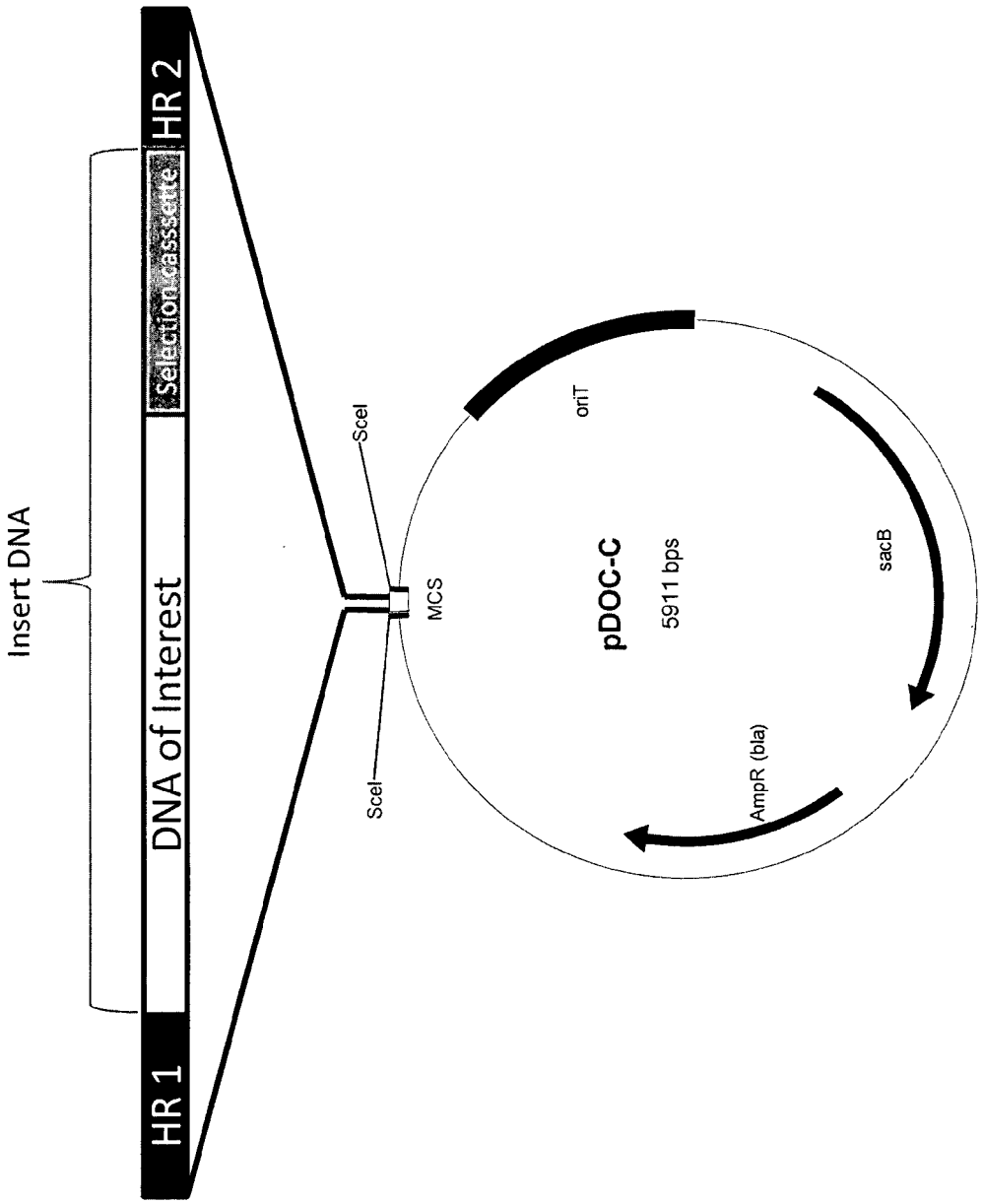

Insert DNA
HR 1
DNA of Interest
Selection cassette
HR 2
SceI
SceI
MCS
oriT
pDOC-C
5911 bps
sacB
AmpR (bla)

FIG. 1

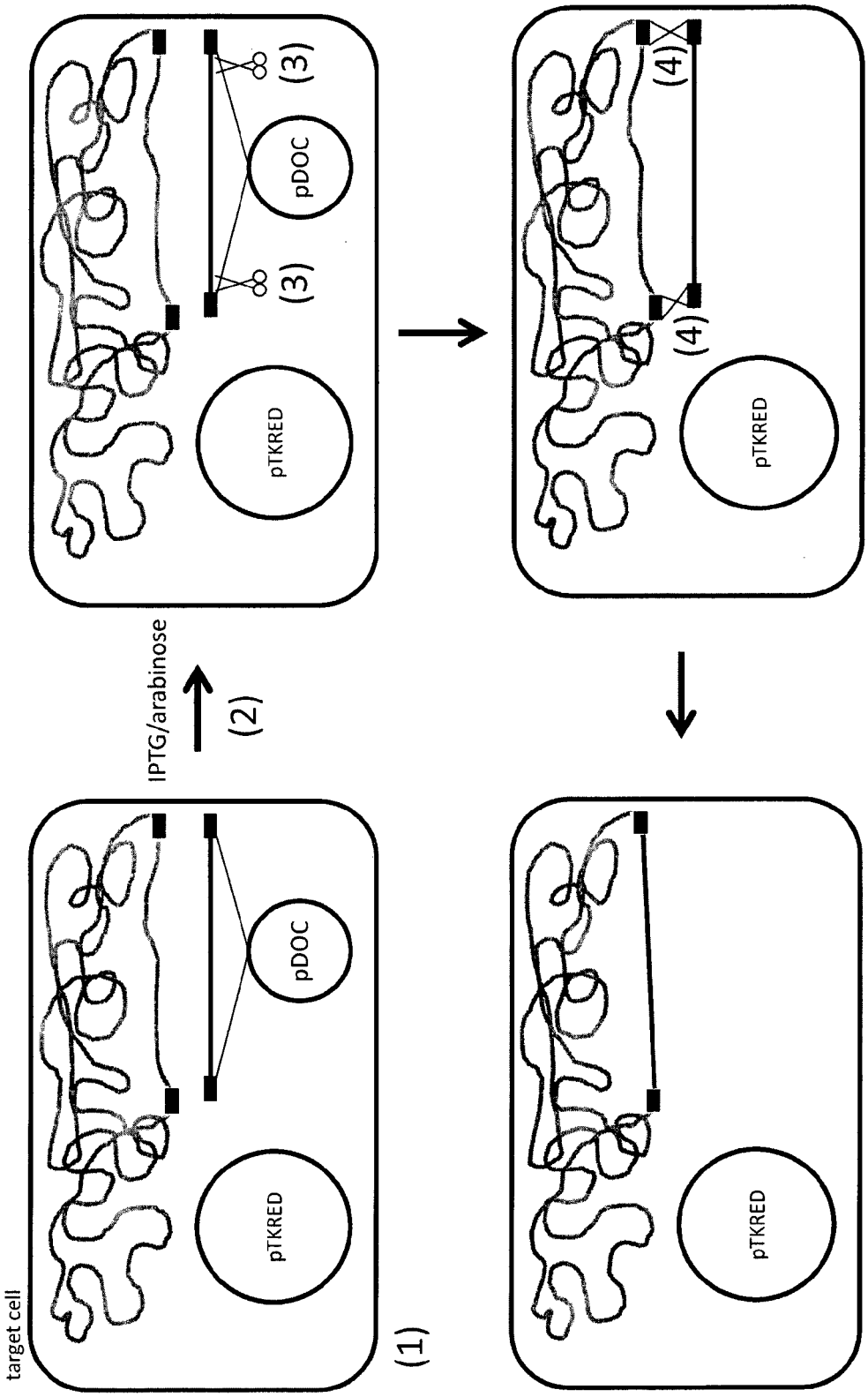


FIG. 2

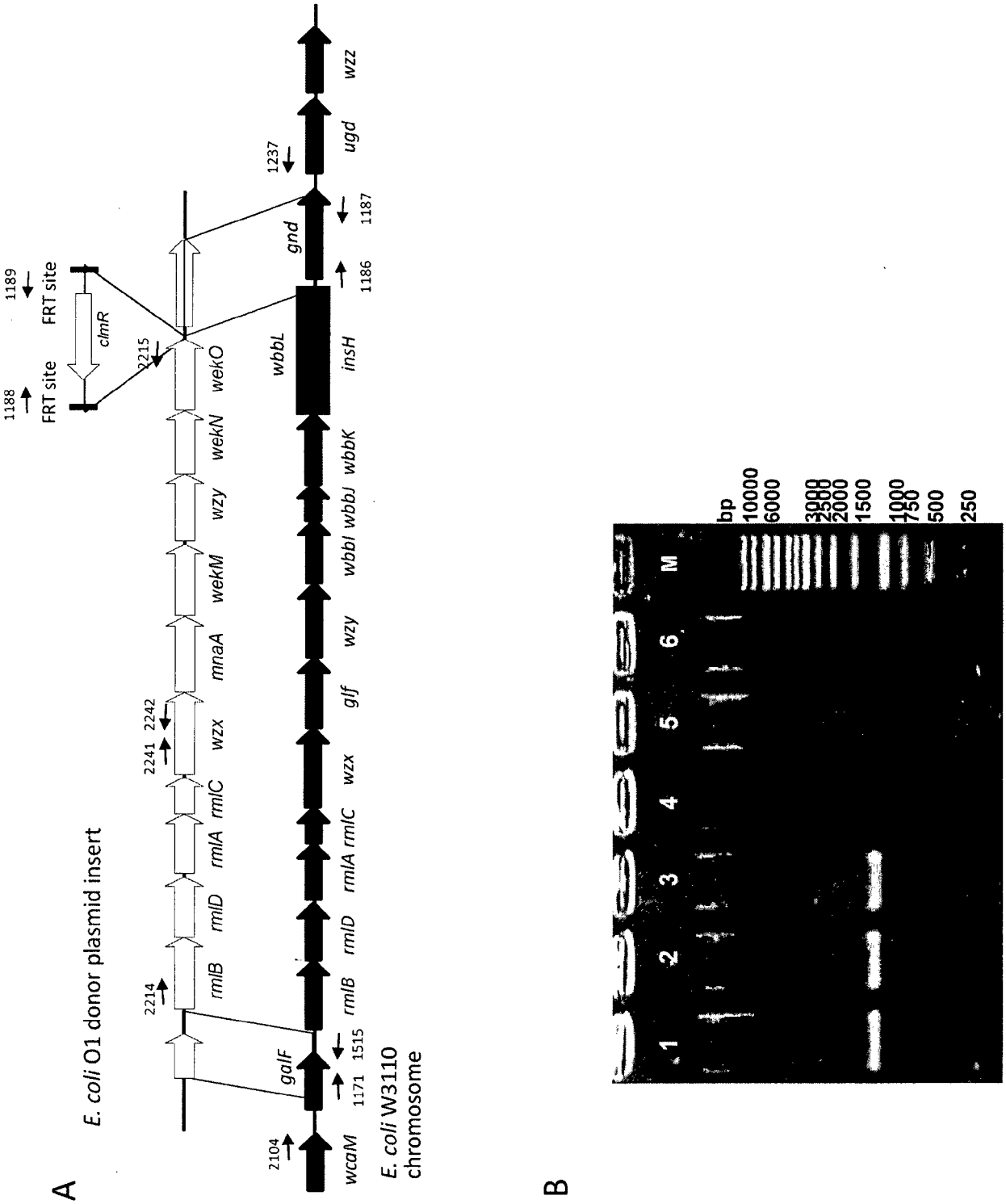
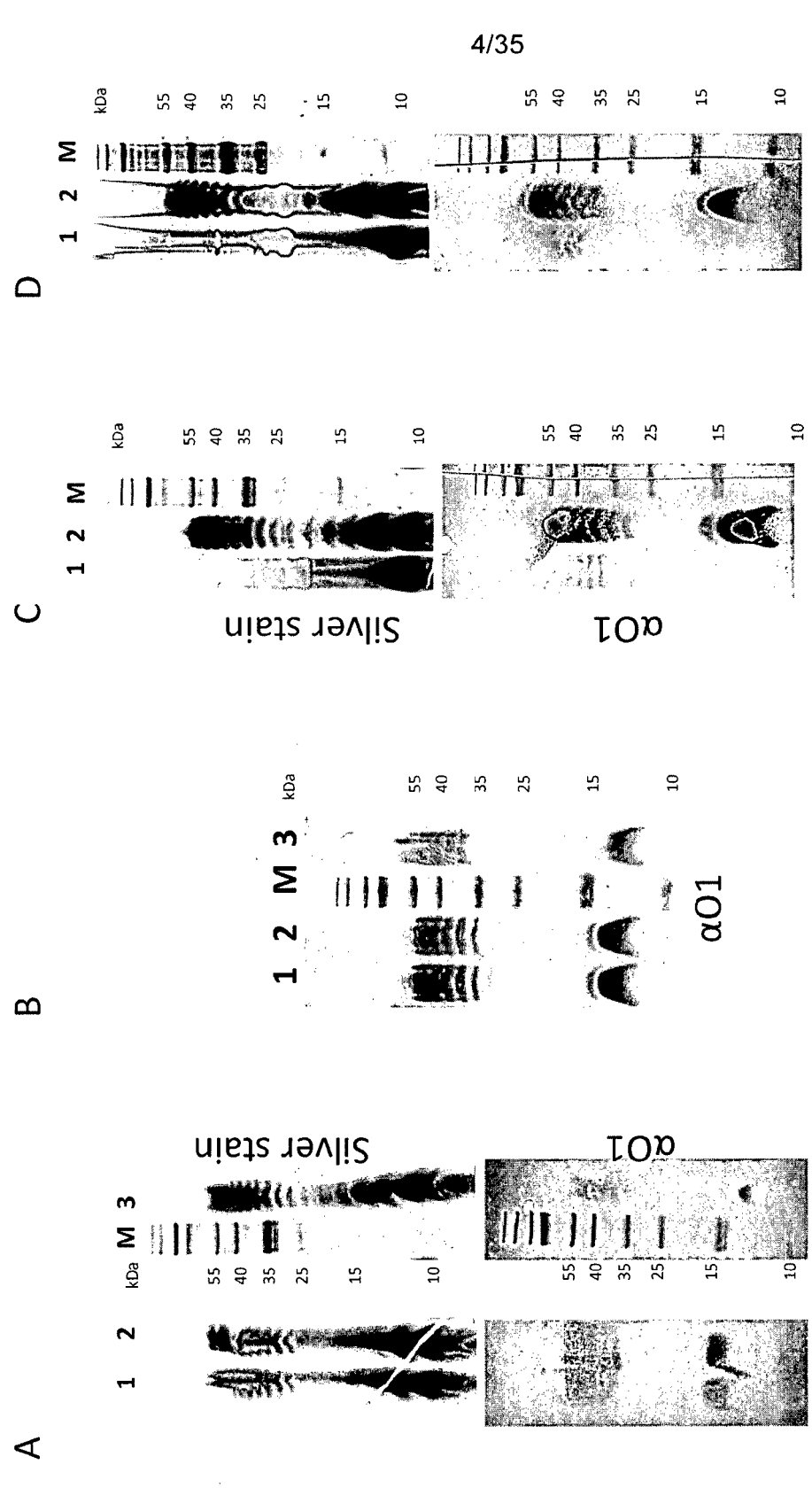

