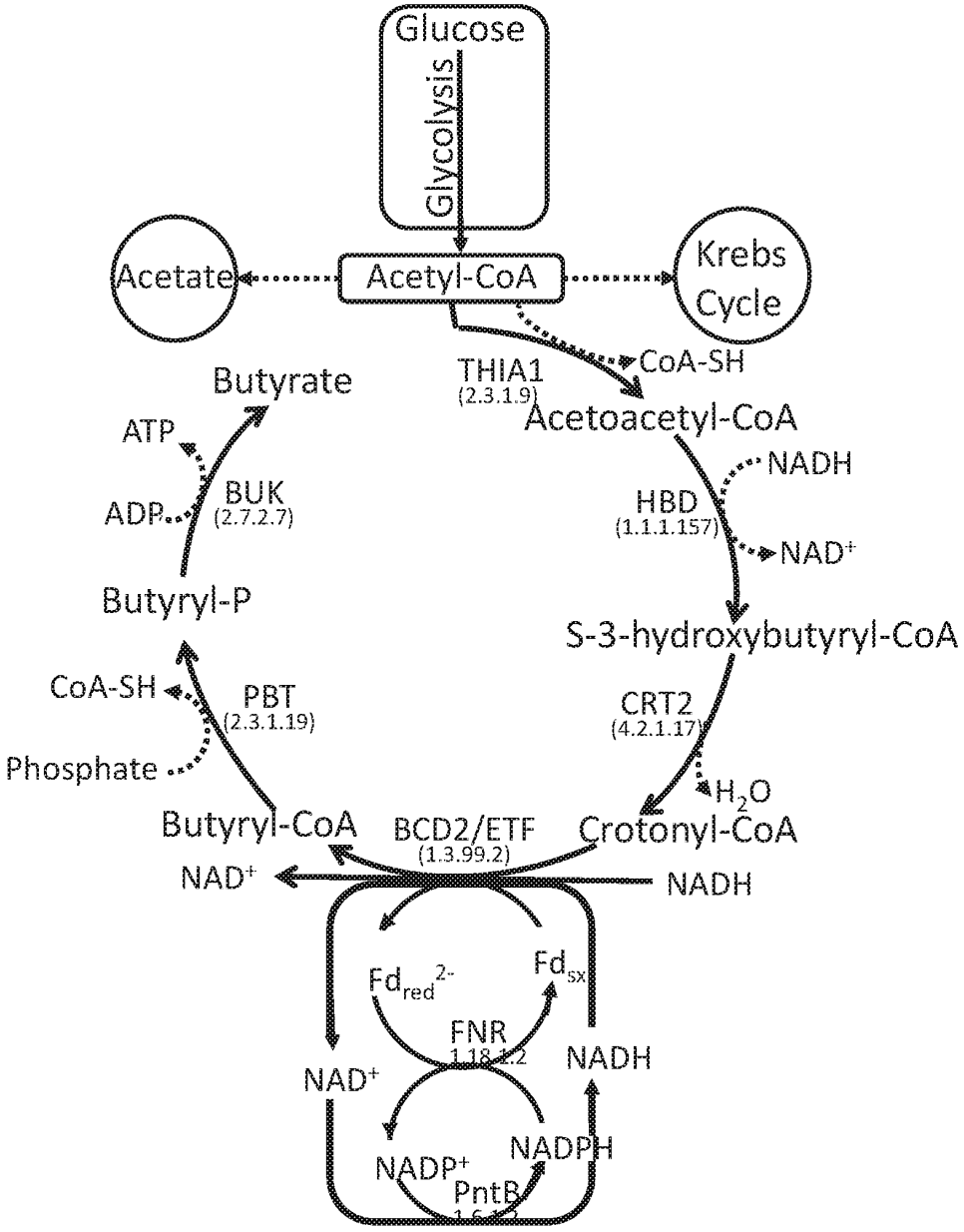

Glucose
Glycolysis
Acetate
Acetyl-CoA
Krebs Cycle
CoA-SH
THIA1
(2.3.1.9)
Acetoacetyl-CoA
NADH
HBD
(1.1.1.157)
NAD+
S-3-hydroxybutyryl-CoA
CRT2
(4.2.1.17)
H2O
Crotonyl-CoA
NADH
BCD2/ETF
(1.3.99.2)
NAD+
Butyryl-CoA
CoA-SH
PBT
(2.3.1.19)
Phosphate
Butyryl-P
ATP
BUK
(2.7.2.7)
ADP
Butyrate
Fdred2-
Fdsx
FNR
1.18.1.2
NAD+
NADH
NADP+
NADPH
PntB

Fig. 1A

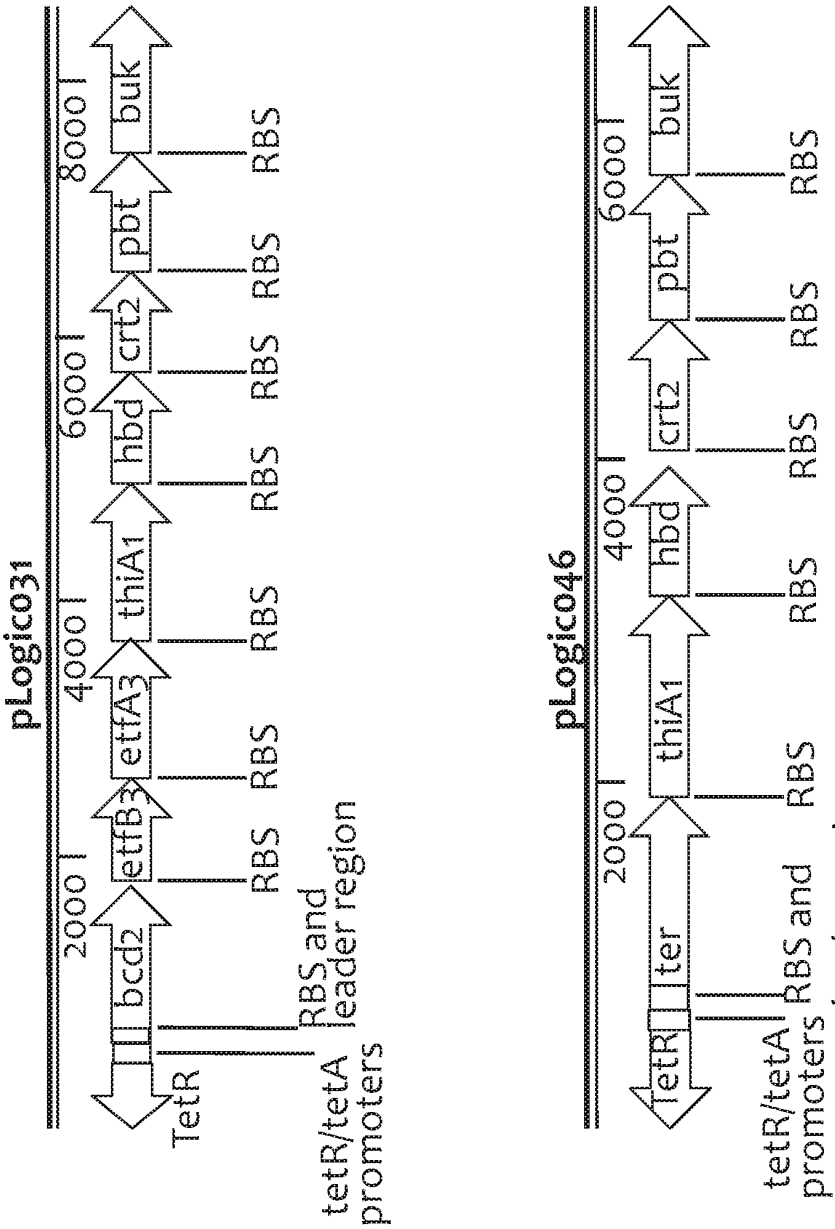

pLogic031
2000
4000
6000
8000
TetR
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
RBS
RBS and leader region
tetR/tetA promoters
pLogic046
2000
4000
6000
tetR
ter
thiA1
hbd
crt2
pbt
buk
RBS
RBS and leader region
tetR/tetA promoters

Fig. 1B

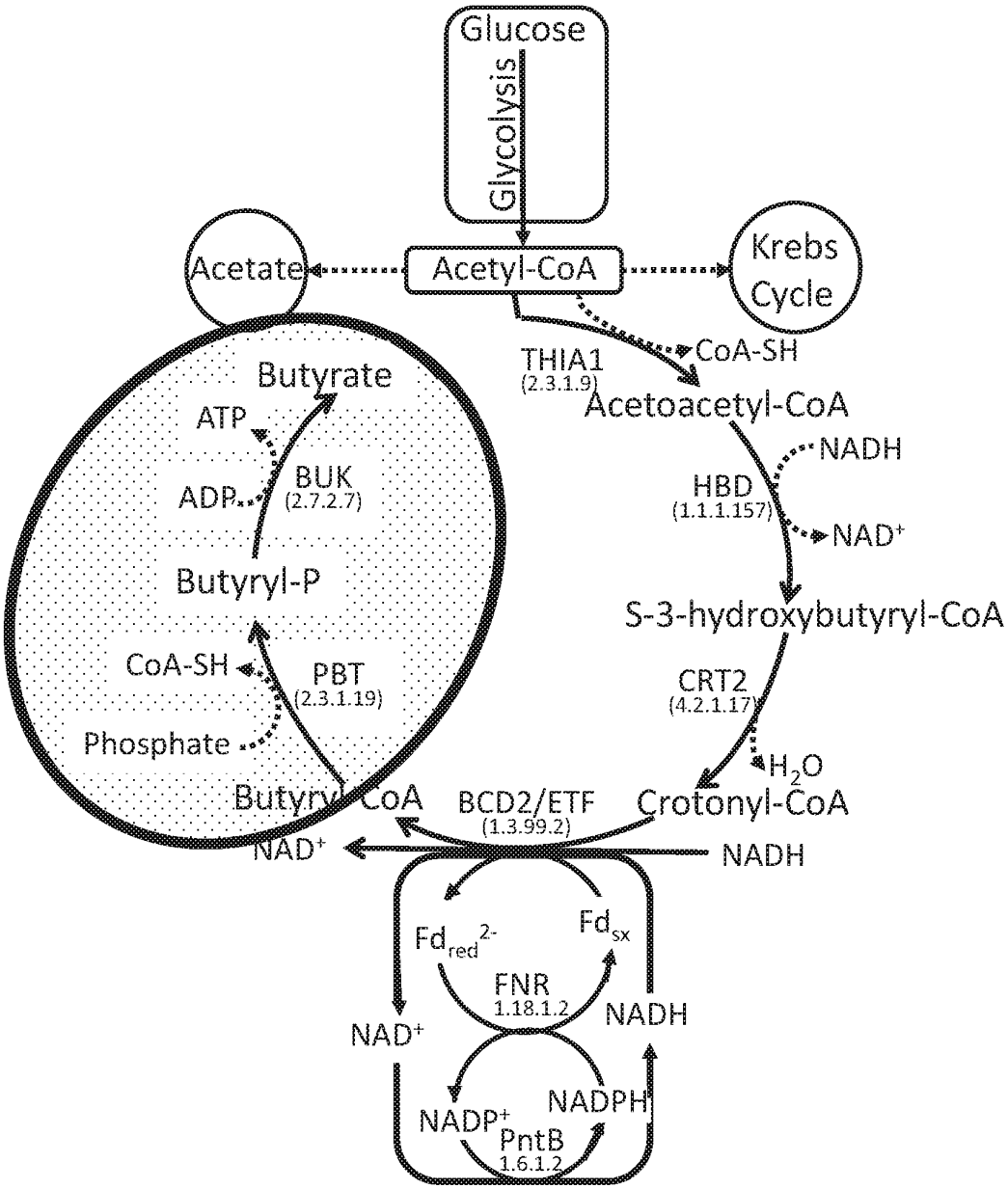

Glucose
Glycolysis
Acetate
Acetyl-CoA
Krebs Cycle
CoA-SH
THIA1
(2.3.1.9)
Acetoacetyl-CoA
NADH
HBD
(1.1.1.157)
NAD+
S-3-hydroxybutyryl-CoA
CRT2
(4.2.1.17)
H2O
Crotonyl-CoA
BCD2/ETF
(1.3.99.2)
NADH
NAD+
Butyryl-CoA
Butyrate
ATP
ADP
BUK
(2.7.2.7)
Butyryl-P
CoA-SH
PBT
(2.3.1.19)
Phosphate
Fdred2-
Fdsx
FNR
1.18.1.2
NADH
NAD+
NADP+
NADPH
PntB
1.6.1.2

Fig. 2

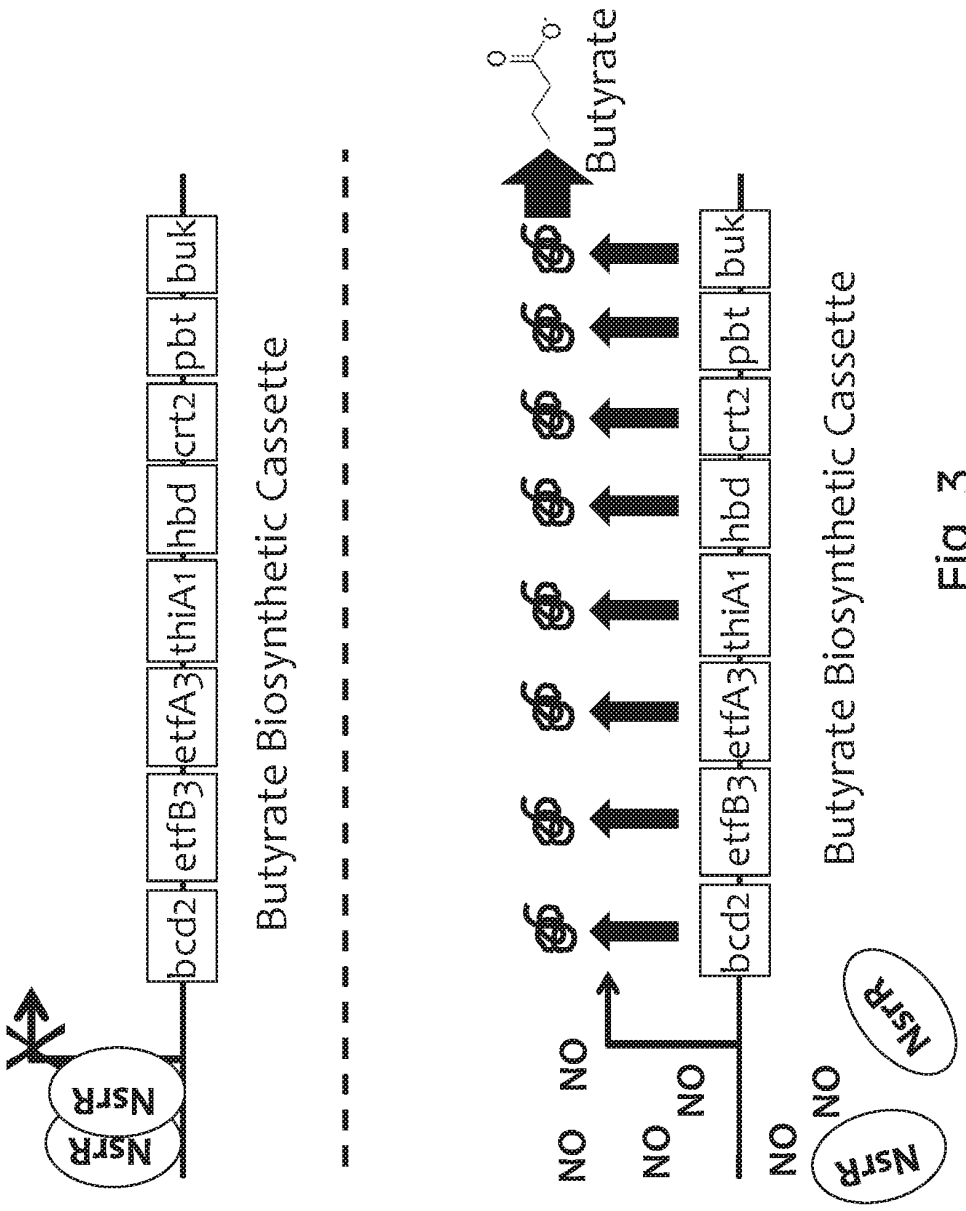

NsrR
NsrR
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette
NO
NO
NO
NO
NO
NO
NsrR
NsrR
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
Butyrate
Butyrate Biosynthetic Cassette

Fig. 3

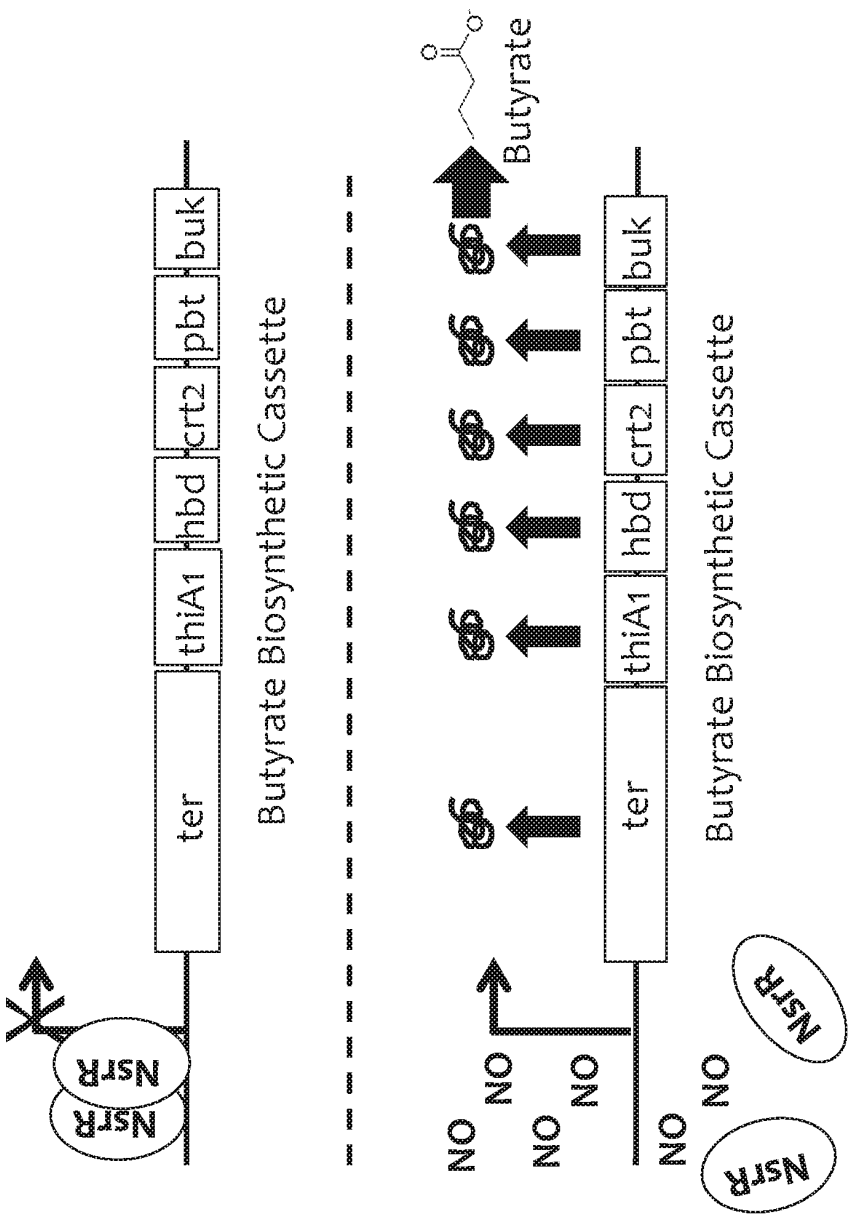

NsrR
NsrR
ter
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette
NO
NO
NO
NO
NO
NO
NsrR
NsrR
ter
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette
Butyrate

Fig. 4

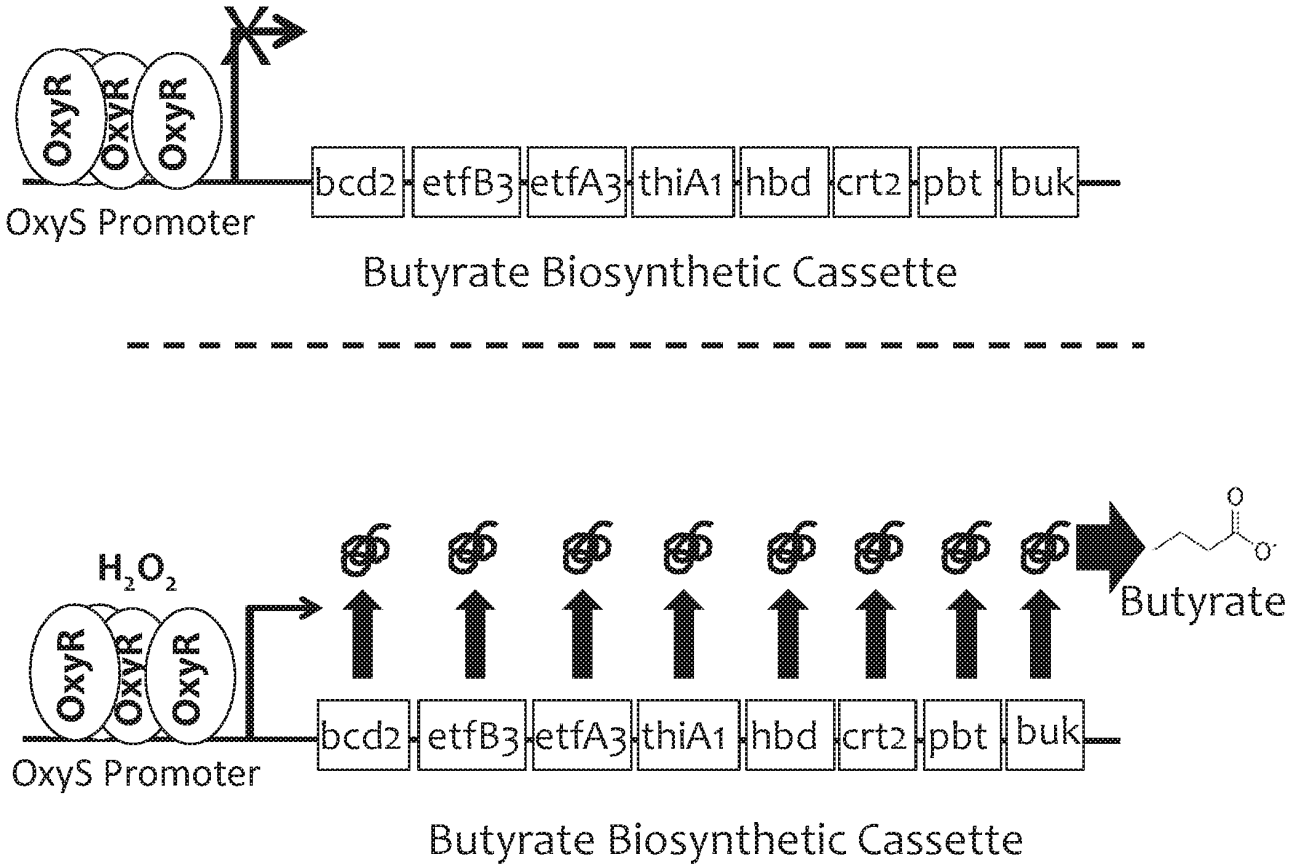

OxyR
OxyR
OxyR
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
OxyS Promoter
Butyrate Biosynthetic Cassette
H2O2
OxyR
OxyR
OxyR
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
Butyrate
OxyS Promoter
Butyrate Biosynthetic Cassette

Fig. 5

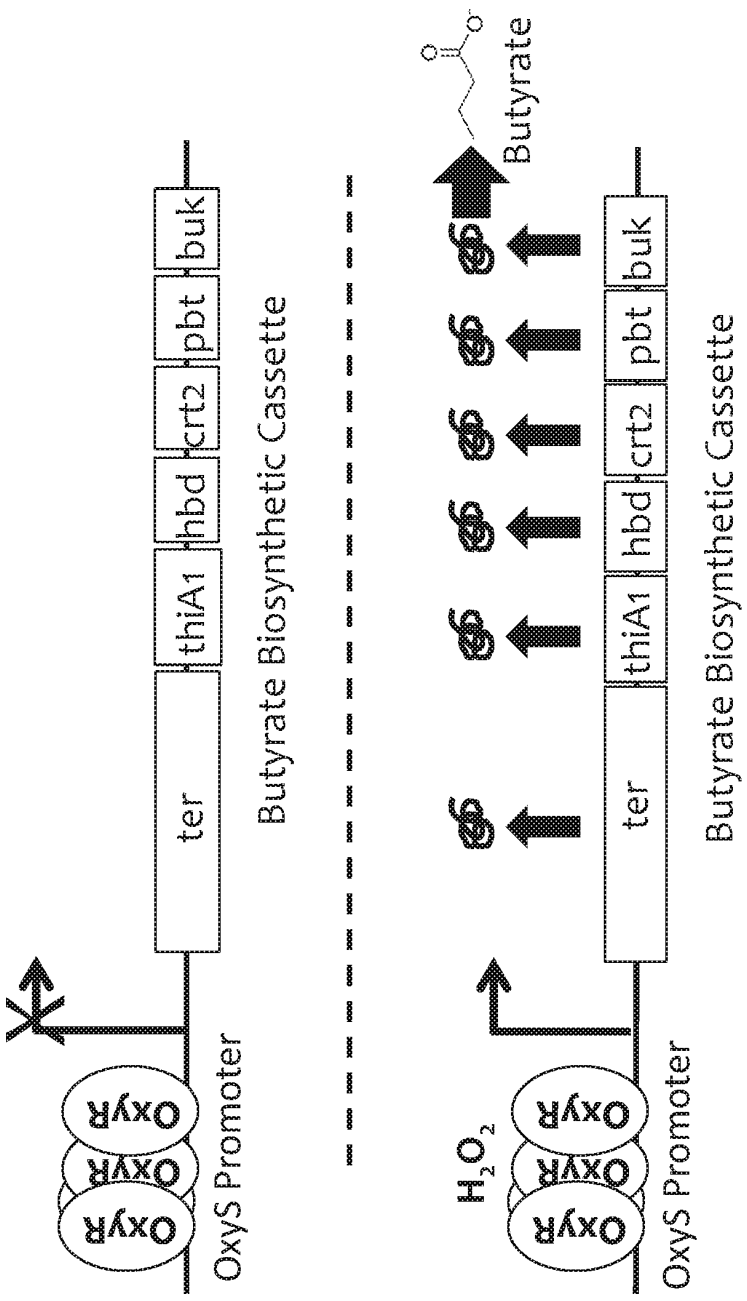

OxyR
OxyR
OxyR
OxyS Promoter
ter
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette
H_2O_2
Butyrate

Fig. 6

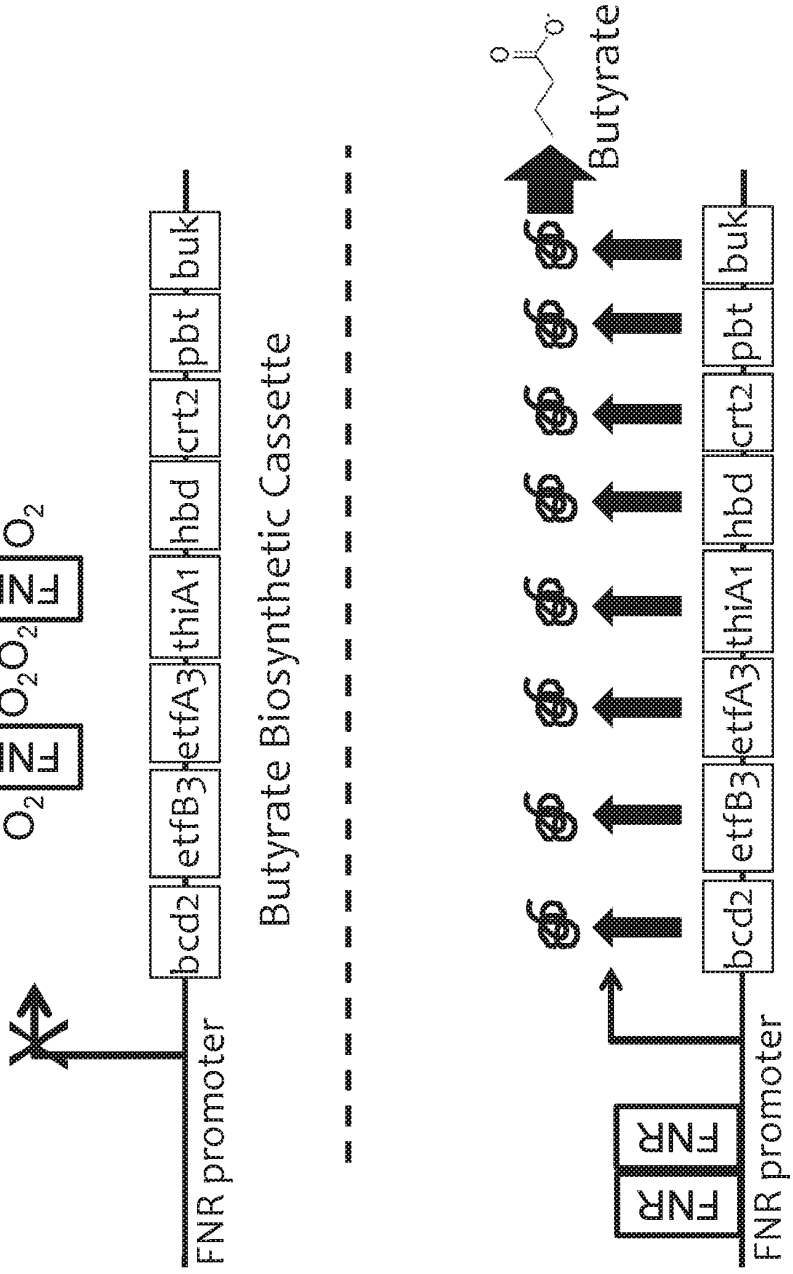

FNR
FNR
O2
O2
O2
O2
FNR promoter
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette
FNR
FNR
FNR promoter
bcd2
etfB3
etfA3
thiA1
hbd
crt2
pbt
buk
Butyrate
Butyrate Biosynthetic Cassette

Fig. 7

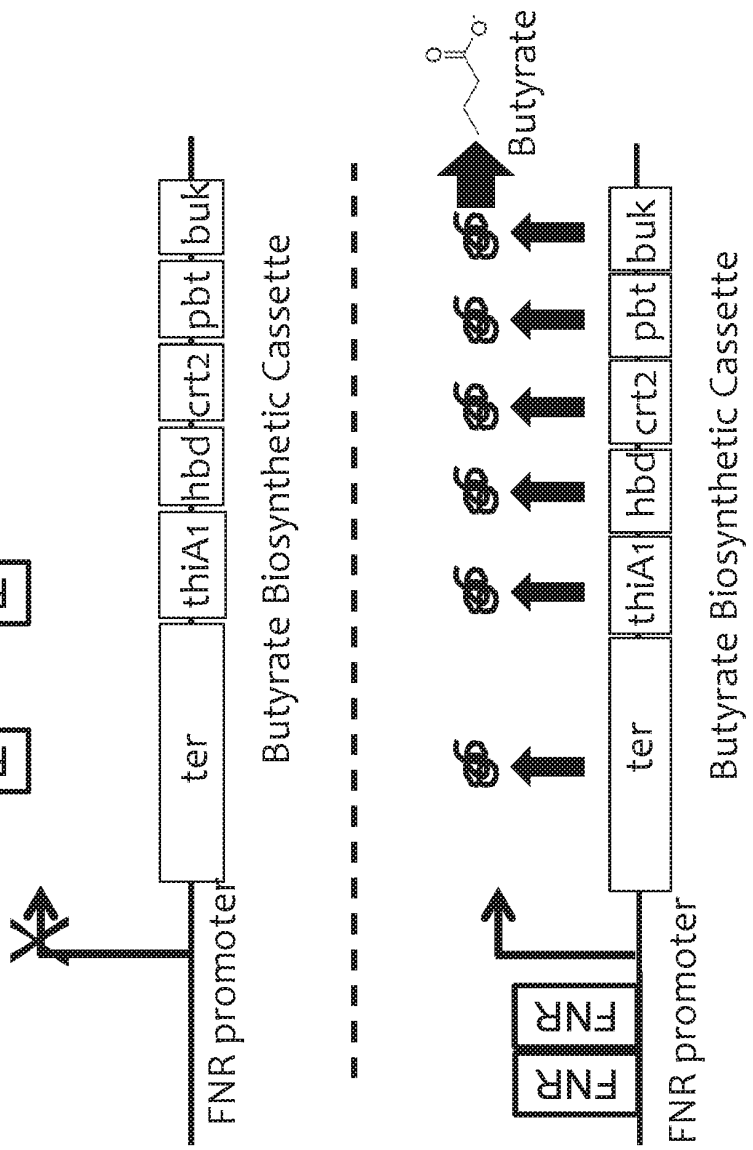

FNR
FNR
O_2
O_2
O_2
O_2
FNR promoter
ter
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette
Butyrate
FNR
FNR
FNR promoter
ter
thiA1
hbd
crt2
pbt
buk
Butyrate Biosynthetic Cassette

