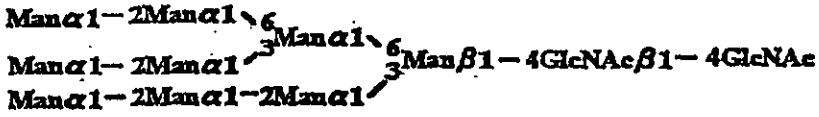
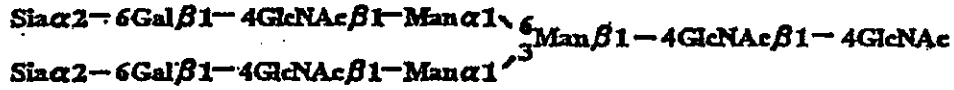
1) High Mannose-Type




2) Complex-Type




3) Hybrid-Type

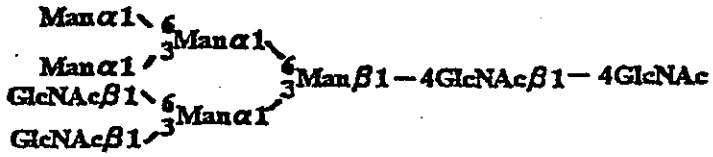



FIG 1

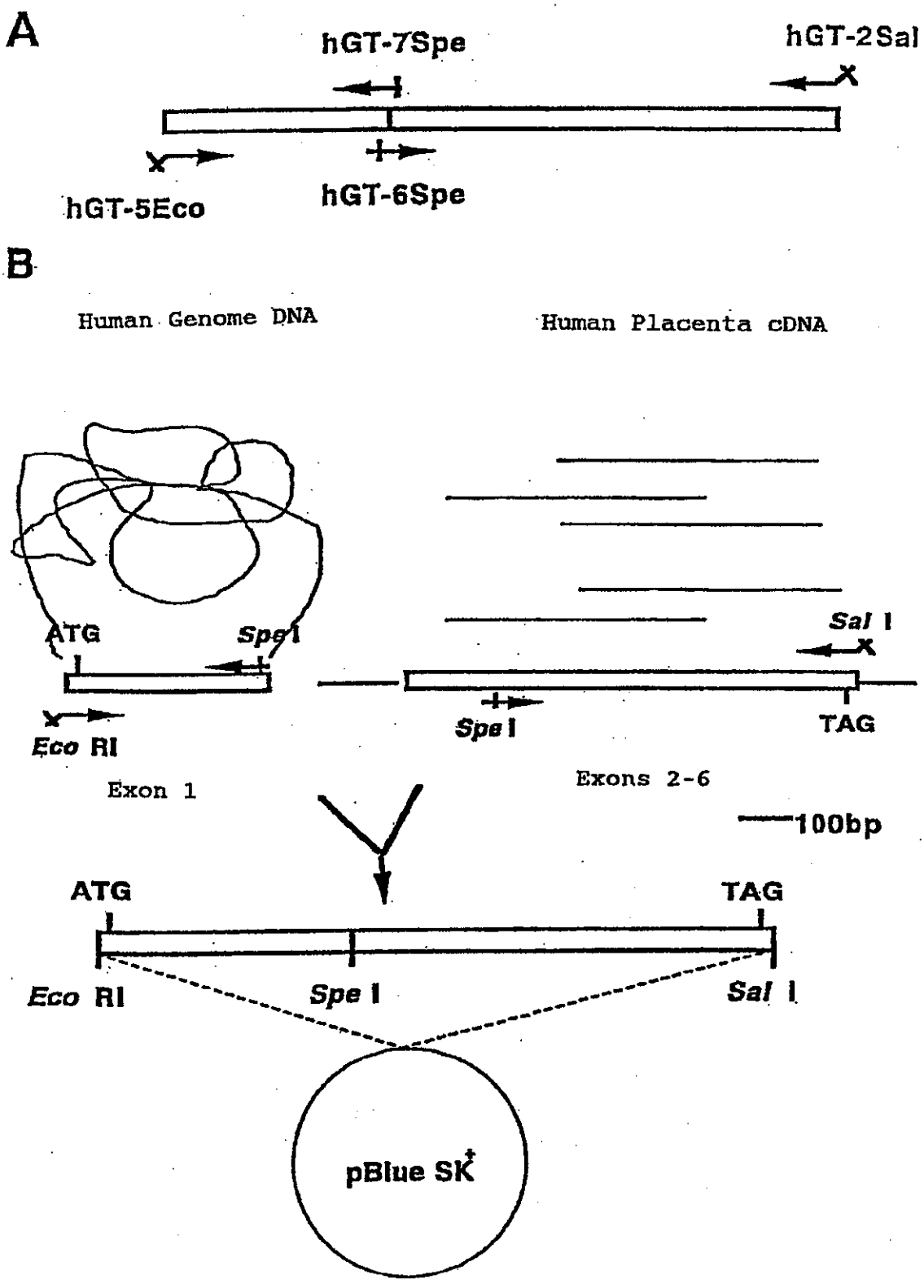

A
hGT-7Spe
hGT-2Sal
hGT-5Eco
hGT-6Spe
B
Human Genome DNA
Human Placenta cDNA
ATG
Spe I
Sal I
Eco RI
Spe I
TAG
Exon 1
Exons 2-6
100bp
ATG
TAG
Eco RI
Spe I
Sal I
pBlue SK+

FIG 2

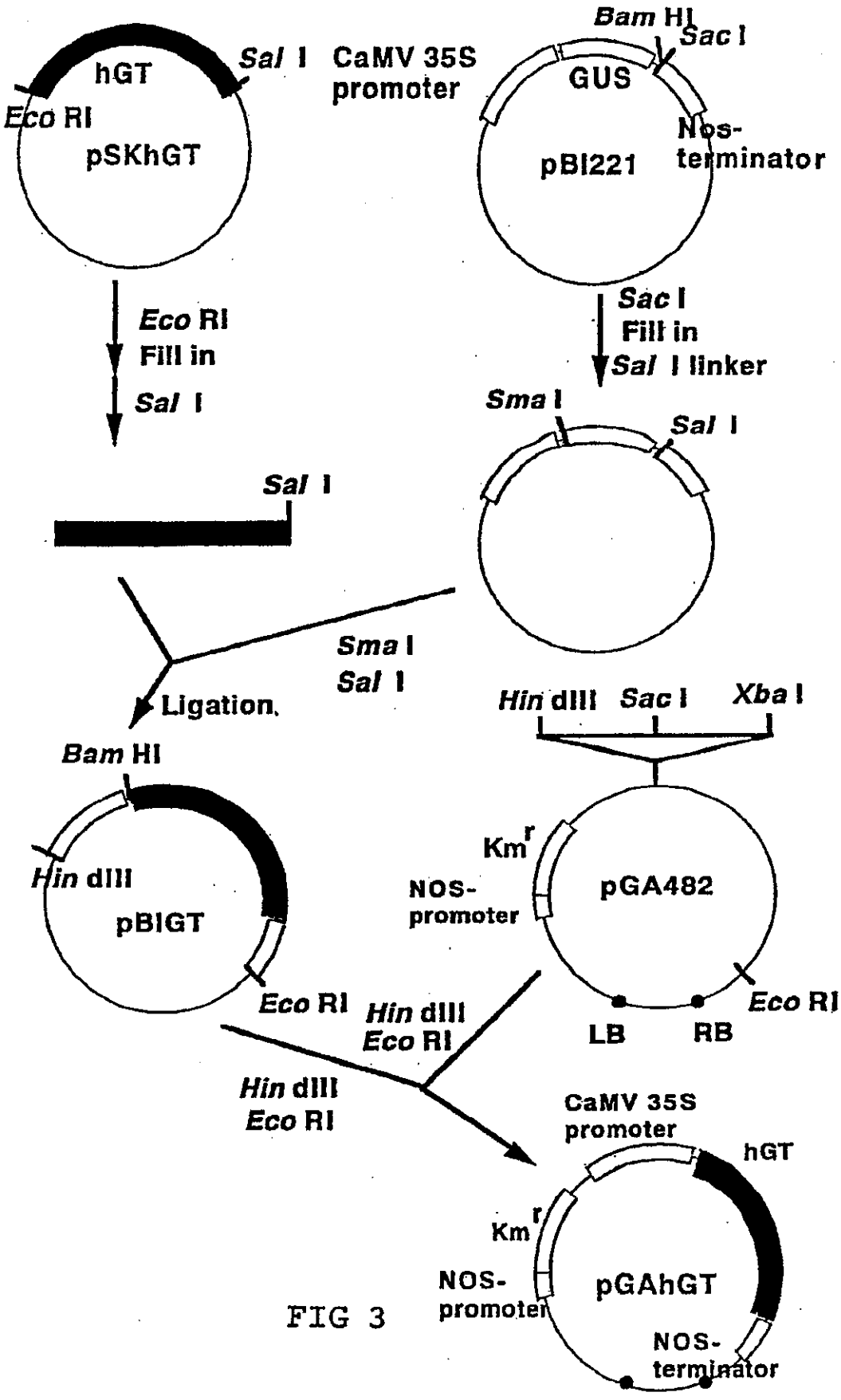

hGT
Sal I
CaMV 35S promoter
Eco RI
pSKhGT
Bam HI
Sac I
GUS
Nos-terminator
pBI221
Eco RI
Fill in
Sal I
Sac I
Fill in
Sal I linker
Sma I
Sal I
Sal I
Sma I
Sal I
Ligation
Hin dIII
Sac I
Xba I
Bam HI
Hin dIII
pBIGT
Eco RI
Km r
NOS-promoter
pGA482
Eco RI
LB
RB
Hin dIII
Eco RI
Hin dIII
Eco RI
CaMV 35S promoter
hGT
Km r
NOS-promoter
pGAhGT
NOS-terminator