A
E. coli O1 donor plasmid insert
E. coli W3110 chromosome
1189
FRT site
1188
FRT site
cmR
2215
2214
2241
2242
2104
1171
1515
1237
1187
1186
wcaM
galF
rmlB
rmlD
rmlA
rmlC
wzx
wzy
wekM
mnaA
wekN
wekO
wbbL
gnd
wbbK
wbbJ
wbbI
glf
insH
ugd
wzz
B
M
1
2
3
4
5
6
bp
10000
6000
3000
2000
1500
1000
750
500
250

FIG. 3



Lane	1	2	3
Strain	W3110ΔrfbO16::rfbO1-cat		upecGVXN140
clone	A	B	

Lane	1	2	3
Strain	W3110ΔrfbO16::rfbO1		upecGVXN140
clone	A	B	

Lane	1	2
Strain	W3110ΔrfbO16::rfbO1ΔwaaL::cat	W3110ΔrfbO16::rfbO1

Lane	1	2
Strain	W3110ΔrfbO16::rfbO1ΔwaaL	W3110ΔrfbO16::rfbO1

FIG. 4

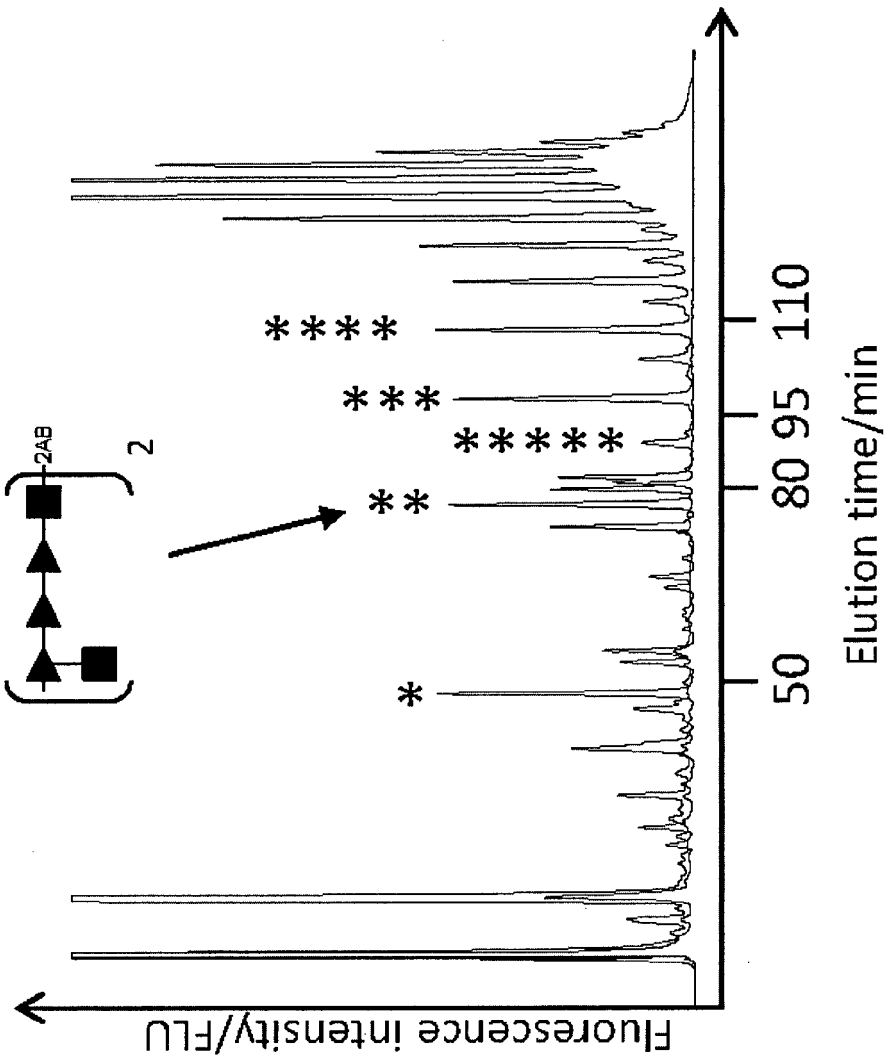

Fluorescence intensity/FLU
Elution time/min
50
80 95
110
*
**
***
****
*****
2AB
2

