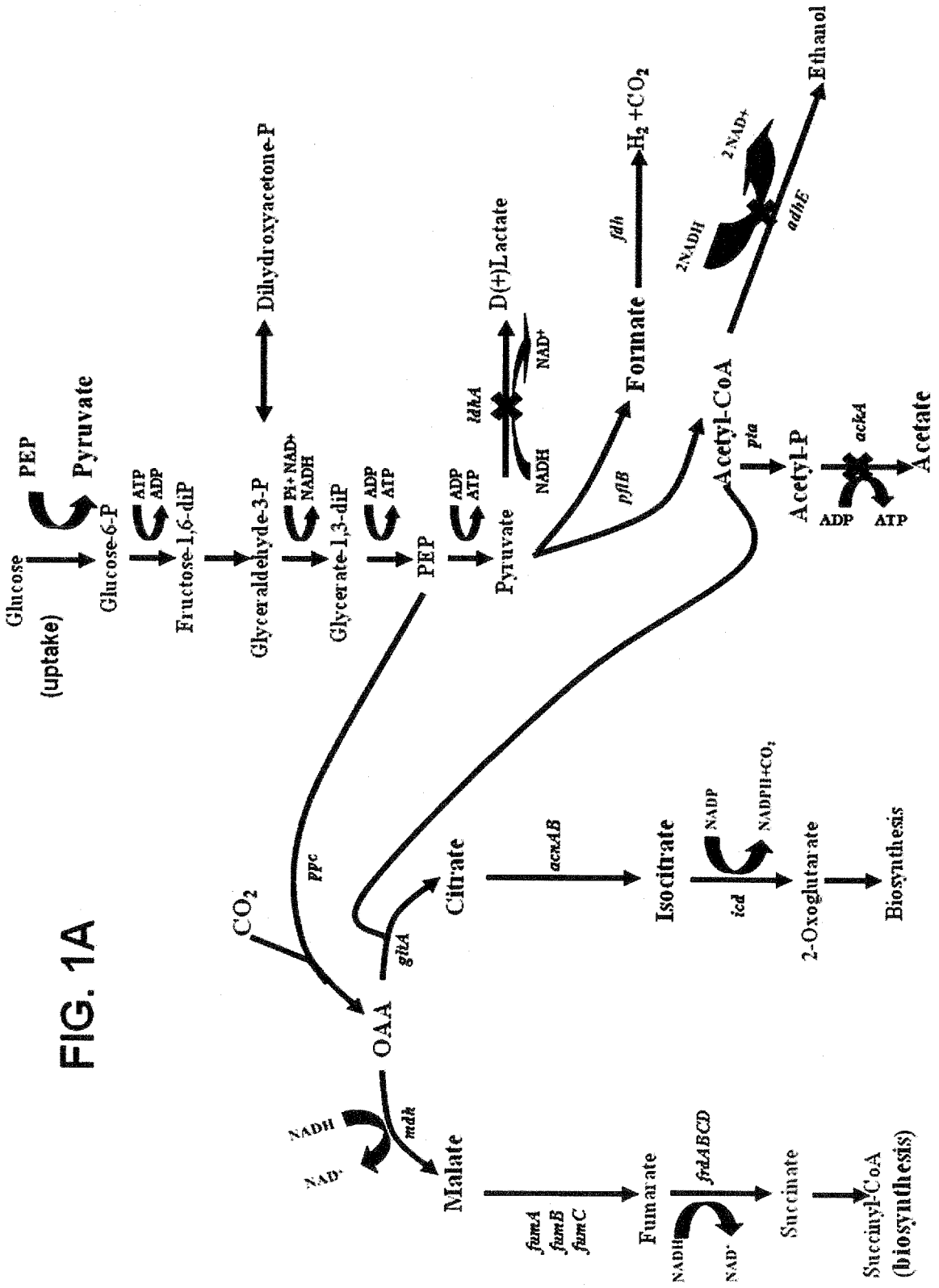
FIG. 1A


Glucose
(uptake)
PEP
Pyruvate
Glucose-6-P
ATP
ADP
Fructose-1,6-diP
Glyceraldehyde-3-P
Dihydroxyacetone-P
Pi+ NAD+
NADH
Glycerate-1,3-diP
ADP
ATP
PEP
ADP
ATP
Pyruvate
ldhA
NADH
NAD+
D(+)Lactate
pflB
Formate
fdh
$H_2 + CO_2$
^2NADH
$^2NAD+$
adhE
Ethanol
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ackA
Acetate
CO_2
ppc
OAA
gltA
Citrate
acnAB
Isocitrate
NADP
icd
NADPH+CO_2
2-Oxoglutarate
Biosynthesis
NADH
NAD+
mdh
Malate
fumA
fumB
fumC
Fumarate
NADH
NAD+
frdABCD
Succinate
Succinyl-CoA
(biosynthesis)

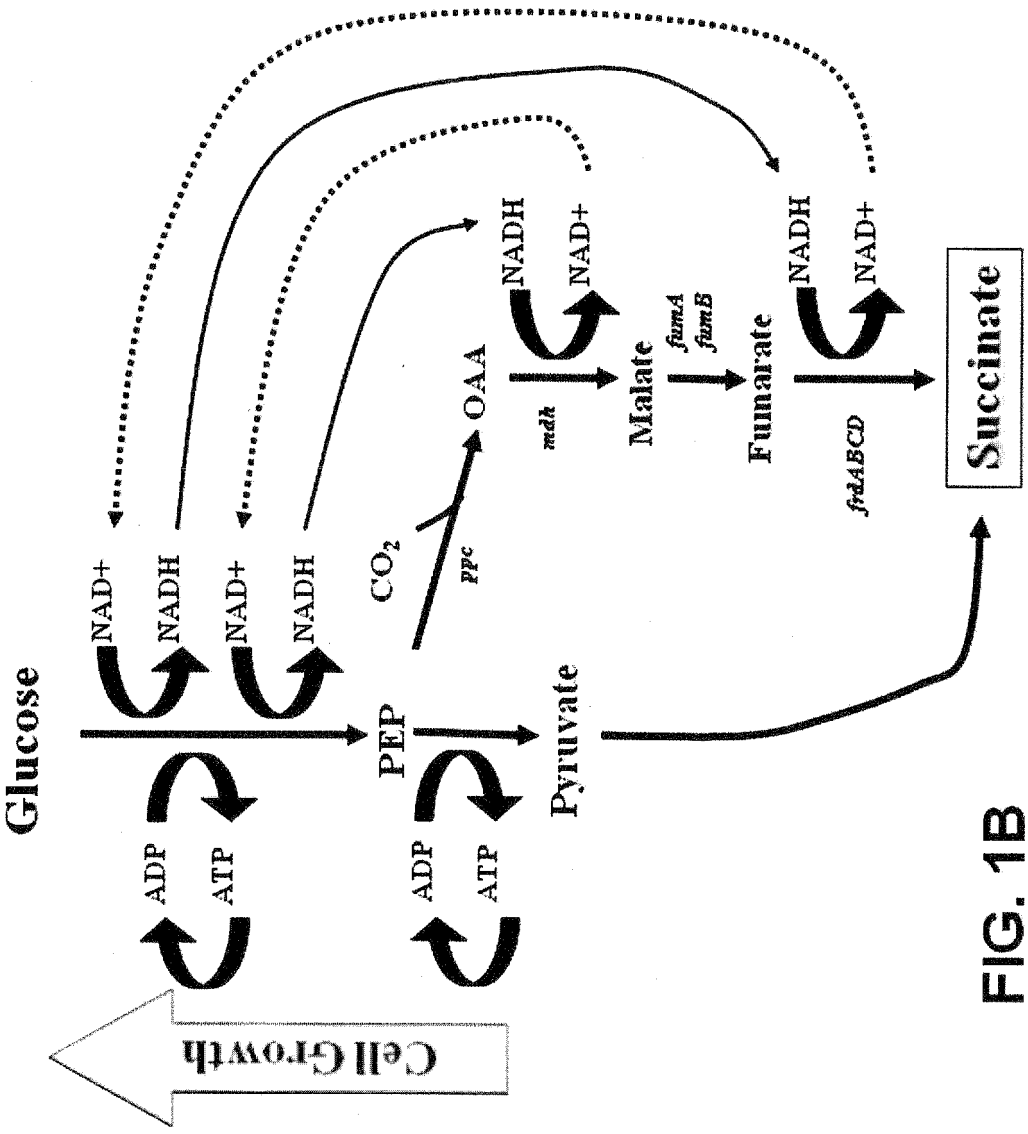

Glucose
NAD+
NADH
NAD+
NADH
ADP
ATP
PEP
ADP
ATP
Pyruvate
CO_2
ppc
OAA
NADH
NAD+
mdh
Malate
fumA
fumB
Fumarate
NADH
NAD+
frdABCD
Succinate
Cell Growth

FIG. 1B

FIG. 2A

FIG. 2B

FIG. 2C

FIG. 2D

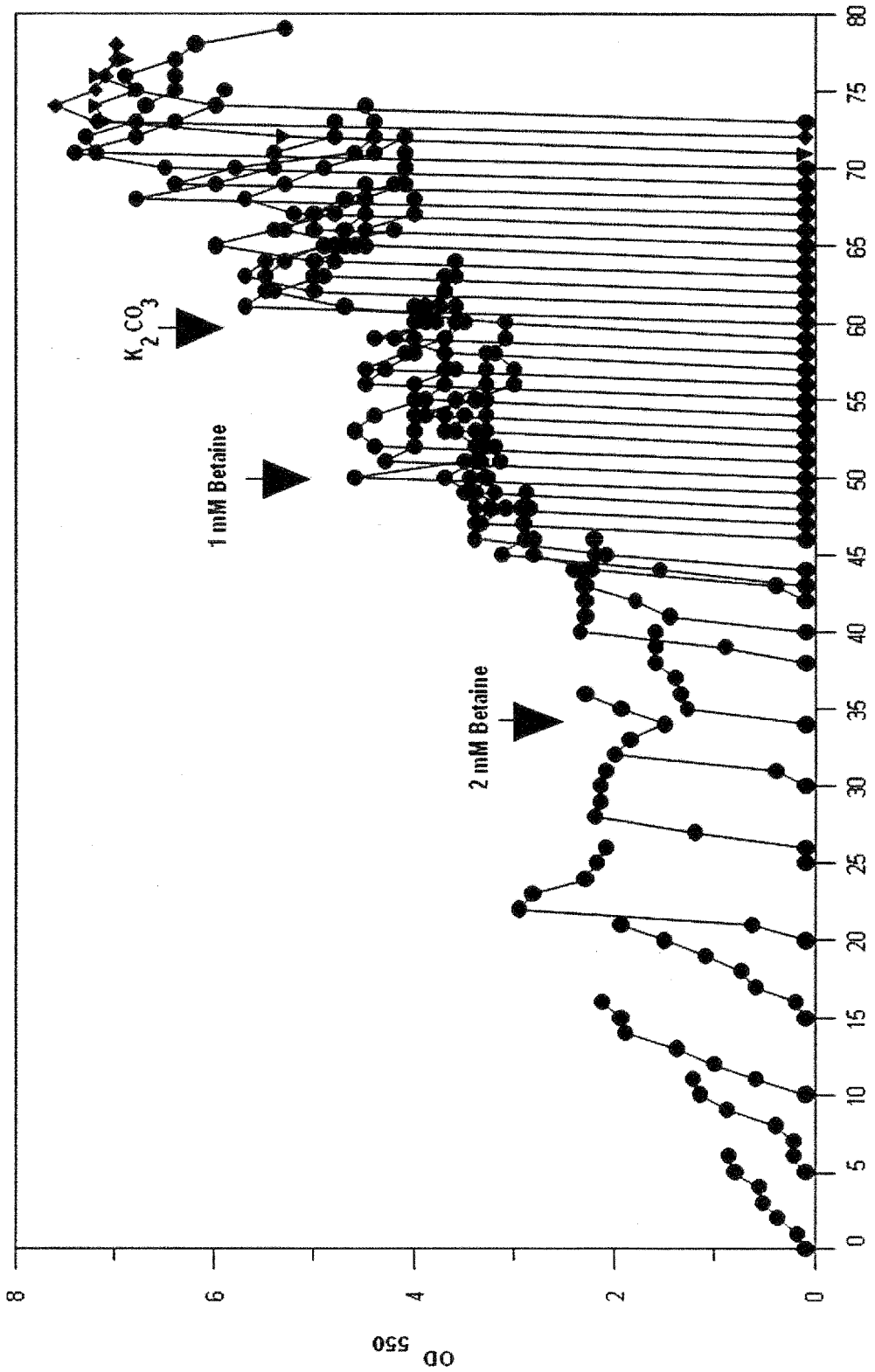

OD 550
0
2
4
6
8
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Time (days)
K_2CO_3
1 mM Betaine
2 mM Betaine

