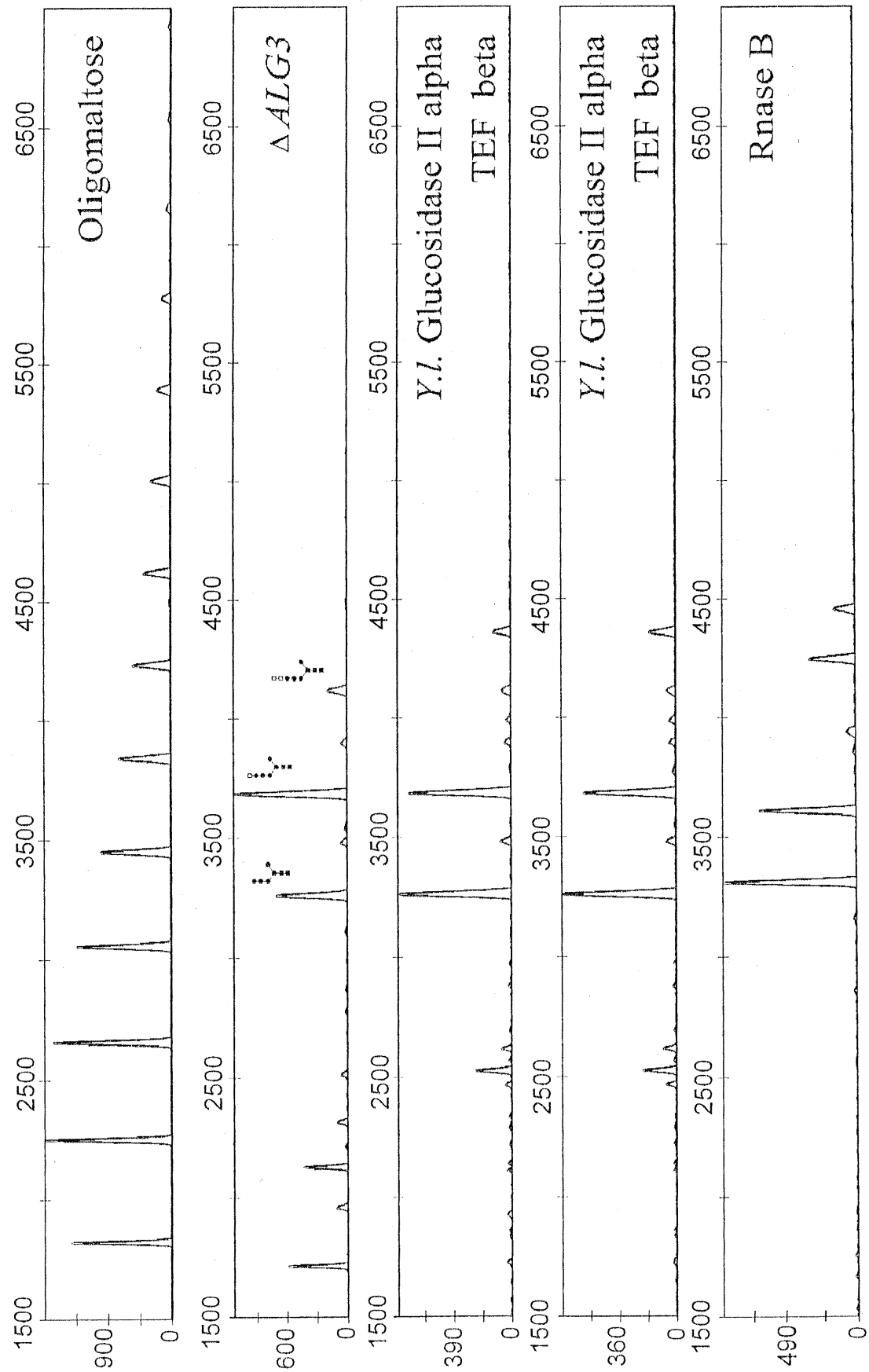

Oligomaltose
ΔALG3
Y.l. Glucosidase II alpha
TEF beta
Y.l. Glucosidase II alpha
TEF beta
Rnase B
1500
2500
3500
4500
5500
6500
900
600
390
360
490
0

FIG. 35

gtaaagcacgagaacttcaagacctgttctcagtctggcttctgtaagcgaaaccgagccttcgccgacgacgccgctg
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FIG.36

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FIG.36(Continuous)