Fig. 8

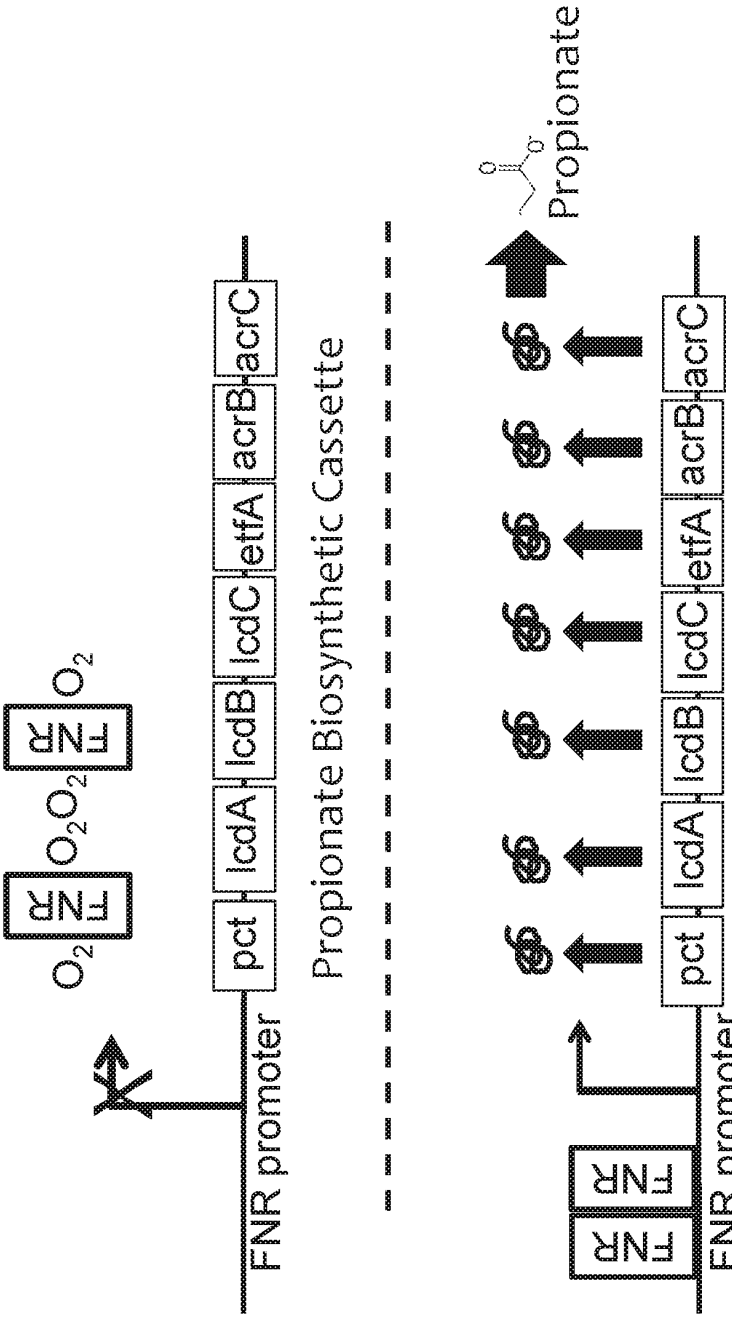

FNR
FNR
O_2
O_2
O_2
O_2
FNR promoter
pct
lcdA
lcdB
lcdC
etfA
acrB
acrC
Propionate Biosynthetic Cassette
Propionate
FNR
FNR
FNR promoter
pct
lcdA
lcdB
lcdC
etfA
acrB
acrC
Propionate Biosynthetic Cassette

Fig. 9

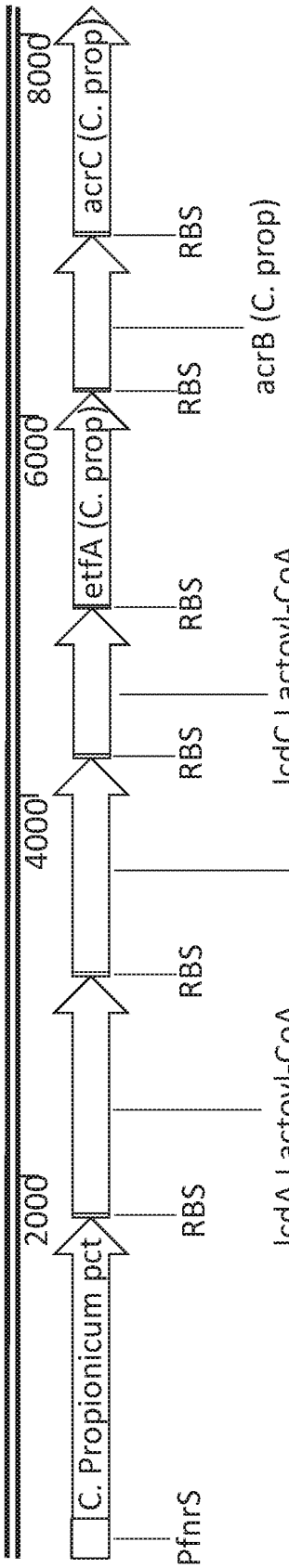

PfnrS
C. Propionicum pct
2000
RBS
lcdA Lactoyl-CoA dehydratase Alpha subunit (C. prop)
RBS
lcdB Lactoyl-CoA dehydratase Beta subunit (C. prop)
4000
RBS
lcdC Lactoyl-CoA dehydratase Gamma subunit (C. prop)
etfA (C. prop)
6000
RBS
acrB (C. prop)
RBS
acrC (C. prop)
8000

Fig. 10

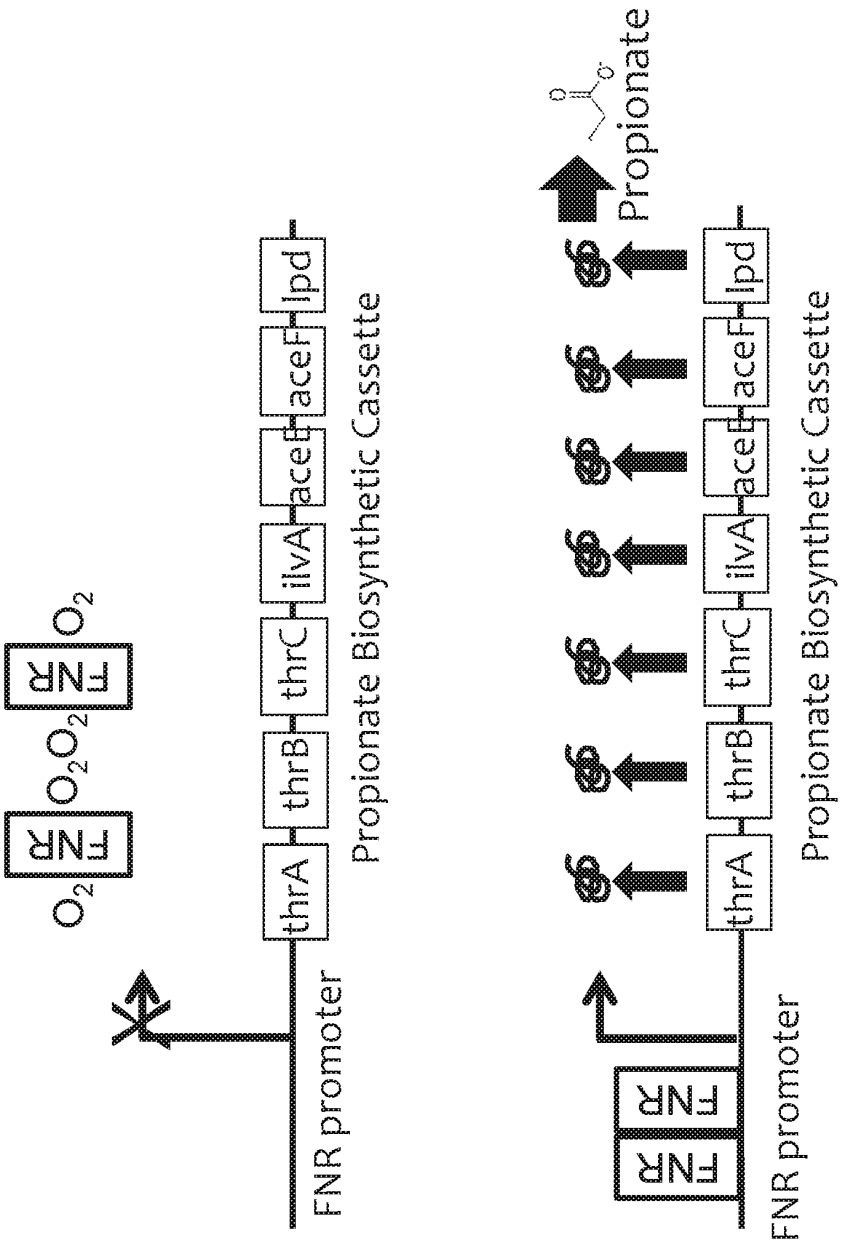

FNR
FNR
O_2 O_2 O_2
O_2
FNR promoter
thrA
thrB
thrC
ilvA
aceE
aceF
lpd
Propionate Biosynthetic Cassette
Propionate
FNR
FNR promoter
thrA
thrB
thrC
ilvA
aceE
aceF
lpd
Propionate Biosynthetic Cassette

Fig. 11

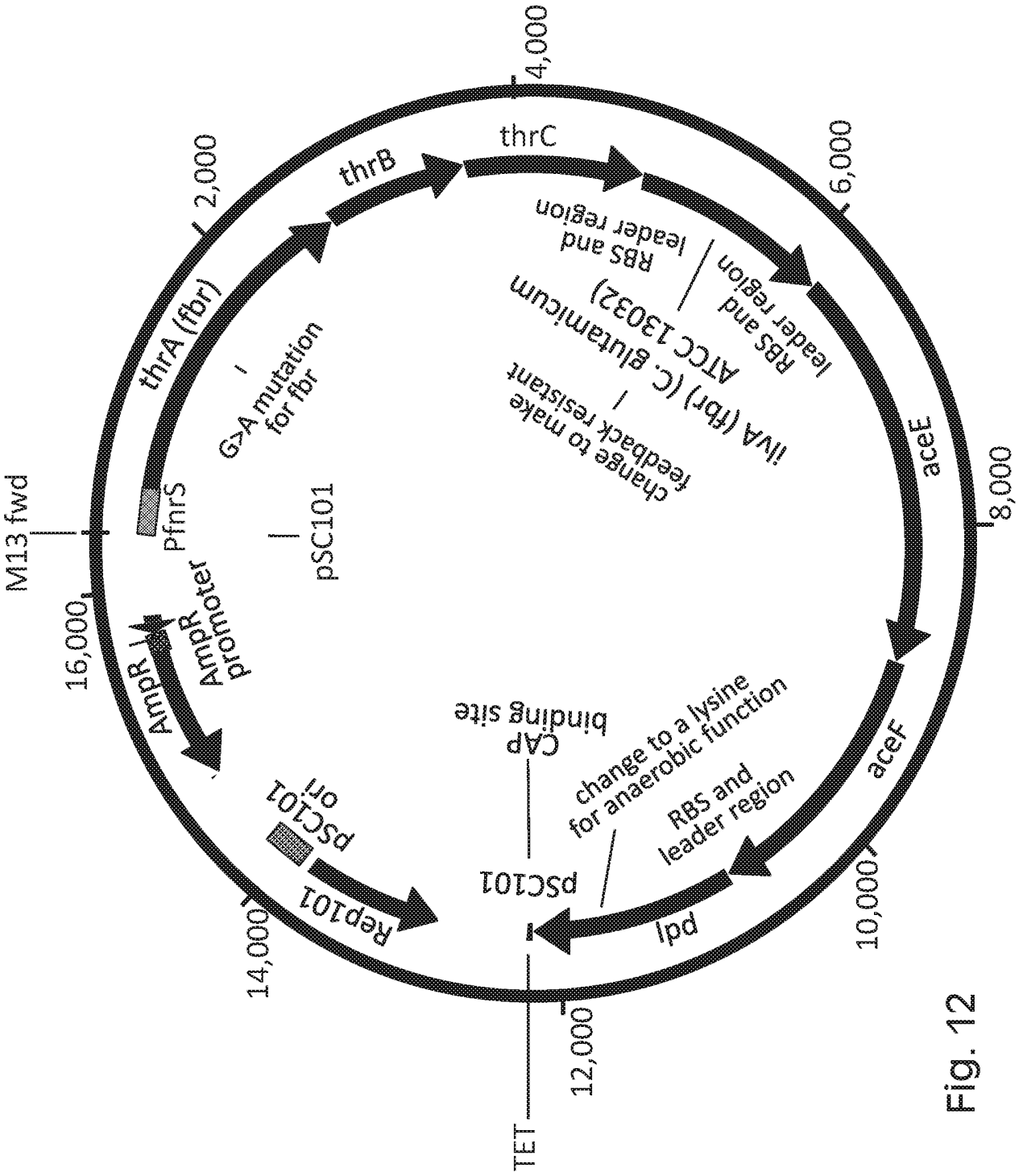

M13 fwd
2,000
4,000
6,000
8,000
10,000
12,000
14,000
16,000
TET
thrA (fbr)
thrB
thrC
aceE
aceF
pdl
Rep101
pSC101 ori
AmpR
AmpR promoter
PfnrS
pSC101
G>A mutation for fbr
change to a lysine for anaerobic function
RBS and leader region
CAP binding site
change to make feedback resistant ilvA (fbr) (C. glutamicum ATCC 13032)
RBS and leader region
RBS and leader region

Fig. 12

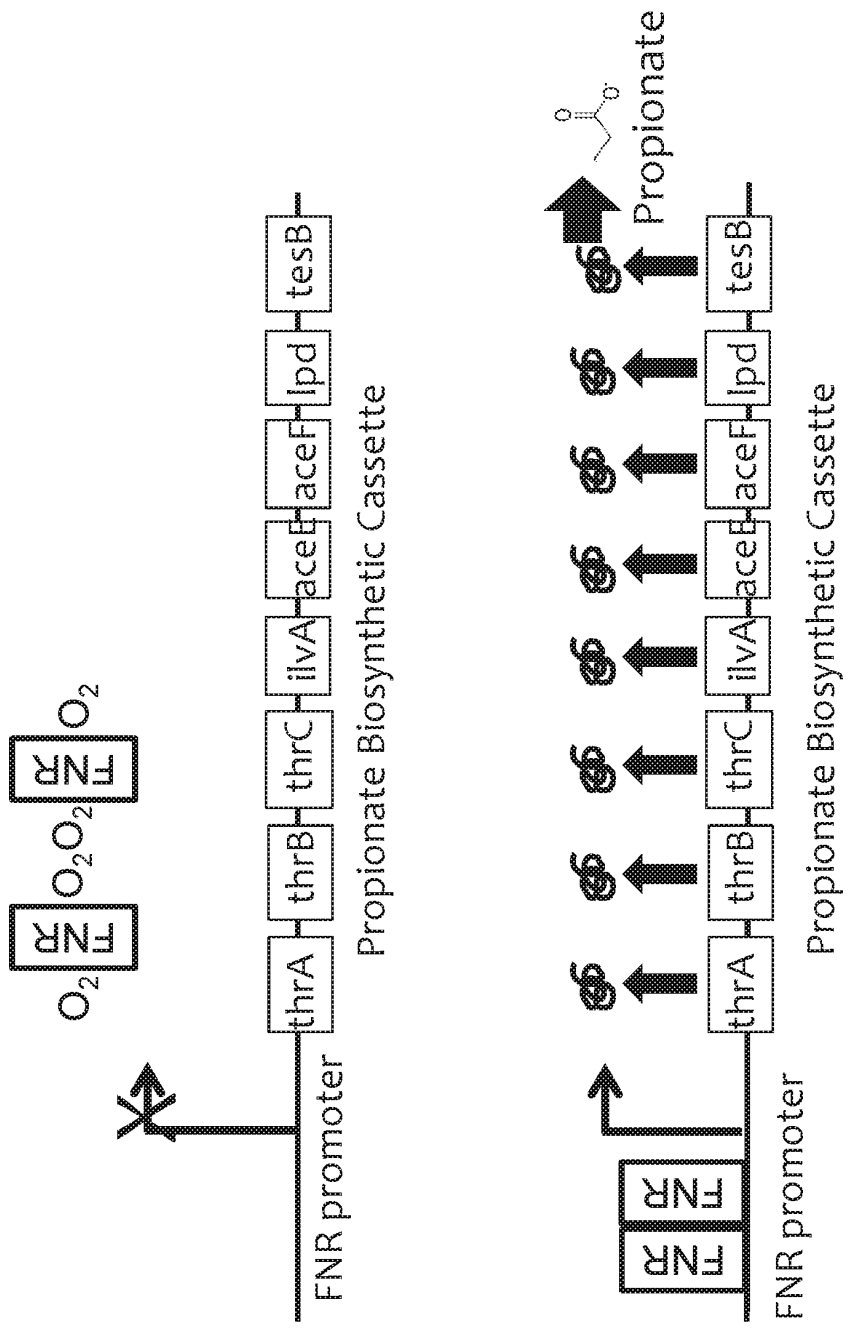

FNR
FNR
O_2
O_2
O_2
O_2
thrA
thrB
thrC
ilvA
aceE
aceF
lpd
tesB
FNR promoter
Propionate Biosynthetic Cassette
Propionate
FNR
FNR
thrA
thrB
thrC
ilvA
aceE
aceF
lpd
tesB
FNR promoter
Propionate Biosynthetic Cassette

Fig. 13

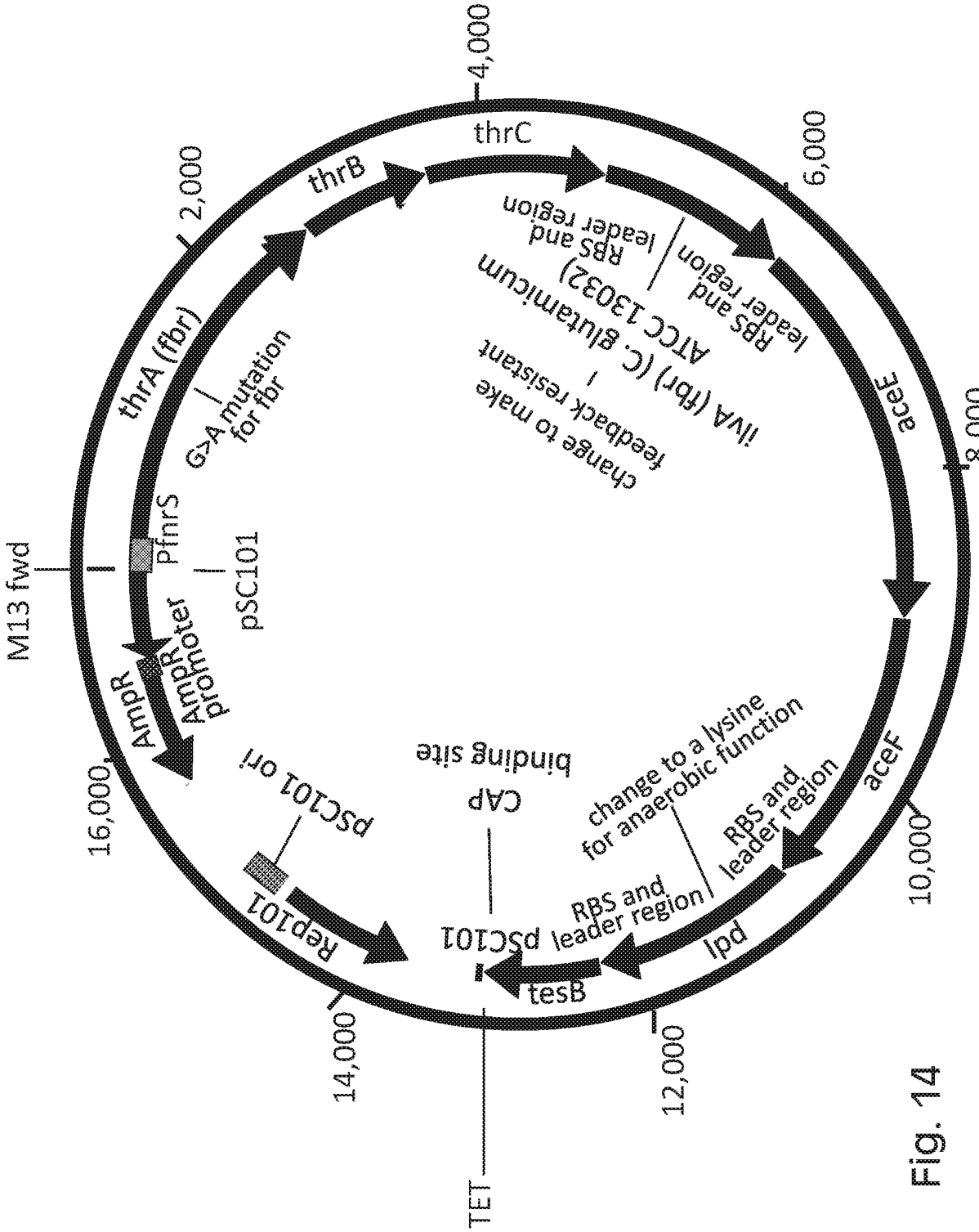

2,000
4,000
6,000
8,000
10,000
12,000
14,000
16,000
M13 fwd
TET
thrA (fbr)
thrB
thrC
RBS and leader region
RBS and leader region
ilvA (fbr) (C. glutamicum ATCC 13032)
change to make feedback resistant
G>A mutation for fbr
PfnrS
pSC101
AmpR
AmpR promoter
pSC101 ori
CAP binding site
change to a lysine for anaerobic function
RBS and leader region
RBS and leader region
aceE
aceF
lpd
tesB
pSC101
Rep101

Fig. 14

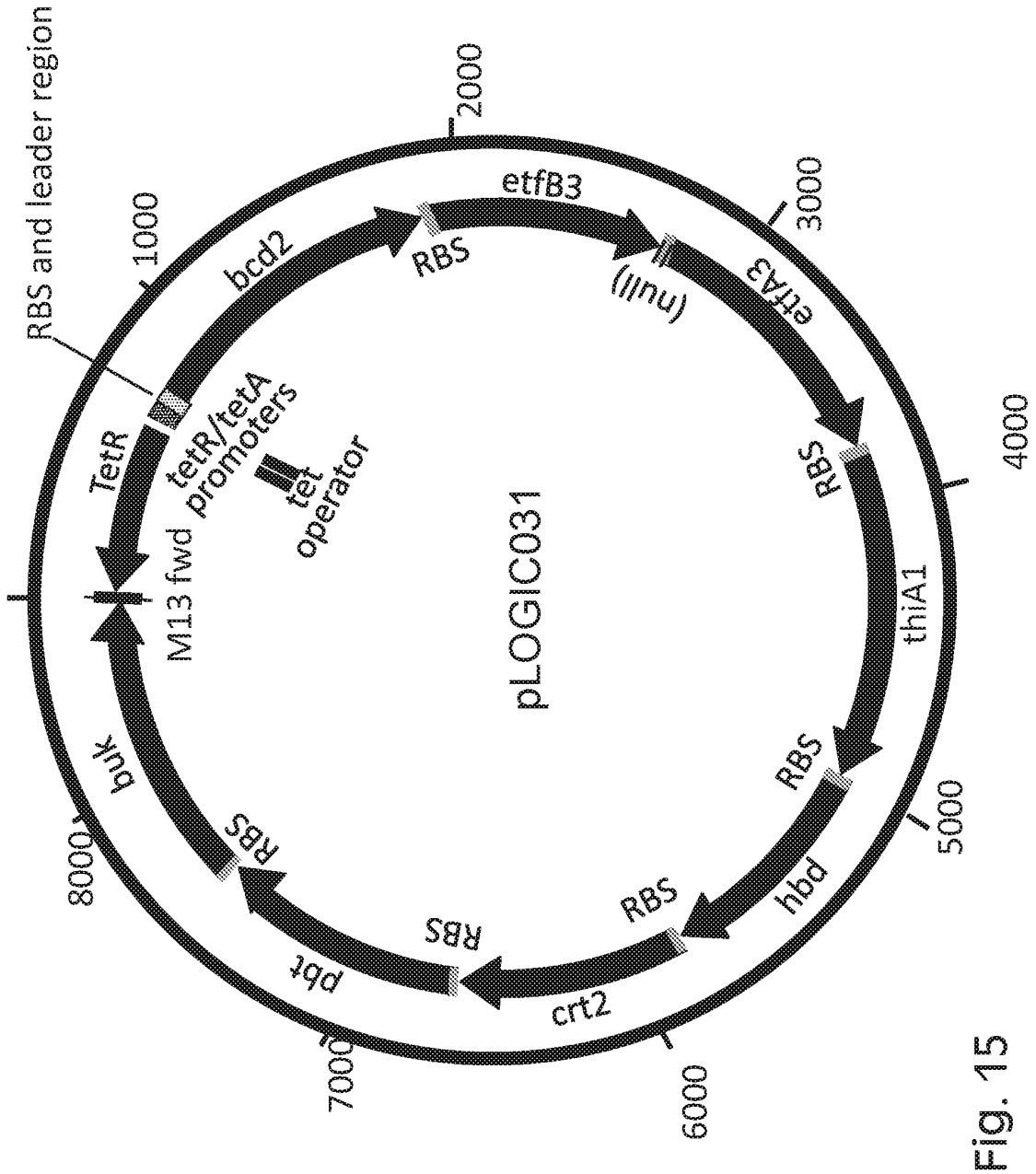

pLOGIC031
RBS and leader region
1000
2000
3000
4000
5000
6000
7000
8000
bcd2
RBS
etfB3
(null)
etfA3
RBS
thiA1
RBS
hbd
RBS
crt2
RBS
pbt
RBS
bujk
M13 fwd
TetR
tetR/tetA promoters
tet operator

Fig. 15

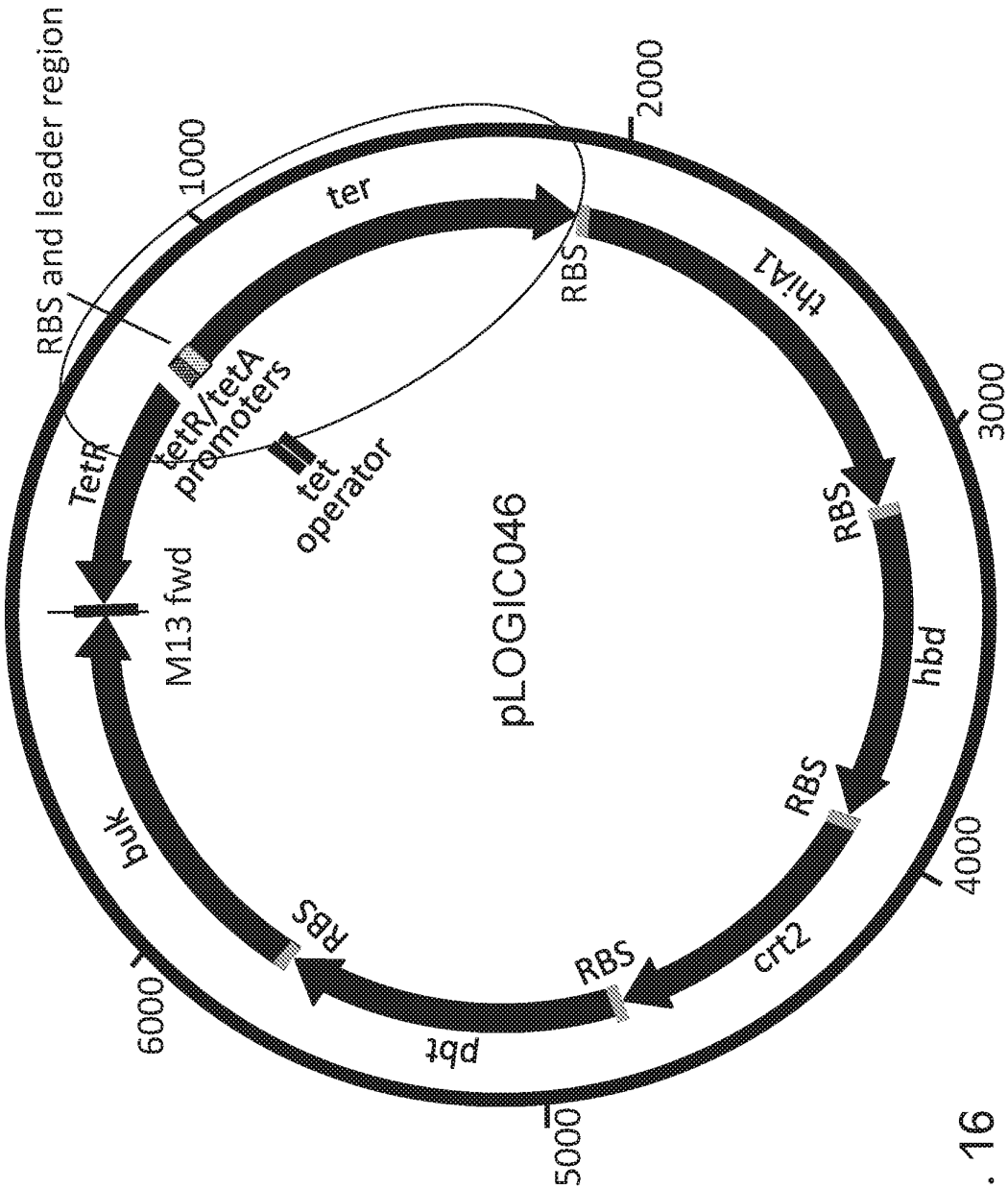

pLOGIC046
1000
2000
3000
4000
5000
6000
ter
RBS
thiA1
RBS
hbd
RBS
crt2
RBS
pbt
RBS
buk
M13 fwd
TetR
tetR/tetA promoters
tet operator
RBS and leader region

Fig. 16

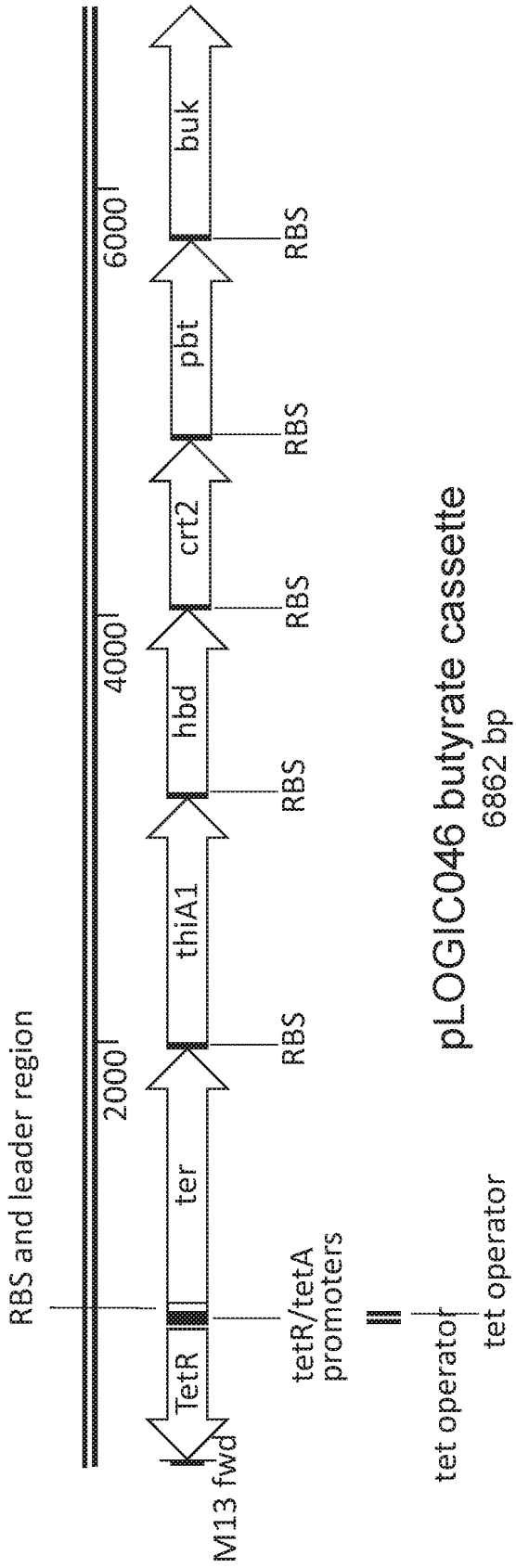

RBS and leader region
2000
4000
6000
M13 fwd
TetR
ter
thiA1
hbd
crt2
pbt
buk
RBS
RBS
RBS
RBS
RBS
tetR/tetA promoters
tet operator
tet operator
pLOGIC046 butyrate cassette
6862 bp