FIG. 3A

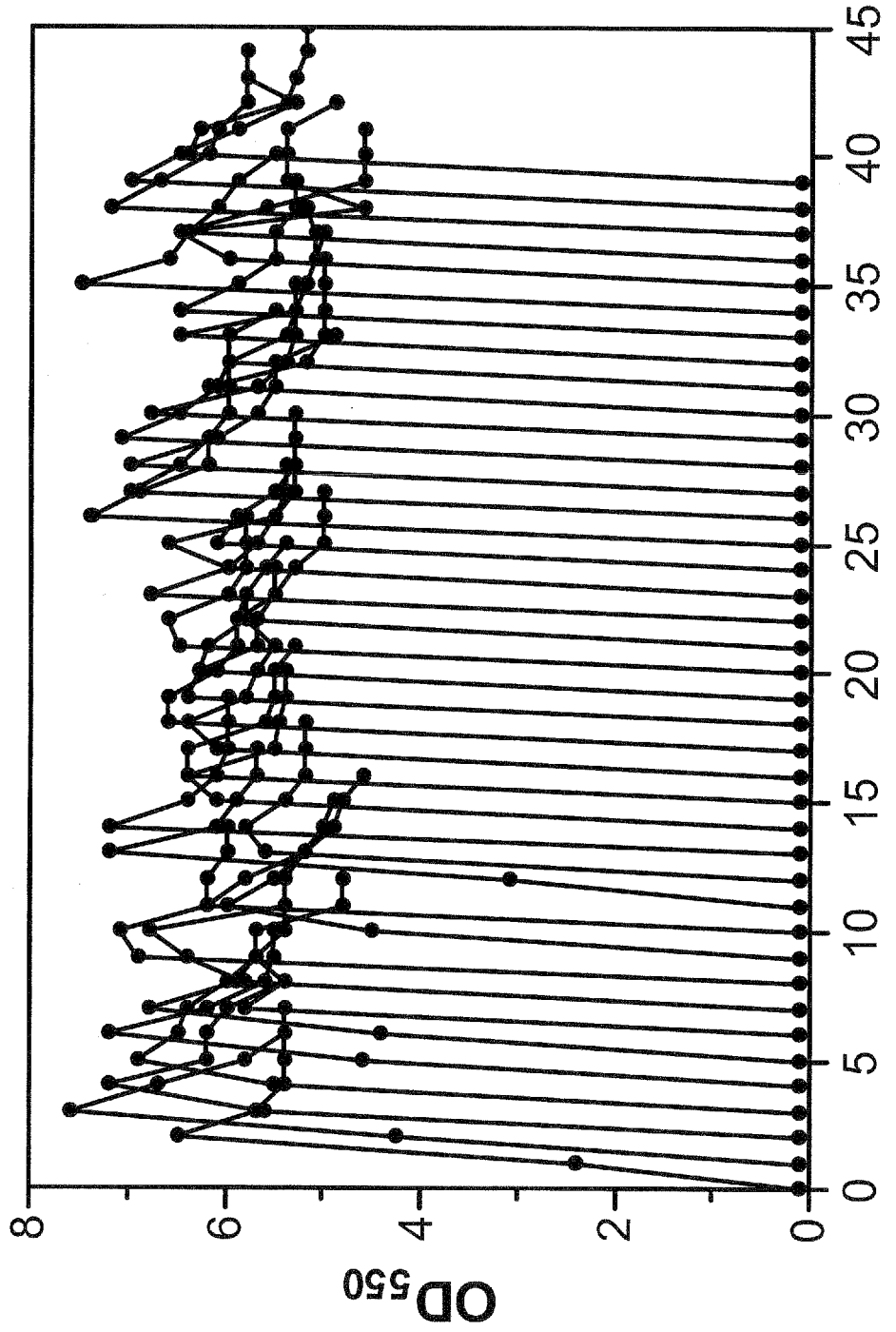


FIG. 3B

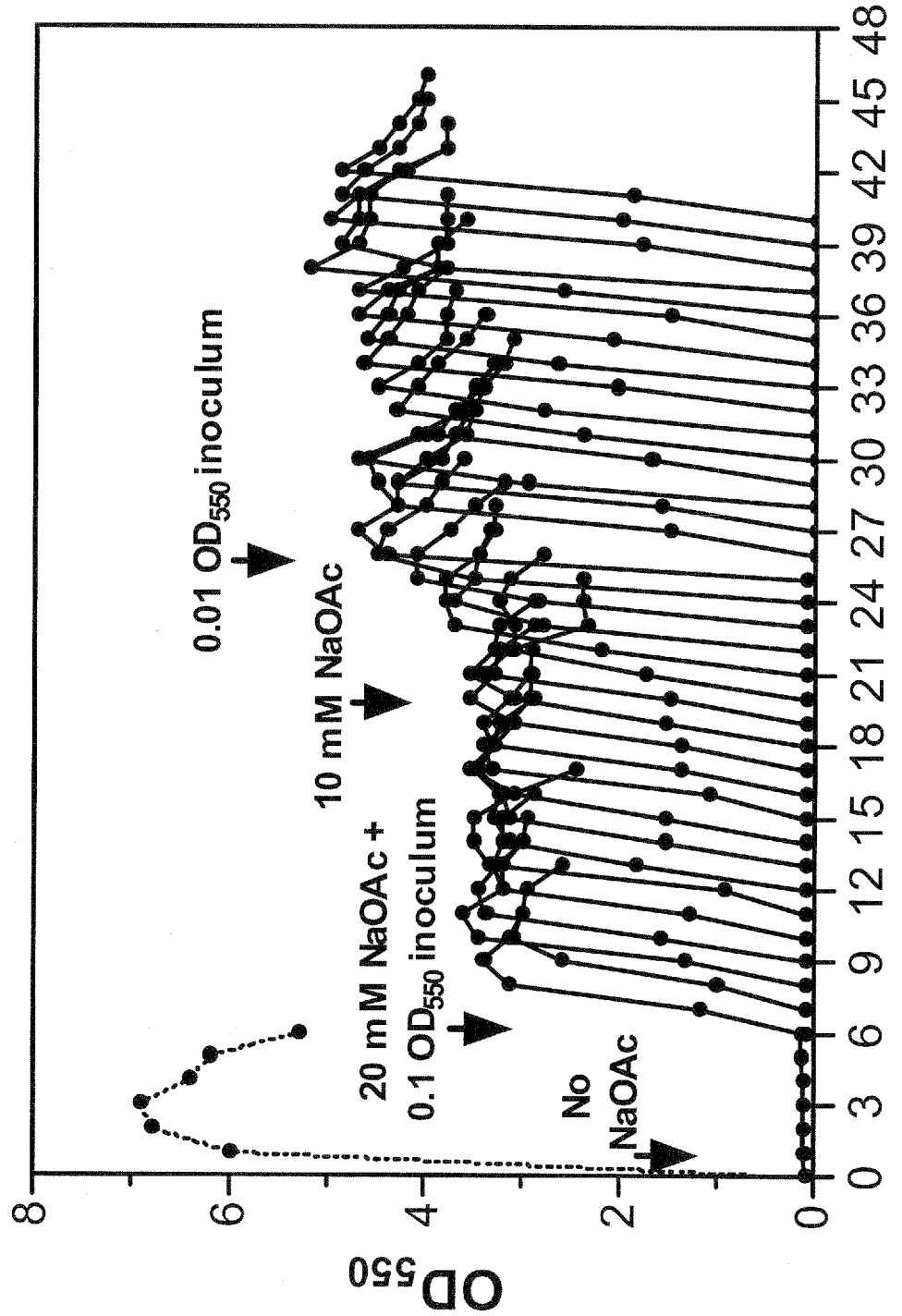

OD550
8
6
4
2
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48
Time (days)
No NaOAc
20 mM NaOAc + 0.1 OD550 inoculum
10 mM NaOAc
0.01 OD550 inoculum

FIG. 3C

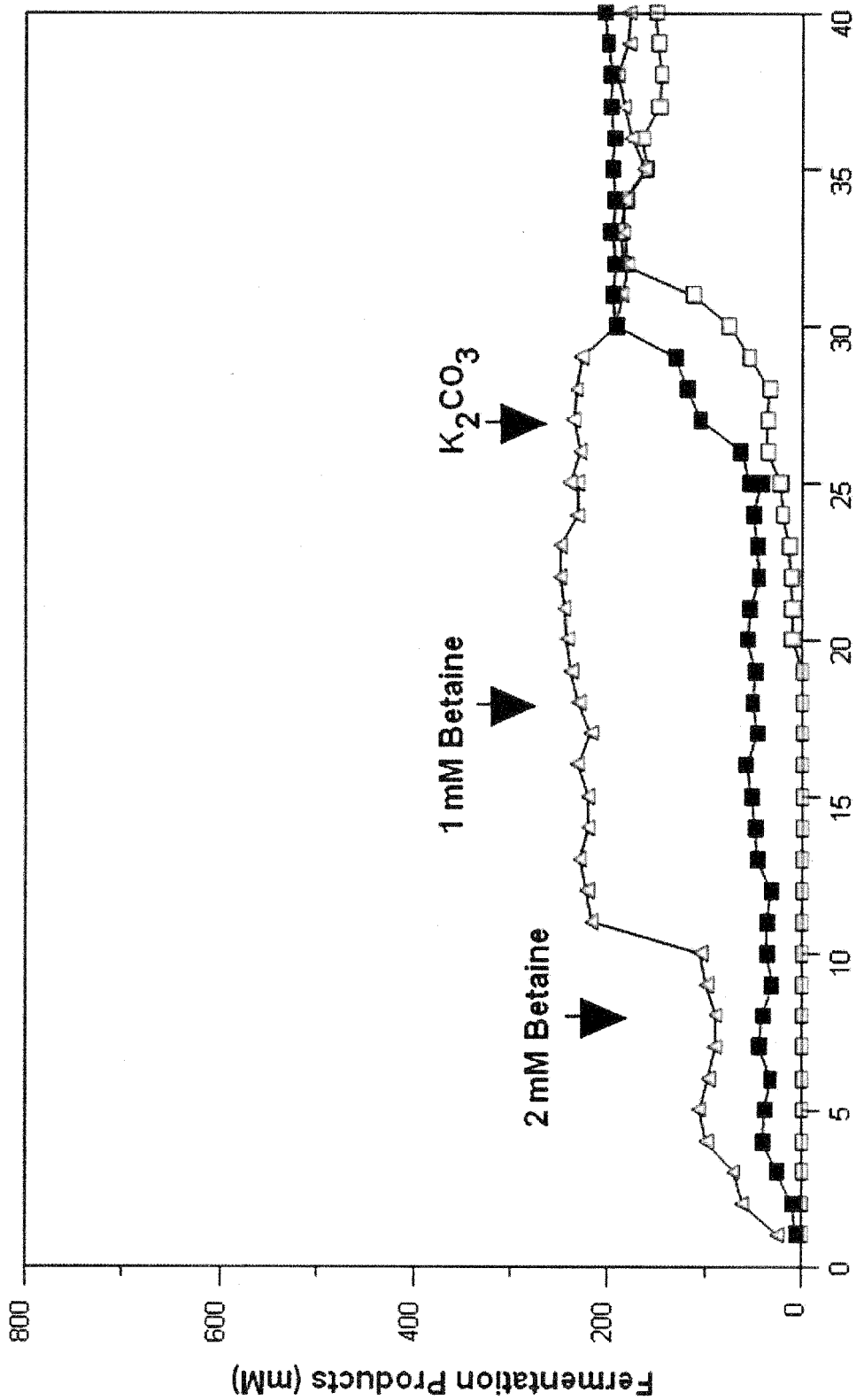

Fermentation Products (mM)
800
600
400
200
0
0
5
10
15
20
25
30
35
40
Number of Transfers
2 mM Betaine
1 mM Betaine
K_2CO_3

FIG. 4A

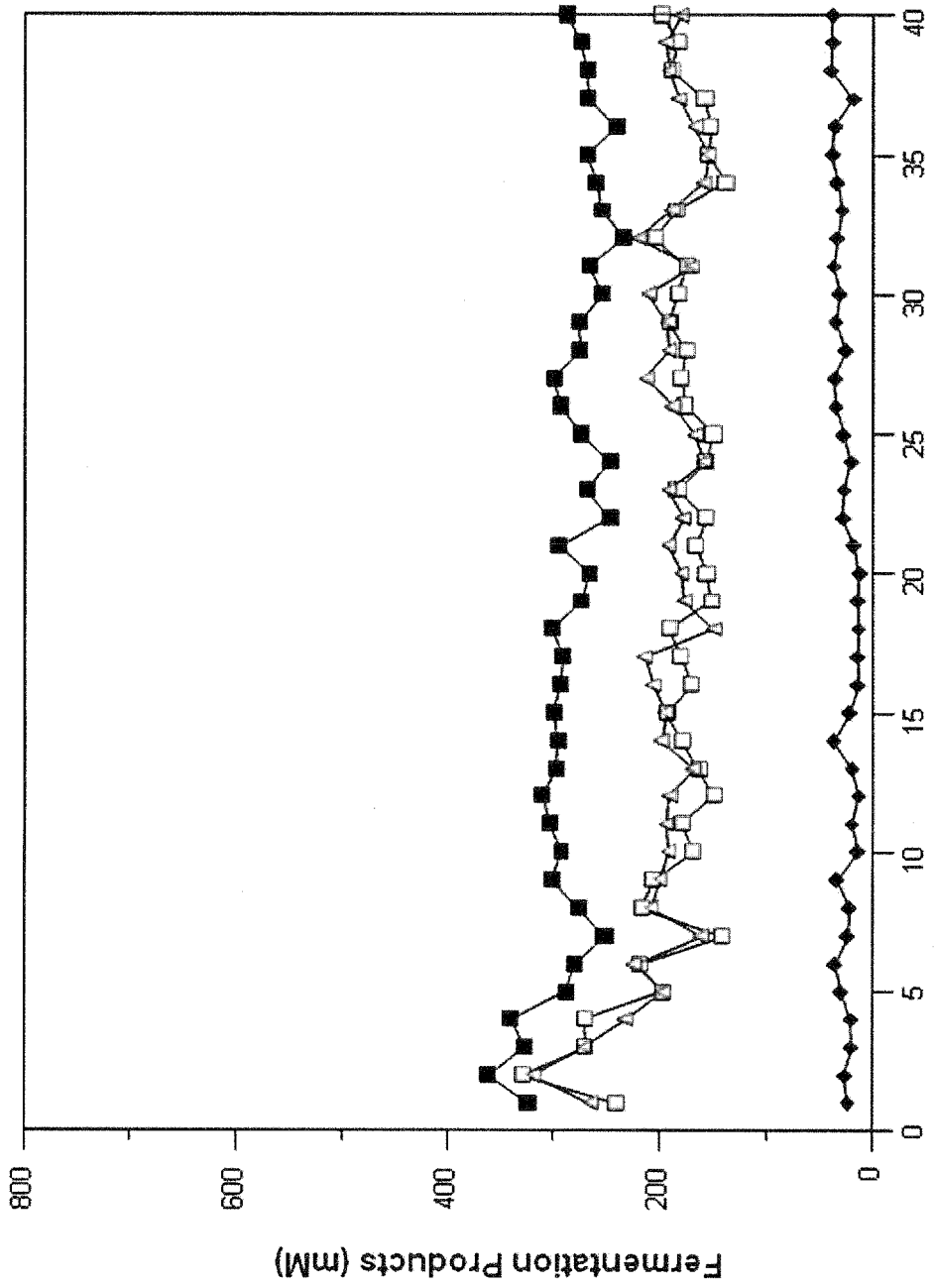

Fermentation Products (mM)
800
600
400
200
0
0
5
10
15
20
25
30
35
40
Number of Transfers