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FIG. 37

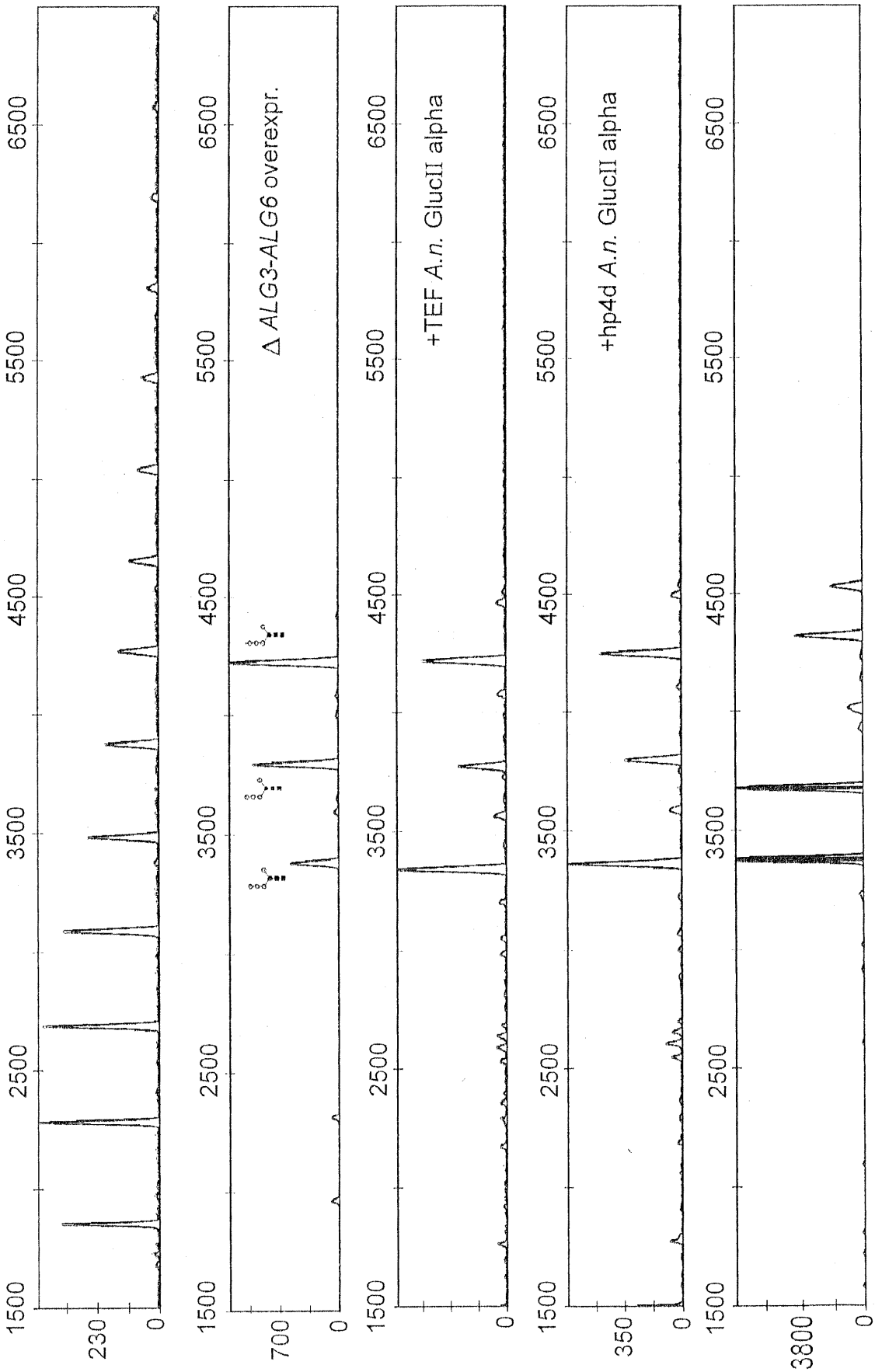

1500
2500
3500
4500
5500
6500
230
0
Δ ALG3-ALG6 overexpr.
700
+TEF A.n. GlucII alpha
+hp4d A.n. GlucII alpha
350
3800