Fig. 17

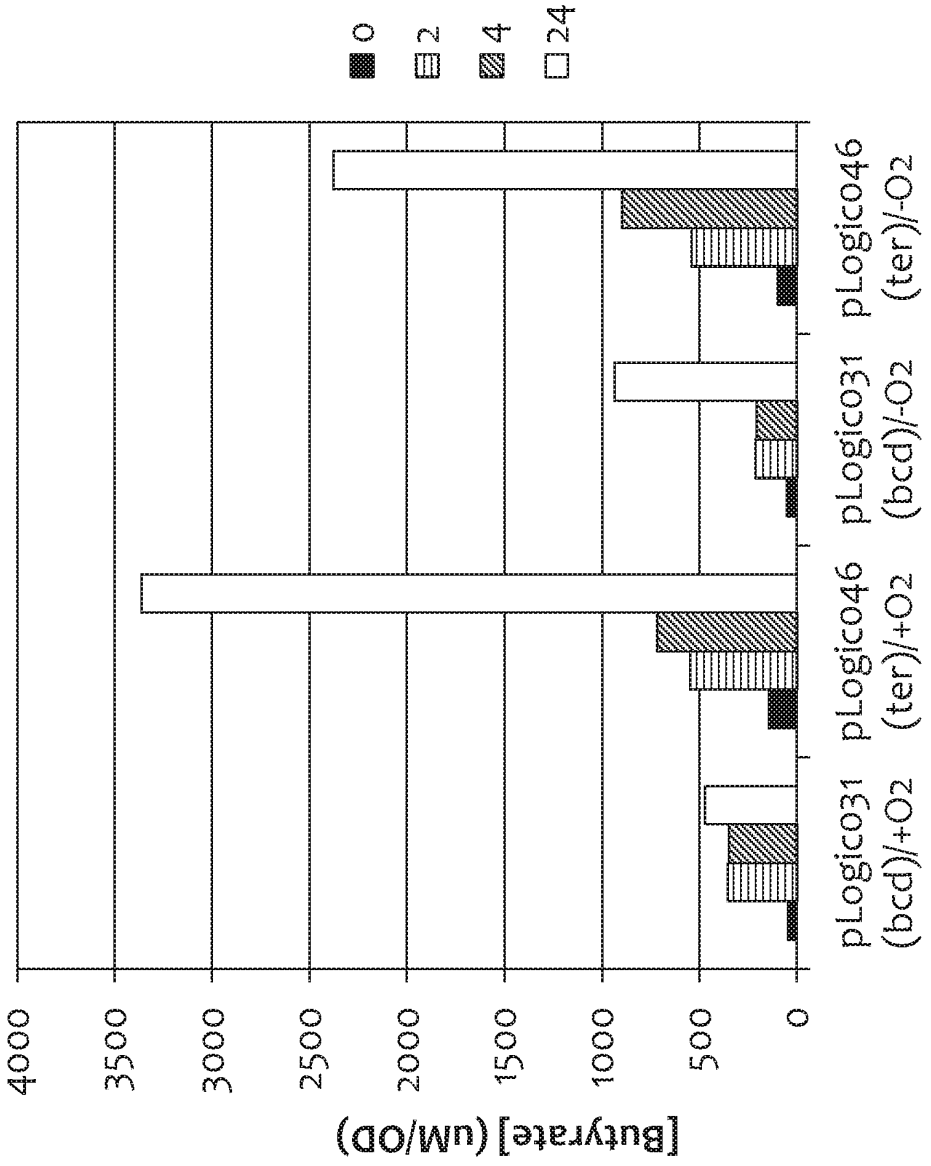
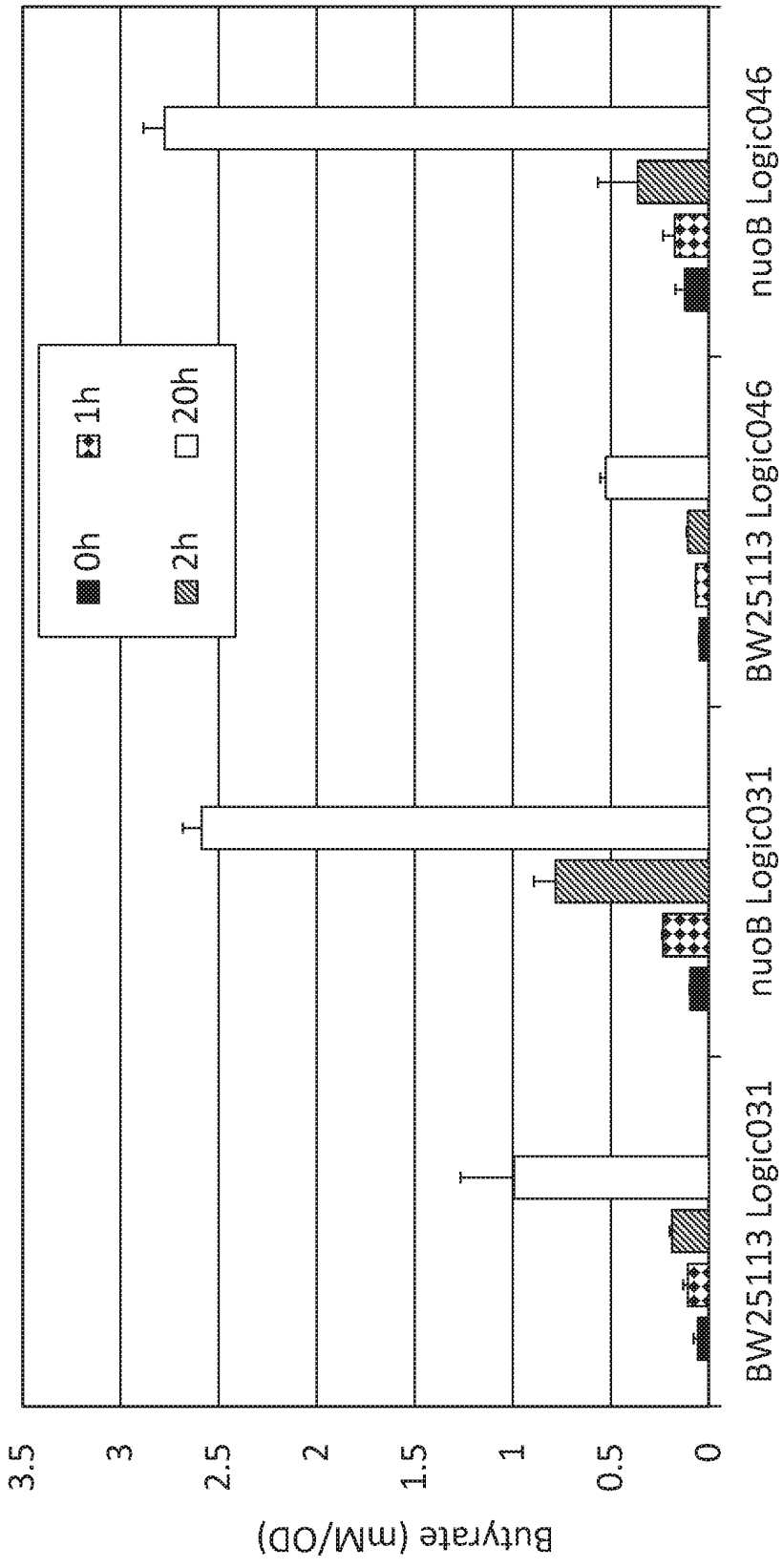

0
2
4
24
[Butyrate] (uM/OD)
4000
3500
3000
2500
2000
1500
1000
500
0
pLogic031 (bcd)/+O2
pLogic046 (ter)/+O2
pLogic031 (bcd)/-O2
pLogic046 (ter)/-O2

Fig. 18



Logic031 – pBR322/*bcd*-butyrate cassette
Logic046 – pBR322/*ter*-butyrate cassette

Fig. 19

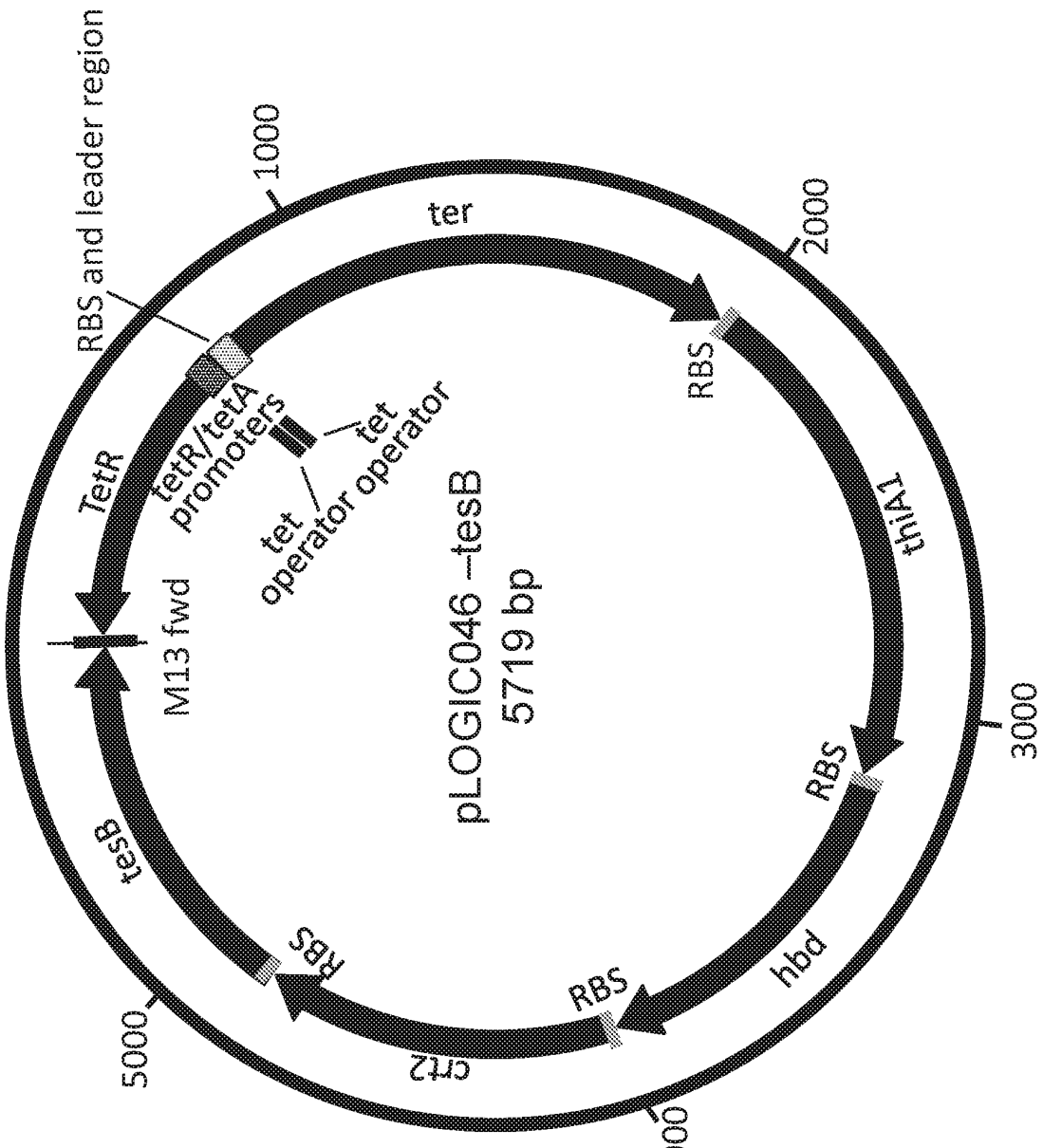

RBS and leader region
1000
ter
2000
RBS
thiA1
RBS
hbd
3000
RBS
4000
crt2
RBS
5000
tesB
M13 fwd
TetR
tetR/tetA promoters
tet operator
tet operator
pLOGIC046 –tesB
5719 bp

Fig. 20

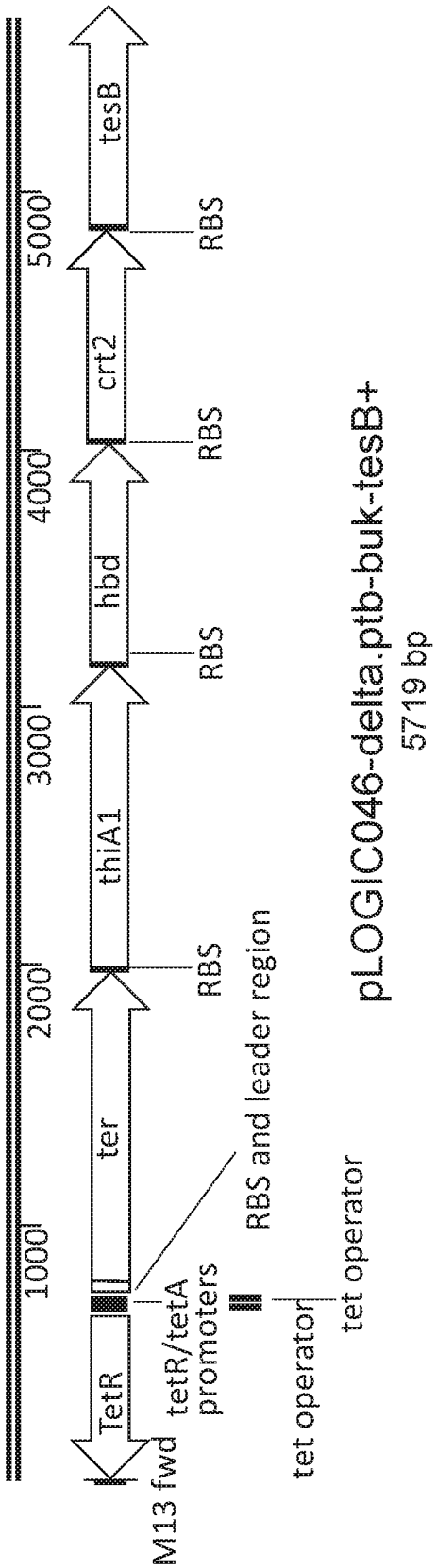

1000
2000
3000
4000
5000
TetR
M13 fwd
tetR/tetA promoters
tet operator
ter
RBS and leader region
tet operator
thiA1
RBS
hbd
RBS
crt2
RBS
tesB
RBS
pLOGIC046-delta.ptb-buk-tesB+
5719 bp

Fig. 21

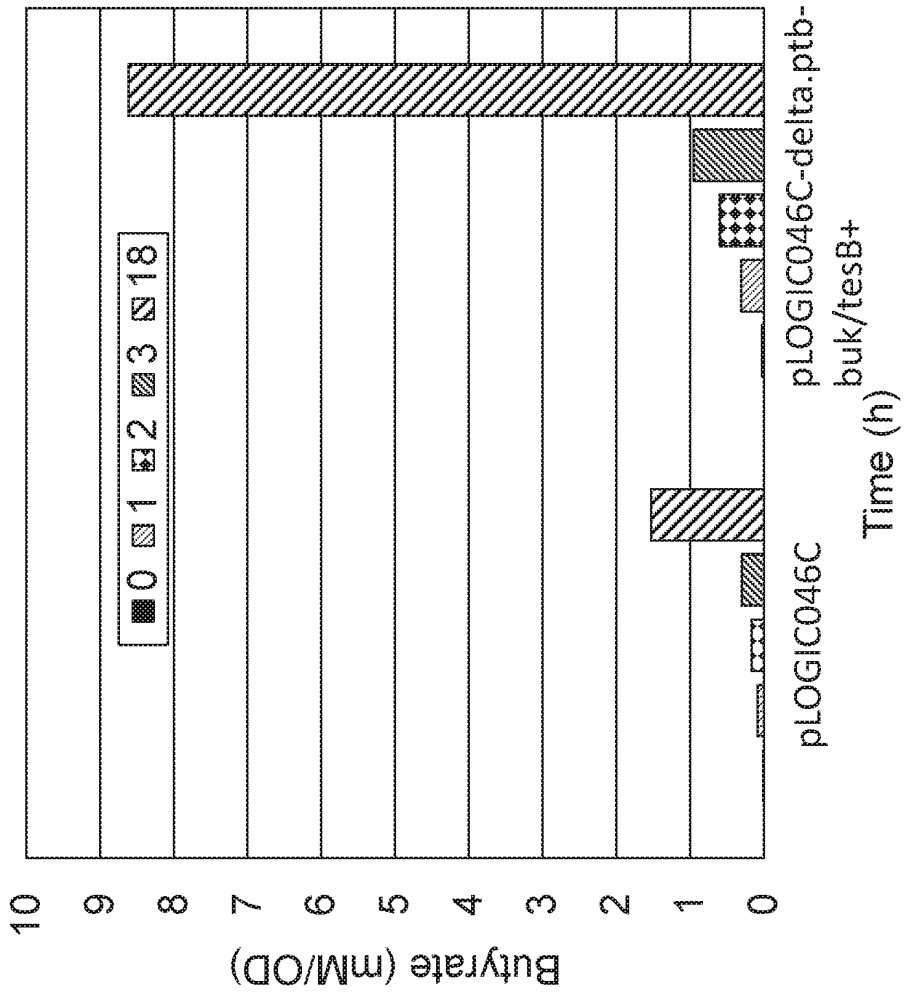

Butyrate (mM/OD)
10
9
8
7
6
5
4
3
2
1
0
0
1
2
3
18
pLOGIC046C
pLOGIC046C-delta.ptb-buk/tesB+
Time (h)

Fig. 22

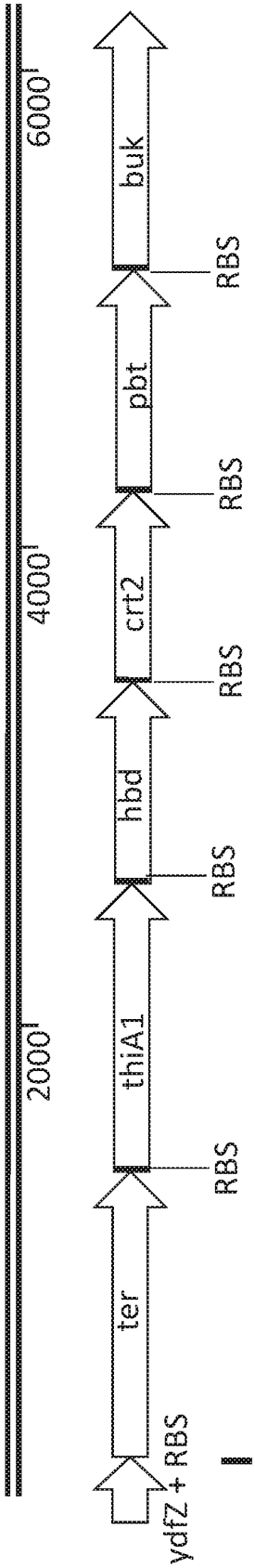

2000
4000
6000
ydfZ + RBS
RBS
ter
RBS
thiA1
RBS
hbd
RBS
crt2
RBS
pbt
RBS
buk
ydfZ-butyrate
6270 bp

Fig. 23

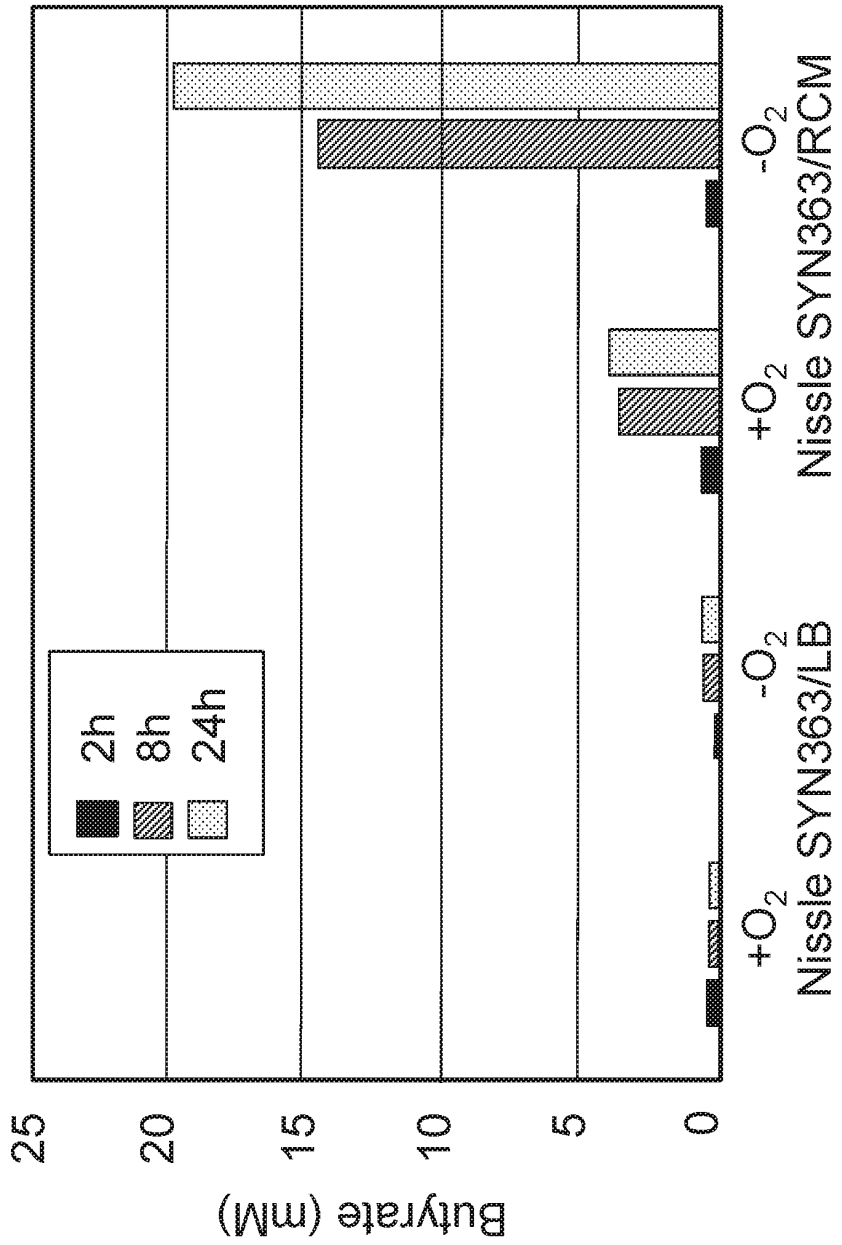

Butyrate (mM)
0
5
10
15
20
25
2h
8h
24h
+O2
-O2
+O2
-O2
Nissle SYN363/LB
Nissle SYN363/RCM

Fig. 24

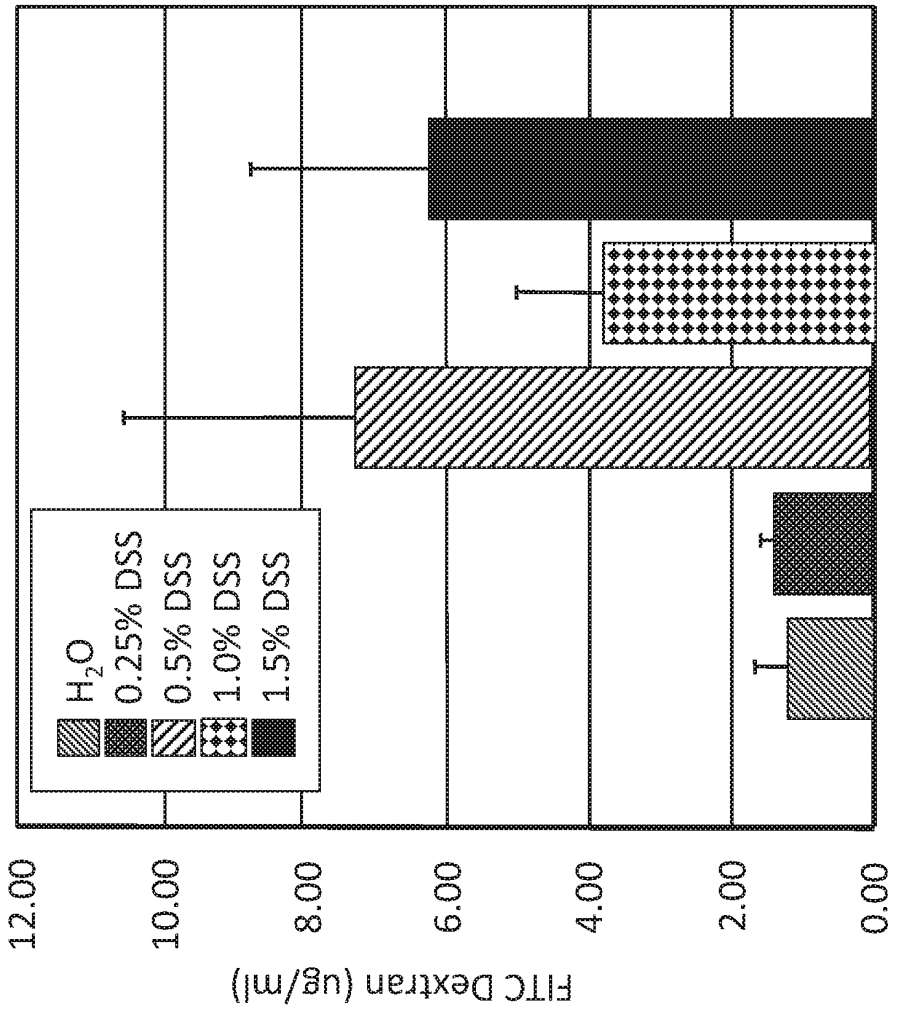

FITC Dextran (ug/ml)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
H2O
0.25% DSS
0.5% DSS
1.0% DSS
1.5% DSS

Fig. 25

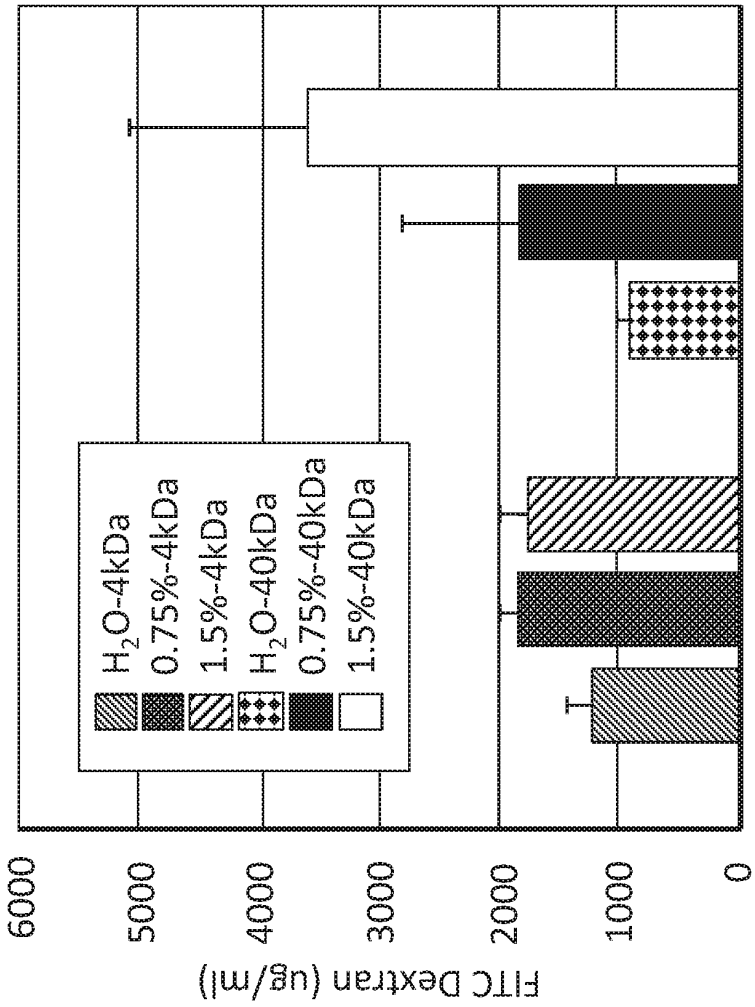

FITC Dextran (ug/ml)
6000
5000
4000
3000
2000
1000
0
H2O-4kDa
0.75%-4kDa
1.5%-4kDa
H2O-40kDa
0.75%-40kDa
1.5%-40kDa

Fig. 26

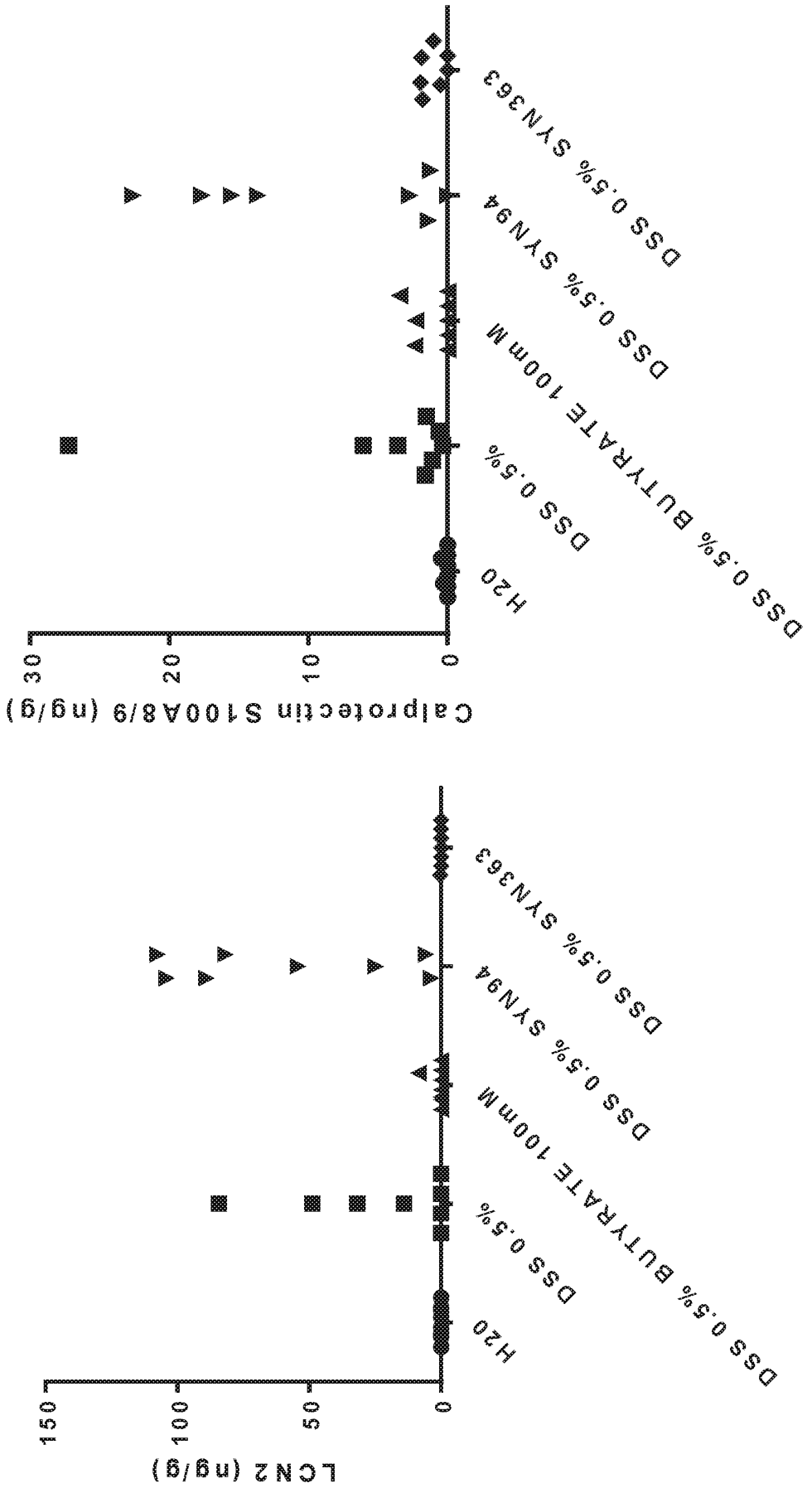

Calprotectin S100A8/9 (ng/g)
0
10
20
30
H20
DSS 0.5%
DSS 0.5% BUTYRATE 100mM
DSS 0.5% SYN94
DSS 0.5% SYN363
LCN2 (ng/g)
0
50
100
150
H20
DSS 0.5%
DSS 0.5% BUTYRATE 100mM
DSS 0.5% SYN94
DSS 0.5% SYN363