FIG. 4B

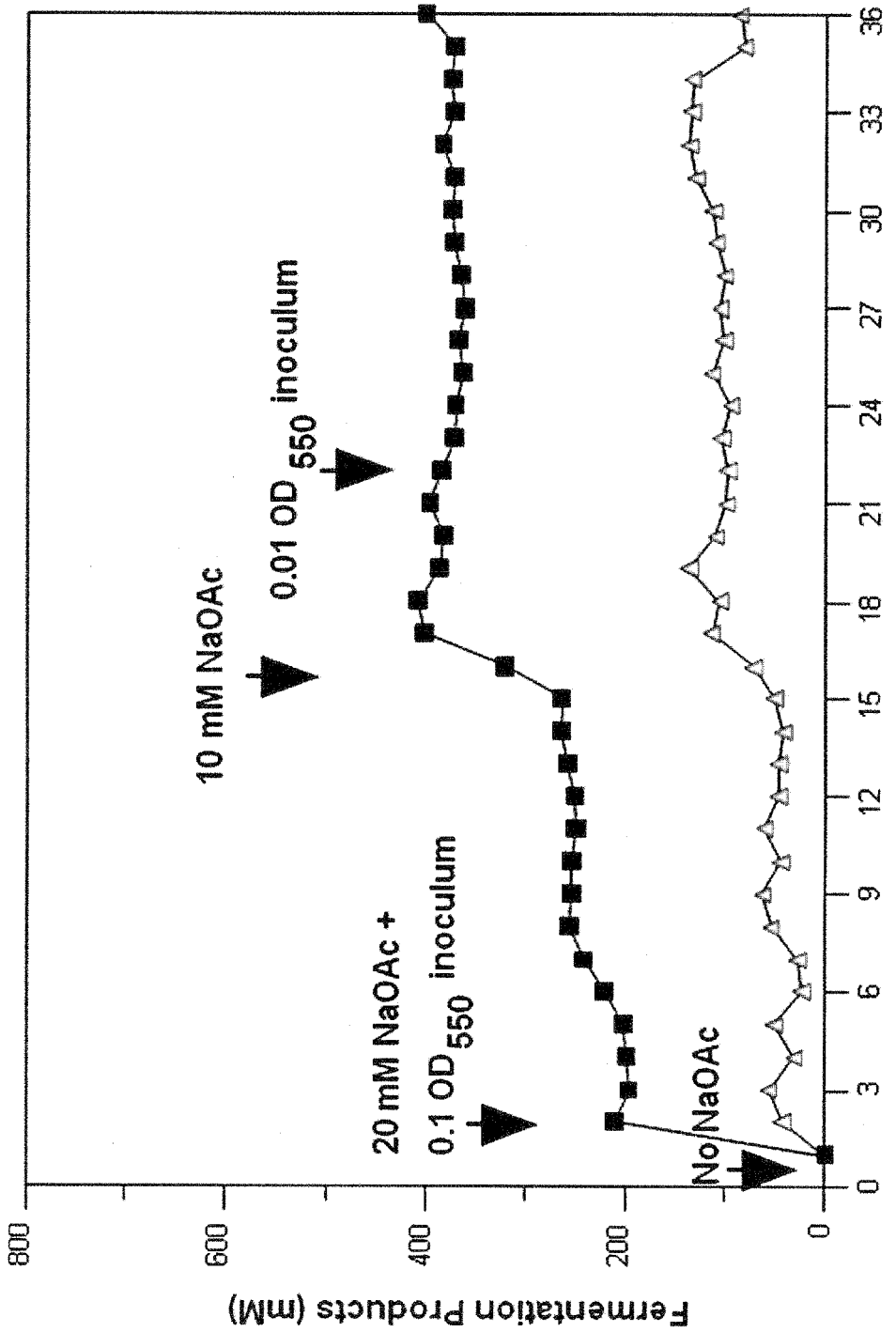

Fermentation Products (mM)
800
600
400
200
0
0
3
6
9
12
15
18
21
24
27
30
33
36
Number of Transfers
No NaOAc
20 mM NaOAc +
0.1 OD 550 inoculum
10 mM NaOAc
0.01 OD 550 inoculum

FIG. 4C

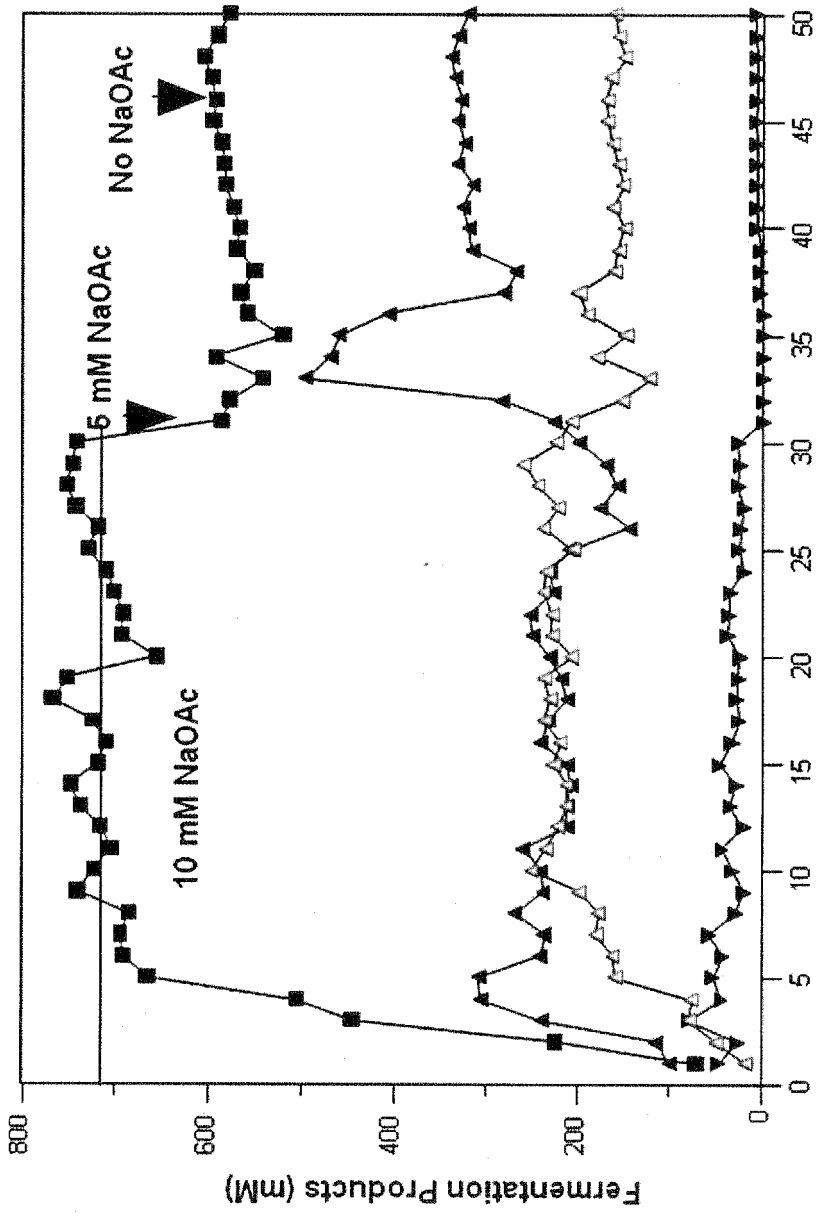

No NaOAc
5 mM NaOAc
10 mM NaOAc
Fermentation Products (mM)
Number of Transfers
0
200
400
600
800
0
5
10
15
20
25
30
35
40
45
50

FIG. 4D

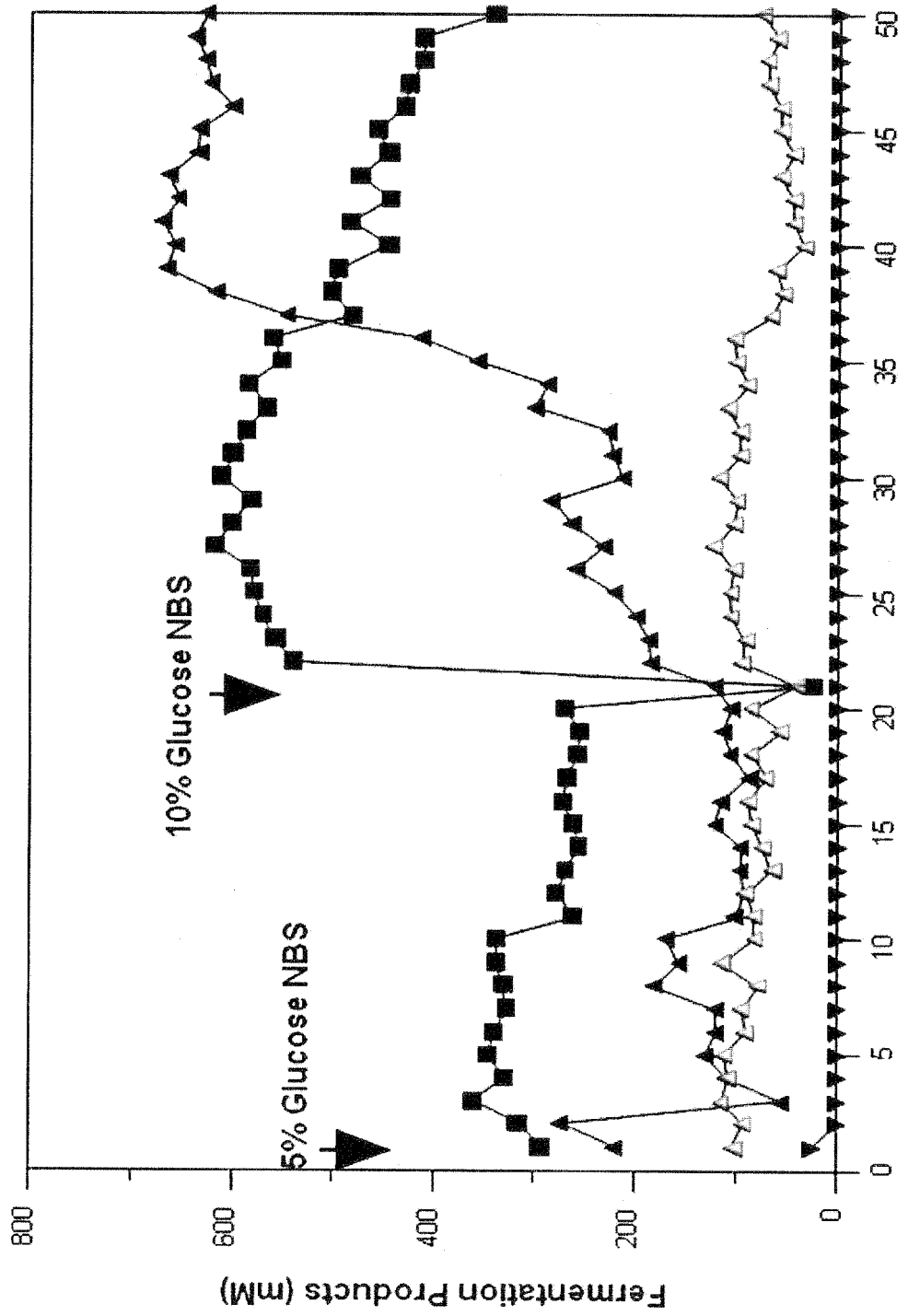

Fermentation Products (mM)
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
50
Number of Transfers
10% Glucose NBS
5% Glucose NBS