FIG. 38

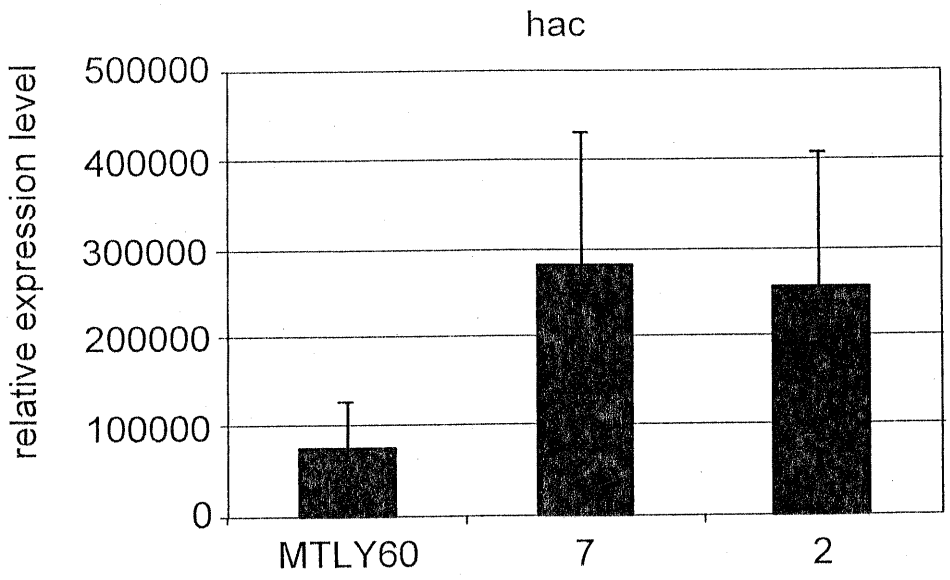


FIG. 39A

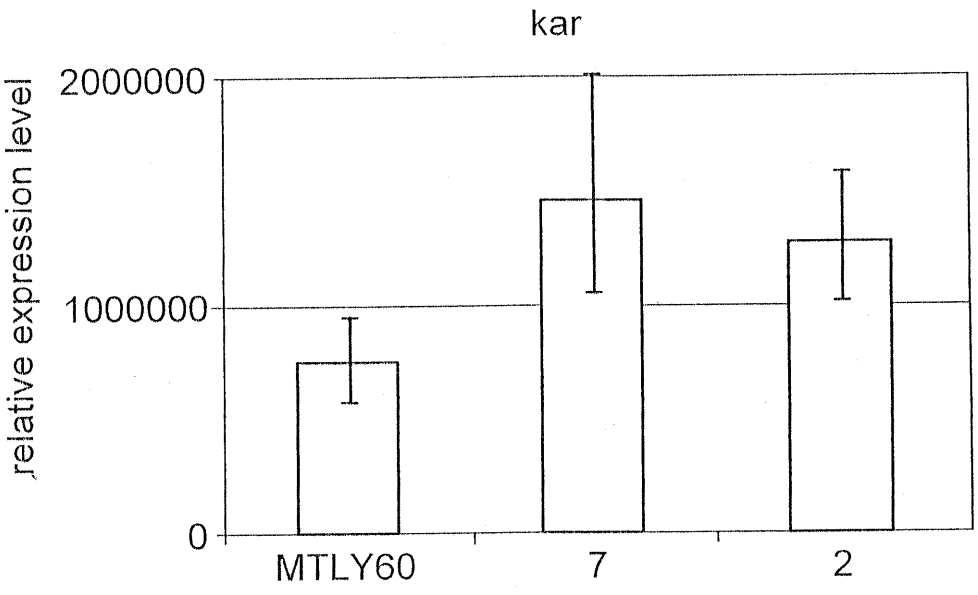
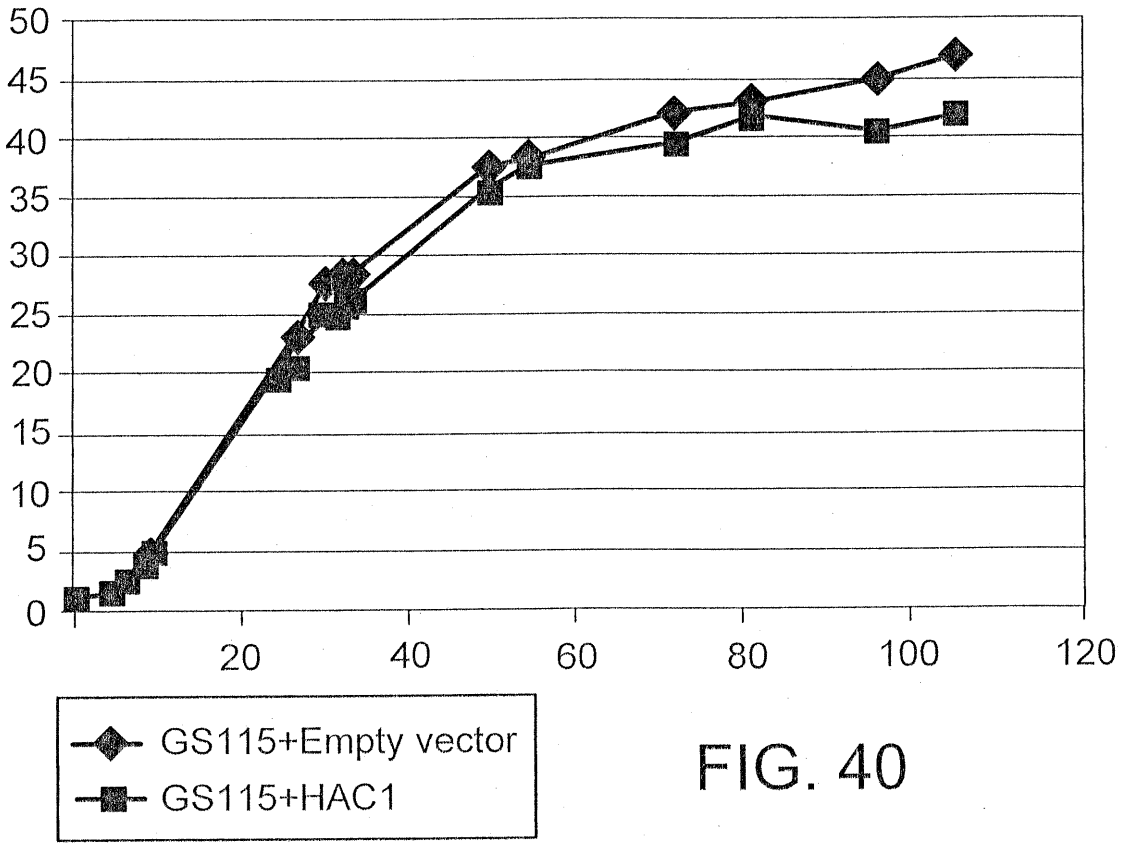