Fig. 27

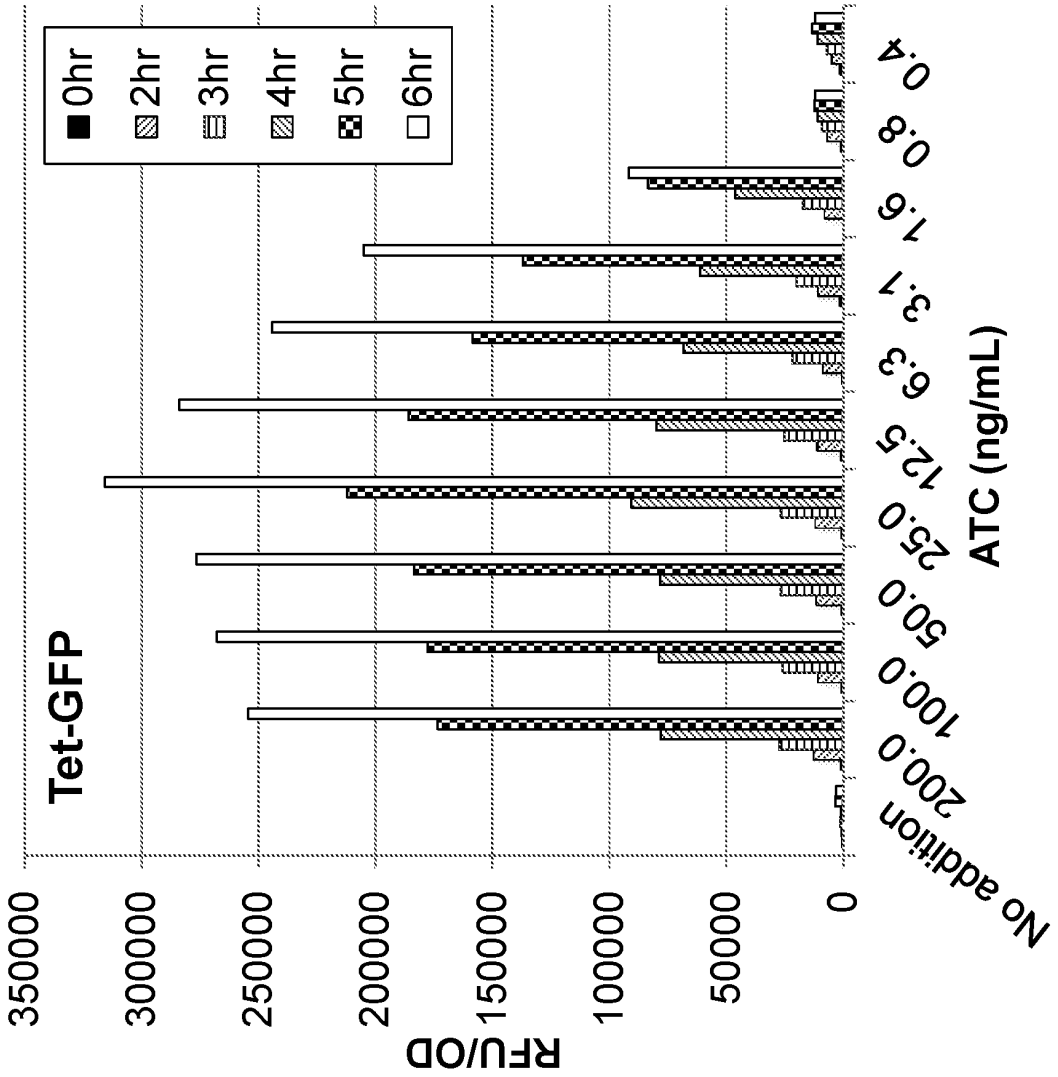

Tet-GFP
0hr
2hr
3hr
4hr
5hr
6hr
RFU/OD
350000
300000
250000
200000
150000
100000
50000
0
No addition
200.0
100.0
50.0
25.0
12.5
6.3
3.1
1.6
0.8
0.4
ATC (ng/mL)

Fig. 28A

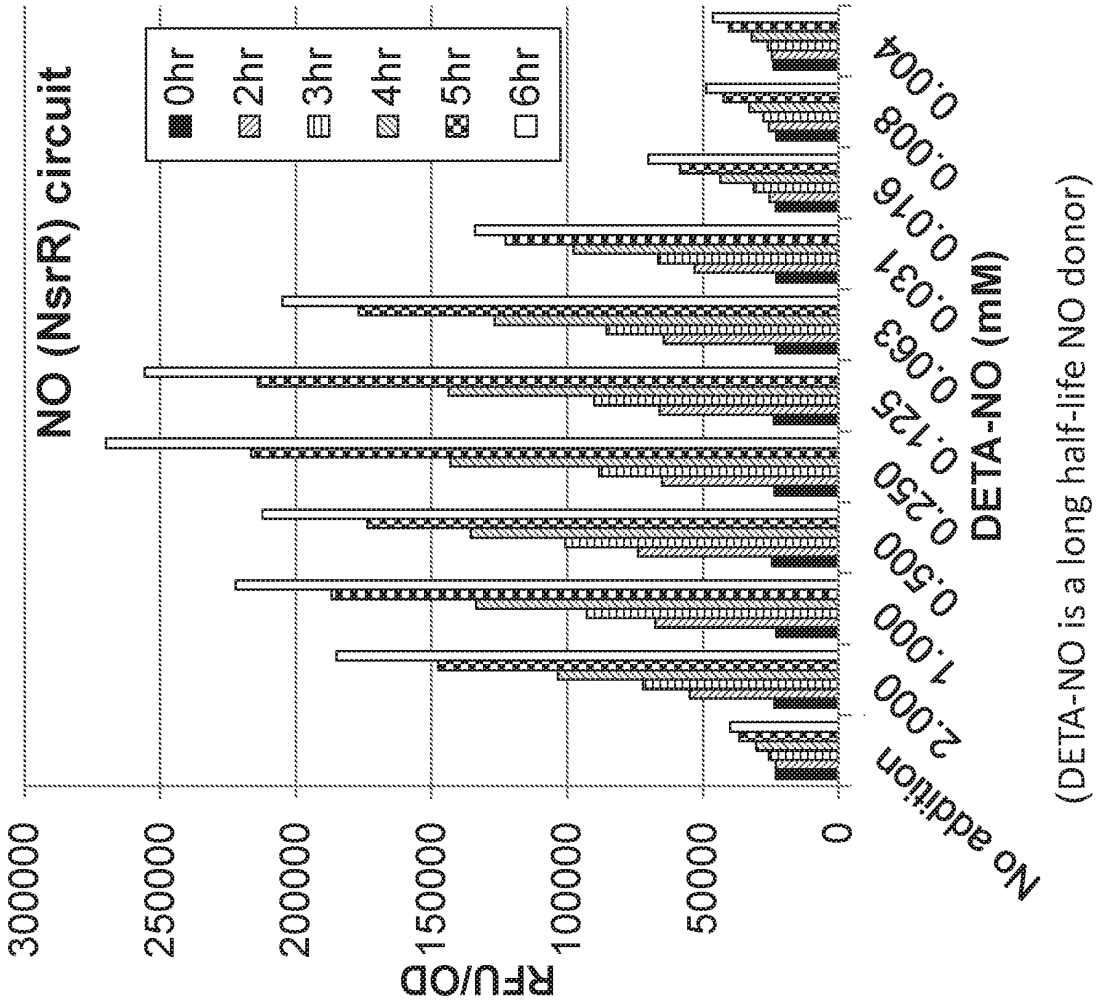

NO (NsrR) circuit
0hr
2hr
3hr
4hr
5hr
6hr
RFU/OD
300000
250000
200000
150000
100000
50000
0
No addition
2.000
1.000
0.500
0.250
0.125
0.063
0.031
0.016
0.008
0.004
DETA-NO (mM)
(DETA-NO is a long half-life NO donor)

Fig. 28B

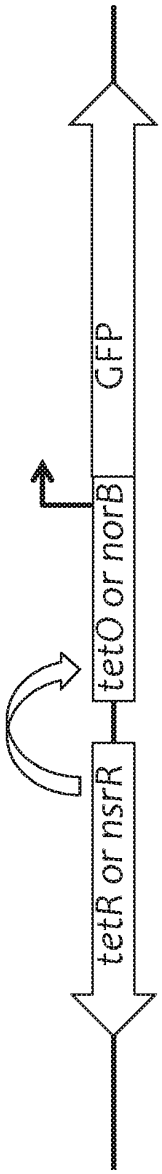


Fig. 28C

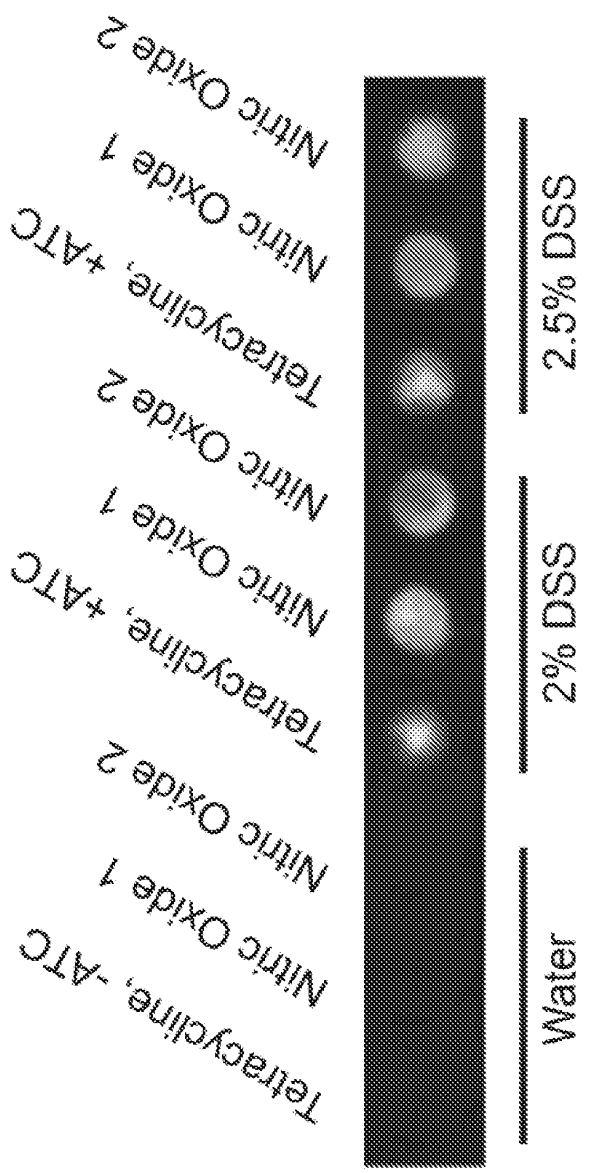
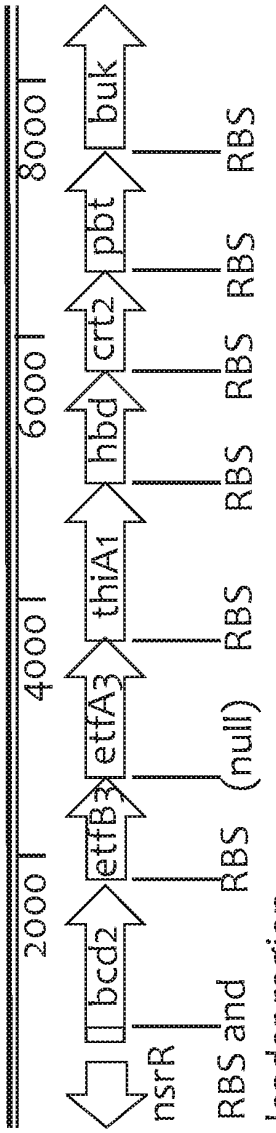
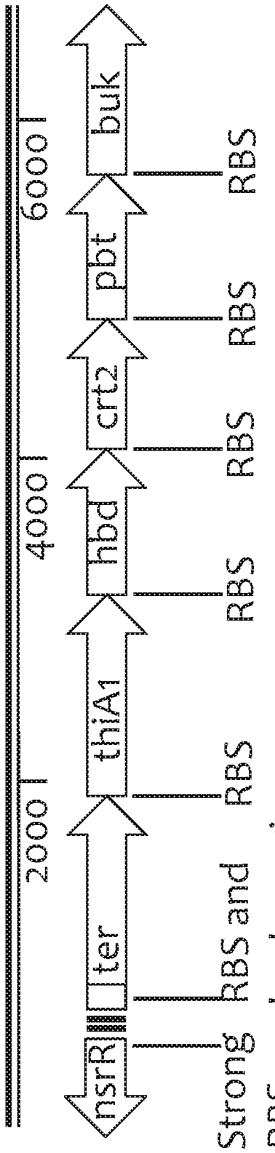

Tetracycline, -ATC
Nitric Oxide 1
Nitric Oxide 2
Tetracycline, +ATC
Nitric Oxide 1
Nitric Oxide 2
Tetracycline, +ATC
Nitric Oxide 1
Nitric Oxide 2
Water
2% DSS
2.5% DSS

Fig. 29



pLogic031-NsrR
8575 bp

Fig. 30



pLogic046-NsrR (2)
6787 bp

Fig. 31

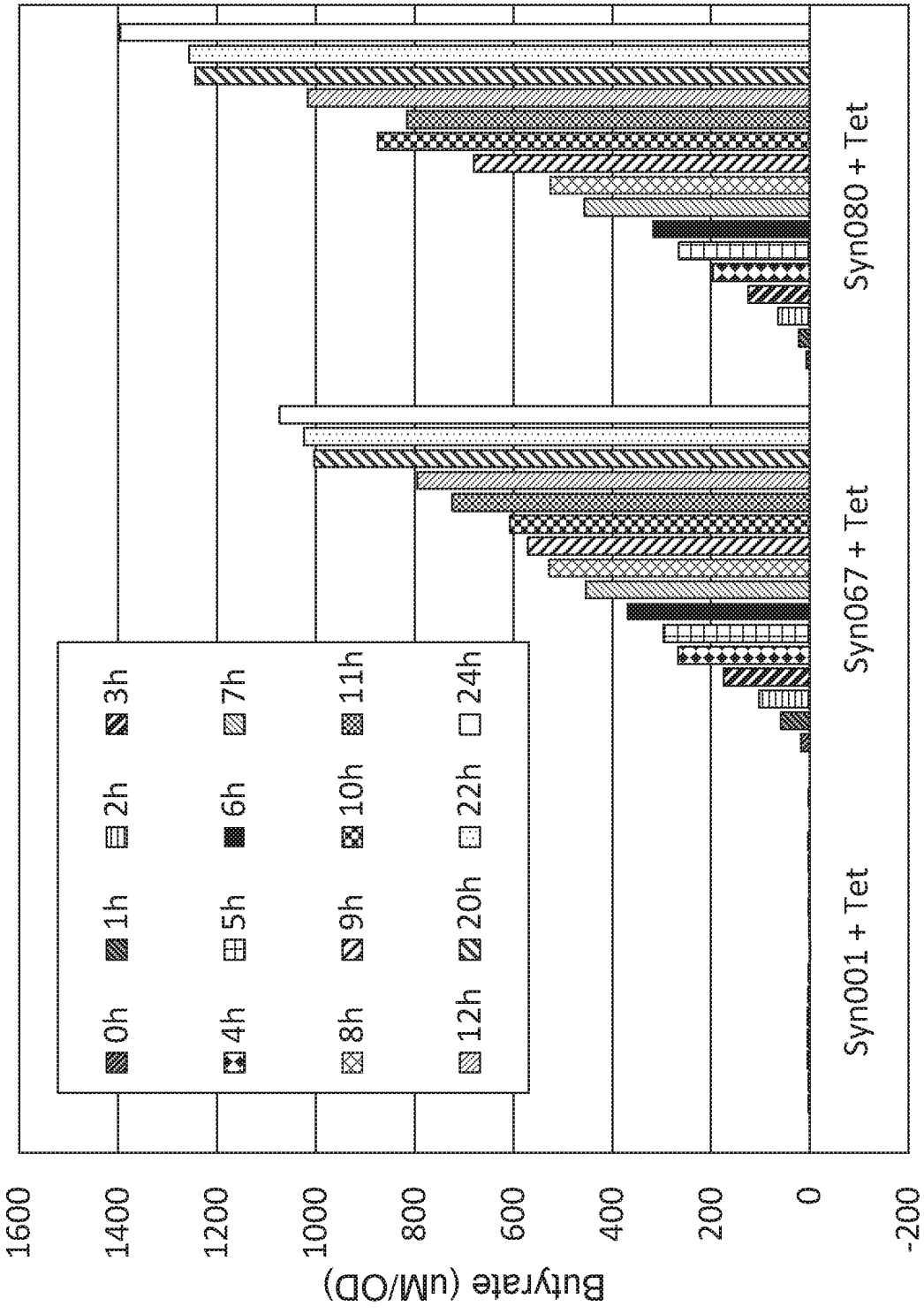

Butyrate (uM/OD)
1600
1400
1200
1000
800
600
400
200
0
-200
Syn001 + Tet
Syn067 + Tet
Syn080 + Tet
0h
1h
2h
3h
4h
5h
6h
7h
8h
9h
10h
11h
12h
20h
22h
24h

Fig. 32

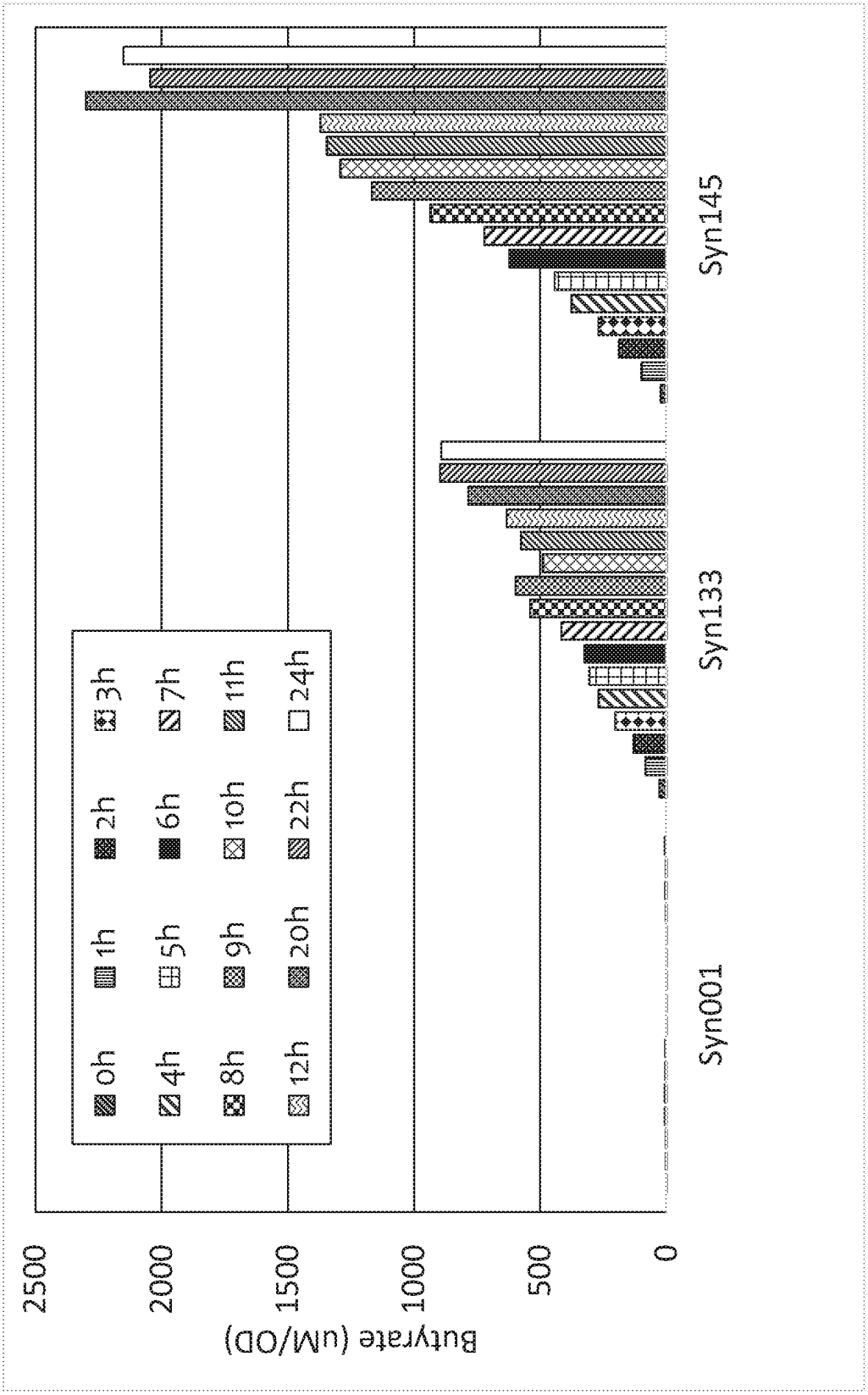

Butyrate (uM/OD)
2500
2000
1500
1000
500
0
Syn001
Syn133
Syn145
0h
1h
2h
3h
4h
5h
6h
7h
8h
9h
10h
11h
12h
20h
22h
24h

Fig. 33

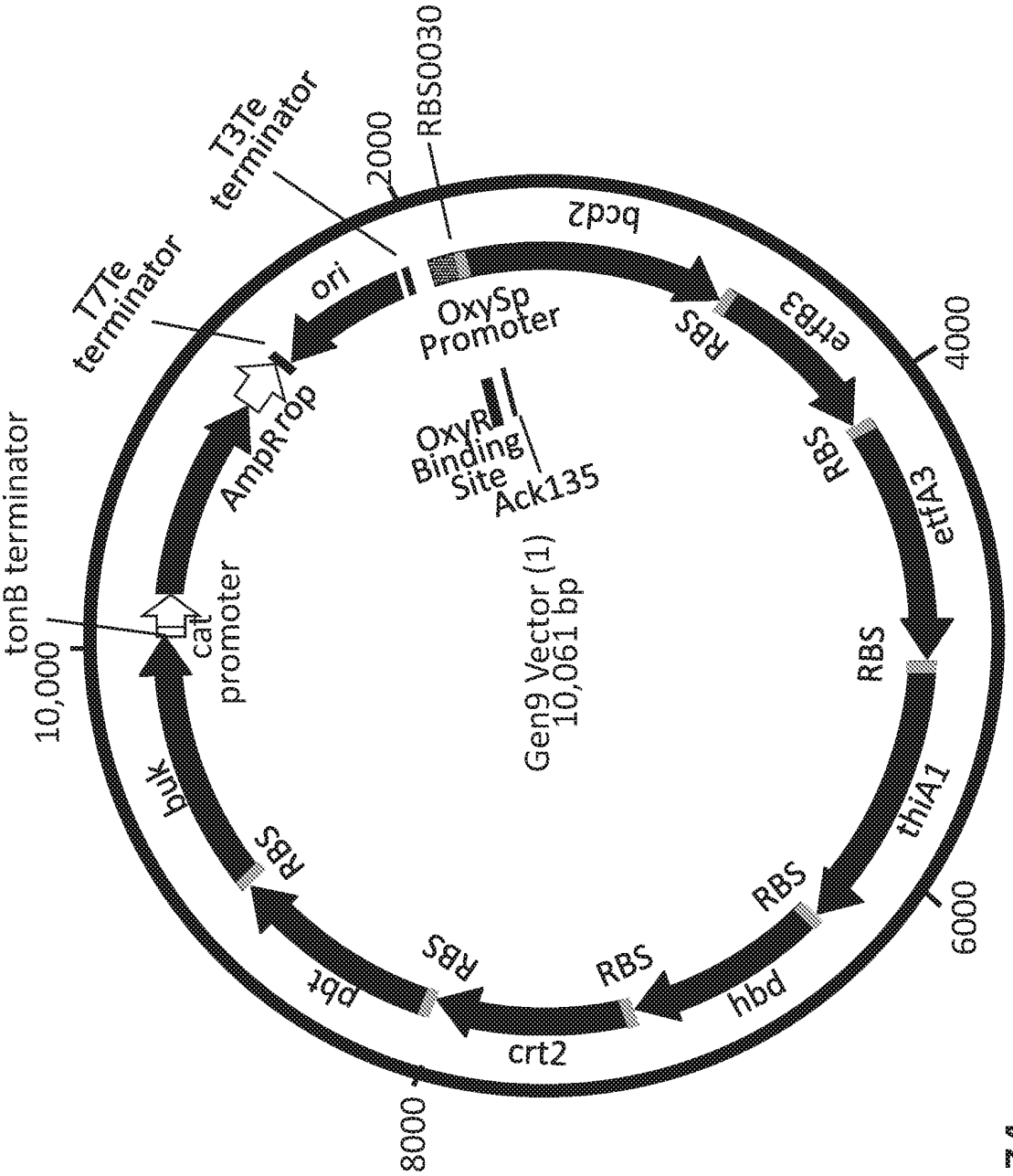

RBS0030
2000
T3Te terminator
T7Te terminator
tonB terminator
10,000
ori
OxySp Promoter
bcd2
RBS
etfB3
4000
AmpR rop
OxyR Binding Site
Ack135
RBS
etfA3
cat promoter
Gen9 Vector (1)
10,061 bp
RBS
thiA1
buk
RBS
RBS
RBS
6000
pbt
RBS
hbd
crt2
8000

Fig. 34

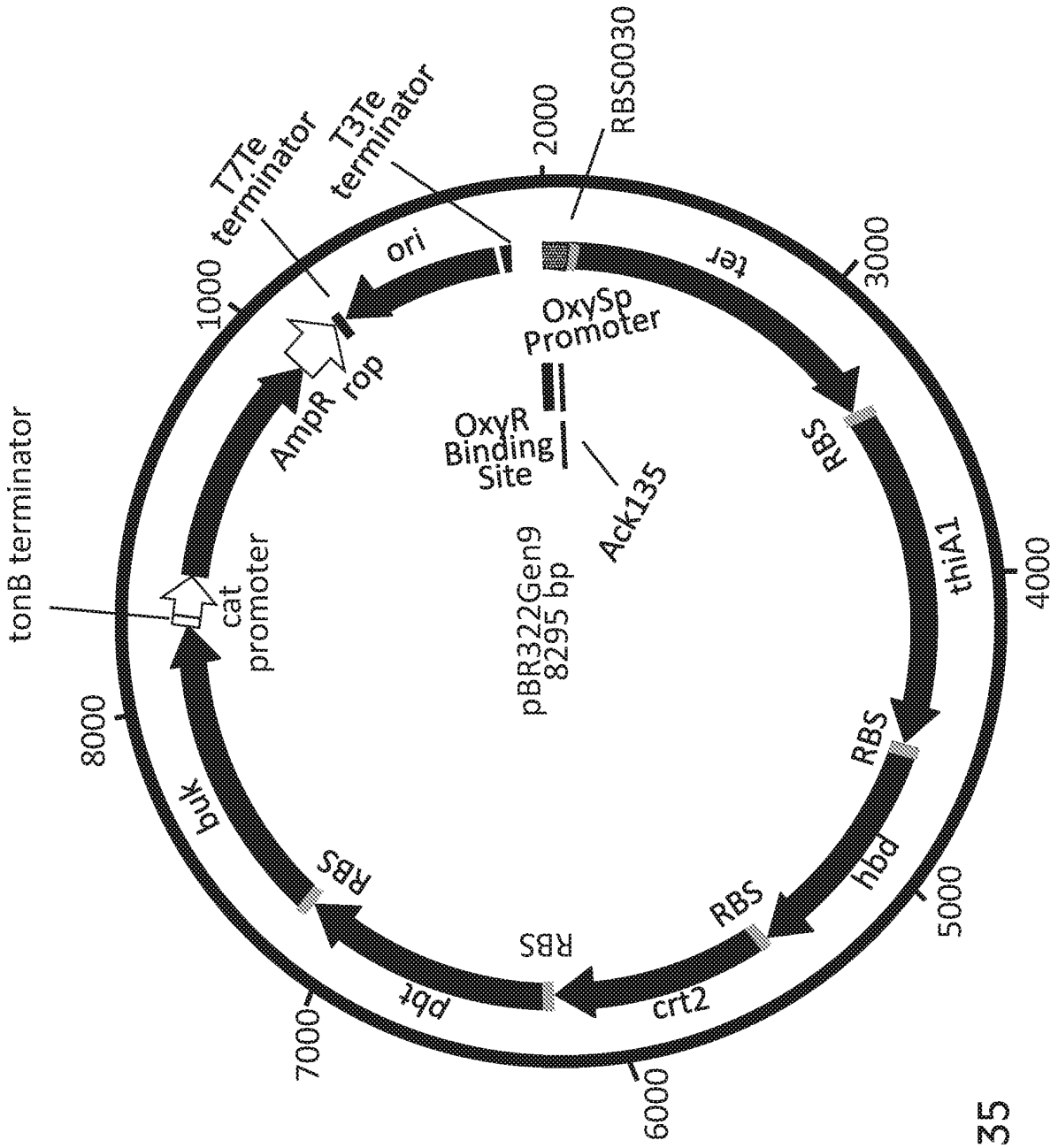

1000
2000
3000
4000
5000
6000
7000
8000
T7Te terminator
T3Te terminator
RBS0030
ori
ter
OxySp Promoter
OxyR Binding Site
Ack135
pBR322Gen9 8295 bp
AmpR rop
cat promoter
tonB terminator
RBS
thiA1
hbd
crt2
pbt
bukk

Fig. 35

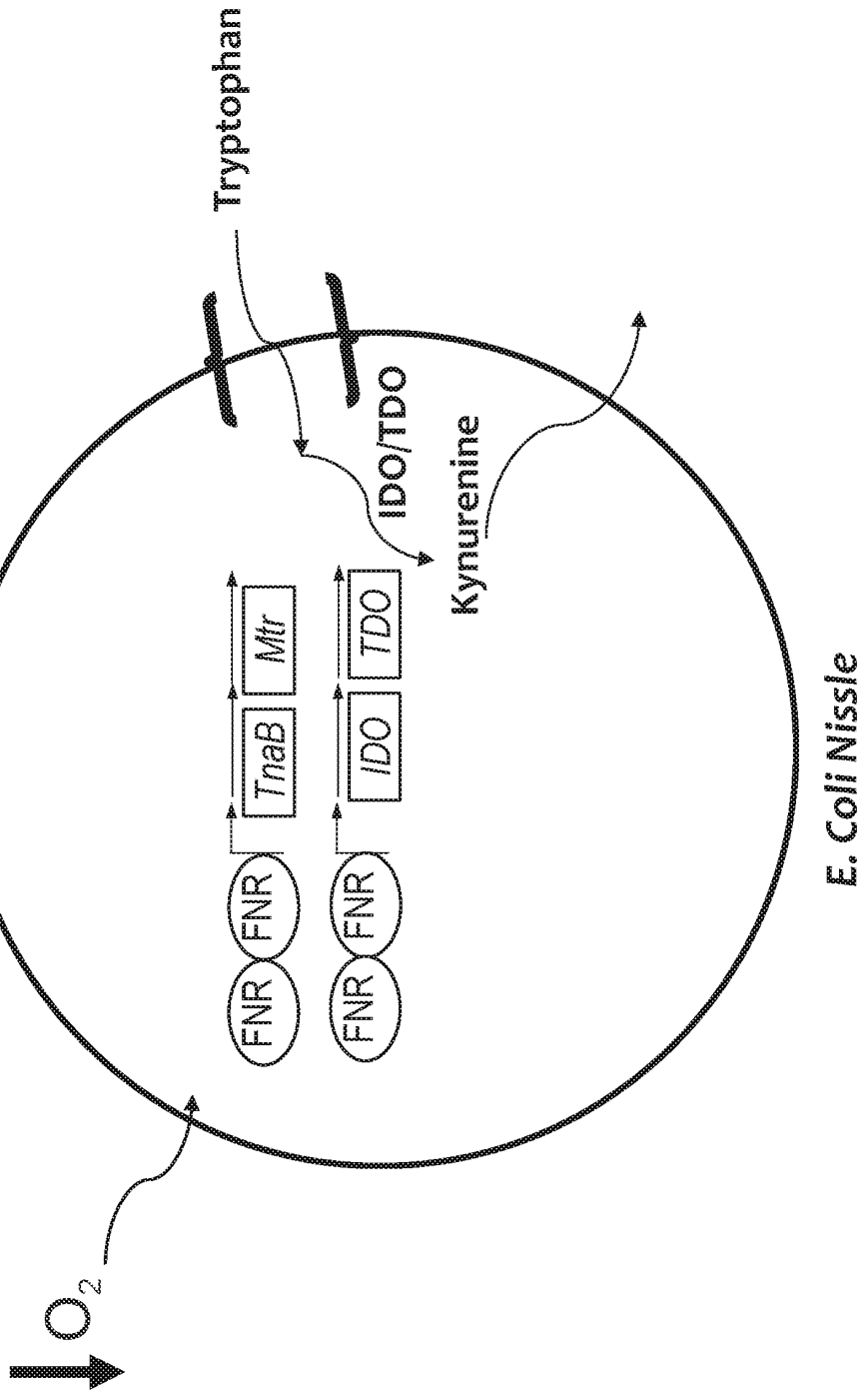

Tryptophan
IDO/TDO
Kynurenine
FNR
FNR
TnaB
Mtr
FNR
FNR
IDO
TDO
O_2
E. Coli Nissle