FIG. 4E

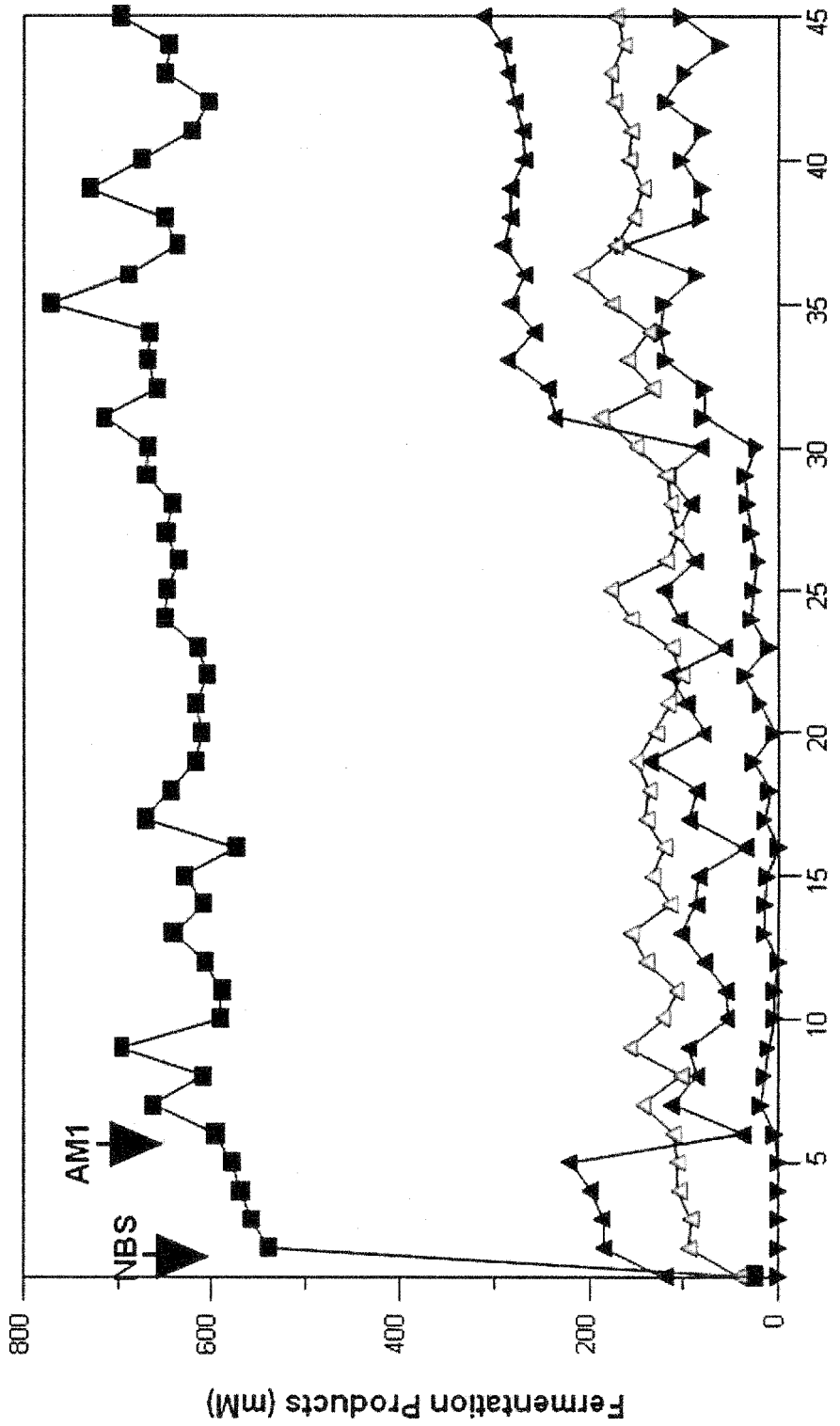

Fermentation Products (mM)
800
600
400
200
0
AM1
NBS
5
10
15
20
25
30
35
40
45
Number of Transfers

FIG. 4F

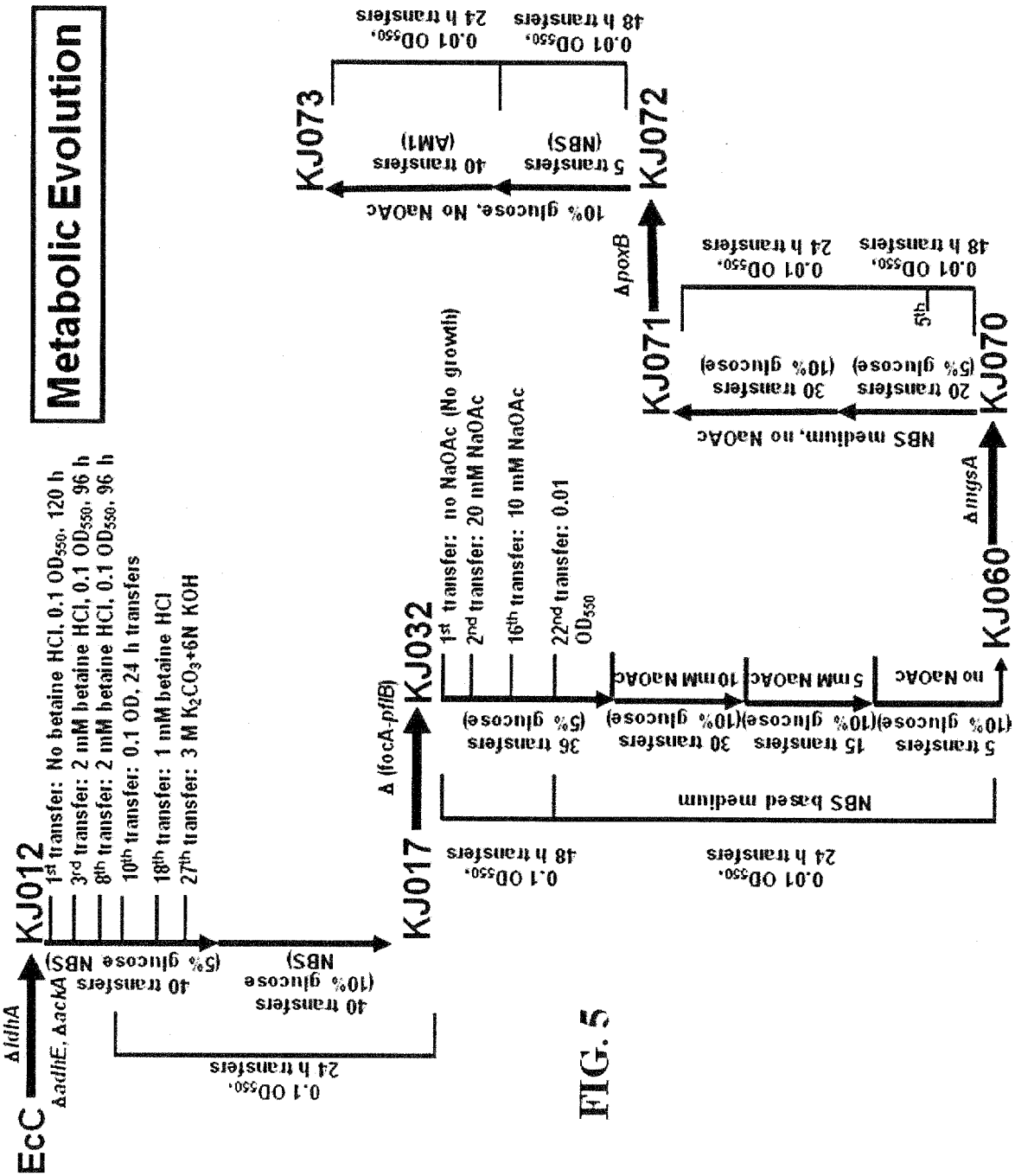
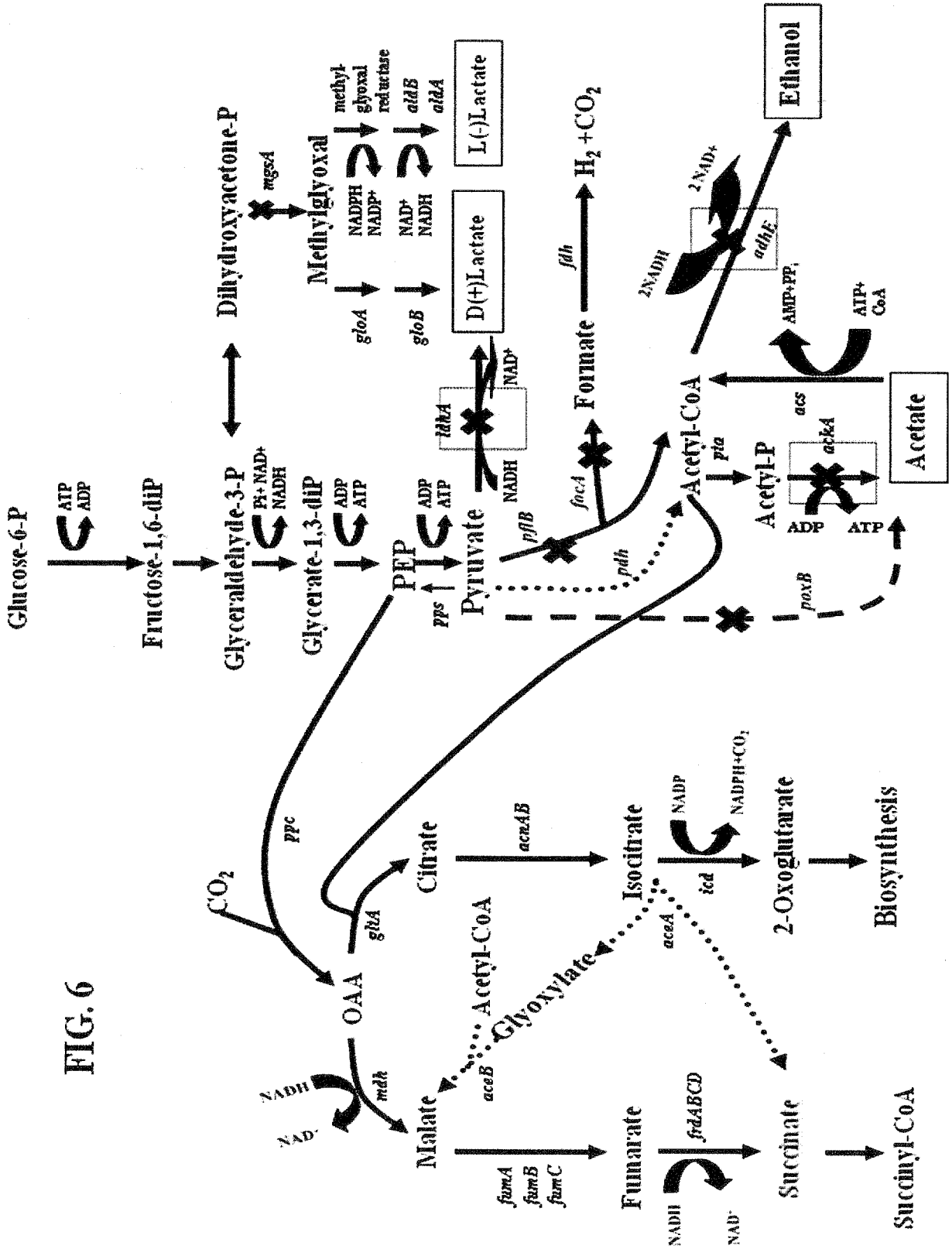

Metabolic Evolution
EcC
ΔadhE, ΔackA
ΔldhA
KJ012
1st transfer: No betaine HCl, 0.1 OD550, 120 h
3rd transfer: 2 mM betaine HCl, 0.1 OD550, 96 h
8th transfer: 2 mM betaine HCl, 0.1 OD550, 96 h
10th transfer: 0.1 OD, 24 h transfers
18th transfer: 1 mM betaine HCl
27th transfer: 3 M K2CO3+6N KOH
10 transfers (5% glucose NBS)
10 transfers (10% glucose NBS)
0.1 OD550, 24 h transfers
KJ017
Δ (focA-pflB)
KJ032
1st transfer: no NaOAc (No growth)
2nd transfer: 20 mM NaOAc
16th transfer: 10 mM NaOAc
22nd transfer: 0.01 OD550
36 transfers (5% glucose)
30 transfers (10% glucose)
15 transfers (10% glucose)
5 transfers (10% glucose)
10 mM NaOAc
5 mM NaOAc
no NaOAc
0.1 OD550, 18 h transfers
0.01 OD550, 24 h transfers
NBS based medium
ΔmgsA
KJ060
KJ070
NBS medium, no NaOAc
20 transfers (5% glucose)
30 transfers (10% glucose)
5th
0.01 OD550, 18 h transfers
0.01 OD550, 24 h transfers
ΔpoxB
KJ071
KJ072
10% glucose, No NaOAc
5 transfers (NBS)
10 transfers (AM1)
0.01 OD550, 18 h transfers
0.01 OD550, 24 h transfers
KJ073

FIG. 5

FIG. 6


Glucose-6-P
ATP
ADP
Fructose-1,6-diP
Glyceraldehyde-3-P
Pi+ NAD+
NADH
Glycerate-1,3-diP
ADP
ATP
PEP
ADP
ATP
Pyruvate
Dihydroxyacetone-P
mgsA
Methylglyoxal
methyl-glyoxal reductase
aldB
aldA
NADPH
NADP+
NAD+
NADH
gloA
gloB
L(-)Lactate
D(+)Lactate
ldhA
NAD+
NADH
pflB
fdh
Formate
$H_2 + CO_2$
focA
pdh
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ackA
acs
AMP+PPi
ATP+ CoA
Acetate
Ethanol
2NADH
2NAD+
adhE
poxB
CO_2
ppc
gltA
Citrate
acnAB
Isocitrate
NADP
NADPH+CO_2
icd
2-Oxoglutarate
Biosynthesis
Glyoxylate
aceA
aceB
Malate
NADH
NAD+
mdh
fumA
fumB
fumC
Fumarate
NADH
NAD+
frdABCD
Succinate
Succinyl-CoA

