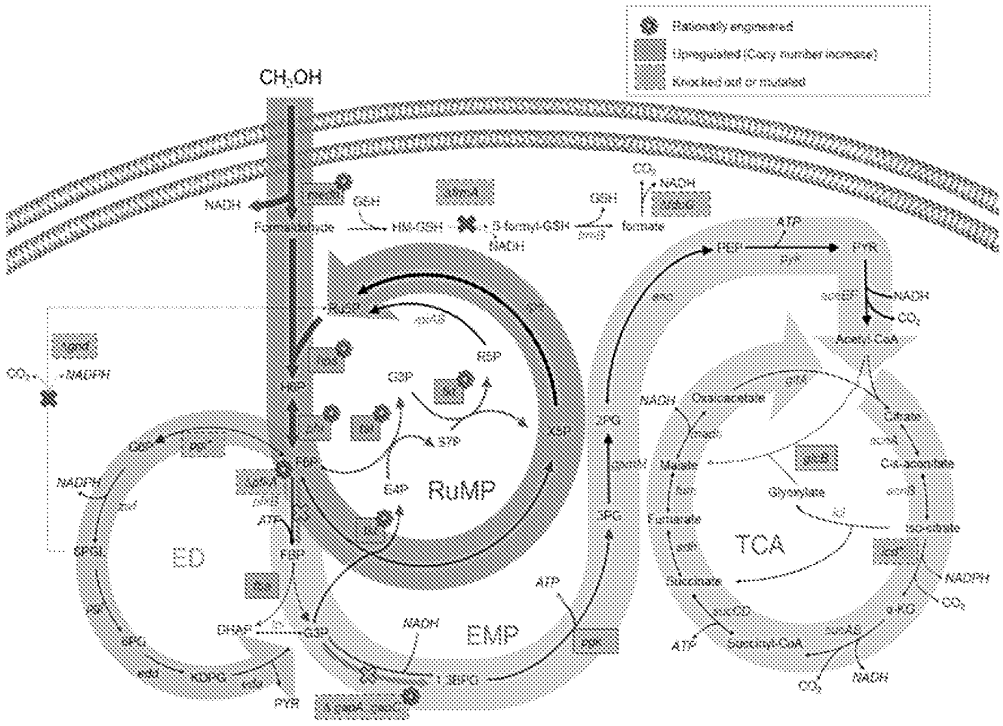
Rationally engineered
Upregulated (Copy number increase)
Knocked out or mutated
CH3OH
NADH
Formaldehyde
HM-GSH
B-formyl-GSH
formate
NADH
CO2
NADH
ATP
PEP
PYR
NADH
CO2
Acetyl-CoA
Oxaloacetate
Citrate
Cis-aconitate
Iso-citrate
Glyoxylate
Malate
Fumarate
Succinate
Succinyl-CoA
TCA
NADH
CO2
NADH
CO2
ATP
NADPH
CO2
NADPH
G6P
F6P
FBP
G3P
DHAP
G3P
NADH
EMP
1,3BPG
PYR
KDPG
6PG
SPGL
ED
ATP
R5P
G3P
X5P
E4P
RuMP
2PG
3PG
ATP

FIG. 1A

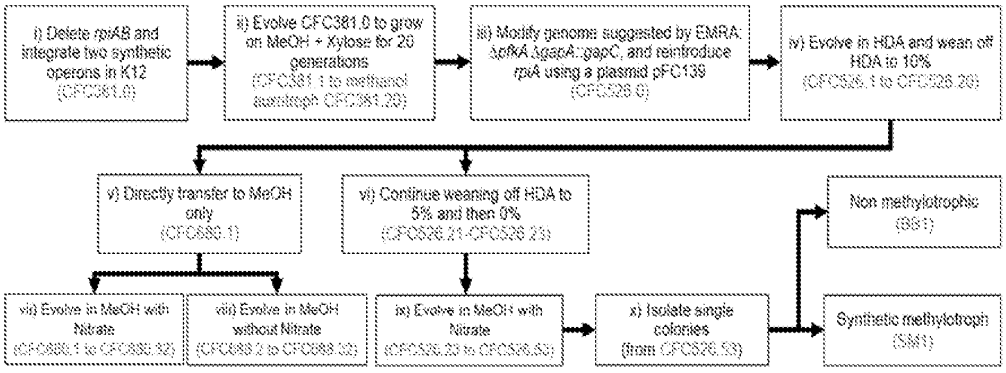
i) Delete rpiAB and integrate two synthetic operons in K12 (CFC381.0)
ii) Evolve CFC381.0 to grow on MeOH + Xylose for 20 generations (CFC381.1 to methanol auxotroph CFC381.20)
iii) Modify genome suggested by EMRA: ΔpfkA ΔgapA::gapC, and reintroduce rpiA using a plasmid pFC139 (CFC526.0)
iv) Evolve in HDA and wean off HDA to 10% (CFC526.1 to CFC526.20)
v) Directly transfer to MeOH only (CFC680.1)
vi) Continue weaning off HDA to 5% and then 0% (CFC526.21-CFC526.23)
vii) Evolve in MeOH with Nitrate (CFC680.1 to CFC680.32)
viii) Evolve in MeOH without Nitrate (CFC688.2 to CFC688.32)
ix) Evolve in MeOH with Nitrate (CFC526.23 to CFC526.53)
x) Isolate single colonies (from CFC526.53)
Non methylotrophic (BB1)
Synthetic methylotroph (SM1)

FIG. 1B

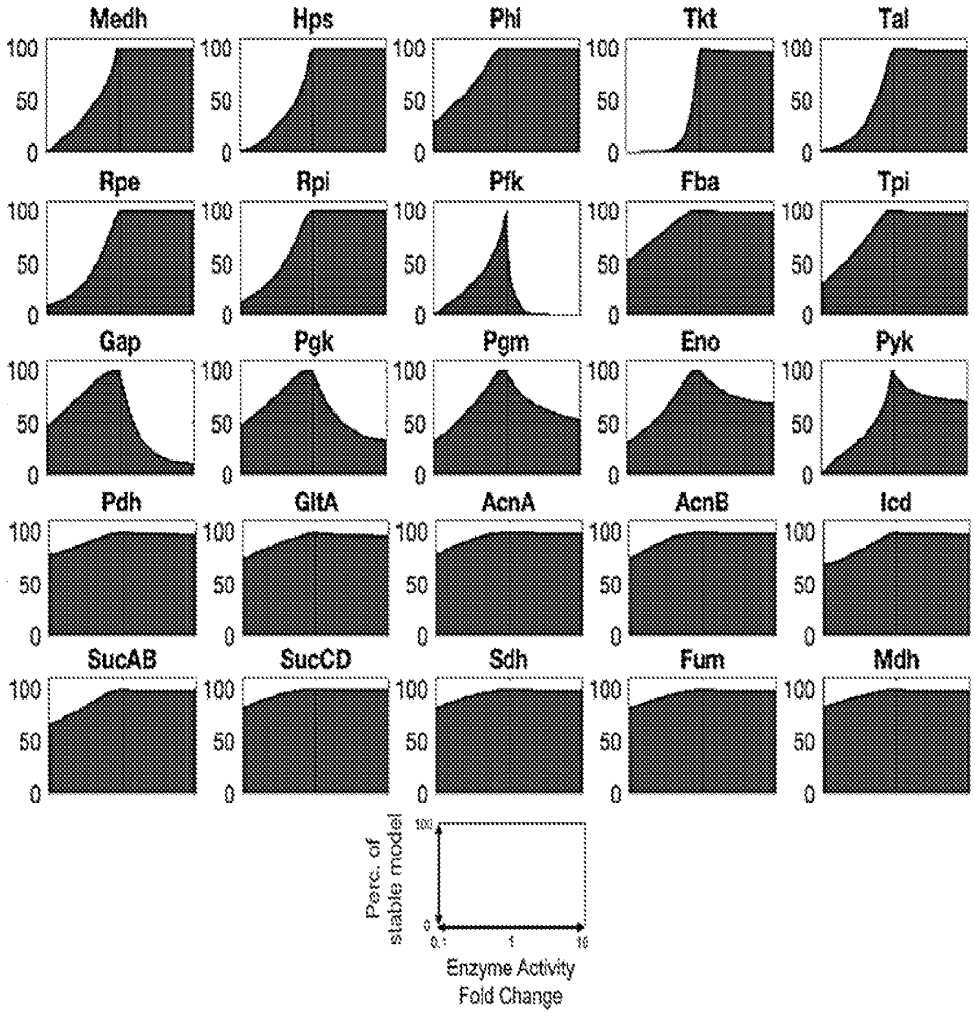
Medh
Hps
Phi
Tkt
Tal
Rpe
Rpi
Pfk
Fba
Tpi
Gap
Pgk
Pgm
Eno
Pyk
Pdh
GltA
AcnA
AcnB
Icd
SucAB
SucCD
Sdh
Fum
Mdh
100
50
0
Perc. of stable model
Enzyme Activity Fold Change

FIG. 2

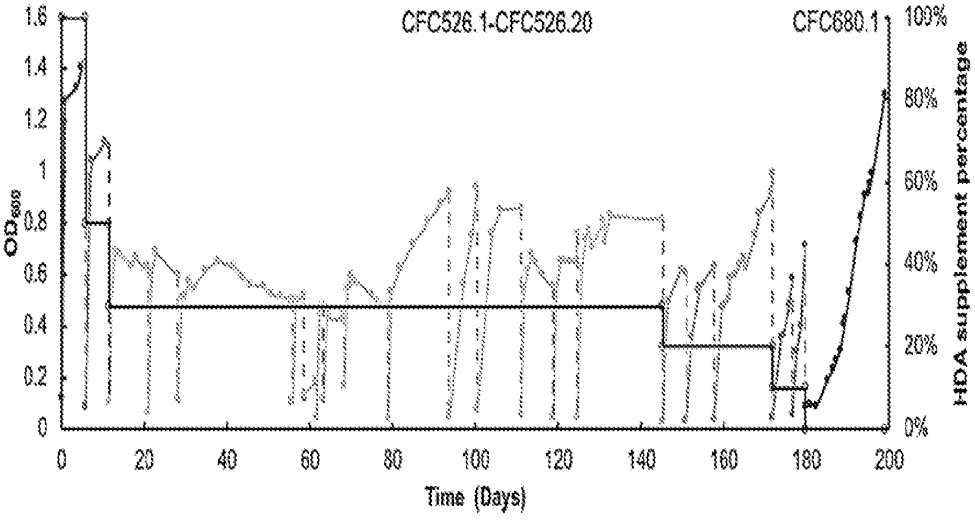
CFC526.1-CFC526.20
CFC680.1
OD600
HDA supplement percentage
Time (Days)
0
20
40
60
80
100
120
140
160
180
200
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
20%
40%
60%
80%
100%

