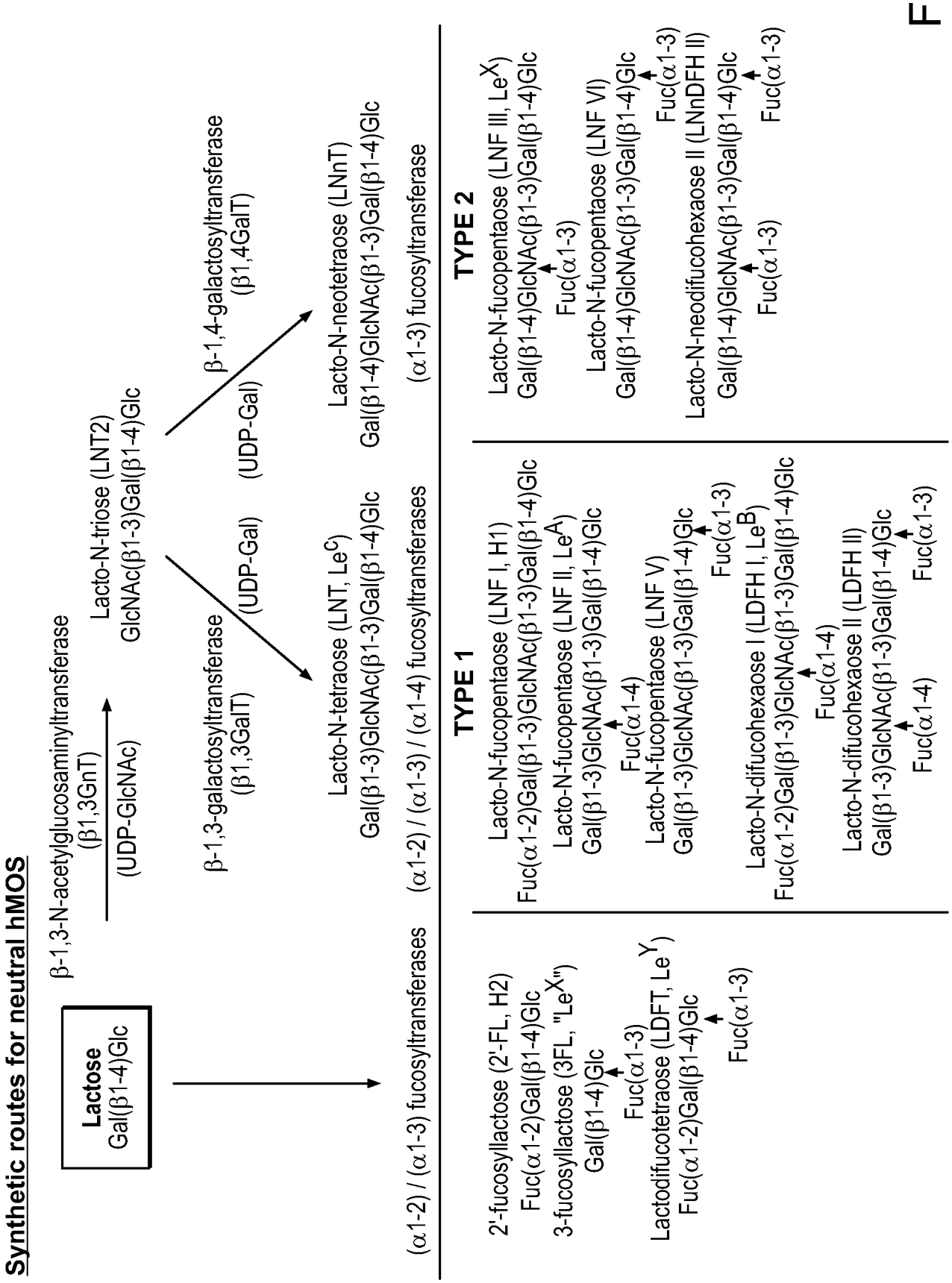

Synthetic routes for neutral hMOS
Lactose
Gal(β1-4)Glc
β-1,3-N-acetylglucosaminyltransferase
(β1,3GnT)
(UDP-GlcNAc)
Lacto-N-triose (LNT2)
GlcNAc(β1-3)Gal(β1-4)Glc
β-1,3-galactosyltransferase
(β1,3GalT)
(UDP-Gal)
β-1,4-galactosyltransferase
(β1,4GalT)
(UDP-Gal)
Lacto-N-tetraose (LNT, Le^C)
Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc
Lacto-N-neotetraose (LNnT)
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
(α1-2) / (α1-3) fucosyltransferases
(α1-2) / (α1-3) / (α1-4) fucosyltransferases
(α1-3) fucosyltransferase
TYPE 1
TYPE 2
2'-fucosyllactose (2'-FL, H2)
Fuc(α1-2)Gal(β1-4)Glc
3-fucosyllactose (3FL, "Le^X")
Gal(β1-4)Glc
Fuc(α1-3)
Lactodifucotetraose (LDFT, Le^Y)
Fuc(α1-2)Gal(β1-4)Glc
Fuc(α1-3)
Lacto-N-fucopentaose (LNF I, H1)
Fuc(α1-2)Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc
Lacto-N-fucopentaose (LNF II, Le^A)
Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc
Fuc(α1-4)
Lacto-N-fucopentaose (LNF V)
Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc
Fuc(α1-3)
Lacto-N-difucohexaose I (LDFH I, Le^B)
Fuc(α1-2)Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc
Fuc(α1-4)
Lacto-N-difucohexaose II (LDFH II)
Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc
Fuc(α1-4)
Fuc(α1-3)
Lacto-N-fucopentaose (LNF III, Le^X)
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
Fuc(α1-3)
Lacto-N-fucopentaose (LNF VI)
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
Fuc(α1-3)
Lacto-N-neodifucohexaose II (LNnDFH II)
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc
Fuc(α1-3)
Fuc(α1-3)

FIG. 1

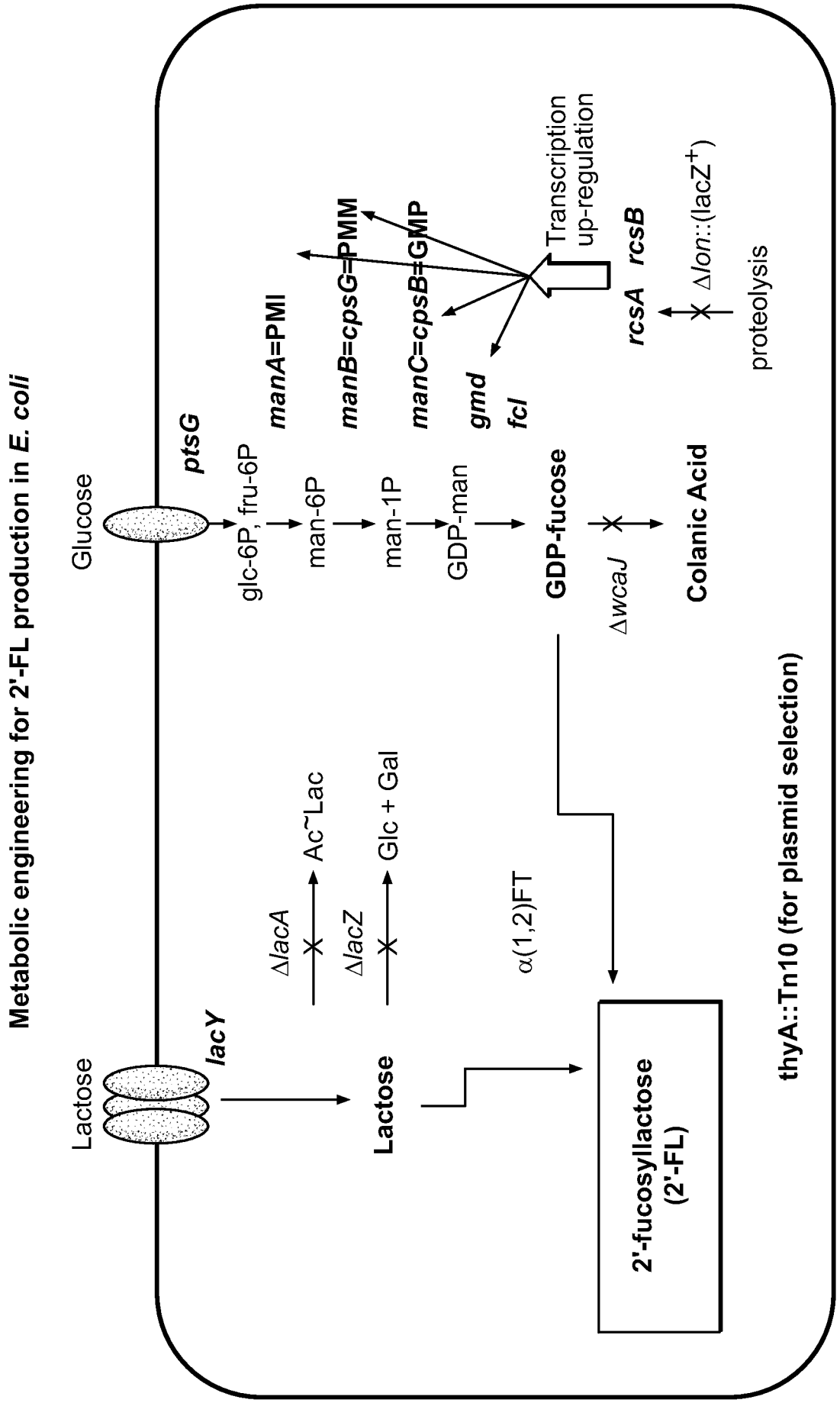

Metabolic engineering for 2'-FL production in E. coli
Glucose
ptsG
glc-6P, fru-6P
manA=PMI
man-6P
manB=cpsG=PMM
man-1P
manC=cpsB=GMP
GDP-man
gmd
fcl
GDP-fucose
Transcription up-regulation
rcsA rcsB
ΔwcaJ
Colanic Acid
Δlon::(lacZ+)
proteolysis
ΔlacA
Ac~Lac
ΔlacZ
Glc + Gal
α(1,2)FT
Lactose
lacY
Lactose
2'-fucosyllactose (2'-FL)
thyA::Tn10 (for plasmid selection)

