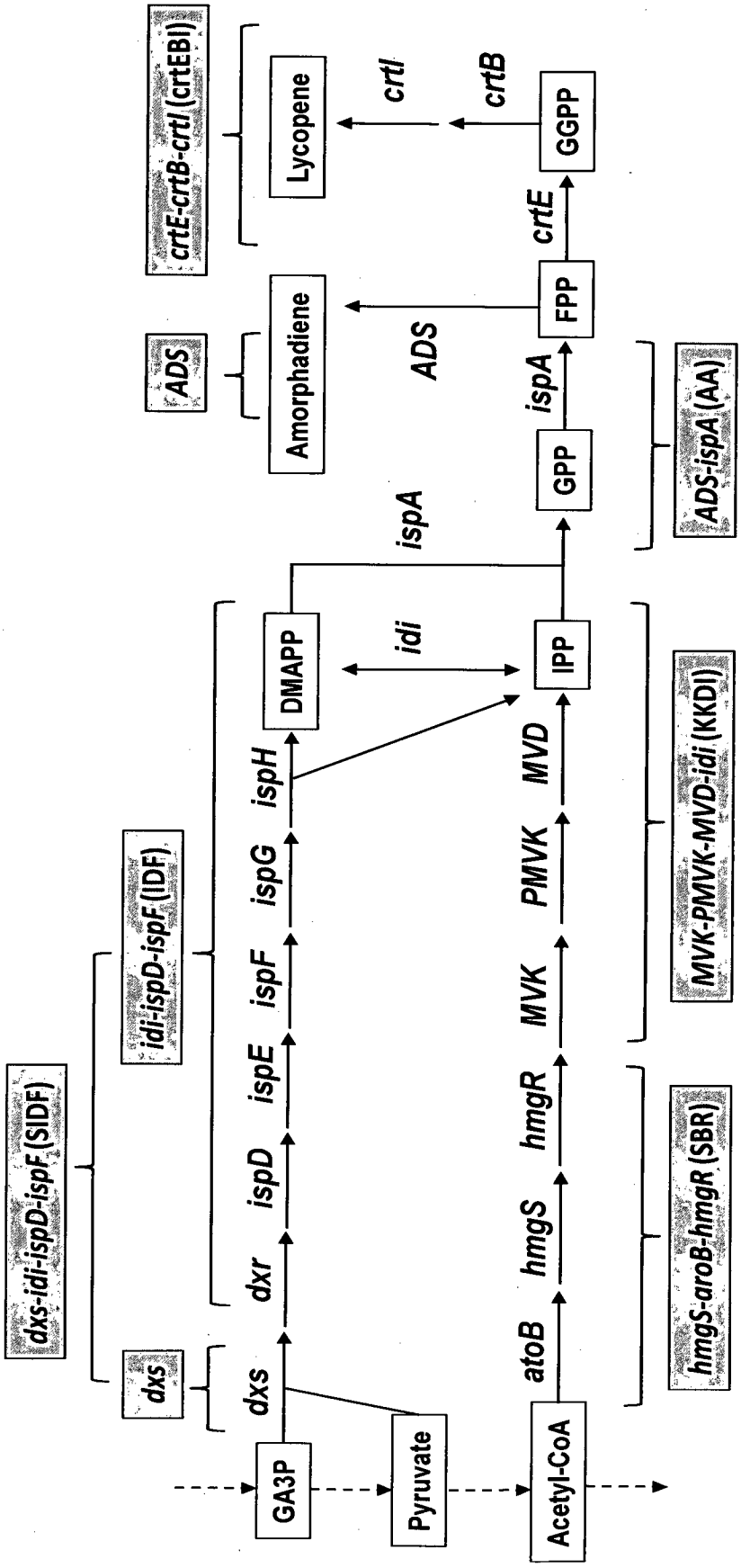

GA3P
Pyruvate
Acetyl-CoA
dxs
dxr
ispD
ispE
ispF
ispG
ispH
DMAPP
idi
IPP
atoB
hmgS
hmgR
MVK
PMVK
MVD
ispA
GPP
ADS
FPP
crtE
crtB
crtI
GGPP
Lycopene
Amorphadiene
ADS
crtE-crtB-crtI (crtEBI)
dxs
dxs-idi-ispD-ispF (SIDF)
idi-ispD-ispF (IDF)
hmgS-aroB-hmgR (SBR)
MVK-PMVK-MVD-idi (KKDI)
ADS-ispA (AA)

FIG. 1

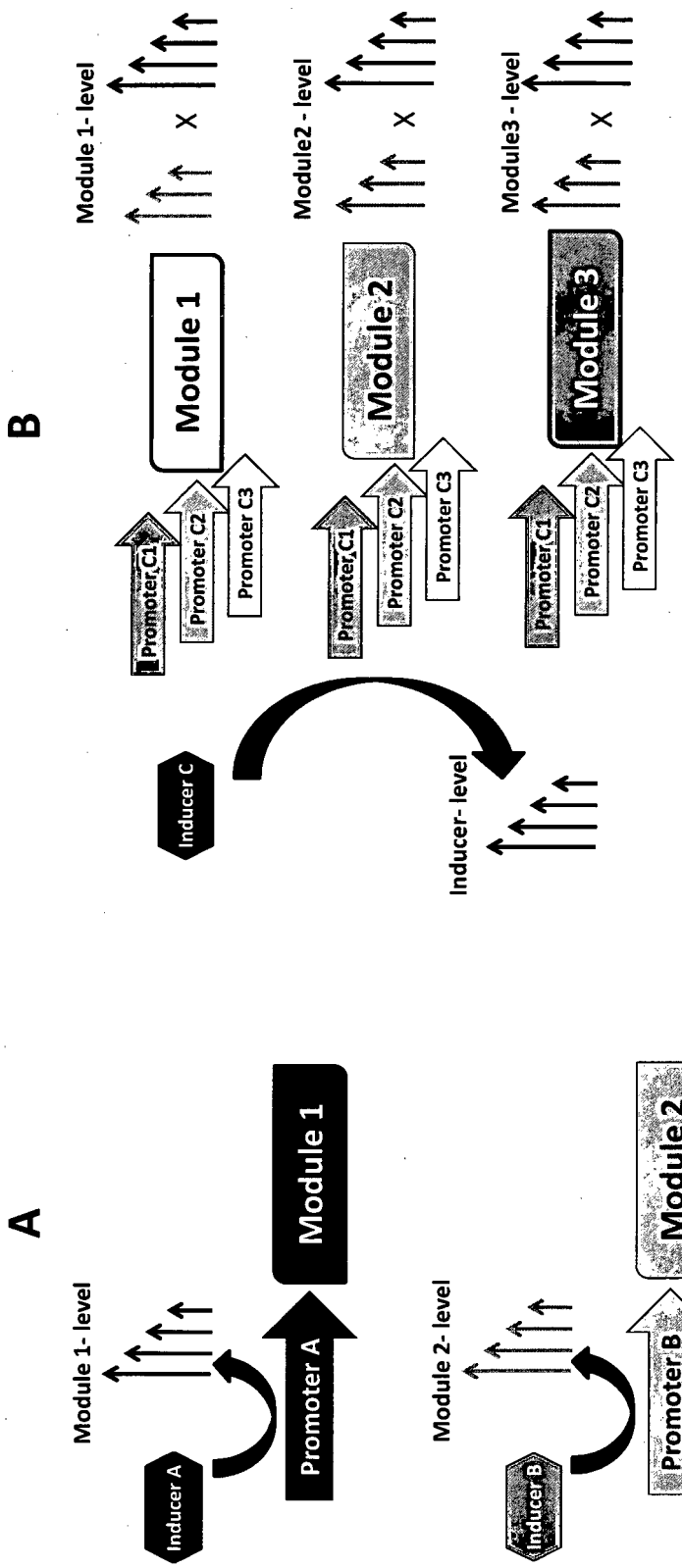

A
Module 1- level
Inducer A
Promoter A
Module 1
Module 2- level
Inducer B
Promoter B
Module 2
B
Module 1- level
Module 1
Promoter C1
Promoter C2
Promoter C3
Module2 - level
Module 2
Promoter C1
Promoter C2
Promoter C3
Module3 - level
Module 3
Promoter C1
Promoter C2
Promoter C3
Inducer C
Inducer- level

FIG. 2

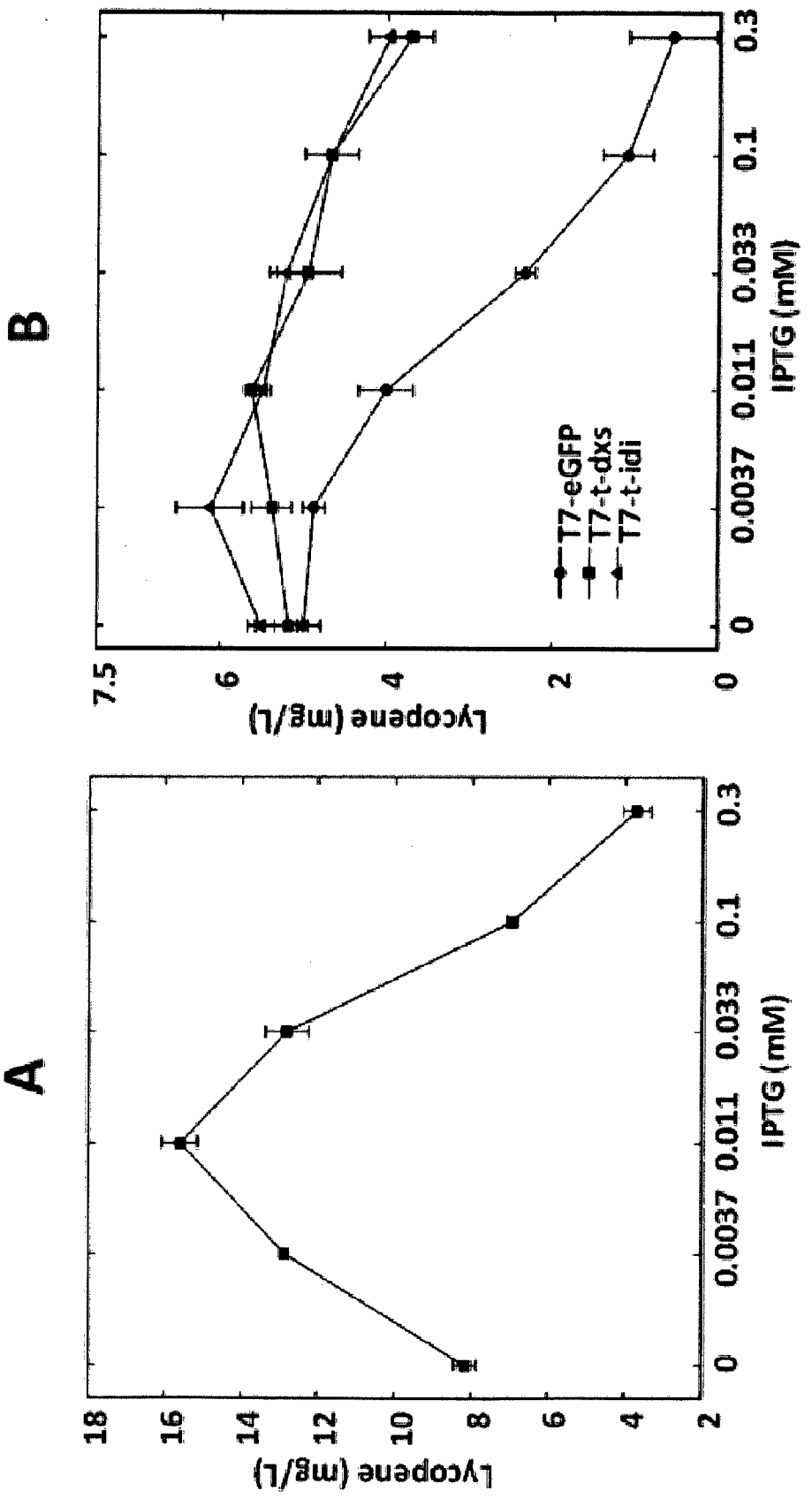

A
Lycopene (mg/L)
18
16
14
12
10
8
6
4
2
0
0.0037
0.011
0.033
0.1
0.3
IPTG (mM)
B
Lycopene (mg/L)
7.5
6
4
2
0
0
0.0037
0.011
0.033
0.1
0.3
IPTG (mM)
T7-eGFP
T7-t-dxs
T7-t-idi

FIG. 3

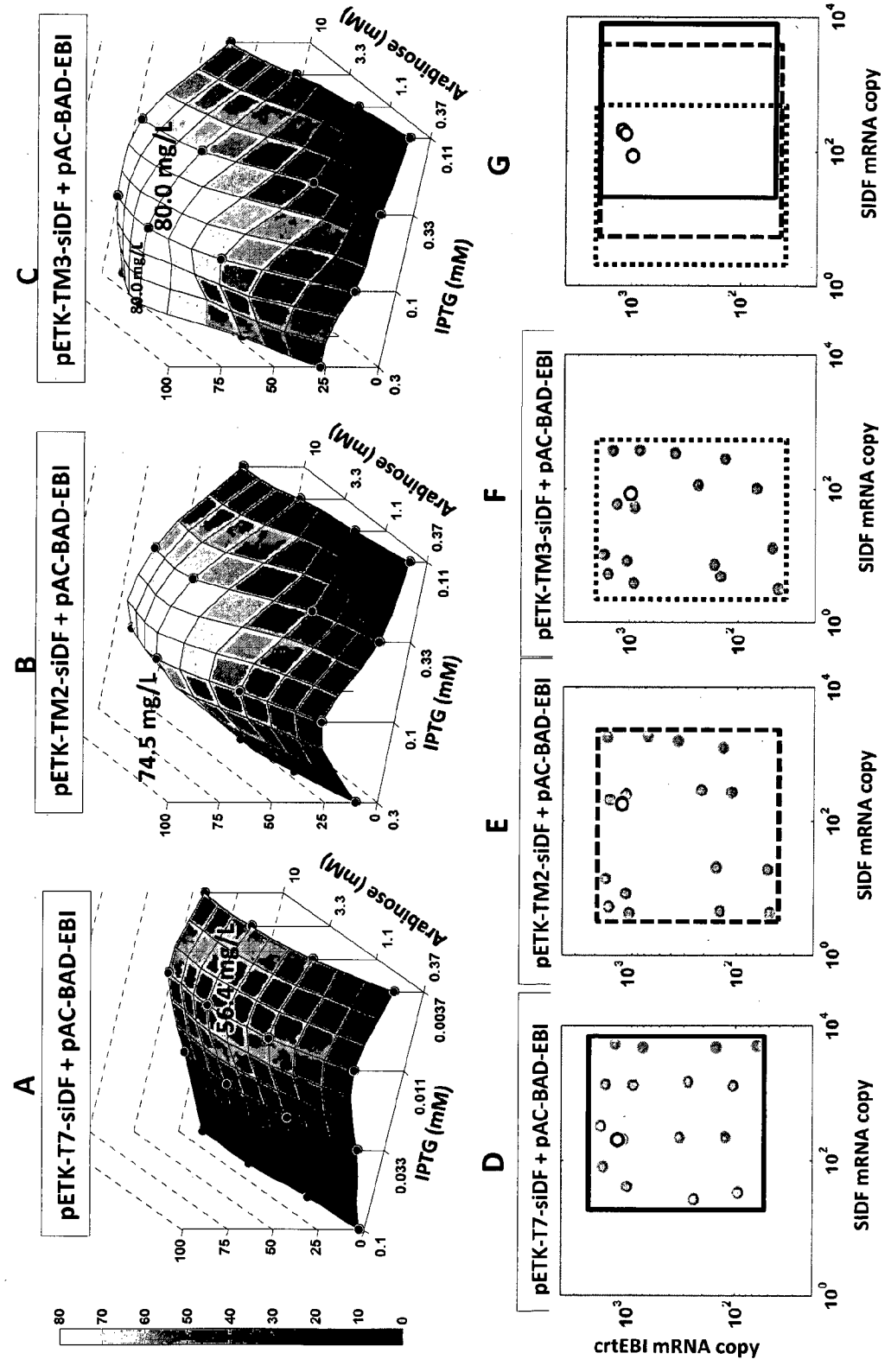

A
pETK-T7-siDF + pAC-BAD-EBI
B
pETK-TM2-siDF + pAC-BAD-EBI
C
pETK-TM3-siDF + pAC-BAD-EBI
56.4 mg/L
74.5 mg/L
80.0 mg/L
Arabinose (mM)
IPTG (mM)
D
E
F
G
pETK-T7-siDF + pAC-BAD-EBI
pETK-TM2-siDF + pAC-BAD-EBI
pETK-TM3-siDF + pAC-BAD-EBI
crtEBI mRNA copy
SIDF mRNA copy