FIG. 3A

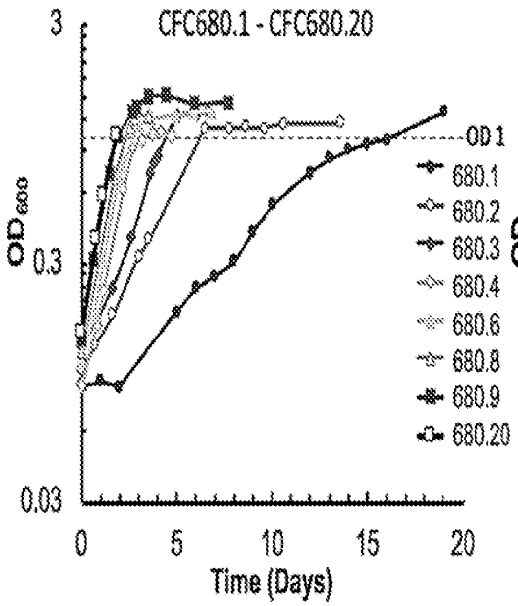

FIG. 3B

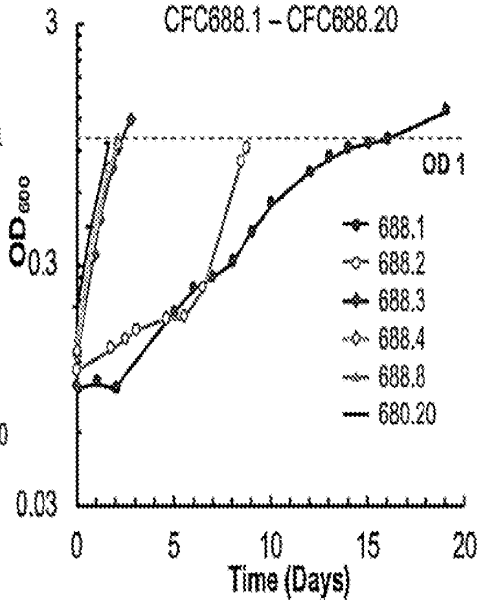

FIG. 3C

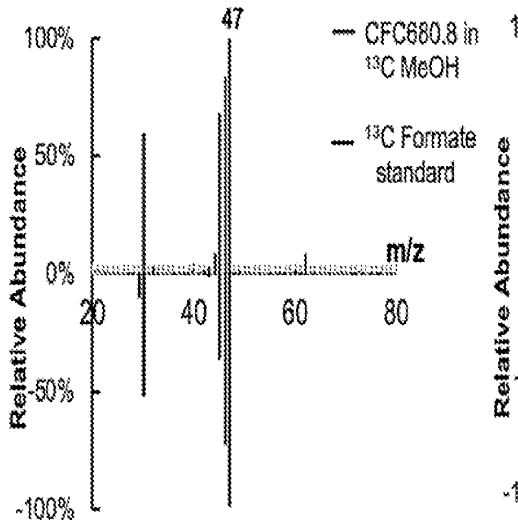

FIG. 3D

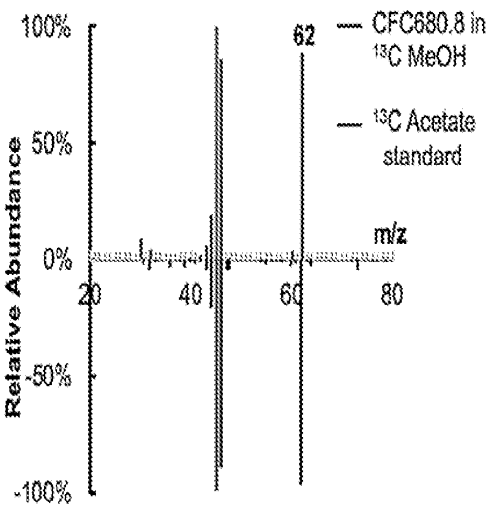

FIG. 3E

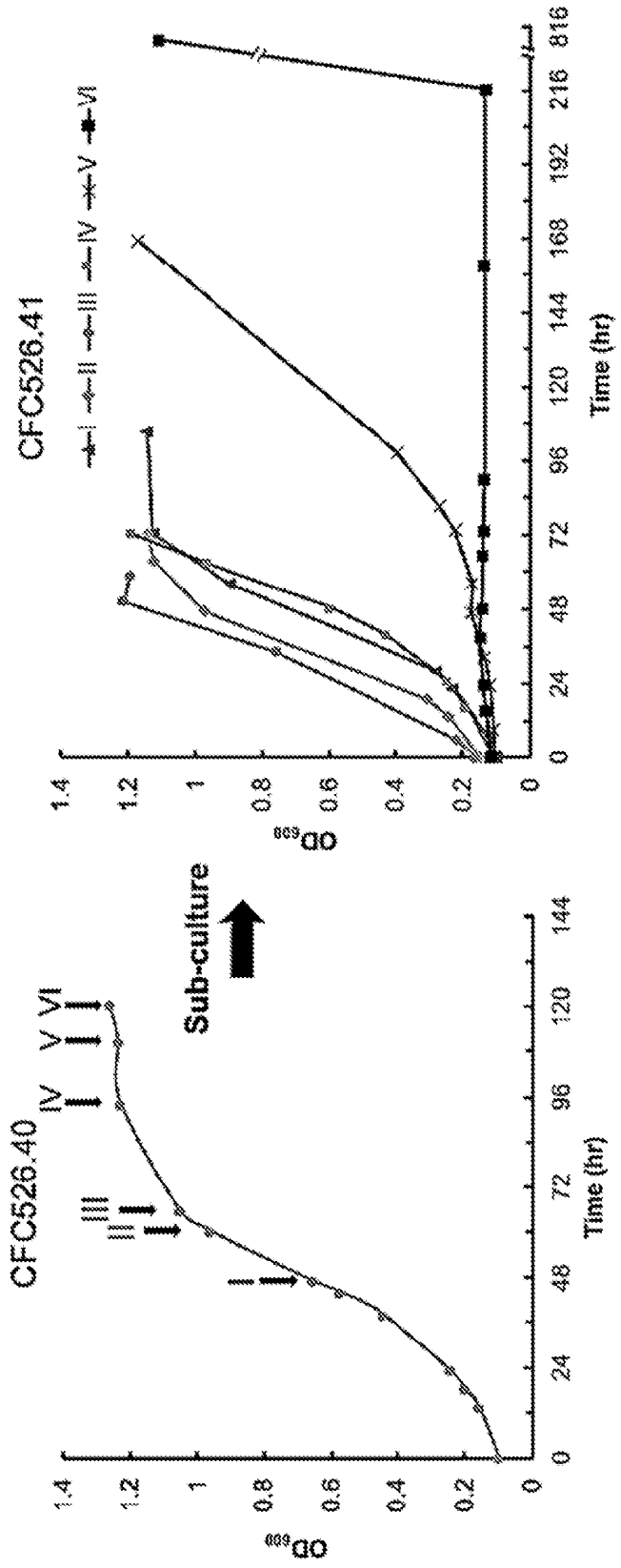
CFC526.41
I II III IV V VI
OD600
Time (hr)
0 24 48 72 96 120 144 168 192 216 816
0 0.2 0.4 0.6 0.8 1 1.2 1.4
Sub-culture
CFC526.40
I II III IV V VI
OD600
Time (hr)
0 24 48 72 96 120 144
0 0.2 0.4 0.6 0.8 1 1.2 1.4

FIG. 4A

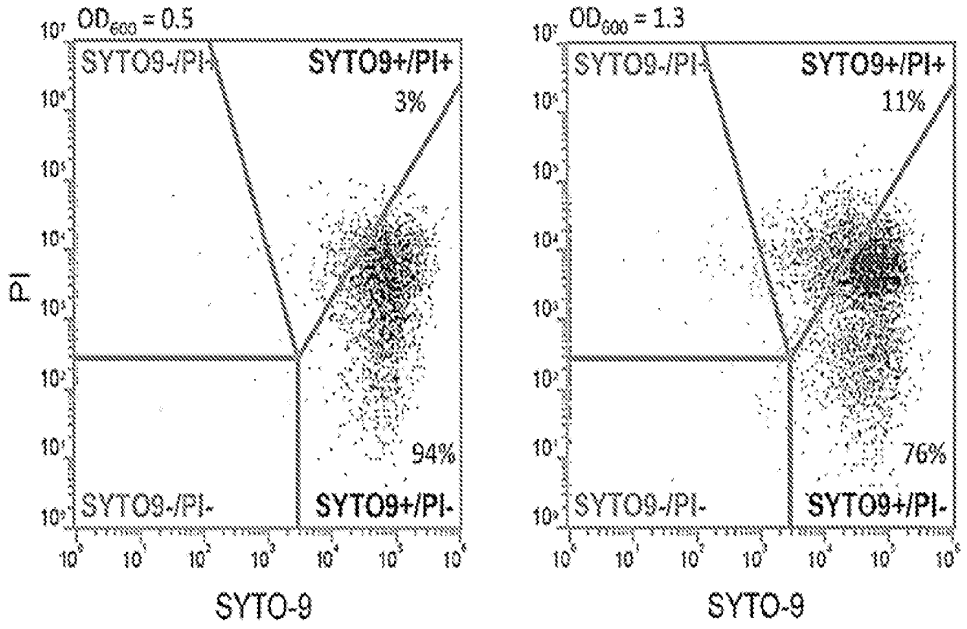
OD600 = 0.5
SYTO9-/PI+
SYTO9+/PI+
3%
94%
SYTO9-/PI-
SYTO9+/PI-
PI
SYTO-9
OD600 = 1.3
SYTO9-/PI+
SYTO9+/PI+
11%
76%
SYTO9-/PI-
SYTO9+/PI-
SYTO-9

FIG. 4B

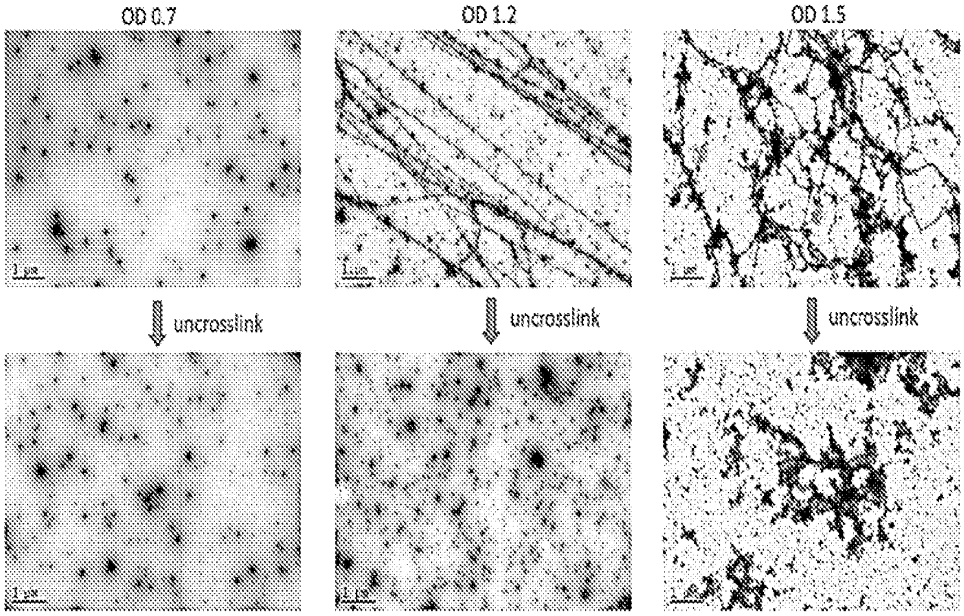
OD 0.7
OD 1.2
OD 1.5
uncrosslink
uncrosslink
uncrosslink

FIG. 4C

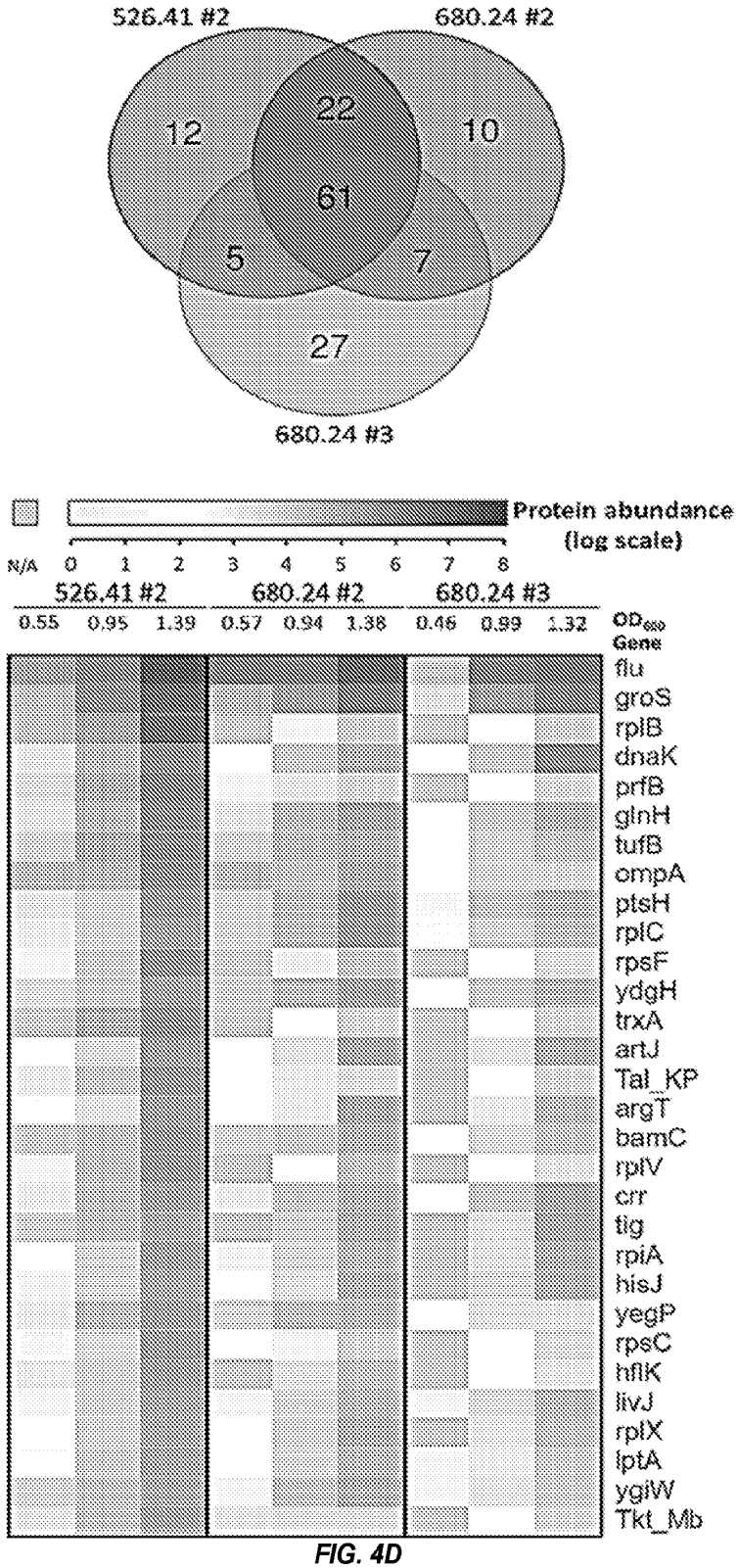
526.41 #2
680.24 #2
680.24 #3
12
22
10
61
5
7
27
Protein abundance
(log scale)
N/A 0 1 2 3 4 5 6 7 8
526.41 #2
680.24 #2
680.24 #3
0.55 0.95 1.39 0.57 0.94 1.38 0.46 0.99 1.32
OD600
Gene
flu
groS
rplB
dnaK
prfB
glnH
tufB
ompA
ptsH
rplC
rpsF
ydgH
trxA
artJ
Tal_KP
argT
bamC
rplV
crr
tig
rpiA
hisJ
yegP
rpsC
hflK
livJ
rplX
lptA
ygiW
Tkt_Mb