FIG 3

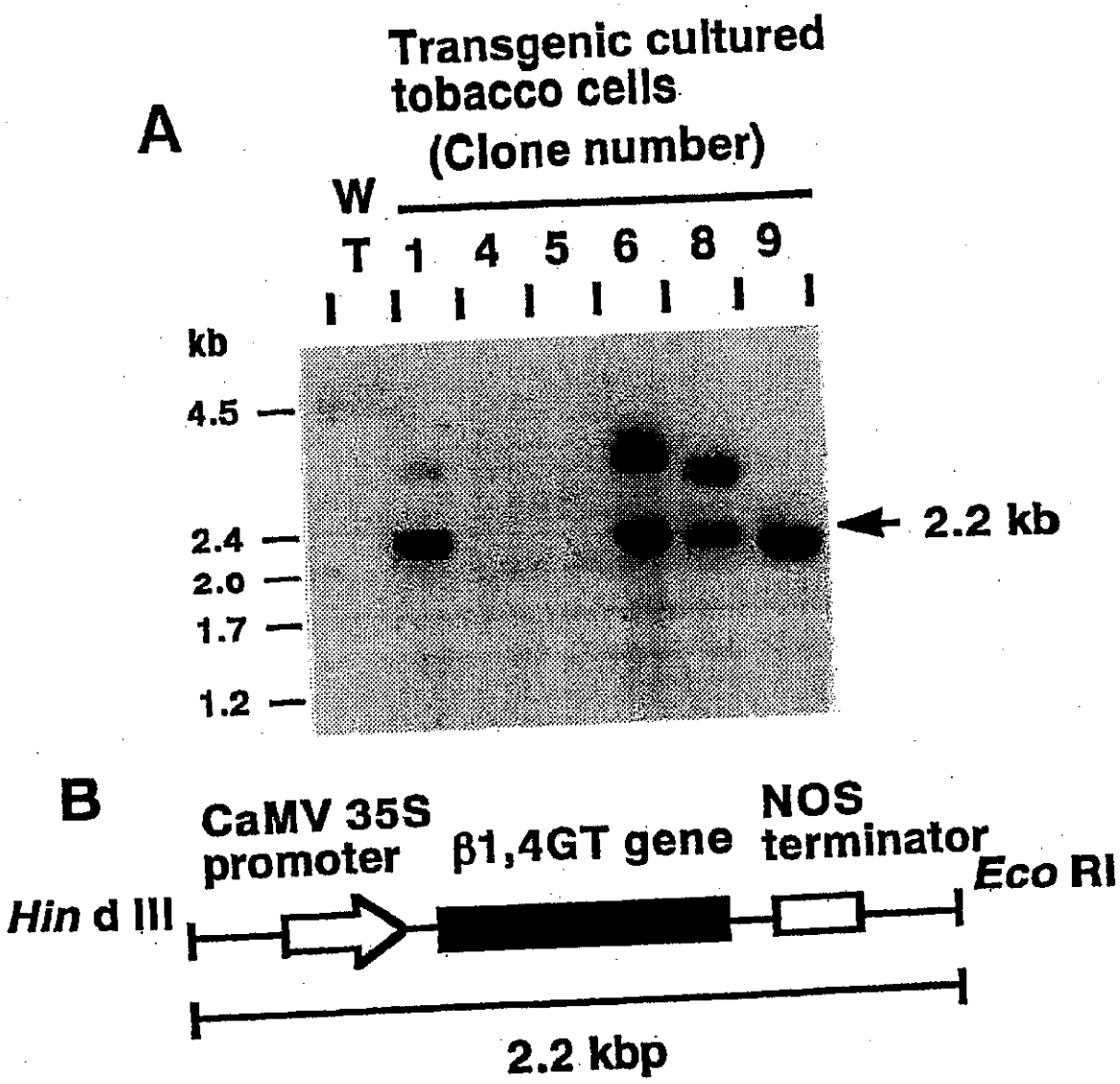

A
Transgenic cultured tobacco cells
(Clone number)
W
T 1 4 5 6 8 9
kb
4.5
2.4
2.0
1.7
1.2
2.2 kb
B
CaMV 35S promoter
β1,4GT gene
NOS terminator
Hin d III
Eco RI
2.2 kbp