FIG. 4

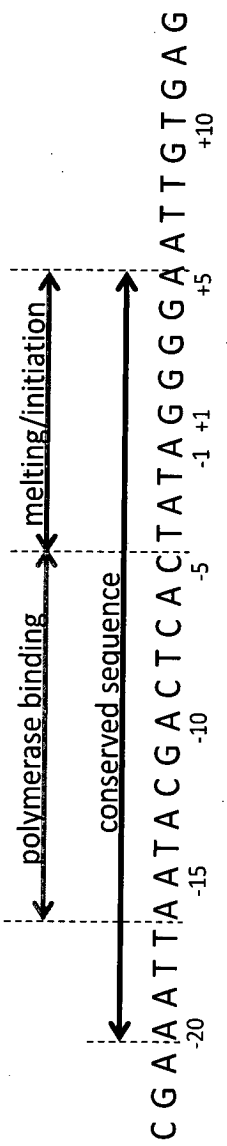

melting/initiation
polymerase binding
conserved sequence
C G A A A T T A A T A C G A C T C A C T A T A G G G G A A T T G T G A G
-20
-15
-10
-5
-1 +1
+5
+10

FIG. 5

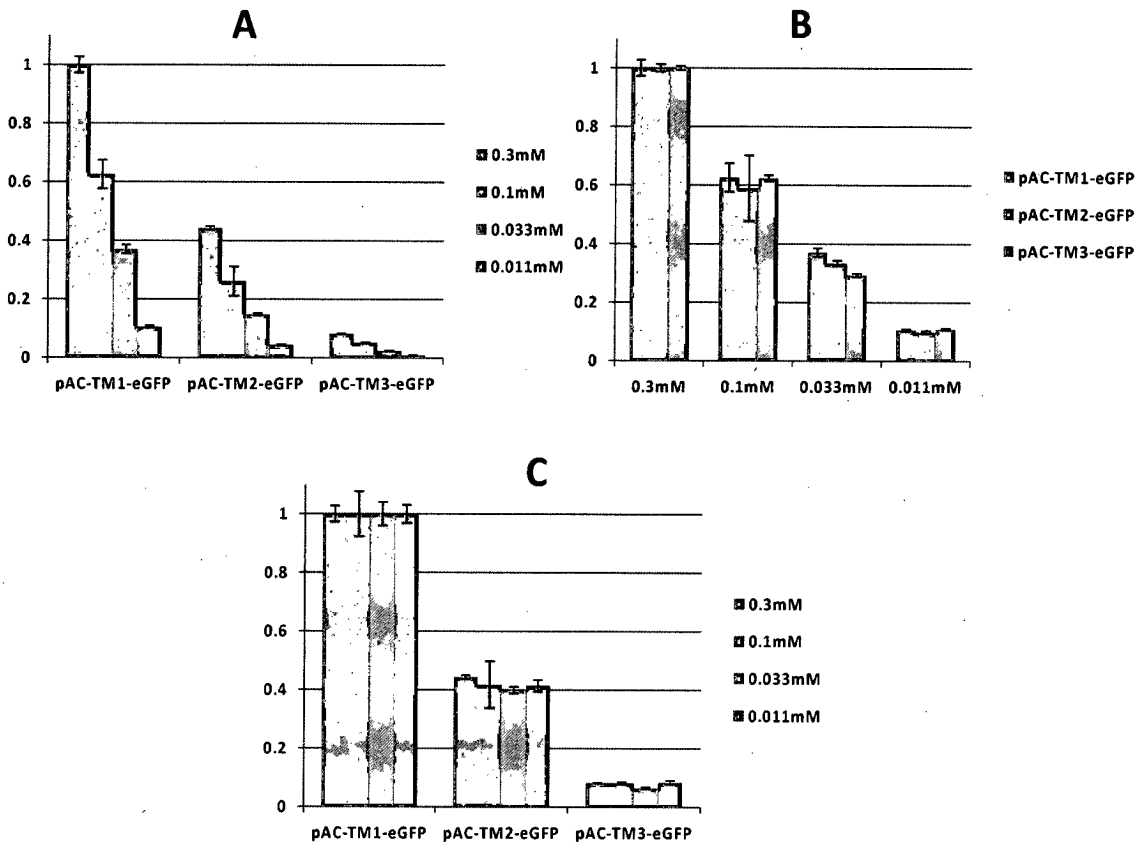

A
1
0.8
0.6
0.4
0.2
0
pAC-TM1-eGFP
pAC-TM2-eGFP
pAC-TM3-eGFP
0.3mM
0.1mM
0.033mM
0.011mM
B
1
0.8
0.6
0.4
0.2
0
0.3mM
0.1mM
0.033mM
0.011mM
pAC-TM1-eGFP
pAC-TM2-eGFP
pAC-TM3-eGFP
C
1
0.8
0.6
0.4
0.2
0
pAC-TM1-eGFP
pAC-TM2-eGFP
pAC-TM3-eGFP
0.3mM
0.1mM
0.033mM
0.011mM

FIG. 6

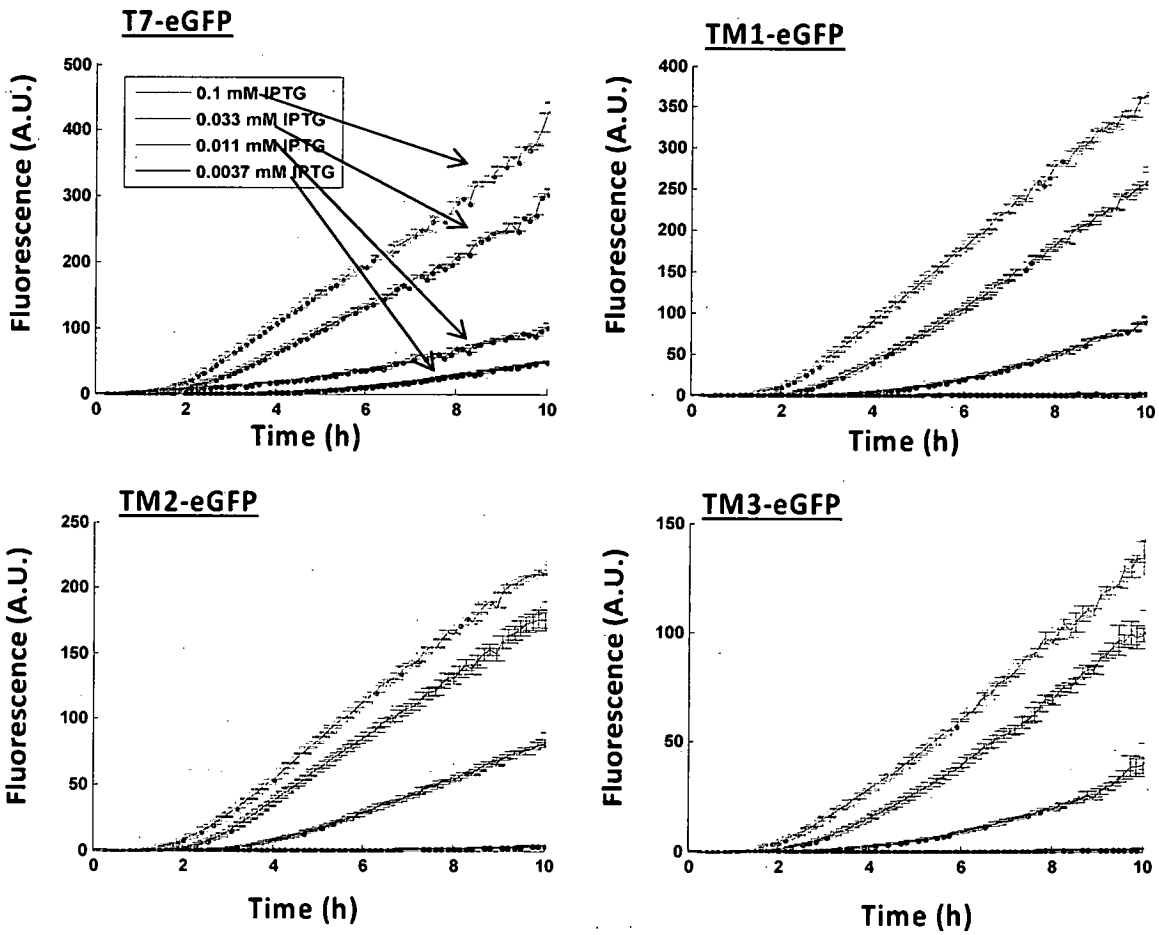

T7-eGFP
0.1 mM IPTG
0.033 mM IPTG
0.011 mM IPTG
0.0037 mM IPTG
Fluorescence (A.U.)
Time (h)
TM1-eGFP
Fluorescence (A.U.)
Time (h)
TM2-eGFP
Fluorescence (A.U.)
Time (h)
TM3-eGFP
Fluorescence (A.U.)
Time (h)

FIG. 7

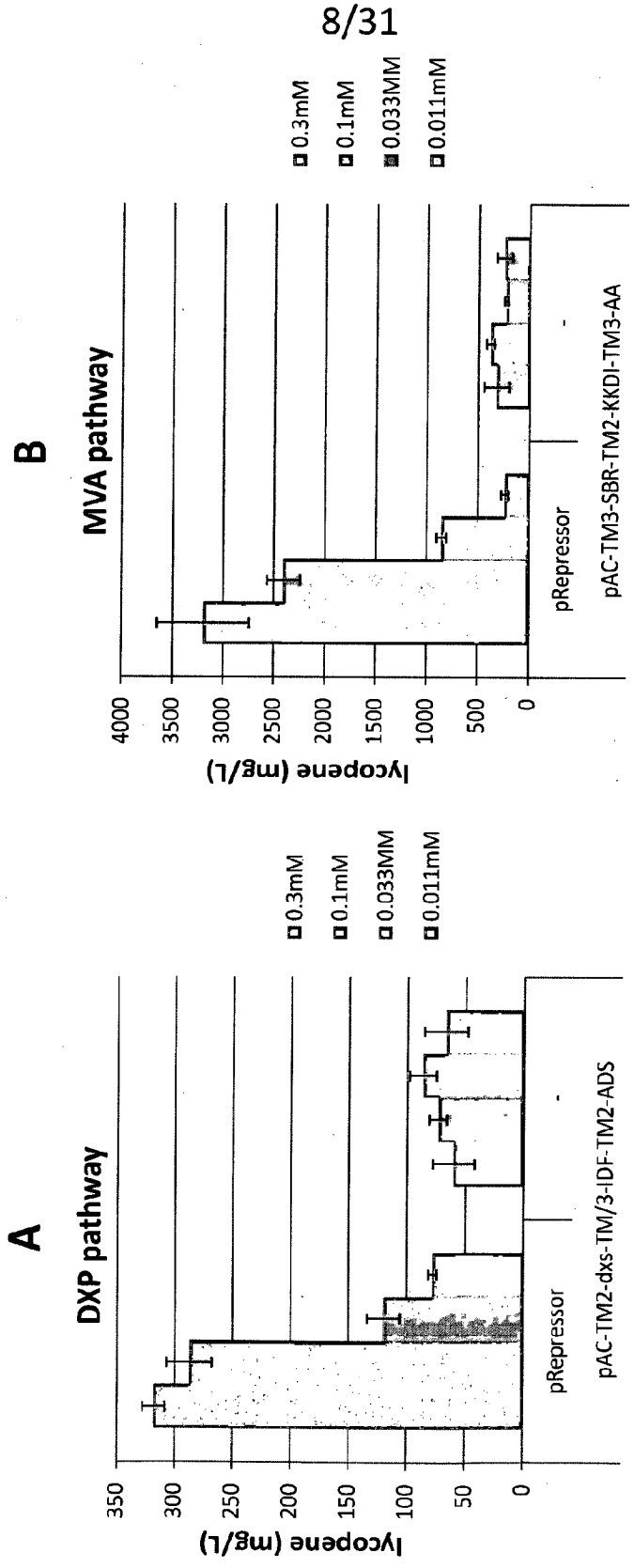

A
DXP pathway
Lycopene (mg/L)
350
300
250
200
150
100
50
0
0.3mM
0.1mM
0.033MM
0.011mM
pRepressor
-
pAC-TM2-dxs-TM/3-IDF-TM2-ADS
B
MVA pathway
Lycopene (mg/L)
4000
3500
3000
2500
2000
1500
1000
500
0
0.3mM
0.1mM
0.033MM
0.011mM
pRepressor
-
pAC-TM3-SBR-TM2-KKDI-TM3-AA