FIG. 2

	1	2	3	4
H.pylori futC (1)		70.10	23.97	22.57
H.mustelae futL (2)	70.10		27.04	21.36
Bacteroides vulgatus futN (3)	23.97	27.04		31.23
E. coli O126 wbgL (4)	22.57	21.36	31.23	

FIG. 3A

H.pylori futC MAFKVVQLCGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDWSDRKMQLE 100
H.mustelae futL MDFKIVQVHGGLGNQMFQYAFAKSLQTHLNIPVLLDTTWFDYGNRELGLH 100
Bacteroides vulgatus futN M- - RLIKVTGGLGNQMFIYAF - YRRMKKYYPKVRIDLSDMMHYKVHYGYE 95
E. coli 0126 wbgL M- - SIIRLQGGLGNQLFQFSFGYALSKINGTPLYFDISHYAENDDHGGYRL 103
H.pylori futC LKPSRLTYFFGYFQDPRYFDAISPLIKQTFTLPPPPENNKNNKKEEEYQL 150
H.mustelae futL FEPSRIAYFHGYFQDPRYFEDISPLIKQTFTLPHPTEH- - - - - - - - - AEQYS 141
Bacteroides vulgatus futN - - - WPLLYFKGFYQSERFFADIKDEVRESFTFDK- - - - - - - - - - NKANSRS 142
E. coli 0126 wbgL AQKWKSKKYIGYWQSEHFFHKHILDLKEFF- IPK- - - - - - - - - - NVSEQAN 150
H.pylori futC FVFCEDLEFT- QNLDLGYPFMDMTTRDKEEEAYWDMLLMQSCQHGIIANS 200
H.mustelae futL FLFCEDLEFV- QNLDLGYPFVDMTTRDGA- - AHWDMMLMQSCKHGIITNS 193
Bacteroides vulgatus futN YIFSDDIAWVKENLP- - LQNAVYIDWNTDELSWQDMMLMSHCKHHIICNS 190
E. coli 0126 wbgL FIFSDDIFWCKENIETLLSKKYNIYYSEDLSQEEDLWLMSLANHHIIANS 199
LFPIDLPYASAKEIAIAKMQHL- - - - - - - - PKLVRDALKCMGFDRVSQEIVFEYEPKL 250
LFPIDLQCASAQQIAAAHMQNL- - - - - - - - PRLVRGALRRMGLGRVSKEIVFEYMPEL 250
MHRVFNLPHTEFCINQP- LKKVIEFL- - - - - FFKKIYERKQAPNSLRAF- - EKK- YF 245
LNNL- QIPEEYLQYTPKLNNIYKFLVRGSRLYPEIFLFLGFCNEFHAYGYDFE- YI 253
CKLSILAA- KNSVFVHIRRGDYVG- - - - - - IGCQLGIDYQKKALEYMAKRVPNMEL 300
RKLSQILAA- KNSVFVHIRRGDYMR- - - - - - LGWQLDISYQLRAIAYMAKRVQNLEL 293
LNMLEILDKDENAVSLHIRRGDYLQPKHWATTGSVCQLPYYQNAIAEMSRRVASPSY 290
LLAAKILESQSSLSIHIRRGDYIKNKTATLTHGVCSLEYYKKALNKIRDLAMIRDV 299
TYSWWAAYLIENPEKIIIGPKHWLFGHENI- - - - - LCKEWVKIESHFEVKSQKYNA* 351
TYSWWAAYLIKNPEKIIIGPSHWIYGNENI- - - - - LCKDWVKIESQFETKS* - - - - - - 337
TFSWWGAWLNPNNDKTVIVPSRWFQHSEAPDIYP- - TGWIKVPVS* - - - - - - - - - - 332
SFSWWGAYLGTSASQIVIYPTPWYDITPKNTYIPIVNHWINVDKHSSC* - - - - - - - - 348