FIG 4

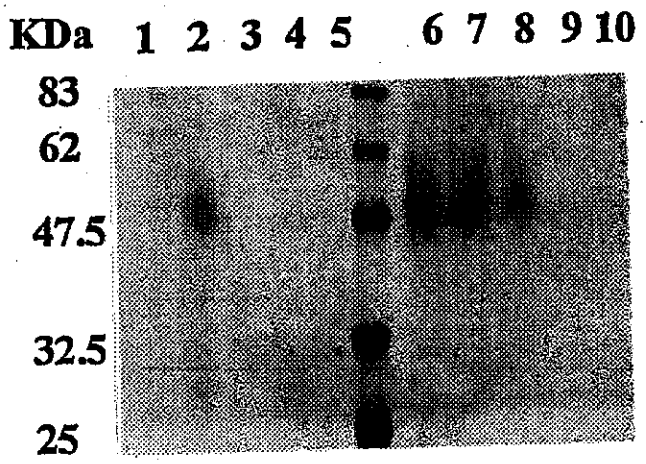



FIG 5

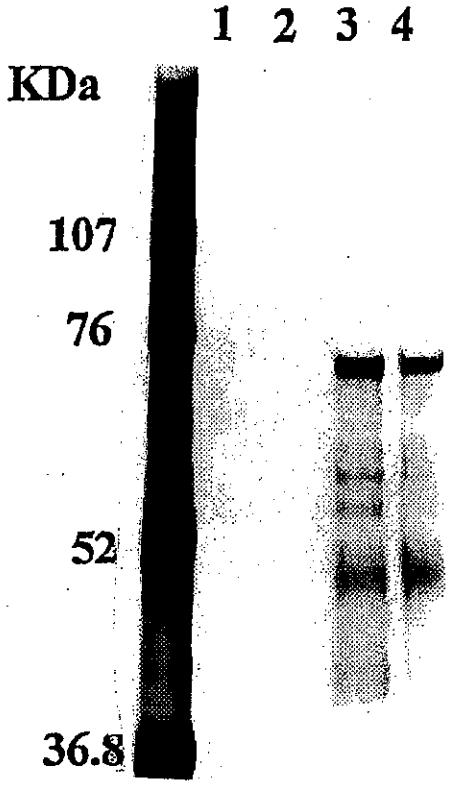



FIG 6

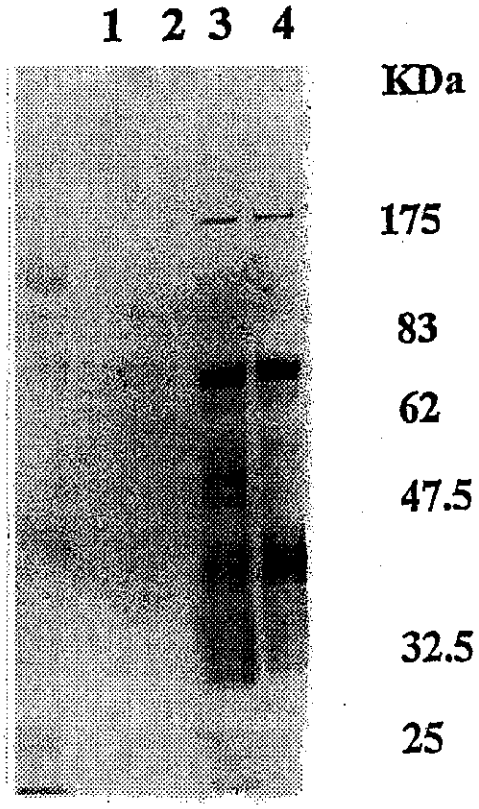



FIG 7

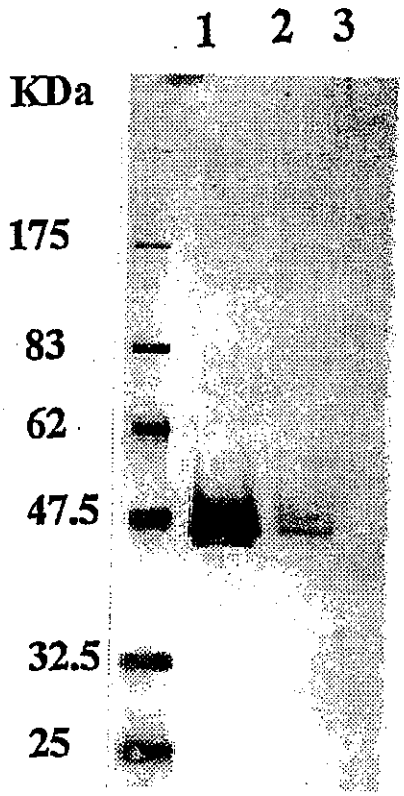



FIG 8

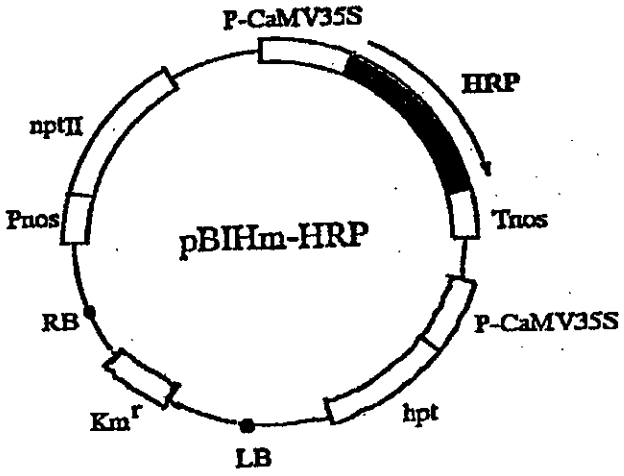

P-CaMV35S
HRP
nptII
Pnos
pBIHm-HRP
Tnos
RB
P-CaMV35S
Km^r
hpt
LB

FIG 9

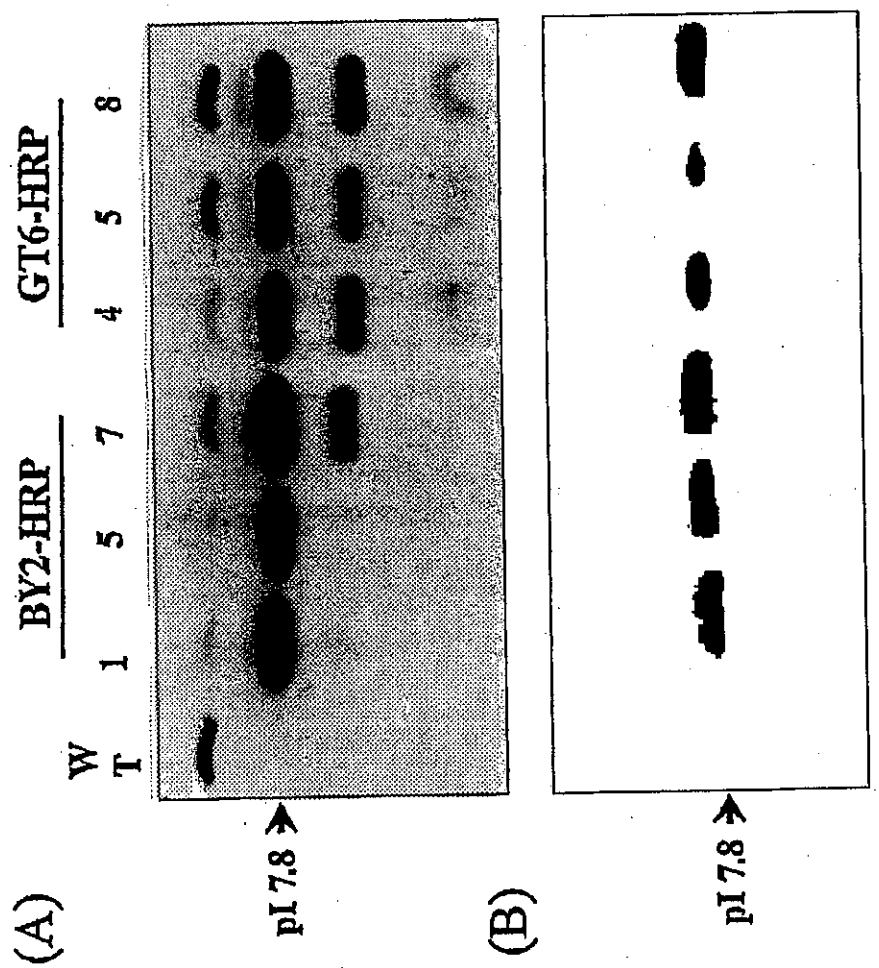



FIG 10

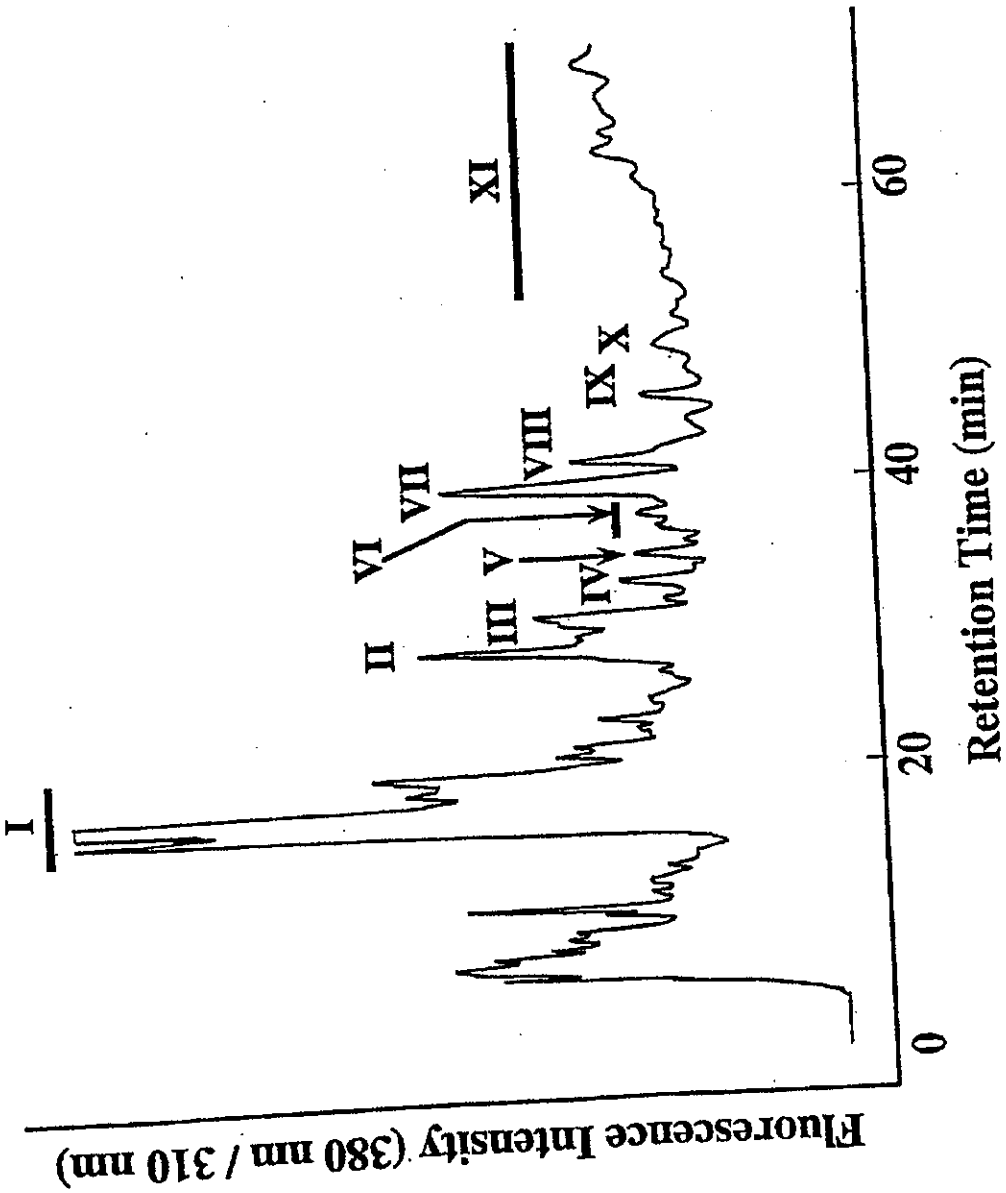

Fluorescence Intensity (380 nm / 310 nm)
Retention Time (min)
0
20
40
60
I
II
III
IV
V
VI
VII
VIII
IX
X
XI