FIG. 4D

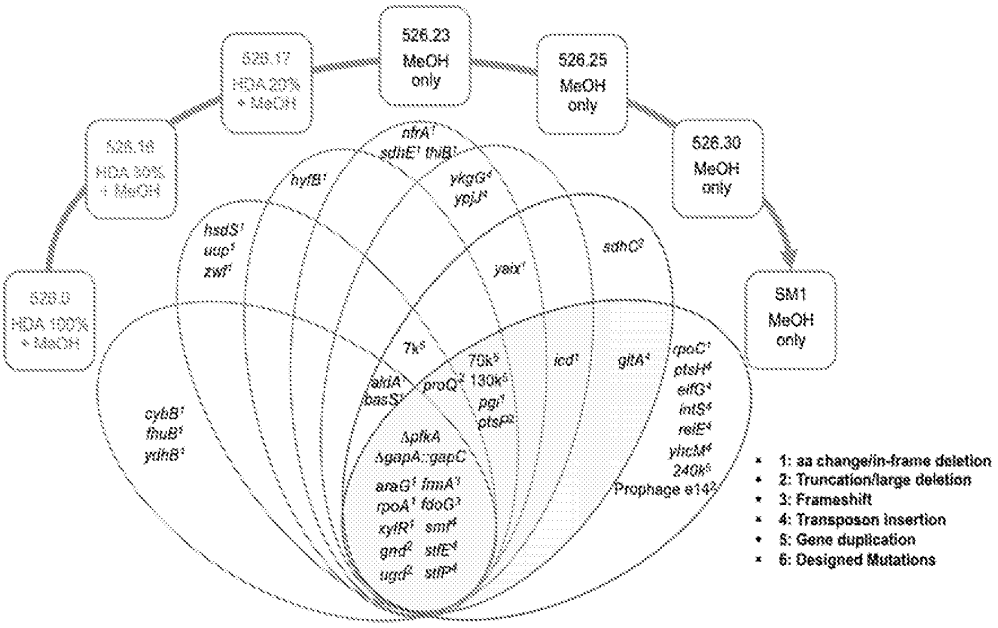
526.23
MeOH
only
526.25
MeOH
only
526.30
MeOH
only
SM1
MeOH
only
526.17
HDA 20%
+ MeOH
526.16
HDA 50%
+ MeOH
526.0
HDA 100%
+ MeOH
nfrA1
sdhE1 thiB1
hyfB1
ykgG4
ypjJ4
hsdS1
uup1
zwf1
sdhC2
yaix1
7k5
70k5
130k5
pgi1
ptsP2
icd1
gltA4
rpoC1
ptsH4
elfG4
intS4
relE4
yhcM4
240k5
Prophage e14
cybB1
fhuB1
ydhB1
ΔpfkA
ΔgapA::gapC
araG1 frmA1
rpoA1 fdoG3
xylR1 smf4
gnd2 stfE4
ugd2 stfP4
1: aa change/in-frame deletion
2: Truncation/large deletion
3: Frameshift
4: Transposon insertion
5: Gene duplication
6: Designed Mutations

FIG. 5A

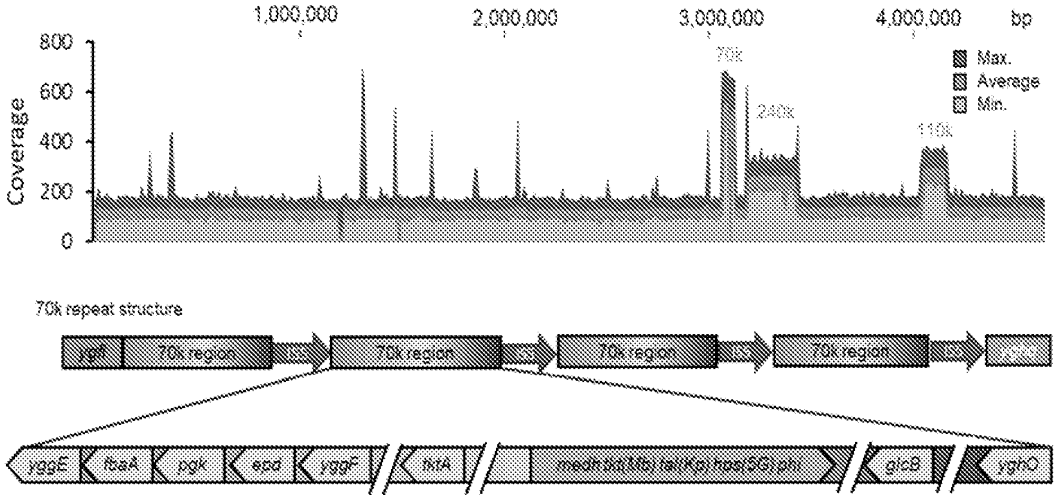
1,000,000
2,000,000
3,000,000
4,000,000
bp
800
600
400
200
0
Coverage
70k
240k
110k
Max.
Average
Min.
70k repeat structure
ygfI
70k region
70k region
70k region
70k region
yggE
fbaA
pgk
epd
yggF
tktA
medh tkt(Mb) tal(Kp) hps(5G) phi
glcB
yghO

FIG. 5B

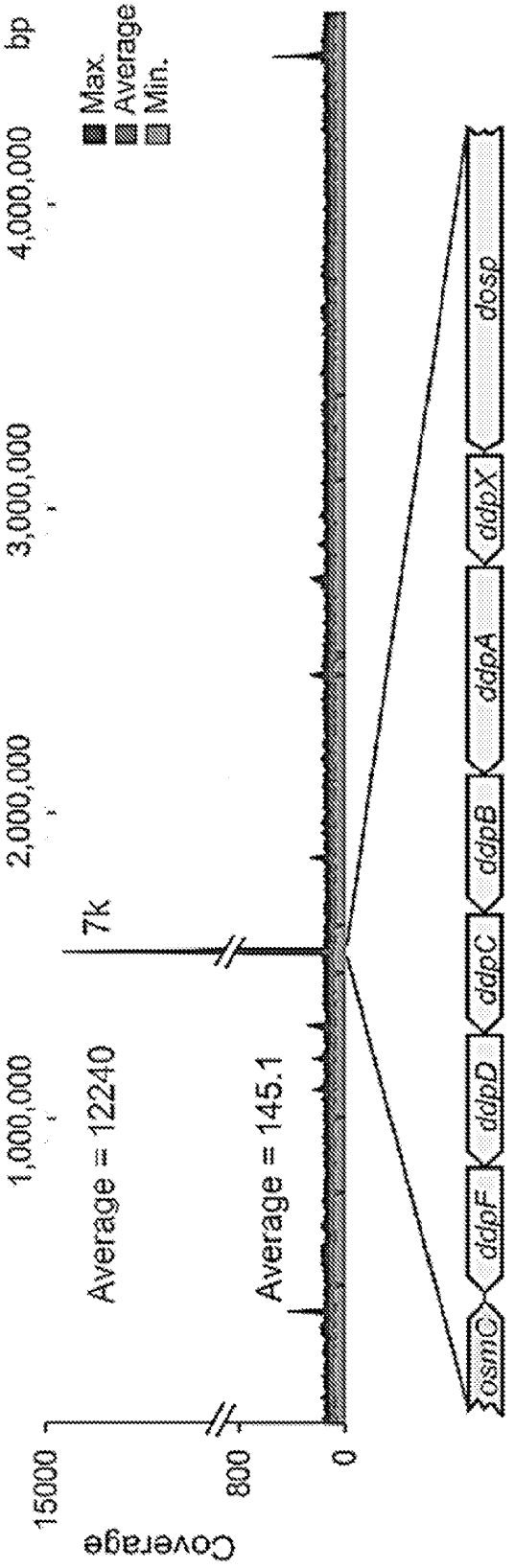
Coverage
15000
800
0
Average = 12240
Average = 145.1
7k
1,000,000
2,000,000
3,000,000
4,000,000
bp
Max.
Average
Min.
osmC
ddpF
ddpD
ddpC
ddpB
ddpA
ddpX
dosp

FIG. 5C

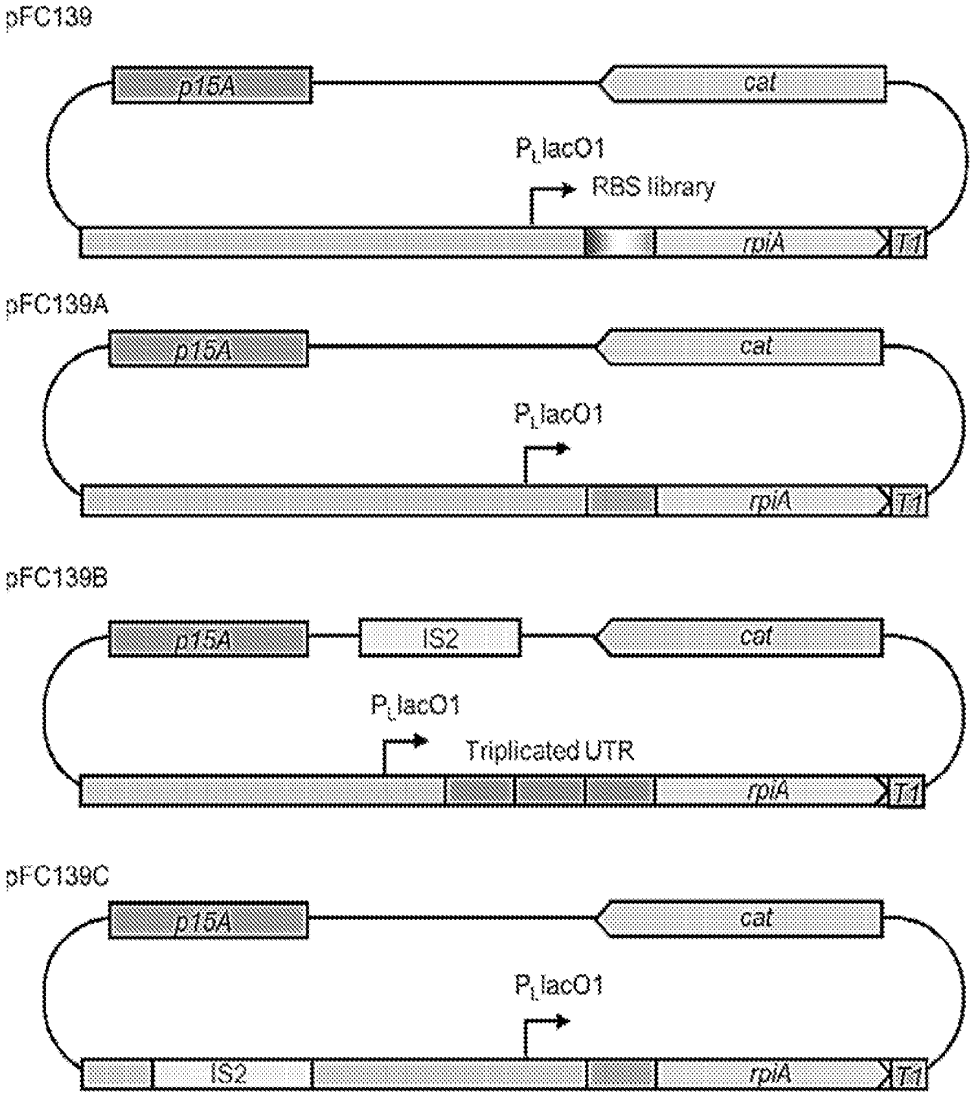
pFC139
p15A
cat
P$_L$lacO1
RBS library
rpiA
T1
pFC139A
p15A
cat
P$_L$lacO1
rpiA
T1
pFC139B
p15A
IS2
cat
P$_L$lacO1
Triplicated UTR
rpiA
T1
pFC139C
p15A
cat
P$_L$lacO1
IS2
rpiA
T1

