Figure 1

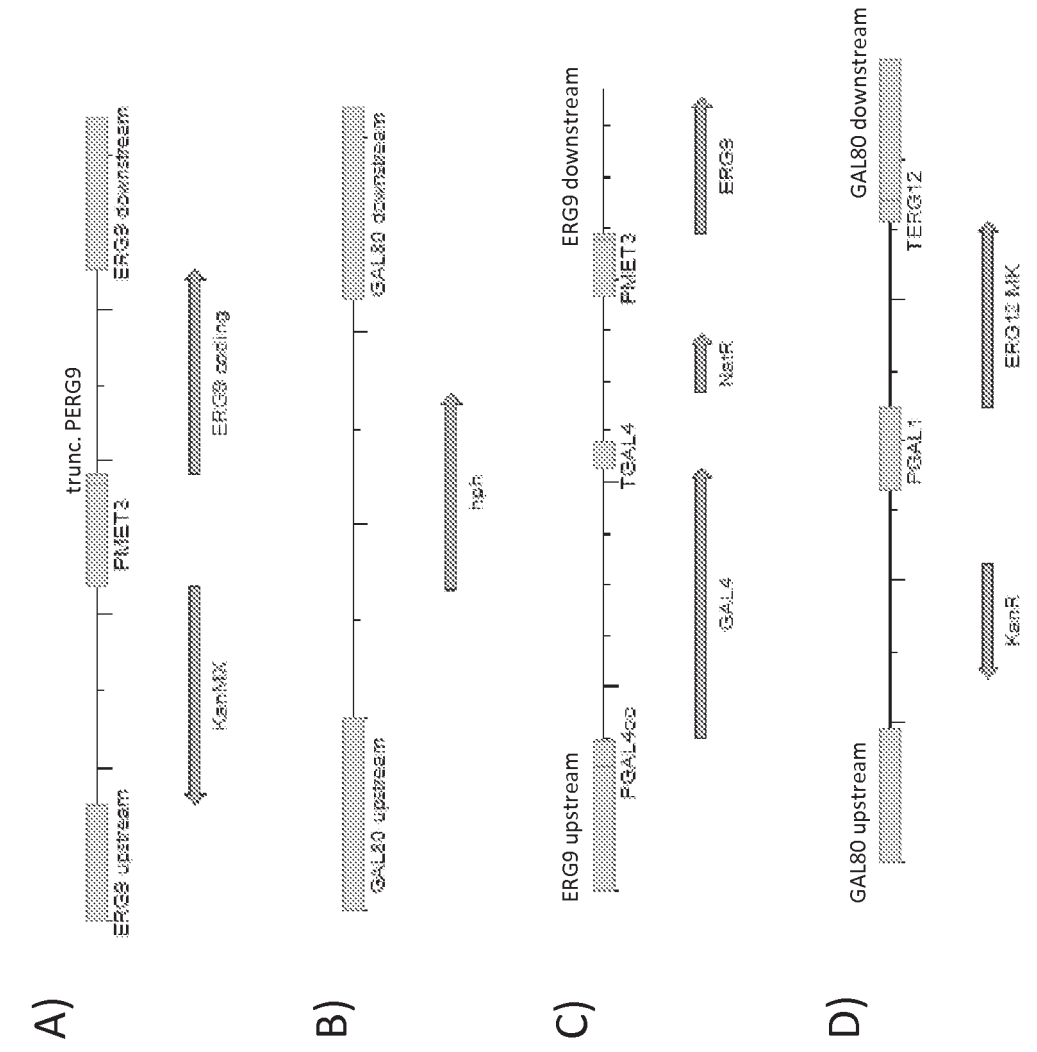

A)
trunc. PERG9
ERG9 upstream
PMET3
ERG9 downstream
KanMX
ERG9 coding
B)
GAL80 upstream
GAL80 downstream
hph
C)
ERG9 upstream
ERG9 downstream
PGAL4cc
TGAL4
PMET3
GAL4
NatR
ERG9
D)
GAL80 upstream
GAL80 downstream
PGAL1
TERG12
KanR
ERG12 MK

Figure 1 (continued)

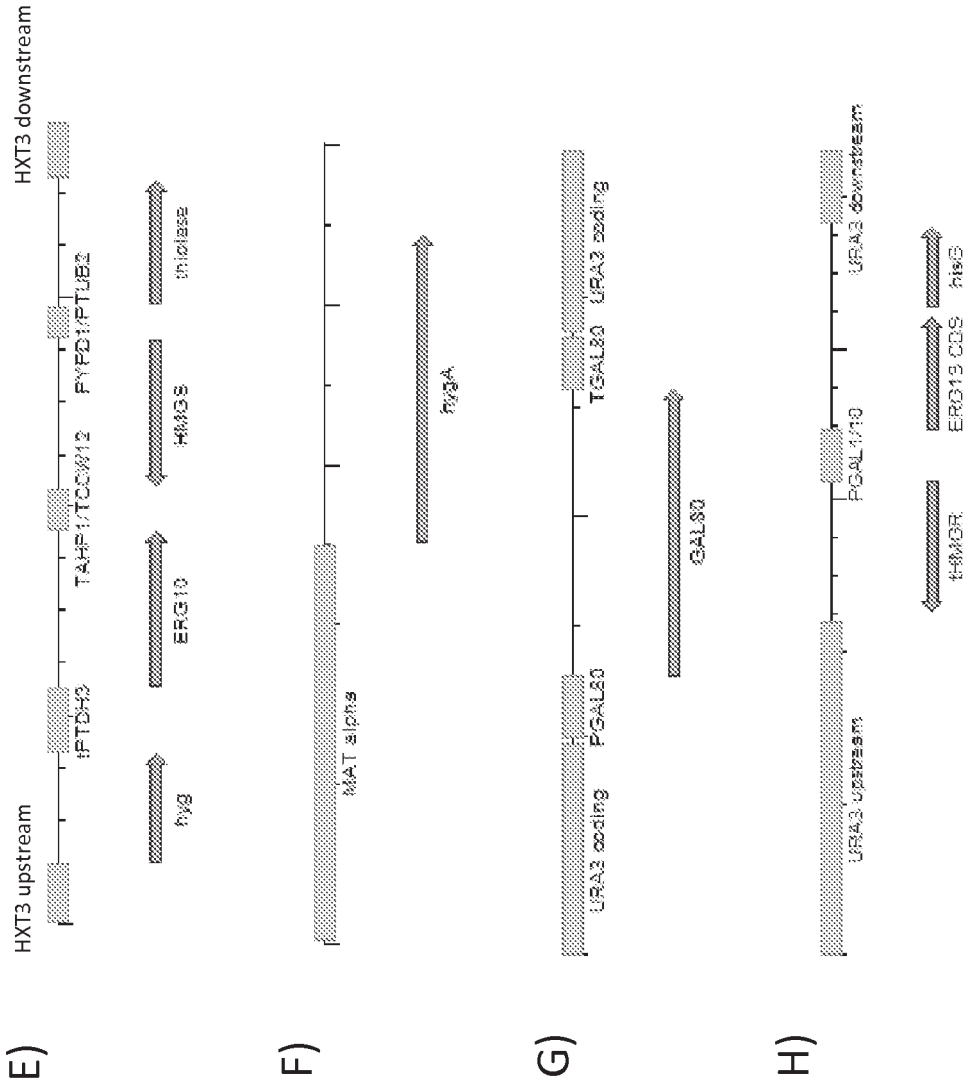


Figure 1 (continued)