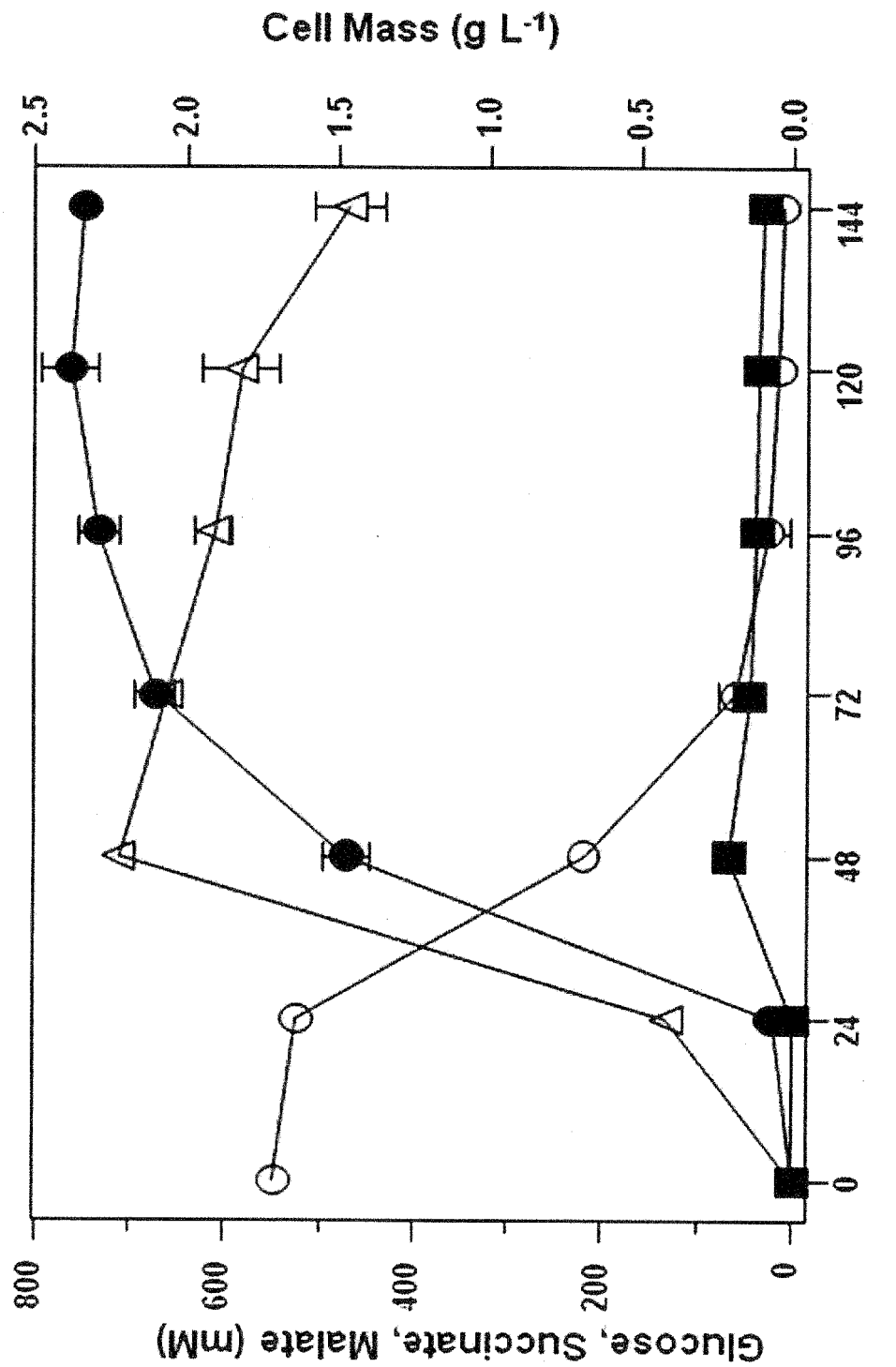

Cell Mass (g L-1)
2.5
2.0
1.5
1.0
0.5
0.0
Glucose, Succinate, Malate (mM)
800
600
400
200
0
Time (h)
0
24
48
72
96
120
144

FIG. 7A

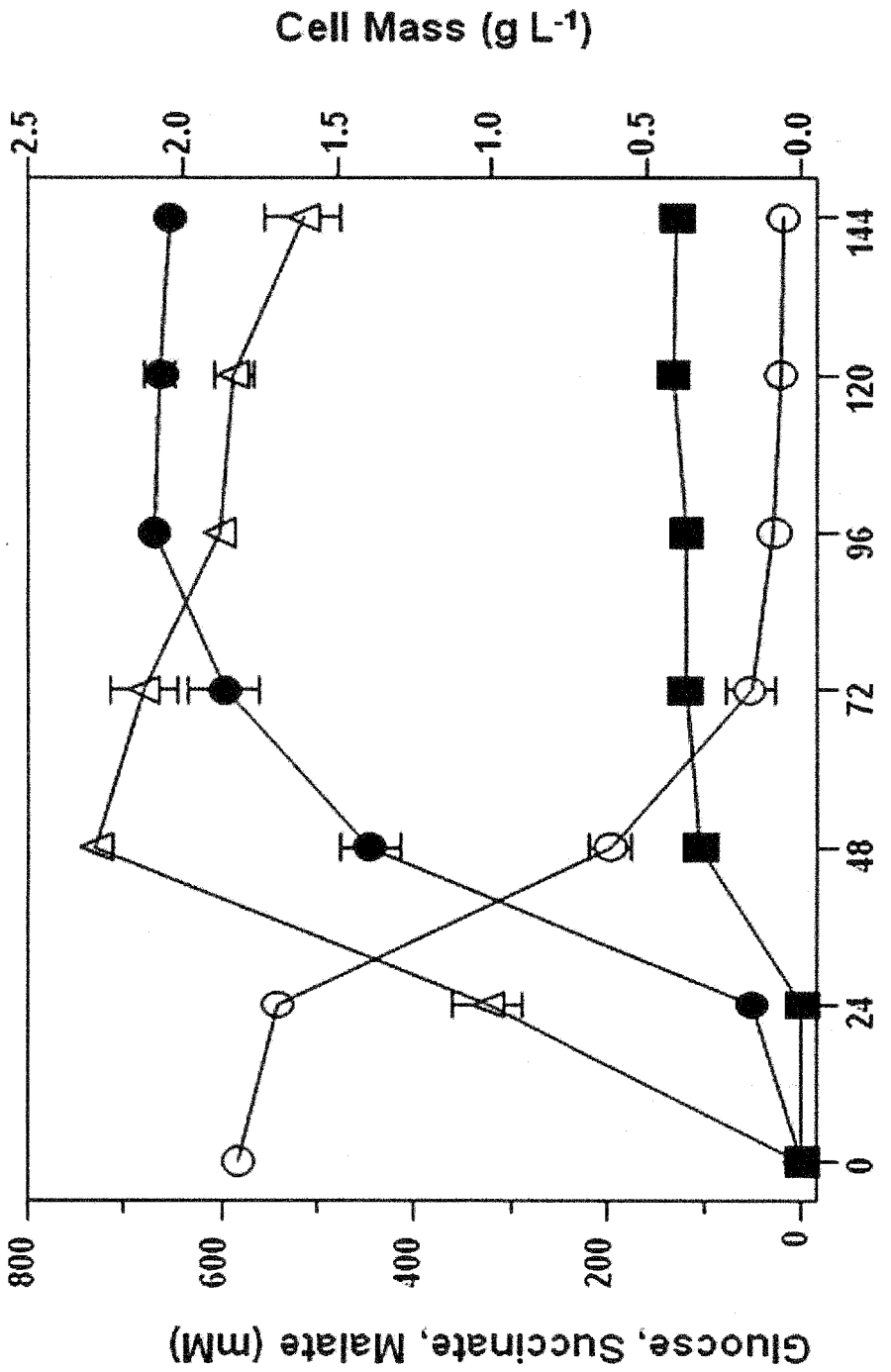

Cell Mass (g L-1)
2.5
2.0
1.5
1.0
0.5
0.0
800
600
400
200
0
Glucose, Succinate, Malate (mM)
0
24
48
72
96
120
144
Time (h)

FIG. 7B

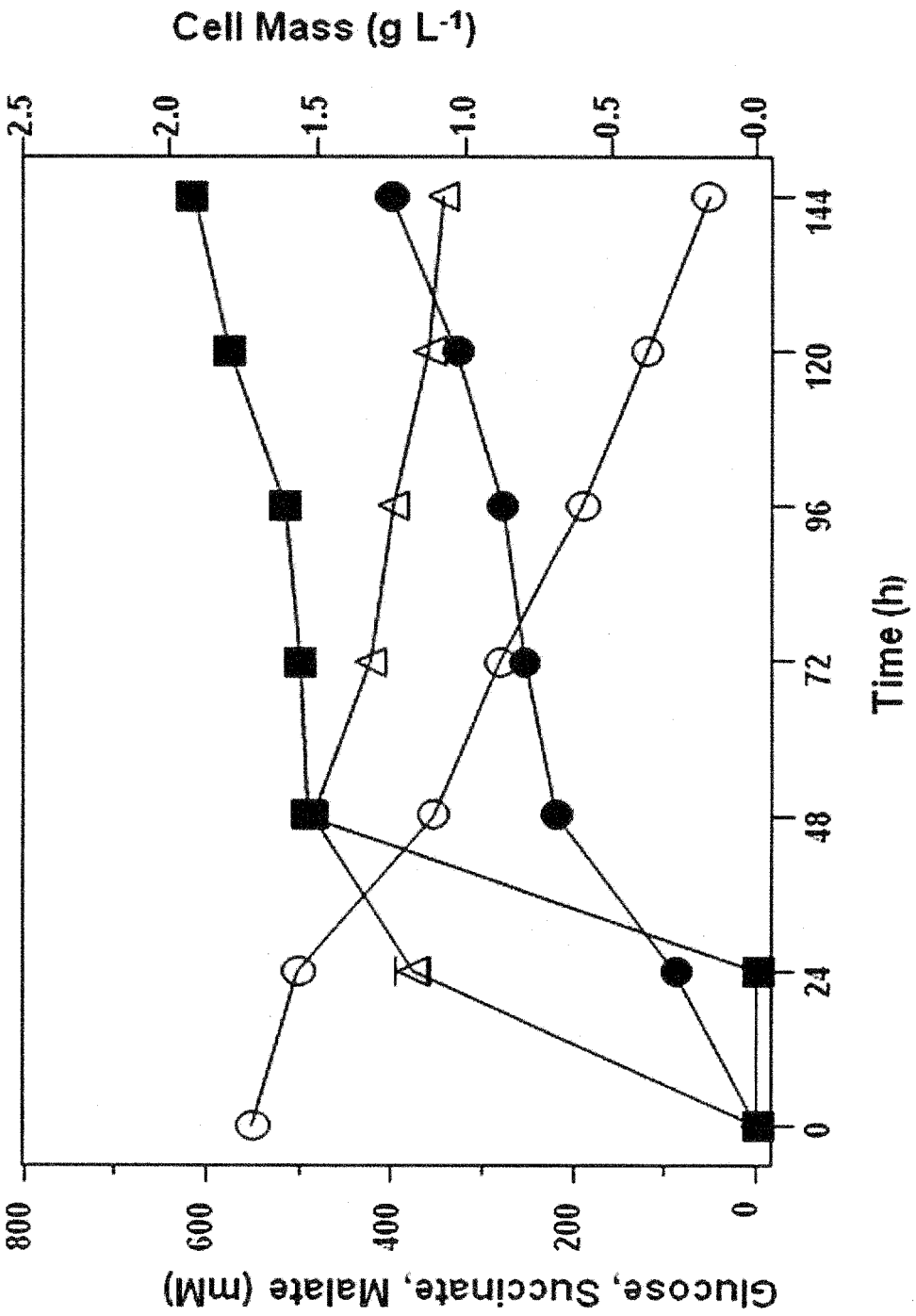

Cell Mass (g L-1)
2.5
2.0
1.5
1.0
0.5
0.0
144
120
96
72
48
24
0
Time (h)
800
600
400
200
0
Glucose, Succinate, Malate (mM)