FIG. 5D

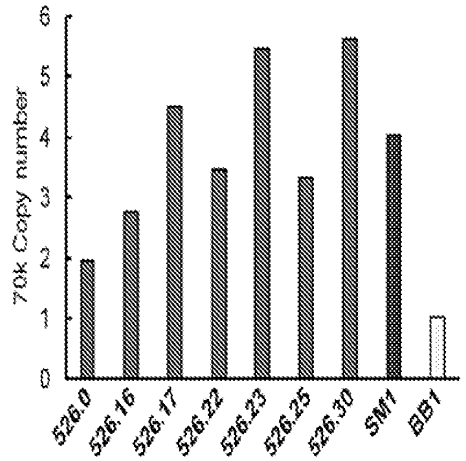

FIG. 6A

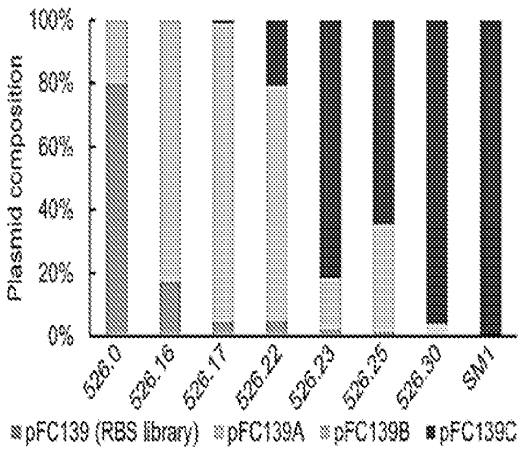

FIG. 6B

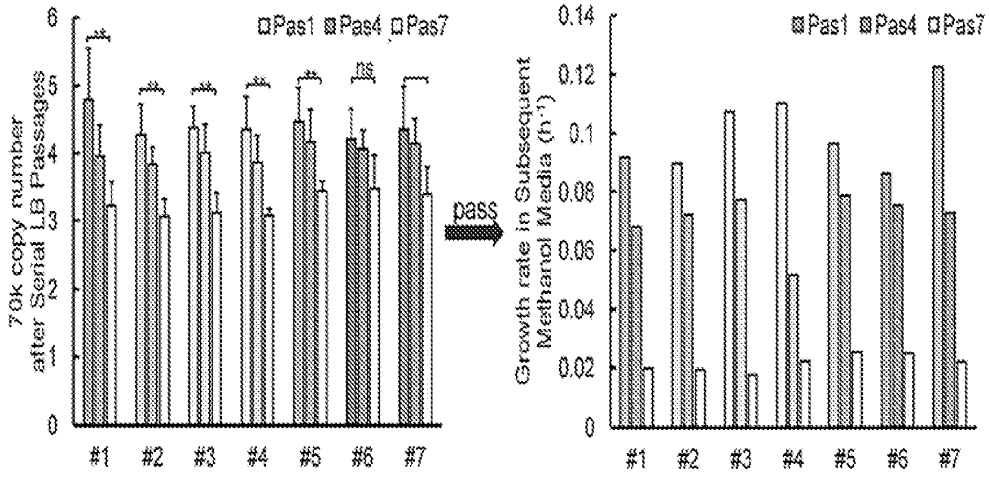

FIG. 6C

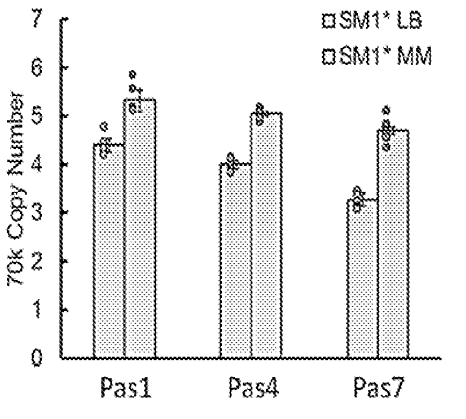

FIG. 6D

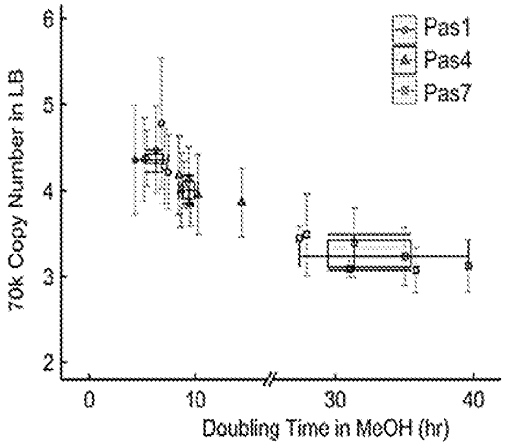

FIG. 6E

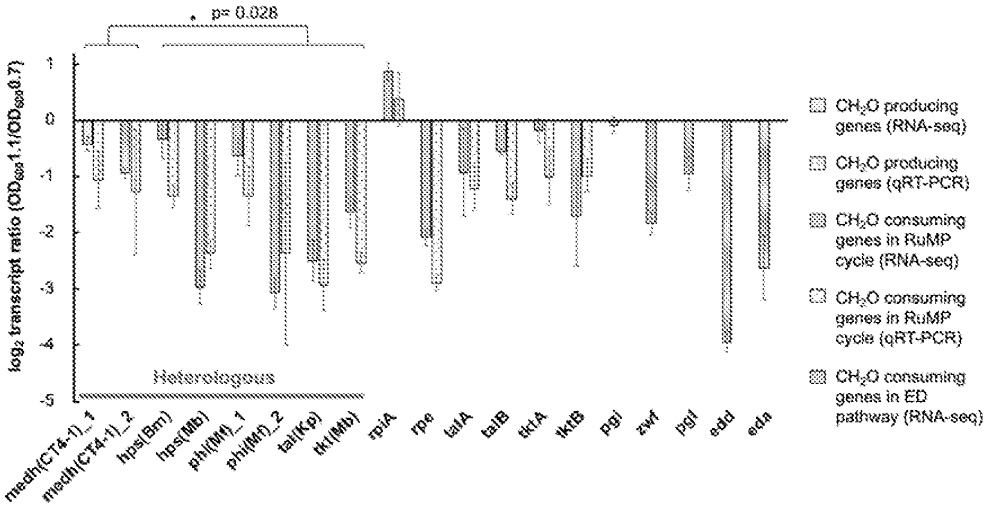
p= 0.028
log2 transcript ratio (OD600 1.1/OD600 0.7)
Heterologous
CH2O producing genes (RNA-seq)
CH2O producing genes (qRT-PCR)
CH2O consuming genes in RuMP cycle (RNA-seq)
CH2O consuming genes in RuMP cycle (qRT-PCR)
CH2O consuming genes in ED pathway (RNA-seq)
medh(CT4-1)_1
medh(CT4-1)_2
hps(Bm)
hps(Mb)
phi(Mf)_1
phi(Mf)_2
tal(Kp)
tkt(Mb)
rpiA
rpe
talA
talB
tktA
tktB
pgl
zwf
pgi
edd
eda

FIG. 7A

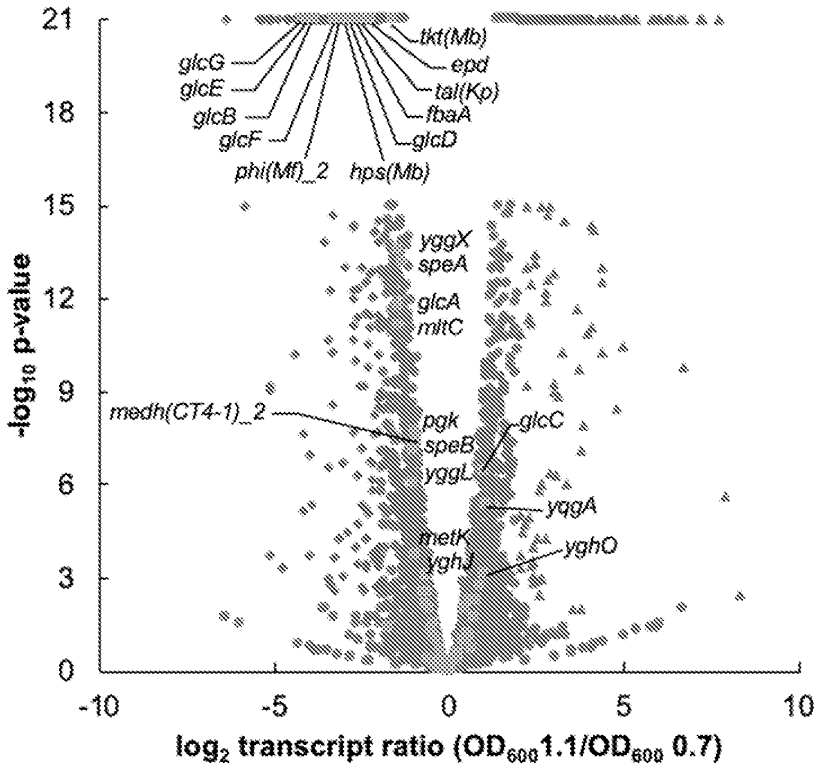
-log10 p-value
log2 transcript ratio (OD600 1.1/OD600 0.7)
tkt(Mb)
epd
tal(Kp)
fbaA
glcD
glcG
glcE
glcB
glcF
phi(Mf)_2
hps(Mb)
yggX
speA
glcA
mltC
medh(CT4-1)_2
pgk
speB
yggL
glcC
yqgA
metK
yghJ
yghO

FIG. 7B

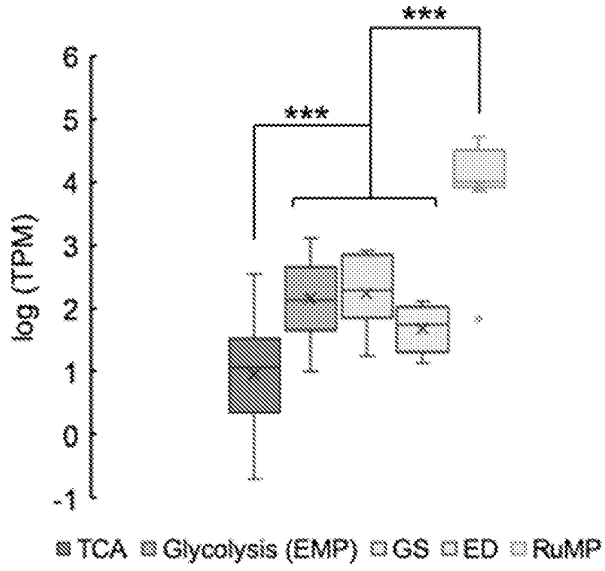
***
***
log (TPM)
6
5
4
3
2
1
0
-1
TCA
Glycolysis (EMP)
GS
ED
RuMP

FIG. 7C

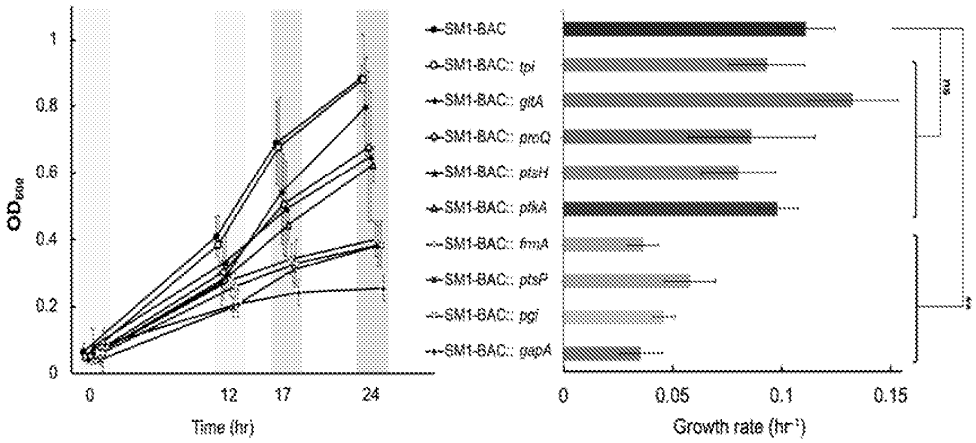
OD600
Time (hr)
SM1-BAC
SM1-BAC:: tpi
SM1-BAC:: gltA
SM1-BAC:: pmQ
SM1-BAC:: ptsH
SM1-BAC:: pfkA
SM1-BAC:: fmA
SM1-BAC:: ptsP
SM1-BAC:: pgi
SM1-BAC:: gapA
Growth rate (hr-1)
ns
*

FIG. 7D

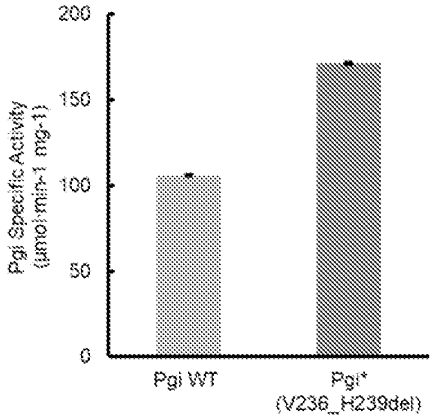

FIG. 7E

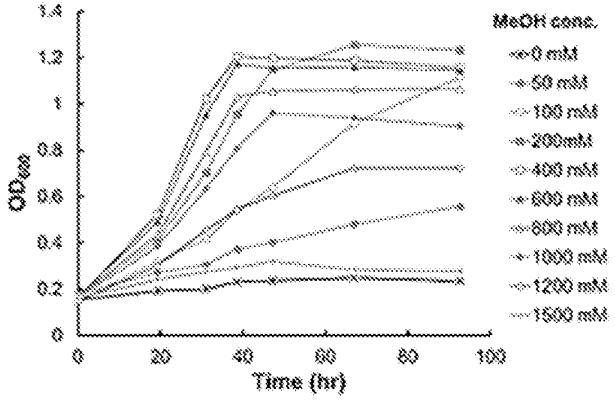

FIG. 7F

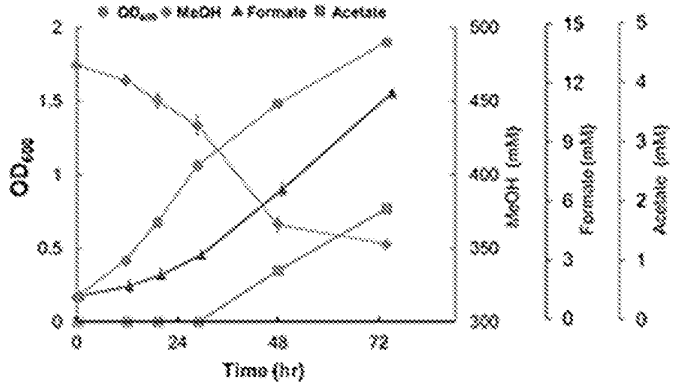

FIG. 7G

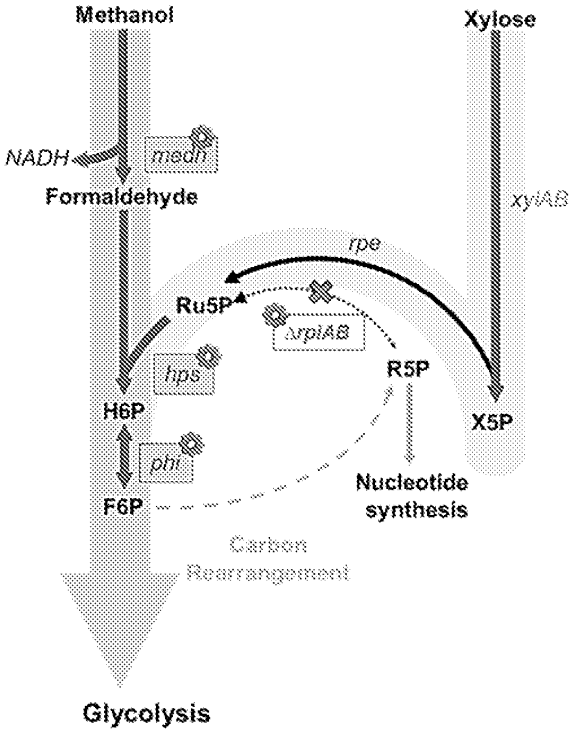
Methanol
Xylose
NADH
mdh
Formaldehyde
xylAB
rpe
Ru5P
ΔrpiAB
hps
R5P
H6P
X5P
phi
F6P
Nucleotide synthesis
Carbon Rearrangement
Glycolysis