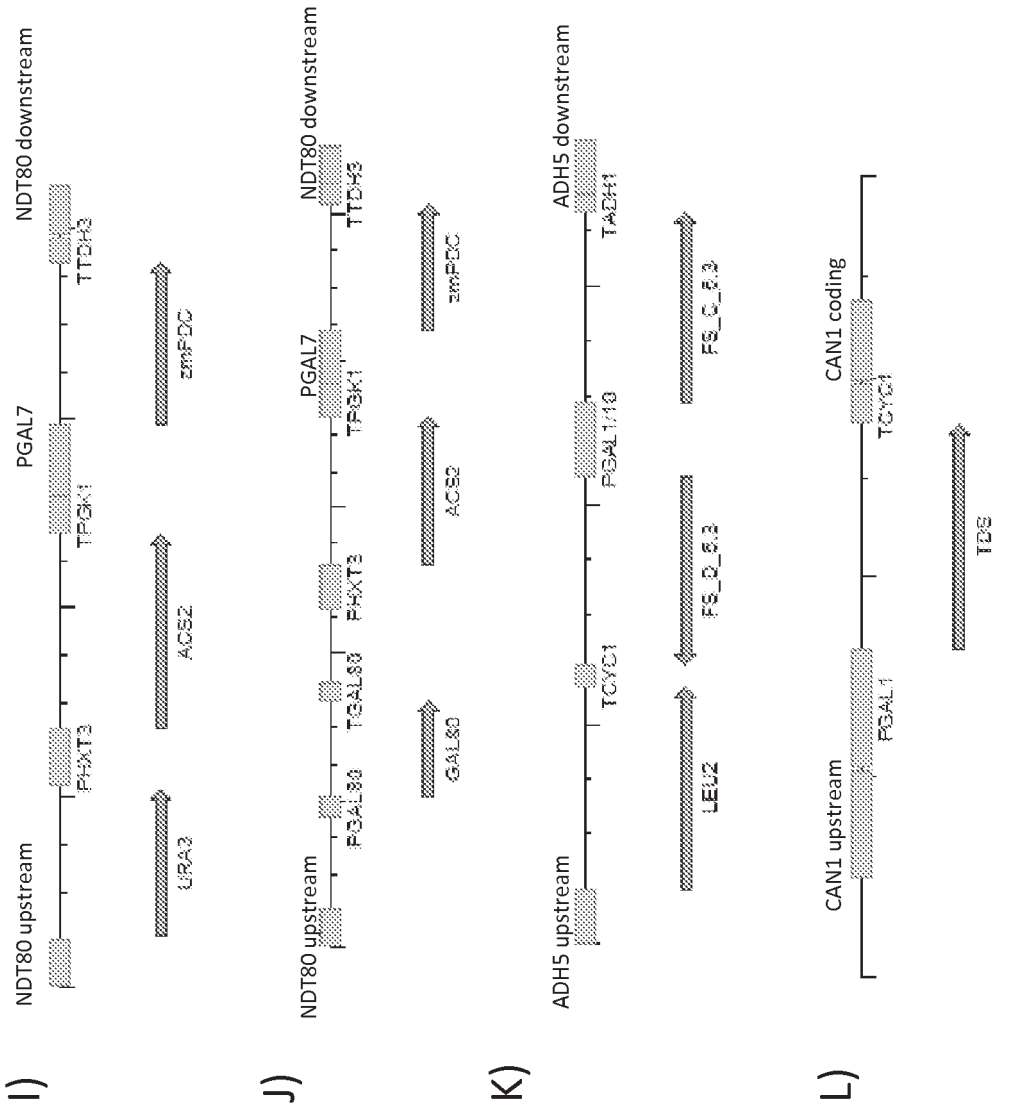


Figure 1 (continued)