FIG. 7C

FIG. 8

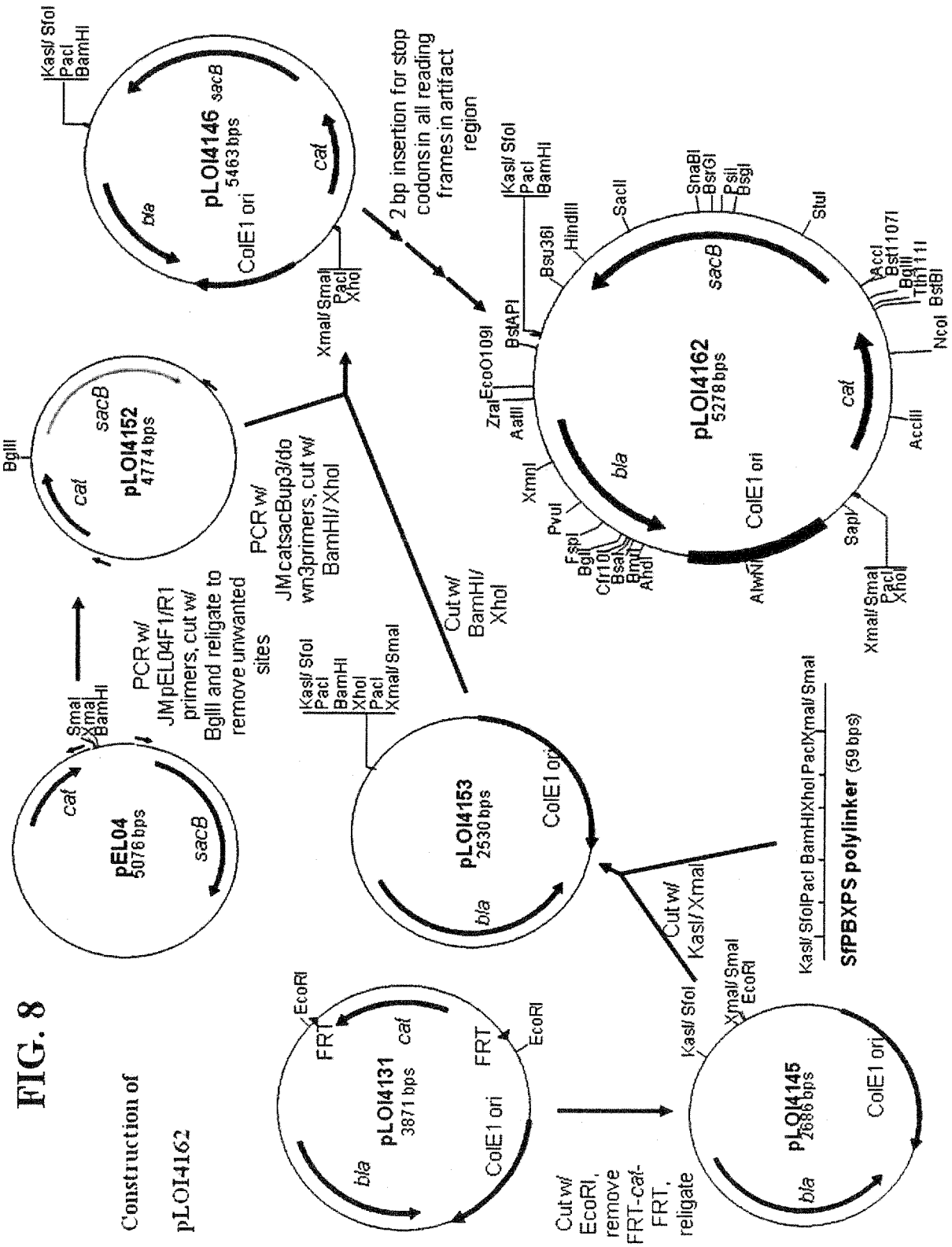
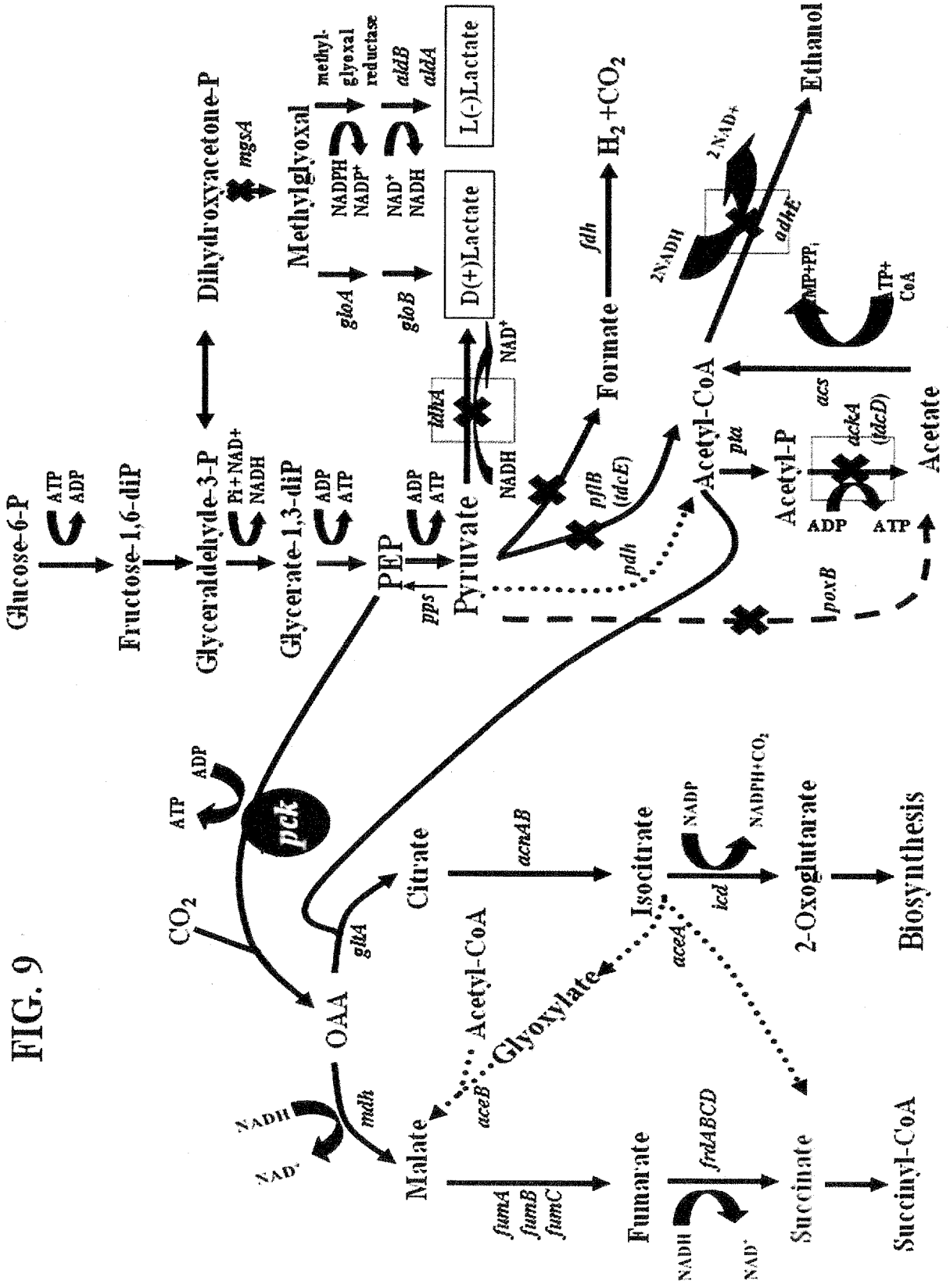

Construction of pLOI4162
pEL04 5076 bps
cat
sacB
SmaI XmaI BamHI
PCR w/ JM pEL04F1/R1 primers, cut w/ BglII and religate to remove unwanted sites
BglII
pLOI4152 4774 bps
PCR w/ JM catsacBup3/do wn3primers, cut w/ BamHI/ XhoI
KasI/ SfoI PacI BamHI XhoI PacI XmaI/ SmaI
pLOI4146 5463 bps
ColE1 ori
bla
XmaI/ SmaI PacI XhoI
2 bp insertion for stop codons in all reading frames in artifact region
Cut w/ BamHI/ XhoI
pLOI4153 2530 bps
pLOI4162 5278 bps
KasI/ SfoI PacI BamHI
ZraI AatII EcoO109I BstAPI Bsu36I HindIII SacII SnaBI BsrGI PsiI BsgI StuI AccI Bst1107I BglII Tth111I NcoI BstBI AccIII
XmnI PvuI FspI BglI Cfr10I BsaI BmtI AhdI
AlwNI
SapI
XmaI/ SmaI PacI XhoI
Cut w/ EcoRI, remove FRT-cat-FRT, religate
pLOI4131 3871 bps
FRT
EcoRI
Cut w/ KasI/ XmaI
KasI/ SfoI
XmaI/ SmaI EcoRI
pLOI4145 2686 bps
KasI/ SfoI PacI BamHI XhoI PacI XmaI/ SmaI
SfPBXPS polylinker (59 bps)

FIG. 9


Glucose-6-P
ATP
ADP
Fructose-1,6-diP
Glyceraldehyde-3-P
Pi+NAD+
NADH
Dihydroxyacetone-P
mgsA
Methylglyoxal
methyl-glyoxal reductase
aldB
aldA
NADPH
NADP+
NAD+
NADH
gloA
gloB
L(-)Lactate
D(+)Lactate
Glycerate-1,3-diP
ADP
ATP
PEP
pps
Pyruvate
ldhA
NADH
NAD+
pflB (ldcE)
pdh
Formate
fdh
H2+CO2
Acetyl-CoA
pta
Acetyl-P
ack4 (ldcD)
acs
Acetate
ADP
ATP
poxB
2NADH
2NAD+
adhE
Ethanol
AMP+PPi
ATP+
CoA
CO2
ATP
ADP
pck
OAA
gltA
Citrate
acnAB
Isocitrate
NADP
NADPH+CO2
icd
aceA
2-Oxoglutarate
Biosynthesis
NADH
NAD+
mdh
Malate
aceB
Glyoxylate
Acetyl-CoA
fumA
fumB
fumC
Fumarate
frdABCD
NADH
NAD+
Succinate
Succinyl-CoA

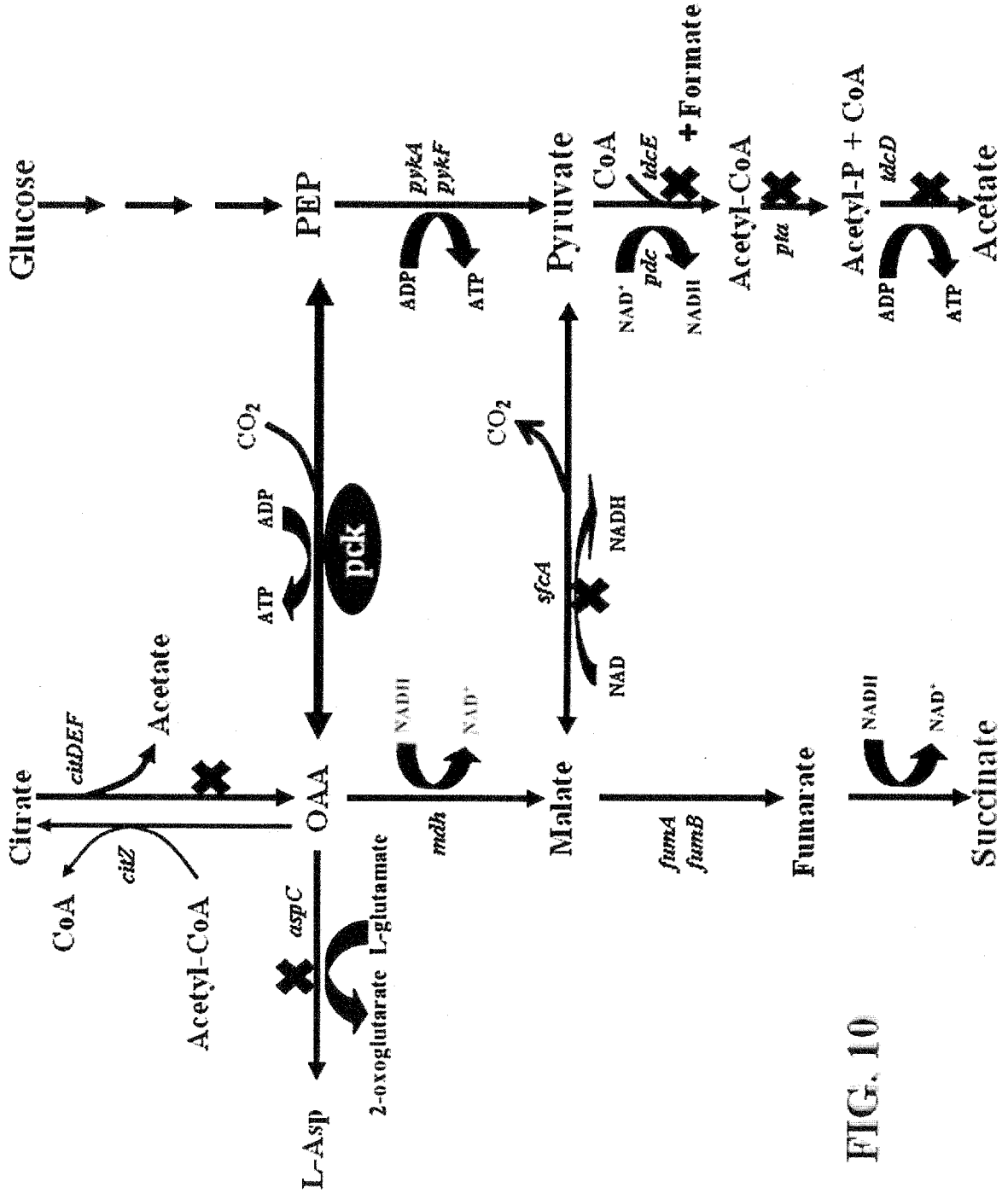

Glucose
PEP
pykA
pykF
ADP
ATP
Pyruvate
CoA
tdcE
+ Formate
NAD'
pdc
NADH
Acetyl-CoA
pta
Acetyl-P + CoA
tdcD
ADP
ATP
Acetate
CO2
ADP
ATP
pck
CO2
sfcA
NADH
NAD
Citrate
citDEF
Acetate
CoA
citZ
Acetyl-CoA
OAA
NADH
NAD'
mdh
Malate
fumA
fumB
Fumarate
NADH
NAD'
Succinate
aspC
L-glutamate
2-oxoglutarate
L-Asp

FIG. 10

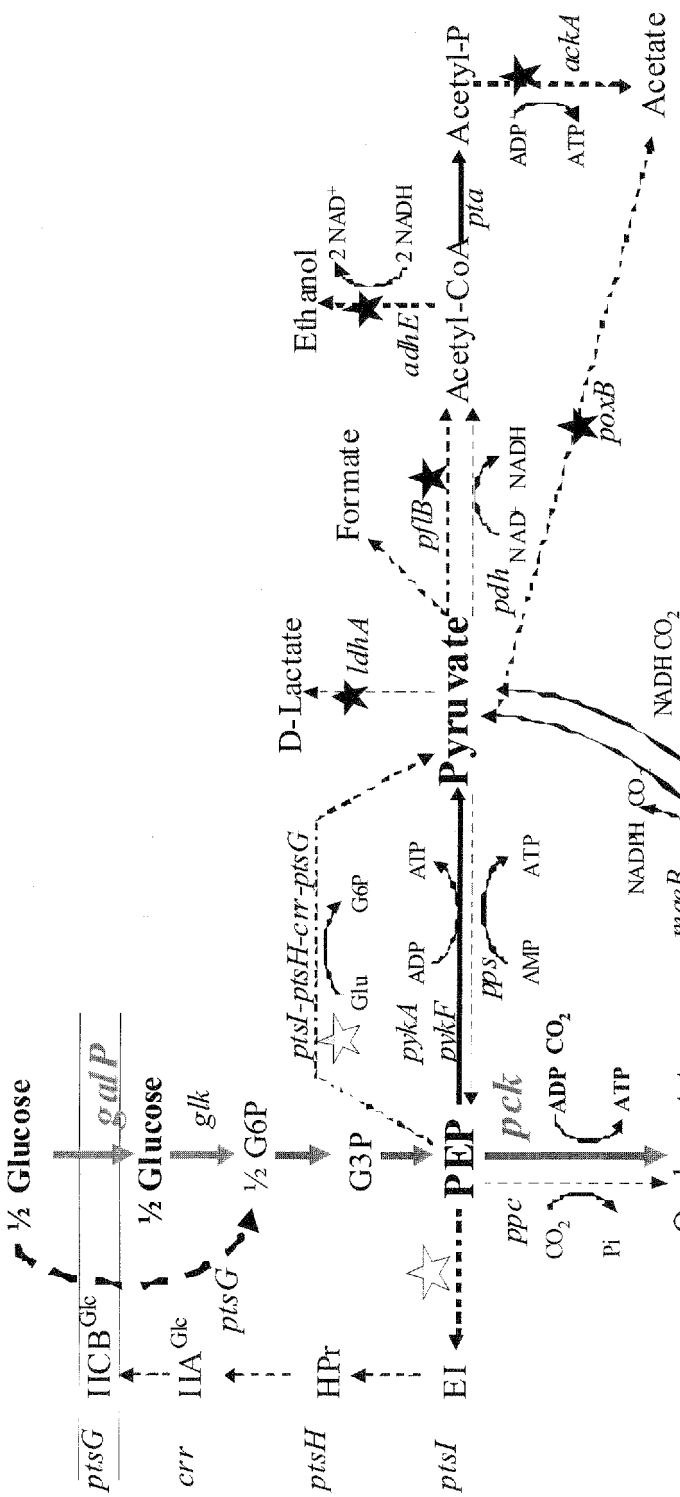

½ Glucose
ptsG
IICB Glc
galP
½ Glucose
crr
IIA Glc
glk
ptsG
½ G6P
ptsH
HPr
ptsI
EI
PEP
ptsI-ptsH-crr-ptsG
Glu
G6P
G3P
pykA
pykF
ADP
ATP
Pyruvate
D-Lactate
ldhA
Formate
pflB
Ethanol
2 NAD+
2 NADH
adhE
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ackA
Acetate
pdh
NAD+
NADH
poxB
pck
ADP CO2
ATP
ppc
CO2
Pi
Oxaloacetate
NADH
NAD+
mdh
Malate
fumABC
H2O
Fumarate
frdABCD
Succinate
ppsA
AMP
ATP
maeB
NADPH
CO2
NADP+
maeA
NADH
CO2
NAD+