FIG 11

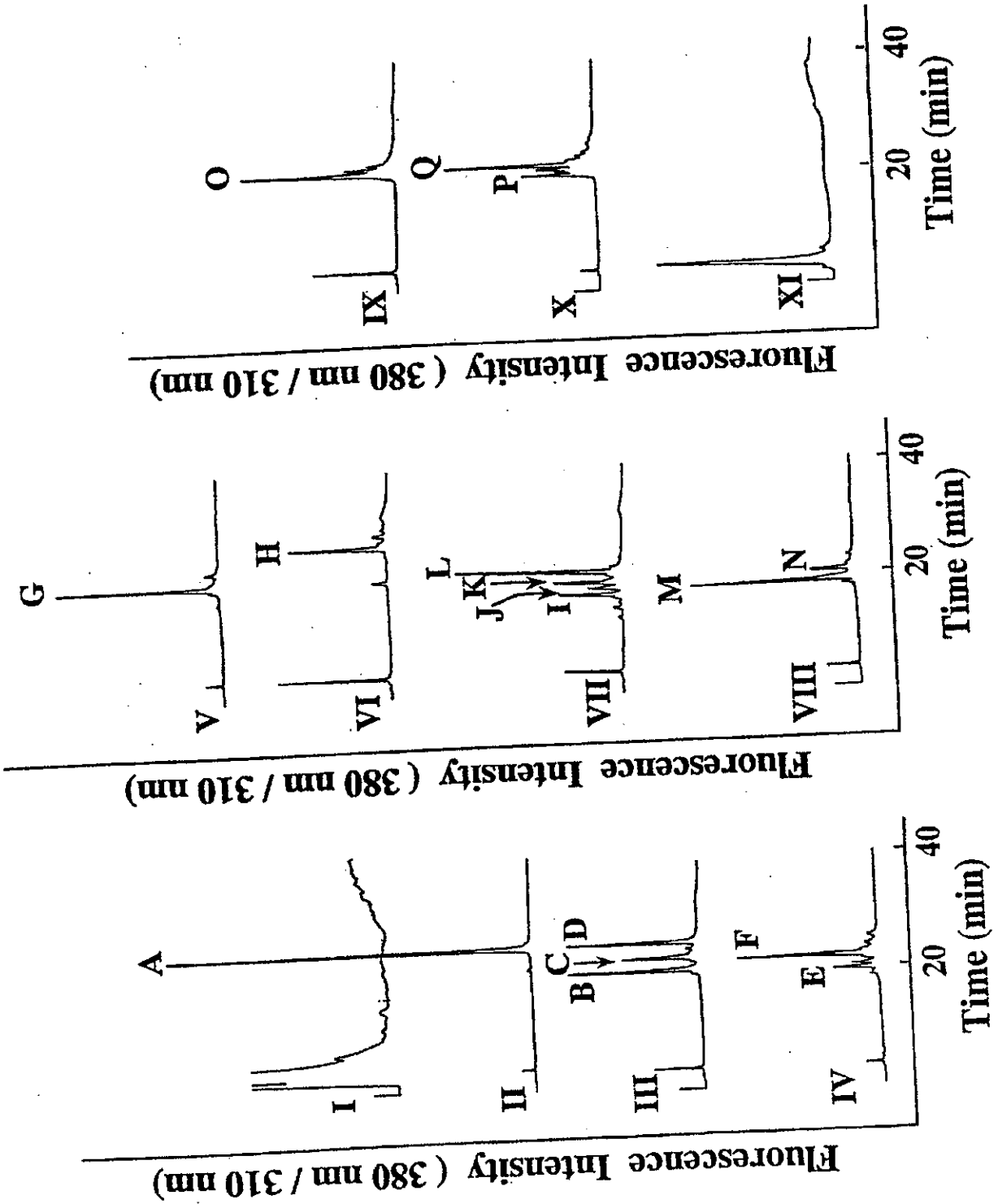

Fluorescence Intensity ( 380 nm / 310 nm)
Time (min)
20
40
A
B
C
D
E
F
I
II
III
IV
G
H
J
K
L
M
N
V
VI
VII
VIII
O
P
Q
IX
X
XI

FIG 12

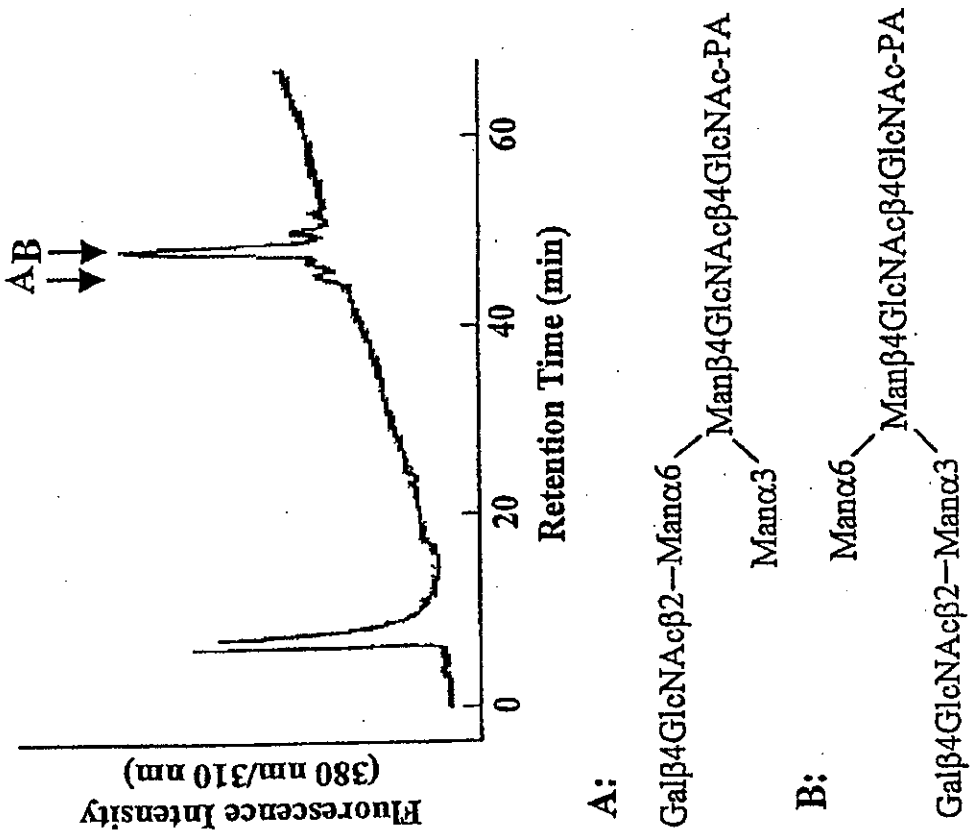

AB
Fluorescence Intensity
(380 nm/310 nm)
0
20
40
60
Retention Time (min)
A:
Galβ4GlcNAcβ2–Manα6
Manβ4GlcNAcβ4GlcNAc-PA
Manα3
B:
Manα6
Manβ4GlcNAcβ4GlcNAc-PA
Galβ4GlcNAcβ2–Manα3

FIG 13

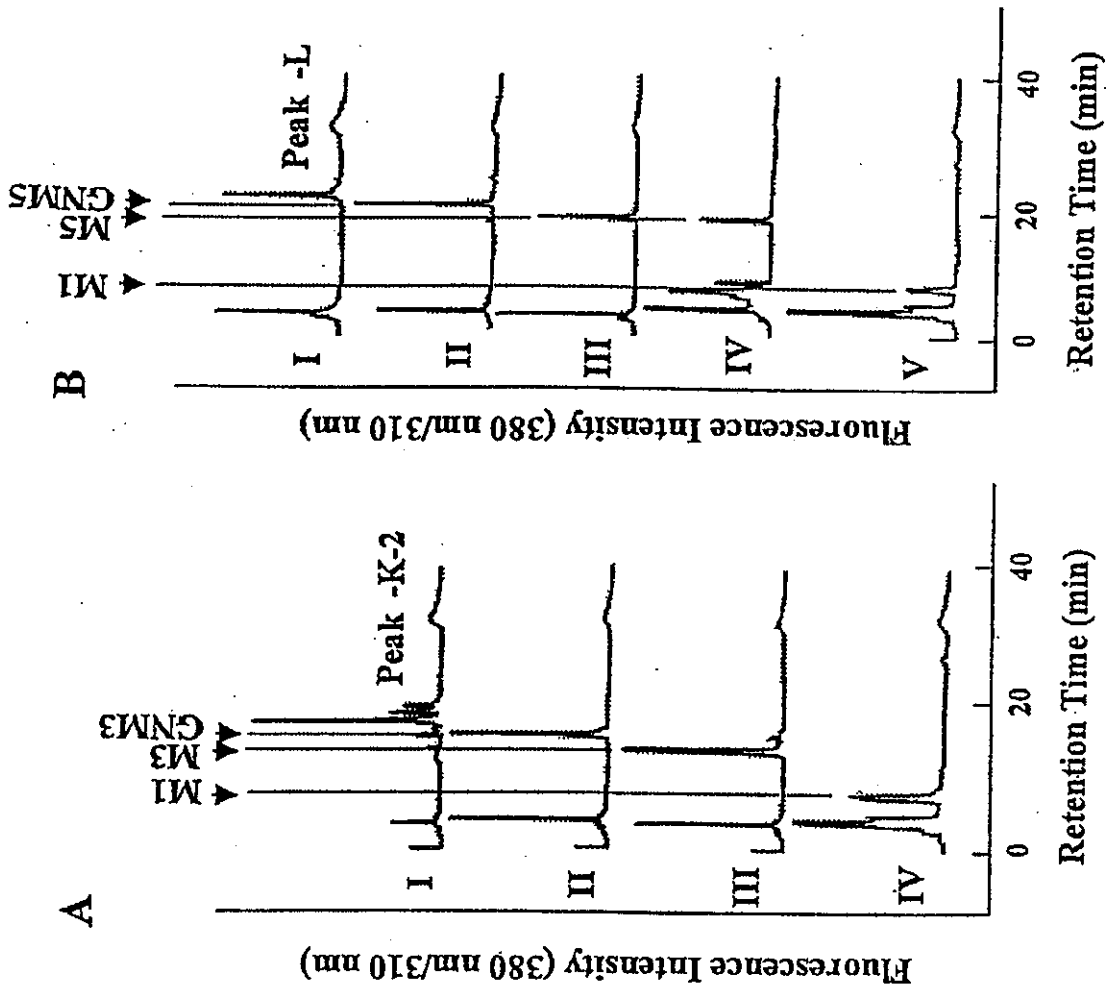

A
B
Fluorescence Intensity (380 nm/310 nm)
Retention Time (min)
0
20
40
I
II
III
IV
V
M1
M3
GNM3
MS
GNMS
Peak -K-2
Peak -L