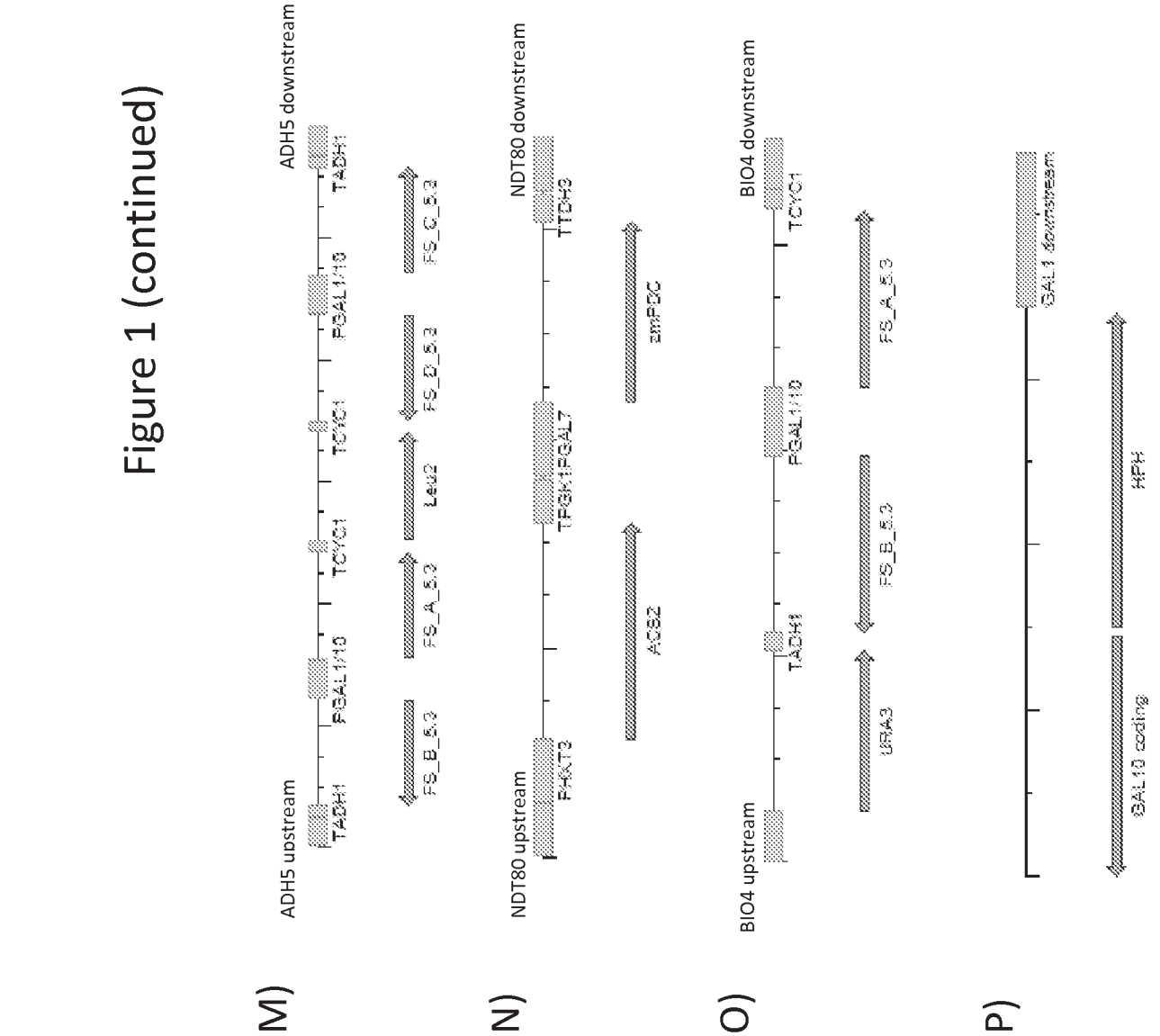


Figure 1 (continued)