FIG. 8

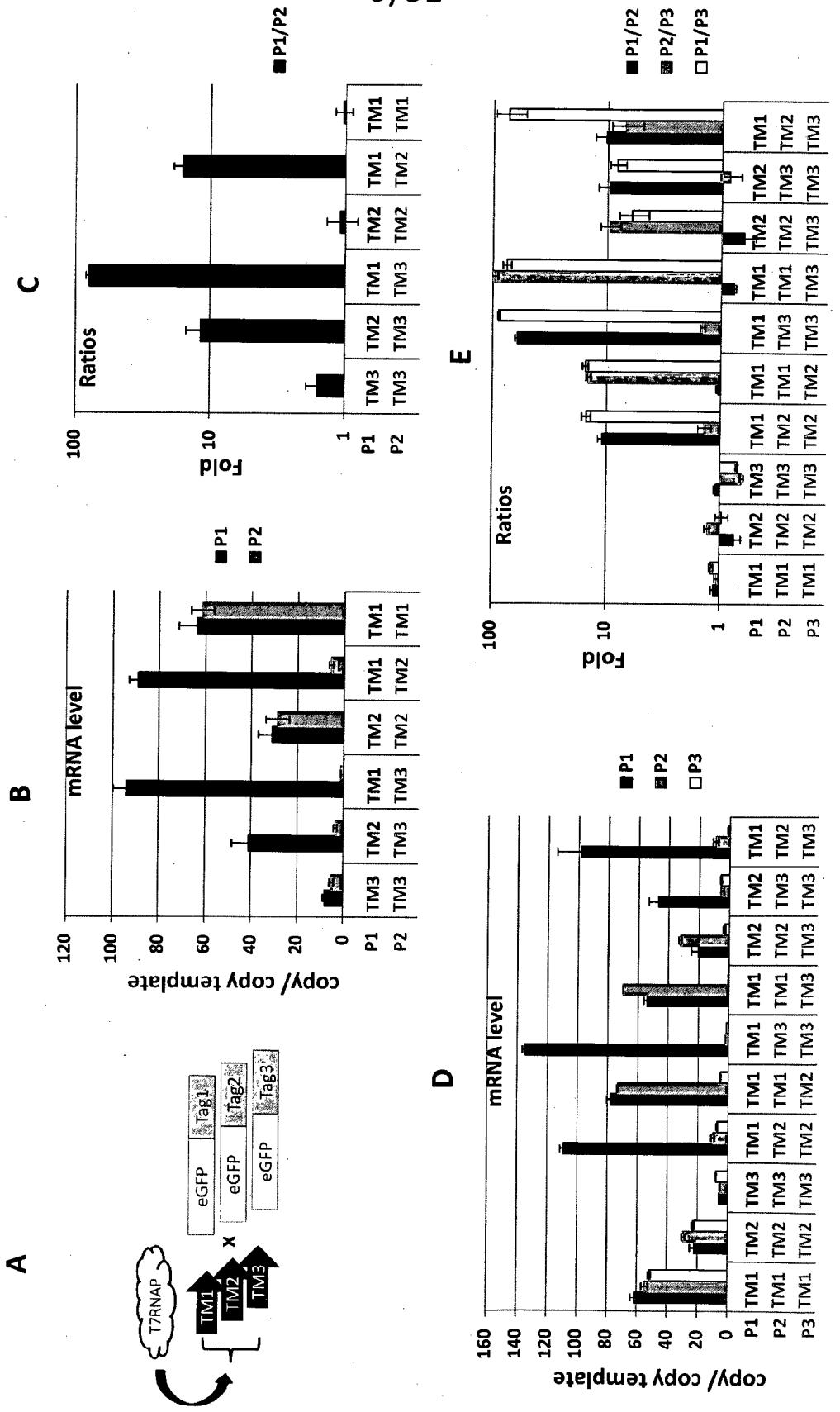

A
T7RNAP
TM1
TM2
TM3
x
eGFP
Tag1
eGFP
Tag2
eGFP
Tag3
B
mRNA level
copy/ copy template
P1
P2
C
Ratios
Fold
P1/P2
D
mRNA level
copy/ copy template
P1
P2
P3
E
Ratios
Fold
P1/P2
P2/P3
P1/P3

FIG. 9

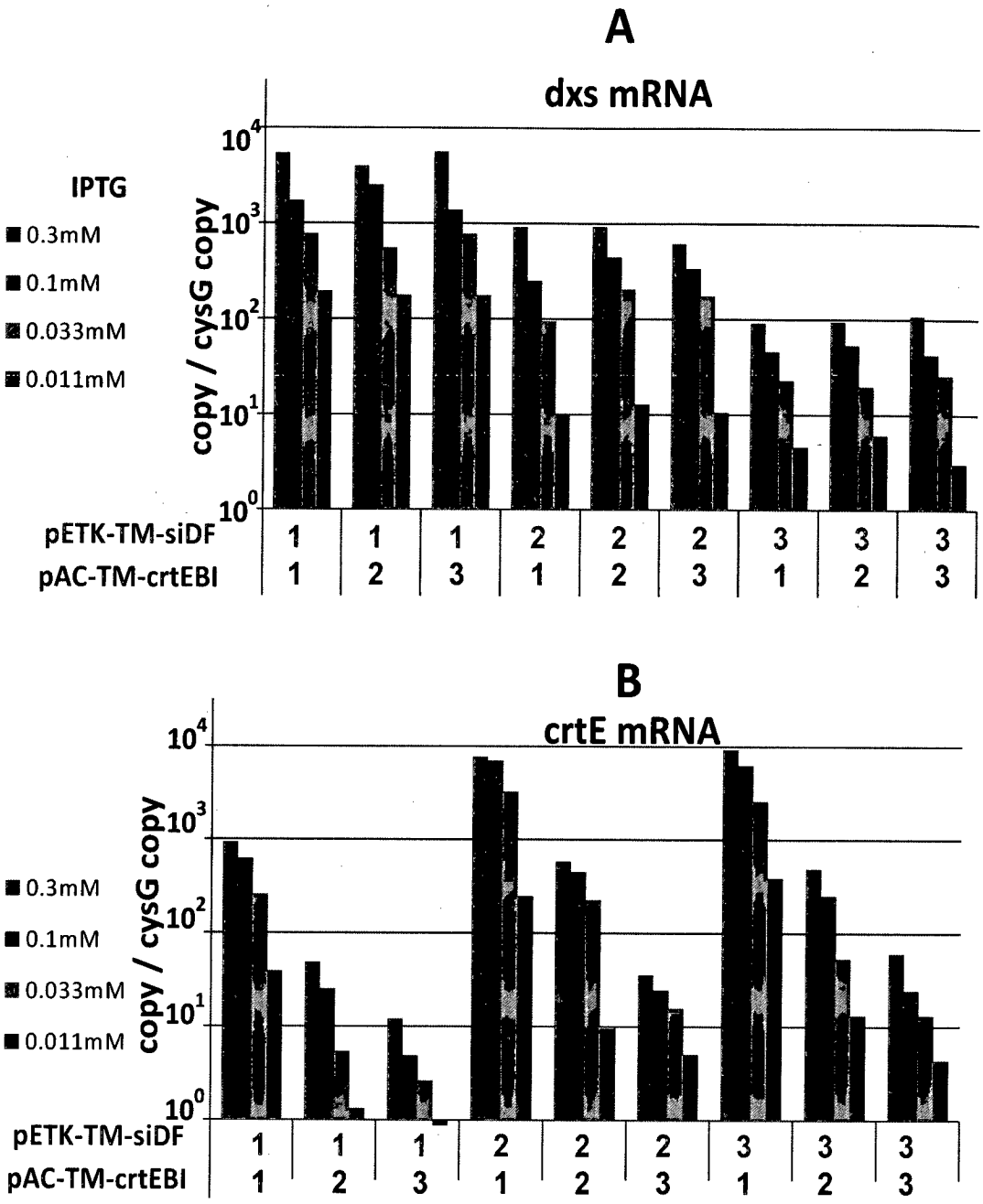

A
dxs mRNA
IPTG
0.3mM
0.1mM
0.033mM
0.011mM
copy / cysG copy
10^4
10^3
10^2
10^1
10^0
pETK-TM-siDF
pAC-TM-crtEBI
1 1
1 2
1 3
2 1
2 2
2 3
3 1
3 2
3 3
B
crtE mRNA
0.3mM
0.1mM
0.033mM
0.011mM
copy / cysG copy
10^4
10^3
10^2
10^1
10^0
pETK-TM-siDF
pAC-TM-crtEBI
1 1
1 2
1 3
2 1
2 2
2 3
3 1
3 2
3 3

FIG. 10

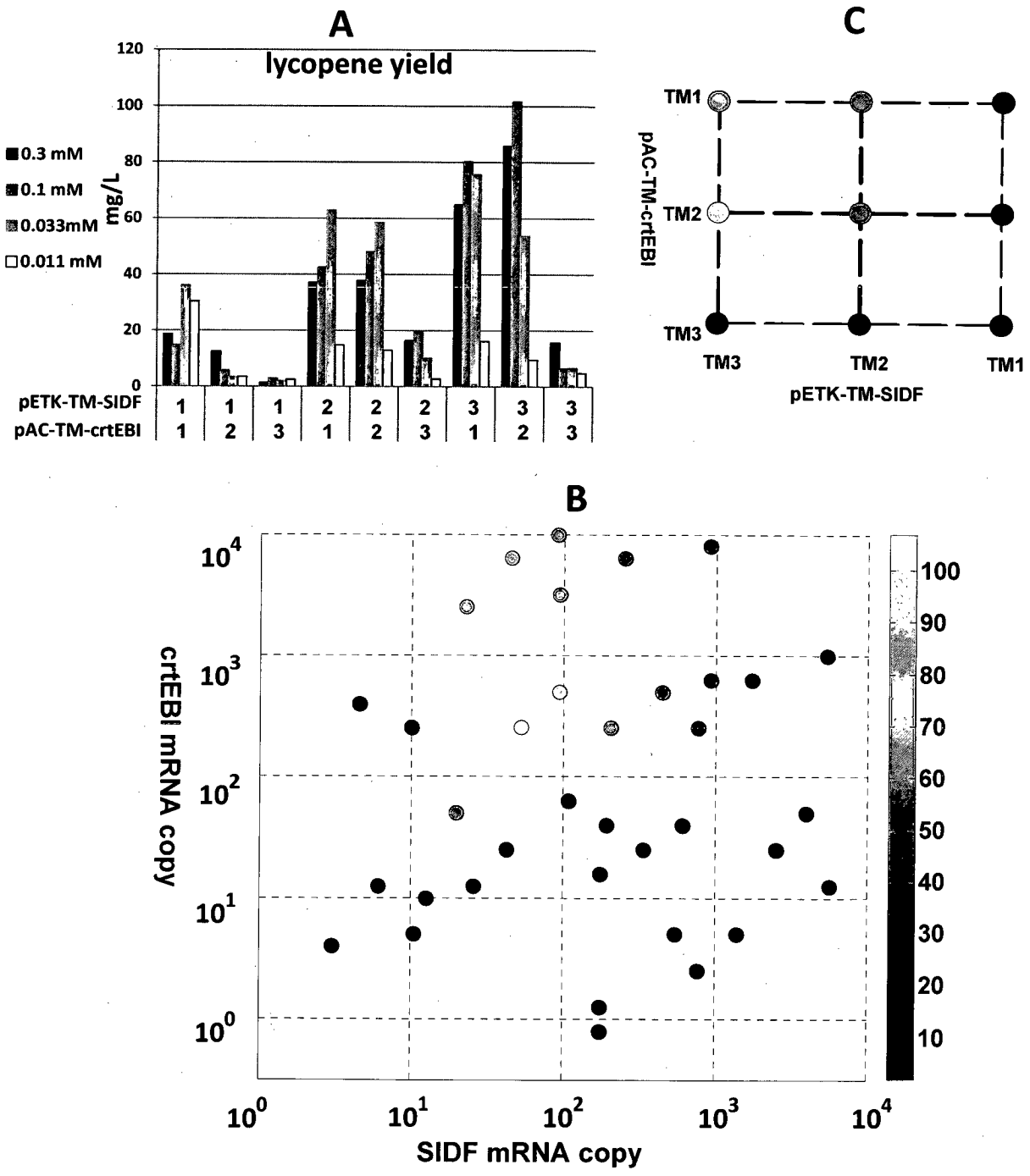

A
lycopene yield
120
100
80
60
40
20
0
mg/L
0.3 mM
0.1 mM
0.033mM
0.011 mM
pETK-TM-SIDF
pAC-TM-crtEBI
1 1
1 2
1 3
2 1
2 2
2 3
3 1
3 2
3 3
C
TM1
TM2
TM3
pAC-TM-crtEBI
TM3
TM2
TM1
pETK-TM-SIDF
B
crtEBI mRNA copy
SIDF mRNA copy
10^0
10^1
10^2
10^3
10^4
100
90
80
70
60
50
40
30
20
10