FIG. 3B

FIG. 4A	FIG. 4B
FIG. 4C	FIG. 4D
FIG. 4E	FIG. 4F

FIG. 4

FIG. 4A

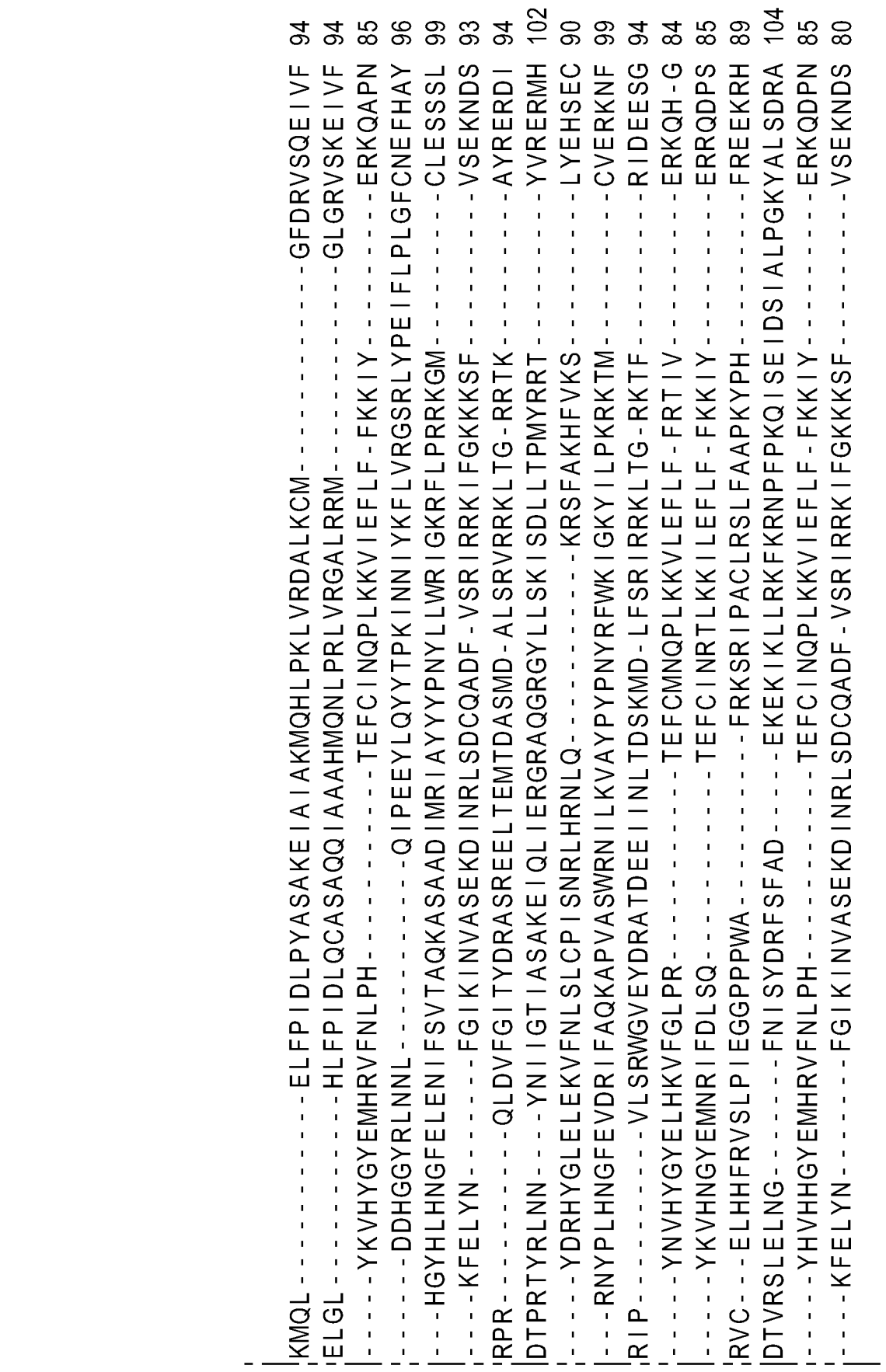

KMQL----------ELFPIDLPYASAKEIAIAKMQHLPKLVRDALKCM----------GFDRVSQEIVF 94
ELGL----------HLFPIDLQCASAQQIAAAHMQNLPRLVRGALRRM----------GLGRVSKEIVF 94
-----YKVHYGYEMHRVFNLPH----------TEFCINQPLKKVIEFLF-FKKIY----------ERKQAPN 85
------DDHGGYRLNNL----------QIPEEYLQYYTPKINNIYKFLVRGSRLYPEIFLPLGFCNEFHAY 96
---HGYHLHNGFELENIFSVTAQKASAADIMRIAYYYPNYLLWRIGKRFLPRRKGM----------CLESSSL 99
----KFELYN----------FGIKINVASEKDINRLSDCQADF-VSRIRRKIFGKKKSF----------VSEKNDS 93
RPR----------QLDVFGITYDRASREELTEMTDASMD-ALSRVRRKLTG-RRTK----------AYRERDI 94
DTPRTYRLNN----YNIIGTIASAKEIQLIERGRAQGRGYLLSKISDLLTPMYRRT----------YVRERMH 102
-----YDRHYGLELEKVFNLSLCPISNRLHRNLQ----------KRSFAKHFVKS----------LYEHSEC 90
---RNYPLHNGFEVDRIFAQKAPVASWRNILKVAYPYPNYRFWKIGKYILPKRKTM----------CVERKNF 99
RIP----------VLSRWGVEYDRATDEEIINLTDSKMD-LFSRIRRKLTG-RKTF----------RIDEESG 94
-----YNVHYGYELHKVFGLPR----------TEFCMNQPLKKVLEFLF-FRTIV----------ERKQH-G 84
-----YKVHNGYEMNRIFDLSQ----------TEFCINRTLKKILEFLF-FKKIY----------ERRQDPS 85
RVC---ELHHFRVSLPIEGGPPPWA----------FRKSRIPACLRSLFAAPKYPH----------FREEKRH 89
DTVRSLELNG----------FNISYDRFSFAD----------EKEKIKLLRKFKRNPFPKQISEIDSIALPGKYALSDRA 104
-----YHVHHGYEMHRVFNLPH----------TEFCINQPLKKVIEFLF-FKKIY----------ERKQDPN 85
----KFELYN----------FGIKINVASEKDINRLSDCQADF-VSRIRRKIFGKKKSF----------VSEKNDS 80

FIG. 4B

```
H.pylori futC      EYEPKLLKPSR-LTYFFGYFQDPRYF--DAISPLIKQTFTLPPPPEN
H.mustelae futL    EYMPELFEPSR-IAYFHGYFQDPRYF--EDISPLIKQTFTLPHPTEH
Bacteroides vulgatus futN  SLRAFEKKYFWPLLYFKGFYQSERFF--ADIKDEVRESFTF----DK
E. coli 0126 wbgL  GYDFEYIAQKWKSKKYIGYWQSEHFFHKHIL--DLKEFFI------P
Prevotella melaninogenica FutO YP_003814512.1  RFDESVLR-QEGNRYFDGYWQDERYF--AAYREKVLKAFTFPAFKRA
Clostridium bolteae+13 FutP WP_002570768.1  CYENDILR-M-DNVYLSGYWQTEKYF--SNTREKLLEDYSF-ALVNS
Lachnospiraceae sp. FutQ WP_009251343.1  NFDPLVME--KDPALLEGCFQSDKYF--RDCEGRVREAYRFRGIESG
Methanosphaerula palustris FutR YP_002467213.1  TFDKAILT-VPDNVYLDGYWQTEKYF--KDIEEILRREVTLKDEPDS
Tannerella sp. FutS WP_021929367.1  EFDEPVYRGLRPYRYYRGYWQNEGYFV--DIEPMIREAFQFNVNILS
Bacteroides caccae FutU WP_005675707.1  SFDAAVLT-RKGDCYYDGYWQHEEYF--CDMKETIWEAFSFPEPVDG
Butyrivibrio FutV WP_022772718.1  KFNPEILE--KENAYLVGYWQCDKYFDDKDVVREIREAFEKKPQE--
Prevotella sp. FutW WP_022481266.1  RMEPYTCQYVWPLVYFKGFYQSERYF--SEVKDEVRECFTF------NP
Parabacteroides johnsonii FutX WP_008155883.1  TLYPYEKRYFWPLLYFKGFYQSERFF--FDIKDDVRKAFSF------NL
Akkermansia muciniphilia FutY YP_001877555  GFDPGLAAPPRRHTYFKGYFQTEQYFL--HCREQLCREFRLKTPLTP
Salmonella enterica FutZ WP_023214330  FYTFETIKNIDKACLFSFYQDAD---LLNKYKQLILPLFELR----DD
Bacteroides sp. FutZA WP_022161880.1  SLRAFEKKYLWPLLYFKGFYQSERFF--ADIKDEVRKAFTF------DS
Clostridium bolteae FutP WP_002570768.1  CYENDILR-M-DNVYLSGYWQTEKYF--SNTREKLLEDYSF-ALVNS
```