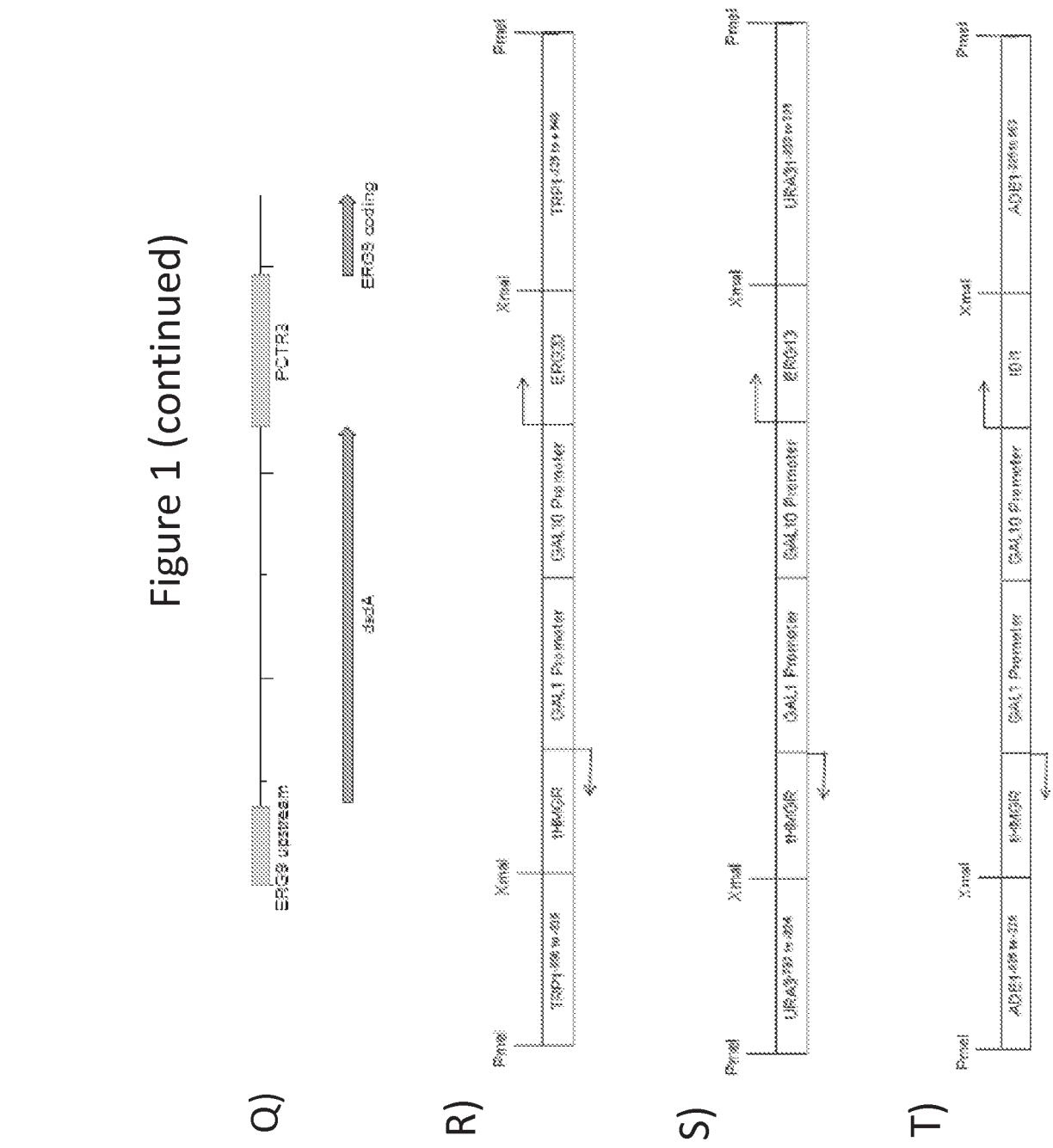
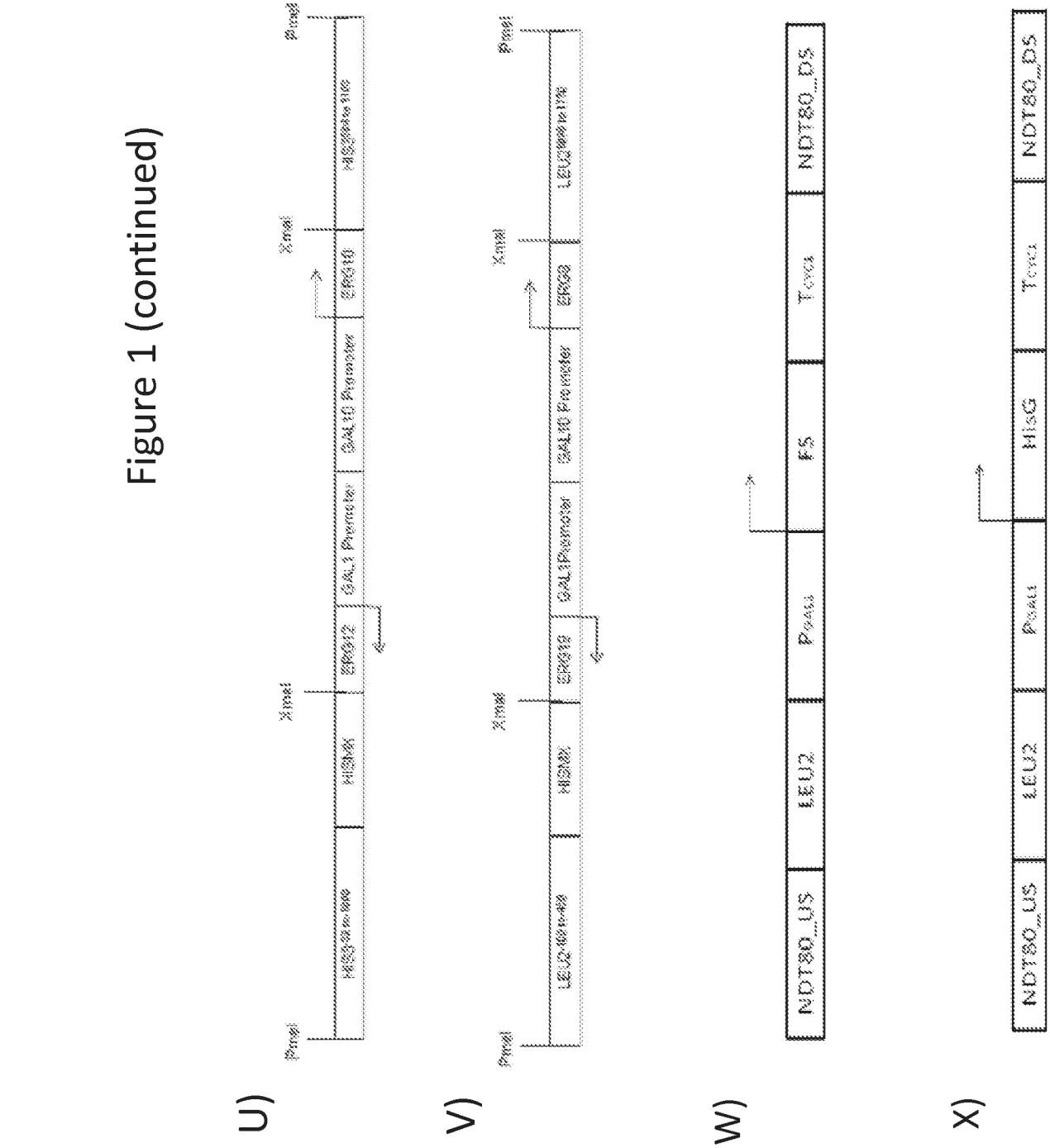