Fig. 36

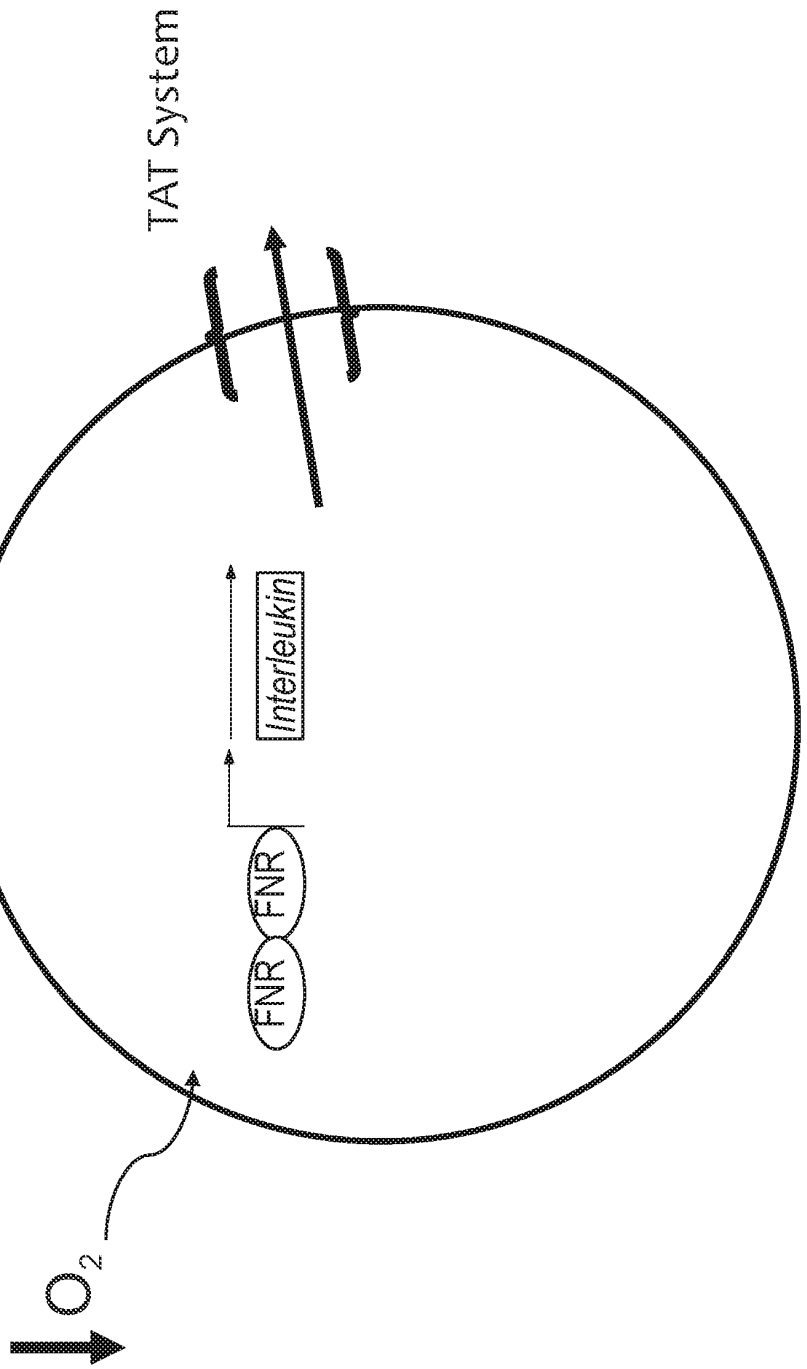


Fig. 37

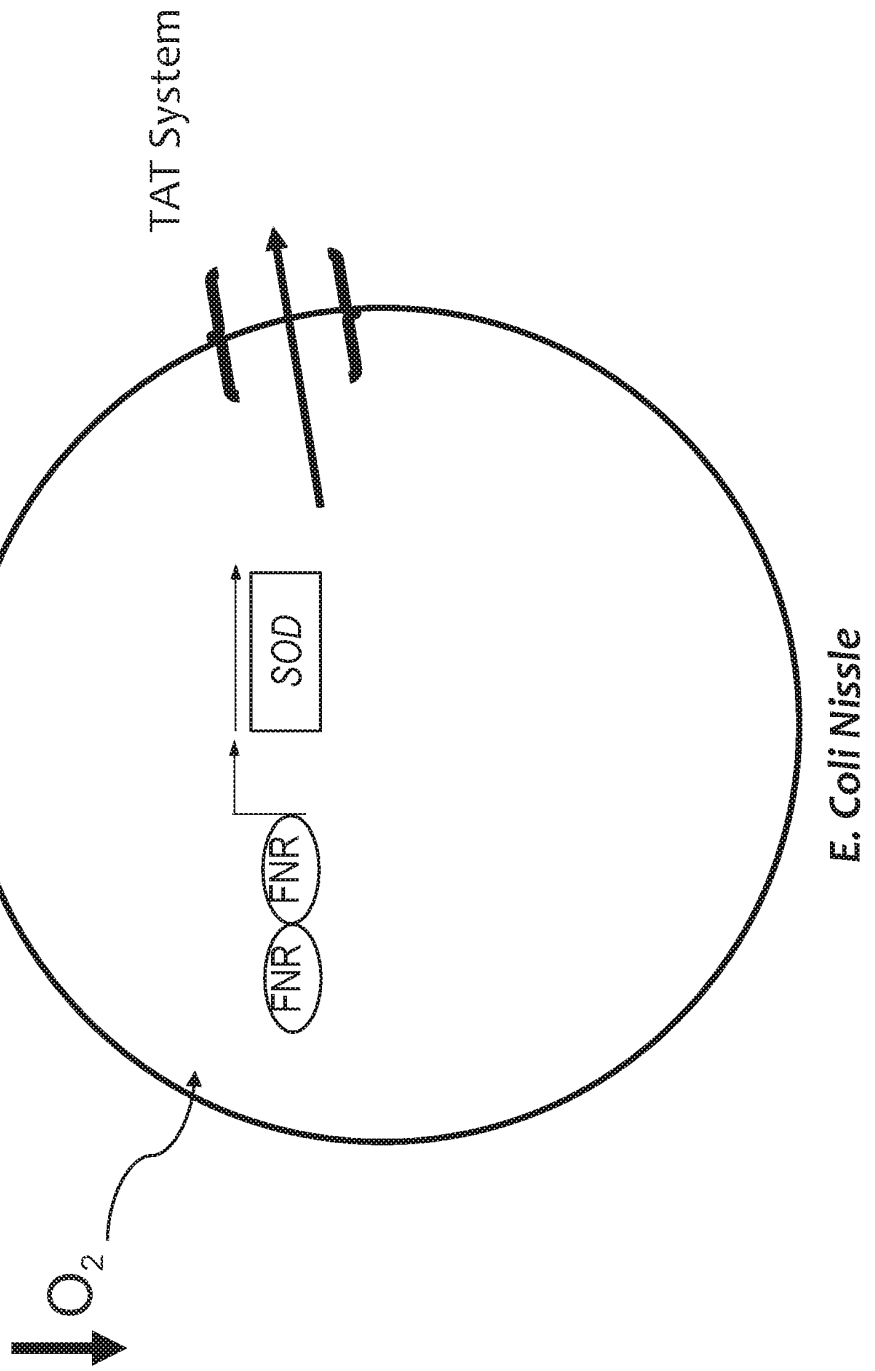


Fig. 38

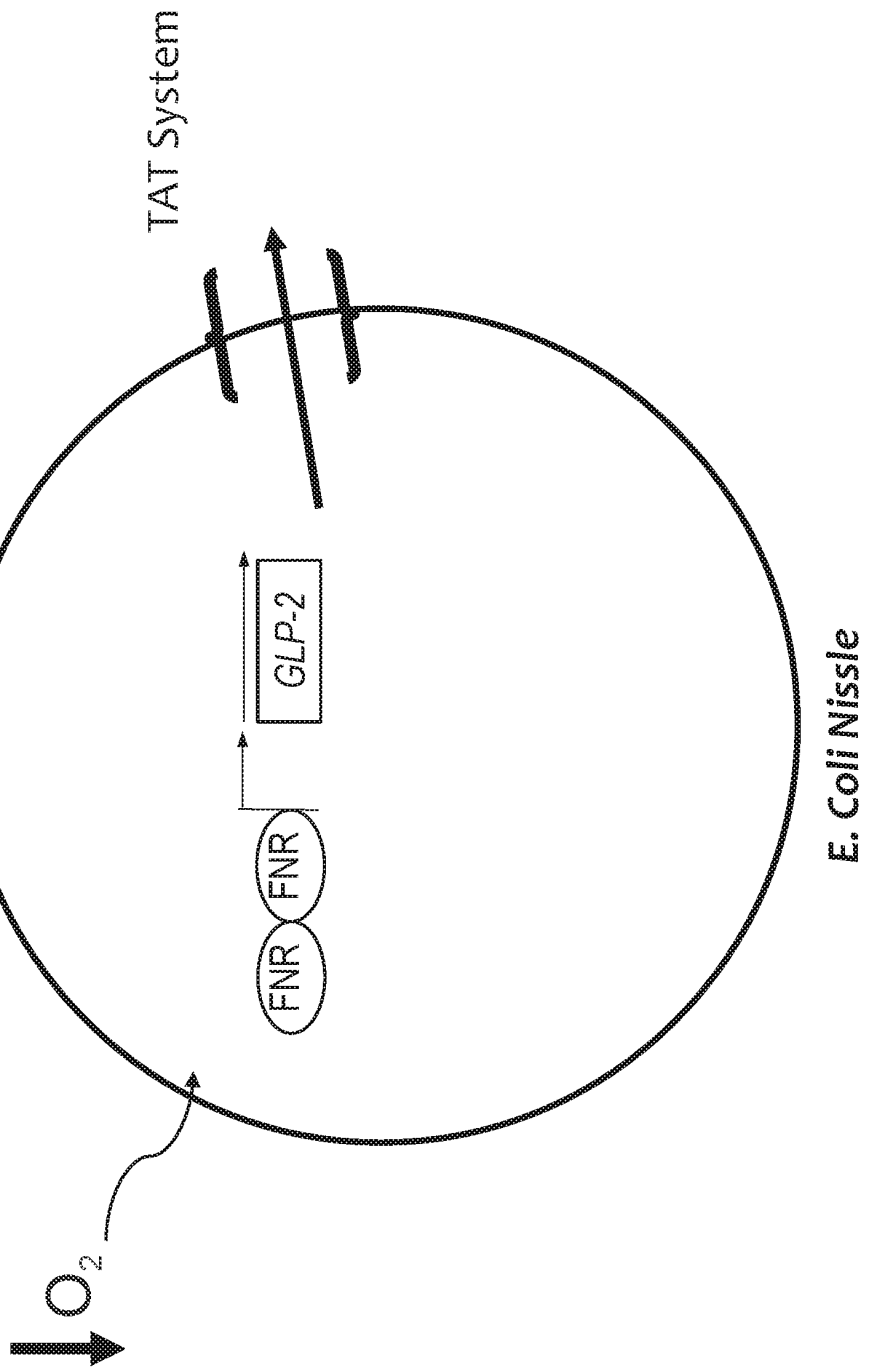


Fig. 39

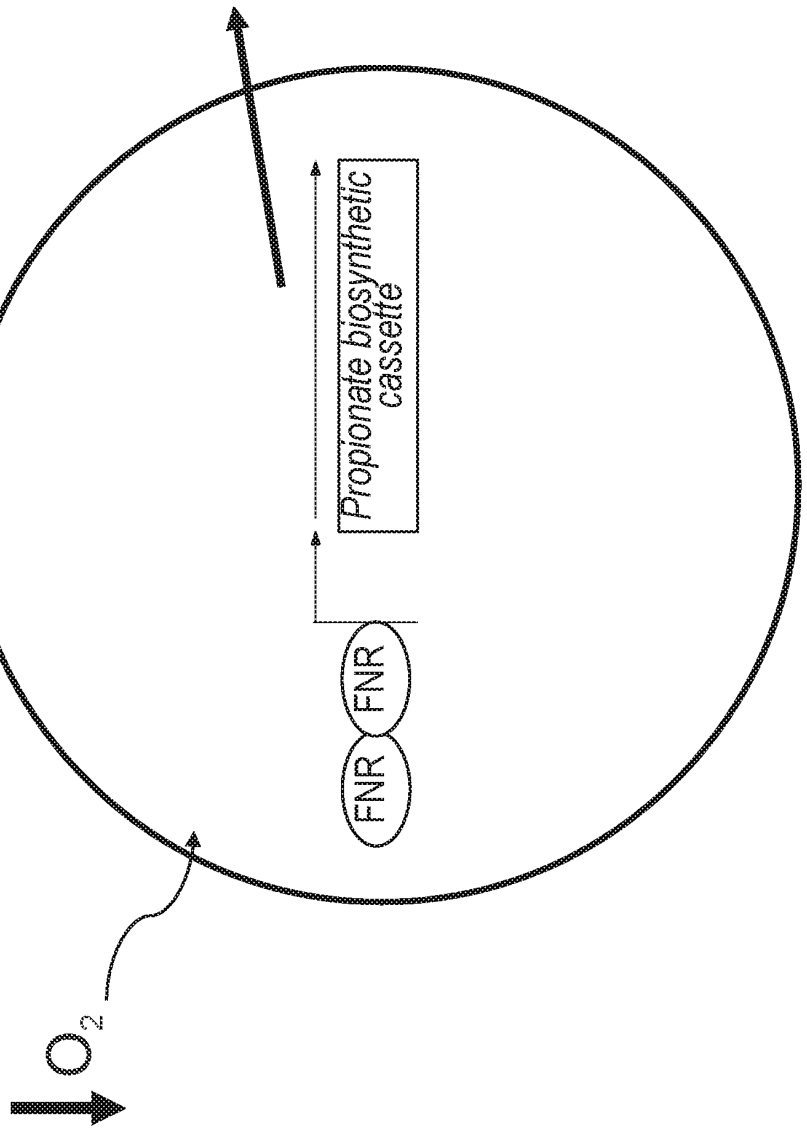


Fig. 40

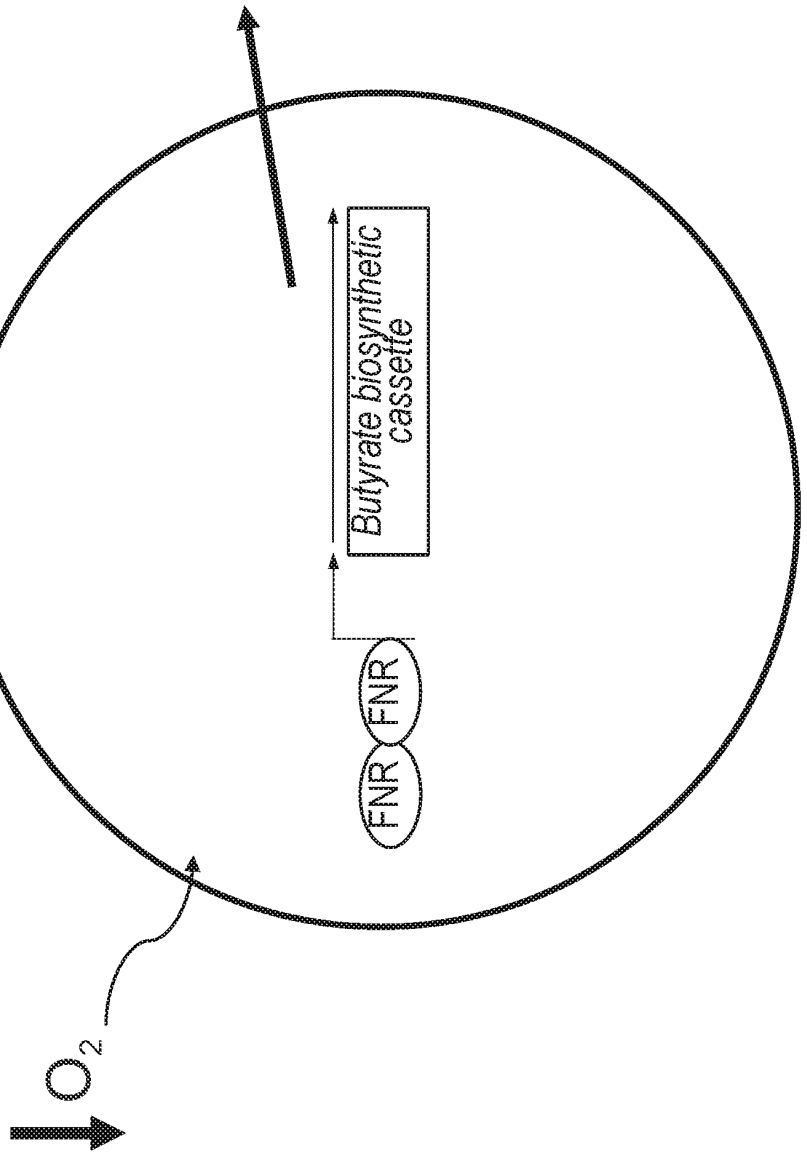


Fig. 41

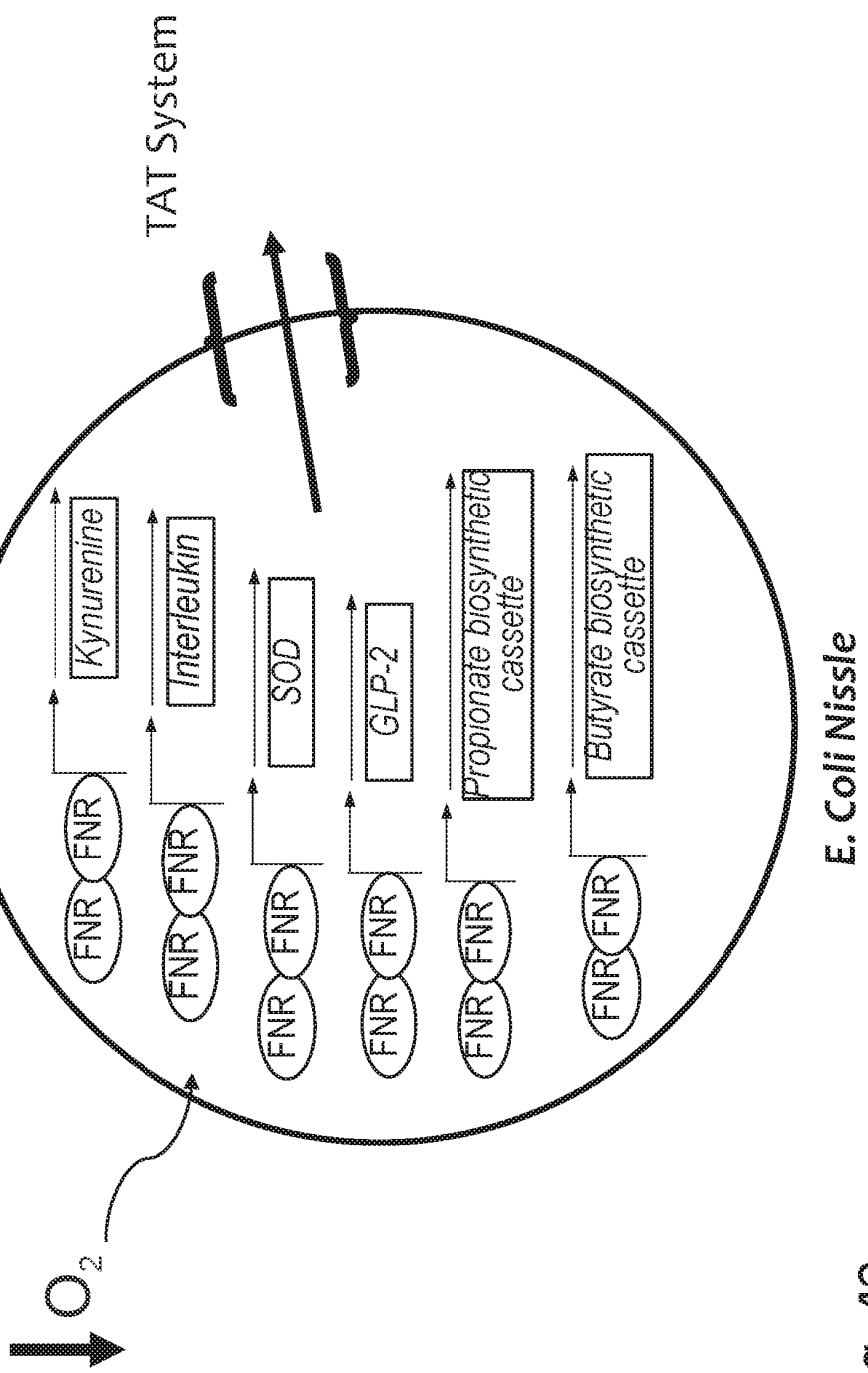

TAT System
Kynurenine
Interleukin
SOD
GLP-2
Propionate biosynthetic cassette
Butyrate biosynthetic cassette
FNR
O_2
E. Coli Nissle

Fig. 42

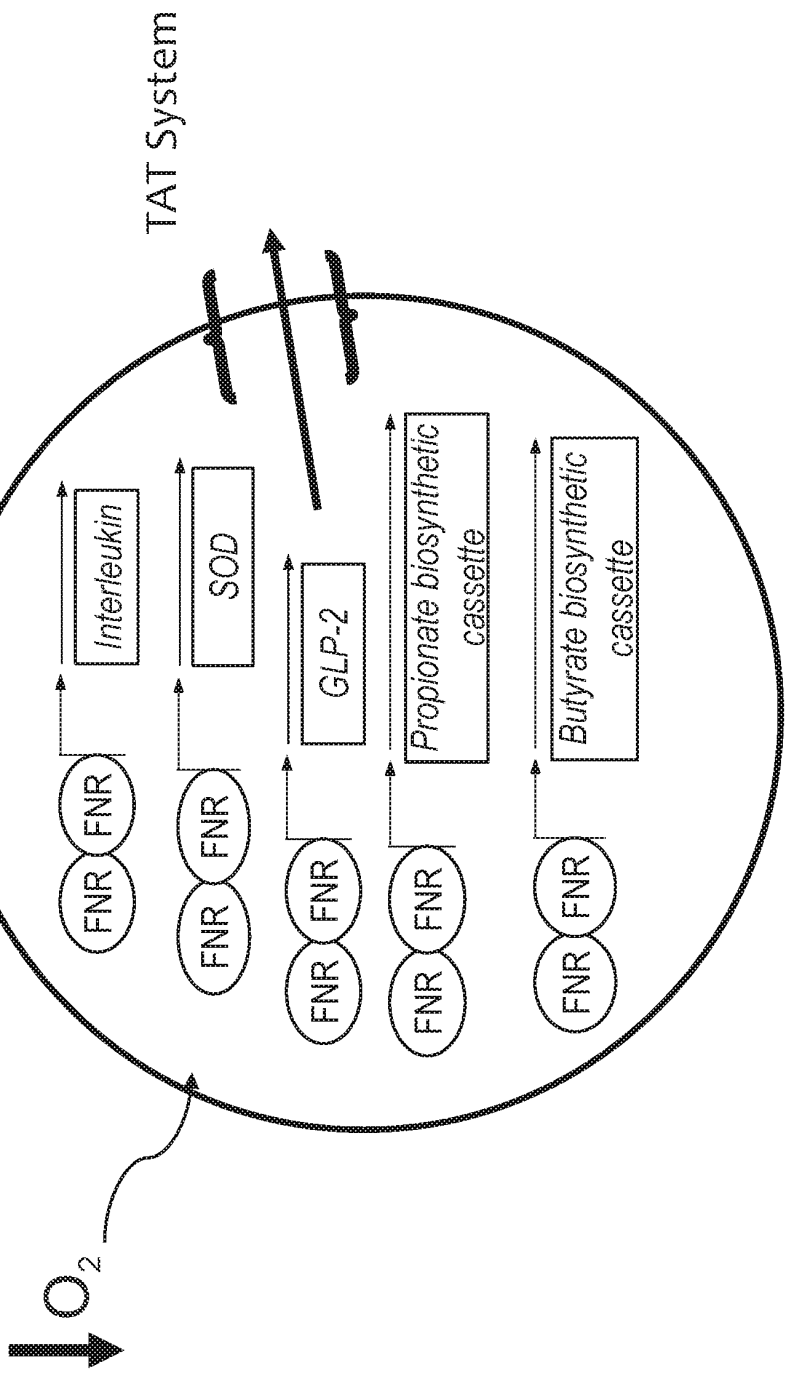

O_2
TAT System
FNR
Interleukin
SOD
GLP-2
Propionate biosynthetic cassette
Butyrate biosynthetic cassette
E. Coli Nissle

Fig. 43

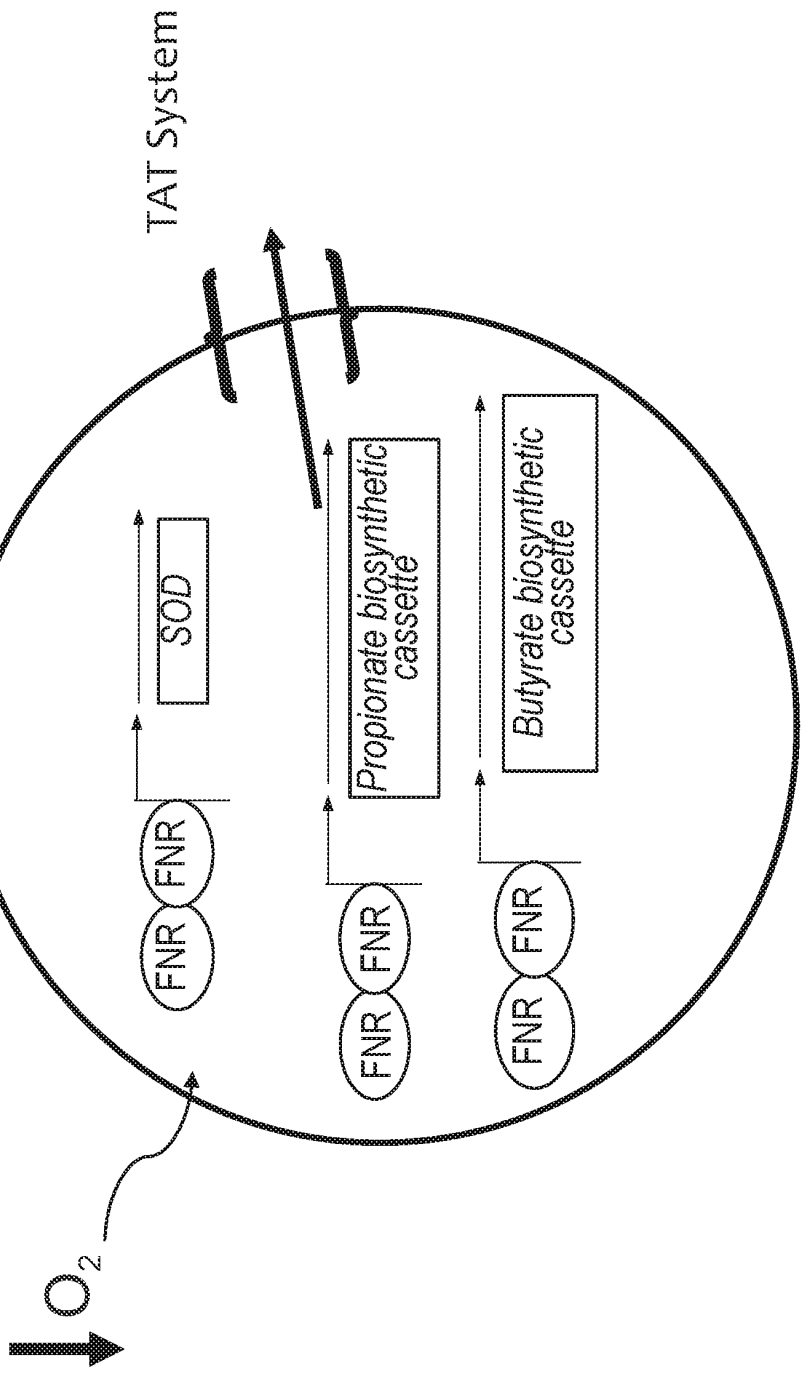

TAT System
SOD
FNR
FNR
Propionate biosynthetic cassette
FNR
FNR
Butyrate biosynthetic cassette
FNR
FNR
E. Coli Nissle
O2

Fig. 44

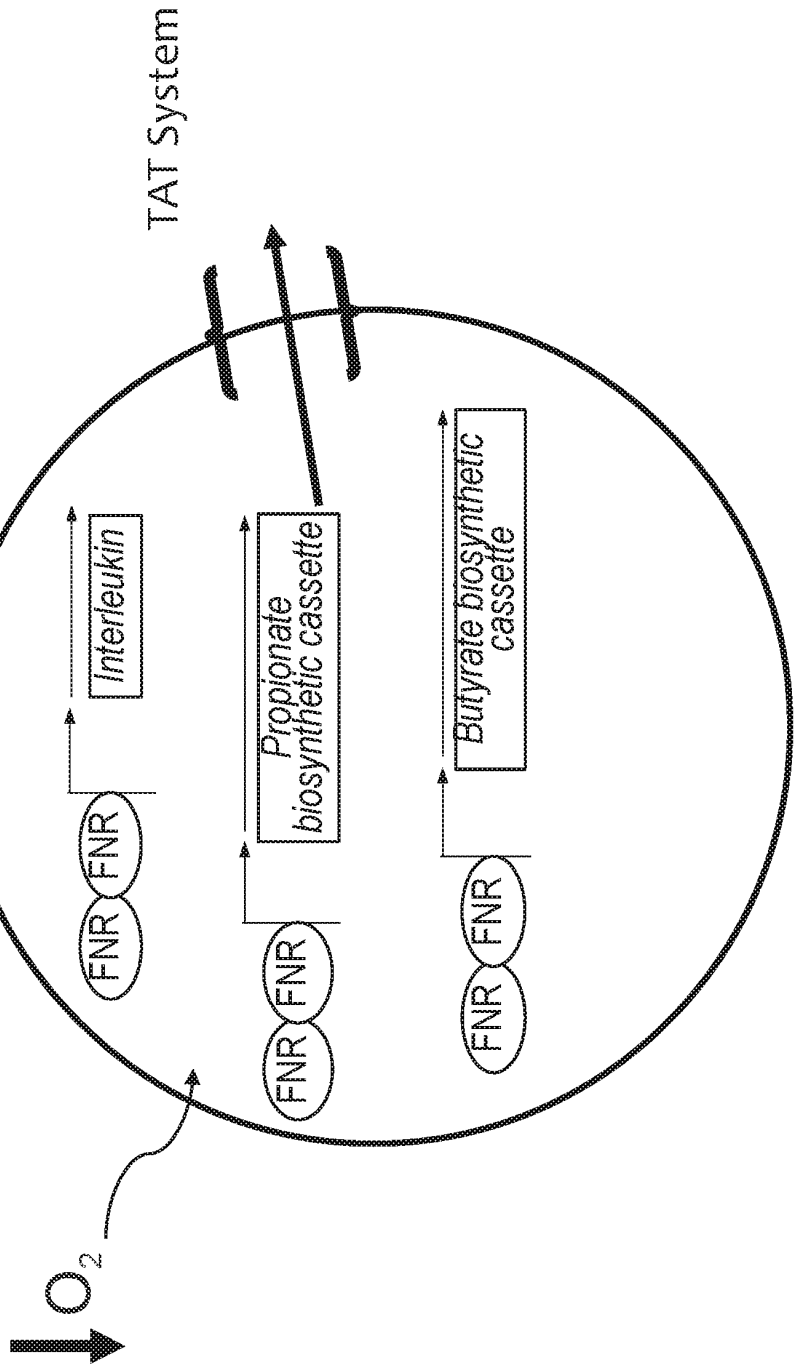

TAT System
Interleukin
Propionate biosynthetic cassette
Butyrate biosynthetic cassette
FNR
FNR
FNR
FNR
FNR
FNR
O_2
E. Coli Nissle