FIG. 4C

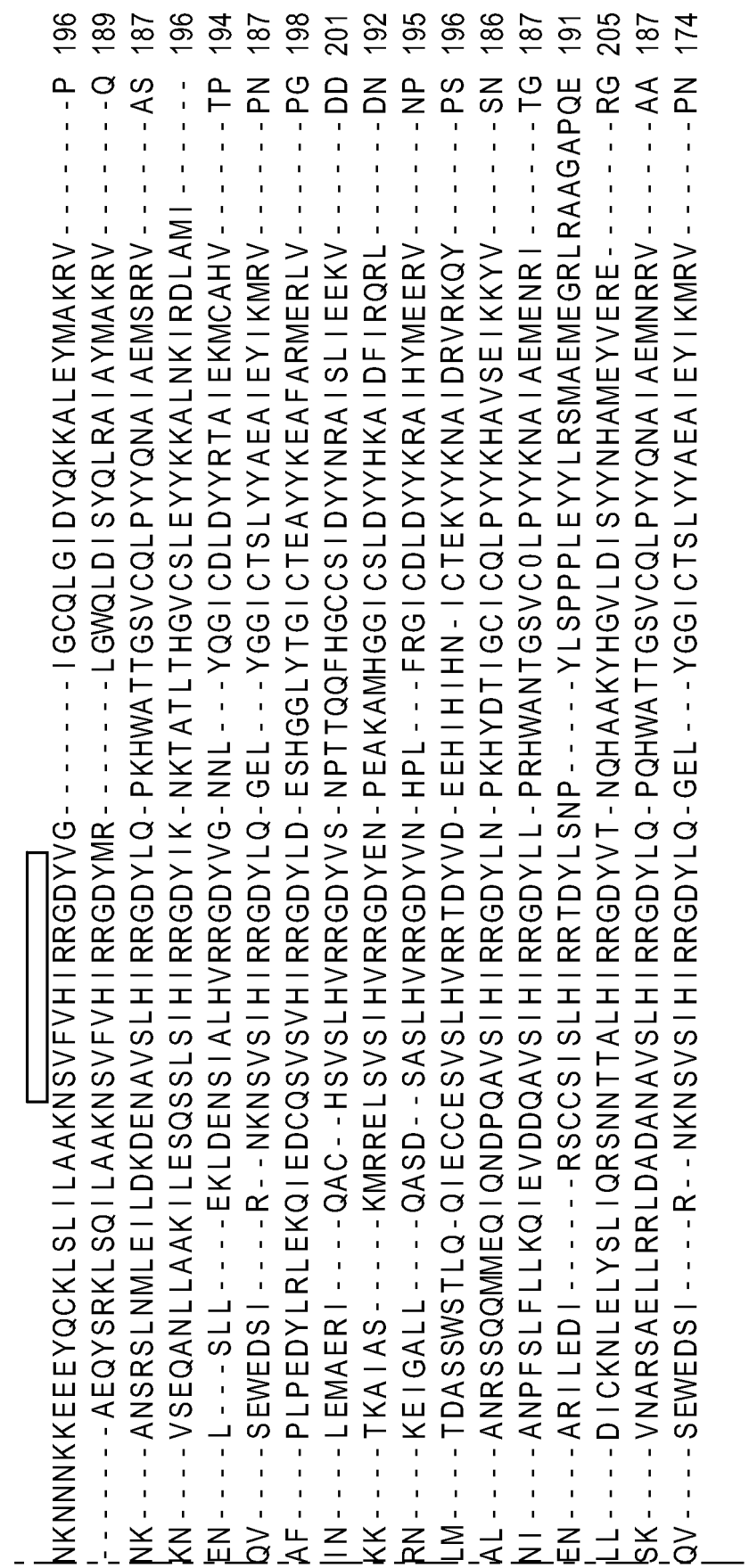


FIG. 4D

```
H.pylori futC      NMELFVFCEDLEFT-QNLDLGYP---FMDMTTRDKEFEAYWDMLLMG
H.mustelae futL    NLELFLFCEDLEFV-QNLDLGYP---FVDMTTRDGA--AHWDMMLMQ
Bacteroides vulgatus futN  PS-YYIFSDDIAWVKENL----PLQNAVYIDWNTDE-DSWQDMMLMS
E. coli 0126 wbgL  -RDVFIFSDDIFWCKENIETLLSKKYNIYYSEDLSQEE---DLWLMS
Prevotella melaninogenica FutO YP_003814512.1  SL-FCIFSNDITWCQQHLQPYLKAP-VVYVTWNTGV-ESYRDMQLMS
Clostridium bolteae+13 FutP WP_002570768.1  AK-FFVFSDDVEWVKQQED----FKGFVIVDRNEYS-SALSDMYLMS
Lachnospiraceae sp. FutQ WP_009251343.1  AR-FFLFSNDPEWTREHFE----SKNCVLVEGSTED-TGYMDLYLMS
Methanosphaerula palustris FutR YP_002467213.1  PS-FFIFSDDLPWAKENLD--IPGEKT-FVAHNGPE-KEYCDLWLMS
Tannerella sp. FutS WP_021929367.1  NICFYLFSDDINWVEENLQL----ENRCIIDWNQGE-DSWQDMYLMS
Bacteroides caccae FutU WP_005675707.1  QL-YCVFSNDMAWCESHLRALLPGKEVVYVDWNKGA-ESYVDMRLMS
Butyrivibrio FutV WP_022772718.1  AV-FFIFTDDKEWCRDHFK----GPNFIVVELEEGDGTDIAEMRLMS
Prevotella sp. FutW WP_022481266.1  PH-FYVFSEDLDWVKANL----PLENAQYIDWNKGA-DSWQDMMLMS
Parabacteroides johnsonii FutX WP_008155883.1  PS-YYVFSDDISWVKENI----PLKKAVYVTWNKGE-DSWQDMMLMS
Akkermansia muciniphilia FutY YP_001877555  SLRYFIFSDDIEWARQNLRPALP---HVHVDINDG-GTGYFDLELMR
Salmonella enterica FutZ WP_023214330  KQNFIIFSDDVRWAQKAFLE----NDNCYVINNSDYDFSAIDMYLMS
Bacteroides sp. FutZA WP_022161880.1  PS-YYVFSDDIAWVKENI----PLQNAVYIDWNKGE-ESWQDMMLMS
Clostridium bolteae FutP WP_002570768.1  AK-FFVFSDDVEWVKQQED----FKGFVIVDRNEYS-SALSDMYLMS
```

FIG. 4E

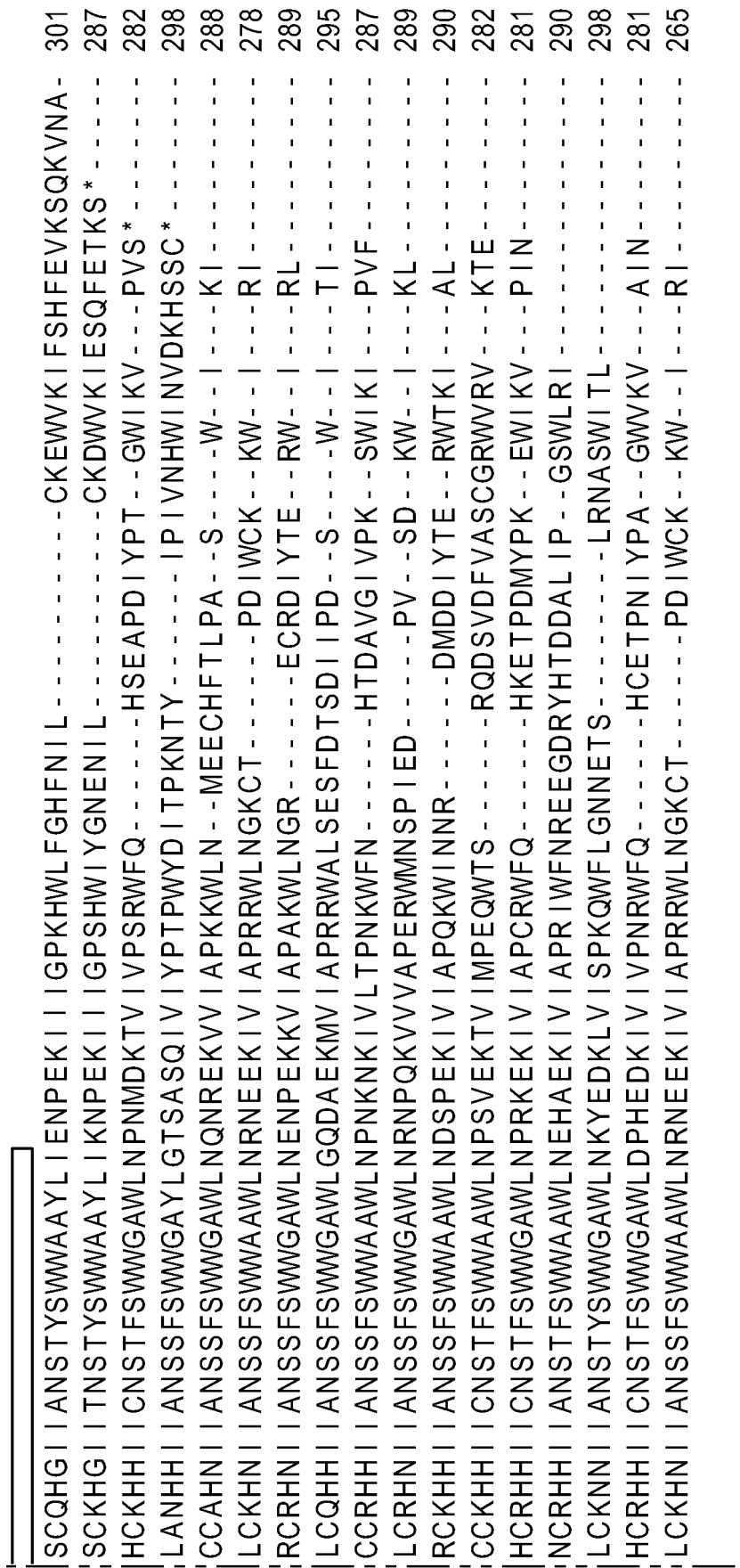

SCQHGIIANSTYSWWAAYLIENPEKIIIGPKHWLFGHFNIL----------CKEWVKIFSHFEVKSQKVNA- 301
SCKHGIITNSTYSWWAAYLIKNPEKIIIGPSHWIYGNENIL----------CKDWVKIESQFETKS*----- 287
HCKHHIICNSTFSWWGAWLNPNMDKTVIVPSRWFQ------HSEAPDIYPT--GWIKV---PVS*------- 282
LANHHIIANSSFSWWGAYLGTSASQIVIYPTPWYDITPKNTY------IPIVNHWINVDKHSSC*------- 298
CCAHNIIANSSFSWWGAWLNQNREKVVIAPKKWLN--MEECHFTLPA--S----W--I---KI--------- 288
LCKHNIIANSSFSWWAAWLNRNEEKIVIAPRRWLNGKCT------PDIWCK--KW--I---RI--------- 278
RCRHNIIANSSFSWWGAWLNENPEKKVIAPAKWLNGR------ECRDIYTE--RW--I---RL--------- 289
LCQHHIIANSSFSWWGAWLGQDAEKMVIAPRRWALSESFDTSDIIPD--S----W--I---TI--------- 295
CCRHHIIANSSFSWWAAWLNPNKNKIVLTPNKWFN------HTDAVGIVPK--SWIKI---PVF------- 287
LCRHNIIANSSFSWWGAWLNRNPQKVVVAPERWMNSPIED------PV--SD--KW--I---KL--------- 289
RCKHHIIANSSFSWWAAWLNDSPEKIVIAPQKWINNR------DMDDIYTE--RWTKI---AL--------- 290
CCKHHIICNSTFSWWAAWLNPSVEKTVIMPEQWTS------RQDSVDFVASCGRWVRV---KTE------- 282
HCRHHIICNSTFSWWGAWLNPRKEKIVIAPCRWFQ------HKETPDMYPK--EWIKV---PIN------- 281
NCRHHIIANSTFSWWAAWLNEHAEKIVIAPRIWFNREEGDRYHTDDALIP--GSWLRI------------- 290
LCKNNIIANSTYSWWGAWLNKYEDKLVISPKQWFLGNNETS----------LRNASWITL------------ 298
HCRHHIICNSTFSWWGAWLDPHEDKIVIVPNRWFQ------HCETPNIYPA--GWVKV---AIN------- 281
LCKHNIIANSSFSWWAAWLNRNEEKIVIAPRRWLNGKCT------PDIWCK--KW--I---RI--------- 265