Figure 1 (continued)



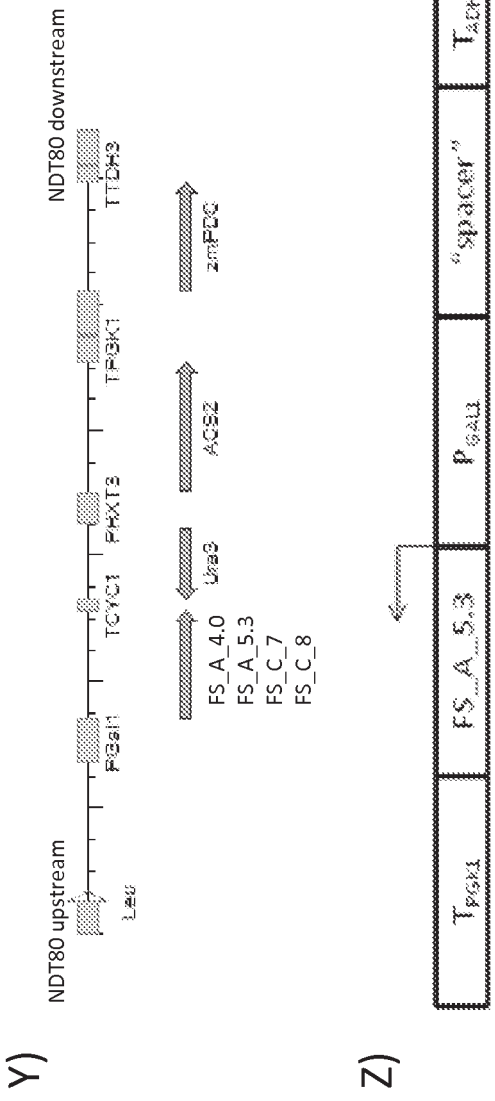
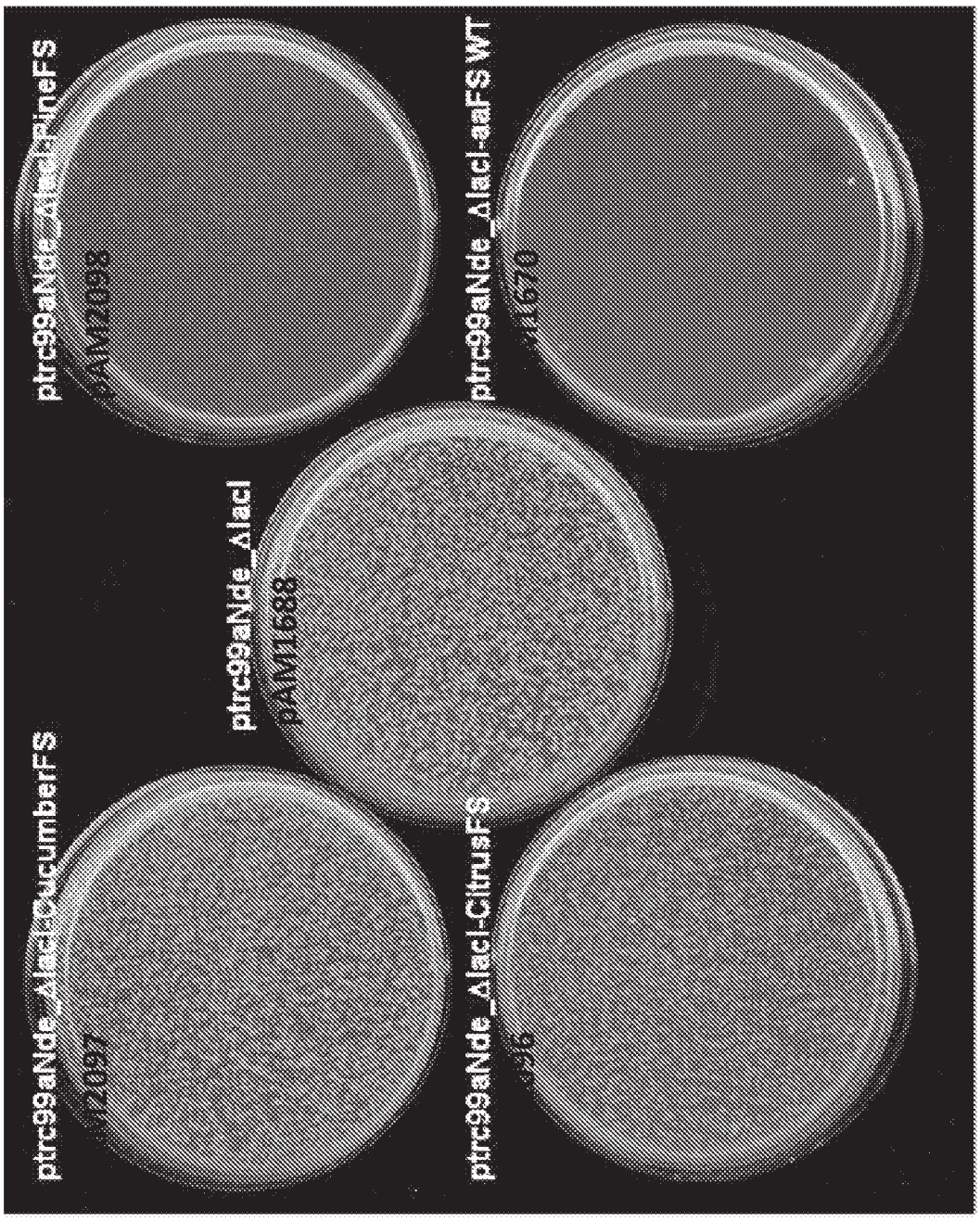
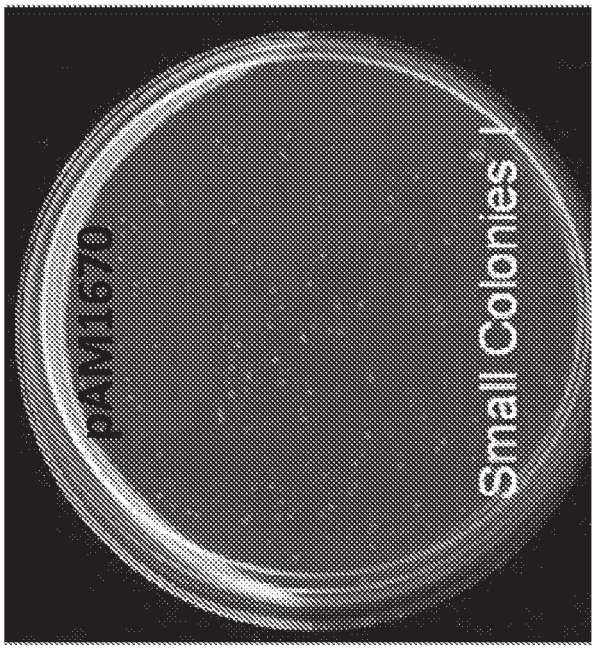