FIG. 11

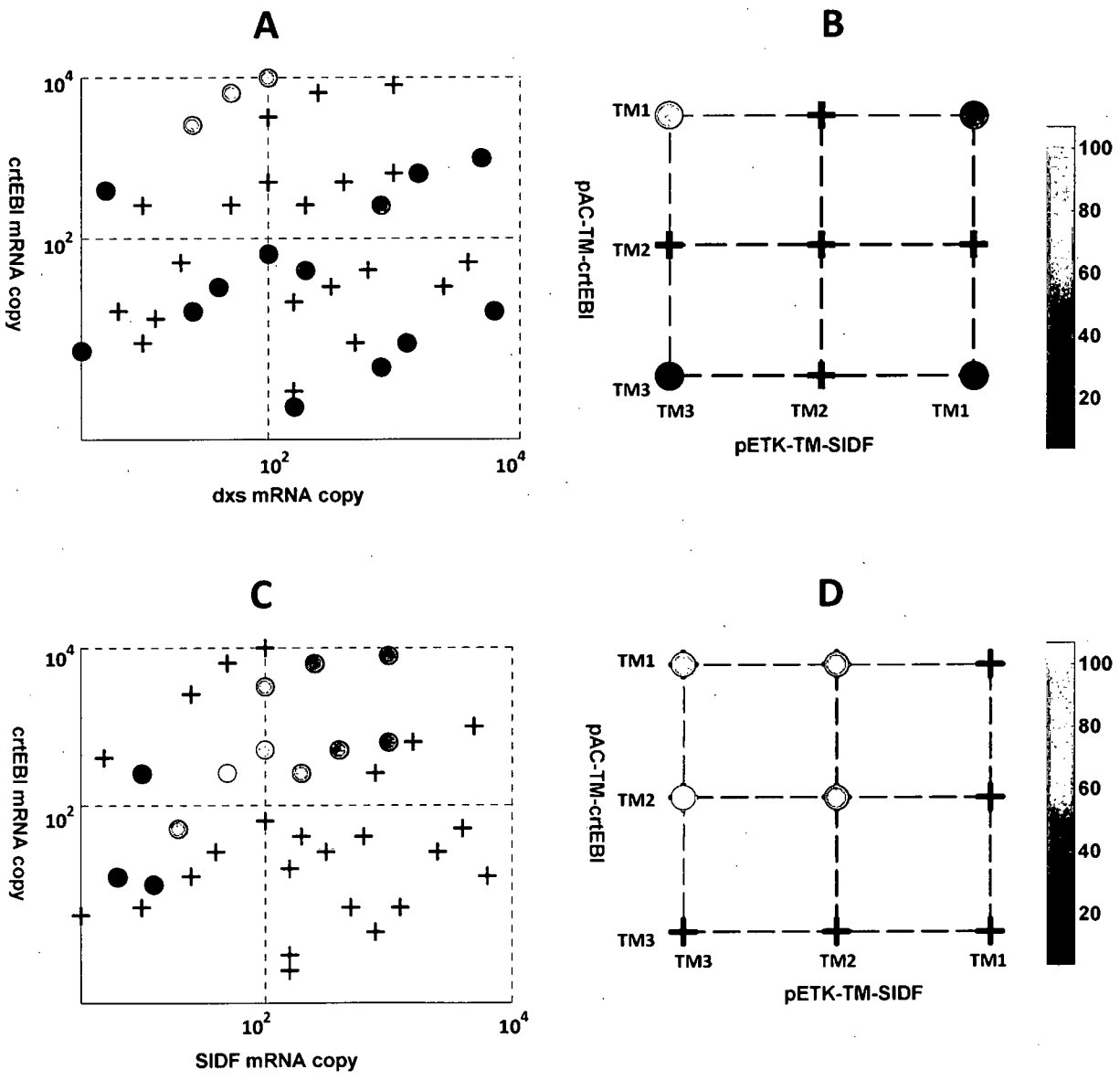

A
B
C
D
crtEBI mRNA copy
dxs mRNA copy
SIDF mRNA copy
pAC-TM-crtEBI
pETK-TM-SIDF
TM1
TM2
TM3
100
80
60
40
20

FIG. 12

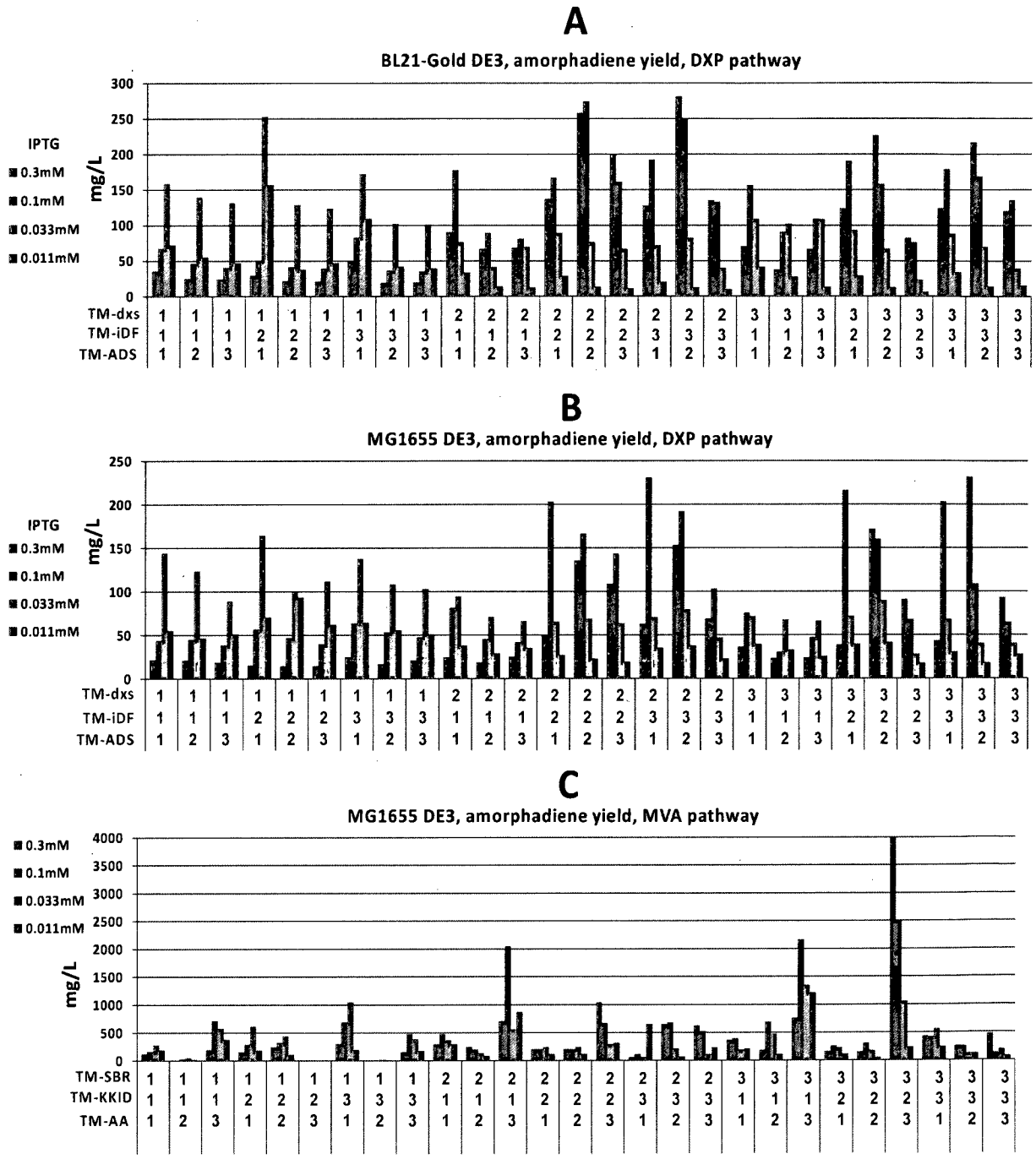

A
BL21-Gold DE3, amorphadiene yield, DXP pathway
IPTG
0.3mM
0.1mM
0.033mM
0.011mM
mg/L
300
250
200
150
100
50
0
TM-dxs
TM-iDF
TM-ADS
B
MG1655 DE3, amorphadiene yield, DXP pathway
IPTG
0.3mM
0.1mM
0.033mM
0.011mM
mg/L
250
200
150
100
50
0
TM-dxs
TM-iDF
TM-ADS
C
MG1655 DE3, amorphadiene yield, MVA pathway
0.3mM
0.1mM
0.033mM
0.011mM
mg/L
4000
3500
3000
2500
2000
1500
1000
500
0
TM-SBR
TM-KKID
TM-AA

FIG. 13

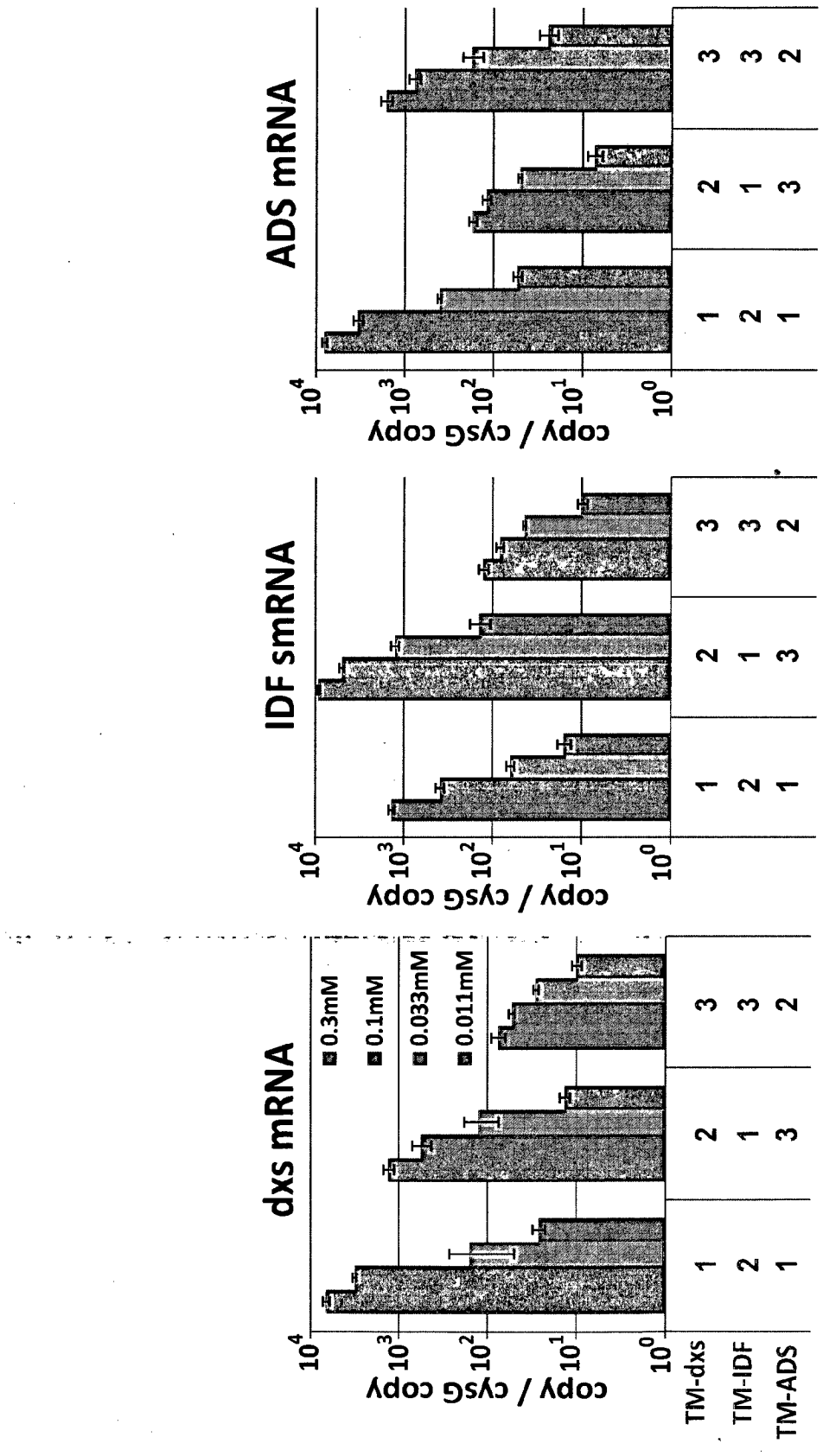

dxs mRNA
IDF smRNA
ADS mRNA
copy / cysG copy
0.3mM
0.1mM
0.033mM
0.011mM
TM-dxs
TM-IDF
TM-ADS

FIG. 14

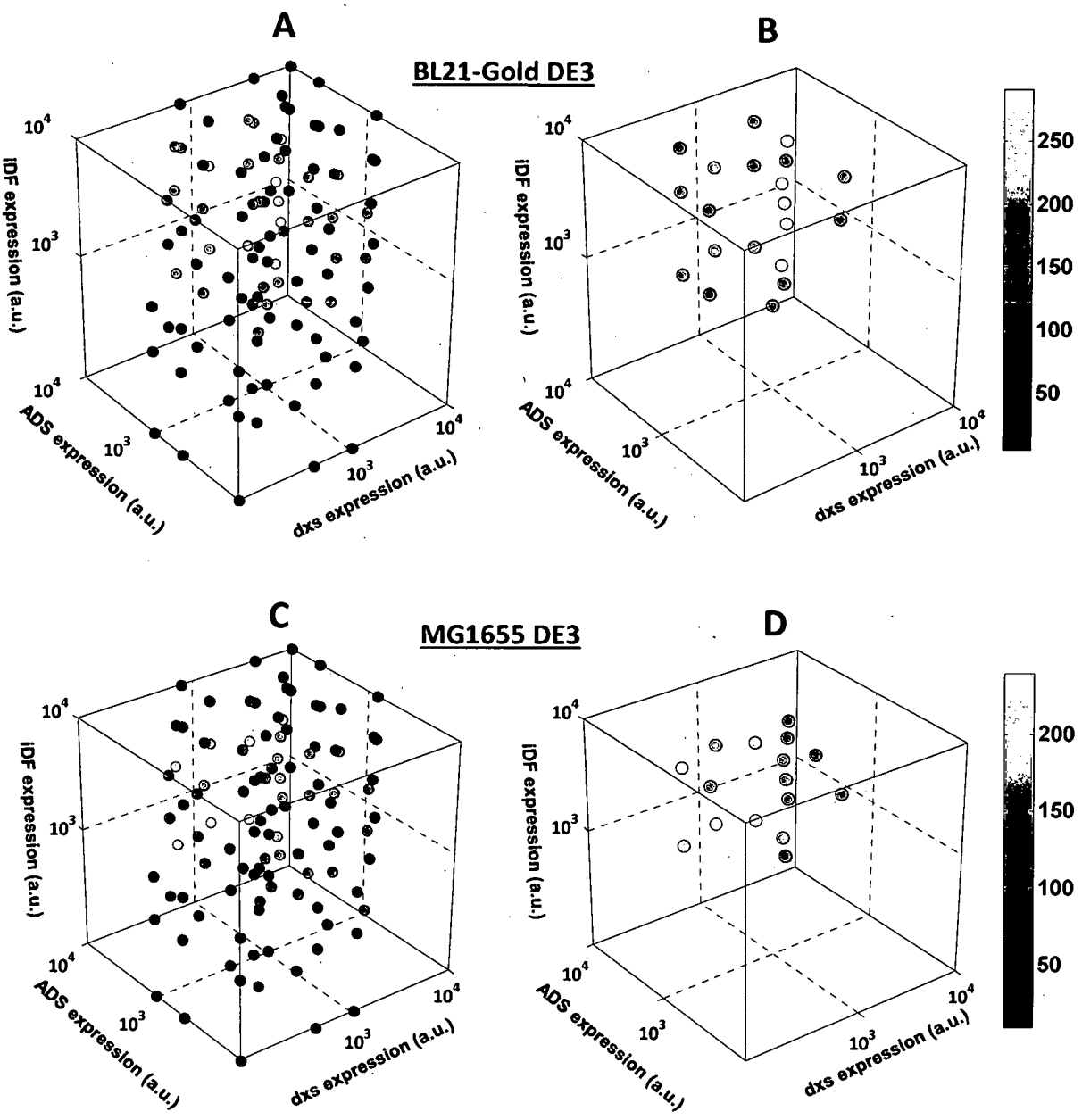

A
BL21-Gold DE3
B
C
MG1655 DE3
D
IDF expression (a.u.)
ADS expression (a.u.)
dxs expression (a.u.)
10^4
10^3
250
200
150
100
50