FIG. 4F

FIG. 5A

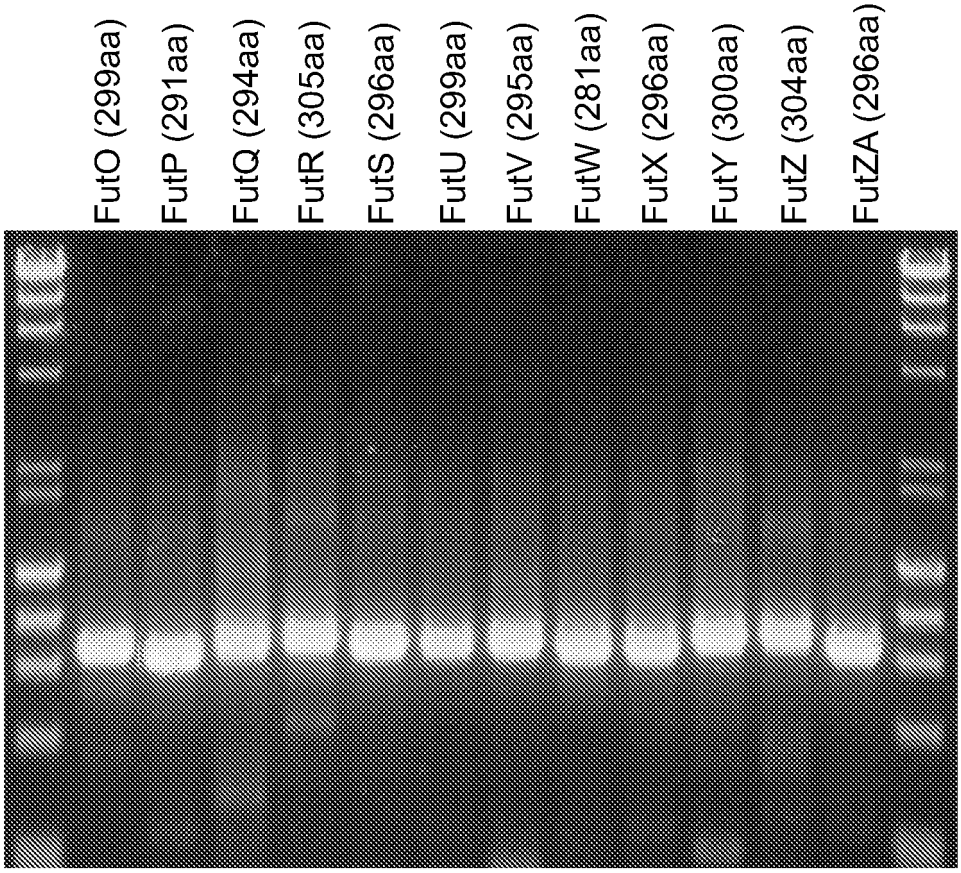
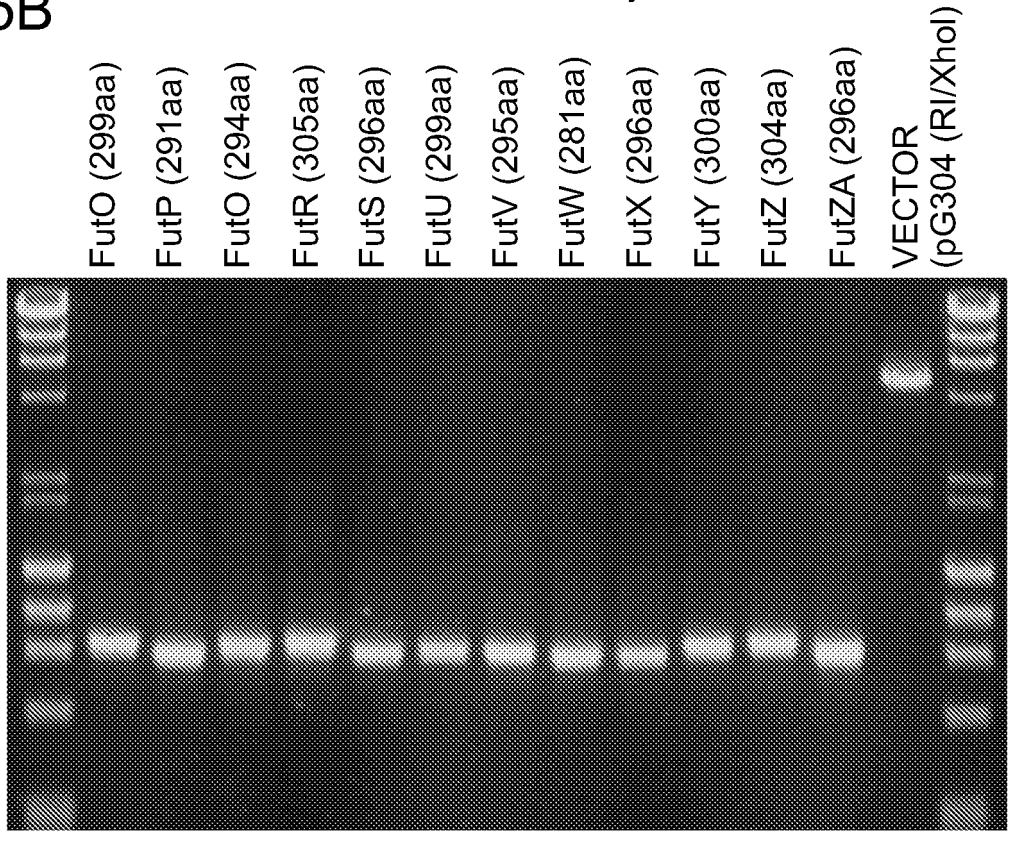

FutO (299aa)
FutP (291aa)
FutQ (294aa)
FutR (305aa)
FutS (296aa)
FutU (299aa)
FutV (295aa)
FutW (281aa)
FutX (296aa)
FutY (300aa)
FutZ (304aa)
FutZA (296aa)

FIG. 5B


Post-Gibson assembly PCR
FutO (299aa)
FutP (291aa)
FutO (294aa)
FutR (305aa)
FutS (296aa)
FutU (299aa)
FutV (295aa)
FutW (281aa)
FutX (296aa)
FutY (300aa)
FutZ (304aa)
FutZA (296aa)
VECTOR (pG304 (RI/XhoI)
Gel-purified RI/XhoI syngene fragment

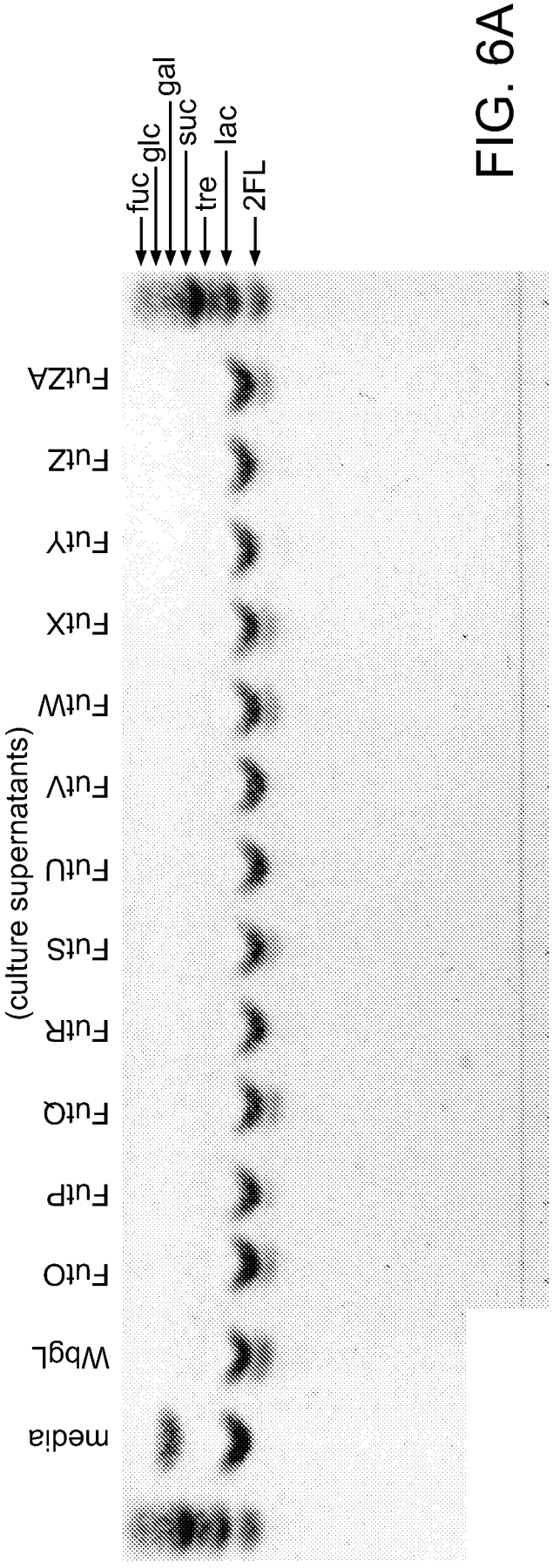

fuc
glc
gal
suc
tre
lac
2FL
(culture supernatants)
media
WbgL
FutO
FutP
FutQ
FutR
FutS
FutU
FutV
FutW
FutX
FutY
FutZ
FutZA