Fig. 45

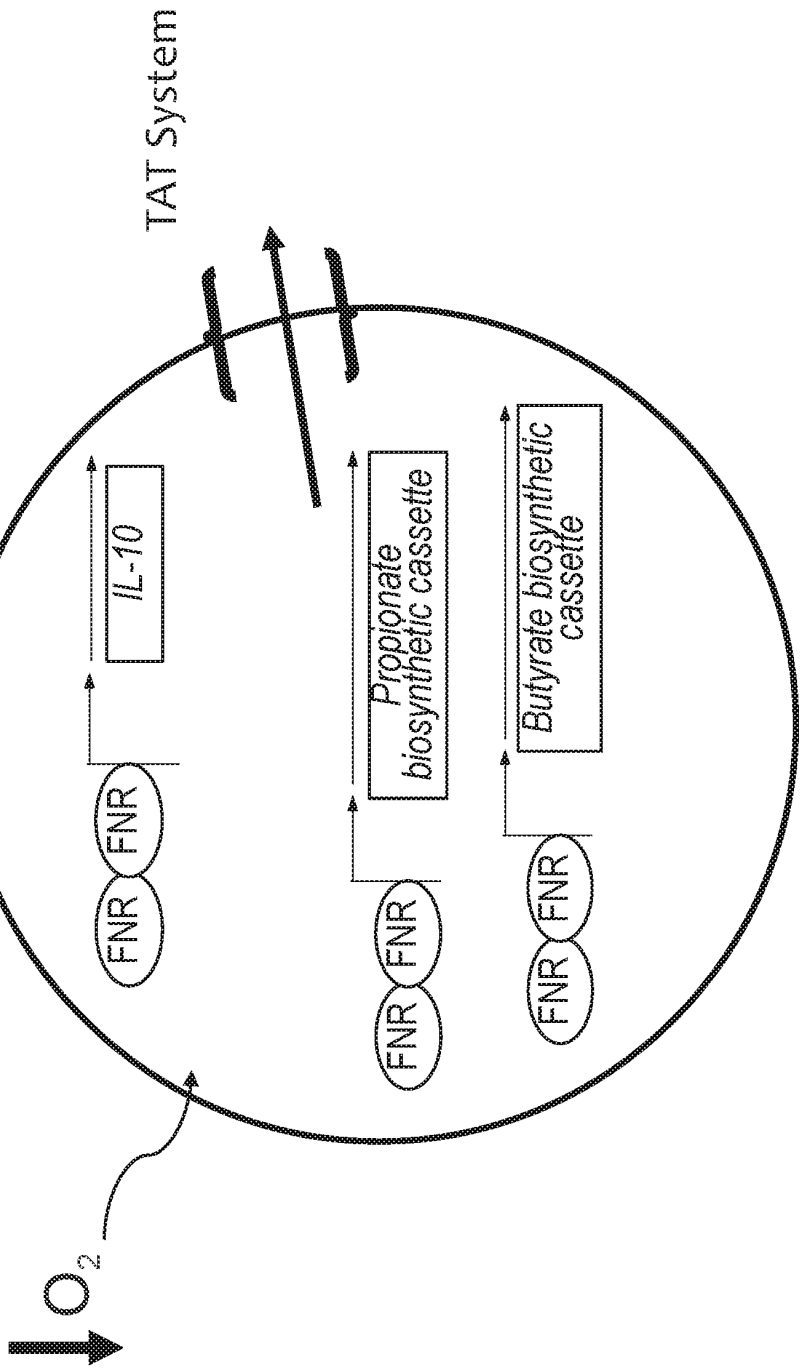

TAT System
IL-10
FNR
FNR
Propionate biosynthetic cassette
FNR
FNR
Butyrate biosynthetic cassette
FNR
FNR
O2
E. Coli Nissle

Fig. 46

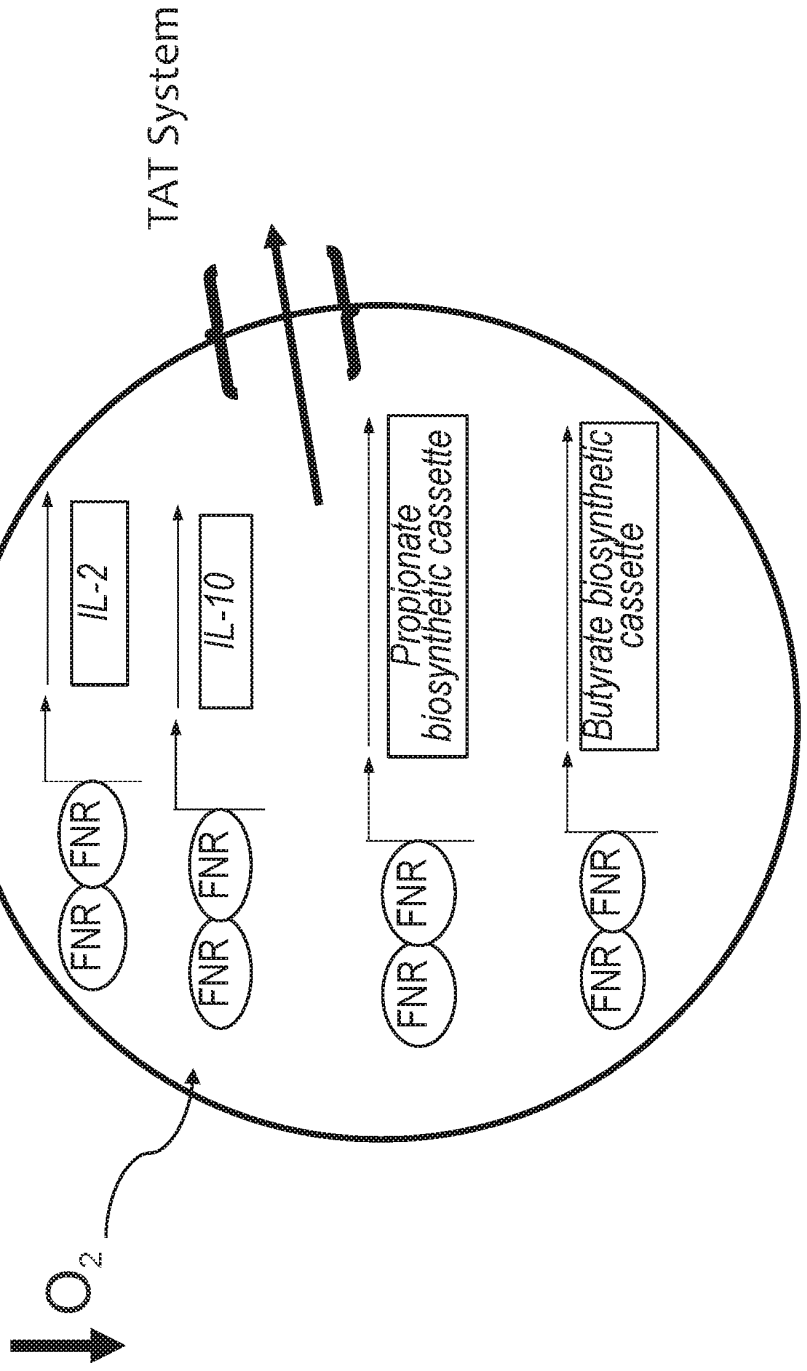

TAT System
IL-2
IL-10
Propionate biosynthetic cassette
Butyrate biosynthetic cassette
FNR
FNR
FNR
FNR
FNR
FNR
FNR
FNR
O2
E. Coli Nissle

Fig. 47

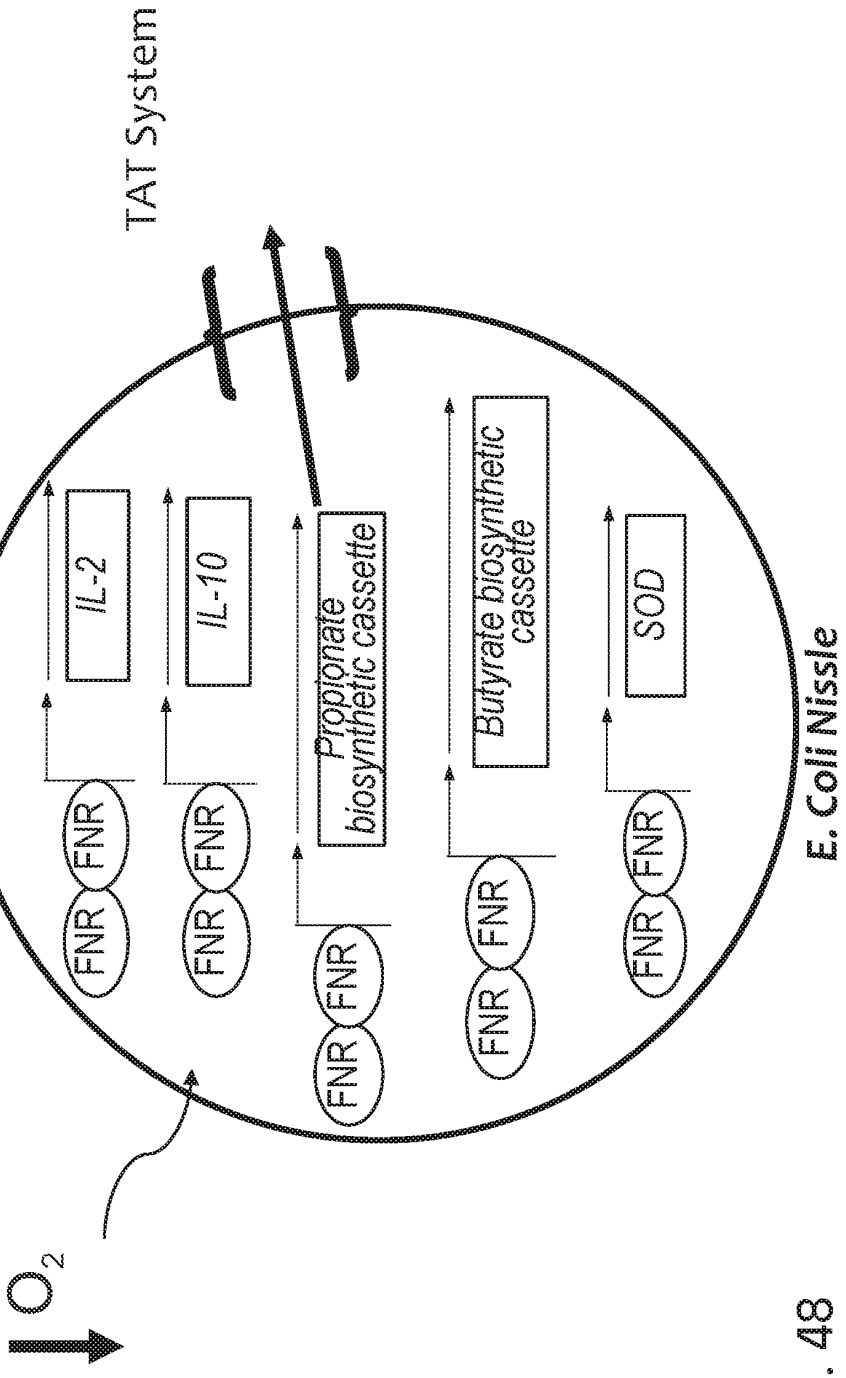

TAT System
IL-2
IL-10
Propionate biosynthetic cassette
Butyrate biosynthetic cassette
SOD
FNR
FNR
FNR
FNR
FNR
FNR
FNR
FNR
FNR
FNR
O_2
E. Coli Nissle

Fig. 48

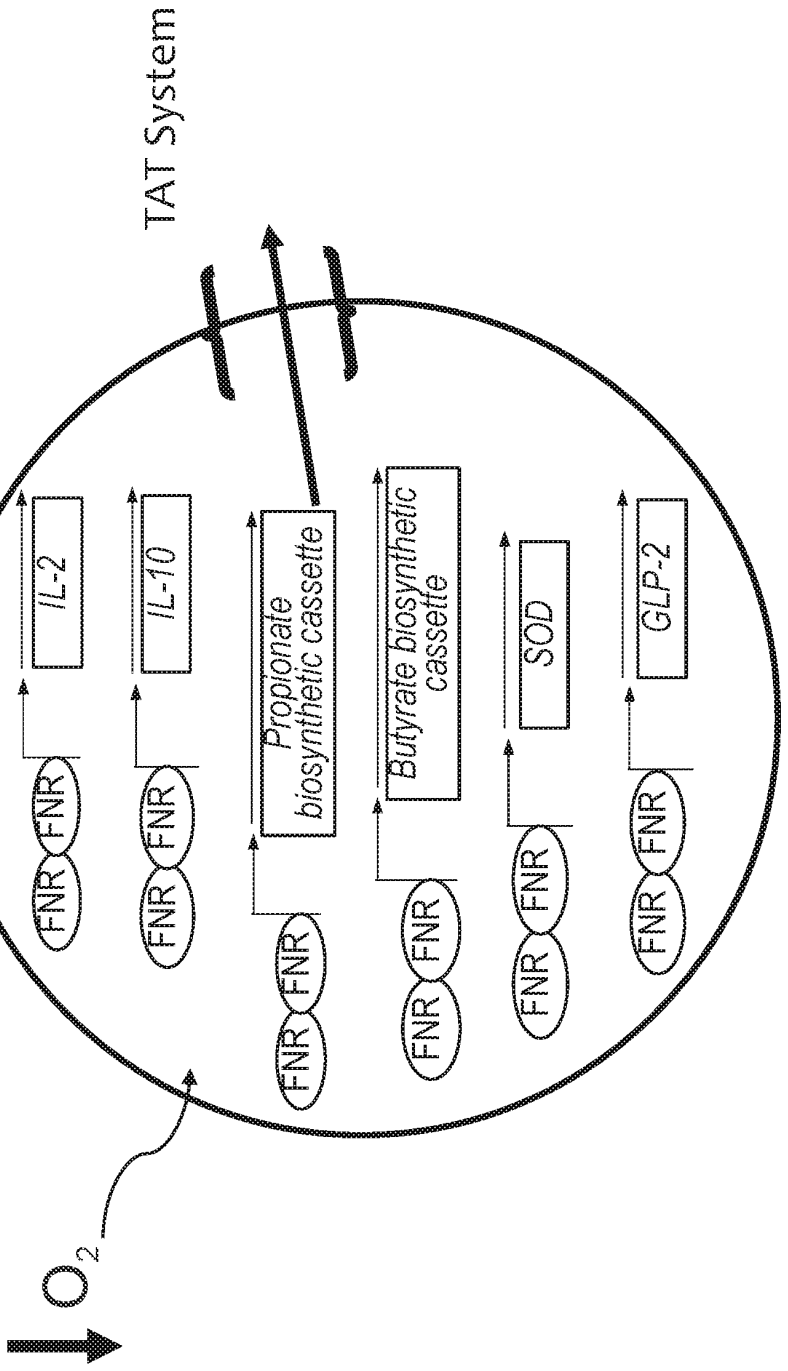

TAT System
O_2
FNR
IL-2
IL-10
Propionate biosynthetic cassette
Butyrate biosynthetic cassette
SOD
GLP-2
E. Coli Nissle

Fig. 49

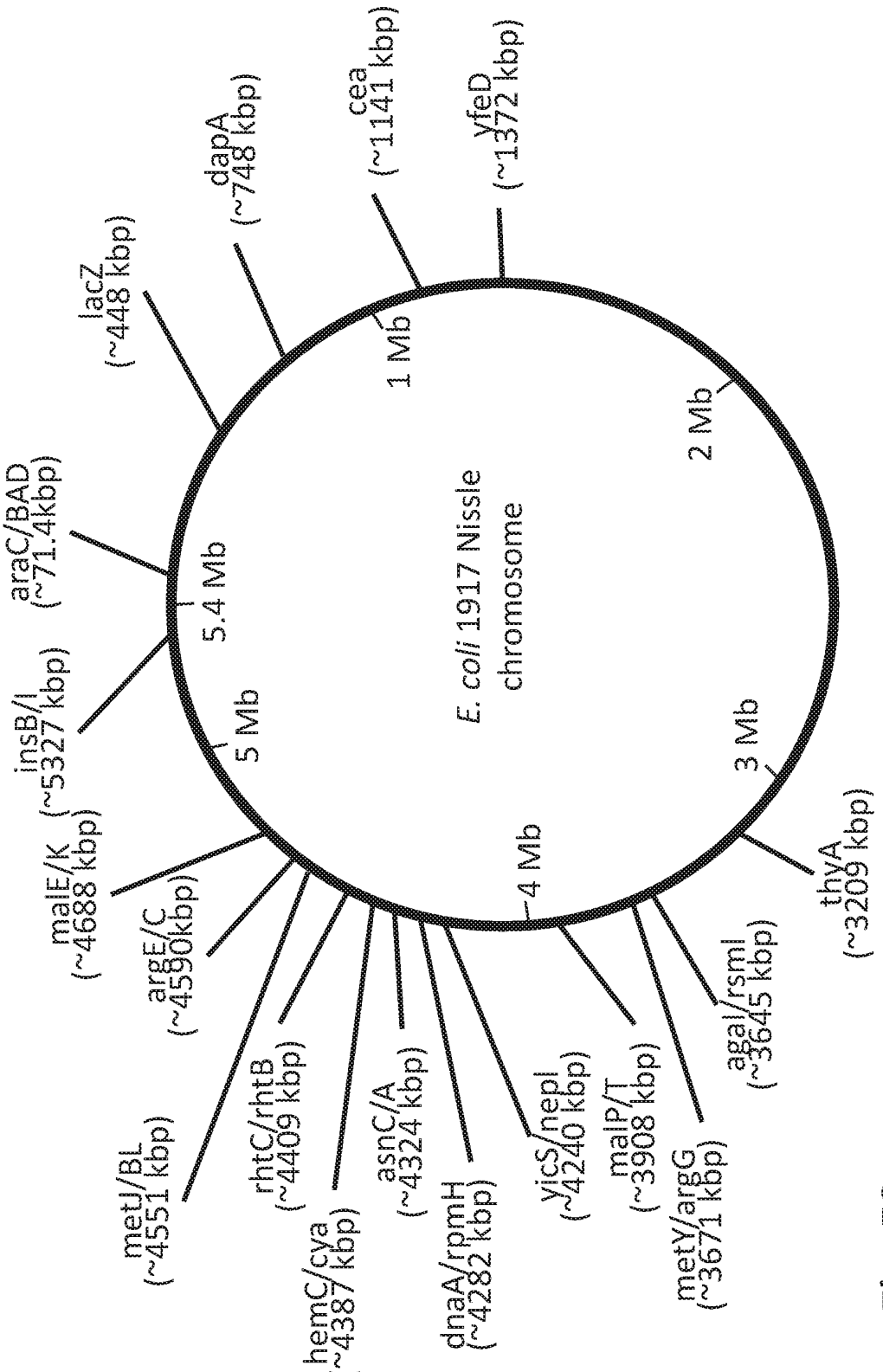

E. coli 1917 Nissle chromosome
1 Mb
2 Mb
3 Mb
4 Mb
5 Mb
5.4 Mb
lacZ (~448 kbp)
dapA (~748 kbp)
cea (~1141 kbp)
yfeD (~1372 kbp)
araC/BAD (~71.4kbp)
insB/I (~5327 kbp)
malE/K (~4688 kbp)
argE/C (~4590kbp)
rhtC/rhtB (~4409 kbp)
hemC/cya (~4387 kbp)
asnC/A (~4324 kbp)
dnaA/rpmH (~4282 kbp)
yicS/nepI (~4240 kbp)
malP/T (~3908 kbp)
metY/argG (~3671 kbp)
agaI/rsmI (~3645 kbp)
thyA (~3209 kbp)
metJ/BL (~4551 kbp)

Fig. 50

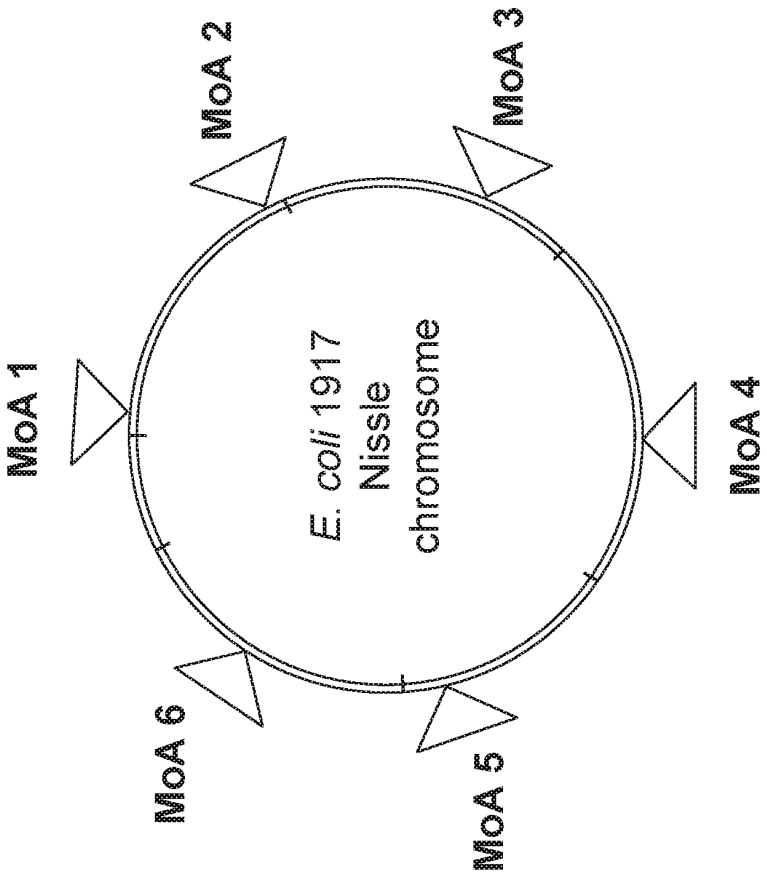


Fig. 51

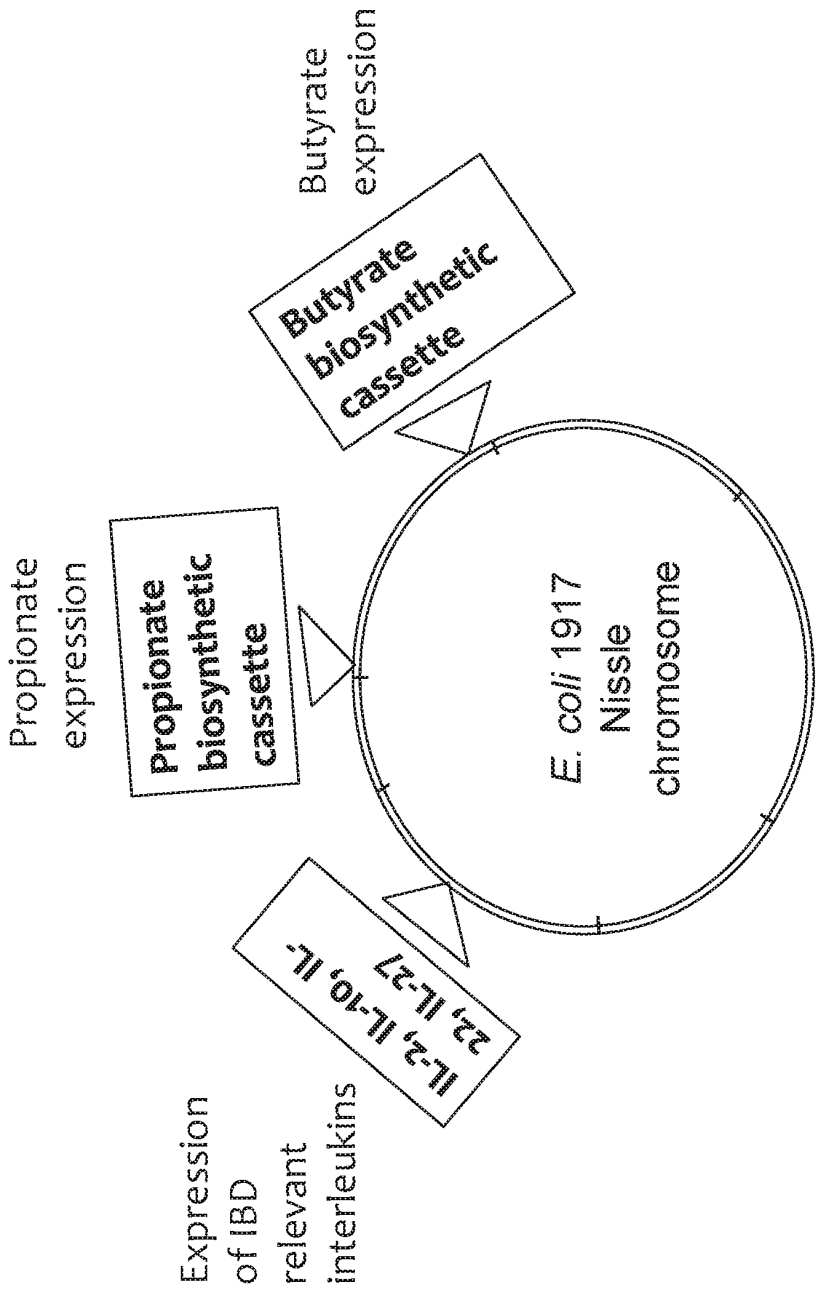

Butyrate expression
Butyrate biosynthetic cassette
Propionate expression
Propionate biosynthetic cassette
E. coli 1917 Nissle chromosome
Expression of IBD relevant interleukins
IL-2, IL-10, IL-22, IL-27

Fig. 52

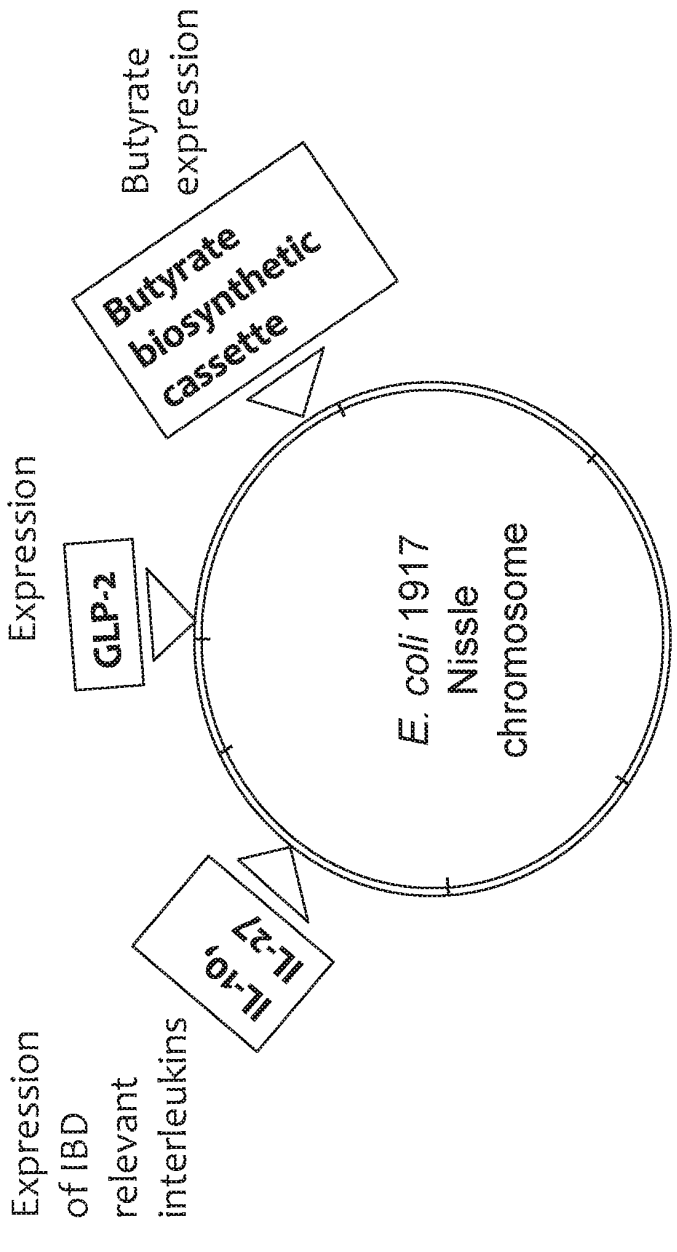

Butyrate expression
Butyrate biosynthetic cassette
GLP-2 Expression
GLP-2
E. coli 1917 Nissle chromosome
Expression of IBD relevant interleukins
IL-10, IL-27

Fig. 53

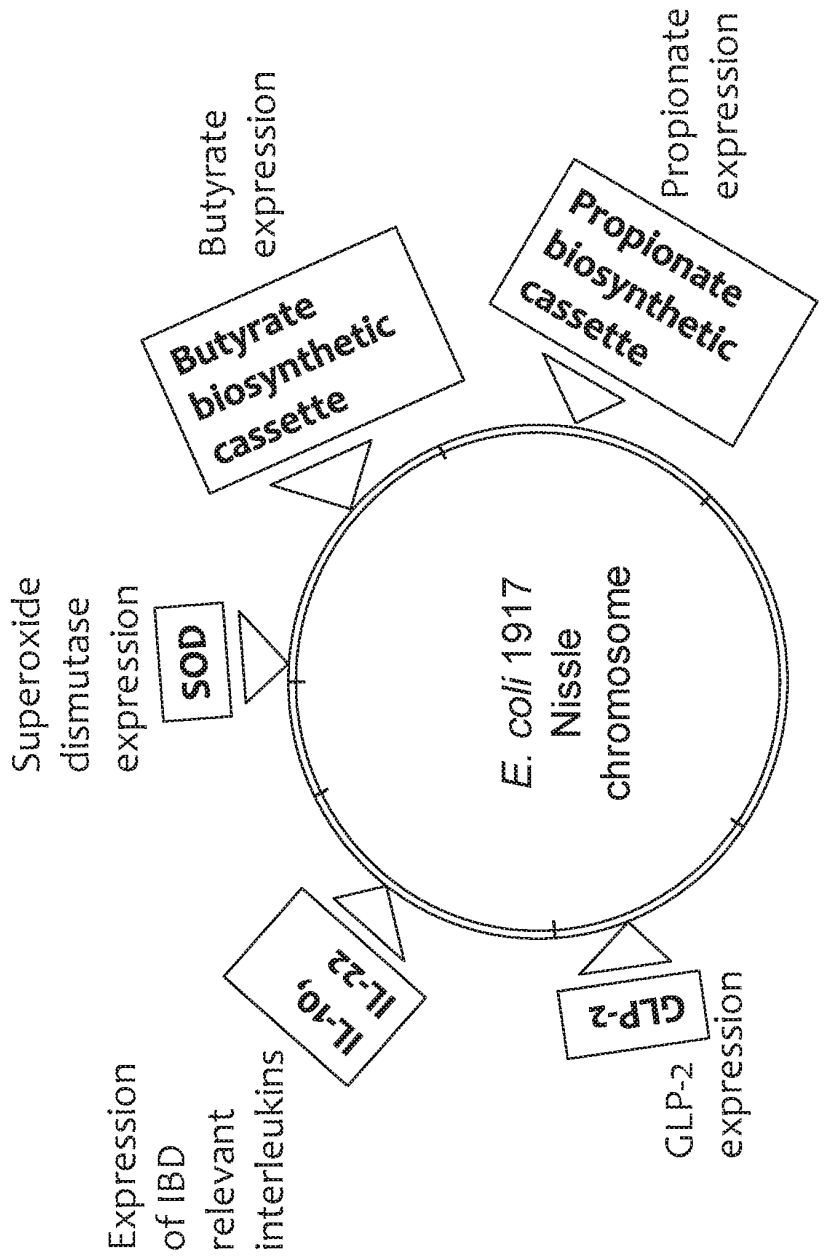

Butyrate expression
Butyrate biosynthetic cassette
Propionate expression
Propionate biosynthetic cassette
Superoxide dismutase expression
SOD
E. coli 1917 Nissle chromosome
Expression of IBD relevant interleukins
IL-10, IL-22
GLP-2
GLP-2 expression