FIG. 15

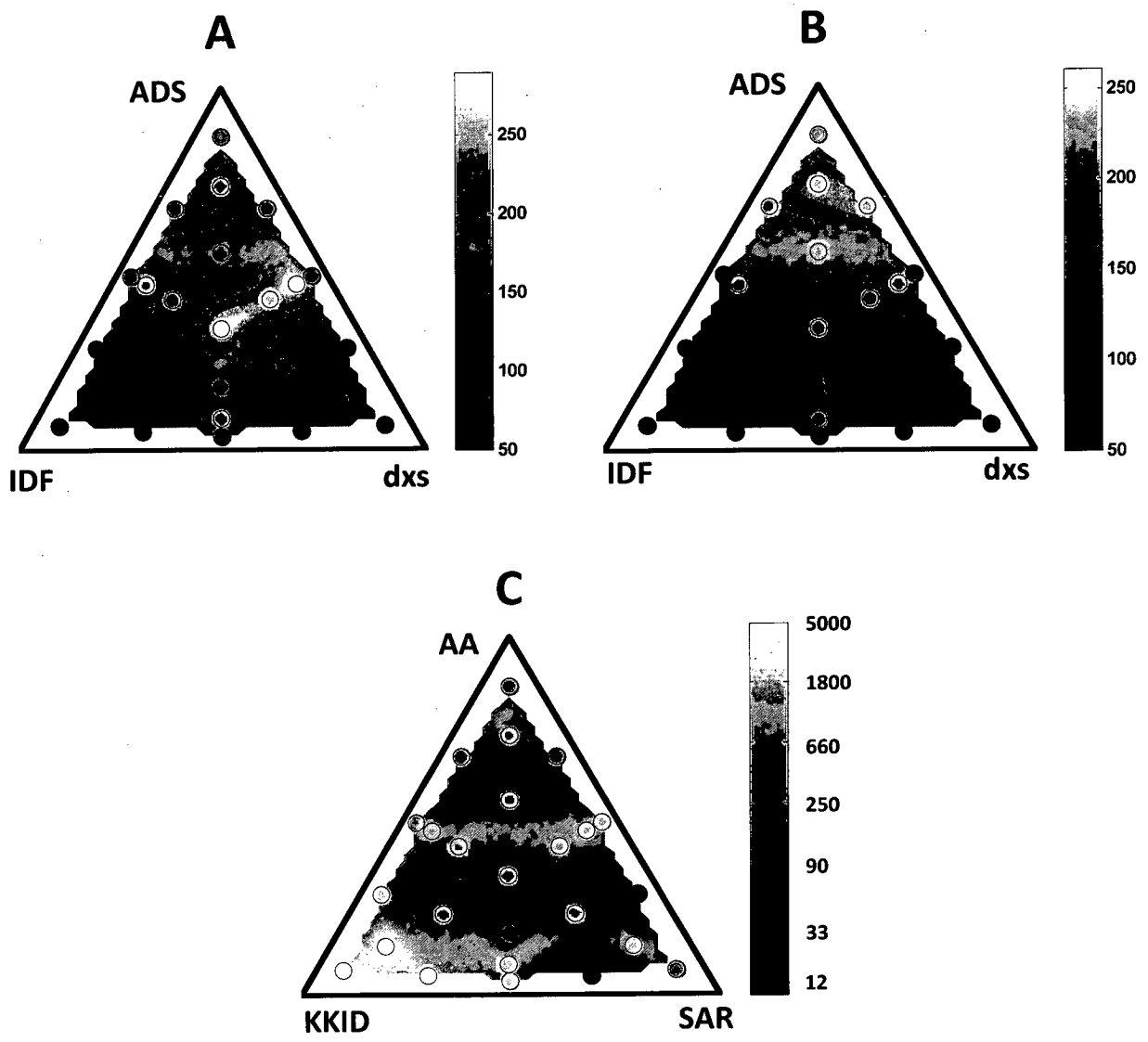

A
ADS
IDF
dxs
250
200
150
100
50
B
ADS
IDF
dxs
250
200
150
100
50
C
AA
KKID
SAR
5000
1800
660
250
90
33
12

FIG. 16

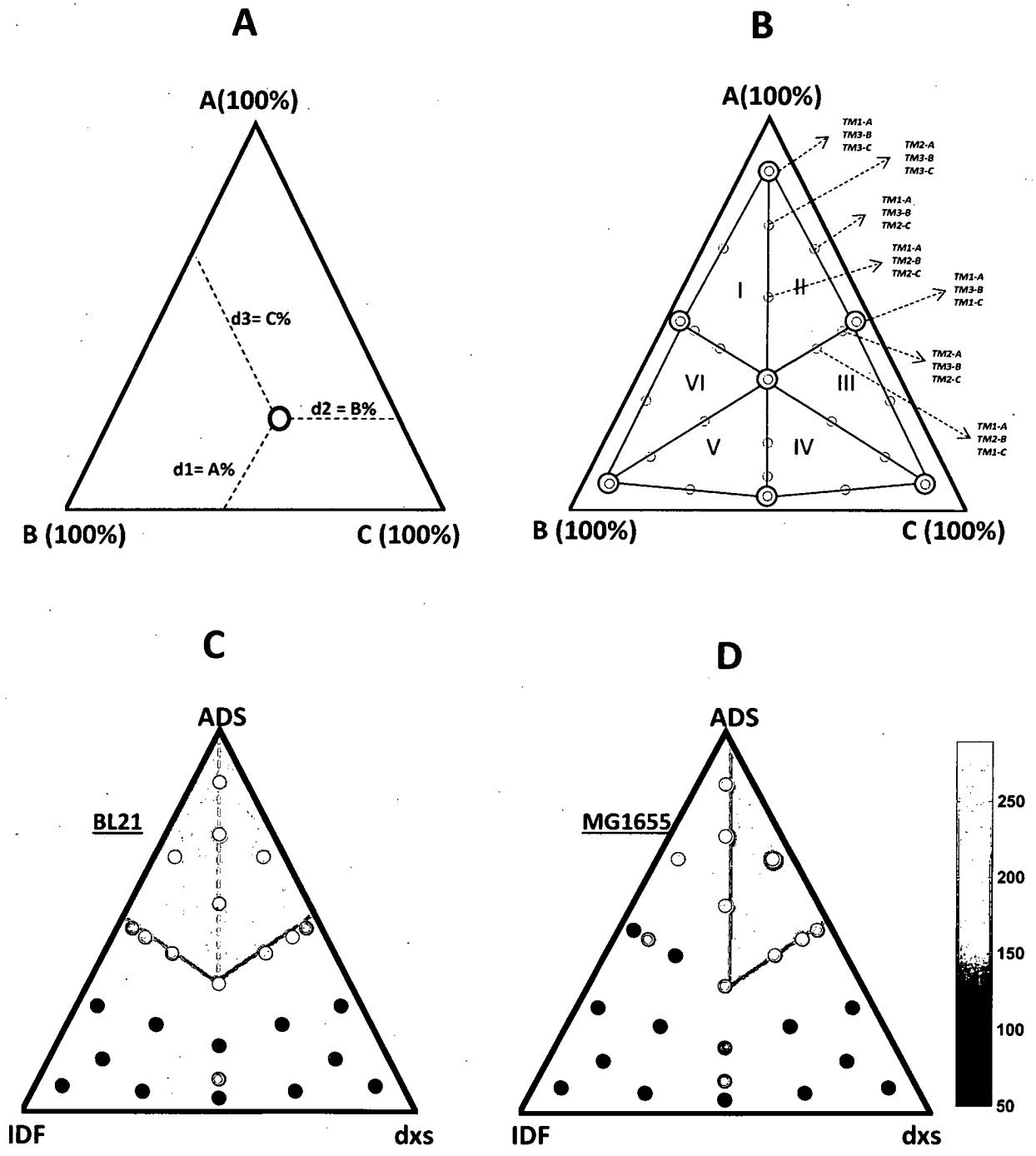

A
A(100%)
d3= C%
d2 = B%
d1= A%
B (100%)
C (100%)
B
A(100%)
TM1-A TM3-B TM3-C
TM2-A TM3-B TM3-C
TM1-A TM3-B TM2-C
TM1-A TM2-B TM2-C
TM1-A TM3-B TM1-C
TM2-A TM3-B TM2-C
TM1-A TM2-B TM1-C
I
II
III
IV
V
VI
B (100%)
C (100%)
C
ADS
BL21
IDF
dxs
D
ADS
MG1655
IDF
dxs
250
200
150
100
50

FIG. 17

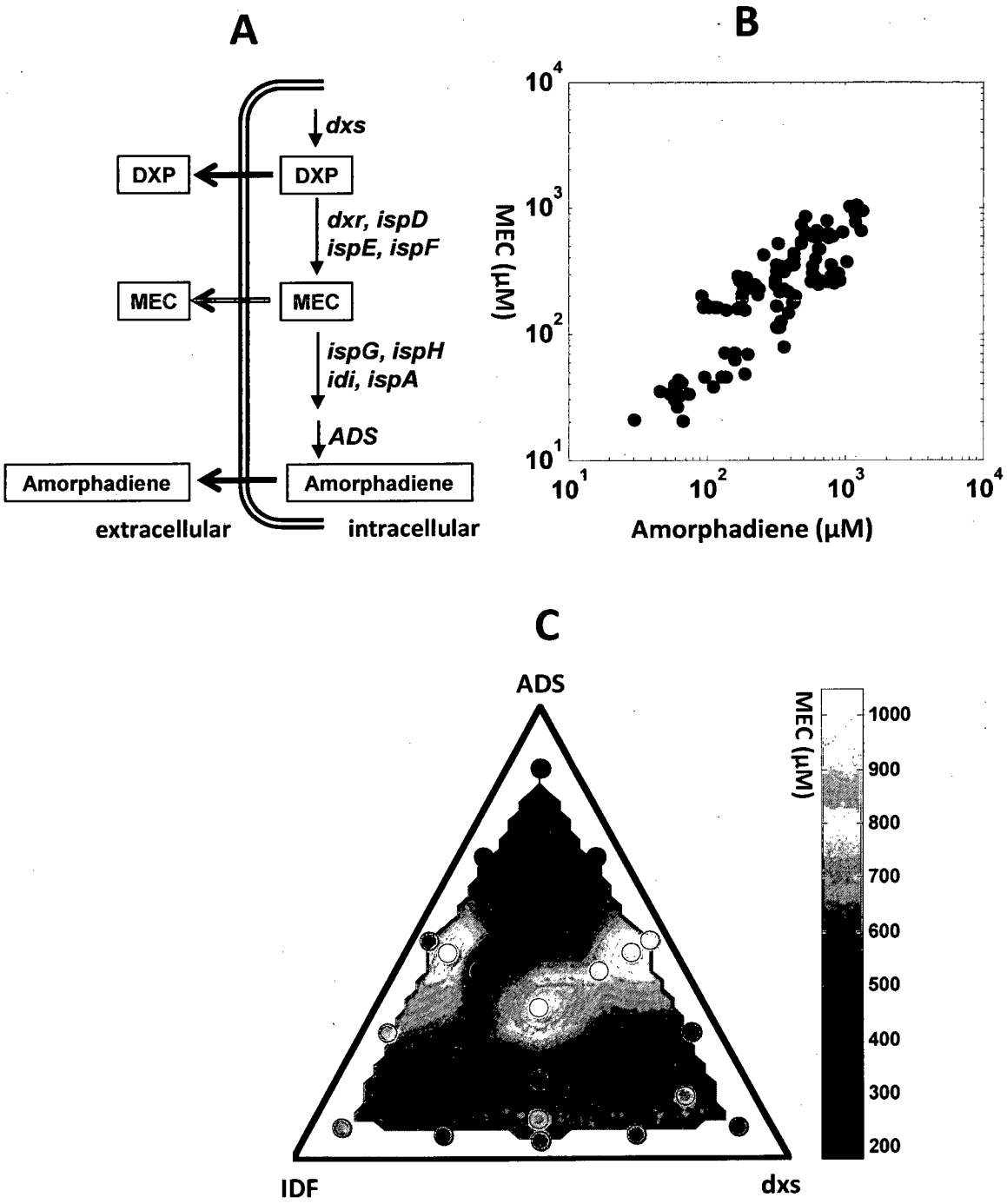

A
dxs
DXP
DXP
dxr, ispD
ispE, ispF
MEC
MEC
ispG, ispH
idi, ispA
ADS
Amorphadiene
Amorphadiene
extracellular
intracellular
B
MEC (μM)
Amorphadiene (μM)
C
ADS
IDF
dxs
MEC (μM)
1000
900
800
700
600
500
400
300
200