FIG. 6A

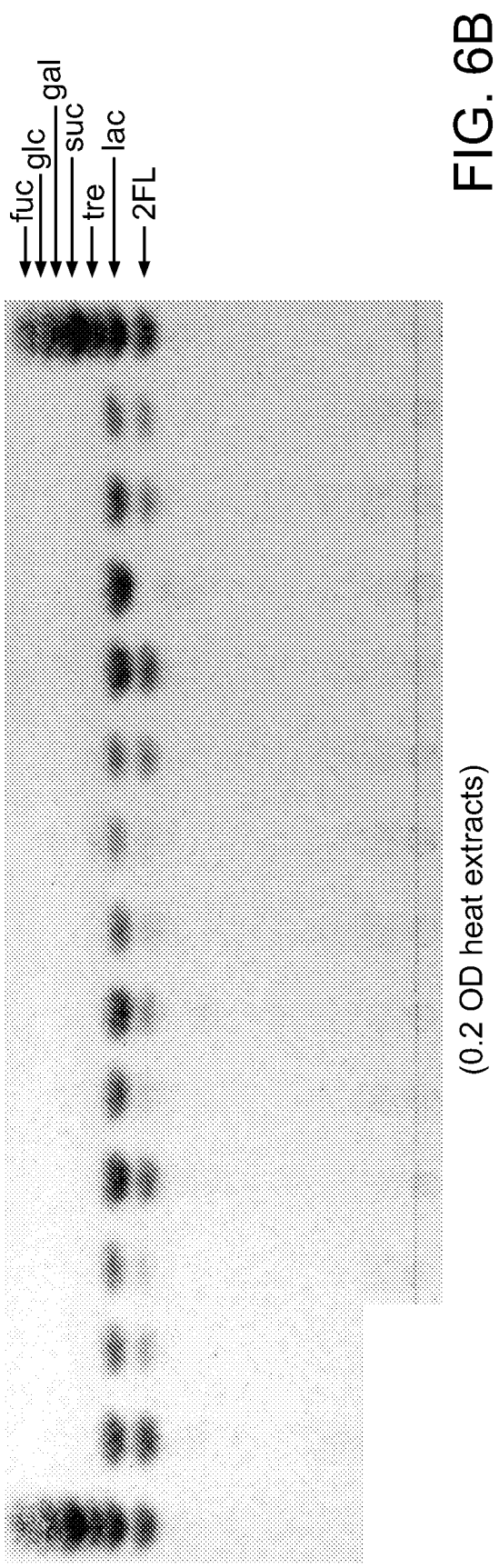
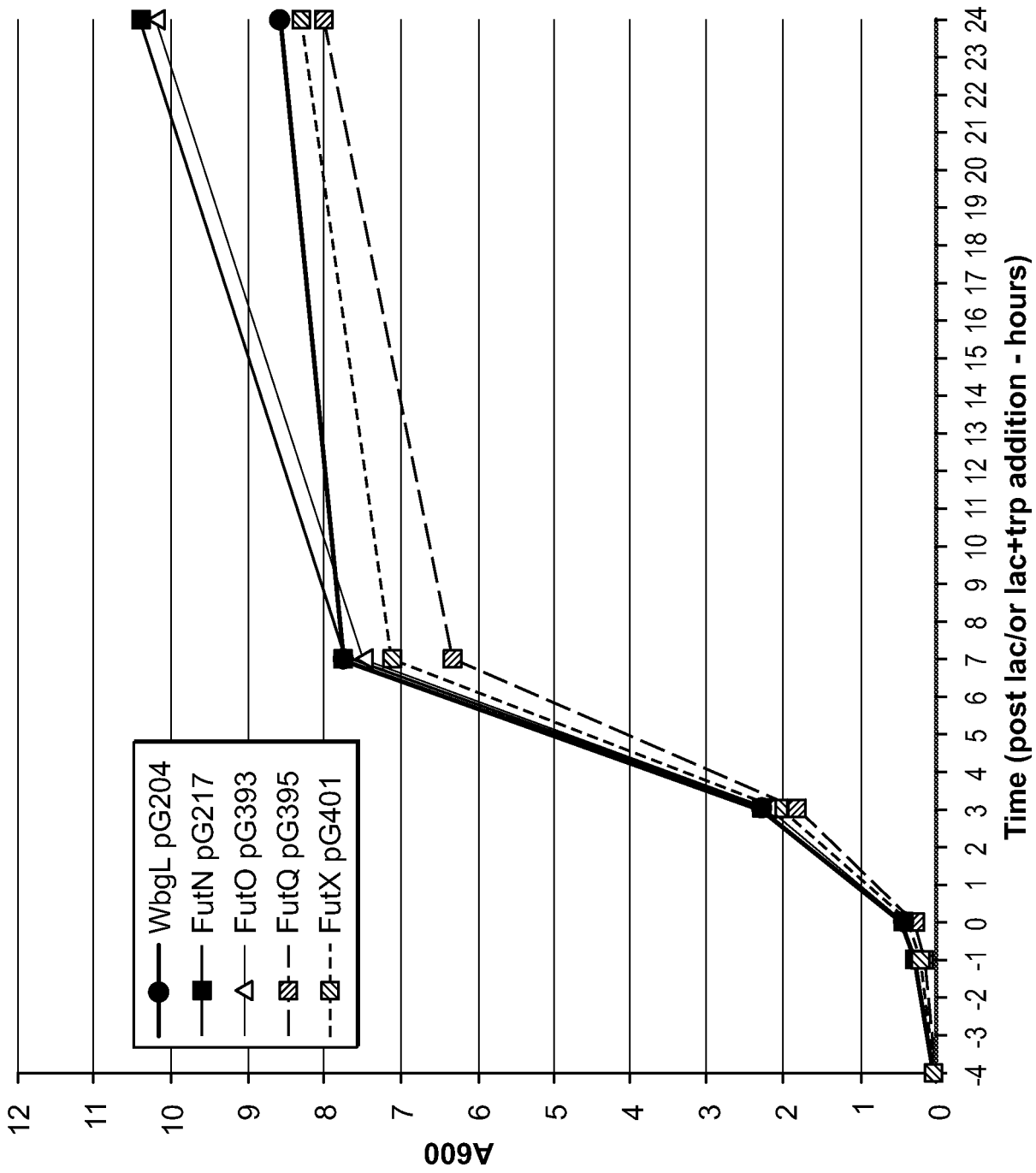

fuc
glc
gal
suc
tre
lac
2FL
(0.2 OD heat extracts)

FIG. 6B

FIG. 7


A600
Time (post lac/or lac+trp addition - hours)
WbgL pG204
FutN pG217
FutO pG393
FutQ pG395
FutX pG401