FIG. 11

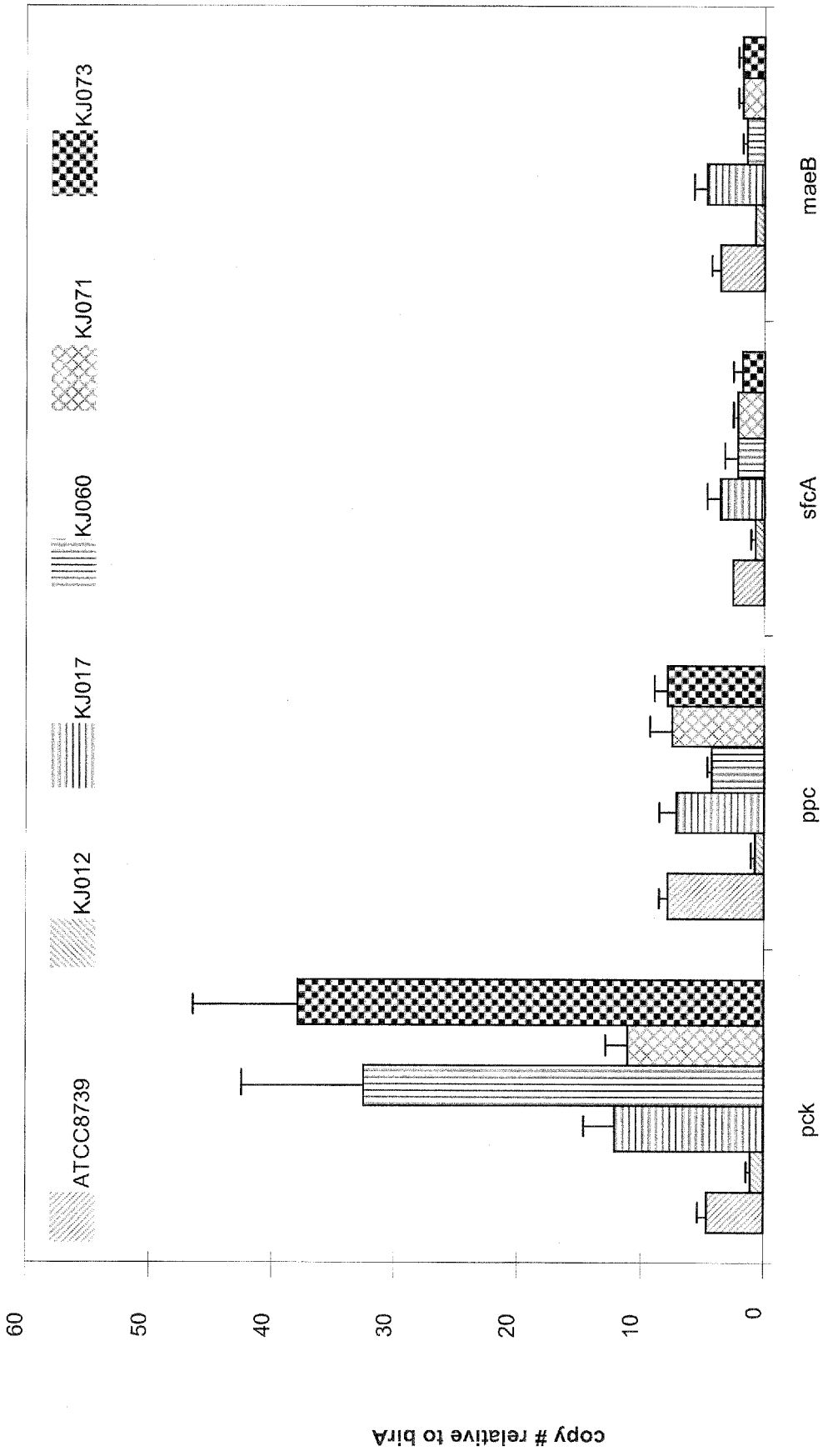

ATCC8739
KJ012
KJ017
KJ060
KJ071
KJ073
pck
ppc
sfcA
maeB
0
10
20
30
40
50
60
copy # relative to birA

FIG. 12A

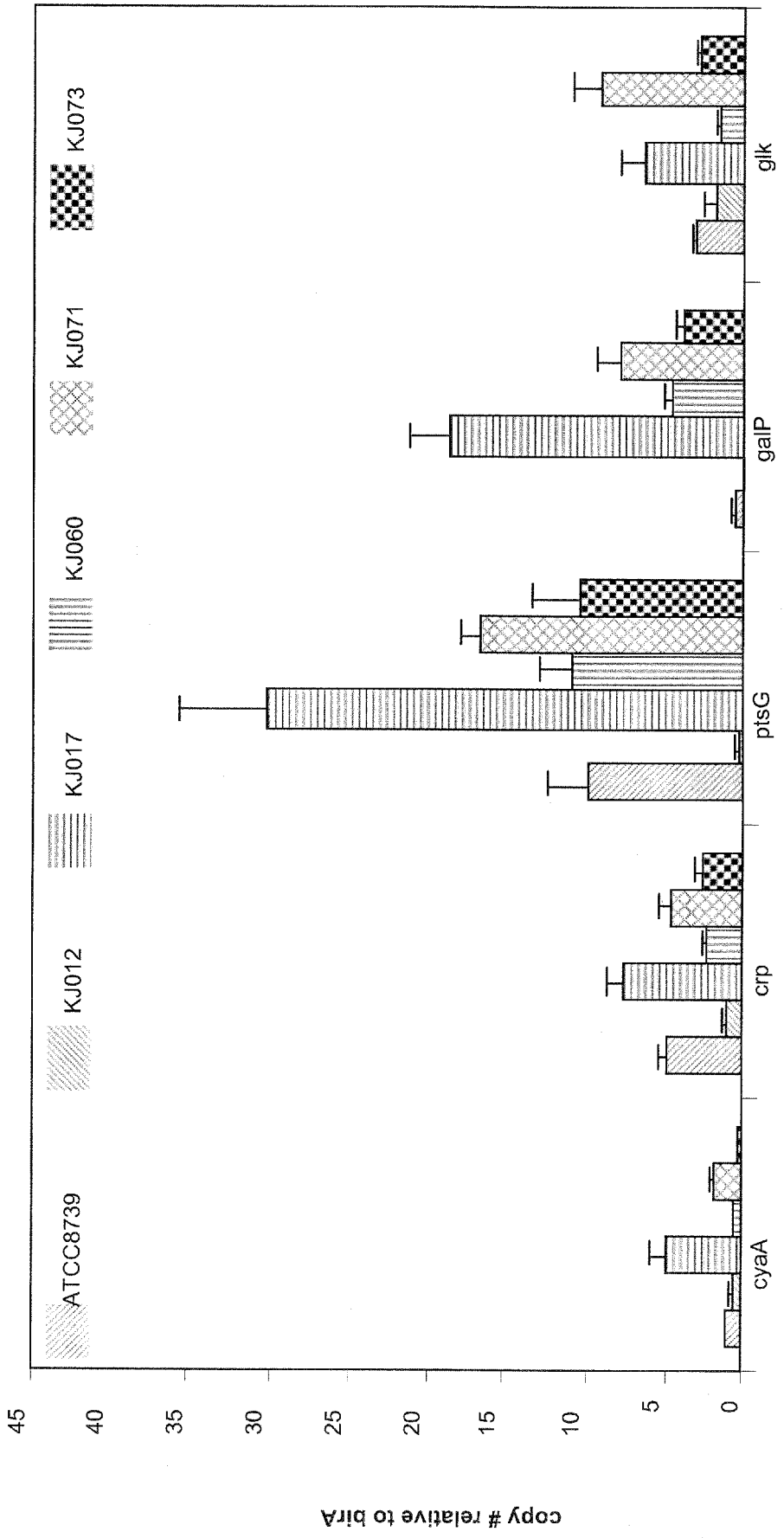

ATCC8739
KJ012
KJ017
KJ060
KJ071
KJ073
copy # relative to birA
0
5
10
15
20
25
30
35
40
45
cyaA
crp
ptsG
galP
glk

FIG. 12B

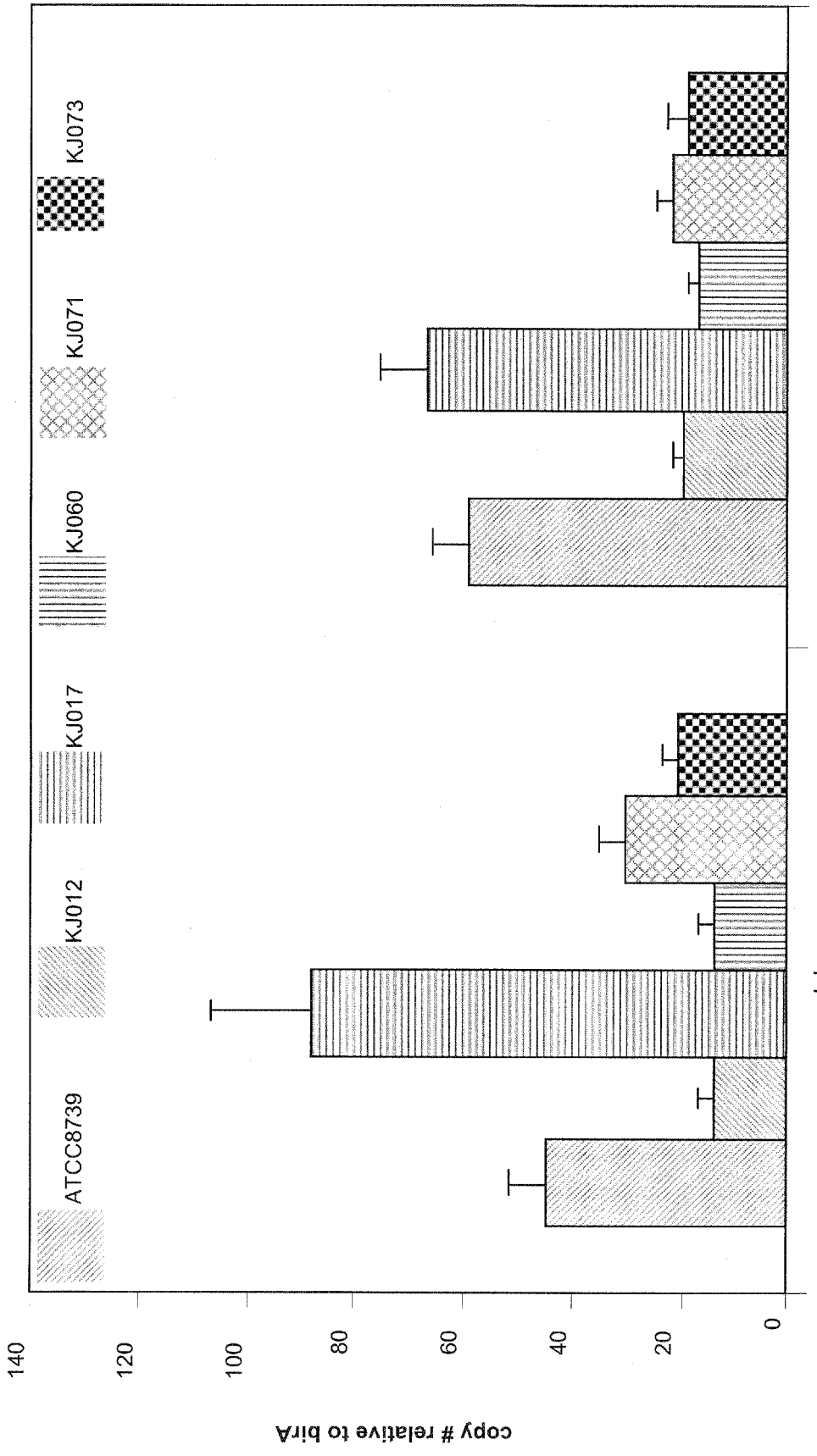

ATCC8739
KJ012
KJ017
KJ060
KJ071
KJ073
copy # relative to birA
0
20
40
60
80
100
120
140
ptsI
crr