FIG. 39B


50
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40
35
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80
100
120
GS115+Empty vector
GS115+HAC1

FIG. 40

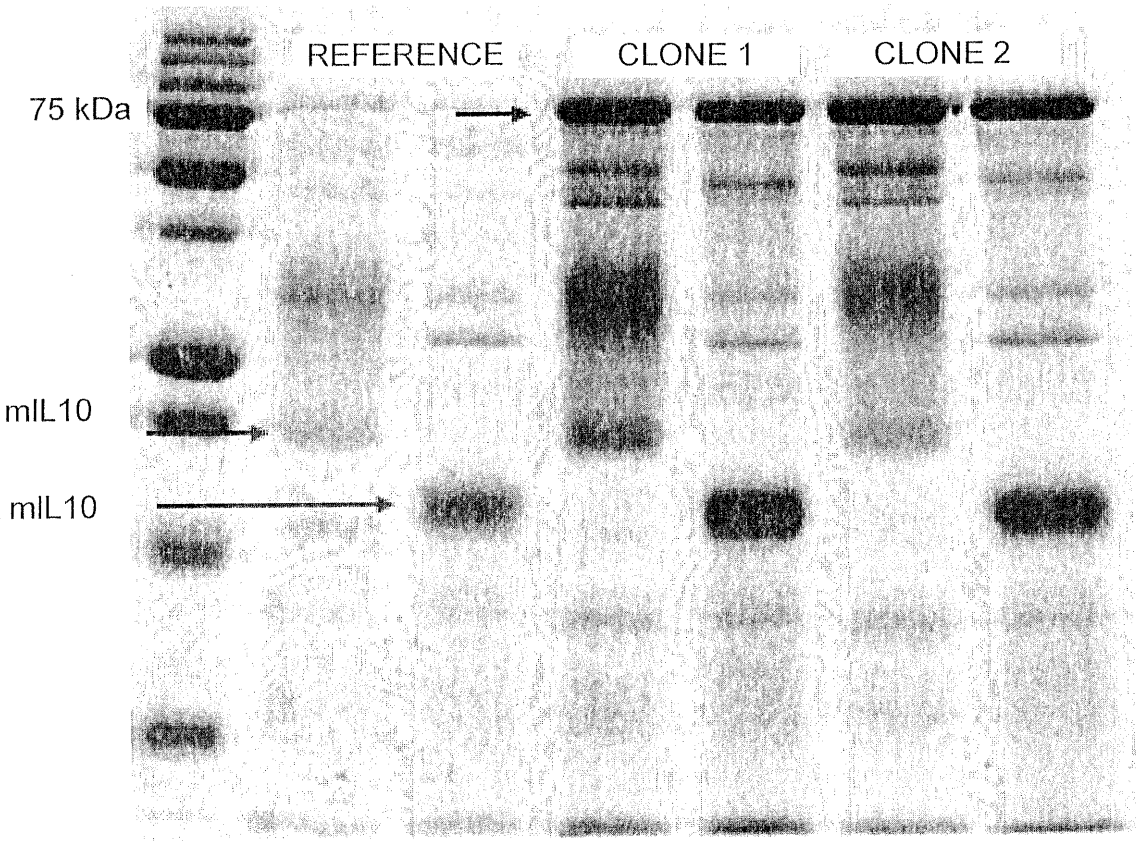

FIG. 41

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FIG. 42

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FIG. 43

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FIG.44

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FIG.44(Continuous)