Y)
NDT80 upstream
NDT80 downstream
Leu2
PGal1
TCYC1
PMXT3
TPGK1
TTDH3
FS_A_4.0
FS_A_5.3
FS_C_7
FS_C_8
Ura3
ACS2
zmPDC
Z)
T_{PGK1}
FS_A_5.3
P_{GAL1}
"spacer"
T_{ADH1}
—TDS-P_{GAL10}

Figure 2


ptrc99aNde_AlacI-CucumberFS
pAM2097
ptrc99aNde_AlacI
pAM1688
ptrc99aNde_AlacI-PineFS
pAM2098
ptrc99aNde_AlacI-CitrusFS
pAM
ptrc99aNde_AlacI-aaFS WT
pAM1670
Small colonies
Small colonies
Similar size colonies as control
Similar size colonies as control



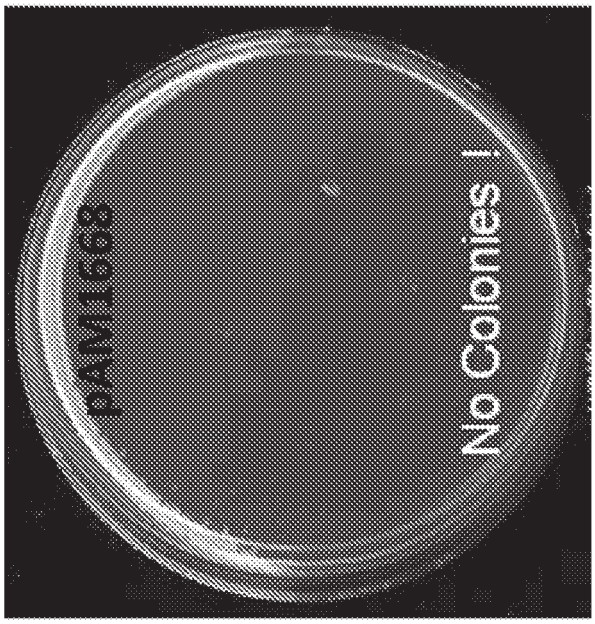


Figure 3

Figure 4

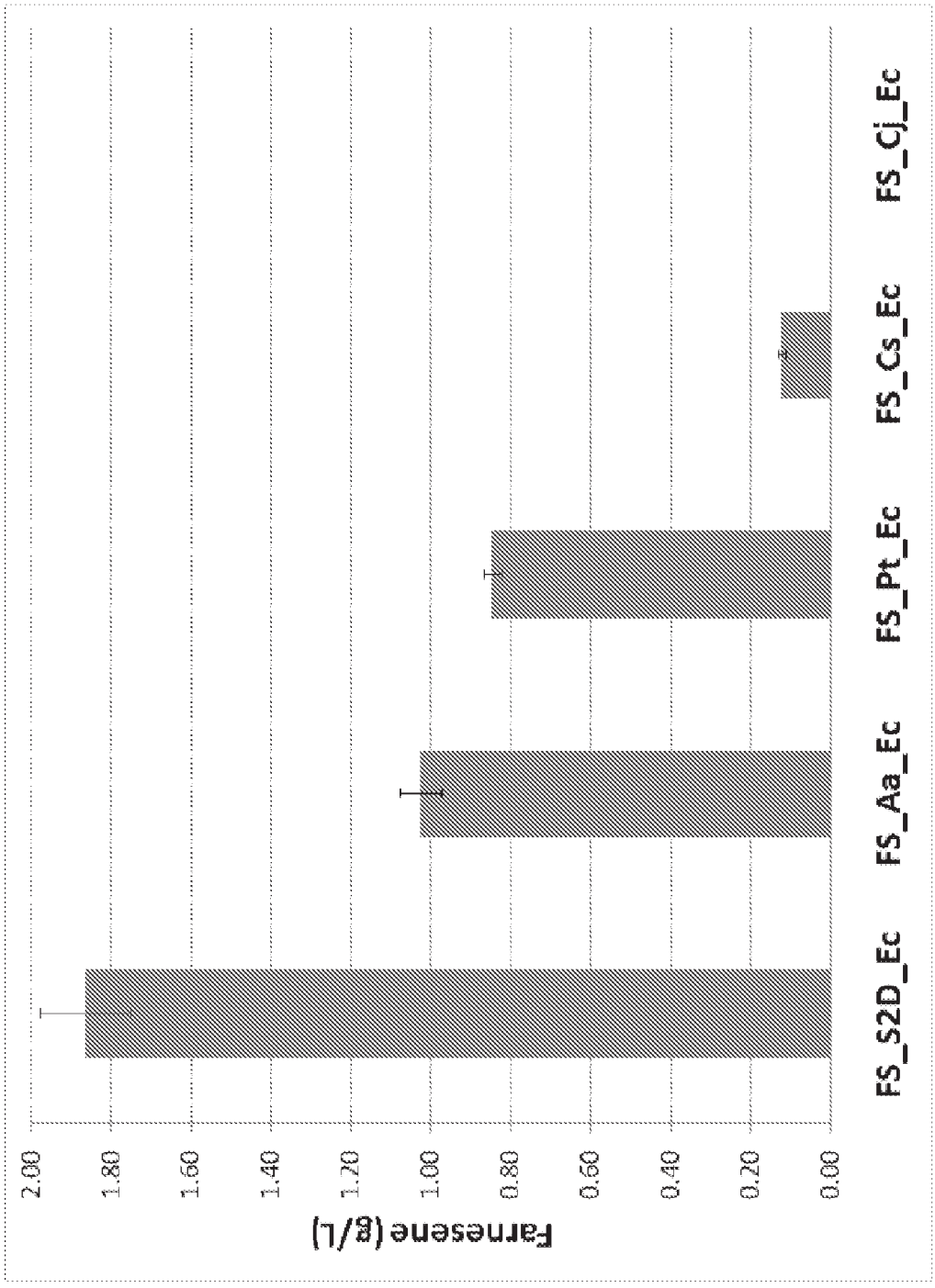

Farnesene (g/L)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
FS_S2D_Ec
FS_Aa_Ec
FS_Pt_Ec
FS_Cs_Ec
FS_Cj_Ec