FIG. 5A

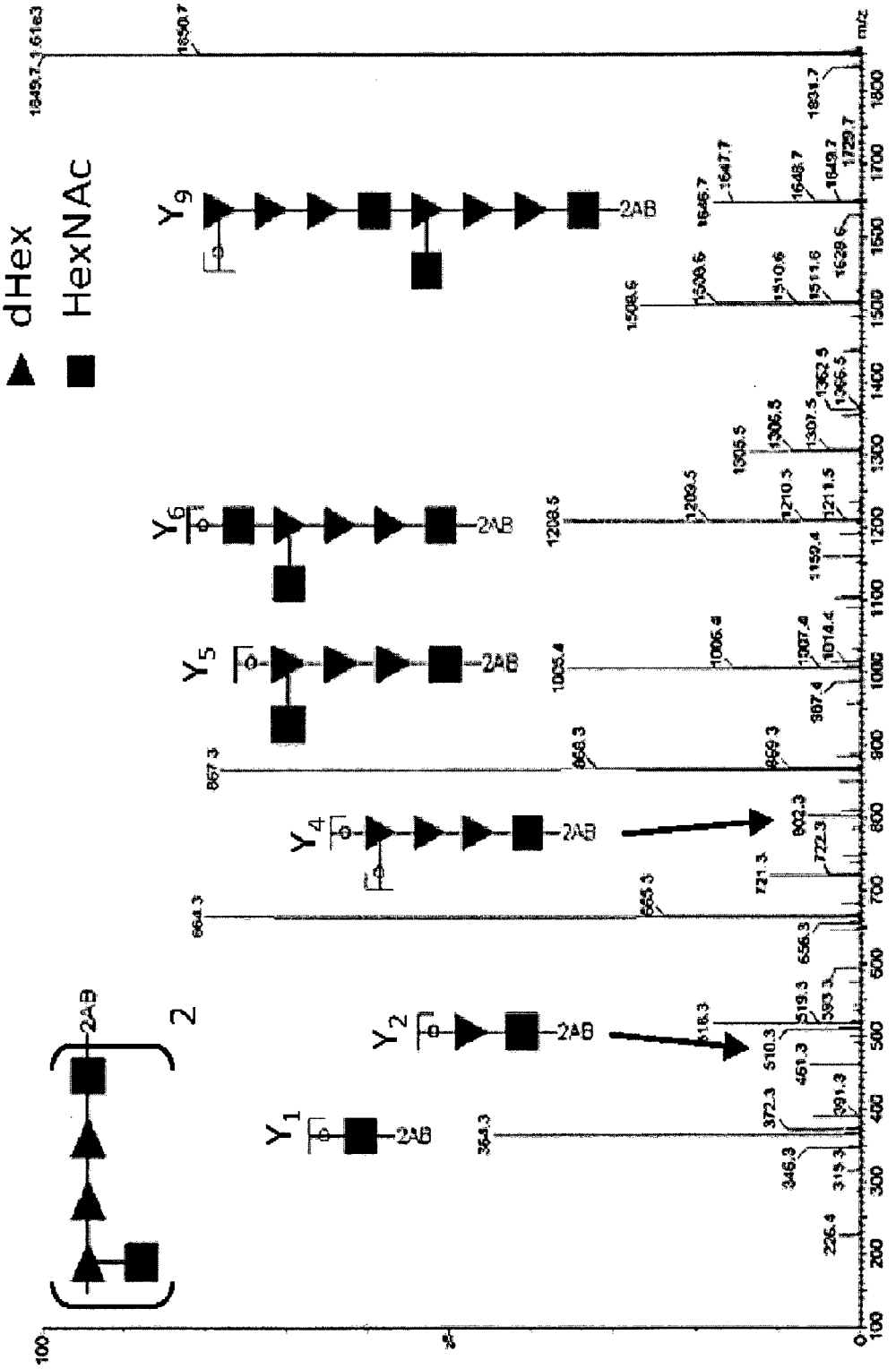


FIG. 5B

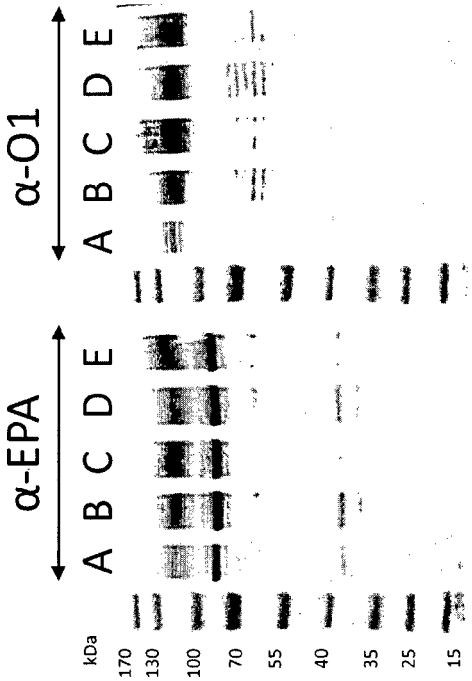


FIG. 6

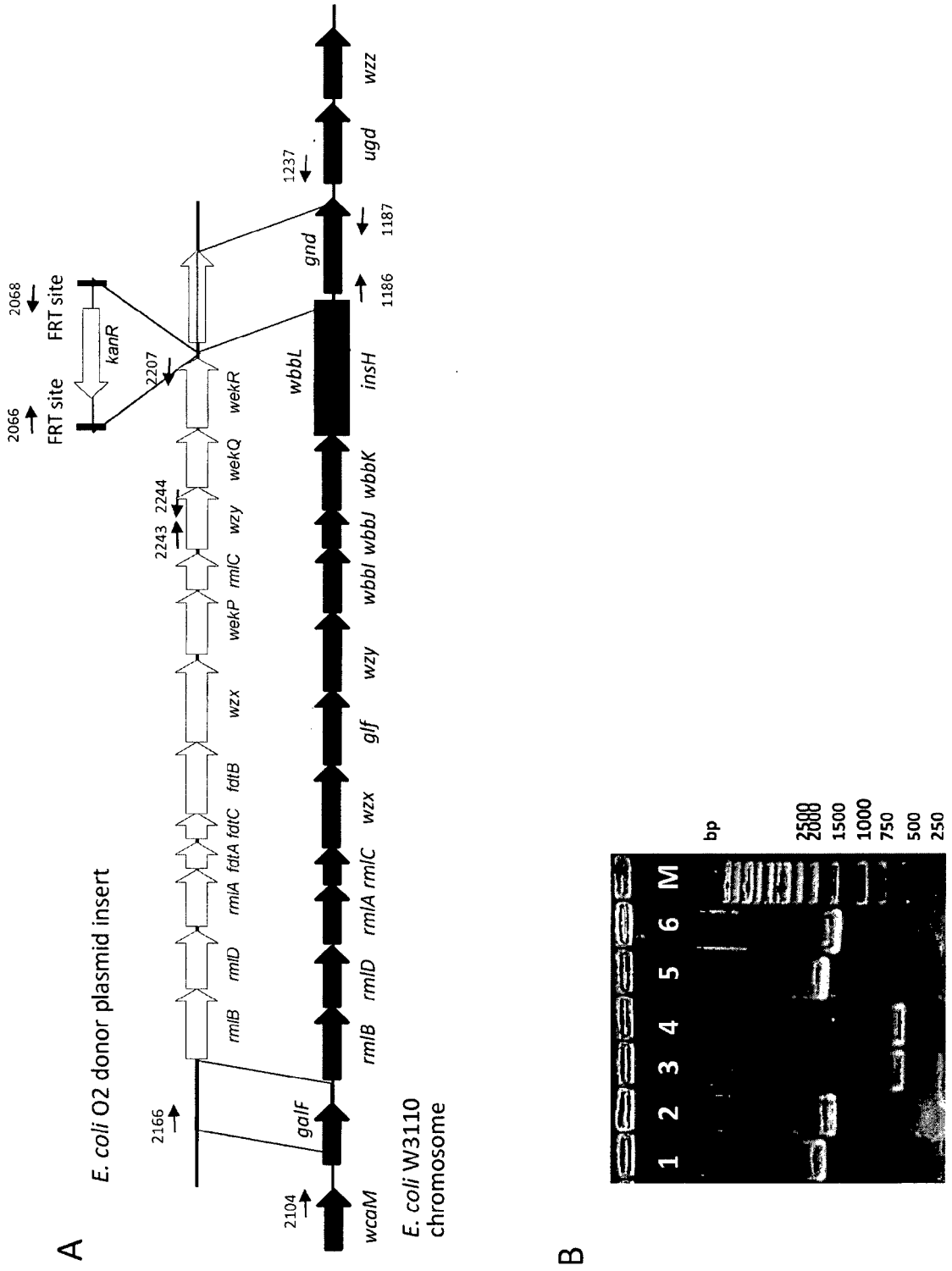
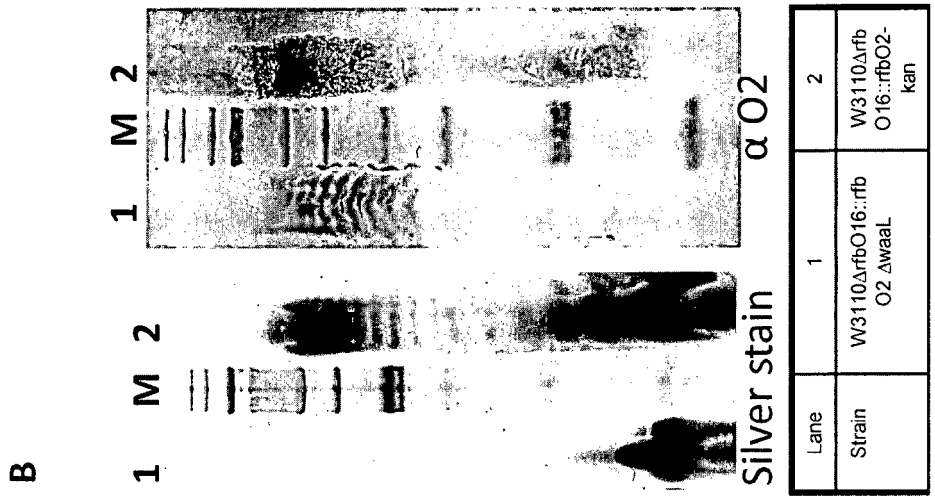

A
E. coli O2 donor plasmid insert
2068
FRT site
kanR
2066
FRT site
2207
2166
galF
2104
wcaM
rmlB rmlD rmlA rmlC wzx glf wzy wbbI wbbJ wbbK insH gnd 1237 1186 ugd 1187 wzz
rmlB rmlD rmlA fdtA fdtC fdtB wzx wekP rmlC wzy 2243 2244 wekQ wekR wbbL
E. coli W3110 chromosome
B
bp
2500
2000
1500
1000
750
500
250
1 2 3 4 5 6 M