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FIG.44(Continuous)

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FIG.45

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FIG.45(Continuous)

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FIG.45(Continuous)

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FIG.46

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FIG.46(Continuous)

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FIG.46(Continuous)

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FIG.47

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FIG.47(Continuous)

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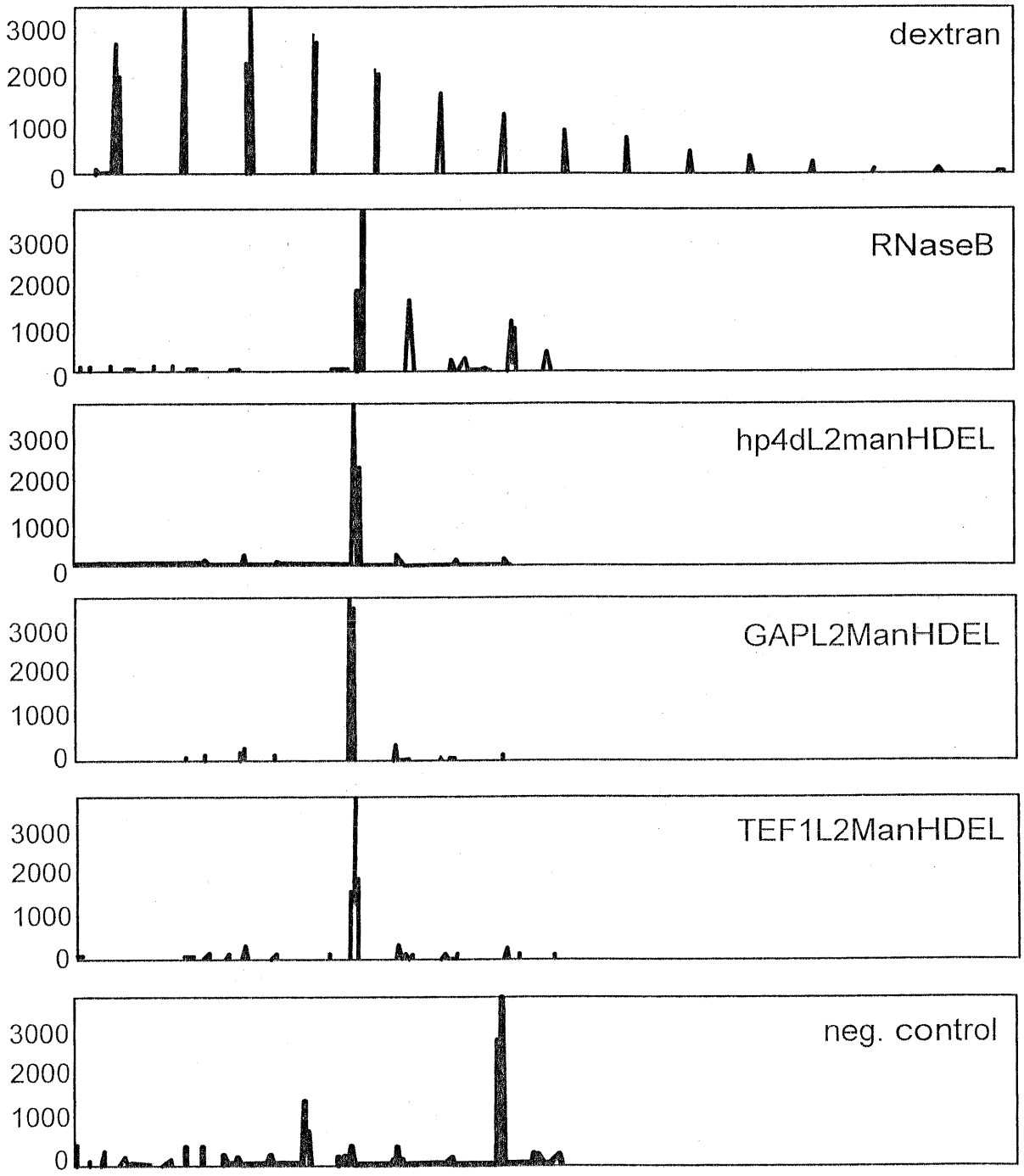
FIG.47(Continuous)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dextran
RNaseB
hp4dL2manHDEL
GAPL2ManHDEL
TEF1L2ManHDEL
neg. control