FIG. 18

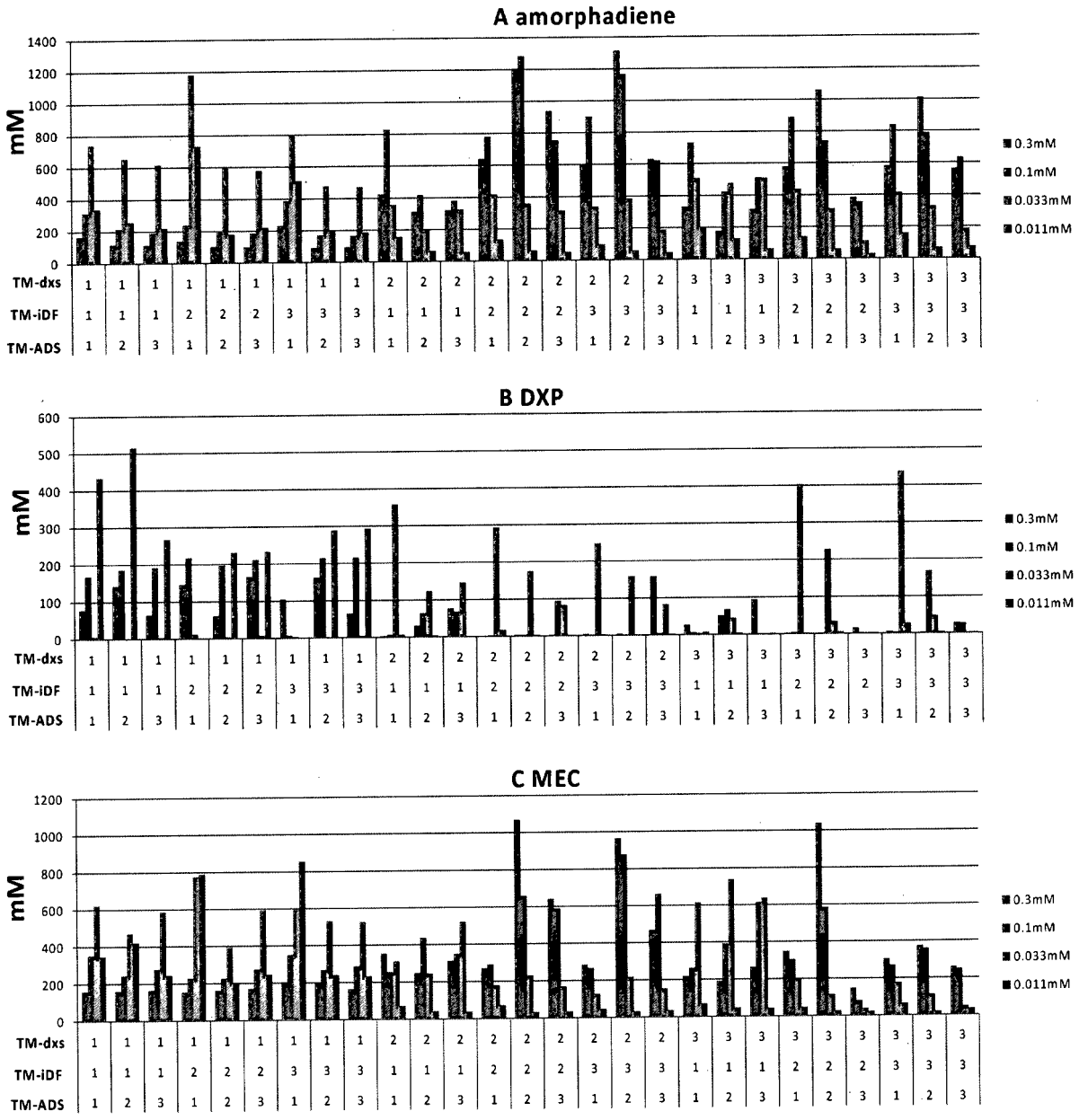

A amorphadiene
mM
1400
1200
1000
800
600
400
200
0
TM-dxs
TM-iDF
TM-ADS
0.3mM
0.1mM
0.033mM
0.011mM
B DXP
mM
600
500
400
300
200
100
0
TM-dxs
TM-iDF
TM-ADS
0.3mM
0.1mM
0.033mM
0.011mM
C MEC
mM
1200
1000
800
600
400
200
0
TM-dxs
TM-iDF
TM-ADS
0.3mM
0.1mM
0.033mM
0.011mM

FIG. 19

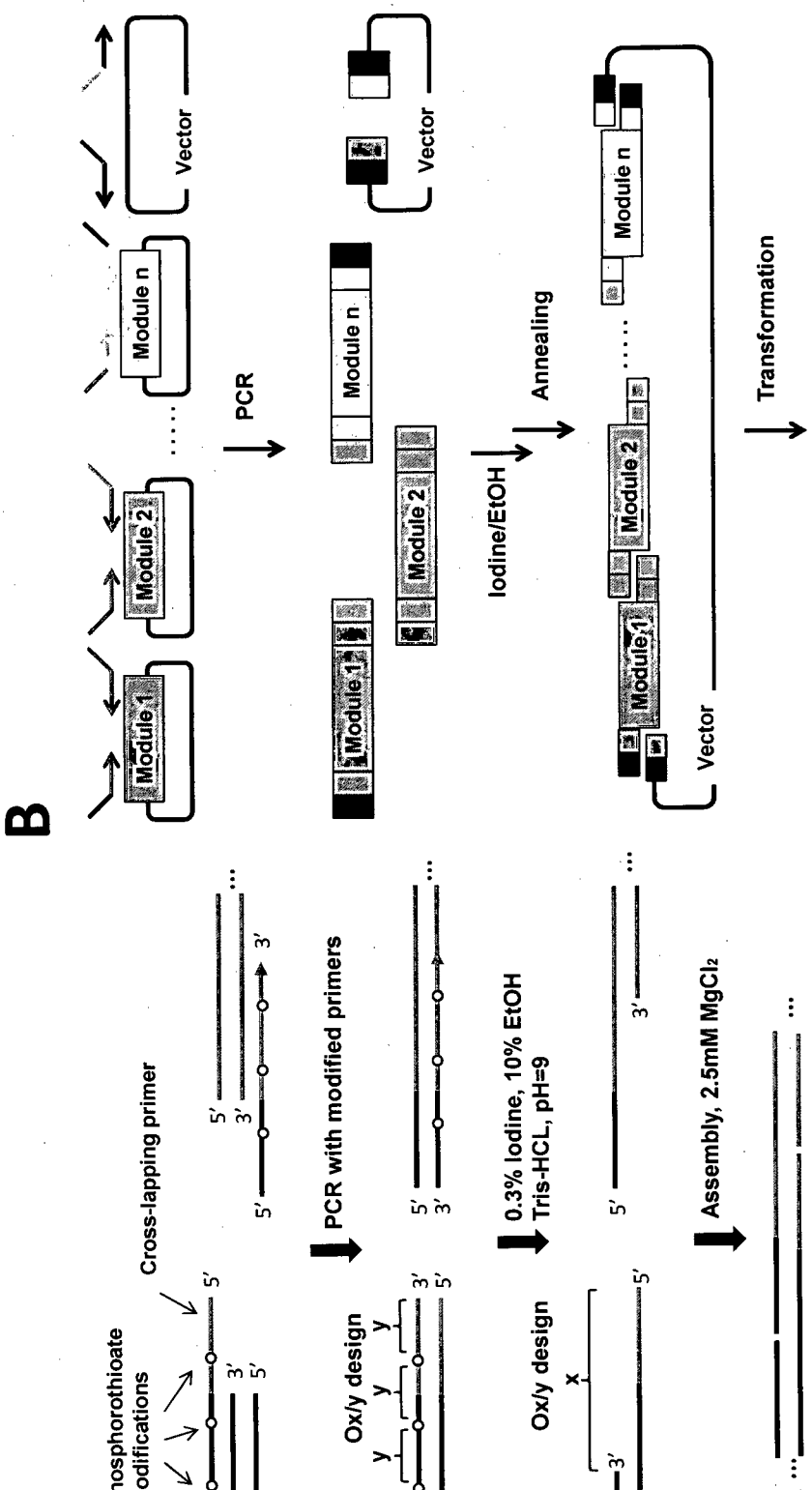

A
Template
Phosphorothioate modifications
Cross-lapping primer
5′
3′
PCR with modified primers
Ox/y design
0.3% Iodine, 10% EtOH
Tris-HCL, pH=9
Assembly, 2.5mM MgCl2
B
Module 1
Module 2
Module n
Vector
PCR
Iodine/EtOH
Annealing
Transformation

FIG. 20

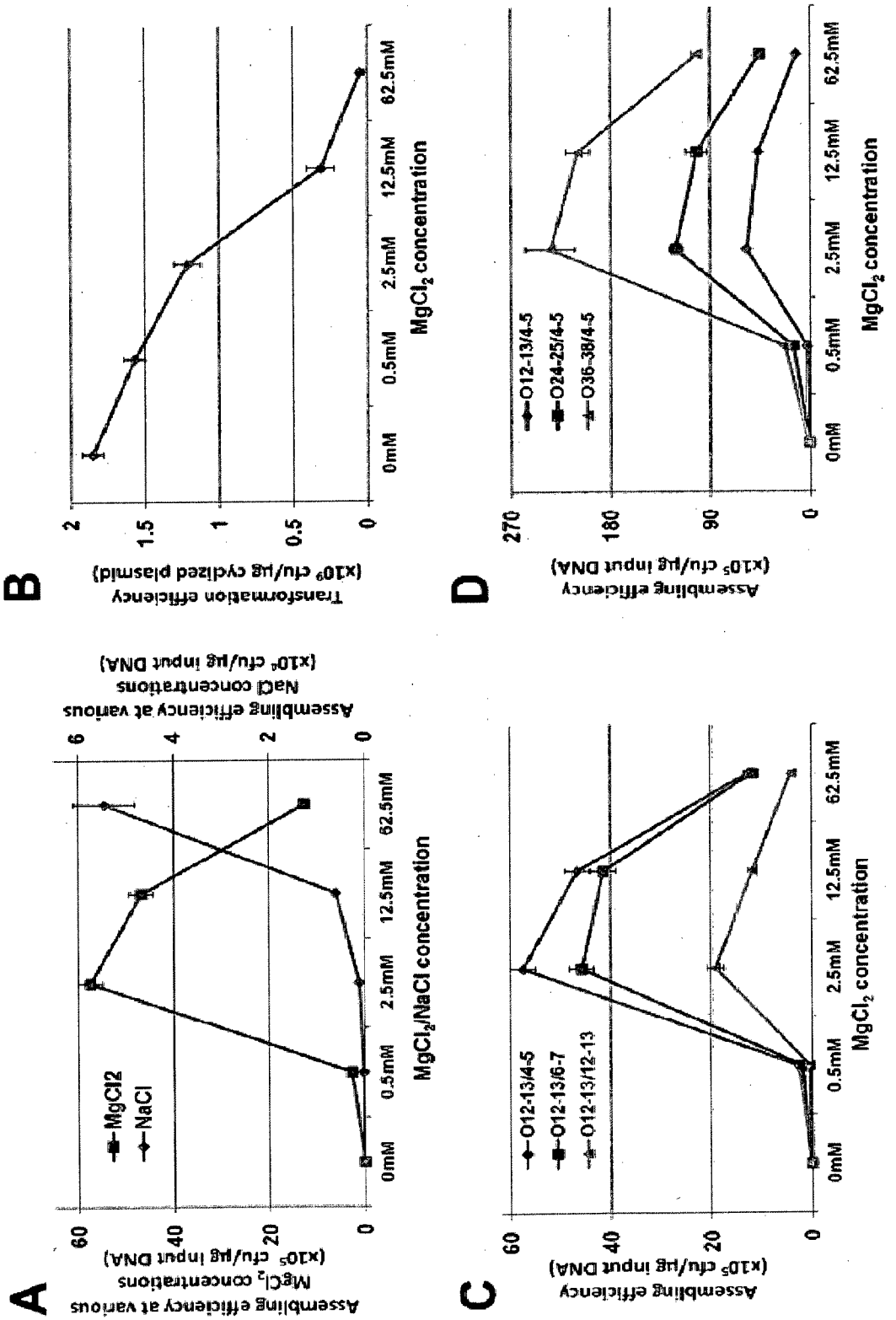

A
Assembling efficiency at various MgCl2 concentrations (x10^5 cfu/μg input DNA)
MgCl2
NaCl
0mM
0.5mM
2.5mM
12.5mM
62.5mM
MgCl2/NaCl concentration
Assembling efficiency at various NaCl concentrations (x10^5 cfu/μg input DNA)
B
Transformation efficiency (x10^9 cfu/μg cyclized plasmid)
MgCl2 concentration
C
Assembling efficiency (x10^5 cfu/μg input DNA)
O12-13/4-5
O12-13/6-7
O12-13/12-13
MgCl2 concentration
D
Assembling efficiency (x10^5 cfu/μg input DNA)
O12-13/4-5
O24-25/4-5
O36-38/4-5
MgCl2 concentration

FIG. 21

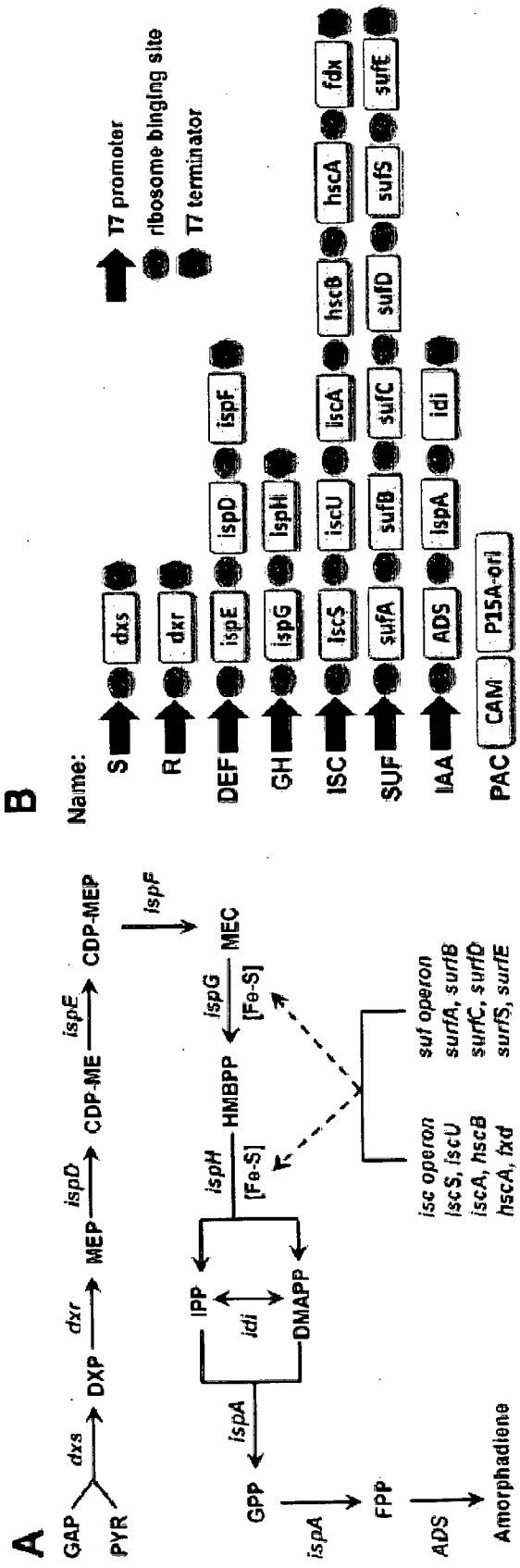

A
GAP
PYR
dxs
DXP
dxr
MEP
ispD
CDP-ME
ispE
CDP-MEP
ispF
MEC
ispG
[Fe-S]
HMBPP
ispH
[Fe-S]
IPP
idi
DMAPP
ispA
GPP
ispA
FPP
ADS
Amorphadiene
isc operon
iscS, iscU
iscA, hscB
hscA, fxd
suf operon
surfA, surfB
surfC, surfD
surfS, surfE
B
Name:
S
R
DEF
GH
ISC
SUF
IAA
PAC
dxs
dxr
ispE
ispD
ispF
ispG
ispH
iscS
iscU
iscA
hscB
hscA
fdx
sufA
sufB
sufC
sufD
sufS
sufE
ADS
ispA
idi
CAM
P15A-ori
T7 promoter
ribosome binding site
T7 terminator