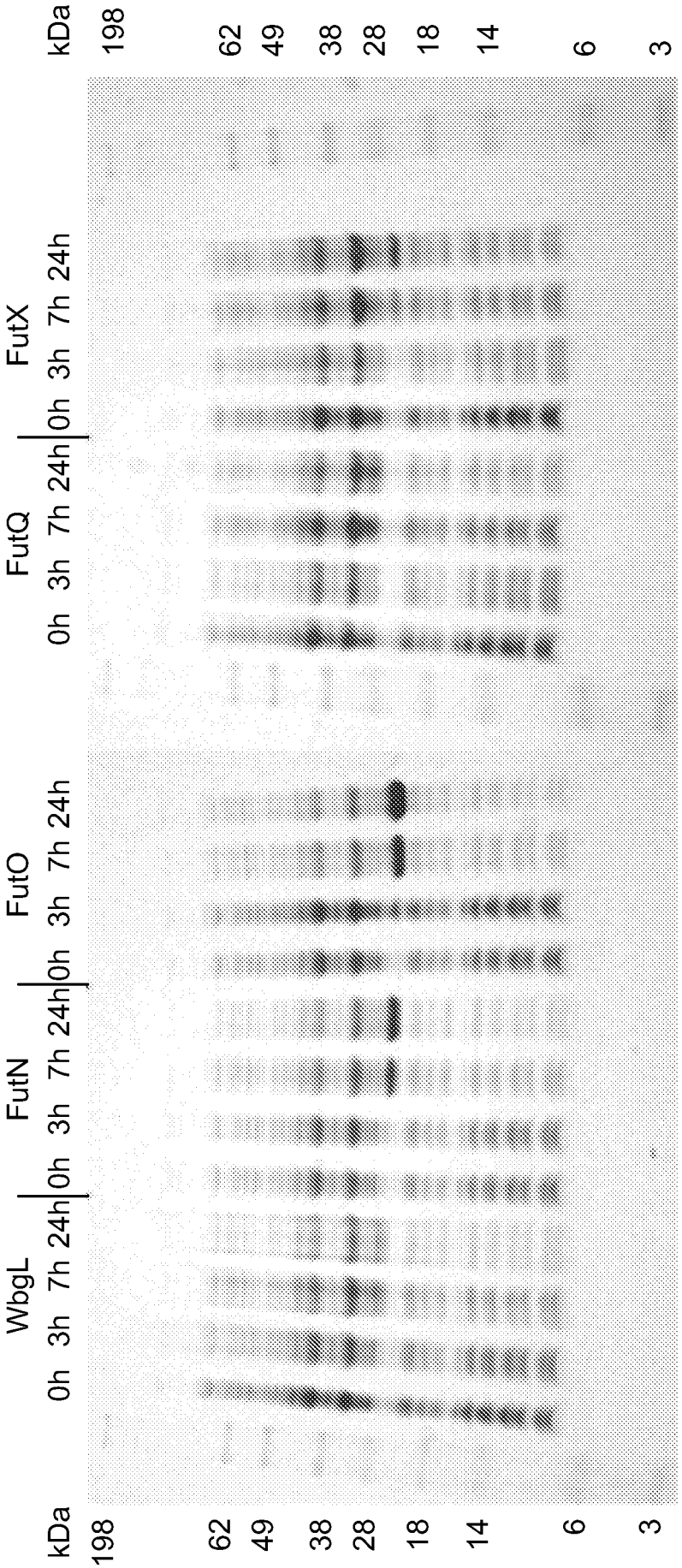


FIG. 8

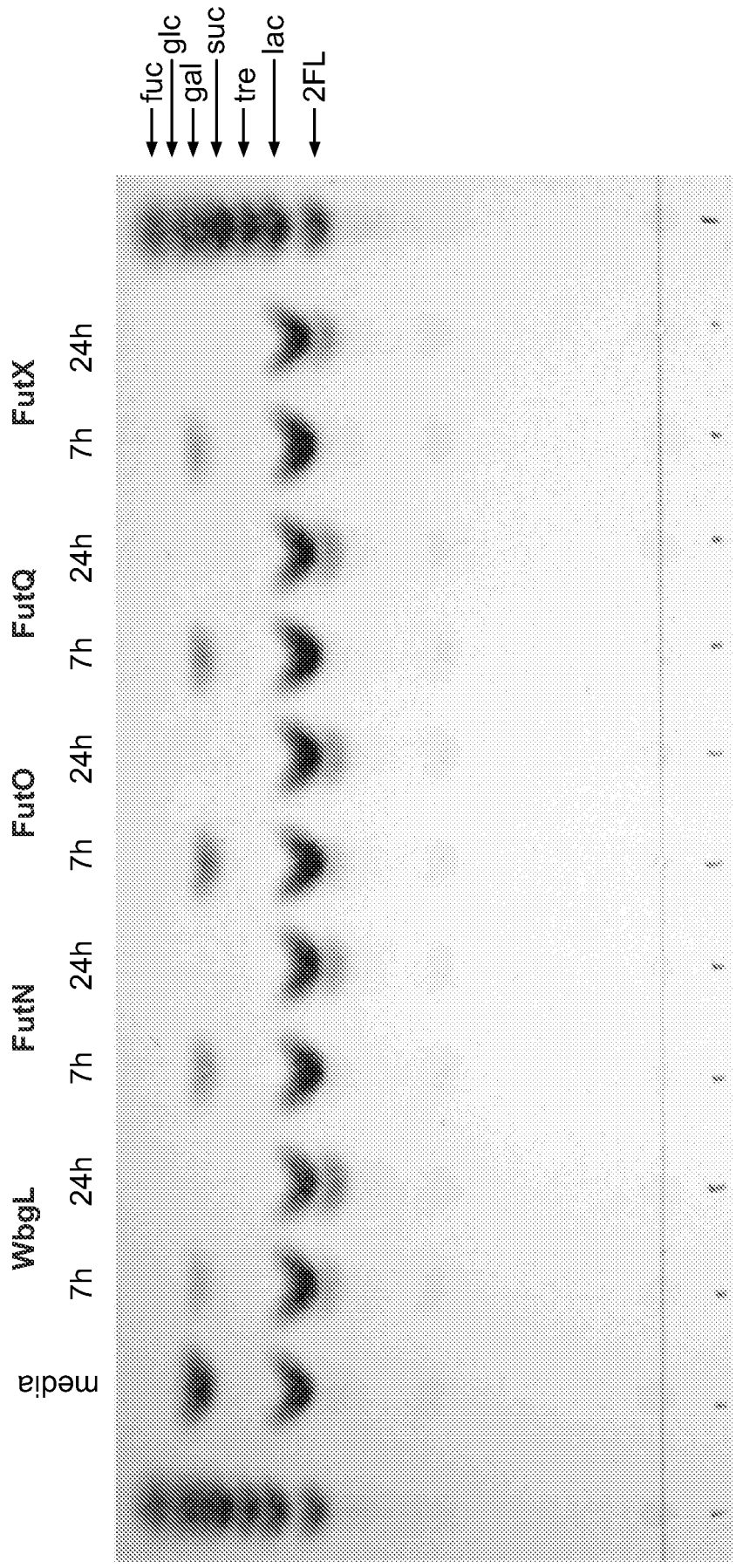
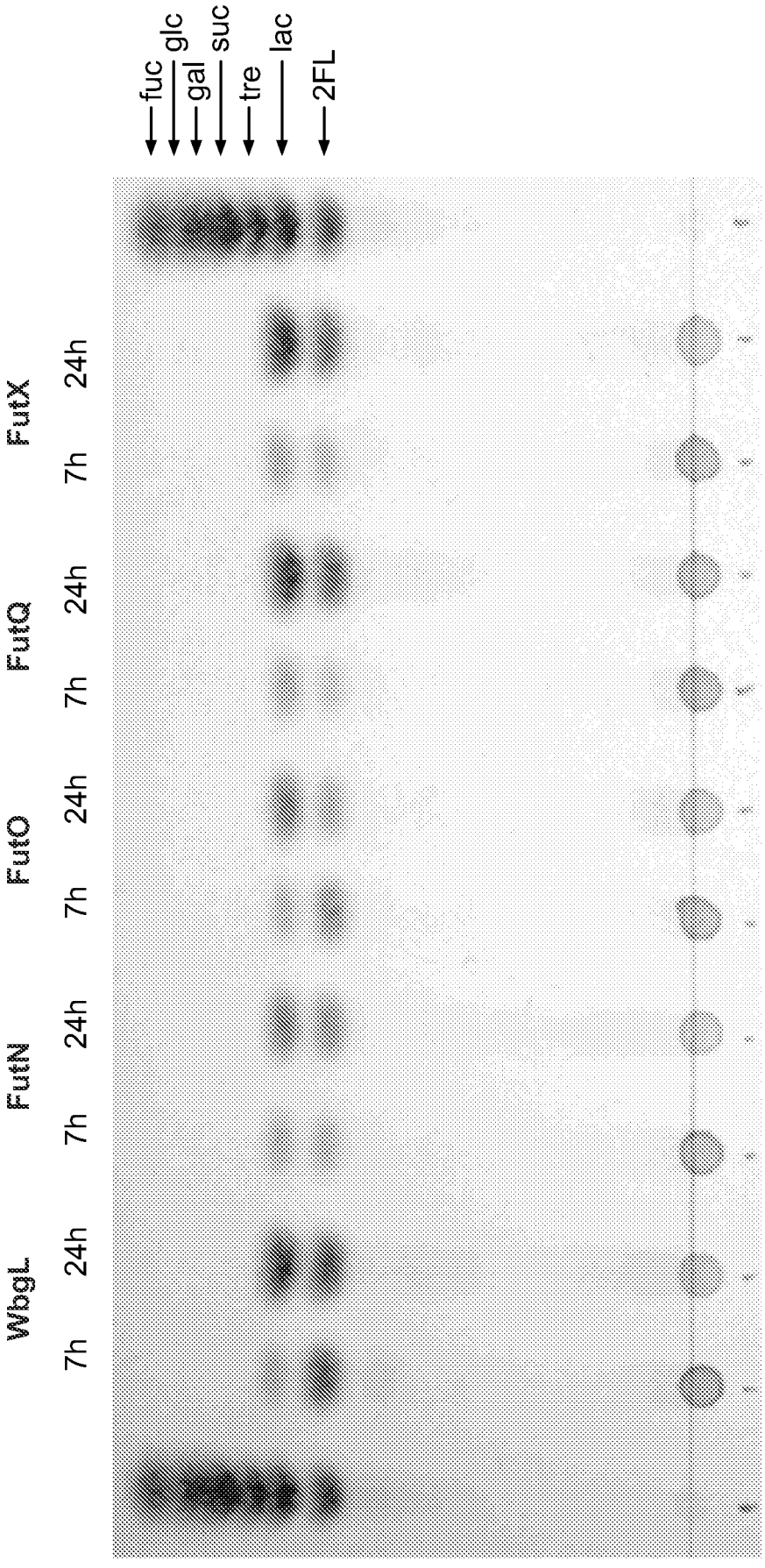