FIG 14

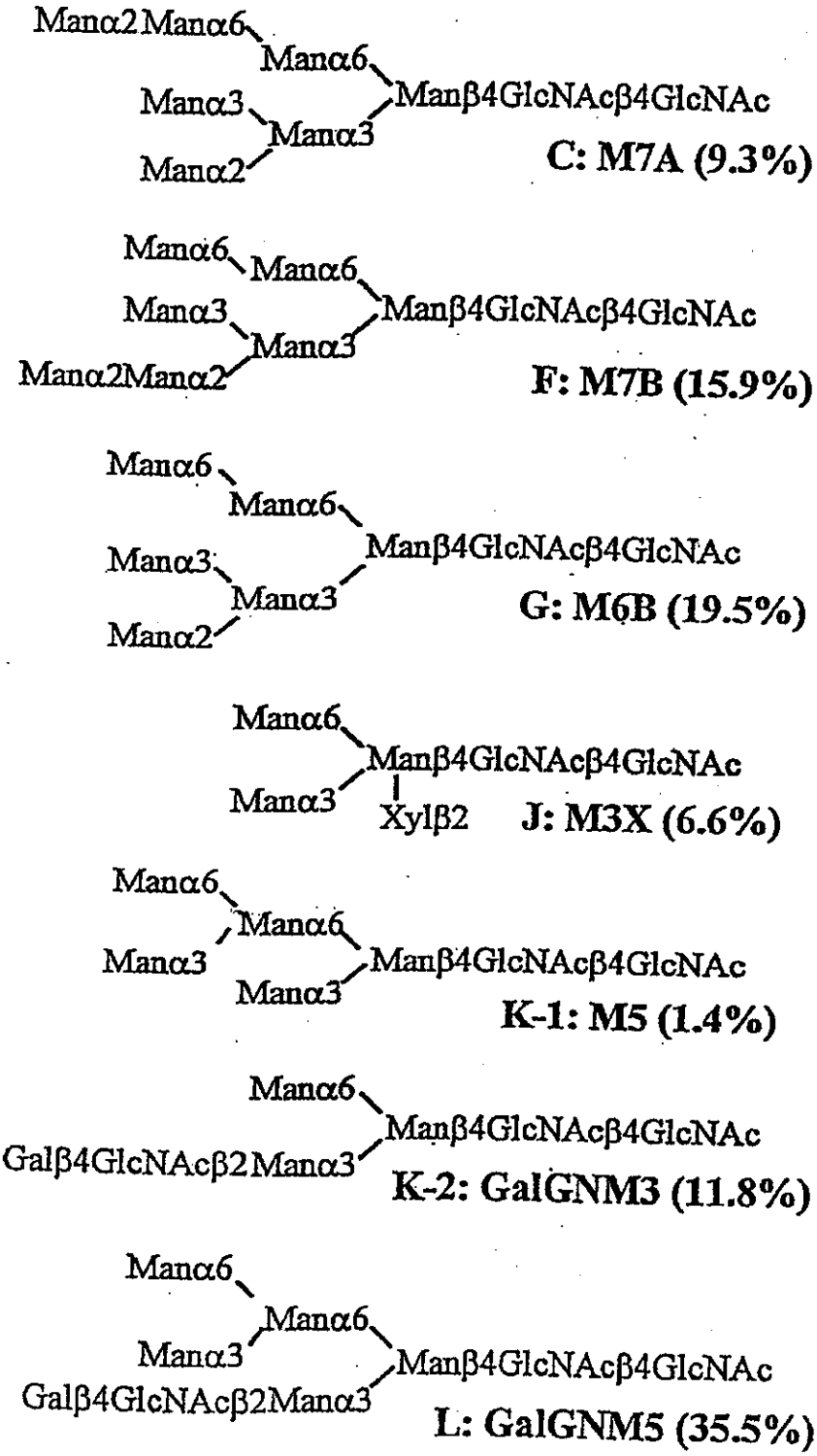

Manα2Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Manα3
Manα2
C: M7A (9.3%)
Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Manα3
Manα2Manα2
F: M7B (15.9%)
Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Manα3
Manα2
G: M6B (19.5%)
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Xylβ2
J: M3X (6.6%)
Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Manα3
K-1: M5 (1.4%)
Manα6
Manβ4GlcNAcβ4GlcNAc
Galβ4GlcNAcβ2Manα3
K-2: GalGNM3 (11.8%)
Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Galβ4GlcNAcβ2Manα3
L: GalGNM5 (35.5%)

FIG 15

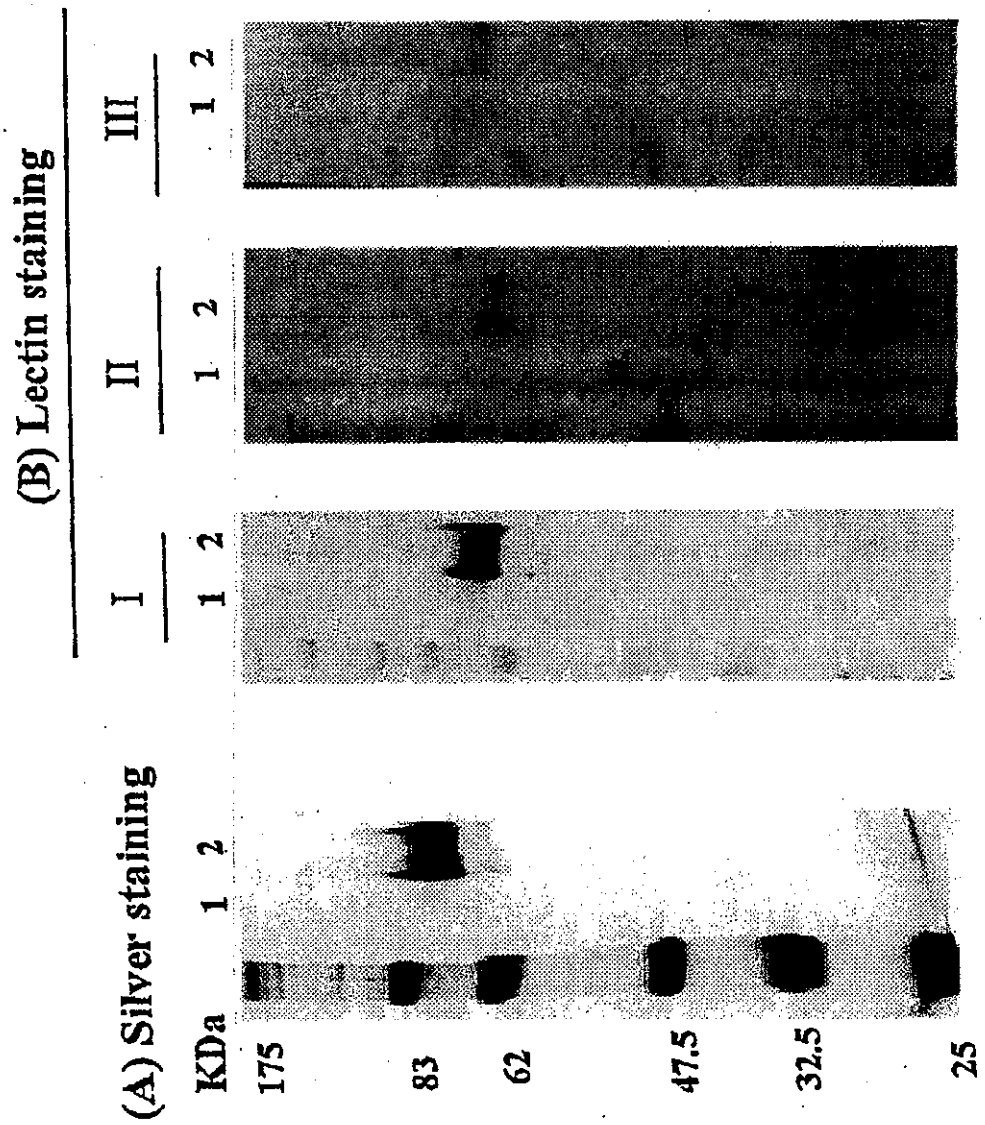

(B) Lectin staining
I
1 2
II
1 2
III
1 2
(A) Silver staining
KDa
1 2
175
83
62
47.5
32.5
25

FIG 16

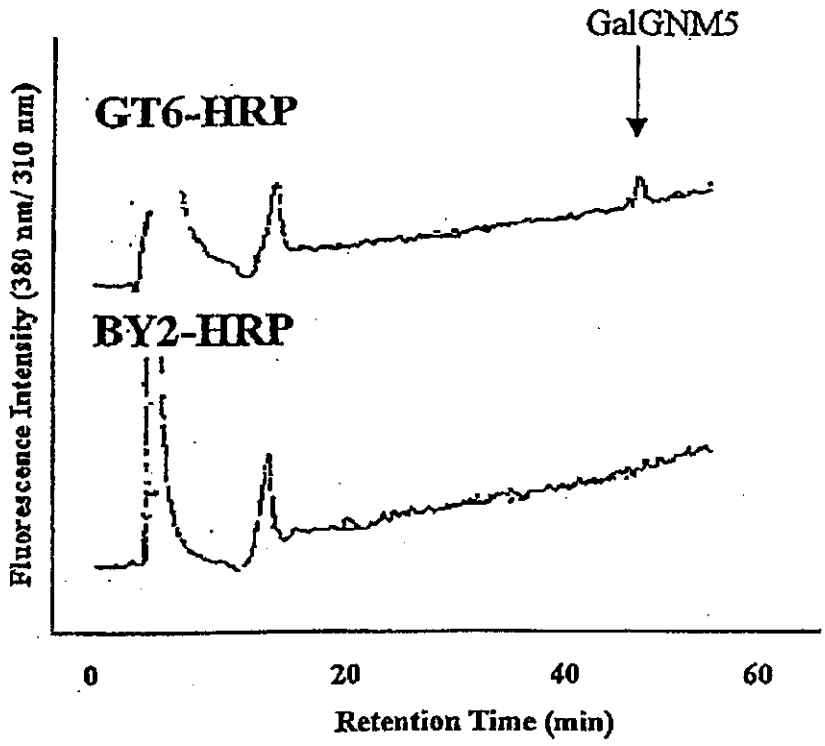

GalGNM5
GT6-HRP
BY2-HRP
Fluorescence Intensity (380 nm/ 310 nm)
0
20
40
60
Retention Time (min)