FIG. 8A

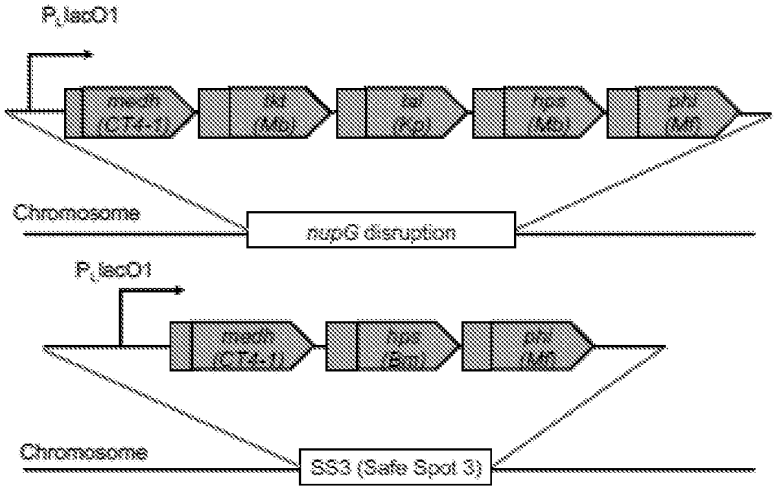

FIG. 8B

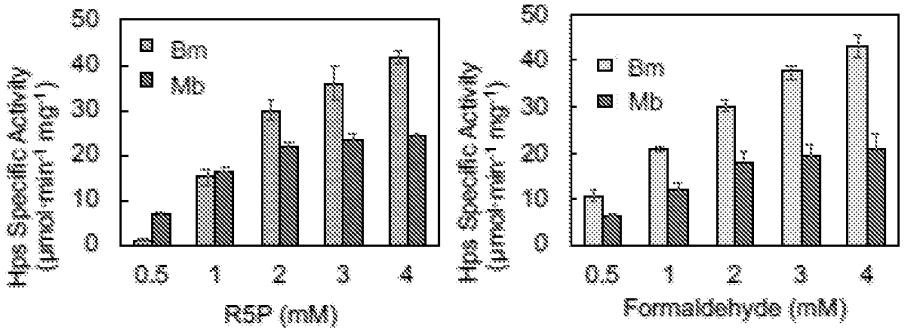

FIG. 8C

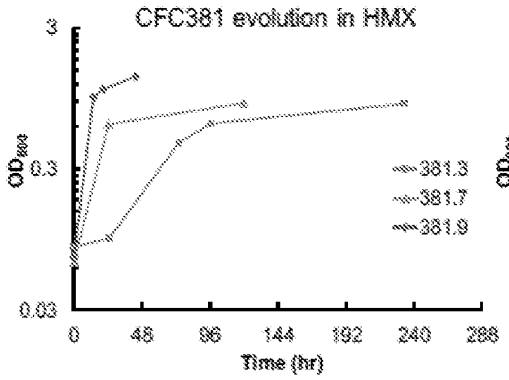

FIG. 8D

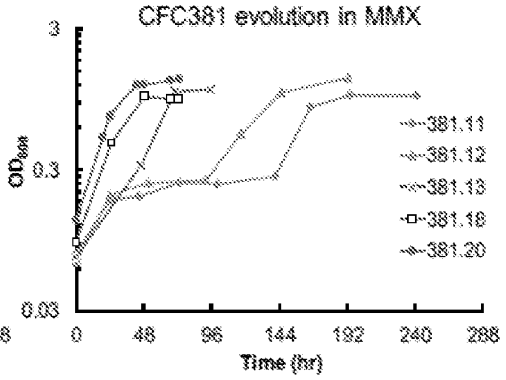

FIG. 8E

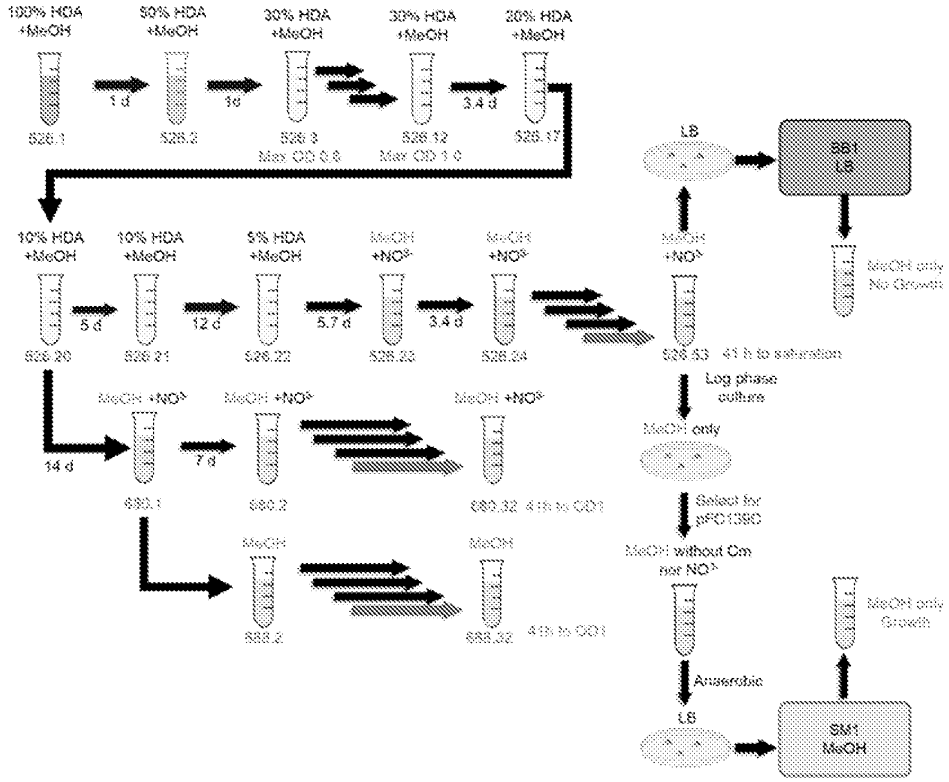
100% HDA +MeOH
50% HDA +MeOH
30% HDA +MeOH
30% HDA +MeOH
20% HDA +MeOH
1 d
1d
3.4 d
526.1
526.2
526.3
526.12
526.17
LB
SM1 LB
10% HDA +MeOH
10% HDA +MeOH
5% HDA +MeOH
MeOH +NO3-
MeOH +NO3-
5 d
12 d
5.7 d
3.4 d
526.20
526.21
526.22
526.23
526.24
526.53
41 h to saturation
Log phase culture
MeOH only
MeOH only No Growth
14 d
7 d
680.1
680.2
680.32
41h to OD1
Select for pFC139D
MeOH without Cm nor NO3-
MeOH
MeOH only Growth
Anaerobic
LB
SM1 MeOH

FIG. 9A

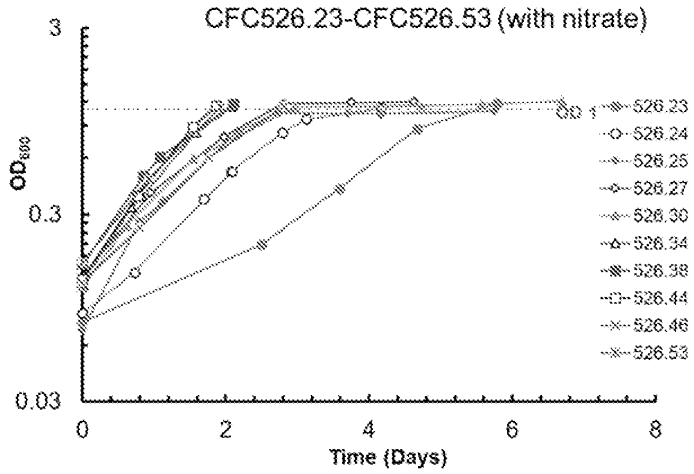
CFC526.23-CFC526.53 (with nitrate)
OD600
3
0.3
0.03
0
2
4
6
8
Time (Days)
OD 1
526.23
526.24
526.25
526.27
526.30
526.34
526.38
526.44
526.46
526.53

FIG. 9B

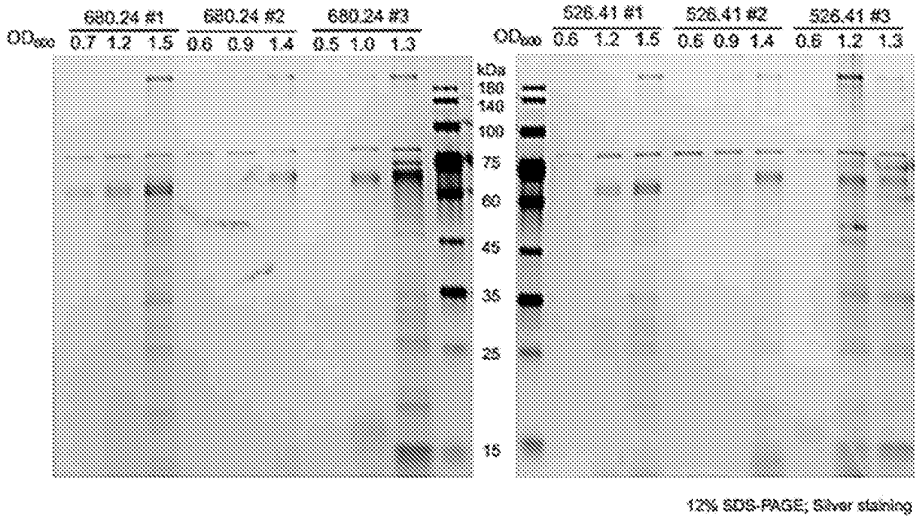

FIG. 10A

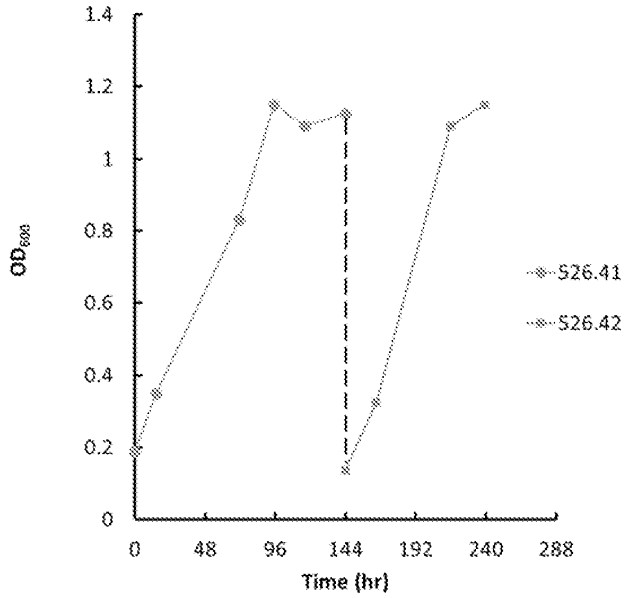

FIG. 10B

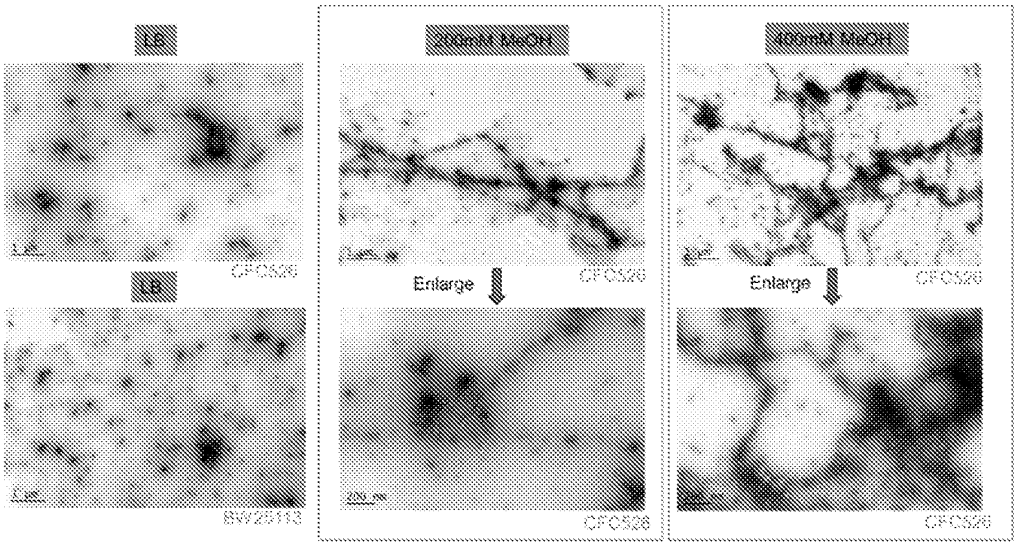
LB
200mM MeOH
400mM MeOH
CFCS26
LB
Enlarge
Enlarge
BW25113
CFCS26
CFCS26
CFCS26