fuc
glc
gal
suc
tre
lac
2FL
media
WbgL
FutN
FutO
FutQ
FutX
7h
24h

FIG. 9A

FIG. 9B



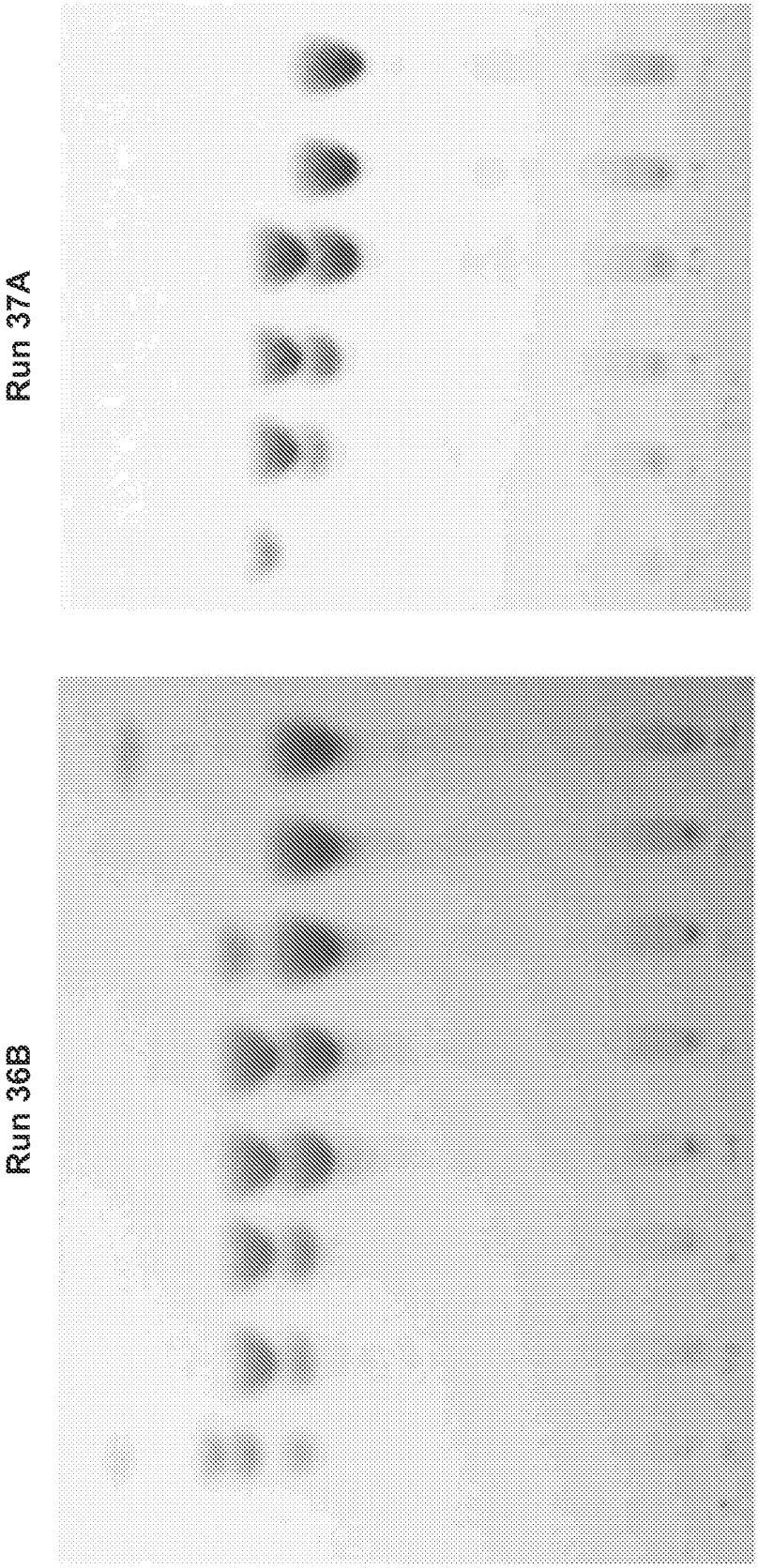


FIG. 10A

FIG. 10B

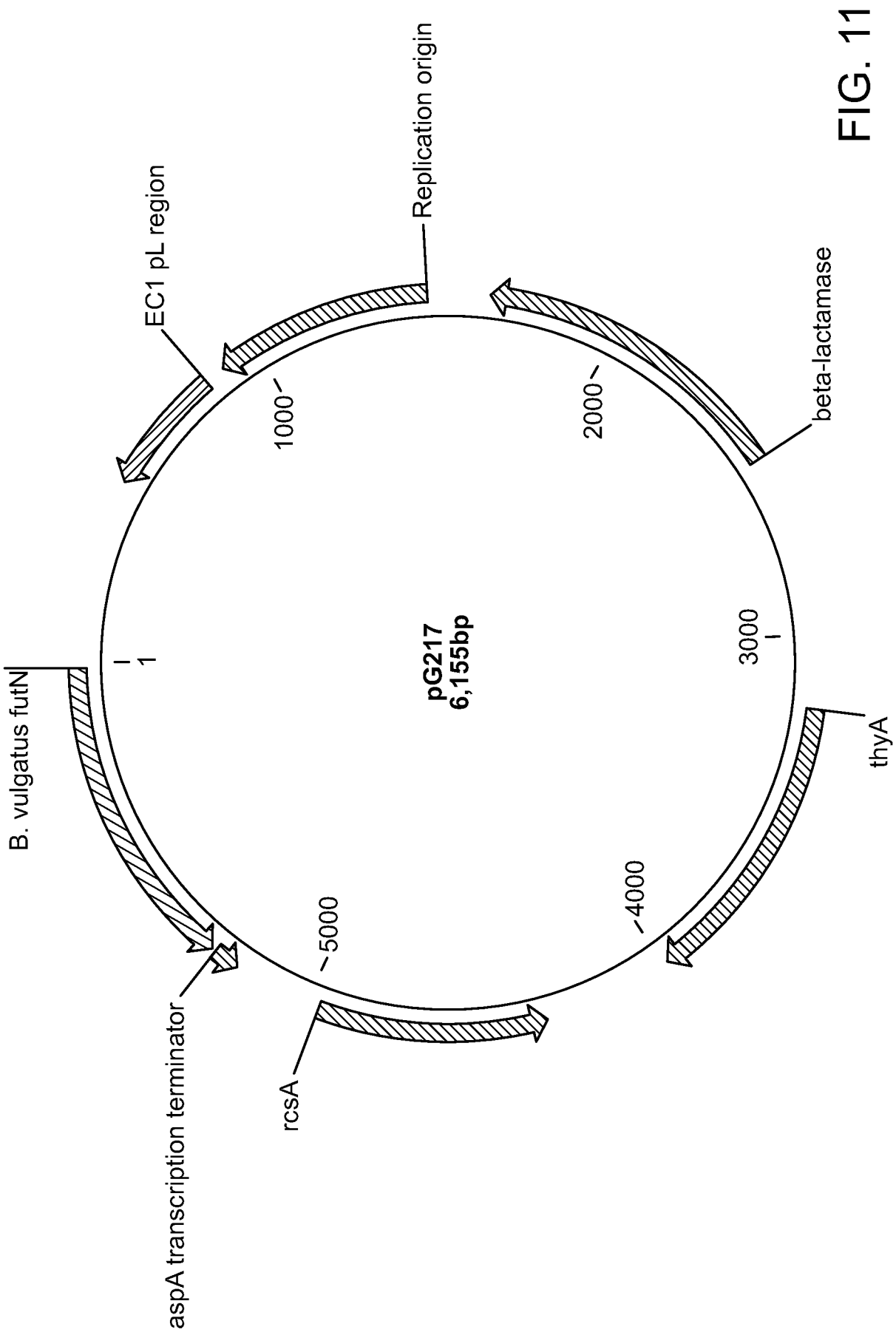

pG217
6,155bp
1
1000
2000
3000
4000
5000
Replication origin
beta-lactamase
thyA
rcsA
aspA transcription terminator
B. vulgatus futN
EC1 pL region

FIG. 11

FIG. 12

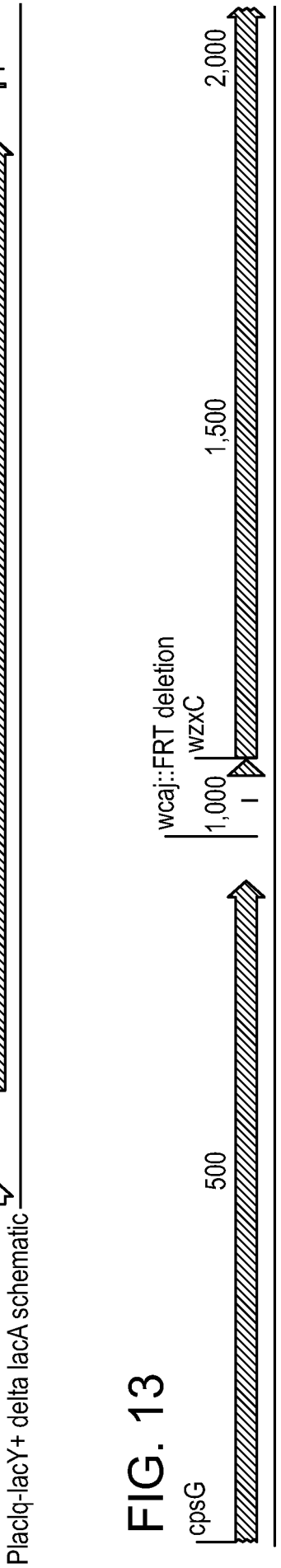


FIG. 13

FIG. 14