FIG. 48

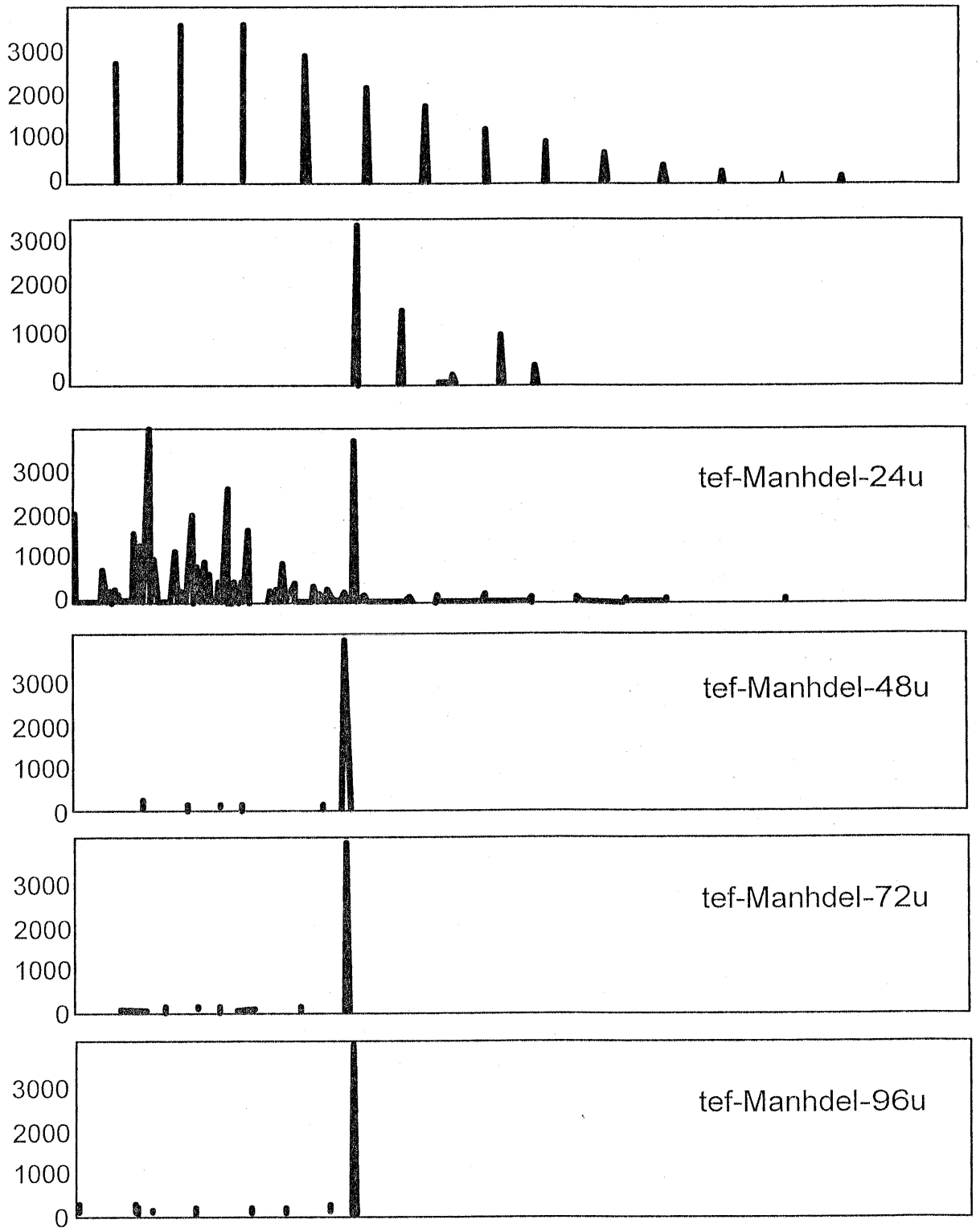
3000
2000
1000
0
tef-Manhdel-24u
tef-Manhdel-48u
tef-Manhdel-72u
tef-Manhdel-96u