FIG. 10C

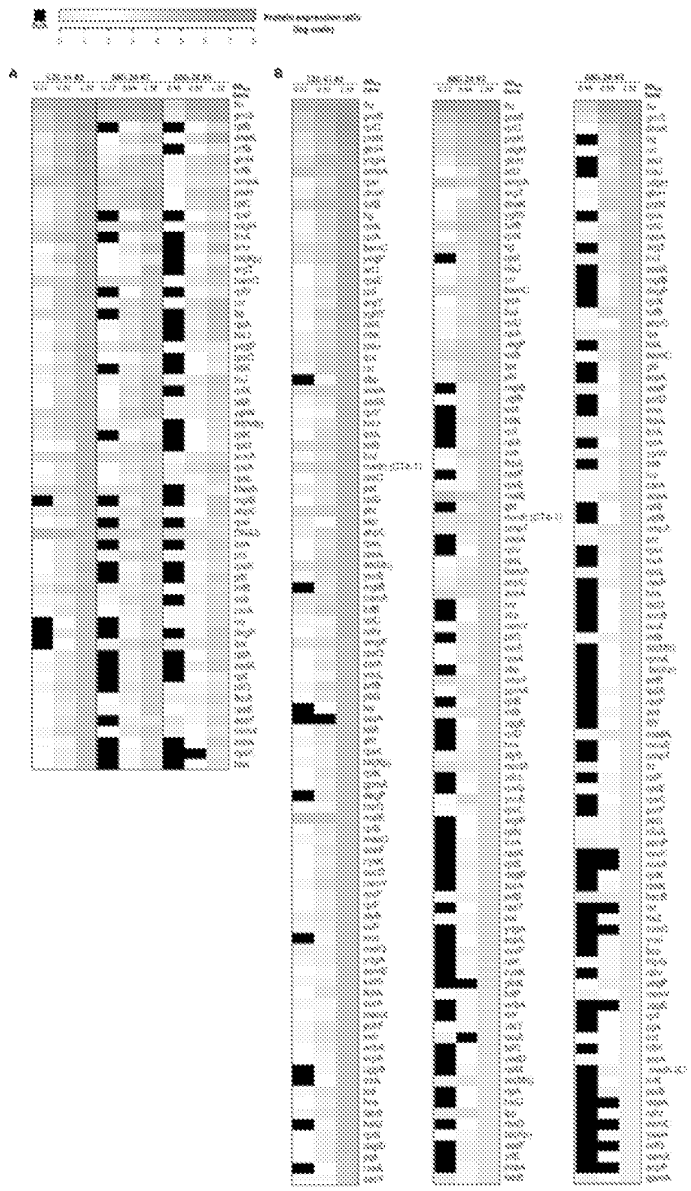

FIG. 11A FIG. 11B

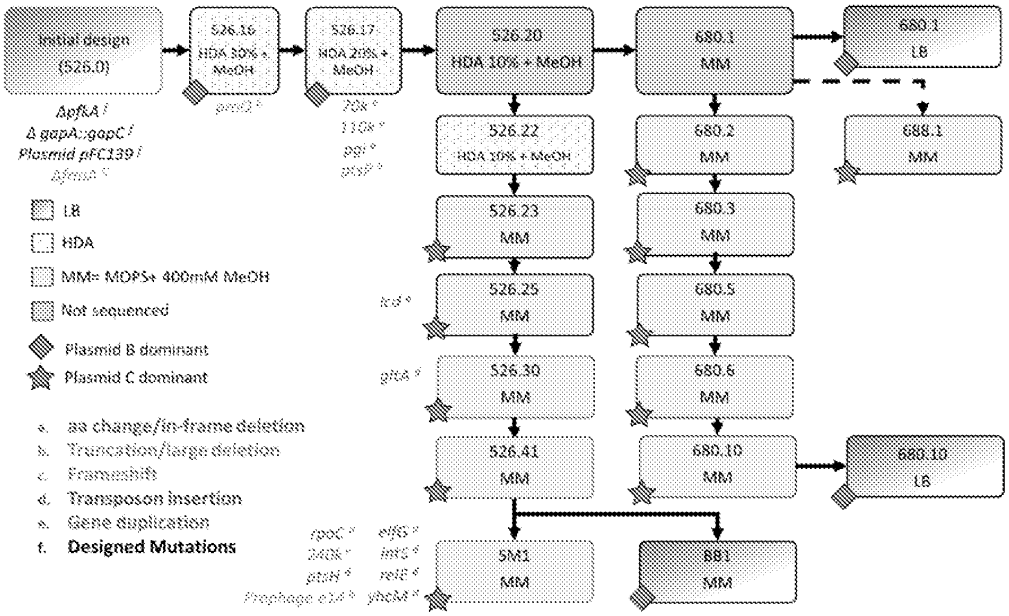

FIG. 12

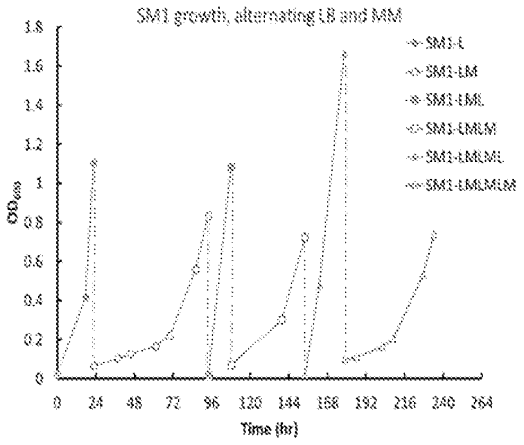

FIG. 13A

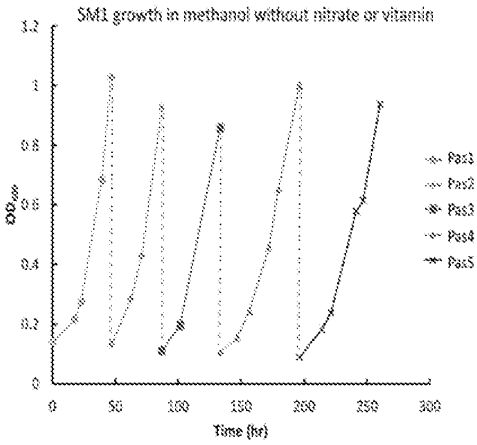

FIG. 13B

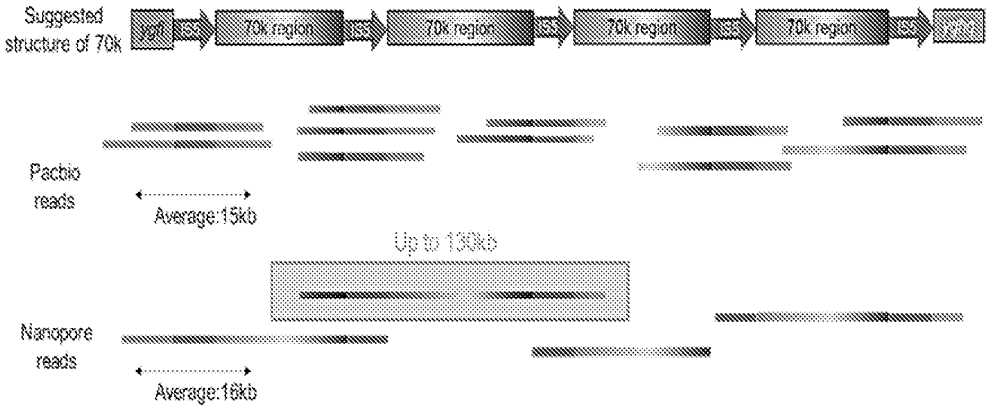

FIG. 14A

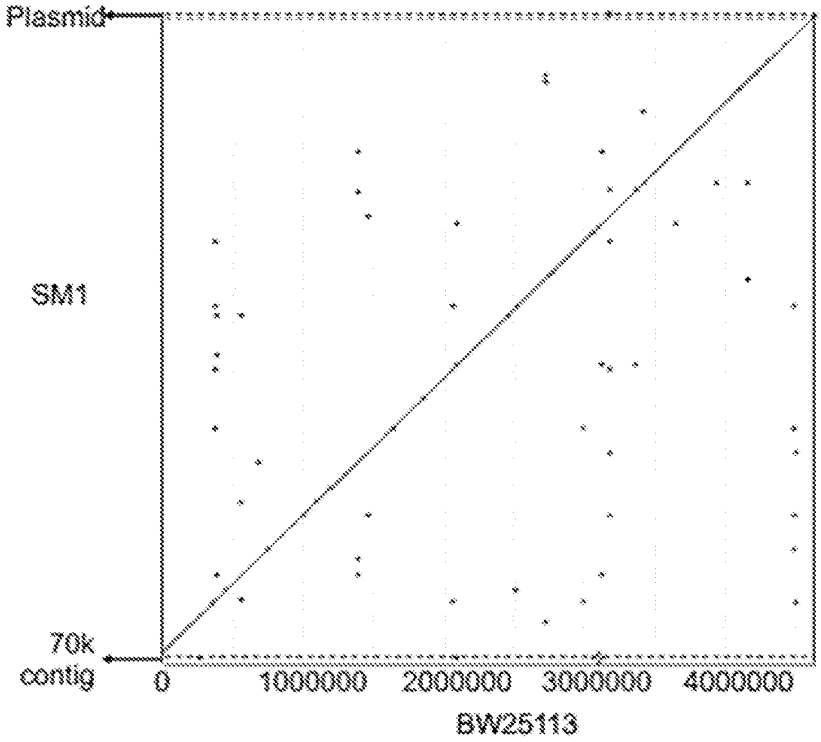

FIG. 14B

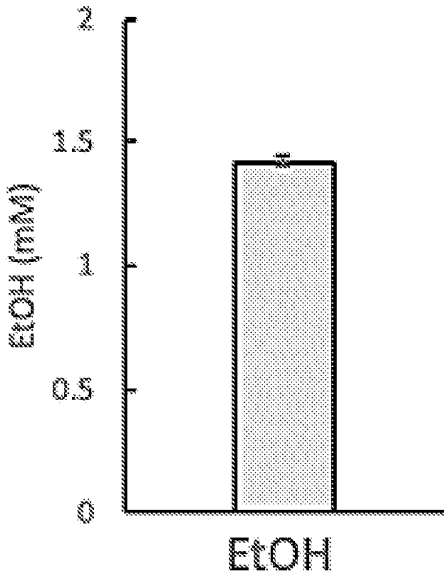

FIG. 15A

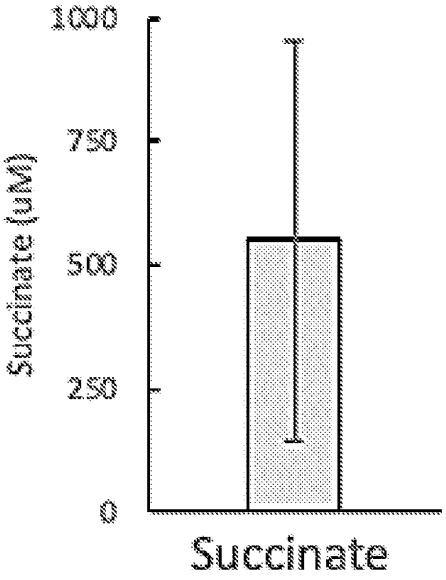

FIG. 15B

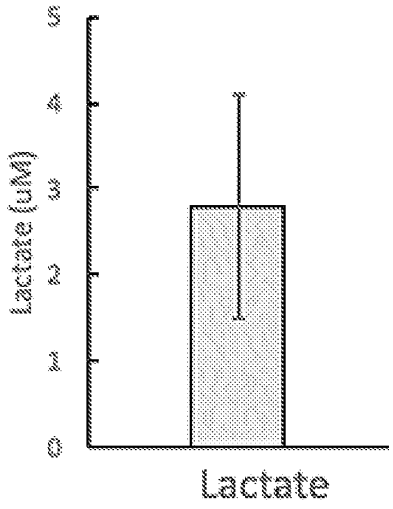

FIG. 15C

Name	0hr Average	24hr Average	0hr SD	24hr SD	Fold Change
(-)-Riboflavin	2.2×10^{3}	5.0×10^{5}	4.4×10^{3}	3.6×10^{4}	227.5
(2R)-6-methylpiperidine-2-carboxylic acid	3.3×10^{5}	2.7×10^{7}	3.1×10^{4}	1.9×10^{6}	83.3
(R)-4-aminoisoxazolidin-3-one	1.3×10^{6}	3.3×10^{7}	7.4×10^{4}	1.0×10^{6}	25.5
1-(4-methoxyphenyl)ethanone	2.1×10^{5}	3.3×10^{6}	5.5×10^{4}	1.8×10^{5}	15.9
1-AMINOCYCLOPROPANE-1-CARBOXYLATE	1.7×10^{6}	8.3×10^{6}	3.3×10^{4}	2.0×10^{6}	48.7
1-Methyladenosine	0	2.2×10^{5}	0	2.0×10^{4}	N/A
1-Methylguanosine	0	2.0×10^{5}	0	2.9×10^{4}	N/A
1,2,3,4-tetrahydro-6-methoxy-1-oxo-beta-carboline	6.9×10^{5}	5.6×10^{5}	2.7×10^{5}	4.1×10^{5}	0.8
10-methylacridone	1.0×10^{6}	4.2×10^{6}	3.3×10^{5}	2.2×10^{5}	3.9
1H-Benzotriazole	1.1×10^{4}	1.1×10^{4}	4.5×10^{3}	9.2×10^{3}	1.0
1h-indole-3-butanoic acid	2.5×10^{5}	3.8×10^{5}	3.1×10^{4}	4.2×10^{4}	1.5
2-{(2H-pyrazolo[3,4-d]pyrimidin-4-yl)amino}ethanol	3.3×10^{6}	3.8×10^{6}	4.9×10^{5}	4.2×10^{5}	1.2
2-(hydroxymethyl)-4(3H)-quinazolinone	2.5×10^{3}	1.3×10^{3}	5.1×10^{3}	2.6×10^{3}	0.5
2-amino-4-hydroxypyrimidine-5-carboxylic acid	1.1×10^{6}	1.4×10^{6}	8.3×10^{5}	4.8×10^{5}	1.3
2-Aminoethanethiol	6.3×10^{3}	9.5×10^{4}	1.5×10^{3}	6.1×10^{4}	15.1
2-AMINOPHENOL	4.7×10^{6}	5.3×10^{6}	2.5×10^{5}	1.7×10^{6}	1.1
2-methyl-4-(pyridin-2-yl)but-3-yn-2-ol	3.0×10^{6}	3.6×10^{6}	2.1×10^{5}	1.6×10^{5}	1.2
2-Methylpyrrolidine	2.8×10^{6}	4.4×10^{8}	4.4×10^{5}	2.4×10^{7}	156.7
2-Phenylacetamide	7.7×10^{5}	1.3×10^{7}	1.3×10^{5}	2.2×10^{6}	17.9
2-Piperidone	5.6×10^{6}	4.2×10^{7}	2.8×10^{5}	3.0×10^{5}	7.5
2'-Deoxyadenosine 5'-monophosphate	4.6×10^{6}	5.8×10^{6}	2.0×10^{5}	1.9×10^{5}	1.3
2'-O-Methyl-5-methylcytidine	0	1.7×10^{6}	0	5.2×10^{4}	N/A
3-(2-hydroxyethyl)quinazolin-4(3H)-one	0	7.3×10^{5}	0	7.3×10^{4}	N/A
3-Amino-1,2,4-triazole	1.0×10^{6}	1.2×10^{6}	4.7×10^{5}	8.2×10^{4}	1.2
3-Methyladenine	1.5×10^{3}	1.1×10^{7}	2.5×10^{3}	1.2×10^{6}	7272.1
3-Pyridinol	9.4×10^{5}	5.1×10^{6}	4.1×10^{5}	4.1×10^{5}	5.4
3,7-dimethyl-6-octenyl acetate	4.4×10^{3}	1.1×10^{4}	1.3×10^{3}	9.9×10^{3}	2.5
4-AMINOBUTYRIC ACID	1.7×10^{6}	2.6×10^{6}	1.5×10^{5}	1.3×10^{5}	1.6
4-aminobutyric acid betaine	4.3×10^{6}	6.5×10^{6}	4.3×10^{5}	4.9×10^{5}	1.5

FIG. 16A

4-hydroxy-4-(pyridin-2-yl)butan-2-one	6.6×10^6	9.0×10^7	8.3×10^5	1.0×10^7	13.6
4-Methyl-5-thiazoleethanol	1.2×10^5	4.6×10^6	7.7×10^3	3.3×10^5	38.4
5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one	4.3×10^5	1.1×10^5	1.5×10^4	1.7×10^4	0.3
5-Methylcytosine	2.3×10^5	1.0×10^7	1.0×10^5	3.3×10^5	44.6
5,6-Dimethylbenzimidazole	5.4×10^4	4.1×10^5	2.7×10^4	2.8×10^5	7.6
5'-S-Methylthioadenosine	3.5×10^3	2.3×10^3	1.2×10^3	2.8×10^3	0.7
6-ethyl-2,3,5-trimethyl-7H-furo[3,2-g]chromen-7-one	2.4×10^4	1.5×10^7	1.3×10^4	8.5×10^5	656.1
6-hydroxybenzofuran-3(2H)-one	3.1×10^7	3.6×10^7	5.0×10^6	4.7×10^6	1.2
7,8-dimethoxy-benzo[d][1,2]oxazin-1-one	6.5×10^3	2.0×10^6	7.9×10^3	3.4×10^5	318.8
8-O-Acetyl shanzhiside methyl ester	7.8×10^5	7.5×10^5	5.3×10^4	4.7×10^4	1.0
Acetylcholine	3.9×10^7	4.8×10^7	1.1×10^6	2.9×10^5	1.2
Adenine	1.9×10^5	6.7×10^6	9.5×10^4	3.8×10^5	34.2
Adenosine	6.0×10^4	1.1×10^7	1.1×10^4	8.7×10^5	196.7
AGMATINE SULFATE	8.7×10^4	3.6×10^3	1.9×10^4	3.8×10^3	0.0
Alanine	1.8×10^5	1.2×10^7	8.6×10^4	1.3×10^5	69.8
alpha-oxo-1h-indole-3-propanoic acid	3.9×10^3	7.8×10^5	7.8×10^3	2.0×10^5	199.9
Anabasamine	3.0×10^4	2.4×10^4	2.5×10^4	1.5×10^4	0.8
Aniline	1.2×10^7	1.5×10^7	1.3×10^6	1.7×10^5	1.2
anthraquinone	1.8×10^5	1.2×10^5	1.4×10^4	2.8×10^5	7.0
Arginine	1.6×10^6	6.8×10^7	4.6×10^5	3.4×10^5	40.4
Benzoic acid	2.0×10^5	6.4×10^6	4.1×10^4	1.1×10^5	31.1
betaine	4.4×10^4	4.7×10^4	3.4×10^4	1.9×10^4	1.1
Cadaverine	1.8×10^5	7.8×10^7	8.3×10^5	5.0×10^5	42.5
Choline	2.8×10^5	2.7×10^5	2.3×10^5	1.8×10^5	1.0
Citrulline	0	3.0×10^5	0	1.3×10^4	N/A
Cowaxanthone B	0	8.8×10^6	0	1.0×10^5	N/A
Creatinine	1.1×10^6	1.0×10^6	4.8×10^5	4.1×10^5	0.9
Cytarabine	0	6.0×10^3	0	7.2×10^3	N/A
Cytosine	2.5×10^4	4.6×10^4	1.6×10^4	3.3×10^4	1.8