FIG 17

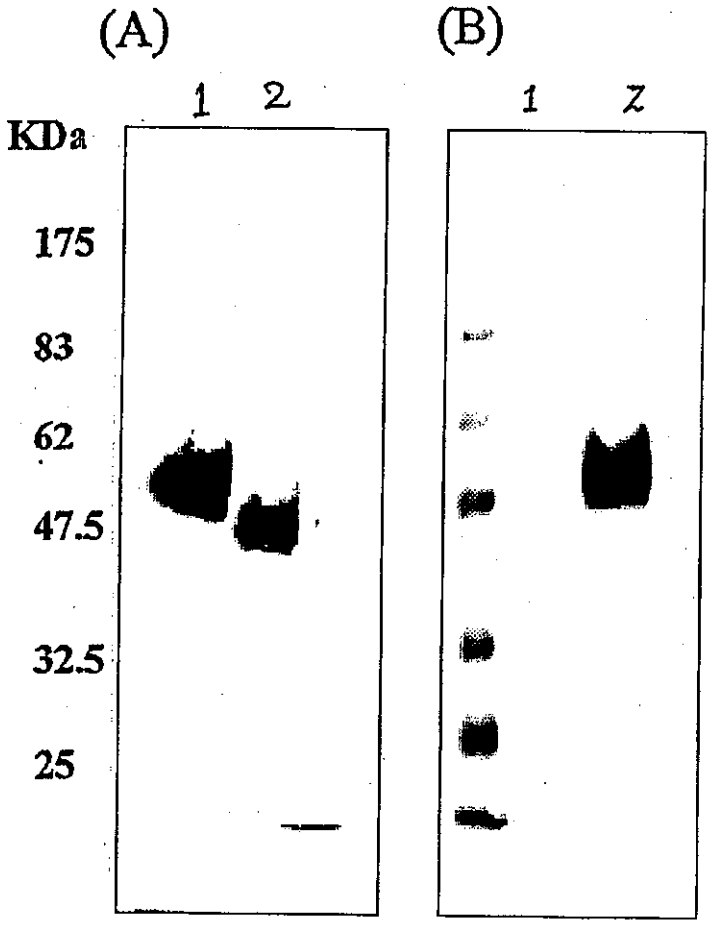



FIG 18

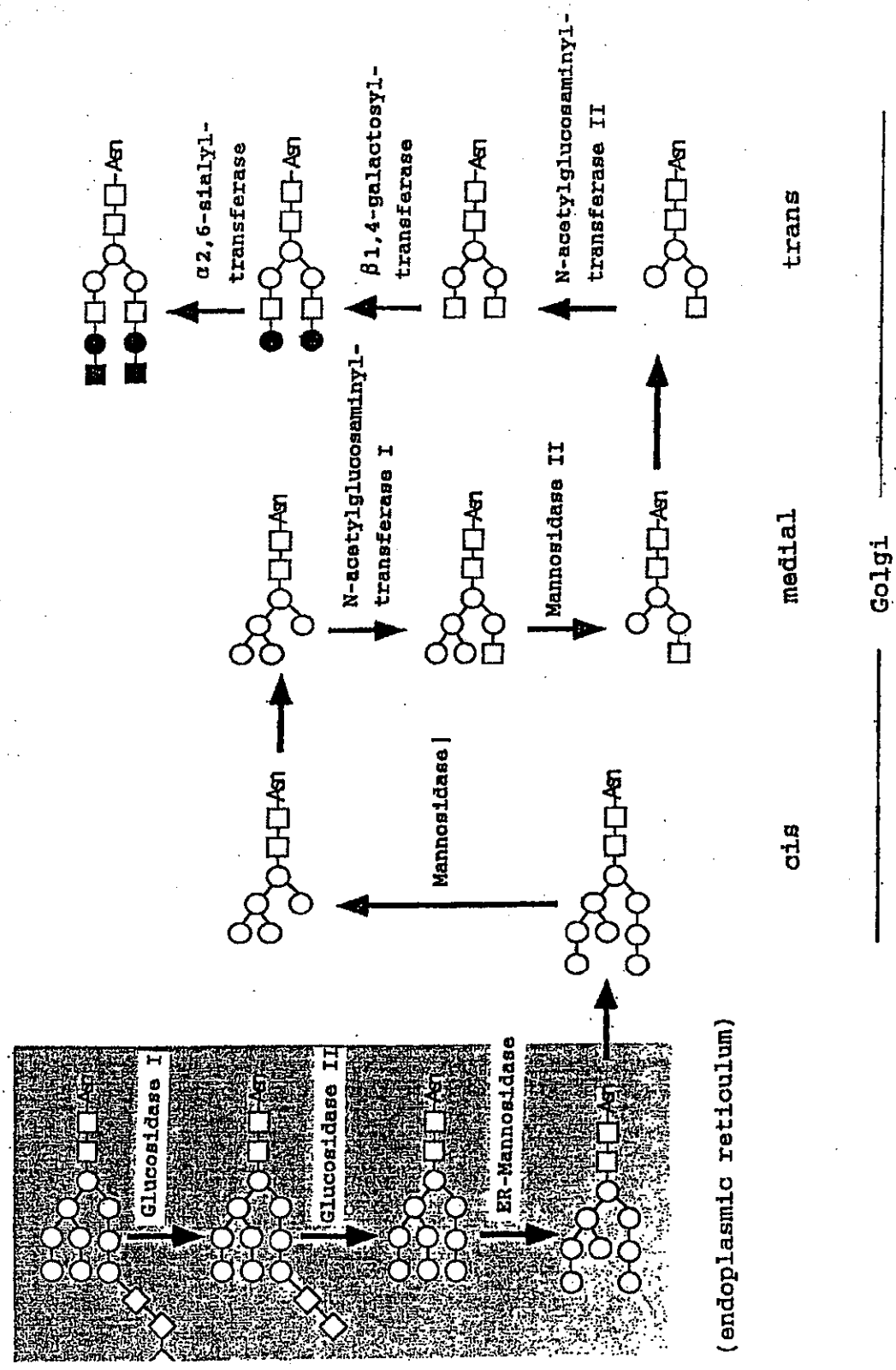

Glucosidase I
Glucosidase II
ER-Mannosidase
Mannosidase I
N-acetylglucosaminyl-transferase I
Mannosidase II
N-acetylglucosaminyl-transferase II
β1,4-galactosyl-transferase
α2,6-sialyl-transferase
Asn
(endoplasmic reticulum)
cis
medial
trans
Golgi

FIG 19

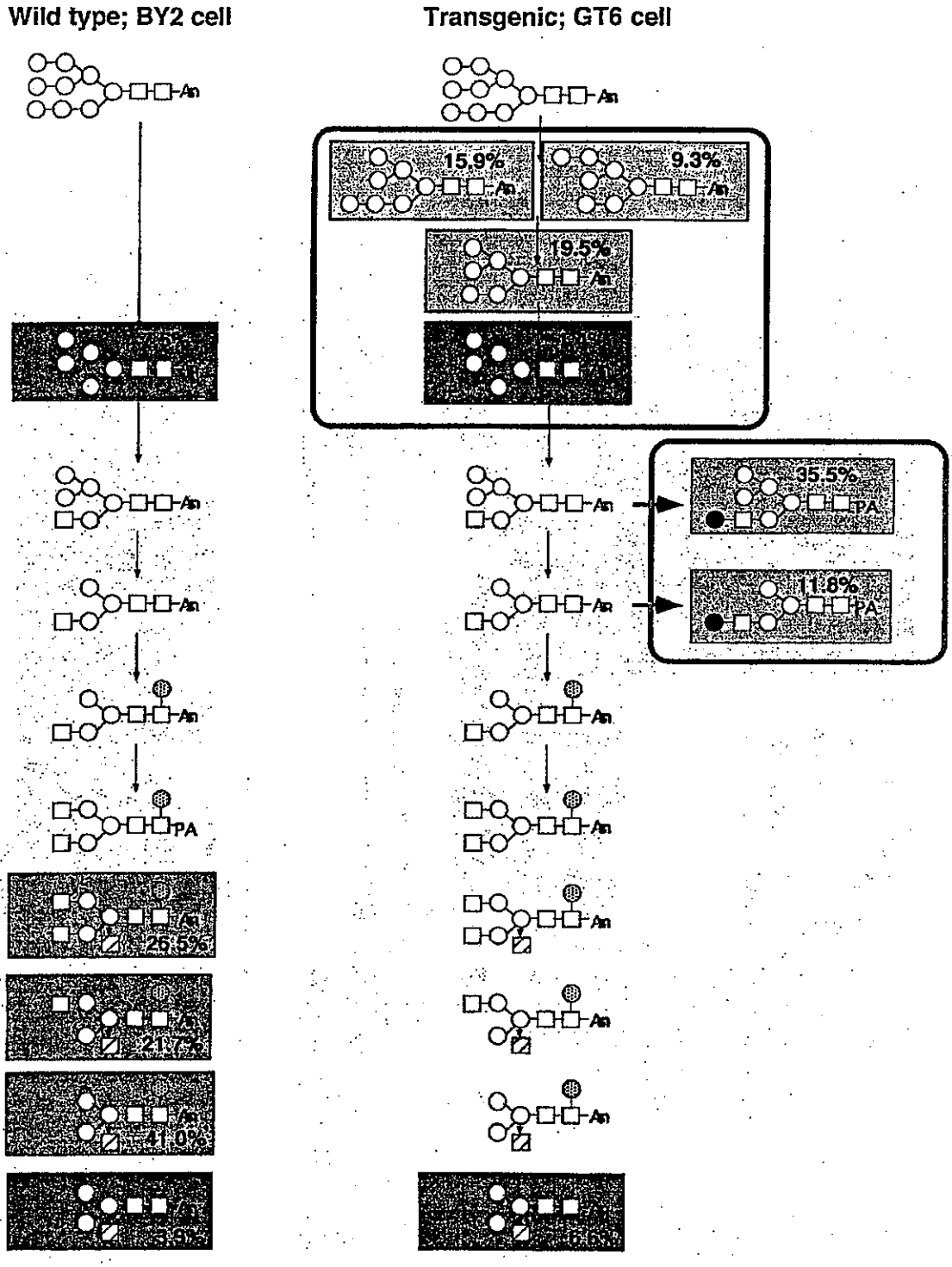

Wild type; BY2 cell
Transgenic; GT6 cell
An
PA
15.9%
9.3%
19.5%
35.5%
11.8%
26.5%
21.7%
41.0%
5.9%
6.6%