FIG. 49

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FIG. 50

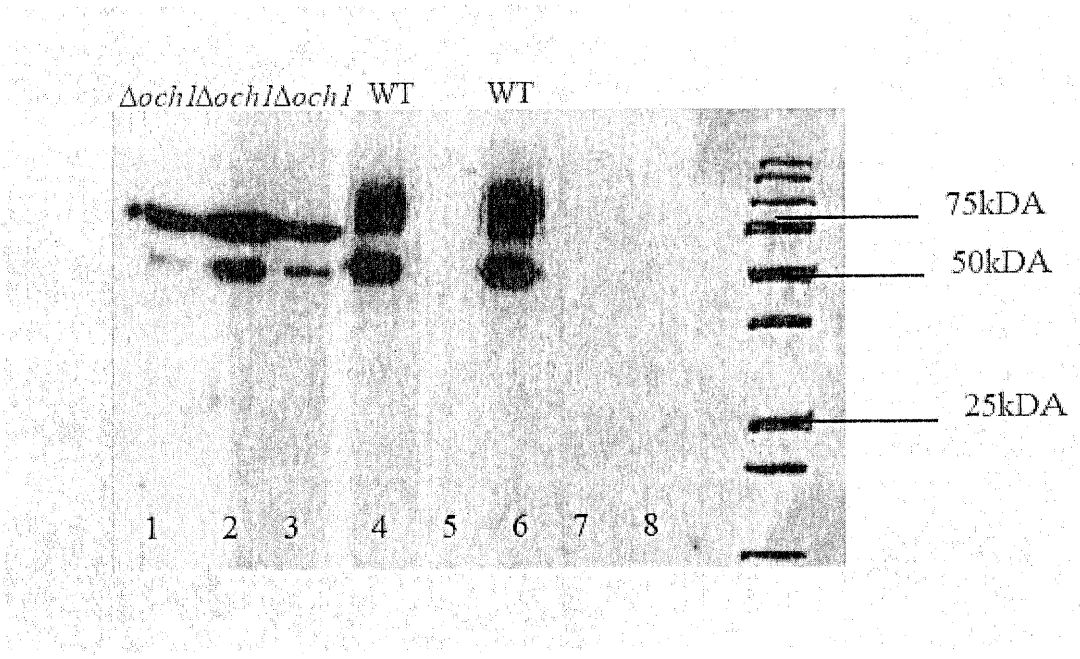

FIG. 51

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FIG. 52

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FIG. 53

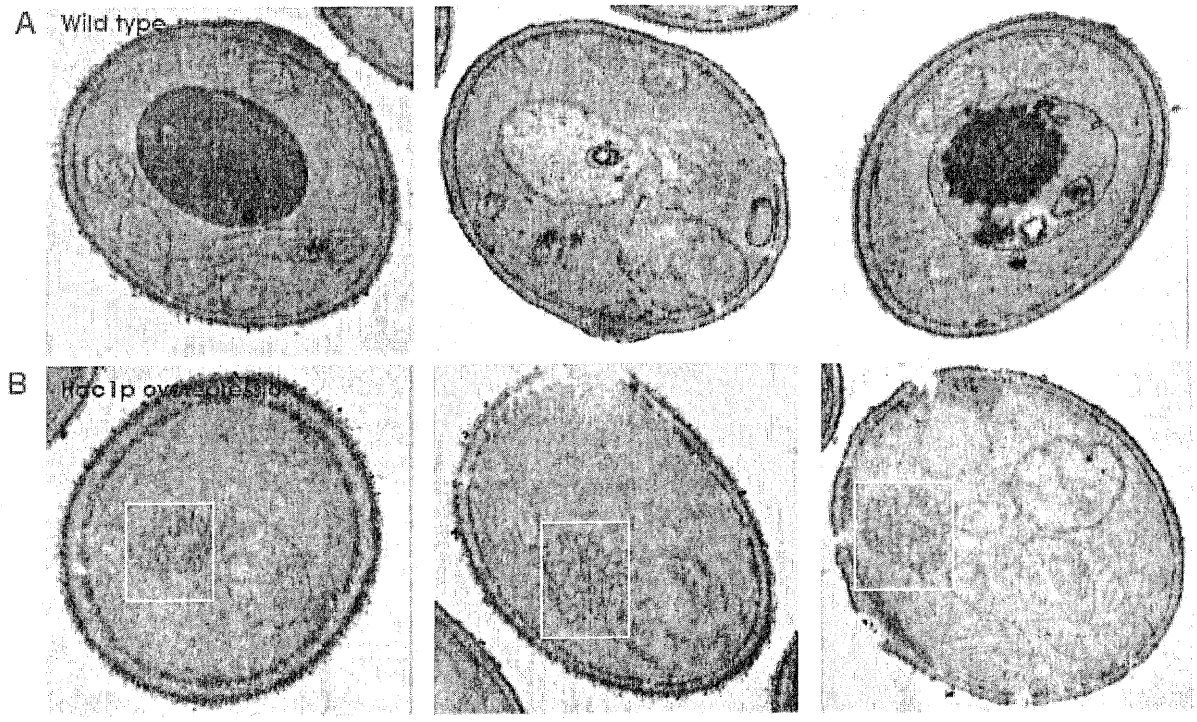