FIG. 7



Lane	1	2
Strain	W3110ΔrfbO16::rfb O2 ΔwaaL	W3110ΔrfbO16::rfbO2-kan



Lane	1	2
Strain	W3110ΔrfbO16::rfbO2-kan	upecGVXN116

FIG. 8

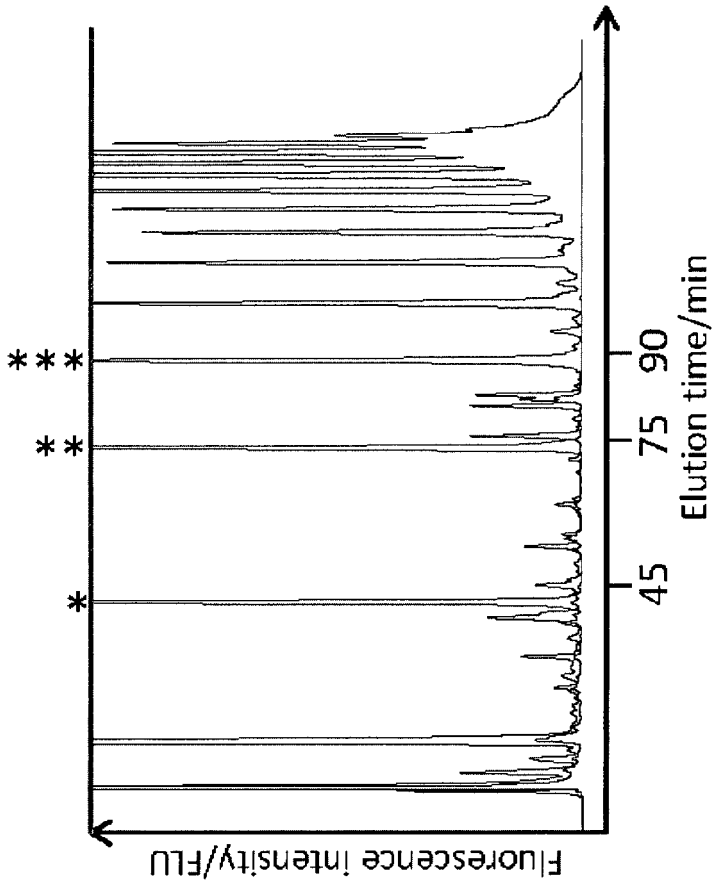


FIG. 9A

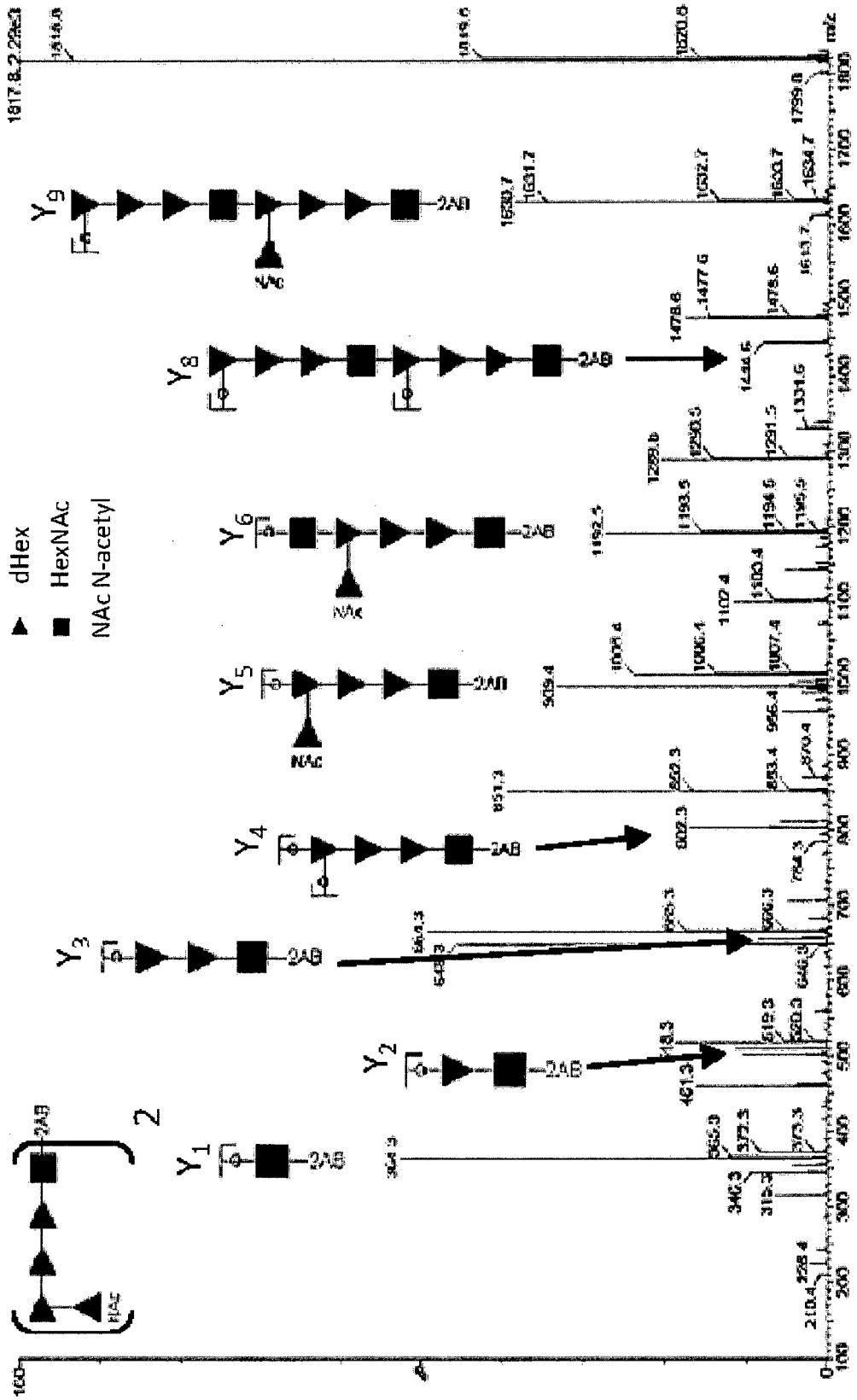


FIG. 9B

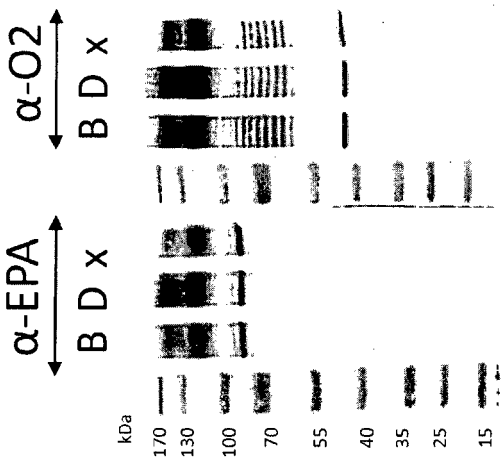


FIG. 10

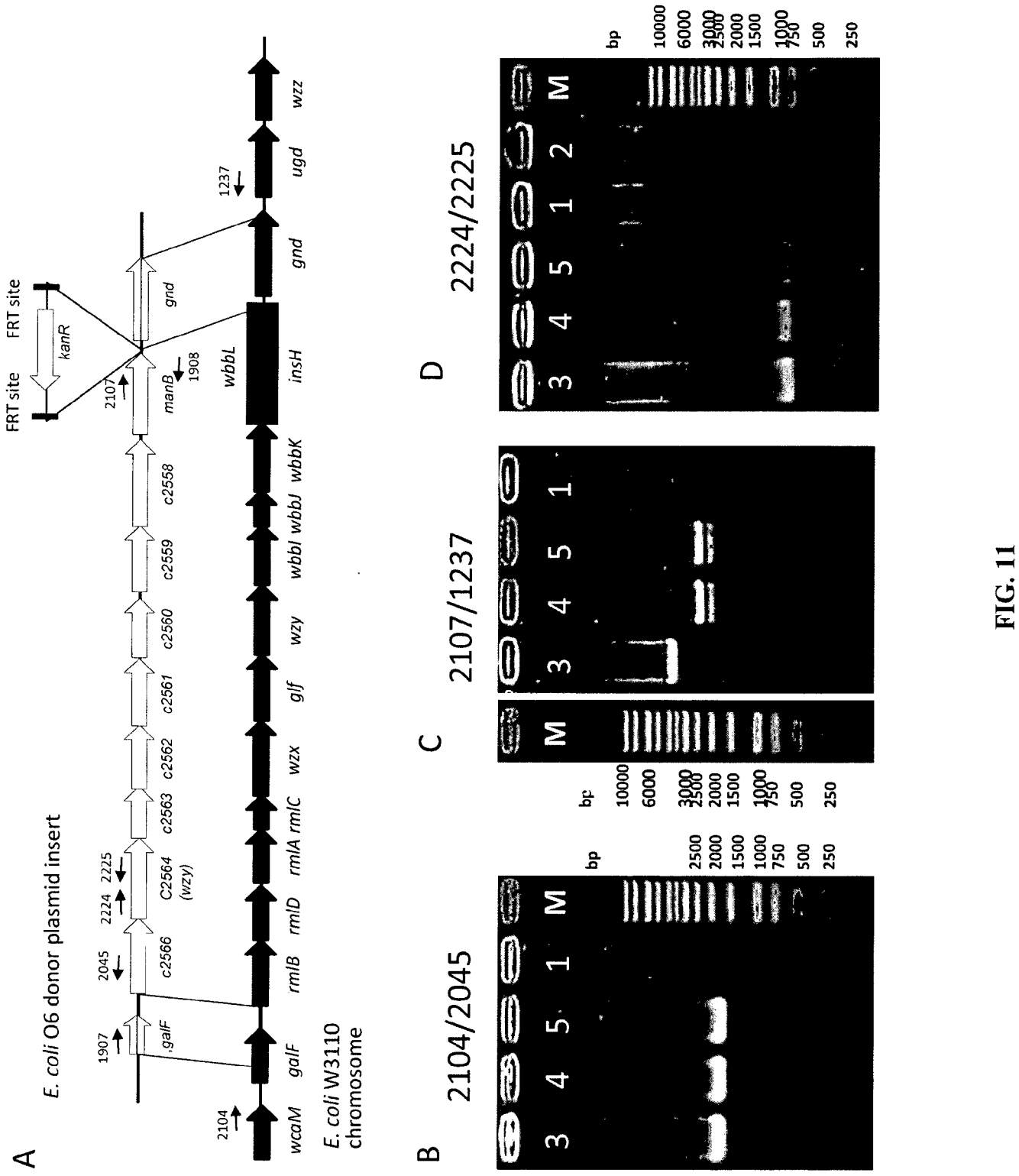
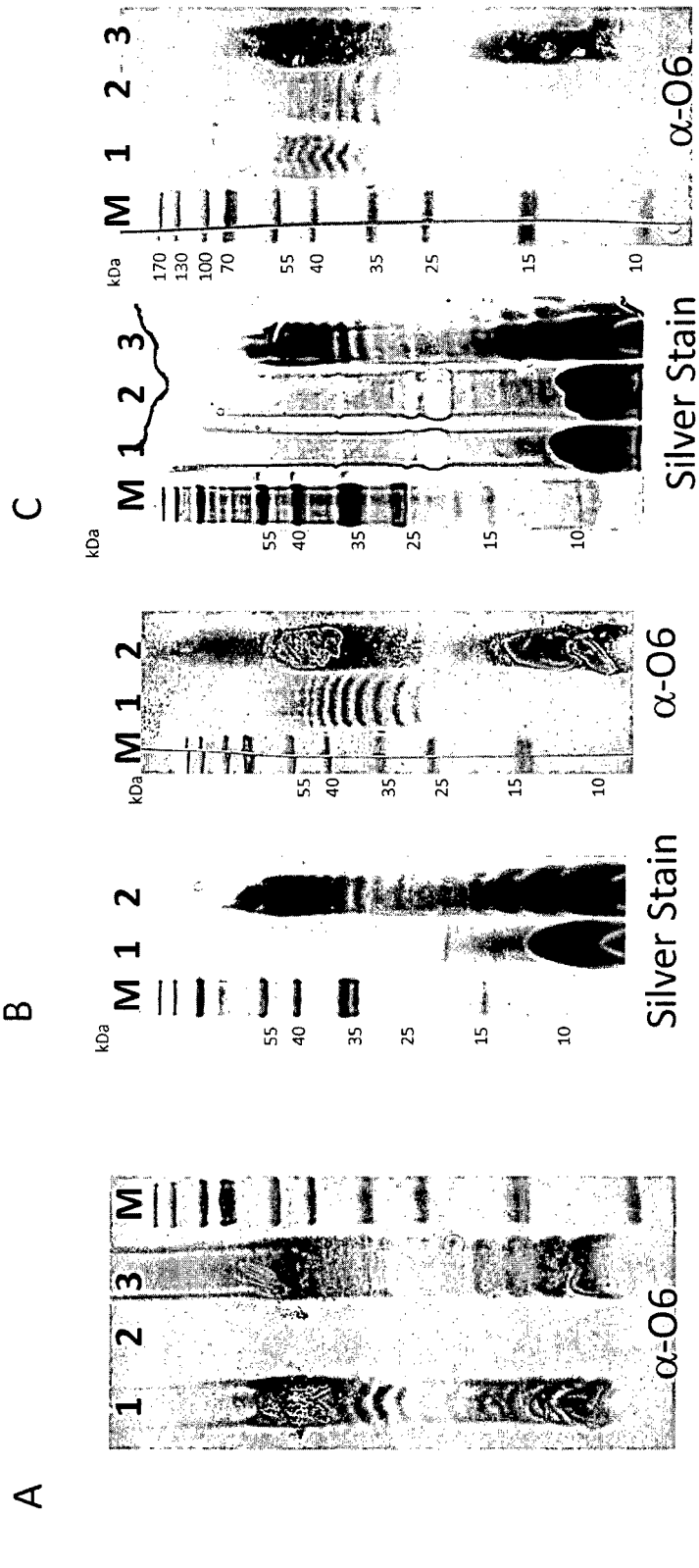

A
E. coli O6 donor plasmid insert
FRT site
FRT site
kanR
1907
2045
2224
2225
2104
2107
1908
1237
wcaM galF ,galF c2566 rmlB C2564 (wzy) rmlD c2563 rmlA c2562 rmlC c2561 wzx c2560 glf c2559 wzy c2558 wbbI wbbJ wbbK wbbL insH gnd gnd ugd wzz
E. coli W3110 chromosome
B
2104/2045
C
2107/1237
D
2224/2225
M
1
2
3
4
5
bp
10000
6000
3000
2500
2000
1500
1000
750
500
250

FIG. 11



Lane	1	2	3
Strain	W3110ΔrfbO16::rfbO6-kan		CCUG27
clone	A	B	

Lane	1	2
Strain	W3110ΔrfbO16::rfbO 6-kan ΔwaaL::cat	W3110ΔrfbO 16::rfbO6-kan

Lane	1	2	3
Strain	W3110ΔrfbO16::rfbO6-kan ΔwaaL (StGVXN4029)		W3110 ΔrfbO16::rfb O6-kan
clone	C	D	

FIG. 12

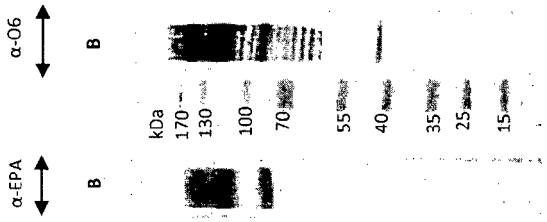
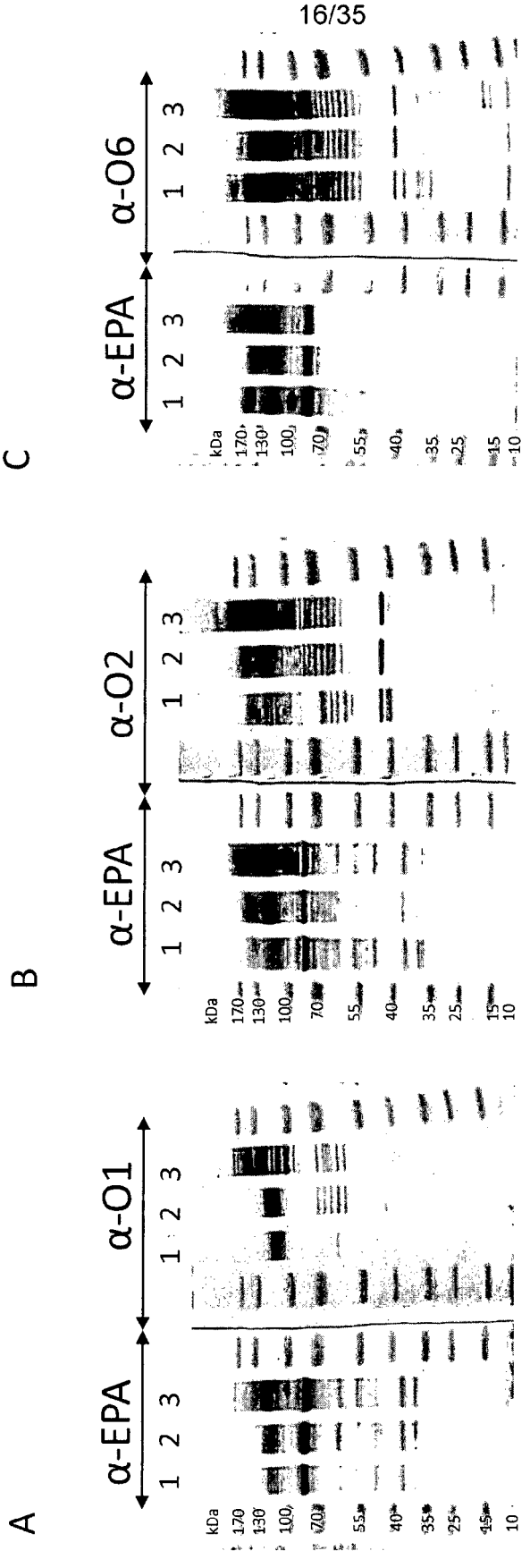


FIG. 13



lane	Strain	Experiment
1	3809	upec_073
2	W3110ΔrfbO 16::rfbO1 ΔwaaL 4026	upec_143
3	W3110ΔwaaL with p947	upec_087

lane	Strain	Experiment
1	3484	upec_073
2	W3110ΔrfbO 16::rfbO2 ΔwaaL 4045	upec_148
3	W3110ΔwaaL with p983	upec_115

lane	Strain	Experiment
1	3686	upec_073
2	W3110ΔrfbO16 ::rfbO6-kan ΔwaaL 4029	upec_143
3	W3110ΔwaaL ΔgtrS ΔwbbIJK ΔwzxO16 ΔwzzE-wecG with p926	upec_087

FIG. 14

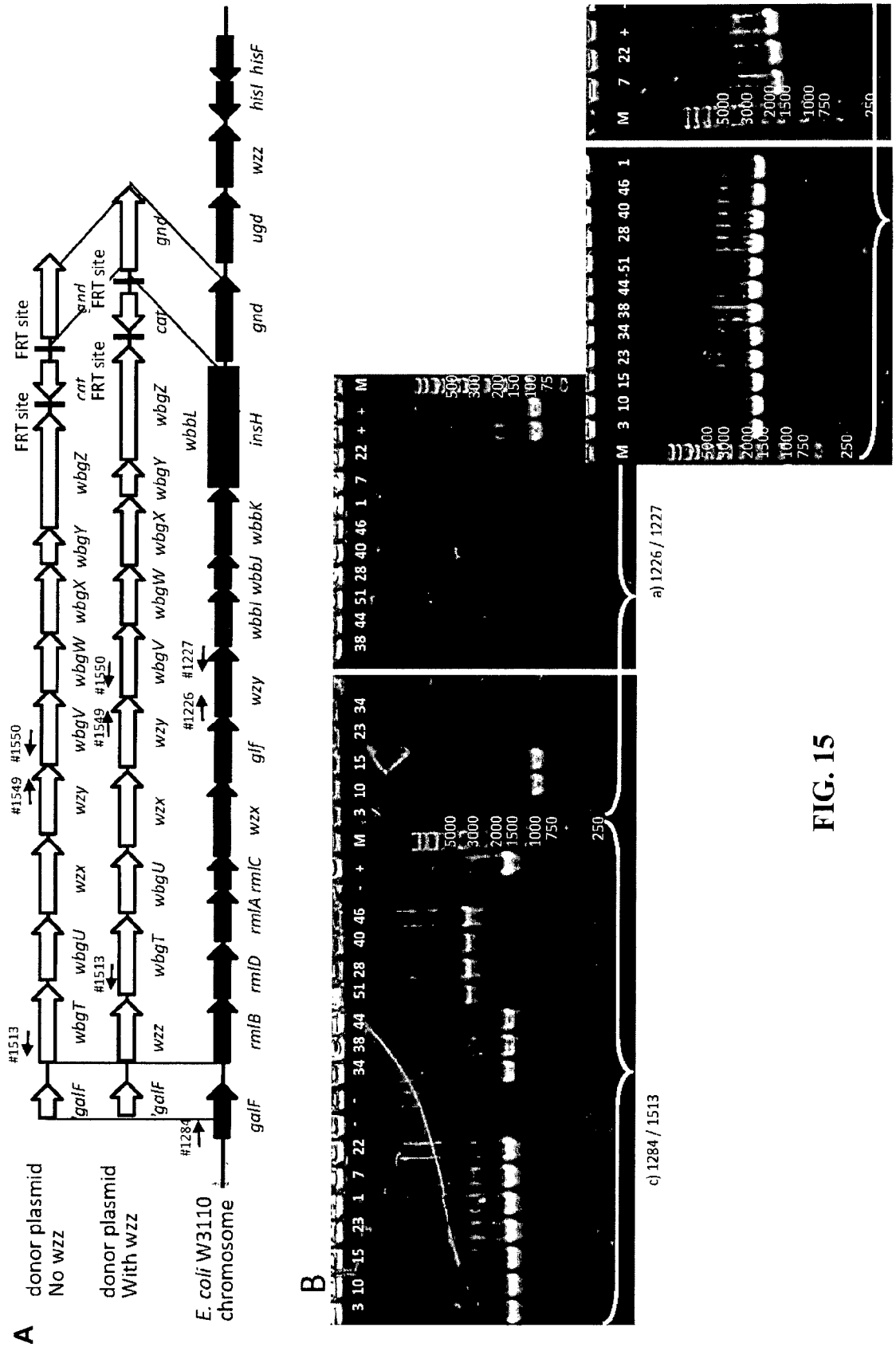

A
donor plasmid
No wzz
donor plasmid
With wzz
E. coli W3110
chromosome
galF
wbgT
wbgU
wzx
wzy
wbgV
wbgW
wbgX
wbgY
wbgZ
cat
FRT site
gnd
wzz
rmlB
rmlD
rmlA
rmlC
wbgU
wbgT
wzx
wzy
glf
wbbI
wbbJ
wbbK
wbbL
insH
ugd
hisI
hisF
#1284
#1513
#1549
#1550
#1226
#1227
B
c) 1284 / 1513
a) 1226 / 1227
b) 1549 / 1550