FIG 20

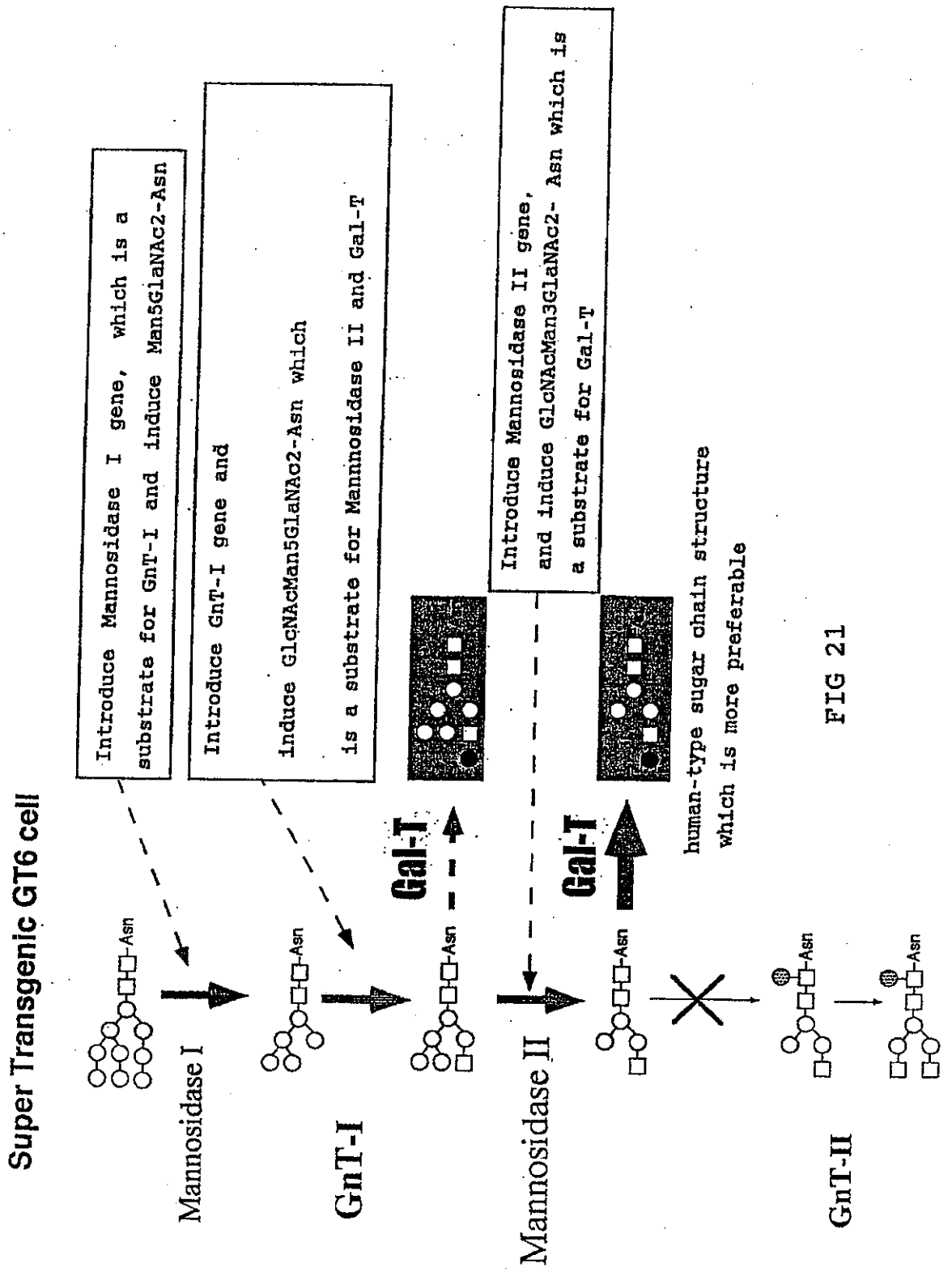

Super Transgenic GT6 cell
Introduce Mannosidase I gene, which is a substrate for GnT-I and induce Man5GlaNAc2-Asn
Introduce GnT-I gene and
induce GlcNAcMan5GlaNAc2-Asn which
is a substrate for Mannosidase II and Gal-T
Introduce Mannosidase II gene,
and induce GlcNAcMan3GlaNAc2- Asn which is
a substrate for Gal-T
Gal-T
Gal-T
human-type sugar chain structure
which is more preferable
Mannosidase I
GnT-I
Mannosidase II
GnT-II
Asn

FIG 21

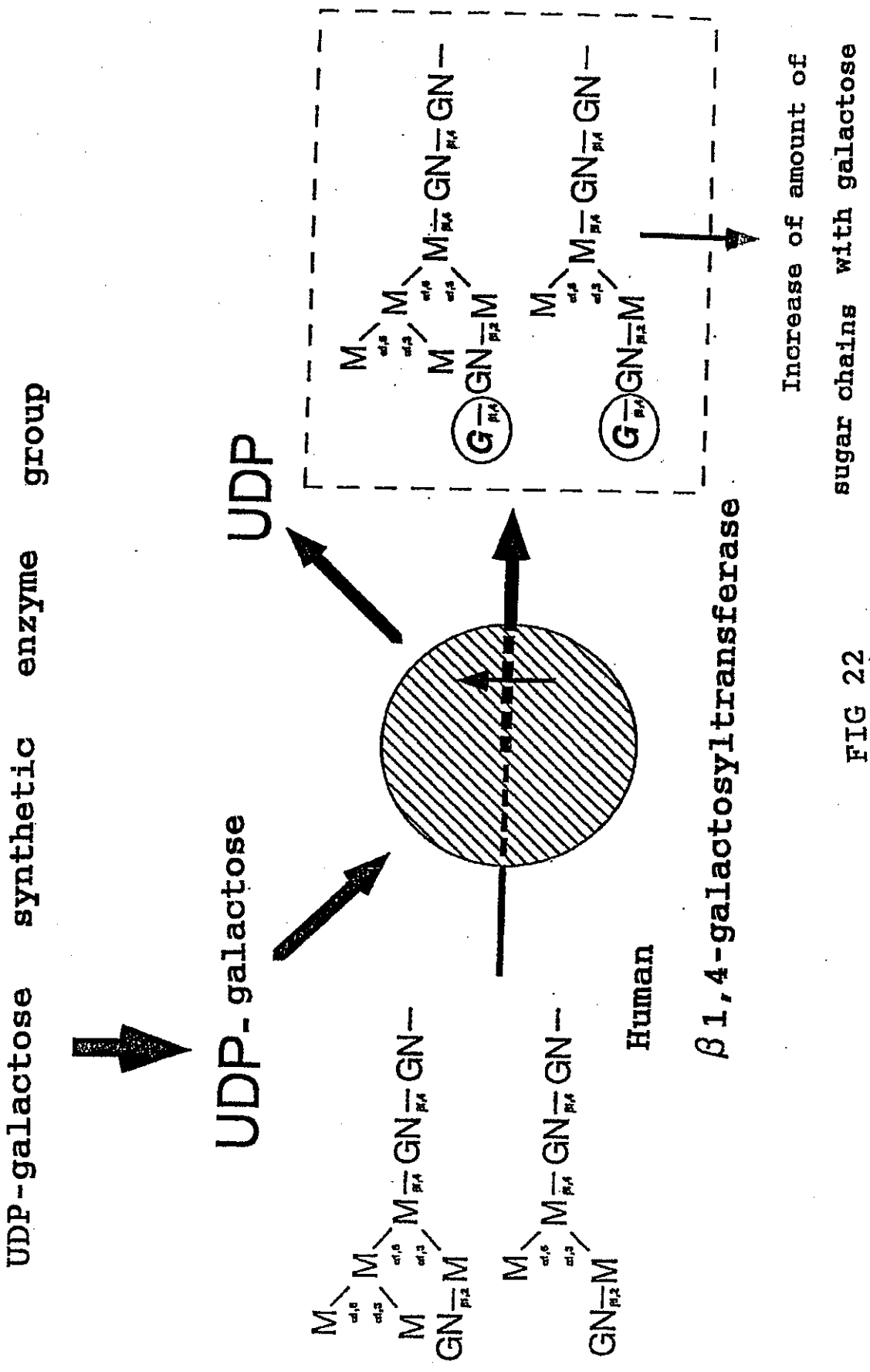

UDP-galactose
synthetic enzyme group
UDP-galactose
UDP
Human
β1,4-galactosyltransferase
Increase of amount of sugar chains with galactose

FIG 22

Sequence Listing

<110> Tatsuji, Seki and Kazuhito, Fujiyama
<120> Methods for production of glycoproteins
having human-type sugar chains
<130> J198080401
<150> JP P1998-350584
<151> 1998-12-09
<160> 6
<170> PatentIn Ver. 2.0

<210> 1
<211> 31
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer hGT-5Eco
<400> 1
aaagaattcg cgatgccagg cgcgcgtccc t 31