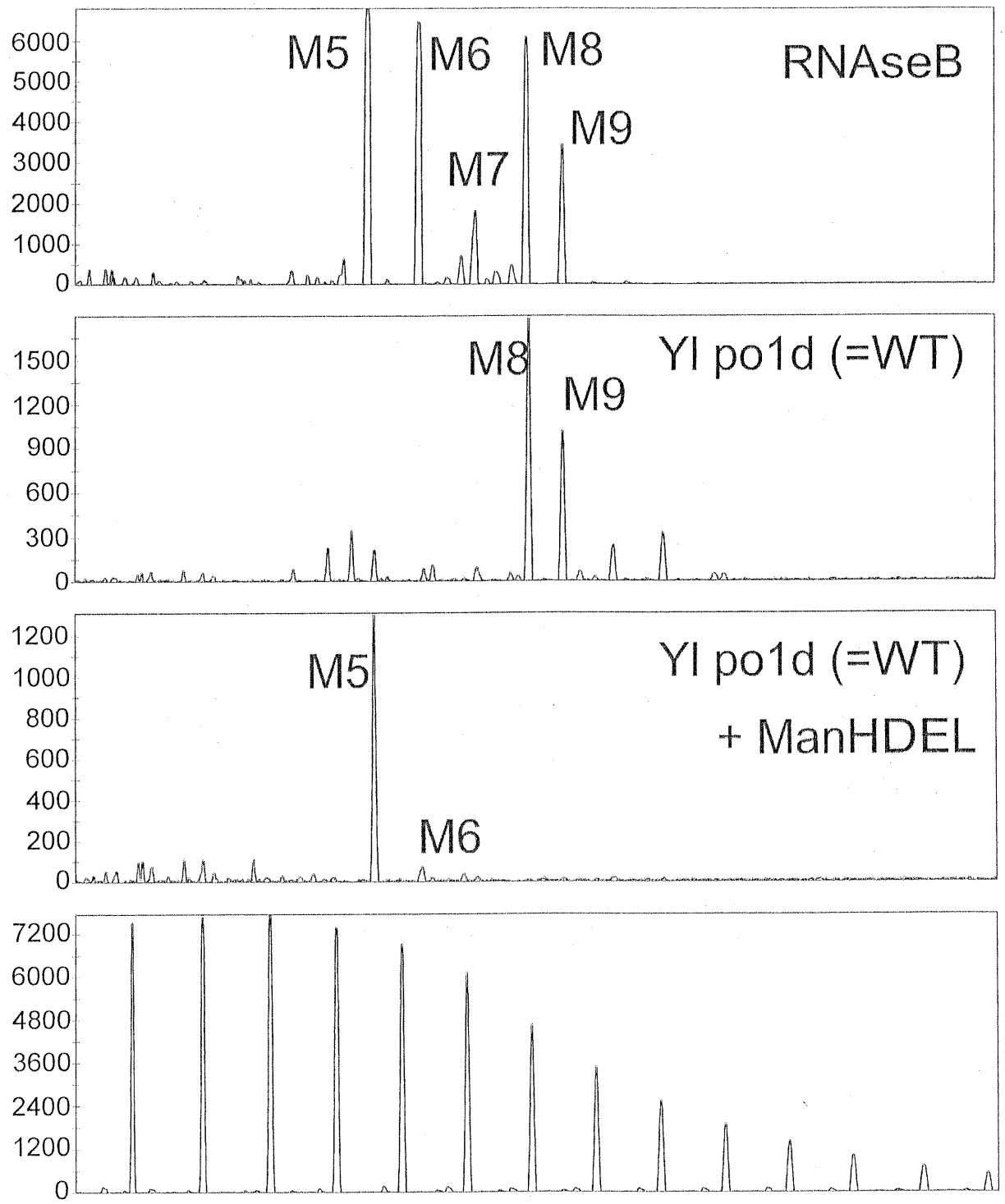


FIG. 54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M9
M7
RNAseB
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300
0
M8
M9
YI po1d (=WT)
1200
1000
800
600
400
200
0
M5
M6
YI po1d (=WT)
+ ManHDEL
7200
6000
4800
3600
2400
1200
0

FIG. 55