FIG. 15

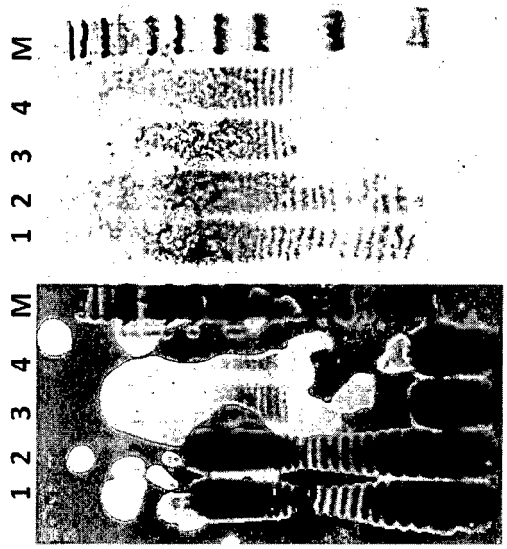
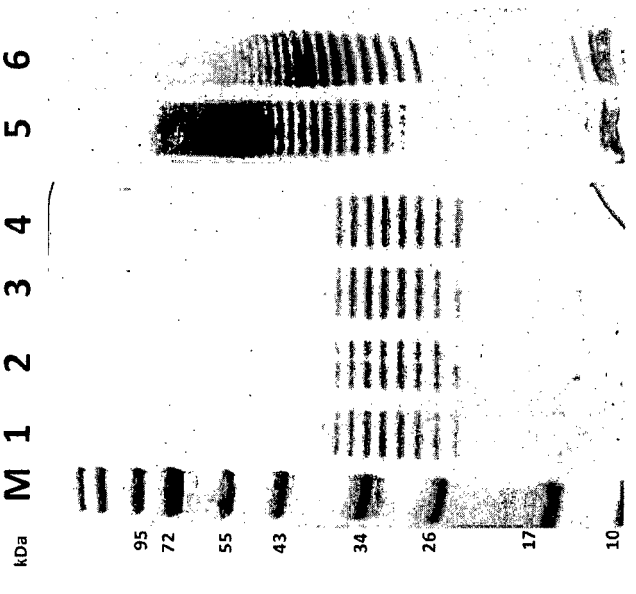
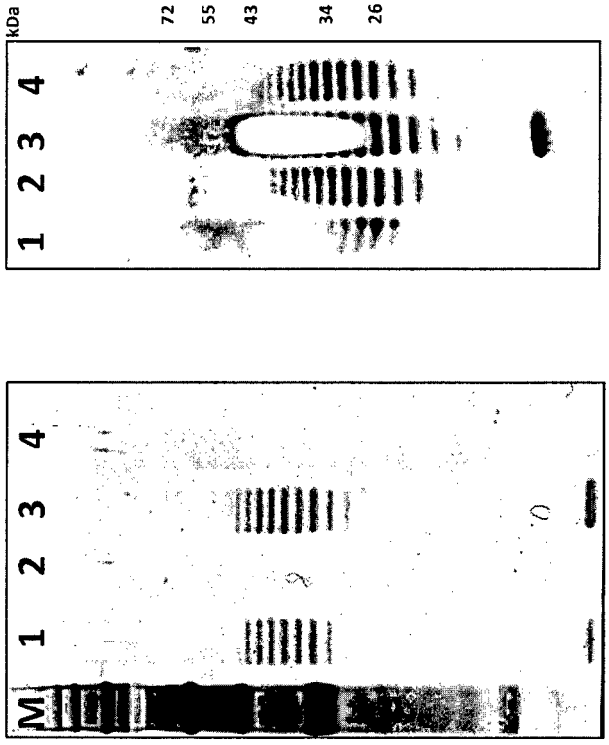


FIG. 16



Lane		1	2	3	4	5	6
sample	M	W3110ΔrfbO16::rfbPaO11-cat ΔwaaL				W3110 ΔwaaL p1012	W3110 ΔwaaL p1013
		clone A	clone B	clone C	clone D	With wzz	w/o wzz

FIG. 17



Lane	1	2	3	4
Strain	W3110 ΔrfbO16::rfbSd1	W3110 ΔwaaL ΔrfbO16::rfbSd1	W3110 ΔrfbO16::rfbSd1-cat	W3110 ΔwaaL ΔrfbO16::rfbSd1-cat

FIG. 18

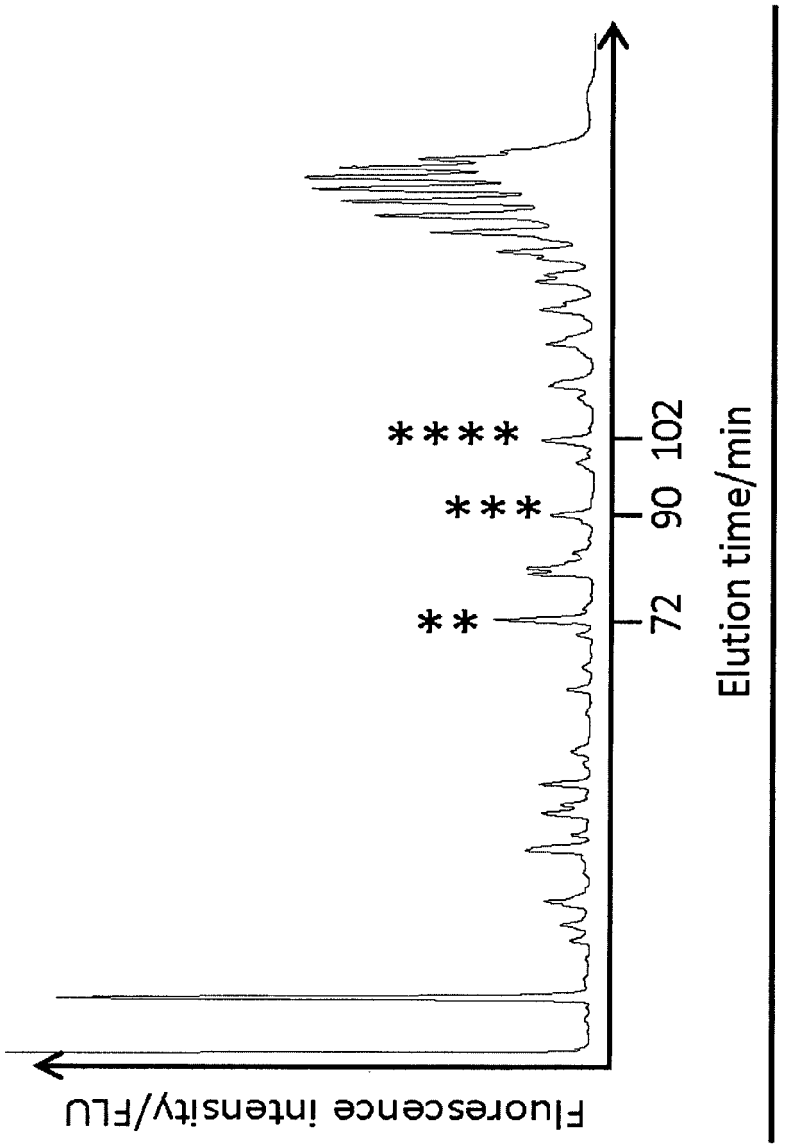


FIG. 19A

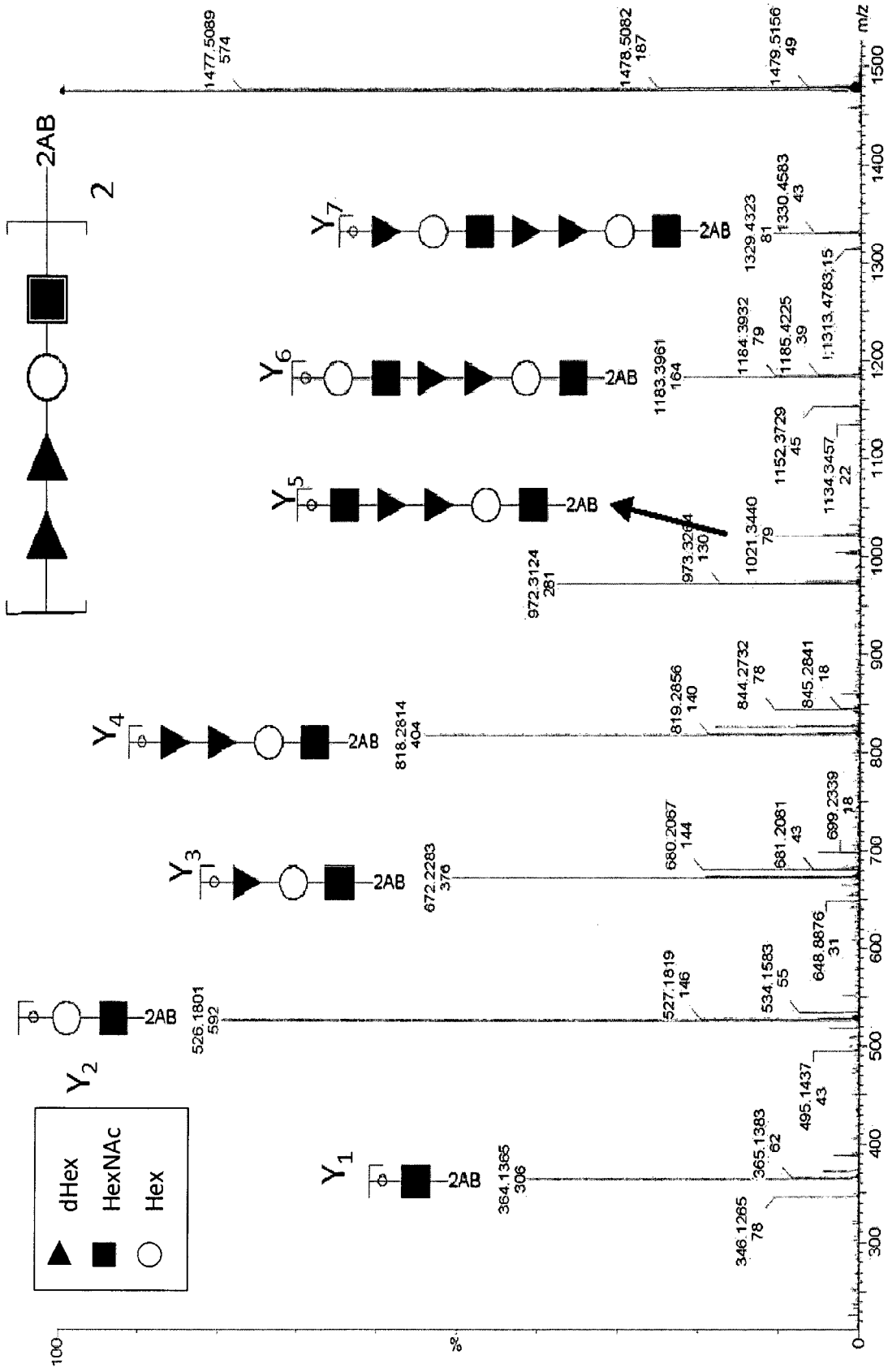


FIG. 19B

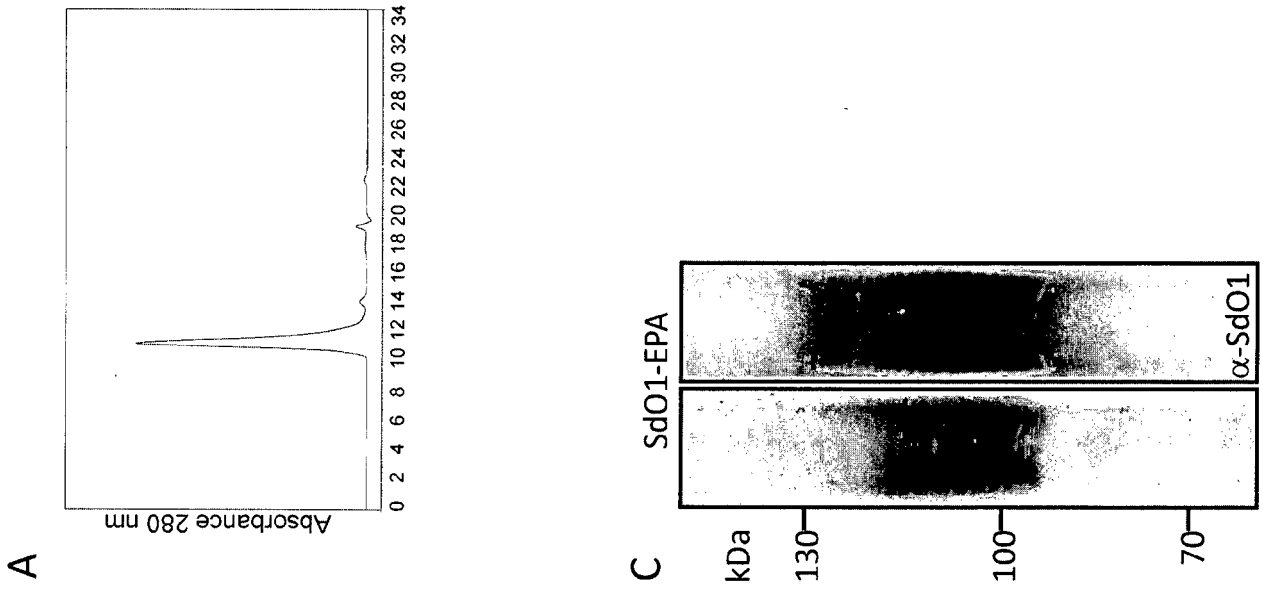

A
Absorbance 280 nm
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
B
Absorbance 250 nm
GlcNAc
Rha
Gal
0.0 5.0 10.0 15.0 20.0 25.0 30.0 35.0 40.0 45.0 50.0
Elution time/ min
C
SdO1-EPA
α-SdO1
kDa
130
100
70
D
MSO119
Hex
HexNAc
dHex
2AB
Fluorescence intensity/FLU
72 90
Elution time/min