<210> 2
<211> 28
<212> DNA
<213> Artificial Sequence
<220>
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<400> 2
tcgatcgcaa aaccatgtgc agctgatg 28

<210> 3
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer hGT-7Spe
<400> 3
acgggactcc tcaggggcga tgatcataa 29

<210> 4
<211> 27
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: primer hGT-6Spe
<400> 4
aagactagtg ggccccatgc tgattga 27

<210> 5
<211> 1158
<212> DNA
<213> Homo sapiens
<220>
<221> CDS
<222> (1)..(1155)
<400> 5

atg cca ggc gcg tcc cta cag cgg gcc tgc cgc ctg ctc gtg gcc gtc 48
Met Pro Gly Ala Ser Leu Gln Arg Ala Cys Arg Leu Leu Val Ala Val
1 5 10 15

tgc gct ctg cac ctt ggc gtc acc ctc gtt tac tac ctg gct ggc cgc 96
Cys Ala Leu His Leu Gly Val Thr Leu Val Tyr Tyr Leu Ala Gly Arg
20 25 30

gac ctg agc cgc ctg ccc caa ctg gtc gga gtc tcc aca ccg ctg cag 144
Asp Leu Ser Arg Leu Pro Gln Leu Val Gly Val Ser Thr Pro Leu Gln
35 40 45

ggc ggc tcg aac agt gcc gcc gcc atc ggg cag tcc tcc ggg gag ctc 192
Gly Gly Ser Asn Ser Ala Ala Ala Ile Gly Gln Ser Ser Gly Glu Leu
50 55 60

cgg acc gga ggg gcc cgg ccg ccg cct cct cta ggc gcc tcc tcc cag 240
Arg Thr Gly Gly Ala Arg Pro Pro Pro Pro Leu Gly Ala Ser Ser Gln
65 70 75 80

ccg cgc ccg ggt ggc gac tcc agc cca gtc gtg gat tct ggc cct ggc 288
Pro Arg Pro Gly Gly Asp Ser Ser Pro Val Val Asp Ser Gly Pro Gly
85 90 95

ccc gct agc aac ttg acc tcg gtc cca gtg ccc cac acc acc gca ctg 336
Pro Ala Ser Asn Leu Thr Ser Val Pro Val Pro His Thr Thr Ala Leu
100 105 110

tcg ctg ccc gcc tgc cct gag gag tcc ccg cta cta gtg ggc ccc atg 384
Ser Leu Pro Ala Cys Pro Glu Glu Ser Pro Leu Leu Val Gly Pro Met
115 120 125

ctg att gag ttt aac atg cct gtg gac ctg gag ctc gtg gca aag cag 432
Leu Ile Glu Phe Asn Met Pro Val Asp Leu Glu Leu Val Ala Lys Gln
130 135 140

aac cca aat gtg aag atg ggc ggc cgc tat gcc ccc agg gac tgc gtc 480
Asn Pro Asn Val Lys Met Gly Gly Arg Tyr Ala Pro Arg Asp Cys Val
145 150 155 160

tct cct cac aag gtg gcc atc atc att cca ttc cgc aac cgg cag gag 528
Ser Pro His Lys Val Ala Ile Ile Ile Pro Phe Arg Asn Arg Gln Glu
165 170 175

cac ctc aag tac tgg cta tat tat ttg cac cca gtc ctg cag cgc cag 576
His Leu Lys Tyr Trp Leu Tyr Tyr Leu His Pro Val Leu Gln Arg Gln
180 185 190

cag ctg gac tat ggc atc tat gtt atc aac cag gcg gga gac act ata 624
Gln Leu Asp Tyr Gly Ile Tyr Val Ile Asn Gln Ala Gly Asp Thr Ile
195 200 205

ttc aat cgt gct aag ctc ctc aat gtt ggc ttt caa gaa gcc ttg aag 672
Phe Asn Arg Ala Lys Leu Leu Asn Val Gly Phe Gln Glu Ala Leu Lys
210 215 220

gac tat gac tac acc tgc ttt gtg ttt agt gac gtg gac ctc att cca 720
Asp Tyr Asp Tyr Thr Cys Phe Val Phe Ser Asp Val Asp Leu Ile Pro
225 230 235 240

atg aat gac cat aat gcg tac agg tgt ttt tca cag cca cgg cac att 768
Met Asn Asp His Asn Ala Tyr Arg Cys Phe Ser Gln Pro Arg His Ile
245 250 255

tcc gtt gca atg gat aag ttt gga ttc agc cta cct tat gtt cag tat 816
Ser Val Ala Met Asp Lys Phe Gly Phe Ser Leu Pro Tyr Val Gln Tyr
260 265 270

ttt gga ggt gtc tct gct cta agt aaa caa cag ttt cta acc atc aat 864
Phe Gly Gly Val Ser Ala Leu Ser Lys Gln Gln Phe Leu Thr Ile Asn
275 280 285

gga ttt cct aat aat tat tgg ggc tgg gga gga gaa gat gat gac att 912
Gly Phe Pro Asn Asn Tyr Trp Gly Trp Gly Gly Glu Asp Asp Asp Ile
290 295 300

ttt aac aga tta gtt ttt aga ggc atg tct ata tct cgc cca aat gct 960

Phe Asn Arg Leu Val Phe Arg Gly Met Ser Ile Ser Arg Pro Asn Ala
305 310 315 320

gtg gtc ggg agg tgt cgc atg atc cgc cac tca aga gac aag aaa aat 1008
Val Val Gly Arg Cys Arg Met Ile Arg His Ser Arg Asp Lys Lys Asn
325 330 335

gaa ccc aat cct cag agg ttt gac cga att gca cac aca aag gag aca 1056
Glu Pro Asn Pro Gln Arg Phe Asp Arg Ile Ala His Thr Lys Glu Thr
340 345 350

atg ctc tct gat ggt ttg aac tca ctc acc tac cag gtg ctg gat gta 1104
Met Leu Ser Asp Gly Leu Asn Ser Leu Thr Tyr Gln Val Leu Asp Val
355 360 365

cag aga tac cca ttg tat acc caa atc aca gtg gac atc ggg aca ccg 1152
Gln Arg Tyr Pro Leu Tyr Thr Gln Ile Thr Val Asp Ile Gly Thr Pro
370 375 380

agc tag 1158

Ser
385

<210> 6
<211> 385
<212> PRT
<213> Homo sapiens
<400> 6

Met Pro Gly Ala Ser Leu Gln Arg Ala Cys Arg Leu Leu Val Ala Val
1 5 10 15

Cys Ala Leu His Leu Gly Val Thr Leu Val Tyr Tyr Leu Ala Gly Arg
20 25 30

Asp Leu Ser Arg Leu Pro Gln Leu Val Gly Val Ser Thr Pro Leu Gln
35 40 45

Gly Gly Ser Asn Ser Ala Ala Ala Ile Gly Gln Ser Ser Gly Glu Leu
50 55 60

Arg Thr Gly Gly Ala Arg Pro Pro Pro Pro Leu Gly Ala Ser Ser Gln
65 70 75 80

Pro Arg Pro Gly Gly Asp Ser Ser Pro Val Val Asp Ser Gly Pro Gly
85 90 95

Pro Ala Ser Asn Leu Thr Ser Val Pro Val Pro His Thr Thr Ala Leu
100 105 110

Ser Leu Pro Ala Cys Pro Glu Glu Ser Pro Leu Leu Val Gly Pro Met
115 120 125

Leu Ile Glu Phe Asn Met Pro Val Asp Leu Glu Leu Val Ala Lys Gln
130 135 140

Asn Pro Asn Val Lys Met Gly Gly Arg Tyr Ala Pro Arg Asp Cys Val
145 150 155 160

Ser Pro His Lys Val Ala Ile Ile Ile Pro Phe Arg Asn Arg Gln Glu
165 170 175

His Leu Lys Tyr Trp Leu Tyr Tyr Leu His Pro Val Leu Gln Arg Gln
180 185 190

Gln Leu Asp Tyr Gly Ile Tyr Val Ile Asn Gln Ala Gly Asp Thr Ile
195 200 205

Phe Asn Arg Ala Lys Leu Leu Asn Val Gly Phe Gln Glu Ala Leu Lys
210 215 220

Asp Tyr Asp Tyr Thr Cys Phe Val Phe Ser Asp Val Asp Leu Ile Pro
225 230 235 240

Met Asn Asp His Asn Ala Tyr Arg Cys Phe Ser Gln Pro Arg His Ile
245 250 255

Ser Val Ala Met Asp Lys Phe Gly Phe Ser Leu Pro Tyr Val Gln Tyr
260 265 270

Phe Gly Gly Val Ser Ala Leu Ser Lys Gln Gln Phe Leu Thr Ile Asn
275 280 285

Gly Phe Pro Asn Asn Tyr Trp Gly Trp Gly Gly Glu Asp Asp Asp Ile
290 295 300

Phe Asn Arg Leu Val Phe Arg Gly Met Ser Ile Ser Arg Pro Asn Ala
305 310 315 320

Val Val Gly Arg Cys Arg Met Ile Arg His Ser Arg Asp Lys Lys Asn
325 330 335

Glu Pro Asn Pro Gln Arg Phe Asp Arg Ile Ala His Thr Lys Glu Thr
340 345 350

Met Leu Ser Asp Gly Leu Asn Ser Leu Thr Tyr Gln Val Leu Asp Val
355 360 365

Gln Arg Tyr Pro Leu Tyr Thr Gln Ile Thr Val Asp Ile Gly Thr Pro
370 375 380

Ser
385