Figure 5

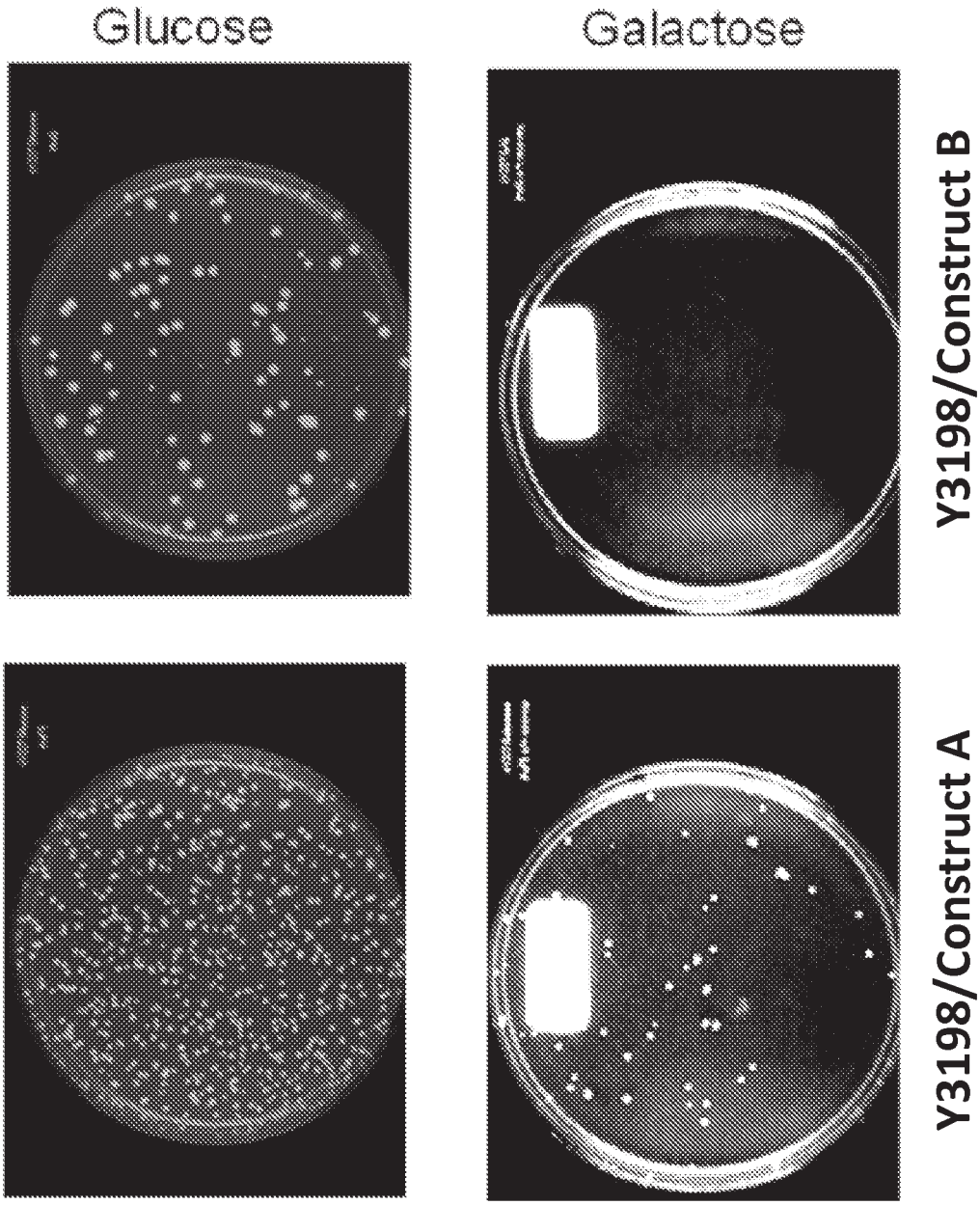


Figure 6

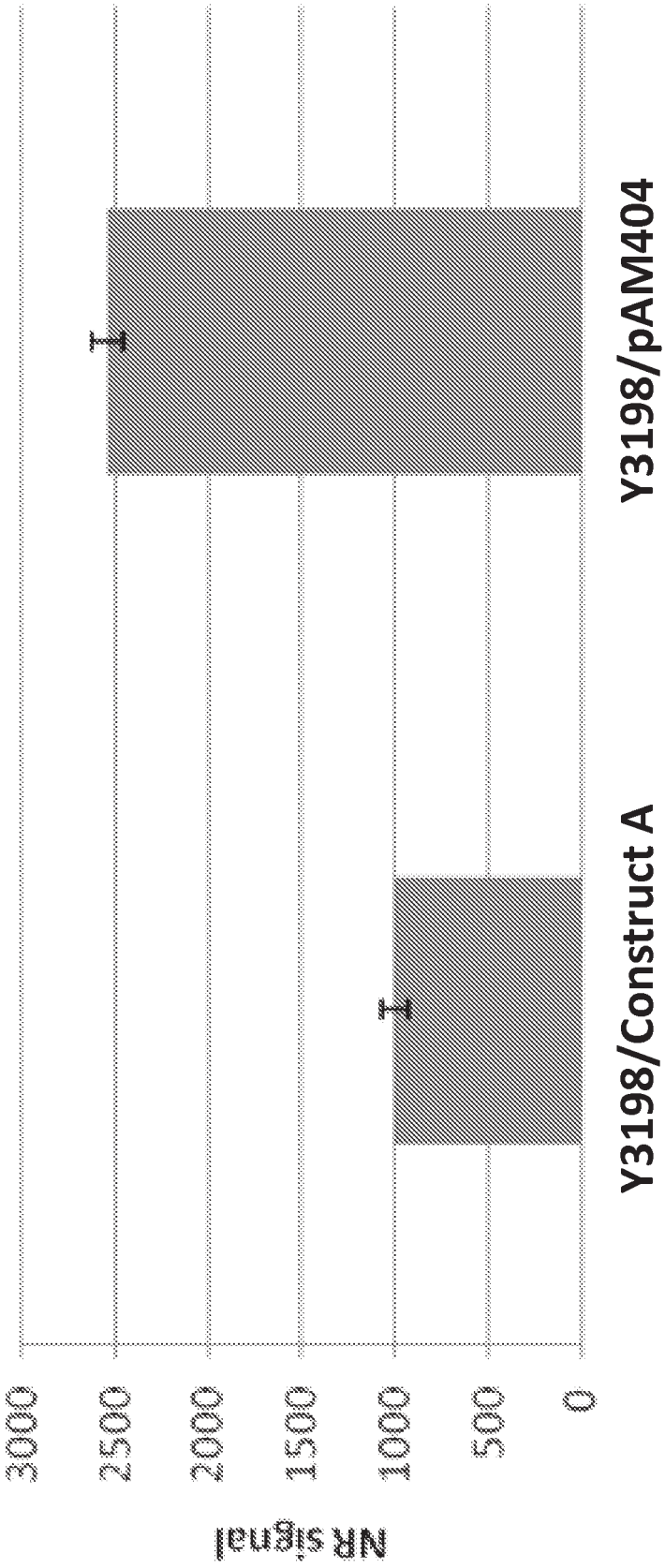

NR signal
3000
2500
2000
1500
1000
500
0
Y3198/Construct A
Y3198/pAM404

Figure 7

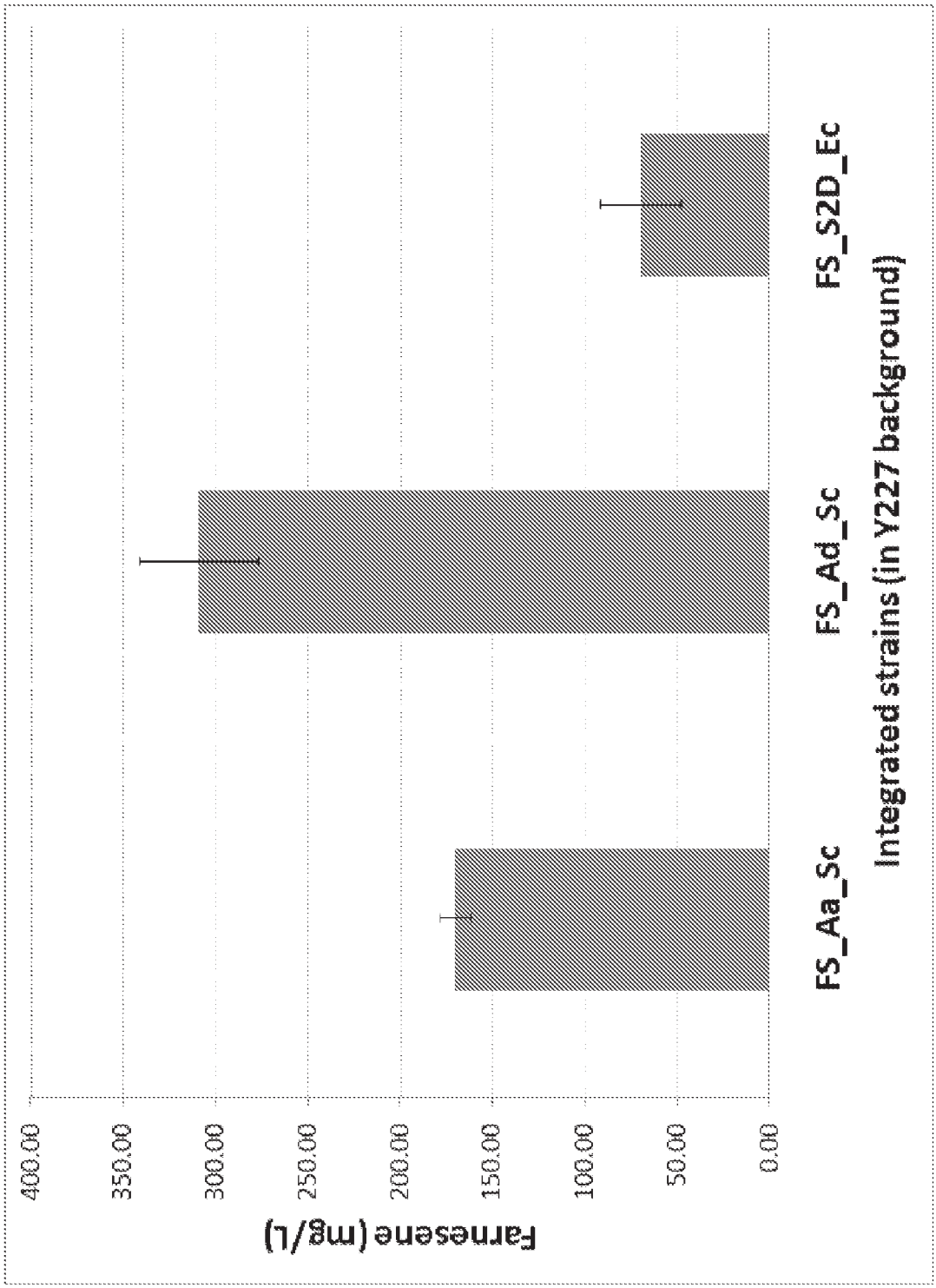

Farnesene (mg/L)
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
FS_Aa_Sc
FS_Ad_Sc
FS_S2D_Ec
Integrated strains (in Y227 background)