FIG. 20

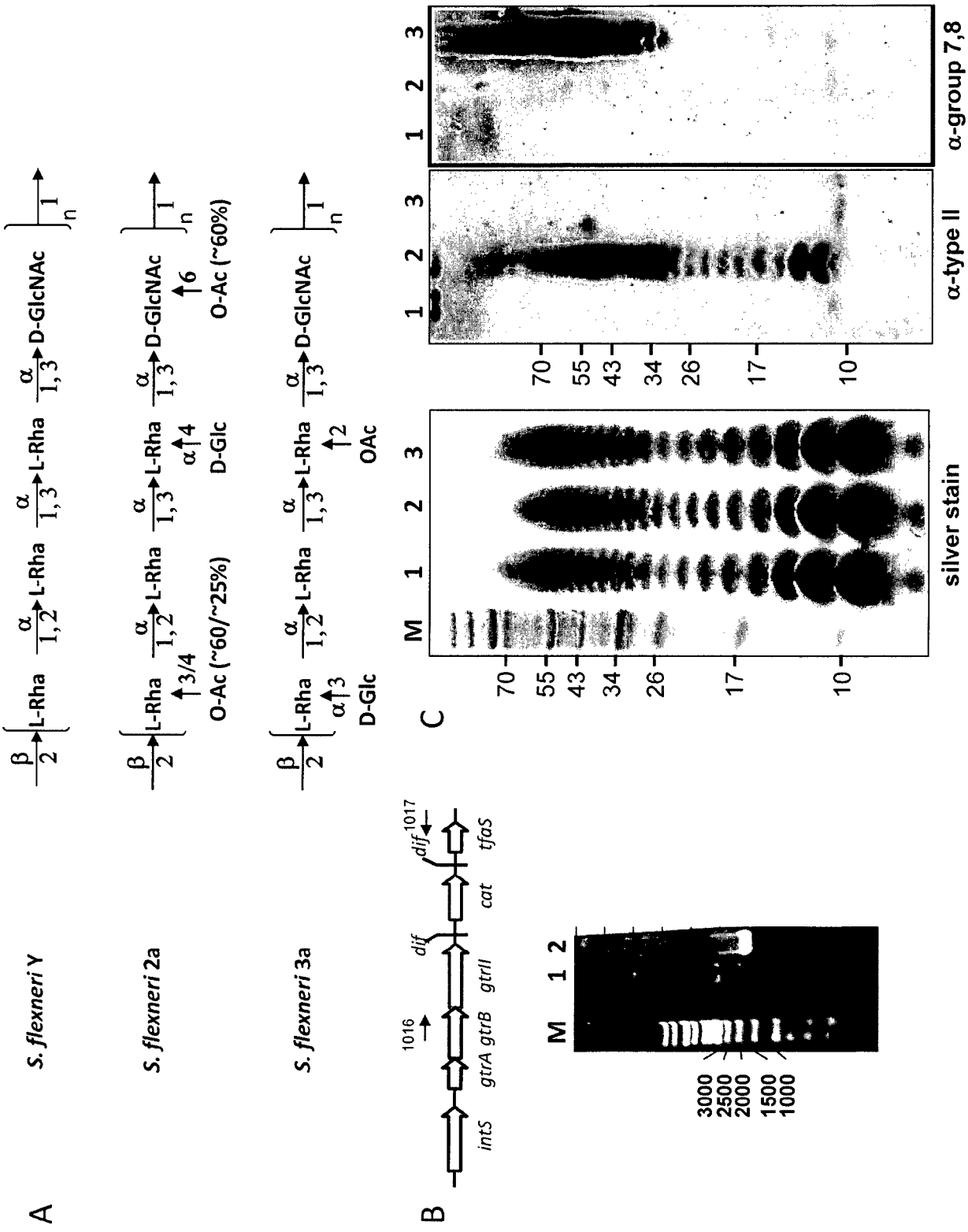

A
S. flexneri Y
S. flexneri 2a
S. flexneri 3a
β
2
L-Rha
α
1,2
L-Rha
α
1,3
L-Rha
α
1,3
D-GlcNAc
1
n
3/4
O-Ac (~60/~25%)
α
4
D-Glc
6
O-Ac (~60%)
3
α
3
D-Glc
2
OAc
B
1016
dif
dif1017
intS
gtrA
gtrB
gtrII
cat
tfaS
M
1
2
3000
2500
2000
1500
1000
C
M
1
2
3
70
55
43
34
26
17
10
silver stain
α-type II
α-group 7,8

FIG. 21

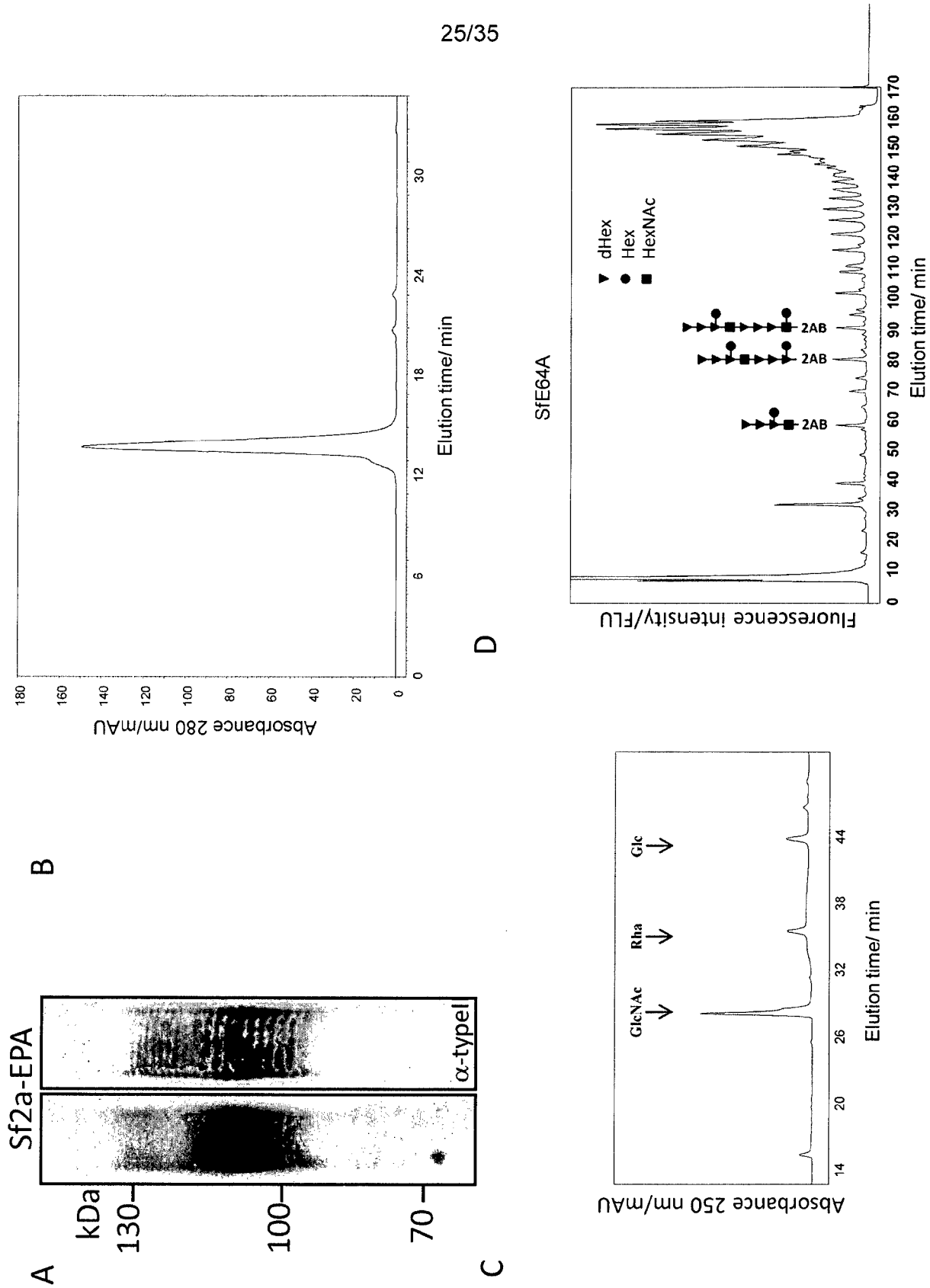

A
Sf2a-EPA
kDa
130
100
70
α-type1
B
Absorbance 280 nm/mAU
Elution time/ min
C
GlcNAc
Rha
Glc
Absorbance 250 nm/mAU
Elution time/ min
D
SfE64A
dHex
Hex
HexNAc
2AB
2AB
2AB
Fluorescence intensity/FLU
Elution time/ min

FIG. 22

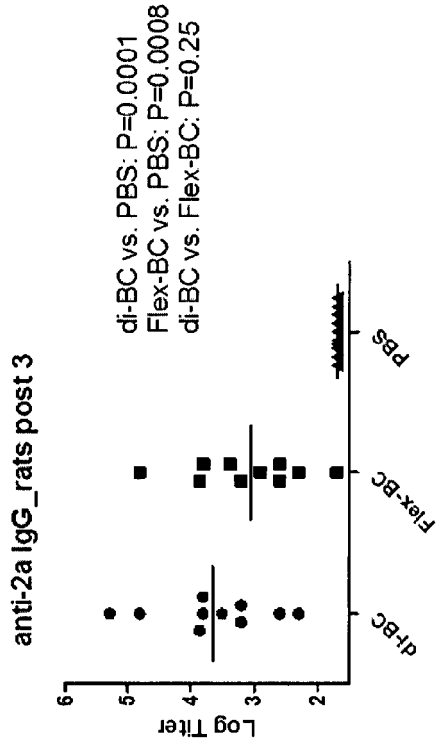

anti-2a IgG_rats post 3
di-BC vs. PBS: P=0.0001
Flex-BC vs. PBS: P=0.0008
di-BC vs. Flex-BC: P=0.25
Log Titer
6
5
4
3
2
di-BC
Flex-BC
PBS