Fig. 54

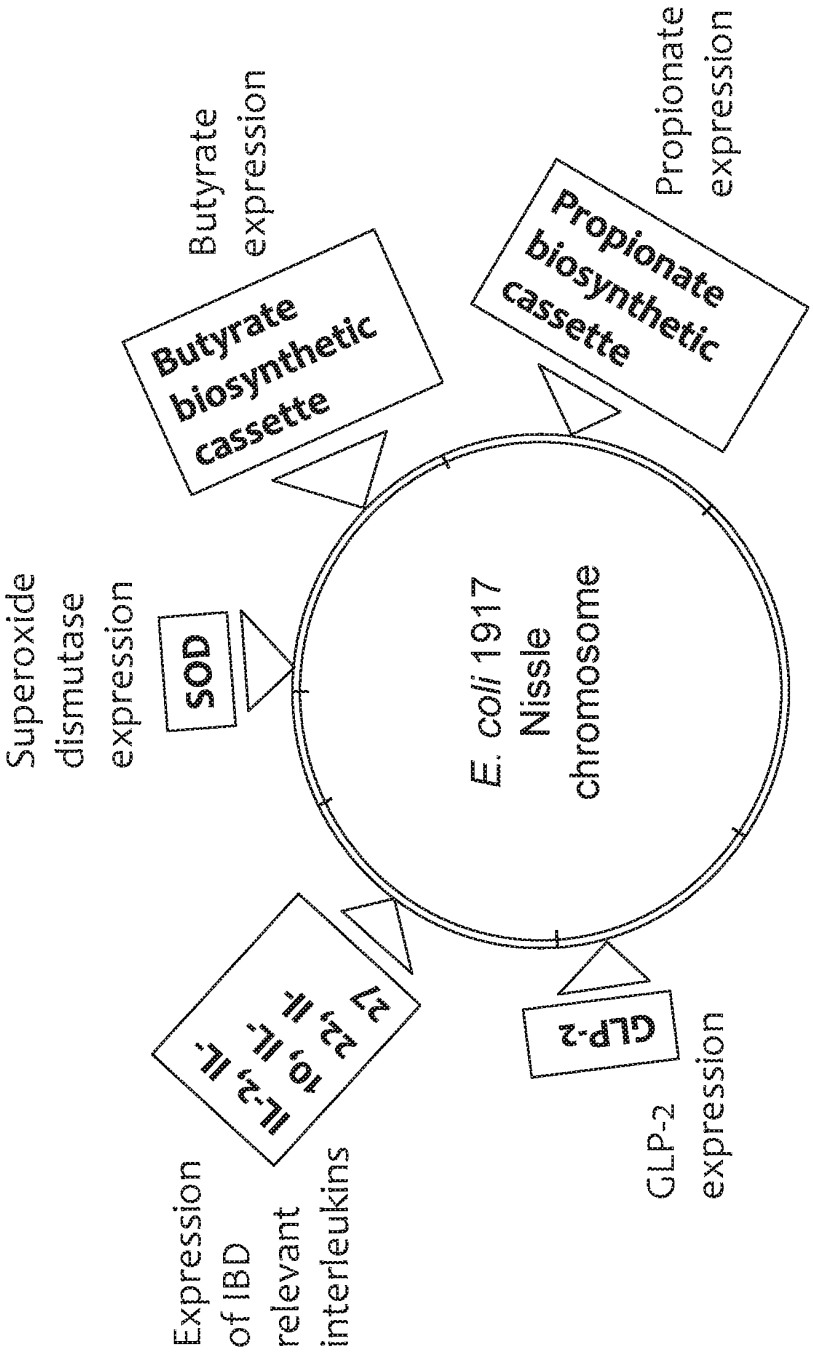

Butyrate expression
Butyrate biosynthetic cassette
Propionate expression
Propionate biosynthetic cassette
Superoxide dismutase expression
SOD
E. coli 1917 Nissle chromosome
IL-2, IL-10, IL-22, IL-27
Expression of IBD relevant interleukins
GLP-2
GLP-2 expression

Fig. 55

Gene	argA	cysE	glnA	glyA	hisB	ilvA	leuB	lysA	metA
AA Auxotroph	Arginine	Cysteine	Glutamine	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine
Pre-Gavage									
24 hours									
48 hours									

Gene	pheA	proA	serA	thrC	trpC	tyrA	ilvD	thyA	uraA	flhD
AA Auxotroph	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine/Isoleucine/Leucine	Thiamine	Uracil	FlhD
Pre-Gavage										
24 hours										
48 hours										

Present
Absent

Fig. 56

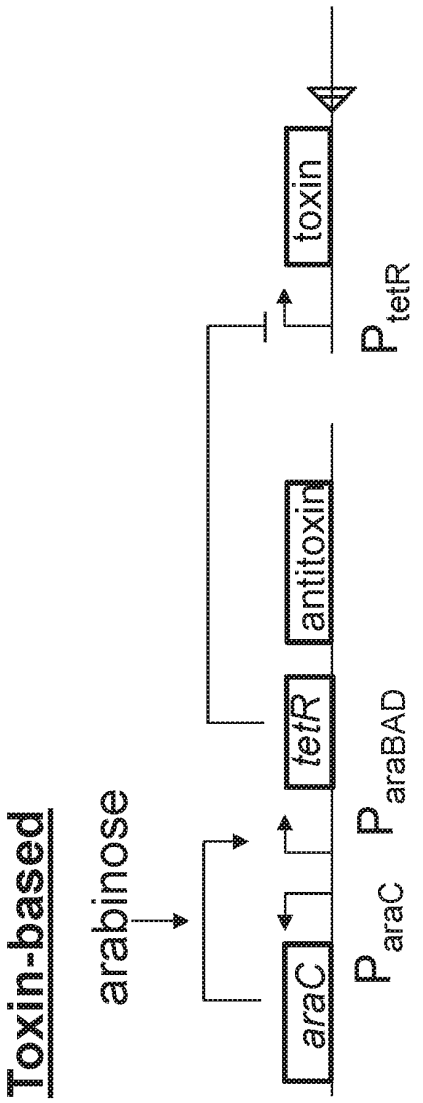

Toxin-based
arabinose
araC
tetR
antitoxin
toxin
P araC
P araBAD
P tetR
Essential gene-based
arabinose
araC
essential
P araC
P araBAD

Fig. 57

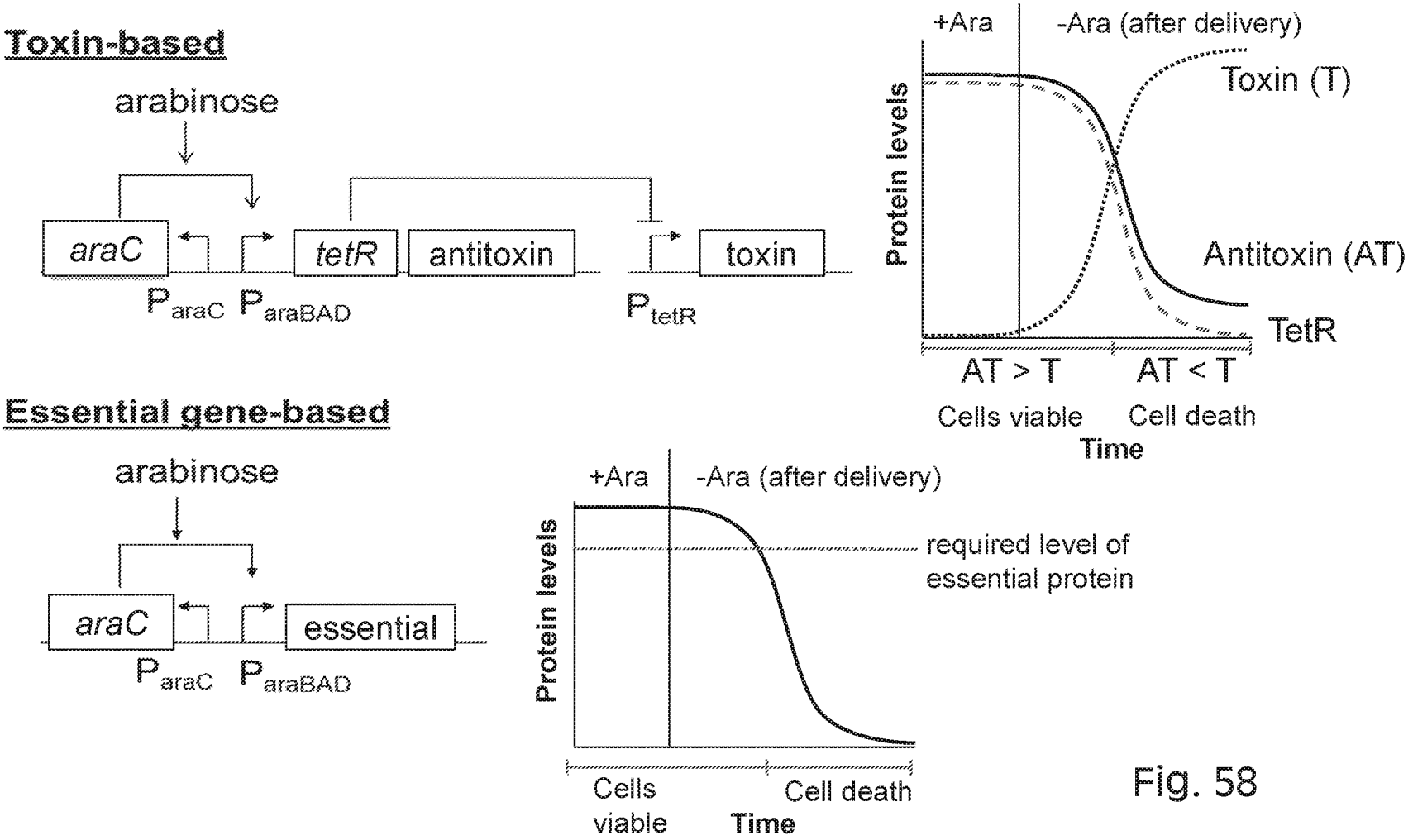

Toxin-based
arabinose
araC
tetR
antitoxin
toxin
P araC
P araBAD
P tetR
+Ara
-Ara (after delivery)
Protein levels
Toxin (T)
Antitoxin (AT)
TetR
AT > T
AT < T
Cells viable
Cell death
Time
Essential gene-based
arabinose
araC
essential
P araC
P araBAD
+Ara
-Ara (after delivery)
Protein levels
required level of
essential protein
Cells
viable
Cell death
Time

Fig. 58

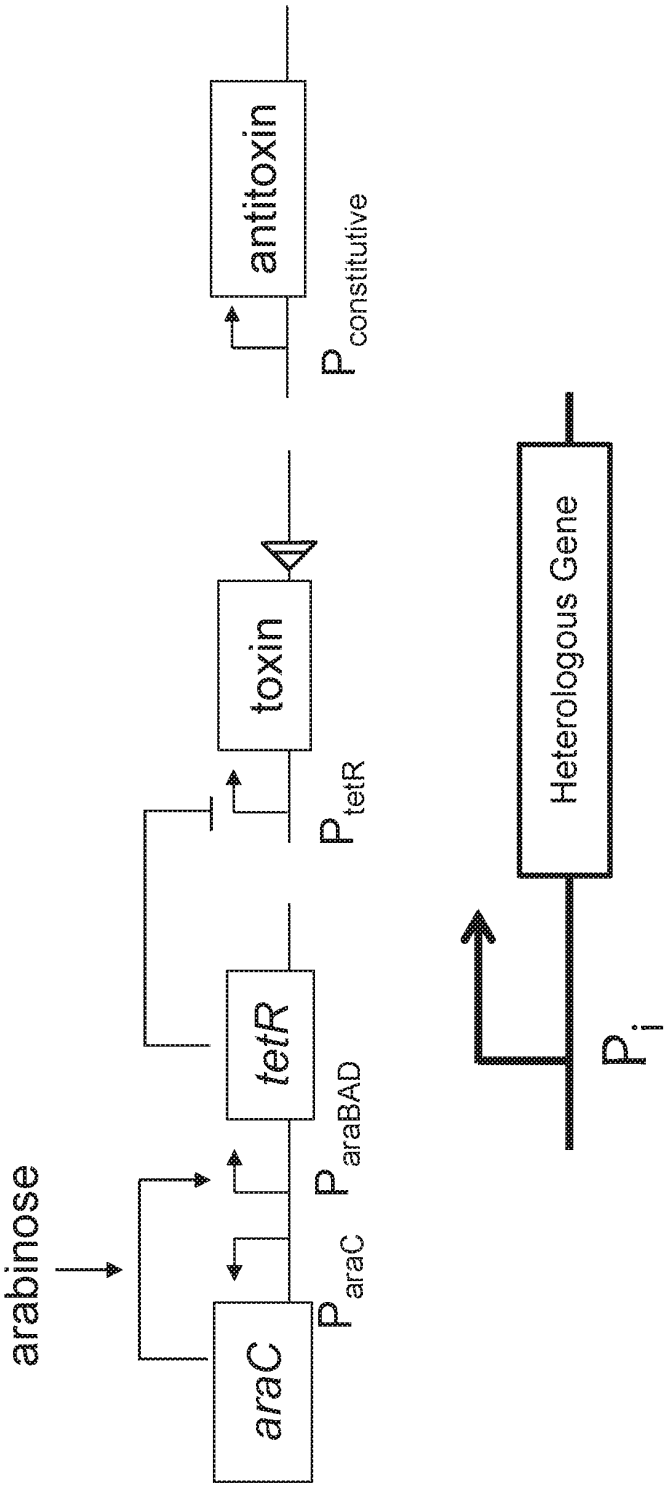

arabinose
araC
P araC
tetR
P araBAD
toxin
P tetR
antitoxin
P constitutive
Heterologous Gene
P i

Fig. 59

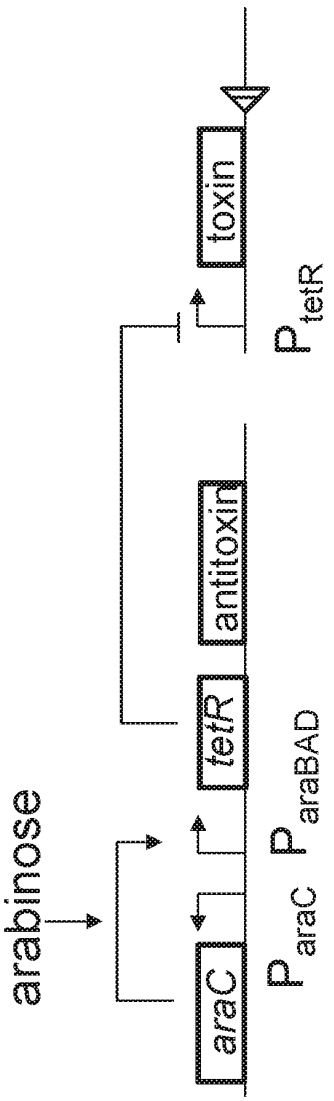


Fig. 60

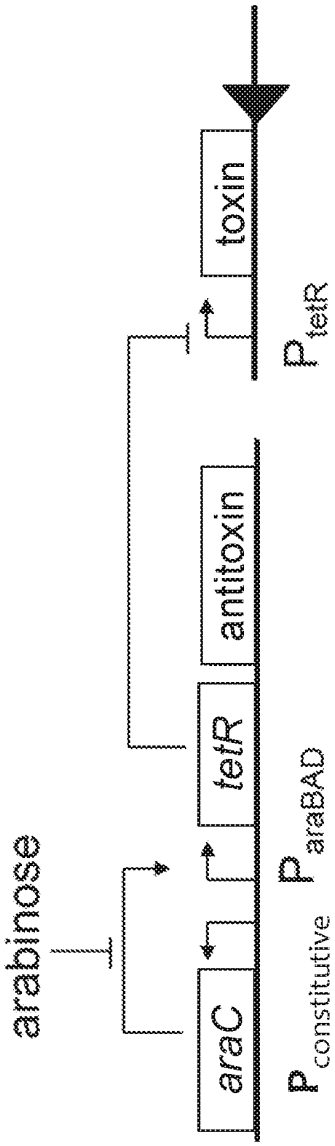

arabinose
araC
tetR
antitoxin
toxin
P constitutive
P araBAD
P tetR

Fig. 61

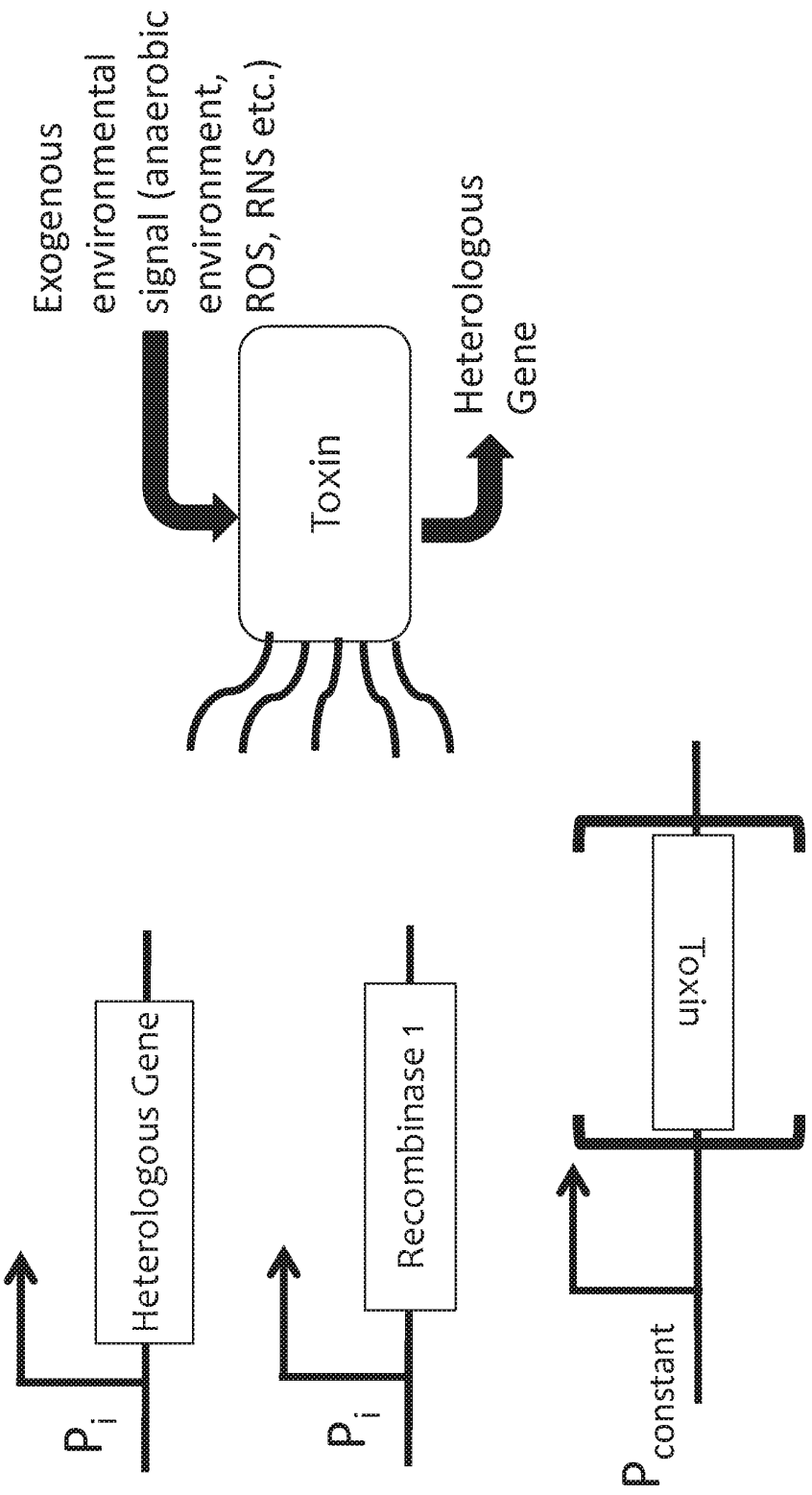

Exogenous environmental signal (anaerobic environment, ROS, RNS etc.)
Toxin
Heterologous Gene
P_i
Heterologous Gene
P_i
Recombinase 1
$P_{constant}$
Toxin

Fig. 62

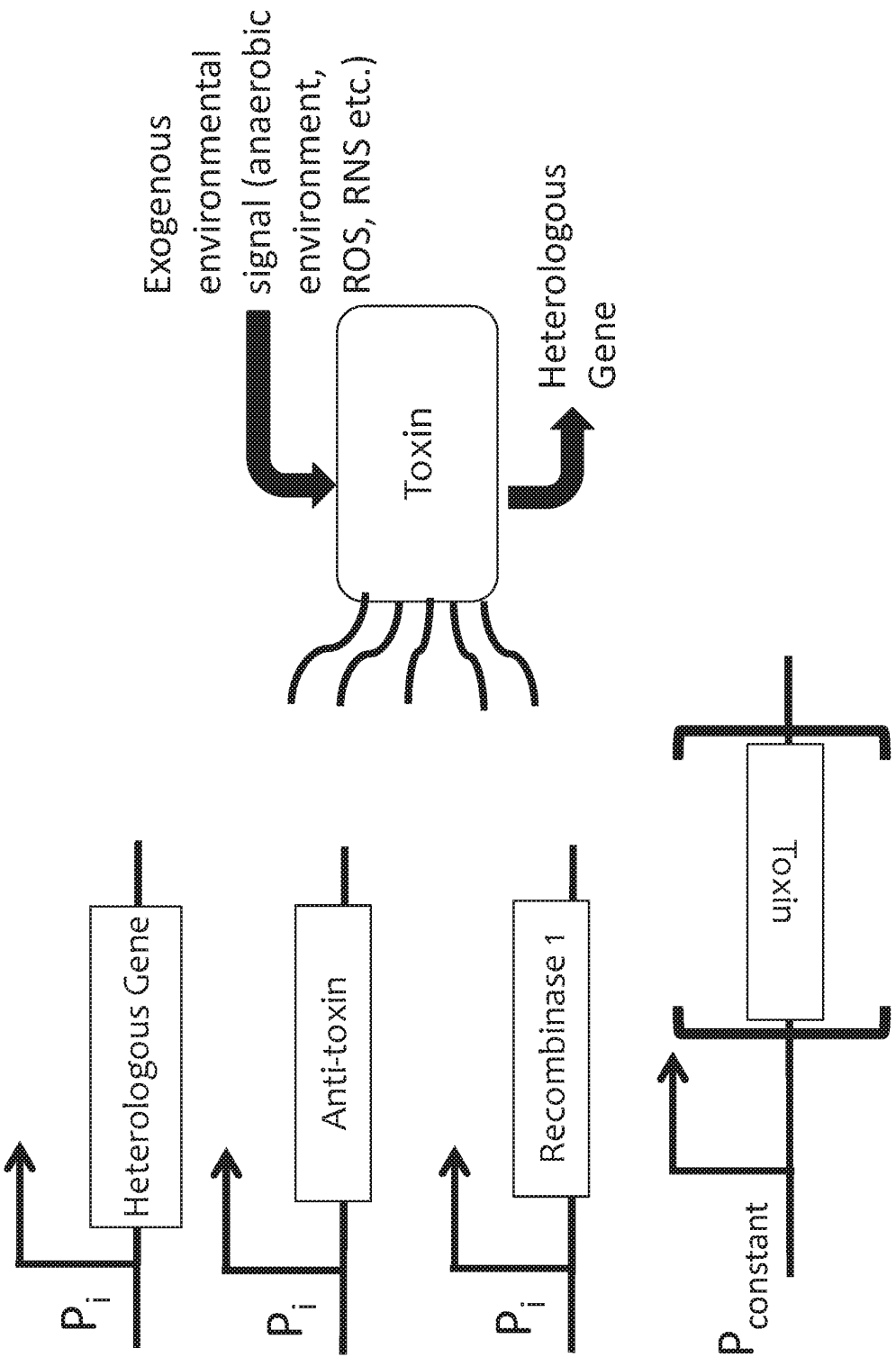
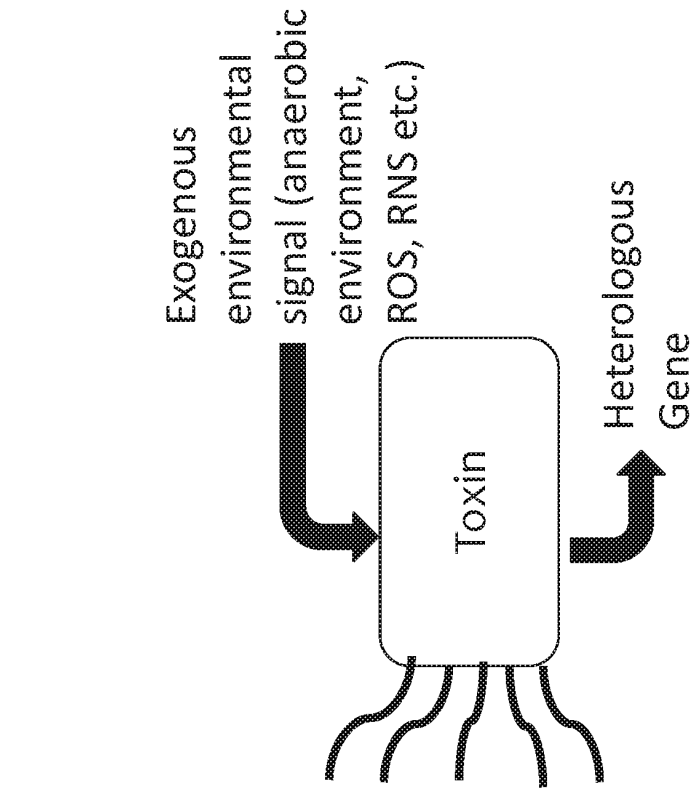

Exogenous environmental signal (anaerobic environment, ROS, RNS etc.)
Toxin
Heterologous Gene
P_i
Heterologous Gene
Anti-toxin
P_i
Anti-toxin
Recombinase 1
P_i
Recombinase 1
Toxin
$P_{constant}$
Toxin

Fig. 63


Exogenous environmental signal (anaerobic environment, ROS, RNS etc.)
Toxin
Heterologous Gene

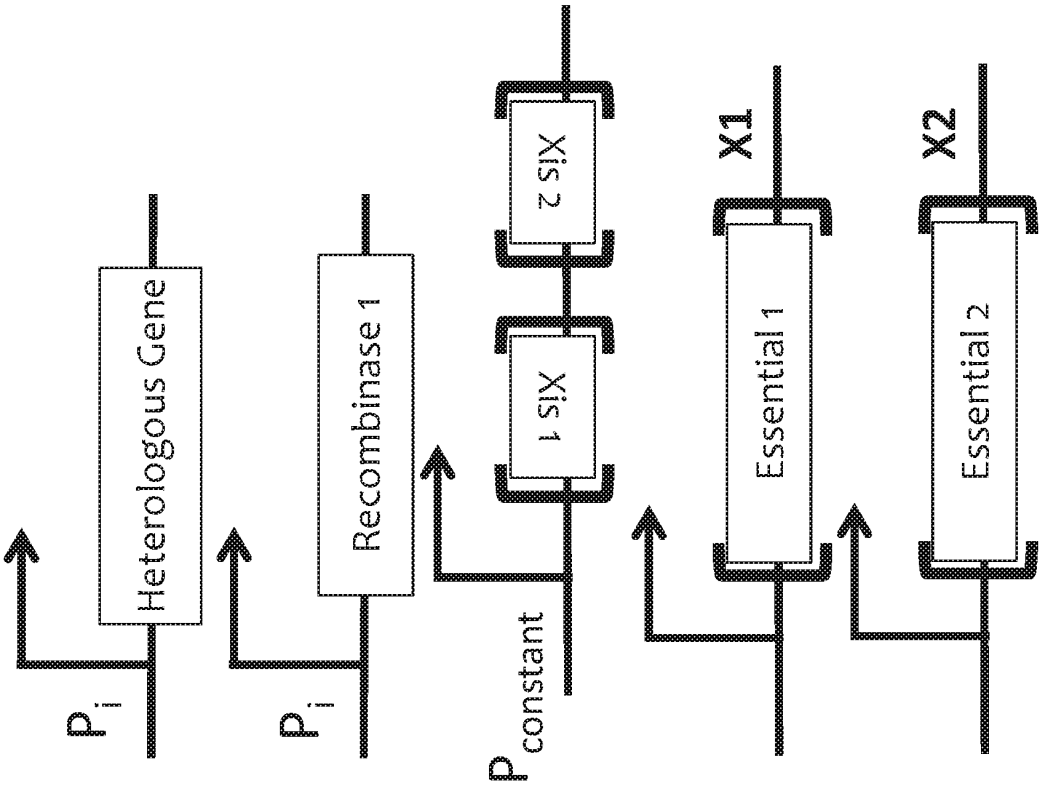

Heterologous Gene
Recombinase 1
Xis 1
Xis 2
Essential 1
Essential 2
X1
X2
P_i
P_i
P_constant

Fig. 64

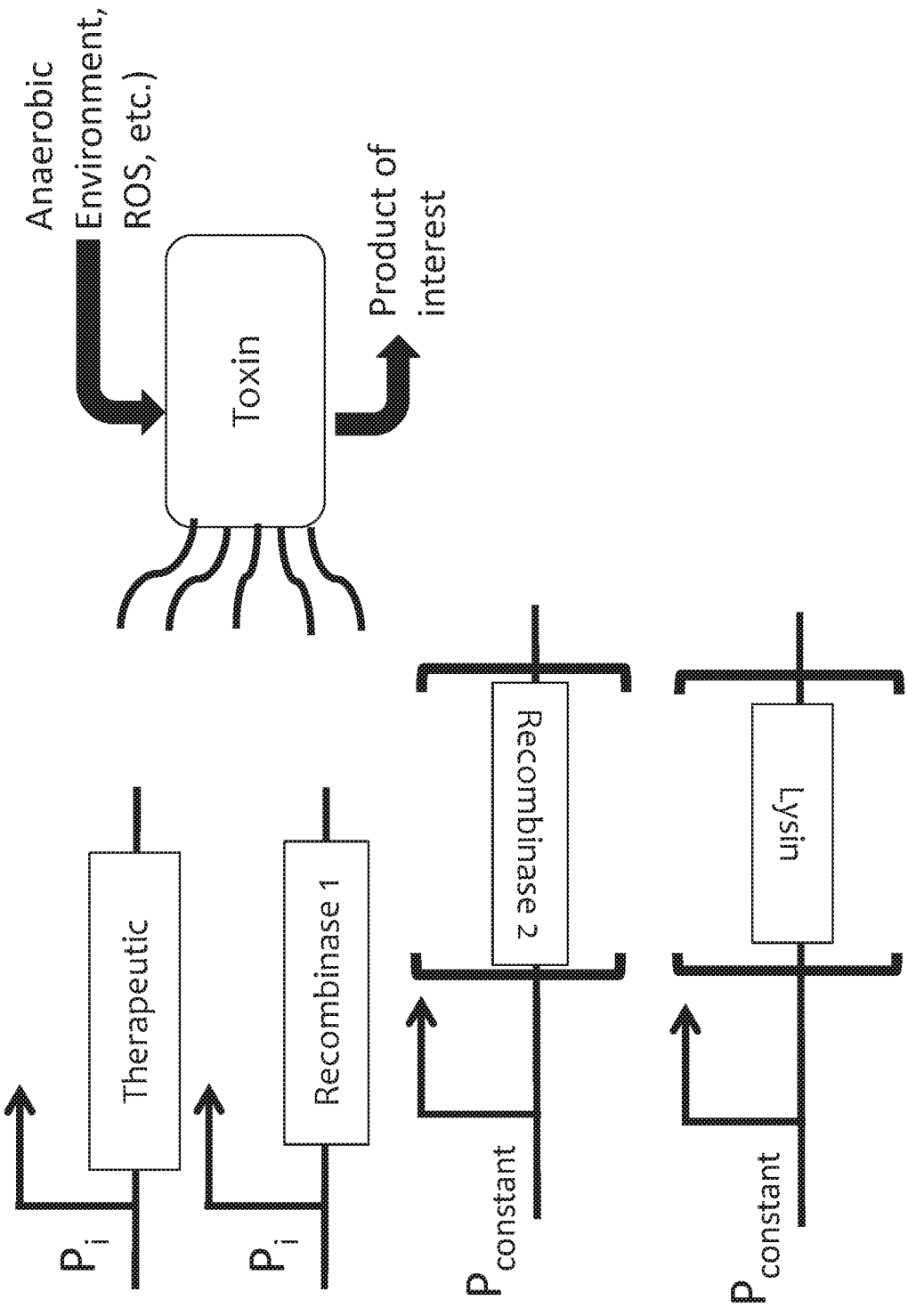

Anaerobic Environment, ROS, etc.)
Toxin
Product of interest
Therapeutic
Recombinase 1
Recombinase 2
Lysin
P_i
P_i
$P_{constant}$
$P_{constant}$