D-(+)-Glucosamine	4.4×10^5	3.1×10^7	1.6×10^6	4.8×10^6	7.1
D-(+)-Glucosamine hydrochloride	5.7×10^6	4.9×10^6	5.9×10^5	2.8×10^5	0.9
D-(+)-Pantothenic acid	9.6×10^5	3.2×10^6	1.8×10^5	2.4×10^5	3.4
D-Ala-D-ala	7.4×10^5	4.8×10^6	7.0×10^4	6.2×10^4	6.6

FIG. 16A (continued)

DEOXYCARNITINE	2.4×10^5	2.2×10^5	7.1×10^4	7.5×10^4	0.9
Desethyl atrazine	8.0×10^4	2.8×10^7	3.2×10^4	2.3×10^6	361.3
desmotroposantonin	0	6.5×10^3	0	4.8×10^3	N/A
DIETHANOLAMINE	4.3×10^5	3.3×10^5	5.4×10^5	1.8×10^5	0.8
Dihydrolycorine	0	9.4×10^2	0	1.8×10^3	N/A
DL-alpha,epsilon-Diaminopimelic acid	1.3×10^4	7.7×10^5	9.1×10^3	8.9×10^4	58.2
Dopamine	3.5×10^5	9.1×10^5	1.8×10^5	1.1×10^5	2.6
Glutathione	7.3×10^5	8.3×10^4	1.0×10^5	2.5×10^4	0.1
Glycerol-2-phosphate	2.1×10^3	9.1×10^2	4.2×10^3	1.8×10^3	0.4
Glycine-Betaine	2.2×10^7	2.9×10^7	3.0×10^6	1.2×10^7	1.3
Guanidinoacetic acid	1.8×10^6	2.3×10^7	7.1×10^4	7.8×10^5	12.3
Heteropeucenin, Methyl Ether	0	2.2×10^5	0	4.5×10^4	N/A
Histidine	6.0×10^5	7.0×10^7	2.2×10^5	3.3×10^6	116.3
honokiol	1.7×10^3	3.5×10^5	3.4×10^3	4.3×10^4	207.9
Hypoxanthine	2.6×10^4	3.1×10^6	9.0×10^3	3.7×10^5	119.1
Indole-3-carbinol	6.1×10^4	1.1×10^5	3.0×10^4	7.2×10^4	1.9
Indole-3-carboxyaldehyde	3.0×10^4	3.3×10^6	3.0×10^4	2.9×10^5	109.5
Indoline	7.6×10^6	2.0×10^8	4.0×10^5	6.9×10^6	26.3
INDOXYL SULFATE	6.5×10^4	2.1×10^6	2.0×10^4	1.8×10^5	32.5
Inosine	0	8.1×10^5	0	3.6×10^4	N/A
Isoleucine	1.5×10^5	5.7×10^7	6.7×10^4	1.3×10^7	385.5
Isonicotinic acid	8.7×10^3	7.0×10^4	3.2×10^3	4.3×10^4	8.1
Ketoisovaleric acid	1.5×10^3	8.0×10^5	3.1×10^3	1.1×10^5	506.6
L-(-)-Phenylalanine	2.0×10^7	1.0×10^8	7.1×10^5	6.3×10^6	5.0
L-(-)-Proline	1.8×10^5	1.8×10^5	8.6×10^4	1.8×10^5	9.9
L-(+)-Arginine	1.4×10^4	2.8×10^5	6.1×10^3	1.4×10^5	190.9
L-5-Oxoproline	3.6×10^5	3.4×10^7	2.0×10^4	6.3×10^5	95.3
L-beta-Homoproline	1.1×10^6	4.9×10^5	1.0×10^5	2.2×10^5	0.4
L-Carnitine	1.2×10^4	1.2×10^4	5.0×10^3	6.7×10^3	1.0
L-Glutamic acid	1.2×10^6	1.1×10^8	9.9×10^4	1.6×10^6	89.5
Leucine	4.2×10^5	5.4×10^7	7.7×10^4	1.3×10^6	129.0
lindenenol	5.4×10^5	7.9×10^5	6.9×10^4	7.1×10^4	1.5
Lysine	2.9×10^5	6.0×10^6	1.2×10^5	2.0×10^5	20.6
Malic acid	0	2.8×10^5	0	2.8×10^5	N/A
Malvidin	6.4×10^5	7.9×10^5	3.3×10^4	4.7×10^4	1.2
Methionine	9.5×10^3	1.6×10^7	1.9×10^4	1.8×10^6	1748.6
Morpholine	4.8×10^8	5.8×10^8	1.5×10^7	1.0×10^7	1.2
Myclobutanil	2.0×10^4	8.0×10^5	9.8×10^3	9.0×10^4	38.4

FIG. 16A (continued)

N-(2-furylmethyl)-9H-purin-6-amine	6.2×10^4	4.3×10^5	1.1×10^4	2.9×10^4	6.8
N-Acetylarginine	0	2.4×10^5	0	5.0×10^4	N/A
N-Acetylhistidine	0	9.1×10^5	0	2.1×10^4	N/A
N-Acetylmuramic Acid	8.5×10^2	2.2×10^6	1.7×10^3	5.3×10^4	2609.4
N-Acetylputrescine	4.4×10^8	1.0×10^9	1.0×10^7	2.6×10^8	2.5
N-epsilon-Acetyllysine	2.6×10^4	5.2×10^5	2.5×10^4	1.2×10^5	20.0
N-Methyl-L-proline	1.5×10^6	8.0×10^5	1.2×10^5	7.1×10^4	0.5
N-Methylanthranilic Acid	1.6×10^6	5.6×10^5	2.1×10^5	3.9×10^5	3.5
N-Methylisoleucine	6.8×10^5	2.3×10^5	2.0×10^5	2.1×10^5	3.5
N-Methylproline	3.1×10^5	4.7×10^6	8.2×10^4	2.1×10^5	15.1
N,N-Dimethylaniline	1.4×10^7	1.8×10^7	1.9×10^6	5.1×10^5	1.2
N,N-Dimethylarginine	1.9×10^3	1.9×10^3	2.5×10^3	2.2×10^3	1.0
N2-Methylguanosine	0	2.6×10^5	0	1.0×10^5	N/A
N8-Acetylspermidine	4.0×10^7	1.2×10^8	2.6×10^6	8.1×10^6	3.1
Nicotinamide	3.6×10^5	4.9×10^7	1.4×10^5	8.9×10^6	135.5
Nordihydroguaiaretic acid	2.9×10^5	3.4×10^5	6.8×10^4	7.2×10^4	1.2
Obtucarbamate A	1.3×10^5	2.7×10^5	2.3×10^4	4.7×10^4	2.0
Ophthalmic acid	2.1×10^3	1.0×10^4	4.3×10^3	8.1×10^3	4.7
Ornithine	6.0×10^5	5.4×10^5	2.7×10^5	1.3×10^5	0.9
p-Coumaraldehyde	1.1×10^5	2.3×10^5	3.1×10^4	1.5×10^5	21.0
Pantethine	0	5.8×10^5	0	5.8×10^5	N/A
Phenylacetaldehyde	7.3×10^4	2.7×10^5	3.1×10^4	9.9×10^4	3.8
Phenylalanine	1.3×10^6	5.1×10^7	1.4×10^5	7.4×10^7	37.5
Phenylglycine	4.3×10^5	8.0×10^6	1.8×10^5	3.0×10^5	18.6
PIPERIDINE	1.2×10^6	1.5×10^6	1.6×10^5	7.9×10^5	1.2
Proline	6.9×10^5	1.6×10^8	8.6×10^4	2.5×10^7	234.6
Propachlor	1.8×10^9	2.0×10^9	6.0×10^7	2.3×10^8	1.1
Propamocarb	4.2×10^5	1.1×10^6	4.5×10^4	9.5×10^4	2.8
PTERIN	1.0×10^6	1.1×10^7	5.4×10^4	3.0×10^5	10.7
Putrescine	6.7×10^6	1.0×10^8	4.6×10^5	1.4×10^7	15.2
Pyridine	5.8×10^6	9.0×10^6	7.2×10^5	7.9×10^6	1.5
Pyridoxal 5-phosphate	3.0×10^5	4.4×10^6	7.3×10^4	1.8×10^5	14.8
Pyridoxamine	1.7×10^5	8.7×10^6	3.6×10^4	2.9×10^5	49.5
PYRIDOXINE	1.9×10^6	1.6×10^7	1.9×10^5	1.5×10^6	8.3
Pyrimidinol	1.3×10^6	1.6×10^6	1.0×10^5	4.5×10^5	1.2
Pyrrolidine	1.0×10^8	9.4×10^8	6.7×10^6	1.3×10^8	9.3
rhein	1.2×10^4	6.6×10^5	5.0×10^3	2.5×10^5	51.7
S-Adenosyl-L-methionine	1.6×10^5	2.6×10^5	1.6×10^4	1.4×10^5	1.6