FIG. 22

FIG. 23A

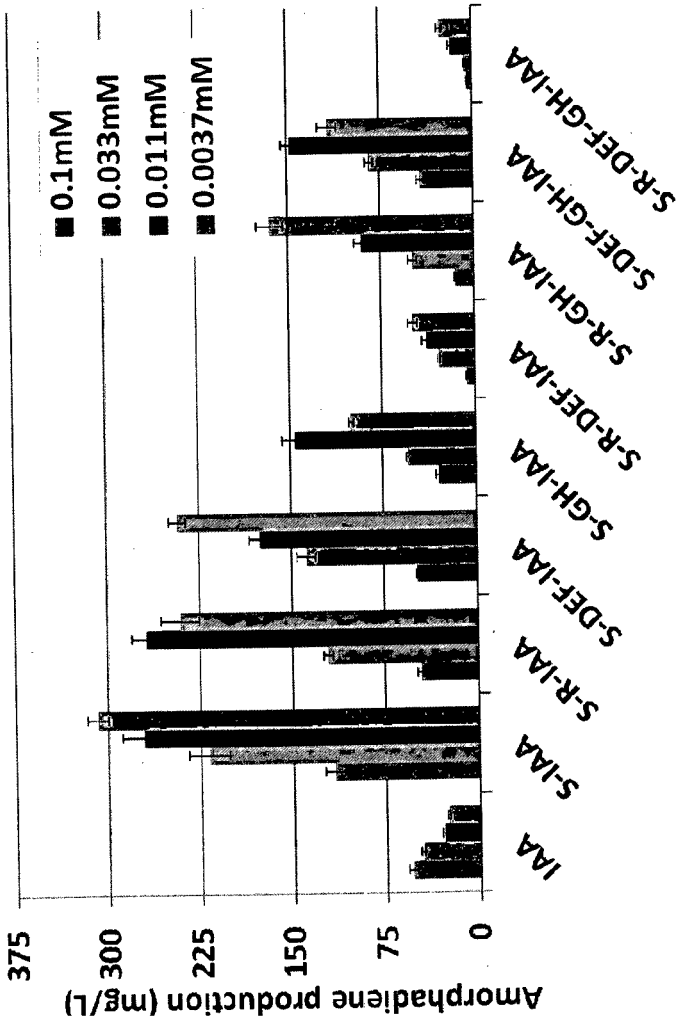


FIG. 23B

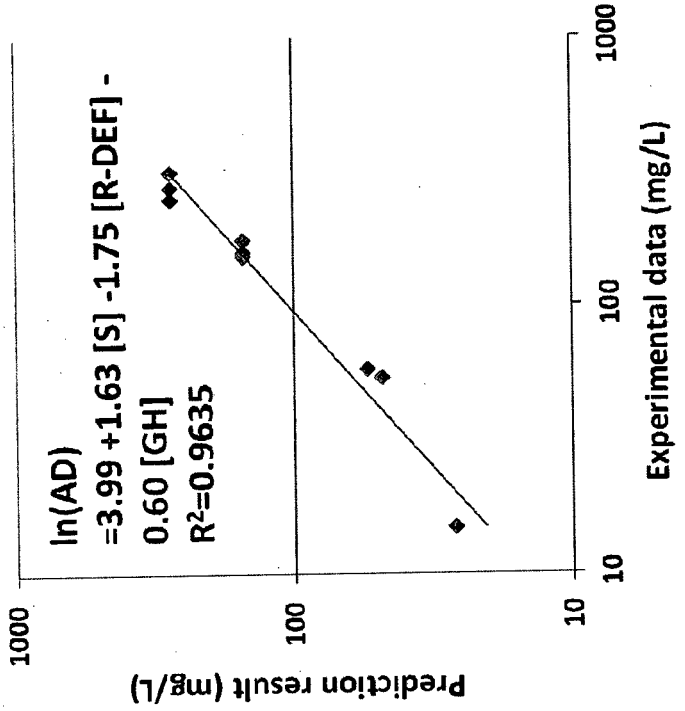


FIG. 23C

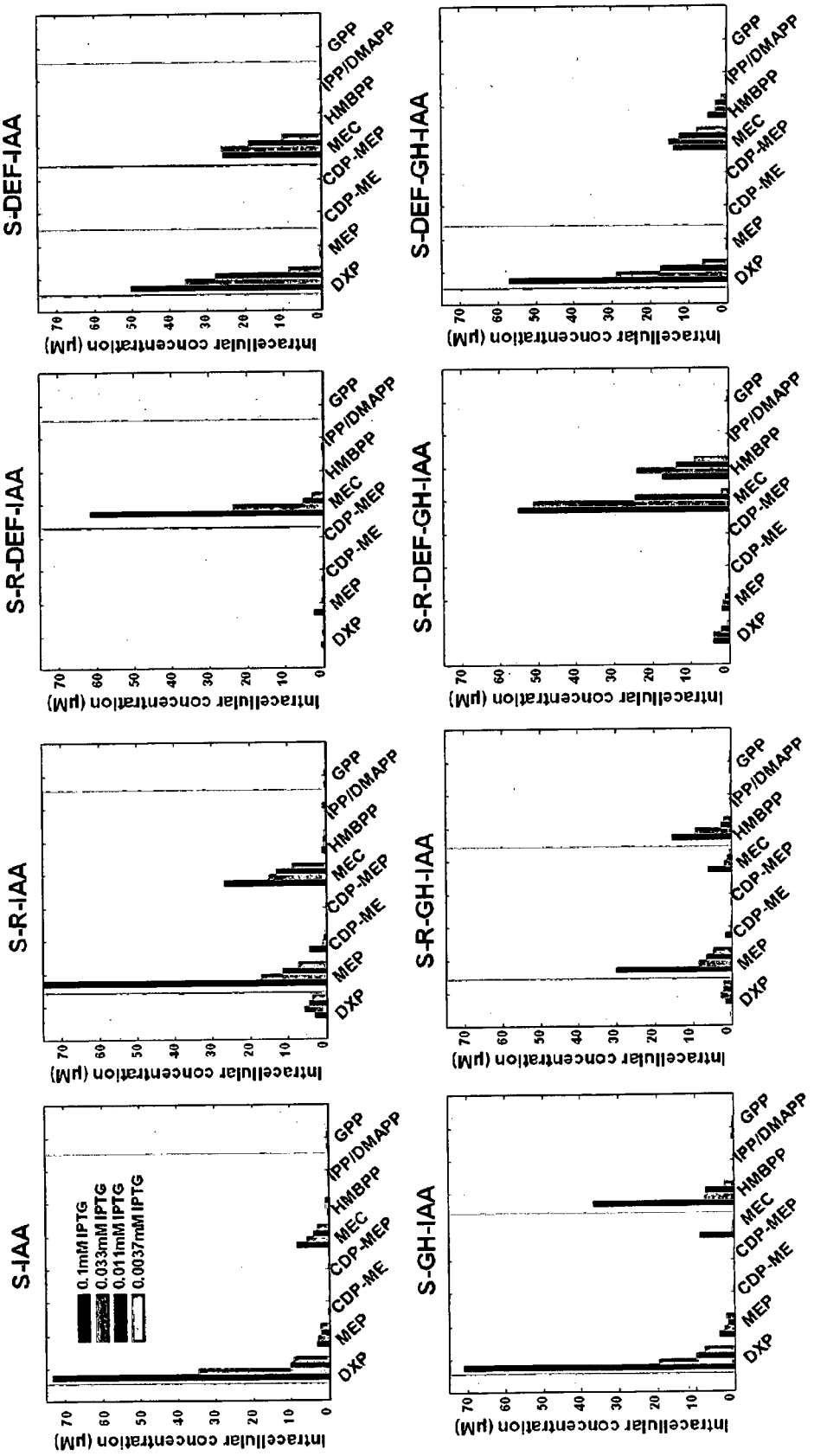

S-IAA
S-R-IAA
S-R-DEF-IAA
S-DEF-IAA
S-GH-IAA
S-R-GH-IAA
S-R-DEF-GH-IAA
S-DEF-GH-IAA
0.1mM IPTG
0.033mM IPTG
0.011mM IPTG
0.0037mM IPTG
DXP
MEP
CDP-ME
CDP-MEP
MEC
HMBPP
IPP/DMAPP
GPP
Intracellular concentration (μM)
0
10
20
30
40
50
60
70

FIG. 24 A intracellular metabolites (µM/OD)

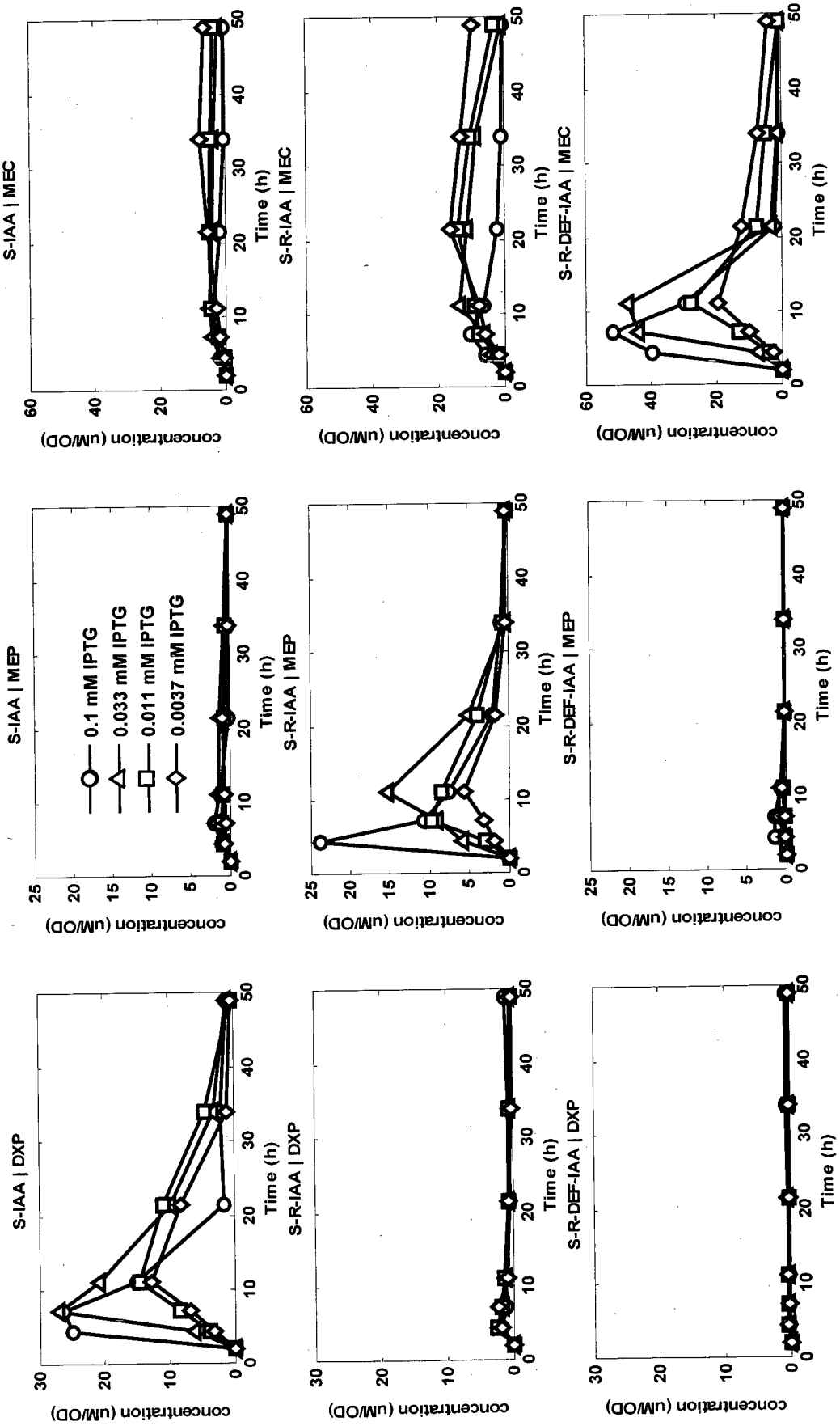
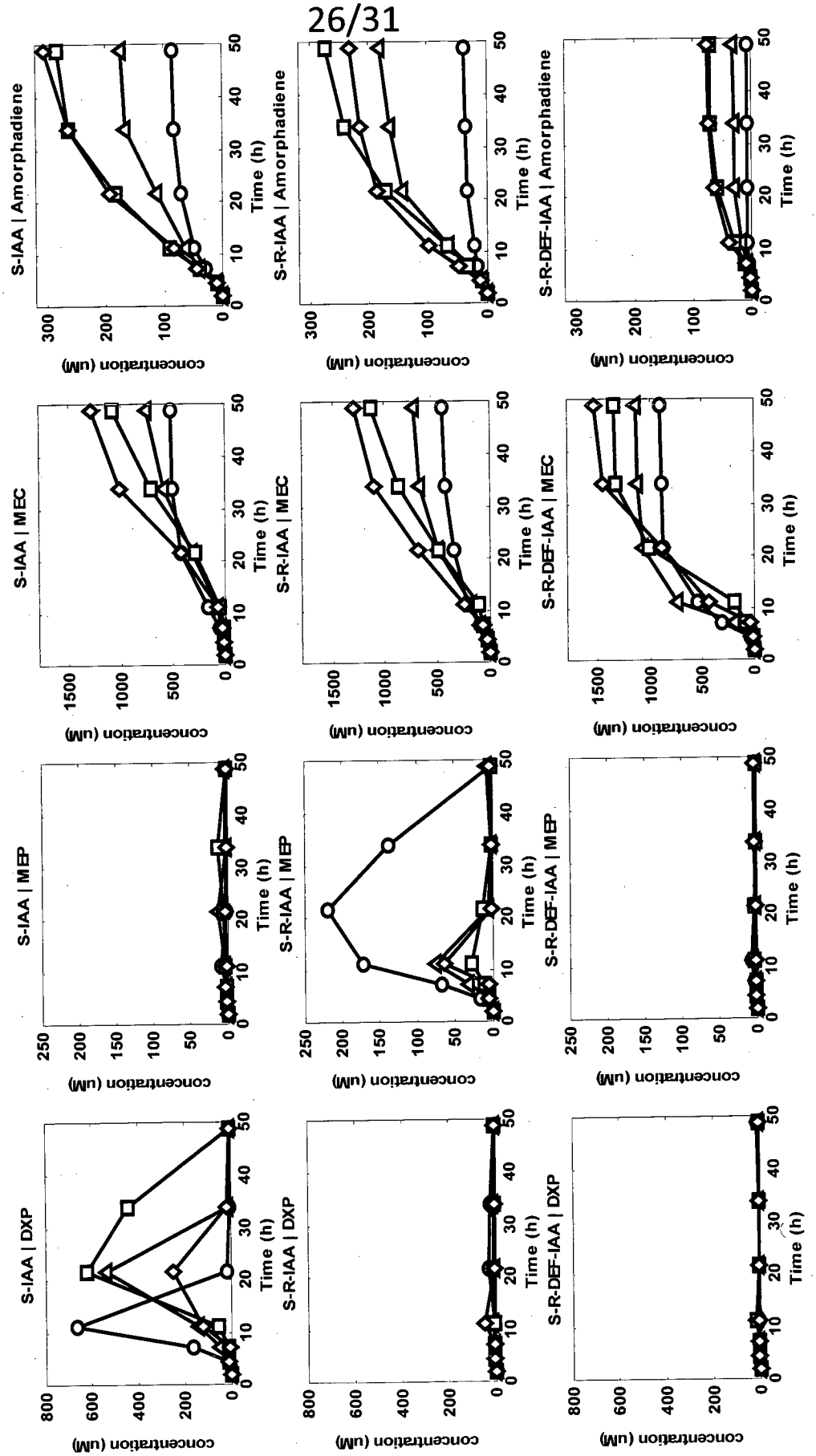