Fig. 65

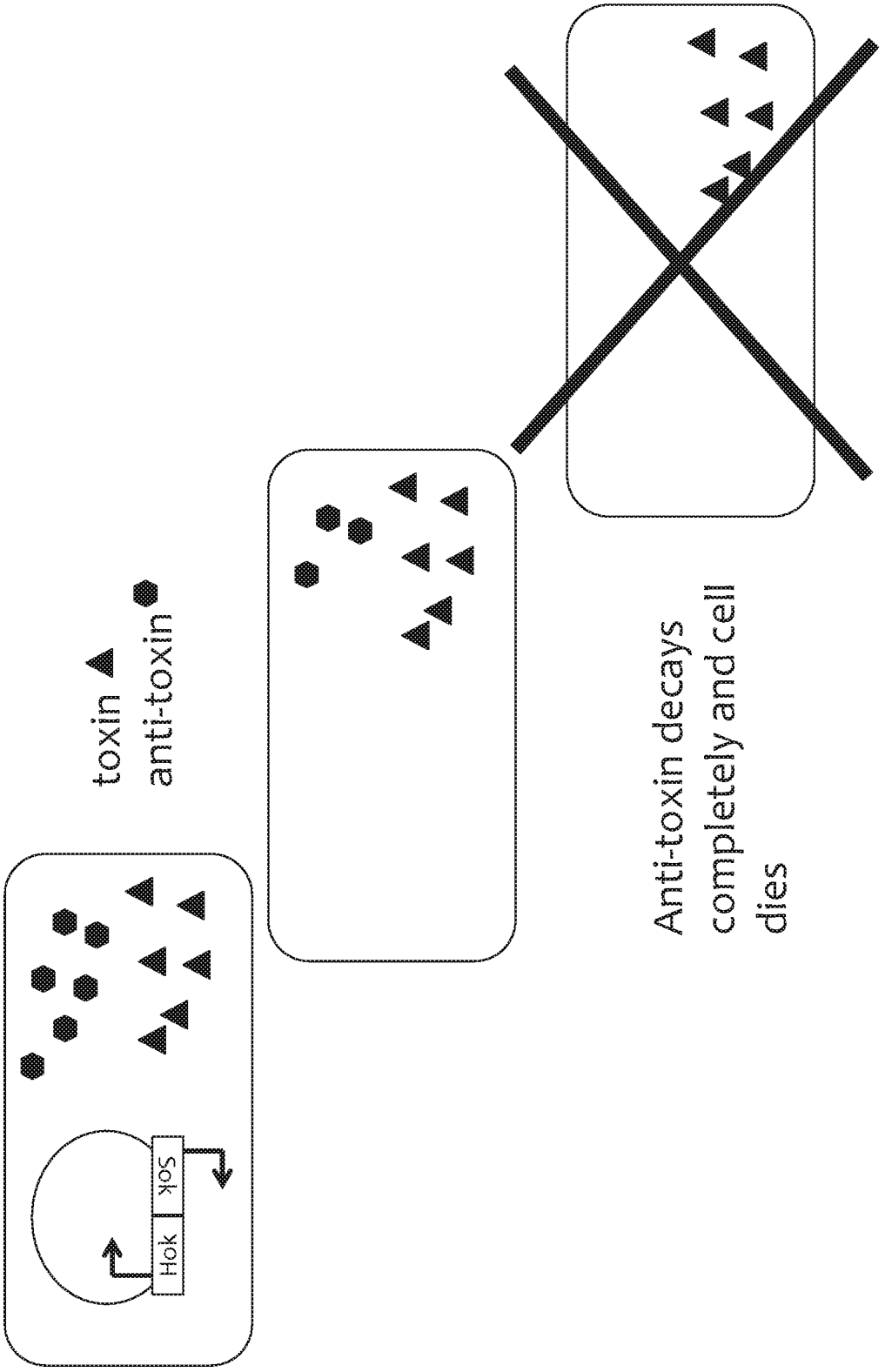

toxin
anti-toxin
Hok
Sok
Anti-toxin decays completely and cell dies

Fig. 66

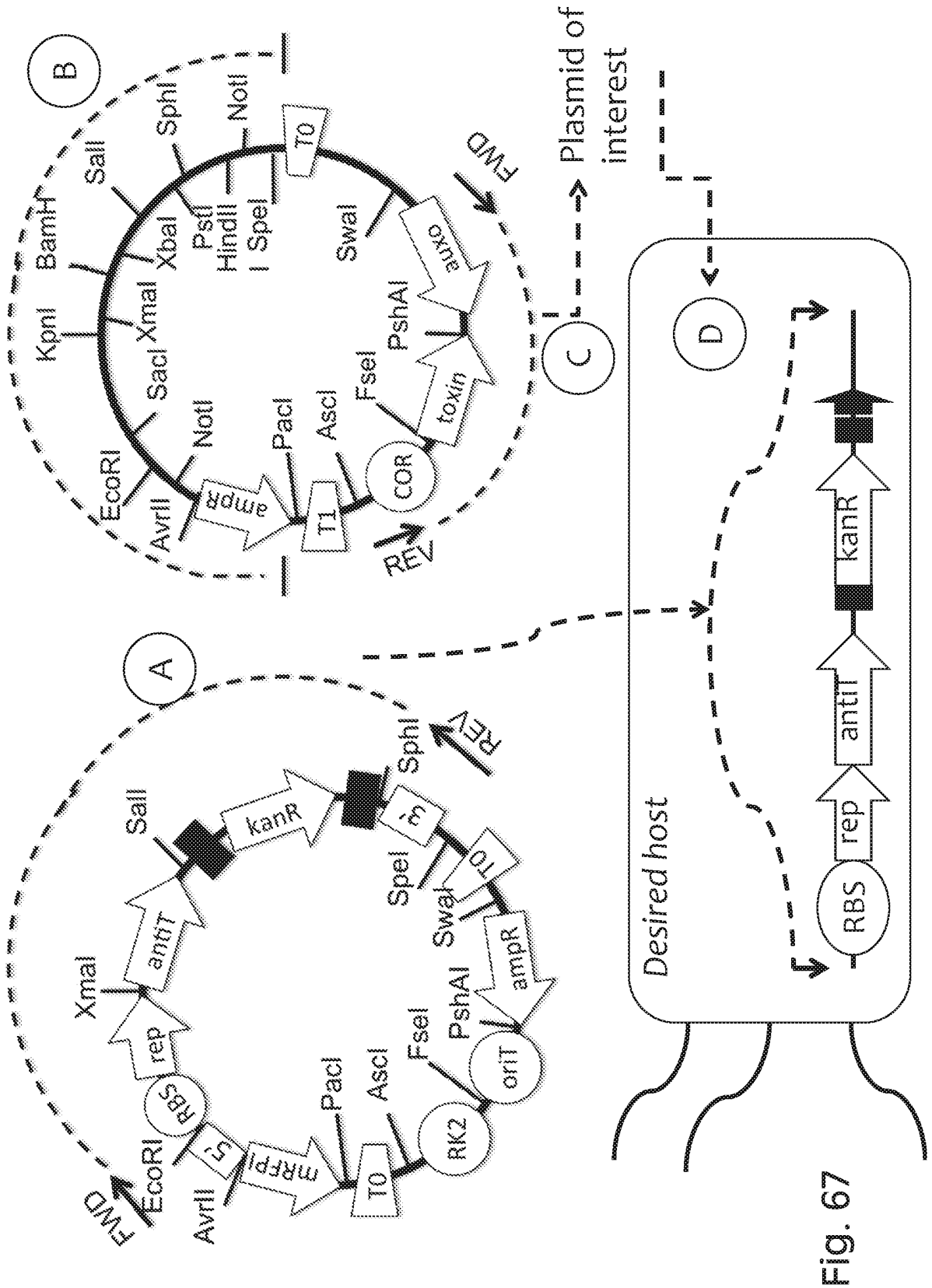

A
B
C
D
Plasmid of interest
Desired host
FWD
REV
EcoRI
AvrII
RBS
rep
antiT
XmaI
SalI
kanR
SphI
REV
λ
T0
SpeI
SwaI
ampR
oriT
PshAI
FseI
AscI
PacI
T0
RK2
mRFPI
S
RBS
rep
antiT
kanR
T0
T1
COR
toxin
PshAI
FseI
AscI
PacI
NotI
ampR
AvrII
EcoRI
SacI
KpnI
XmaI
XbaI
BamH
SalI
PstI
HindIII
SpeI
NotI
SphI
T0
SwaI
axe
FWD
REV

Fig. 67

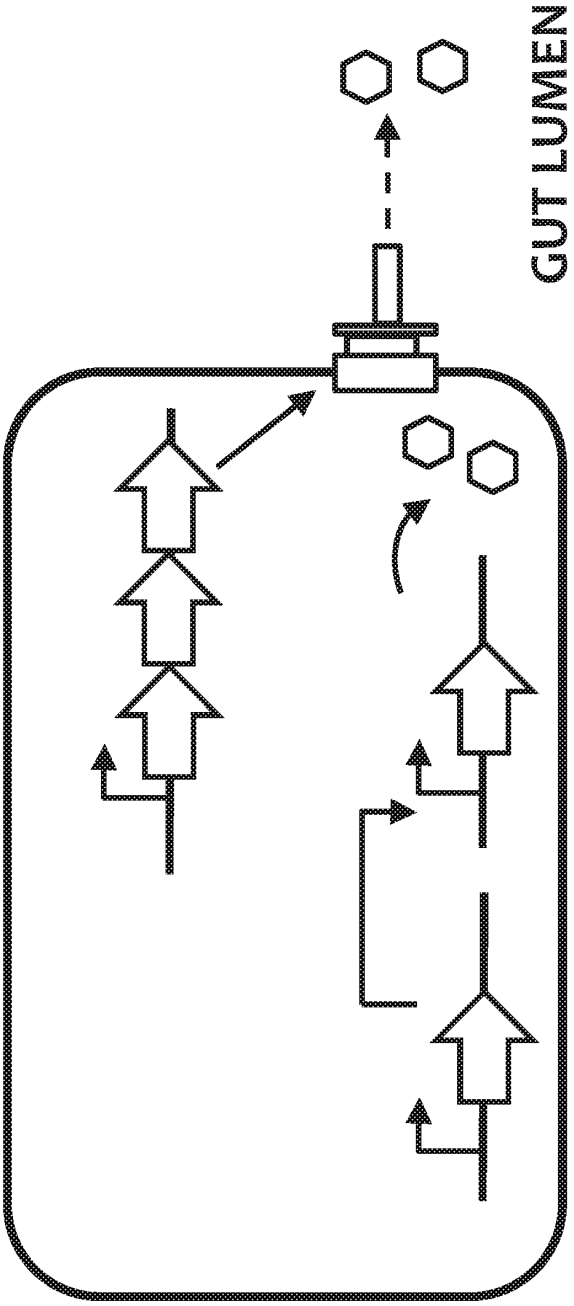


Fig. 68

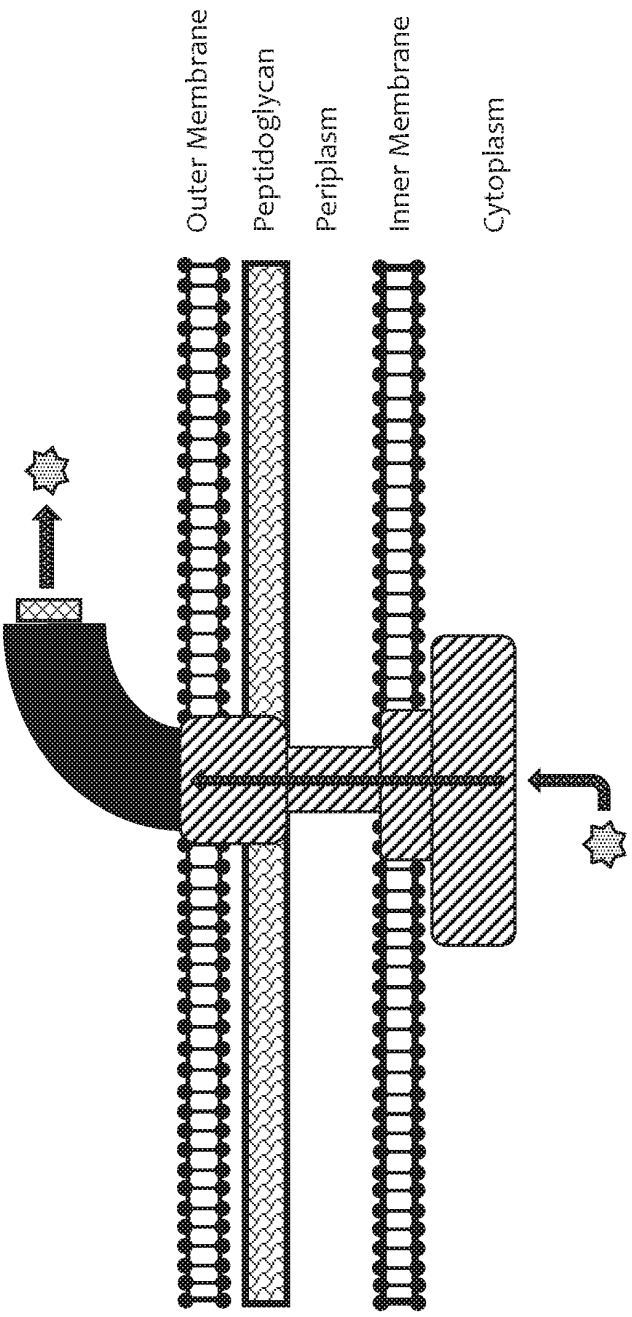

Outer Membrane
Peptidoglycan
Periplasm
Inner Membrane
Cytoplasm

Fig. 69

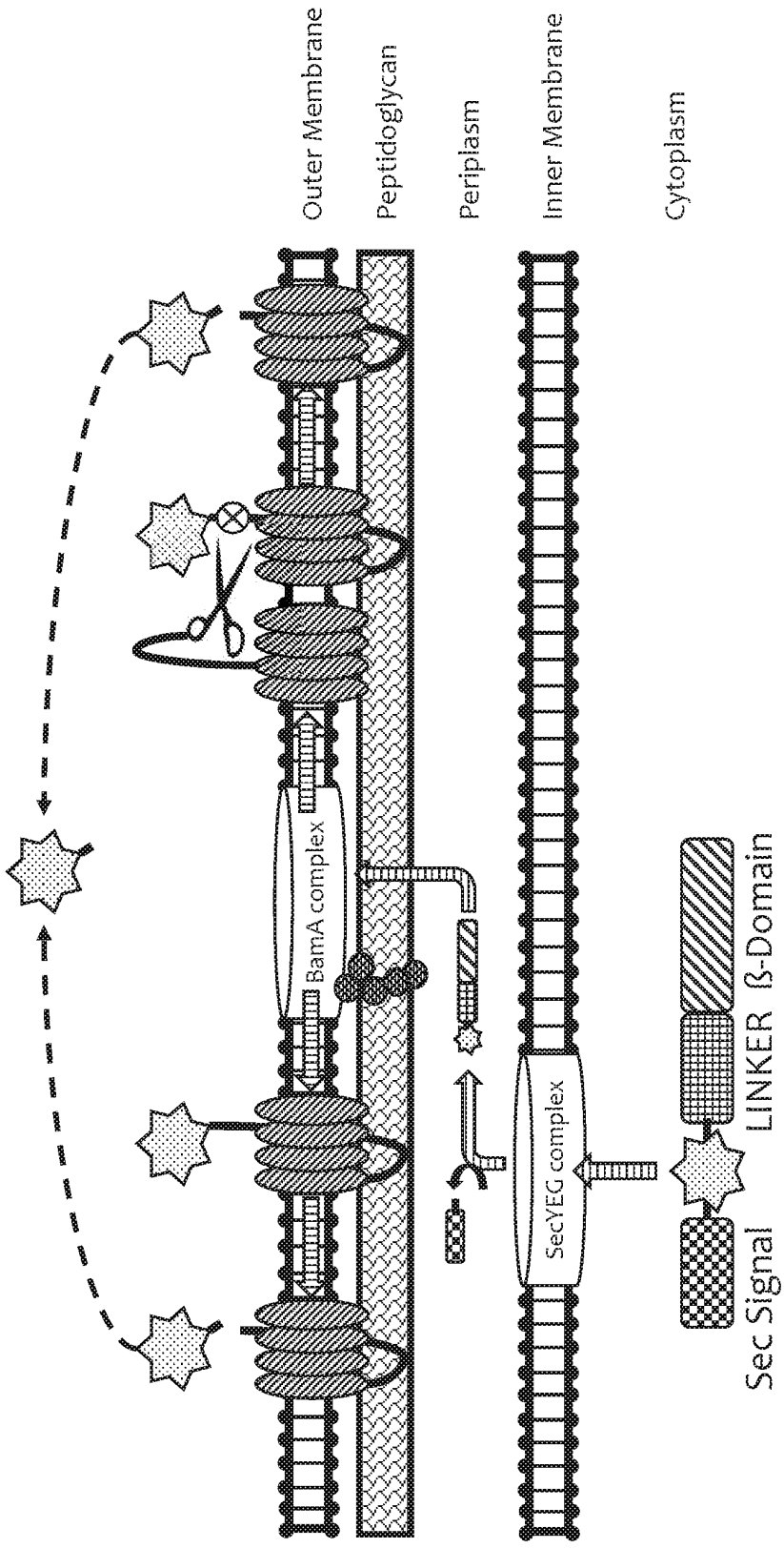

Outer Membrane
Peptidoglycan
Periplasm
Inner Membrane
Cytoplasm
BamA complex
SecYEG complex
Sec Signal
LINKER
β-Domain

Fig. 70

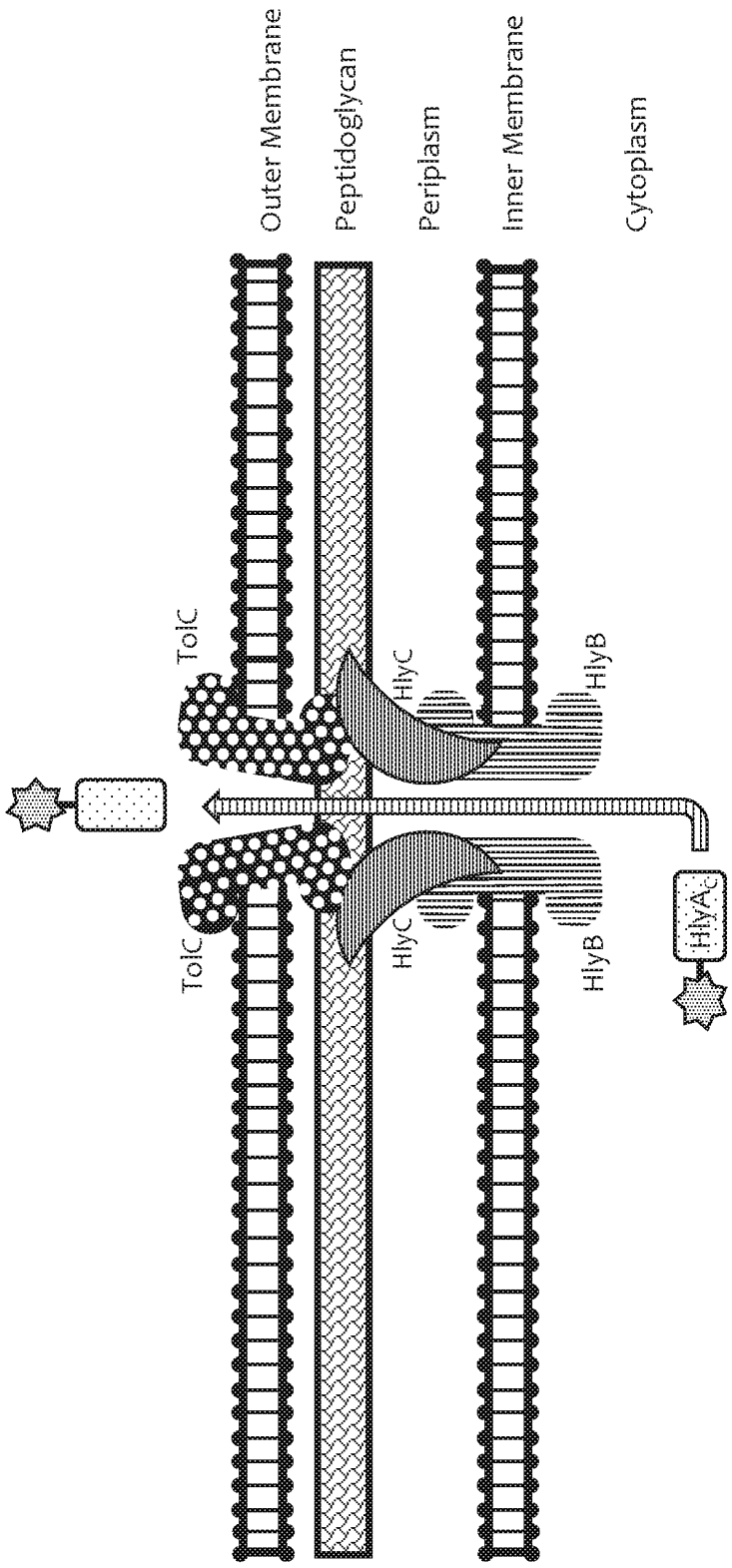

Outer Membrane
Peptidoglycan
Periplasm
Inner Membrane
Cytoplasm
TolC
HlyC
HlyB
TolC
HlyC
HlyB
HlyA

Fig. 71

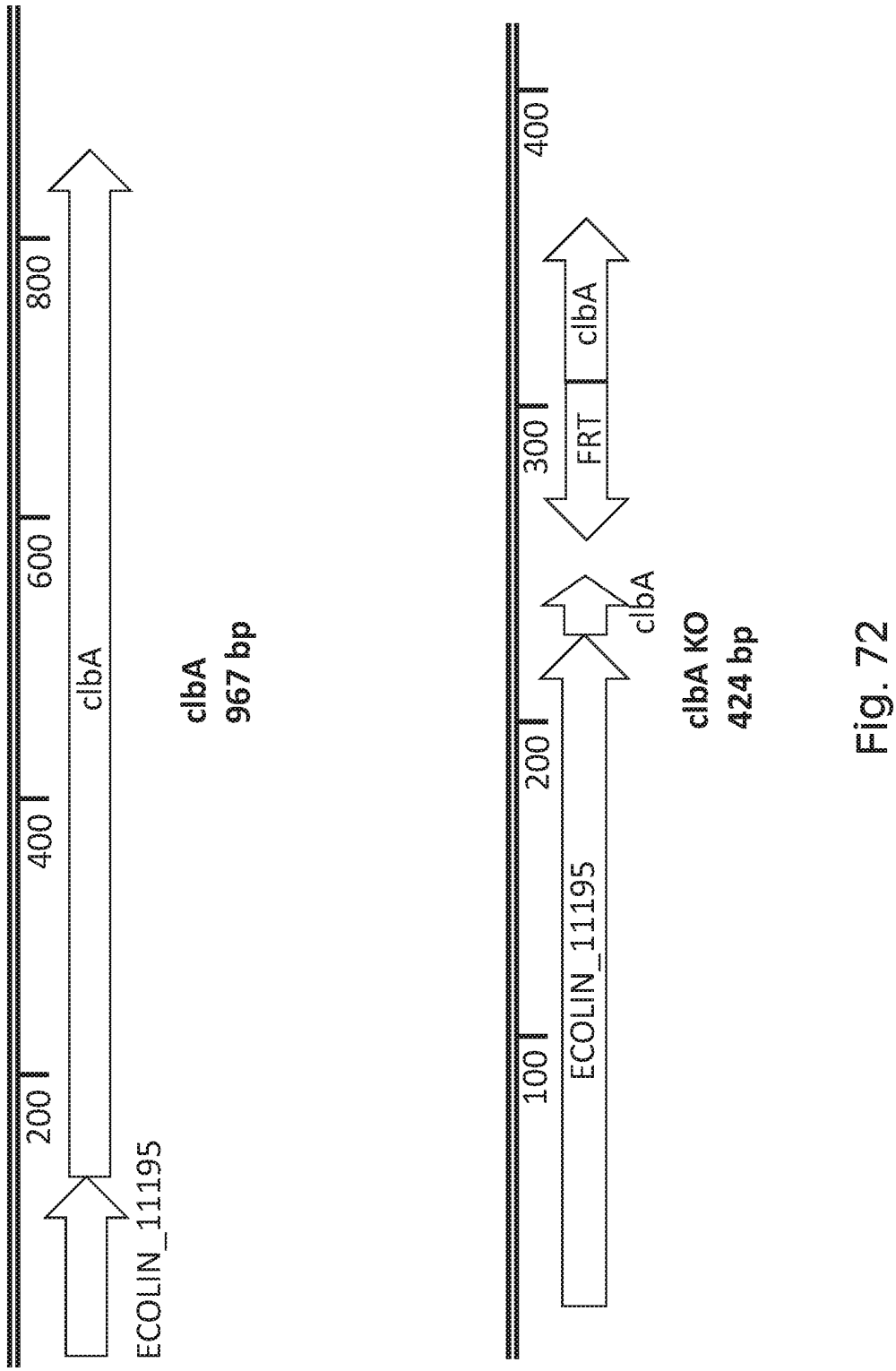

200
400
600
800
clbA
ECOLIN_11195
clbA
967 bp
100
200
300
400
ECOLIN_11195
clbA
FRT
clbA
clbA KO
424 bp

Fig. 72

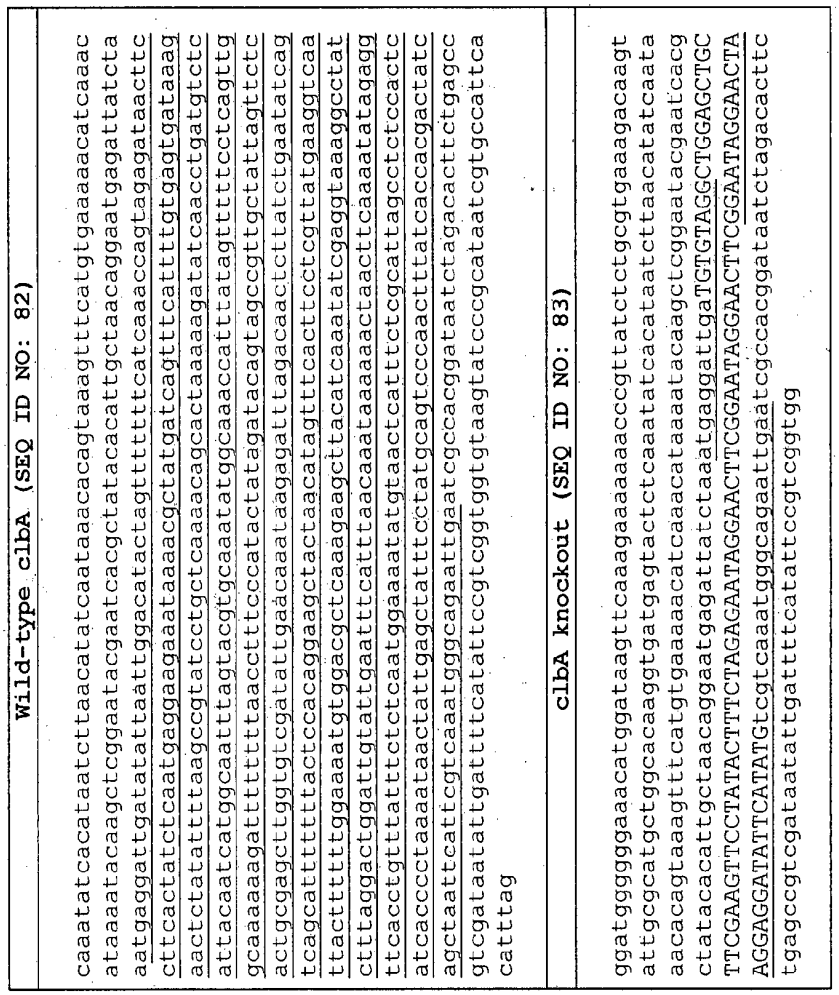


Fig. 73

RECTIFIED SHEET (RULE 91) ISA/EP

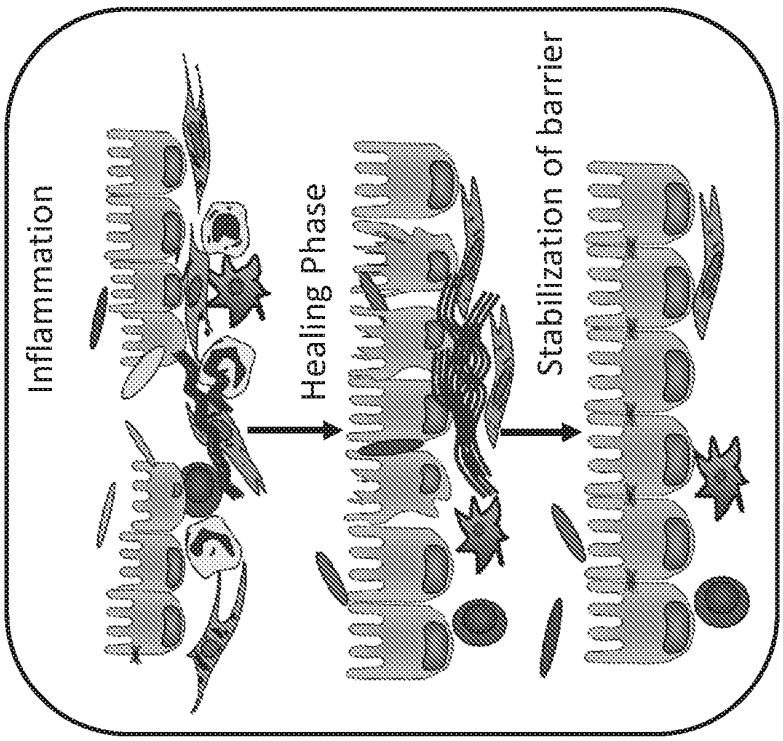


Fig. 74

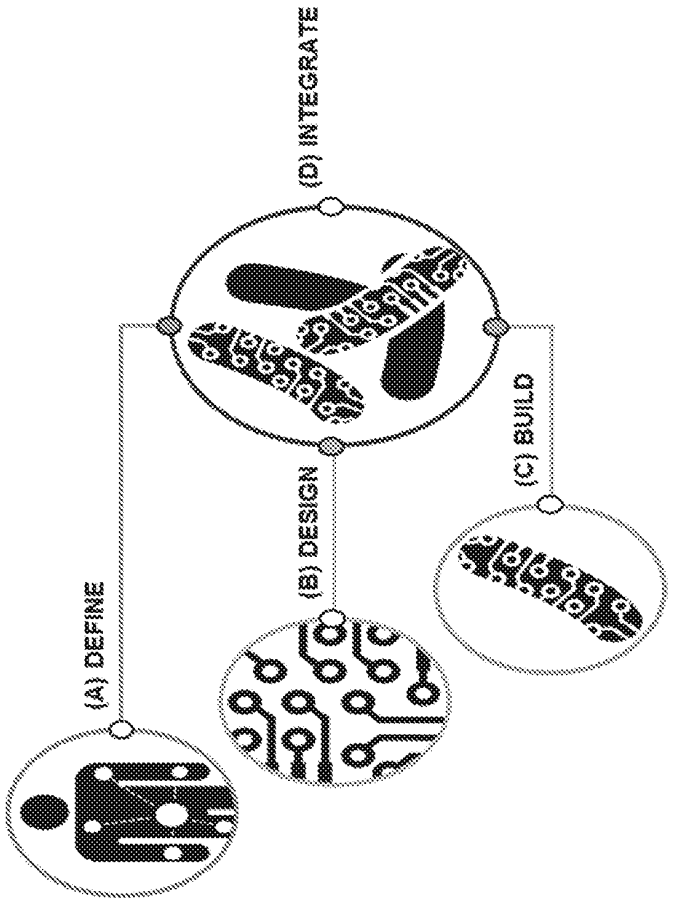


Fig. 75

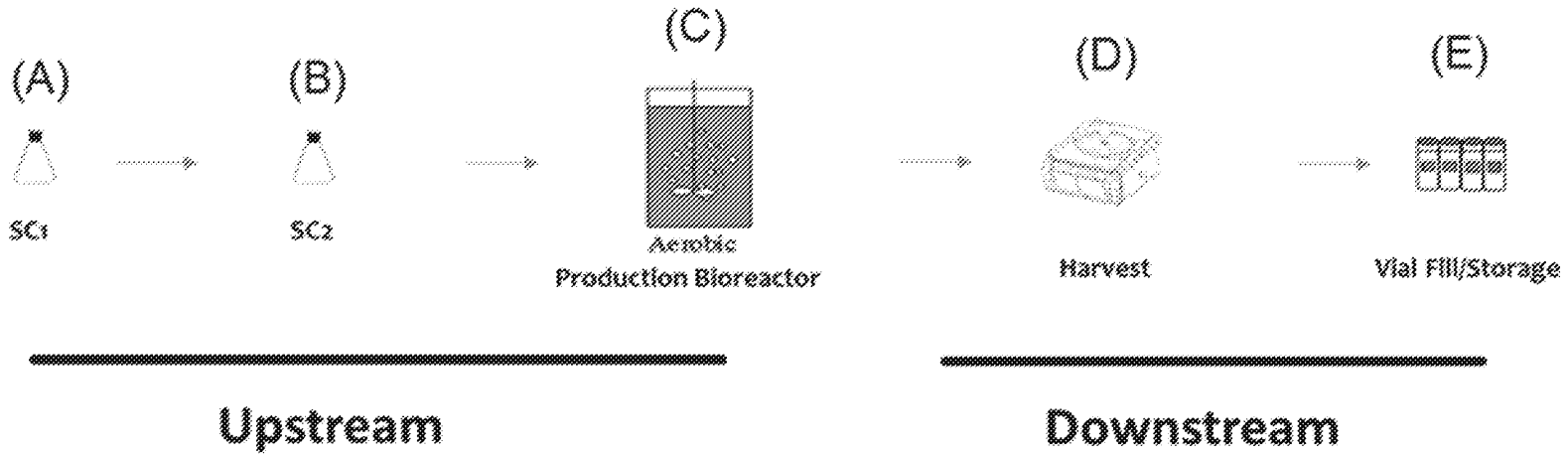

(A)
(B)
(C)
(D)
(E)
SC1
SC2
Aerobic
Production Bioreactor
Harvest
Vial Fill/Storage
Upstream
Downstream

Fig. 76