FIG. 16A (continued)

Sec-O-glucosylhamaudol	0	1.9×10^5	0	2.2×10^4	N/A
securinine	0	7.0×10^5	0	3.2×10^4	N/A
Shanzhiside	6.7×10^5	6.8×10^4	4.5×10^4	2.2×10^4	0.1
TETRAMETHYLENEDIAMINE	7.1×10^5	1.1×10^5	1.1×10^5	6.4×10^4	1.7
Thiamine; LC-ESI-QTOF; MS2; CE	2.4×10^5	7.9×10^6	6.4×10^3	5.4×10^5	32.3
Threonine	2.4×10^4	4.0×10^6	1.3×10^4	3.5×10^5	167.2
Trachelanthine	7.4×10^5	9.2×10^5	7.7×10^4	5.9×10^4	1.2
trans-4-Hydroxy-L-proline	2.3×10^6	2.7×10^6	1.5×10^5	2.4×10^5	1.1
Triethanolamine	6.0×10^7	2.7×10^7	3.2×10^6	3.3×10^6	0.5
Trimethylamine N-oxide	7.2×10^5	1.2×10^7	4.7×10^4	5.1×10^5	16.5
TRYPTOPHAN	1.3×10^4	1.3×10^7	1.2×10^4	1.0×10^6	1014.0
Tryptophanol	8.8×10^3	2.4×10^6	3.3×10^3	2.5×10^5	273.1
Tyrosine	1.0×10^6	2.9×10^7	1.2×10^5	4.9×10^6	27.7
UROCANIC ACID	3.0×10^6	2.4×10^6	7.3×10^5	8.7×10^4	0.8
Wogonin	3.7×10^4	2.1×10^6	1.0×10^4	8.2×10^4	57.2
Xanthine	1.7×10^4	1.5×10^7	1.3×10^4	4.2×10^6	884.7
Xanthosine	2.8×10^5	6.1×10^4	4.0×10^4	2.5×10^4	0.2
XANTHURENIC ACID	7.3×10^5	8.6×10^5	1.0×10^5	3.8×10^4	1.2

FIG. 16A (continued)

Name	0hr Average	24hr Average	0hr SD	24hr SD	Fold Change
(-)-Citramalic acid	1.2×10^5	5.8×10^6	1.1×10^4	4.3×10^4	4.6
1,4-Butynediol	4.5×10^4	4.6×10^4	2.2×10^4	1.2×10^4	1.0
2-Hydroxy-4-methylpentanoic acid	2.5×10^6	2.8×10^7	1.7×10^5	1.8×10^6	11.5
2-Oxobutyric acid	4.2×10^5	5.0×10^5	3.2×10^5	7.6×10^4	1.2
2',4'-Dihydroxychalcone	4.0×10^4	1.9×10^6	7.3×10^3	1.3×10^5	47.0
2',4',6'-Trihydroxydihydrochalcone	0	2.0×10^3	0	2.8×10^3	N/A
3-Hydroxymandelic acid	1.1×10^7	1.3×10^7	4.6×10^5	2.6×10^6	1.1
3-Phenyllactic acid	3.7×10^6	1.1×10^7	1.1×10^5	1.4×10^6	3.0
3,4-Dihydroxybenzoic acid	7.8×10^5	3.3×10^6	1.6×10^4	1.3×10^6	4.3
3'-O-Methylguanosine	0	2.4×10^5	0	2.7×10^4	N/A
4-Hydroxy-6-Methylpyran-2-One	7.0×10^3	4.9×10^3	4.2×10^3	2.1×10^3	0.7
4-Hydroxybenzaldehyde	1.1×10^6	6.7×10^5	8.8×10^4	1.6×10^5	0.6
4,4'-Thiodiphenol	1.3×10^5	1.1×10^5	4.8×10^4	3.4×10^4	0.9
6,7-DIHYDROXYCOUMARIN	1.1×10^4	1.6×10^6	1.0×10^4	8.4×10^4	139.5
8-Methylsulfinyloctyl isothiocyanate	4.3×10^2	6.6×10^2	8.7×10^2	1.3×10^3	1.5
Acetylleucine	9.9×10^4	5.0×10^5	1.6×10^4	2.8×10^4	5.1
Adenine	8.9×10^5	8.9×10^6	1.0×10^5	3.1×10^5	10.0
Ala-Ala	5.4×10^5	2.8×10^6	6.6×10^4	2.4×10^5	5.2
Ala-Ile	5.0×10^2	7.9×10^2	2.4×10^2	4.6×10^2	1.6
alpha-L-(-)-Fucose 1-phosphate bis(cyclohexylammonium) salt	2.9×10^3	5.6×10^3	2.1×10^3	3.8×10^3	1.9
Aspartate	3.1×10^5	2.0×10^7	4.5×10^4	1.9×10^6	64.1
Caffeic acid	4.5×10^4	1.4×10^5	9.2×10^3	1.3×10^4	3.2
Catechol	4.7×10^4	2.2×10^5	1.0×10^4	8.8×10^4	4.8
D-(+)-Malic acid	9.0×10^5	2.8×10^6	8.9×10^4	1.4×10^5	3.2
D-(+)-Pantothenic acid	1.0×10^6	5.6×10^6	6.7×10^4	7.5×10^4	5.2
D-GLYCERIC ACID	4.6×10^5	2.9×10^5	5.3×10^4	6.0×10^4	0.6
D-Ribose 5-phosphate	6.7×10^2	1.0×10^3	4.7×10^2	1.1×10^3	1.5
D-Sorbitol	1.1×10^5	1.3×10^6	2.6×10^4	9.9×10^4	11.7
D-Sorbitol 6-phosphate	1.5×10^5	1.2×10^5	1.4×10^4	1.7×10^5	8.1
Dihydroxy-Valerate	2.6×10^5	3.5×10^5	3.2×10^4	1.7×10^4	1.3
DL-2-Hydroxyvaleric acid	7.7×10^5	6.5×10^6	9.1×10^4	2.4×10^5	8.4
DL-3-(4-Hydroxyphenyl)lactic acid	3.7×10^5	2.3×10^6	3.6×10^4	2.3×10^5	6.3
DL-3-Aminoisobutyric acid	1.1×10^5	5.9×10^6	1.2×10^4	5.4×10^5	53.9

FIG. 16B

DL-beta-Hydroxybutyric acid	1.2×10^5	5.1×10^6	2.8×10^4	2.9×10^5	42.7
Dodecylbenzenesulfonic acid	6.6×10^5	4.6×10^5	7.8×10^3	1.2×10^4	0.7
Fumaric acid	1.0×10^6	7.8×10^5	3.2×10^4	6.2×10^4	0.8
Glucose	1.4×10^4	7.4×10^3	6.1×10^3	2.9×10^3	0.5
GLUTARIC ACID	4.5×10^5	6.1×10^5	4.7×10^4	2.6×10^4	1.3
Glycerol-2-phosphate	1.1×10^5	9.5×10^6	1.8×10^4	9.9×10^5	82.2
GUANINE	9.6×10^3	1.5×10^5	5.0×10^3	1.0×10^4	16.0
Guanosine	1.8×10^3	0	2.9×10^3	0	0.0
Hydroxymethylglutaric acid	1.1×10^5	1.0×10^6	6.6×10^4	4.4×10^4	0.9
Hypoxanthine	1.4×10^7	2.1×10^8	8.7×10^5	1.4×10^7	14.9
Inosine	1.8×10^4	3.8×10^5	2.0×10^3	3.5×10^4	20.4
Itaconic acid	8.4×10^4	1.5×10^5	2.2×10^4	5.2×10^4	1.8
Ketoleucine	1.3×10^5	1.5×10^5	3.9×10^4	4.8×10^4	1.2
L-(-)-Phenylalanine	7.5×10^5	1.7×10^7	2.7×10^5	4.2×10^5	23.8
L-5-Oxoproline	3.0×10^5	6.6×10^6	1.1×10^5	4.8×10^5	22.2
L-Arginine	5.4×10^2	3.2×10^6	1.0×10^3	4.2×10^5	6023.4
L-Glutamic acid	1.8×10^6	8.1×10^7	1.4×10^5	7.1×10^6	45.5
L-Histidine	1.2×10^4	1.5×10^7	7.5×10^3	1.6×10^6	1292.2
L-Leucine	3.0×10^5	5.7×10^6	2.0×10^3	3.5×10^5	187.4
L-Norvaline	1.4×10^4	3.4×10^6	9.4×10^3	3.0×10^5	233.0
L-Threonic acid hemicalcium salt	2.8×10^4	1.9×10^6	2.6×10^4	4.1×10^5	69.5
L-Tyrosine	1.8×10^5	4.3×10^6	1.9×10^4	3.2×10^5	23.9
Lactic acid	9.4×10^6	6.5×10^7	1.6×10^6	7.2×10^6	6.9
Methylsuccinic acid	1.2×10^5	2.5×10^6	1.6×10^4	1.7×10^5	21.3
N-Acetyl-DL-glutamic acid	1.1×10^6	1.6×10^6	1.0×10^5	1.9×10^5	1.5
N-Acetylaspartate	9.0×10^3	9.5×10^4	4.8×10^3	1.3×10^4	10.6
N-acetylphenylalanine	4.5×10^5	3.2×10^6	1.5×10^4	2.9×10^5	7.3
NIACIN	2.4×10^6	1.4×10^7	2.4×10^5	9.0×10^5	5.8
O-Phosphoethanolamine	2.9×10^4	9.0×10^5	1.2×10^4	1.2×10^5	30.9
Orotic acid	1.9×10^6	4.2×10^6	2.4×10^5	4.4×10^5	2.1
ORTHO-AMINOBENZOIC ACID	4.7×10^4	9.7×10^4	1.1×10^4	1.6×10^4	2.0
p-Coumaraldehyde	2.3×10^4	1.3×10^6	2.6×10^4	3.1×10^5	59.7
p-Coumaraldehyde; PlaSMA ID-92	2.3×10^5	7.3×10^5	7.8×10^3	1.1×10^5	3.2
P-HYDROXYBENZOIC ACID	7.3×10^4	9.0×10^5	1.7×10^4	8.0×10^4	12.3
Pantethine	9.3×10^1	6.6×10^5	1.8×10^2	1.7×10^4	7093.1
Paracetamol	1.8×10^4	2.4×10^5	2.5×10^3	2.5×10^4	13.6
Phosphoric acid	6.4×10^7	1.5×10^8	4.6×10^6	7.1×10^6	2.3

FIG. 16B (continued)

PYRIDOXINE	8.9×10^4	1.2×10^5	6.7×10^3	1.5×10^5	14.2
Serine	1.0×10^5	1.6×10^6	3.4×10^4	1.8×10^5	16.1
Succinic acid	1.5×10^8	4.3×10^8	5.0×10^5	2.6×10^7	2.8
Succinic acid	3.7×10^6	8.7×10^5	1.0×10^6	3.6×10^6	2.3
Succinic acid (not validated); PlaSMA ID-26	6.4×10^5	2.4×10^5	2.2×10^5	8.6×10^5	3.8
Threonine	1.0×10^4	9.8×10^5	9.4×10^3	1.0×10^5	97.3
Thymidine-5`-monophosphate	0	5.7×10^2	0	1.1×10^3	N/A
TRANS-ORTHO-COUMARIC ACID	1.5×10^4	8.6×10^4	4.6×10^3	1.0×10^4	5.5
Trehalose	1.2×10^4	7.0×10^3	5.1×10^3	3.7×10^3	0.6
Trifluoroacetic acid	6.7×10^6	6.2×10^6	6.8×10^5	4.6×10^5	0.9
Triptophenolide	8.5×10^5	5.6×10^5	5.0×10^4	3.7×10^4	0.7
URACIL	2.7×10^6	5.0×10^6	2.7×10^5	5.7×10^5	1.9
Uric acid	7.7×10^3	1.6×10^6	4.2×10^3	5.6×10^4	215.6
Uridine	2.2×10^4	7.3×10^4	5.8×10^3	6.7×10^3	3.3
UROCANIC ACID	3.5×10^5	3.5×10^5	5.5×10^4	4.7×10^4	1.0
Xanthine	4.2×10^7	1.1×10^8	5.1×10^6	4.9×10^6	2.8

FIG. 16B (continued)