FIG. 23

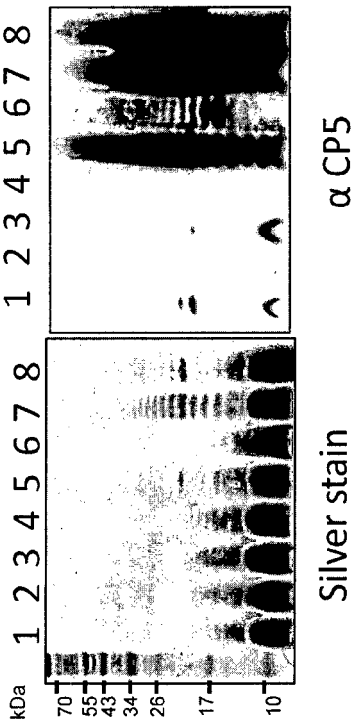


FIG. 24

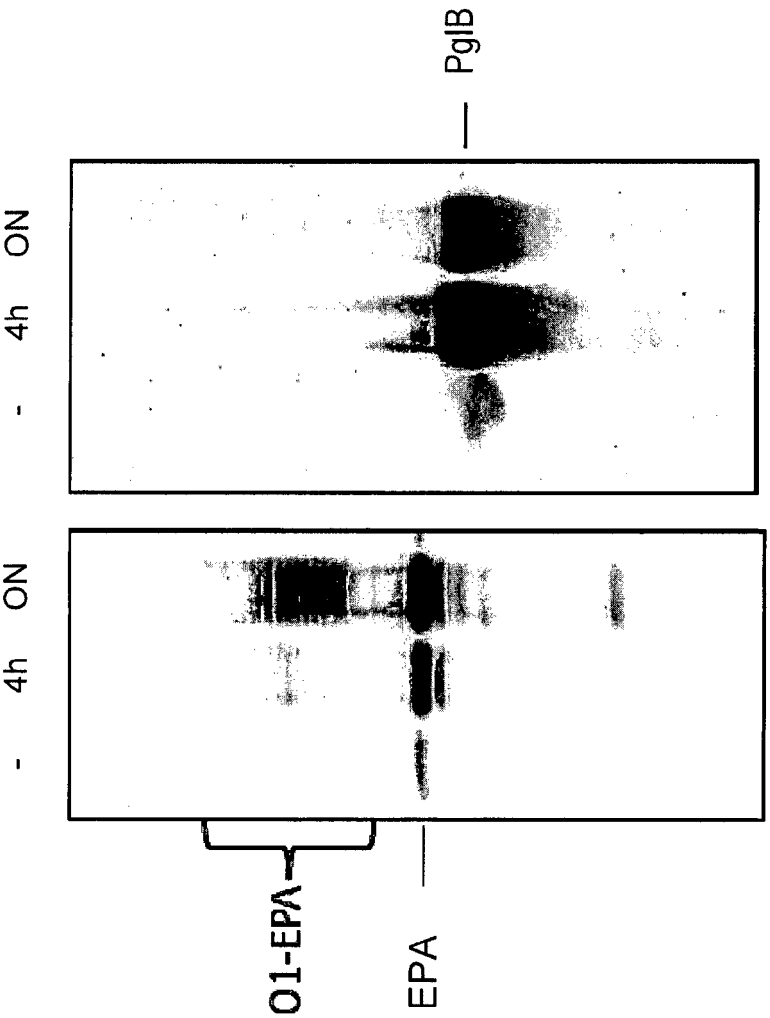


FIG. 25

O1-EPA Purification

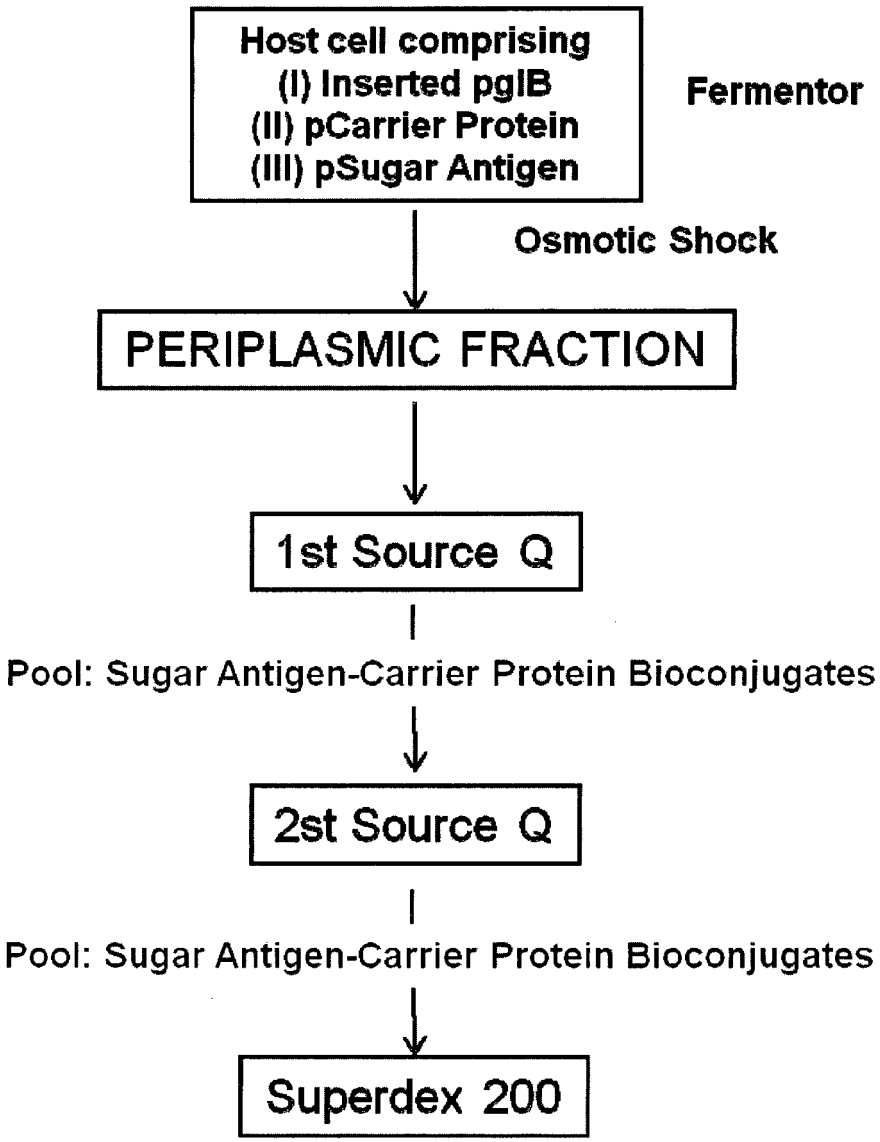


FIG. 26

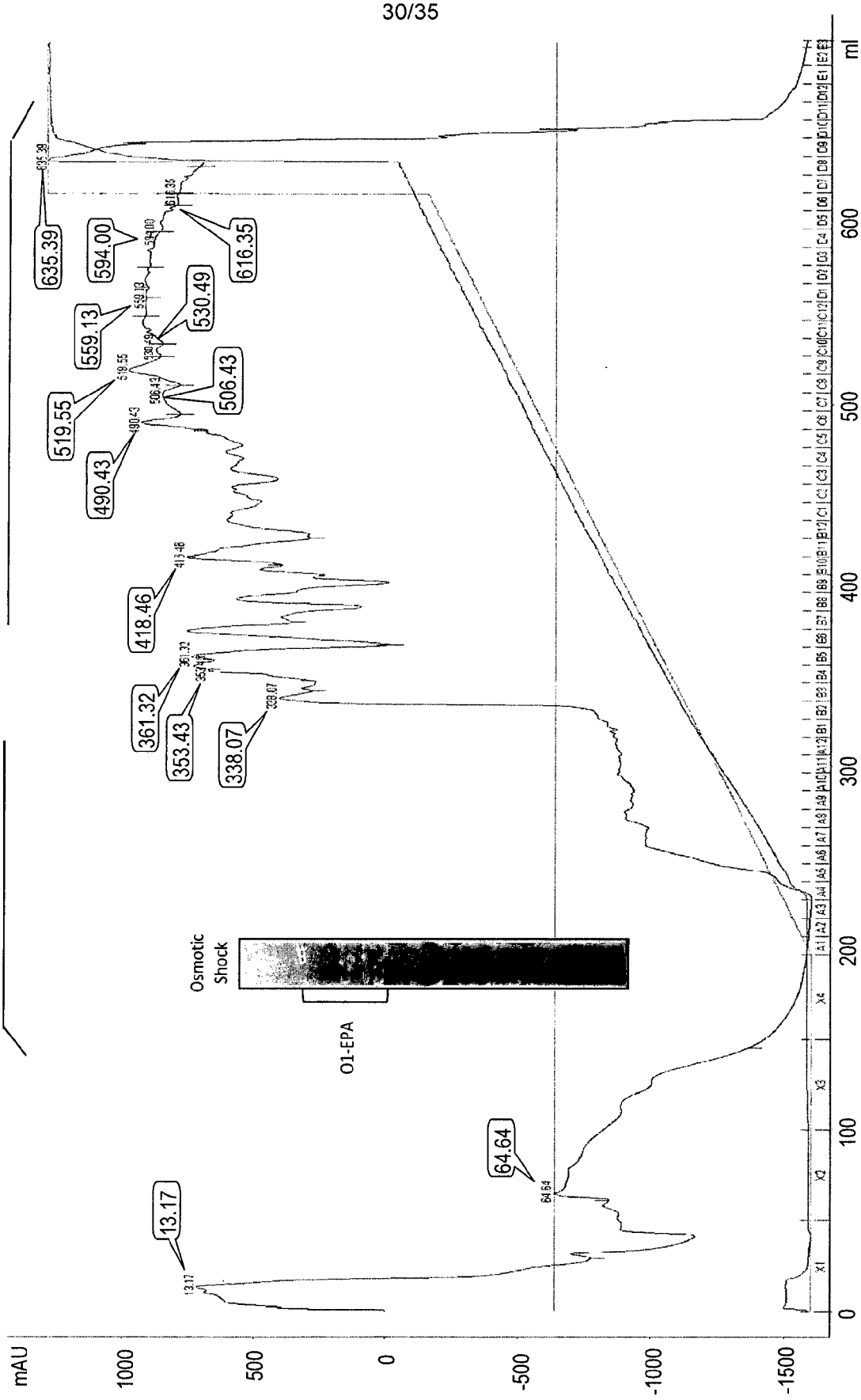

mAU
1000
500
0
-500
-1000
-1500
ml
0
100
200
300
400
500
600
635.39
594.00
616.35
559.13
530.49
519.55
506.43
490.43
418.46
361.32
353.43
338.07
64.64
13.17
Osmotic Shock
O1-EPA

FIG. 27

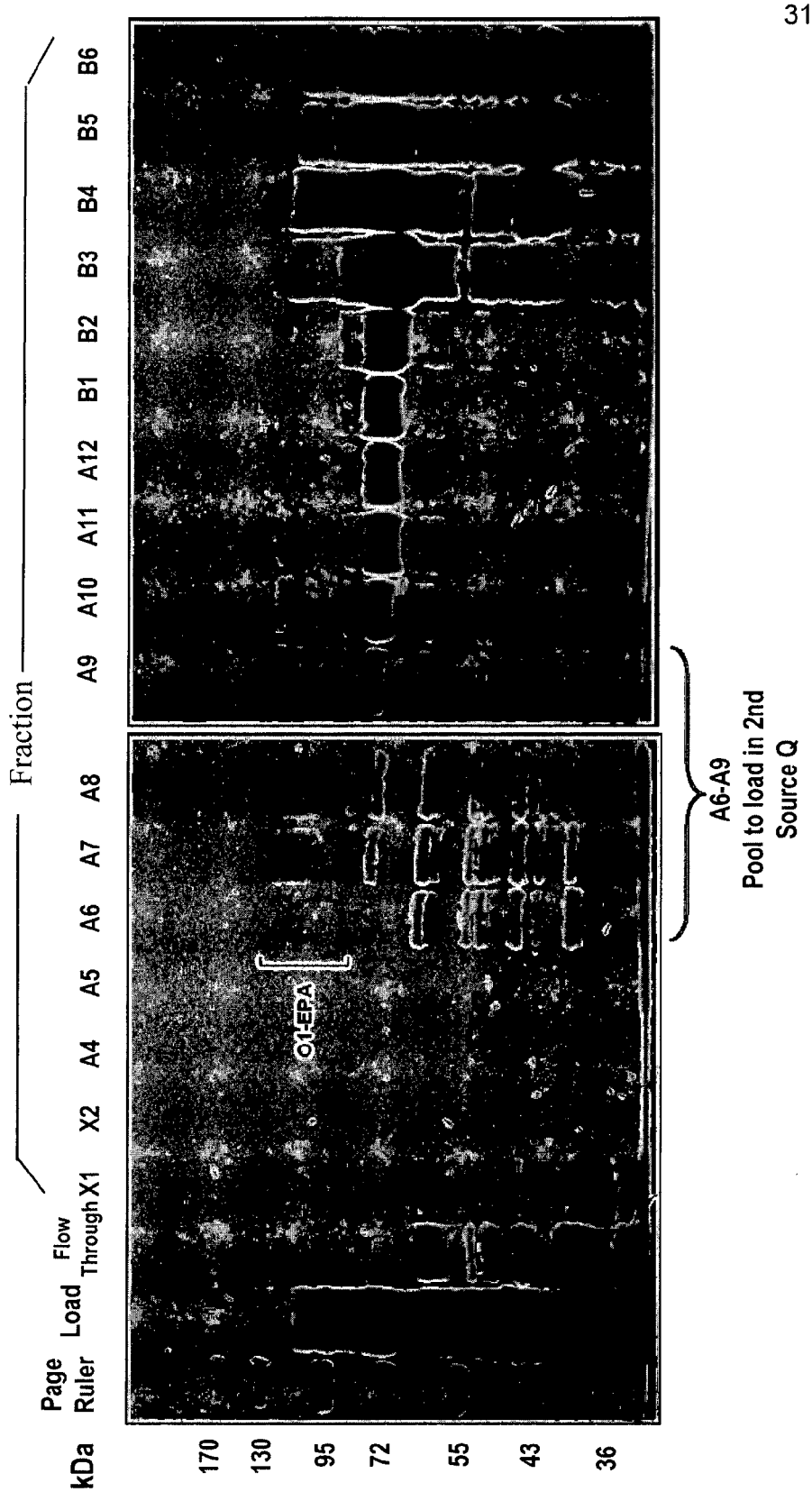

kDa
Page Ruler
Load
Flow Through
X1
X2
A4
A5
A6
A7
A8
A9
A10
A11
A12
B1
B2
B3
B4
B5
B6
Fraction
170
130
95
72
55
43
36
Q1-EPA
A6-A9
Pool to load in 2nd
Source Q

FIG. 28

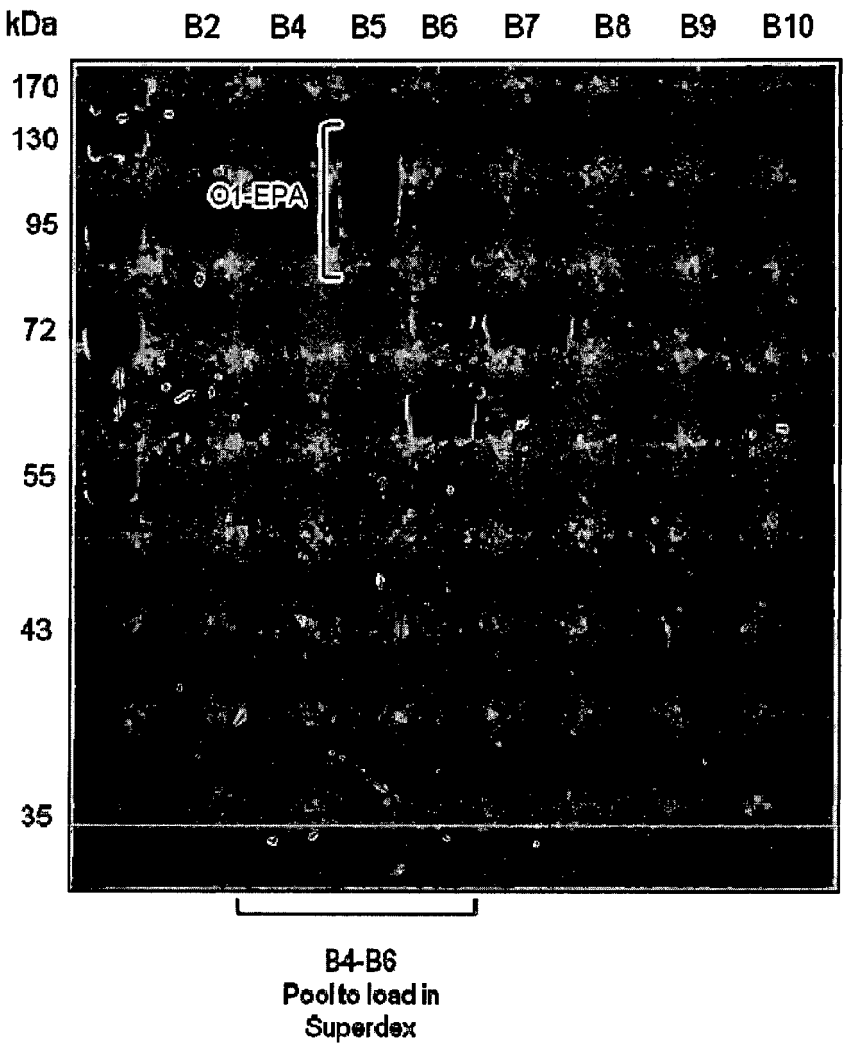

kDa
B2
B4
B5
B6
B7
B8
B9
B10
170
130
95
72
55
43
35
O1-EPA
B4-B6
Pool to load in
Superdex