FIG. 12C

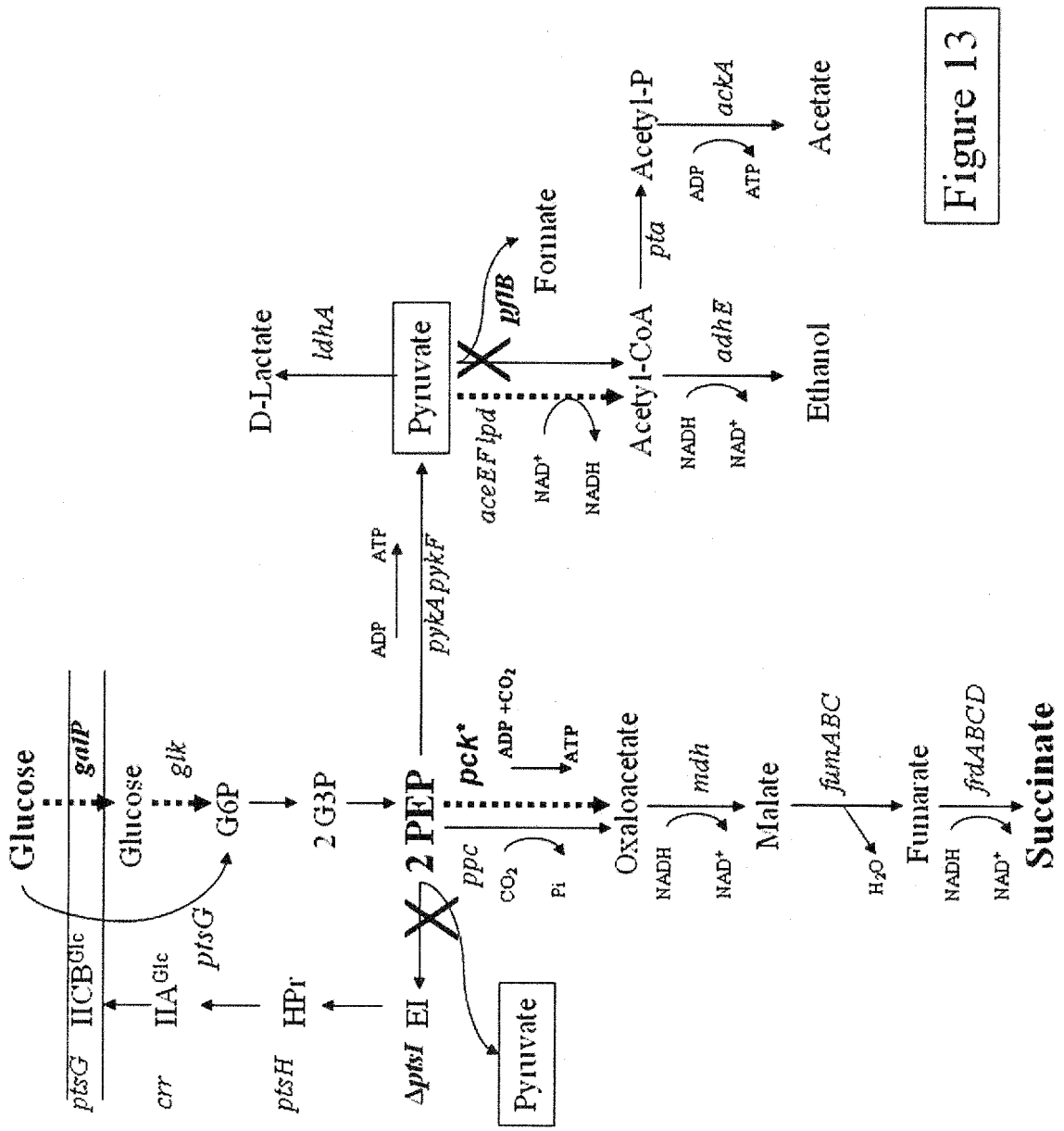

Glucose
ptsG
galP
Glucose
glk
G6P
ptsG
IICB^Glc
IIA^Glc
crr
ptsH
HPr
ΔptsI
EI
2 G3P
2 PEP
pck*
ppc
CO2
ADP +CO2
ATP
Pi
Oxaloacetate
NADH
NAD+
mdh
Malate
fumABC
H2O
Fumarate
NADH
NAD+
frdABCD
Succinate
Pyruvate
ADP
ATP
pykA pykF
D-Lactate
ldhA
Pyruvate
aceEF lpd
pflB
Formate
NAD+
NADH
Acetyl-CoA
pta
Acetyl-P
ADP
ATP
ackA
Acetate
NADH
NAD+
adhE
Ethanol

Figure 13

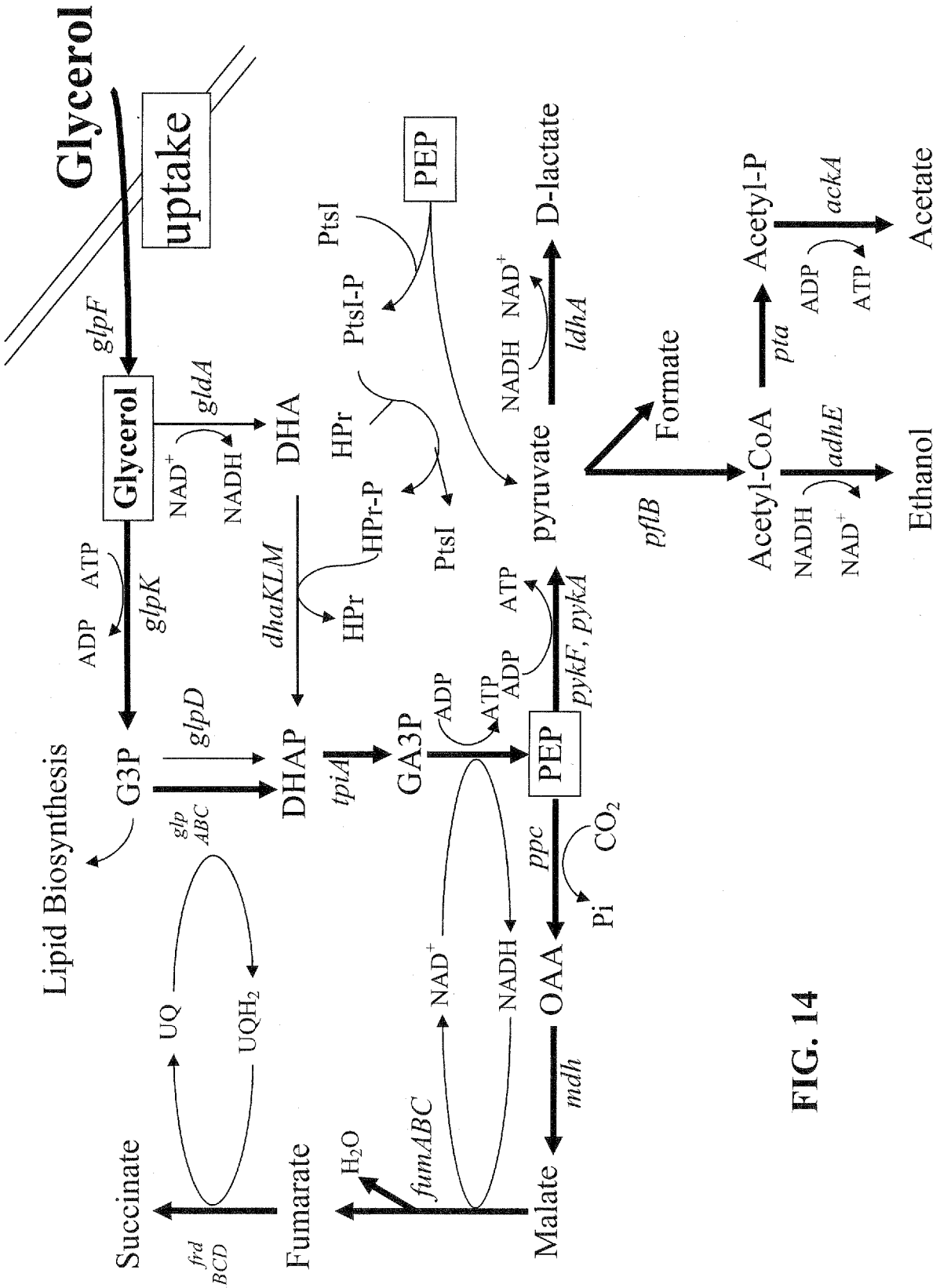

Glycerol
uptake
glpF
Glycerol
gldA
NAD+
NADH
DHA
ATP
ADP
glpK
dhaKLM
HPr
HPr-P
PtsI
PtsI-P
PEP
G3P
glpD
DHAP
tpiA
GA3P
ADP
ATP
PEP
pykF, pykA
pyruvate
NADH
NAD+
D-lactate
ldhA
Lipid Biosynthesis
glp ABC
UQ
UQH2
ppc
Pi
CO2
Formate
pflB
Acetyl-CoA
pta
Acetyl-P
ackA
ADP
ATP
NADH
NAD+
adhE
Ethanol
Acetate
Succinate
frd ABCD
Fumarate
H2O
fumABC
NAD+
NADH
OAA
mdh
Malate

FIG. 14

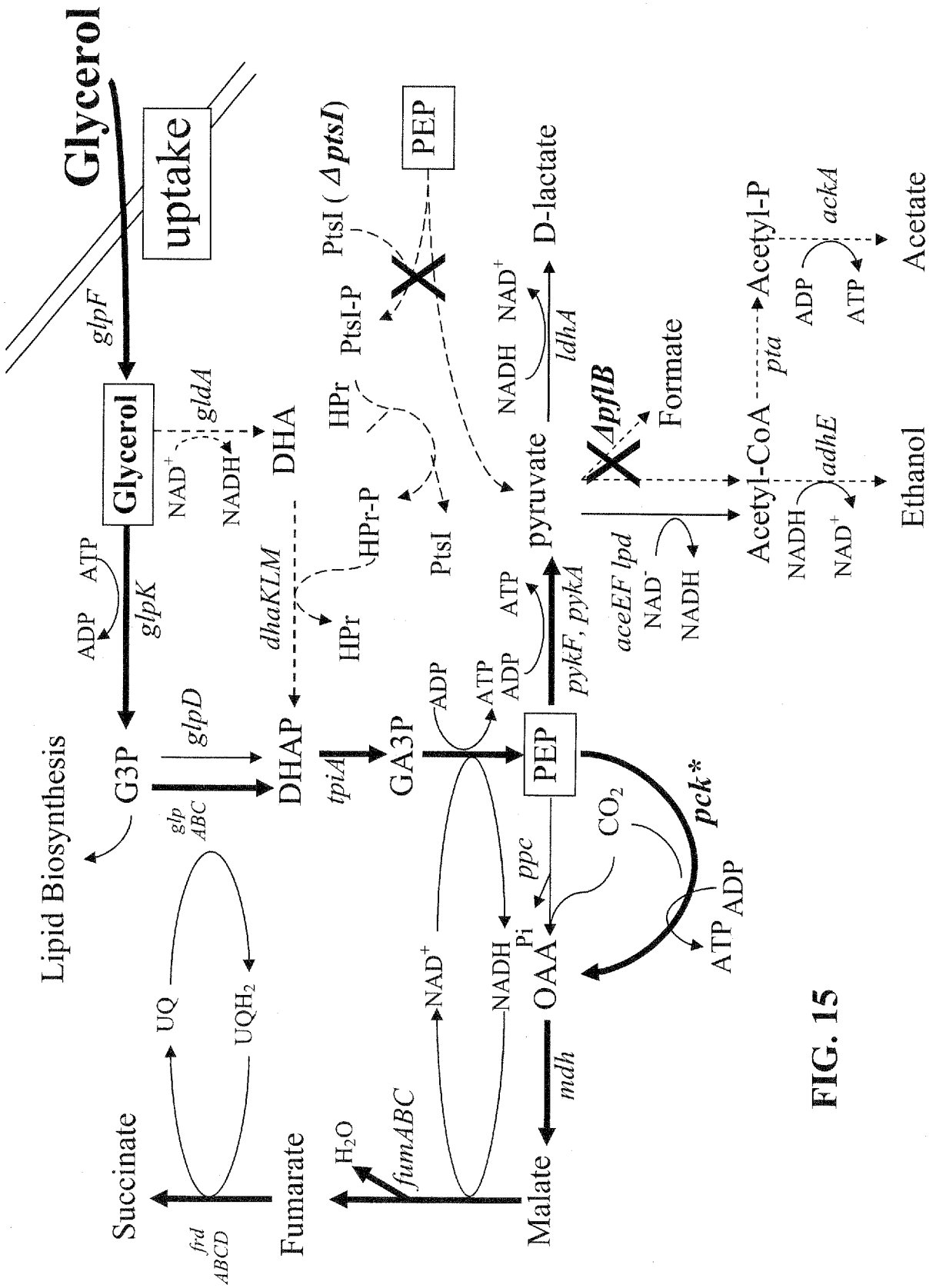

Glycerol
uptake
glpF
Glycerol
NAD+
gldA
NADH
DHA
ADP
ATP
glpK
dhaKLM
HPr
PtsI-P
PtsI (ΔptsI)
PEP
HPr-P
PtsI
NADH
NAD+
D-lactate
ldhA
ΔpflB
Formate
Acetyl-CoA
pta
Acetyl-P
ackA
ADP
ATP
Acetate
NADH
adhE
NAD+
Ethanol
Lipid Biosynthesis
G3P
glpD
DHAP
tpiA
GA3P
ADP
ATP
ADP
ATP
PEP
pykF, pykA
pyruvate
aceEF lpd
NAD+
NADH
glp ABC
ppc
CO2
pck*
ATP
ADP
UQ
UQH2
NAD+
NADH
Pi
OAA
mdh
Succinate
frd ABCD
Fumarate
H2O
fumABC
Malate

FIG. 15