FIG. 24B extracellular concentration (μM)



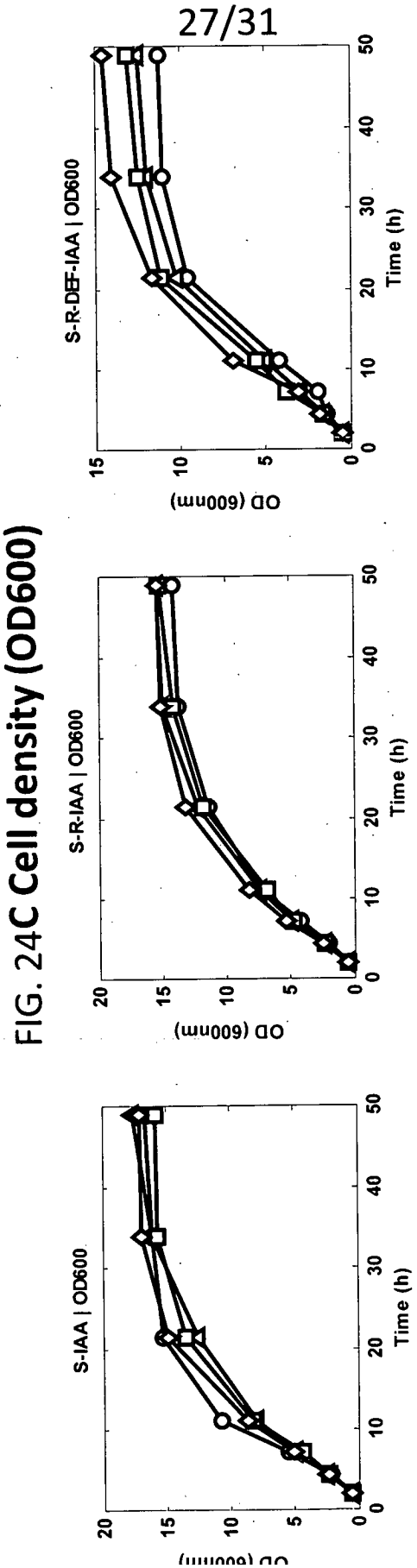


FIG. 24C Cell density (OD600)

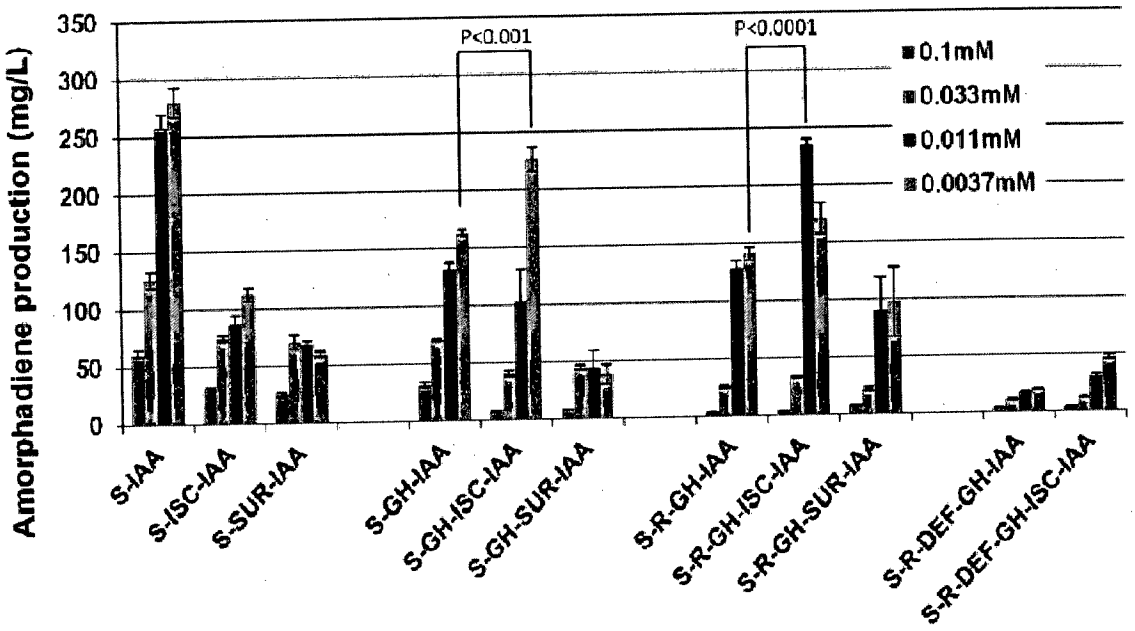

Amorphadiene production (mg/L)
350
300
250
200
150
100
50
0
P<0.001
P<0.0001
0.1mM
0.033mM
0.011mM
0.0037mM
S-IAA
S-ISC-IAA
S-SUR-IAA
S-GH-IAA
S-GH-ISC-IAA
S-GH-SUR-IAA
S-R-GH-IAA
S-R-GH-ISC-IAA
S-R-GH-SUR-IAA
S-R-DEF-GH-IAA
S-R-DEF-GH-ISC-IAA

FIG. 25

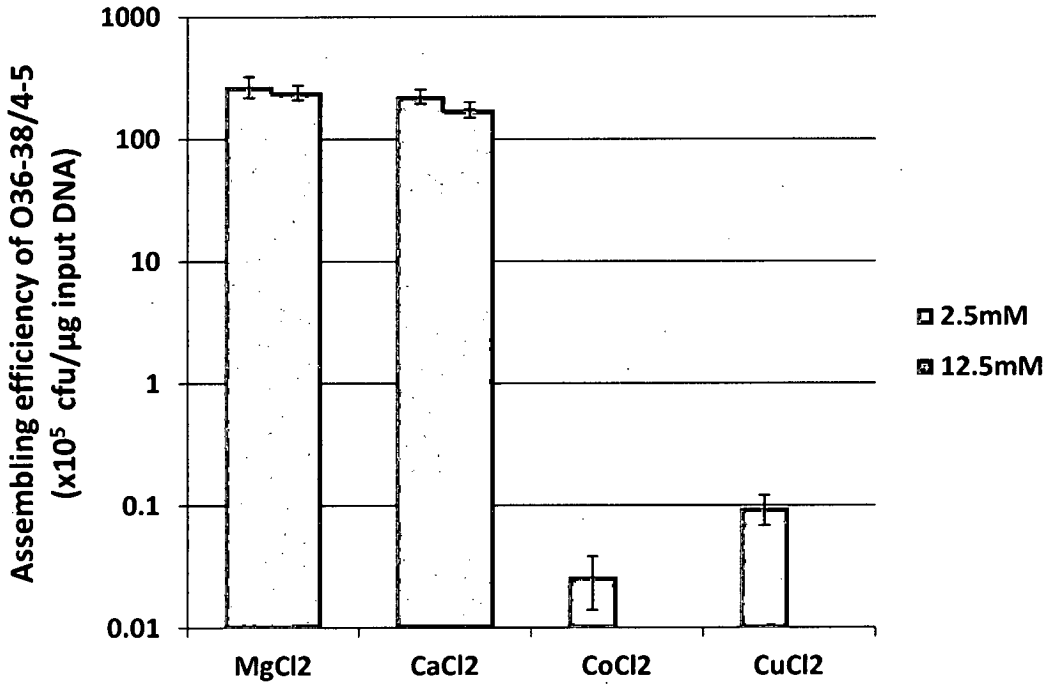

Assembling efficiency of O36-38/4-5
(x10^5 cfu/µg input DNA)
1000
100
10
1
0.1
0.01
MgCl2
CaCl2
CoCl2
CuCl2
2.5mM
12.5mM

FIG. 26

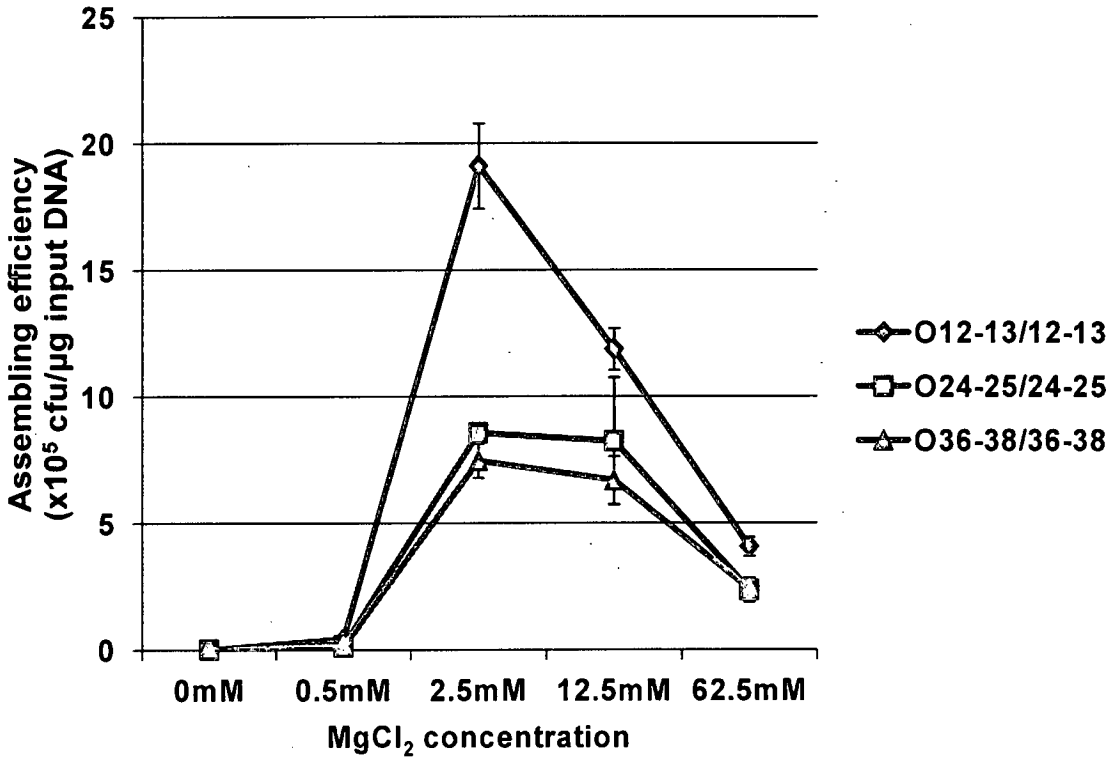

25
20
15
10
5
0
Assembling efficiency
(x10⁵ cfu/µg input DNA)
0mM
0.5mM
2.5mM
12.5mM
62.5mM
MgCl2 concentration
O12-13/12-13
O24-25/24-25
O36-38/36-38

FIG. 27

atgtctctgactgaggaaaaaccaatccgtccgatcgcaaactttccgccgagcatc
tggggtgatcagttcctgatctaccagaagcaggtcgagcaaggcgtagaacagatc
gtgaacgacctgaaaaaggaggtacgtcagctgctgaaagaggctctggacatcccg
atgaaacatgcgaacctgctgaagctgattgatgagattcagcgcctgggtatccca
taccacttcgaacgtgaaattgatcacgcgctgcagtgtatttatgaaacgtatggt
gataactggaacggtgaccgtagctccctgtggttccgtctgatgcgtaagcagggt
tattacgtgacctgcgacgtcttcaacaactacaaagacaagaacggcgcgtttaaa
cagagcctggcgaatgacgttgaaggcctgctggagctgtatgaagcaacctctatg
cgtgttccgggcgaaatcatcctggaagatgctctgggtttcacccgttctcgcctg
tctatcatgaccaaggacgcattttccactaacccggccctgttcaccgaaatccag
cgtgcgctgaaacagcctctgtggaagcgtctgccgcgtatcgaggcggcacagtac
atcccgttctatcagcaacaggattcccacaacaaaaccctgctgaaactggcgaaa
ctggaatttaacctgctgcaatccctgcacaaagaagaactgtctcatgtttgcaaa
tggtggaaagccttcgacatcaagaaaaacgcgccgtgcctgcgcgaccgtatcgta
gaatgctatttctggggcctgggctccggttatgagcctcaatactctcgtgctcgc
gtattcttcaccaaagcggtagctgtgatcaccctgatcgacgatacctacgatgct
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acttgcctggataccctgccggaatacatgaaaccgatctataaactgttcatggat
acttataccgaaatggaagagttcctggcaaaagaaggtcgcactgatctgttcaac
tgtggcaaggagtttgtgaaagaattcgtccgcaacctgatggtggaggcgaagtgg
gcaaacgagggtcacatcccgaccaccgaagaacacgacccggtagttatcatcacc
ggtggcgcaaacctgctgactaccacttgctacctgggtatgtccgacatttttacg
aaagaaagcgttgaatgggcagtgtctgcgcctccgctgttccgttactctggtatc
ctgggccgtcgcctgaacgatctgatgacccataaagccgagcaggaacgtaaacac
tccagcagctctctggaatcttacatgaaagaatacaacgttaacgaagagtatgcg
cagaccctgatctacaaagaggttgaggatgtatggaaagacatcaaccgtgagtac
ctgactactaaaaacatcccacgcccgctgctgatggcagtgatttatctgtgccag
tttctggaagtgcagtatgcgggcaaagataacttcacccgtatgggtgacgaatac
aaacacctgatcaagtccctgctggtttacccaatgtccatc

FIG. 28