FIG. 29

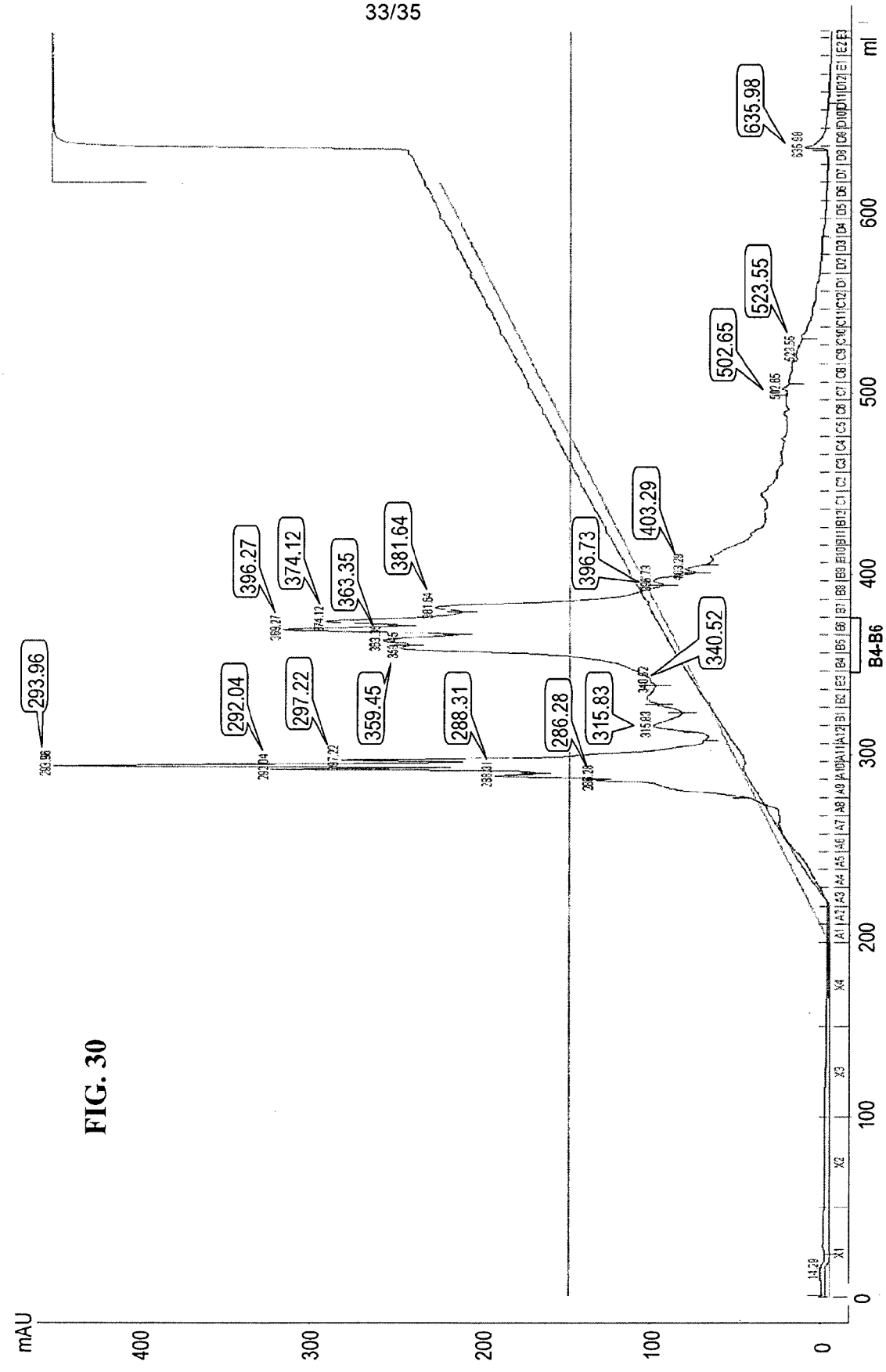

293.96
292.04
297.22
359.45
288.31
286.28
315.83
340.52
396.73
403.29
502.65
523.55
635.98
396.27
374.12
363.35
381.64
B4-B6
mAU
ml

FIG. 30

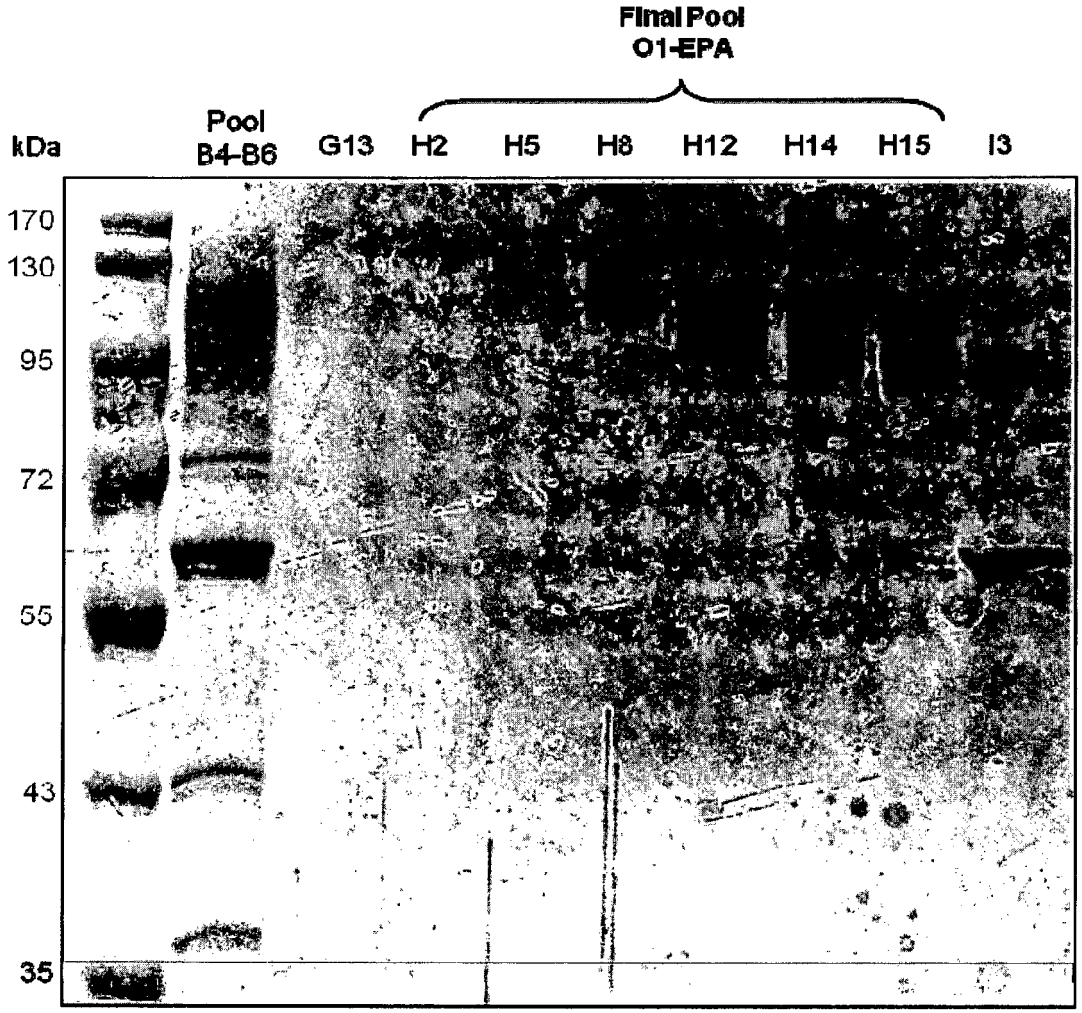

Final Pool
O1-EPA
kDa
Pool
B4-B6
G13
H2
H5
H8
H12
H14
H15
I3
170
130
95
72
55
43
35

FIG. 31

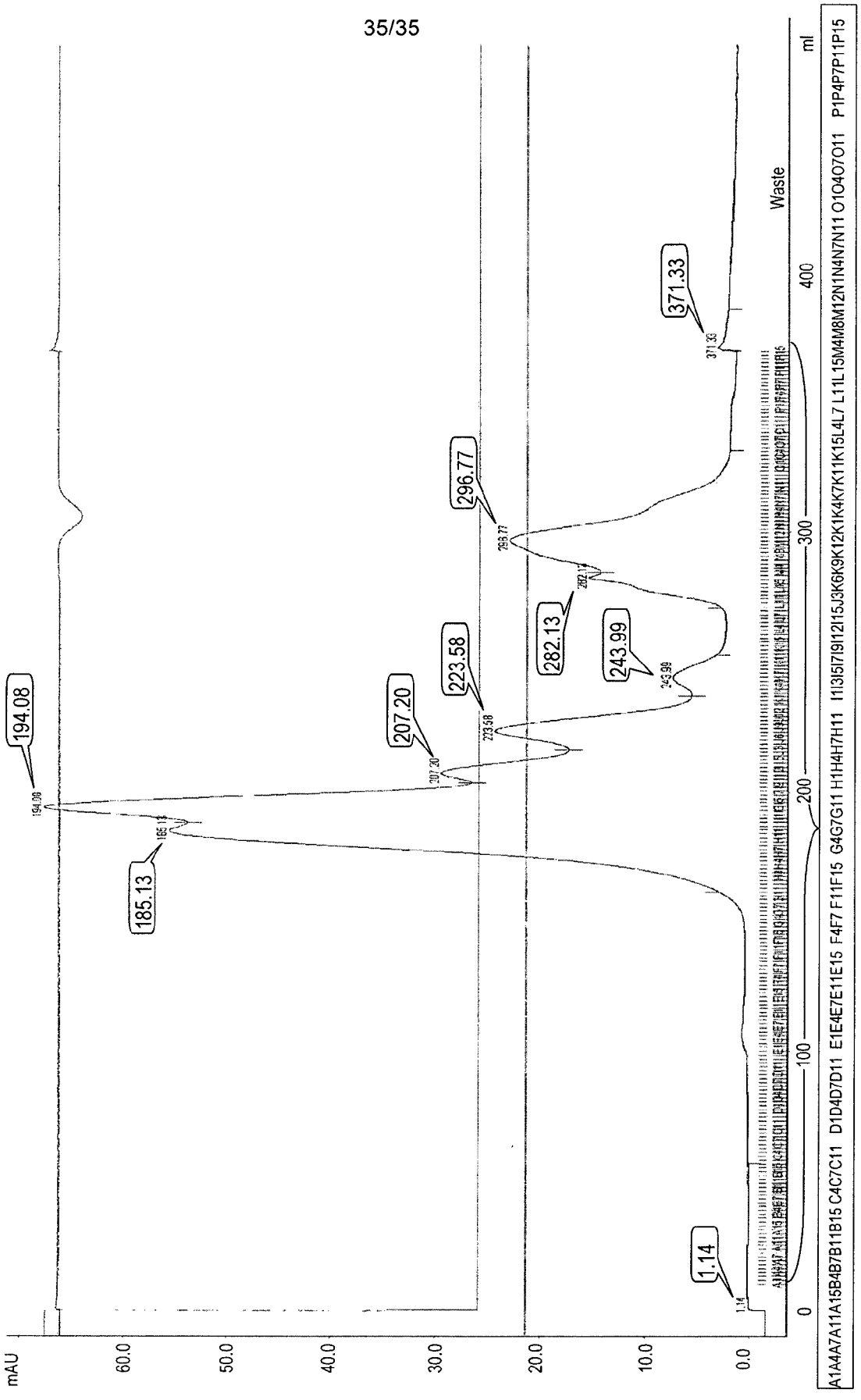

194.08
185.13
207.20
223.58
282.13
243.99
296.77
371.33
1.14
mAU
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
100
200
300
400
ml
Waste
A1A4A7A11A15B4B7B11B15 C4C7C11 D1D4D7D11 E1E4E7E11E15 F4F7 F11F15 G4G7G11 H1H4H7H11 I1I3I5I7I9I12I15J3K6K9K12K1K4K7K11K15L4L7 L11L15M4M8M12N1N4N7N11 O1O4O7O11 P1P4P7P11P15

FIG. 32