Figure 8

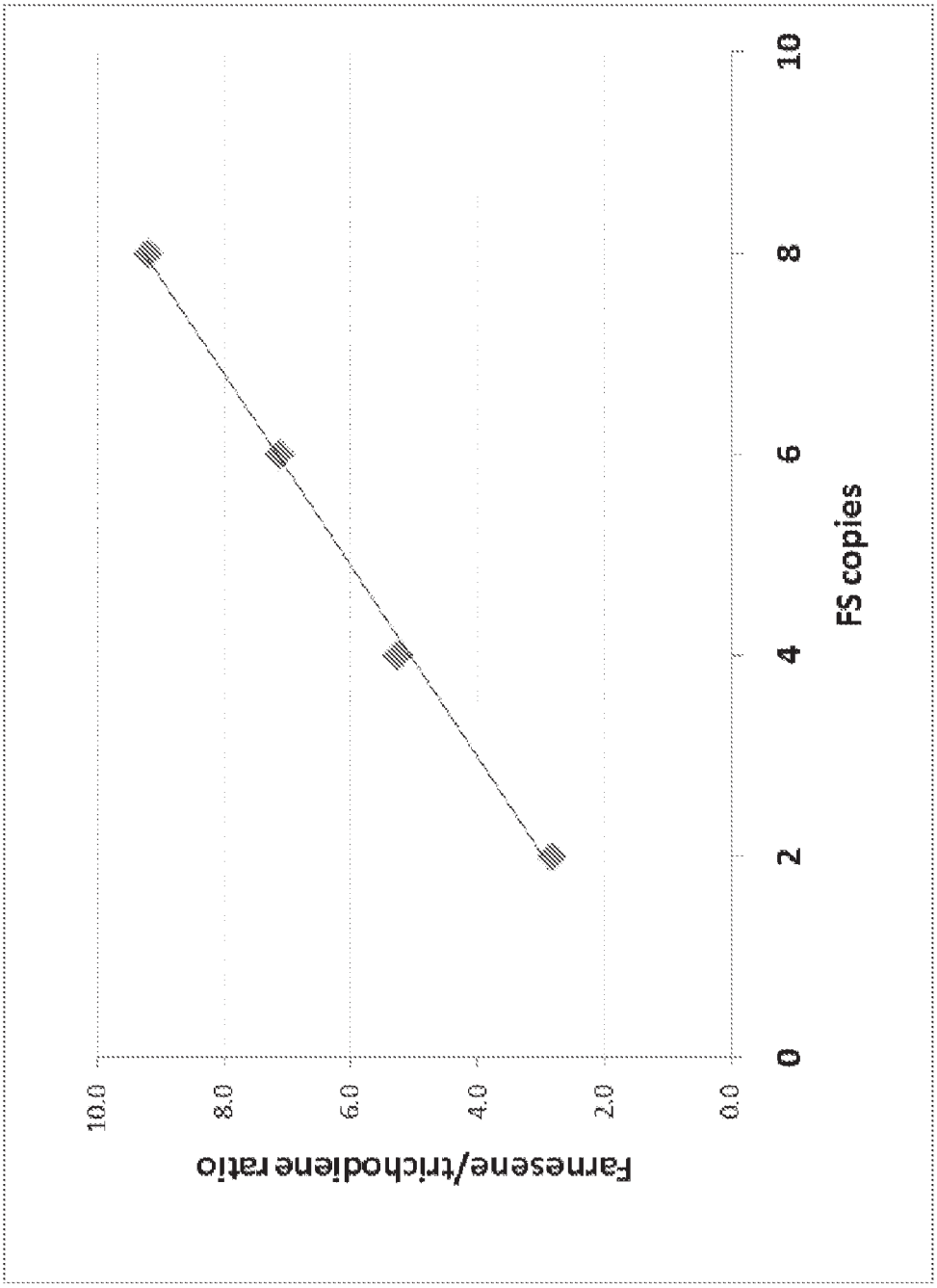

Farnesene/trichodiene ratio
10.0
8.0
6.0
4.0
2.0
0.0
0
2
4
6
8
10
FS copies

Figure 9

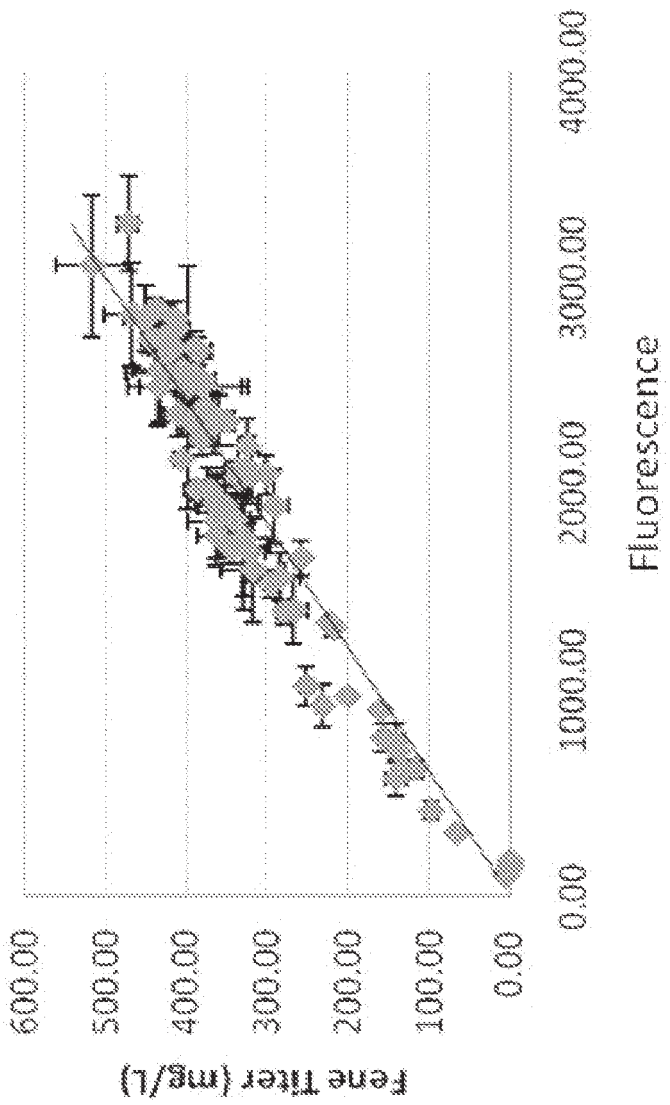

y = 0.1665x
R² = 0.9233
Fene Titer (mg/L)
600.00
500.00
400.00
300.00
200.00
100.00
0.00
0.00
1000.00
2000.00
3000.00
4000.00
Fluorescence

Figure 10

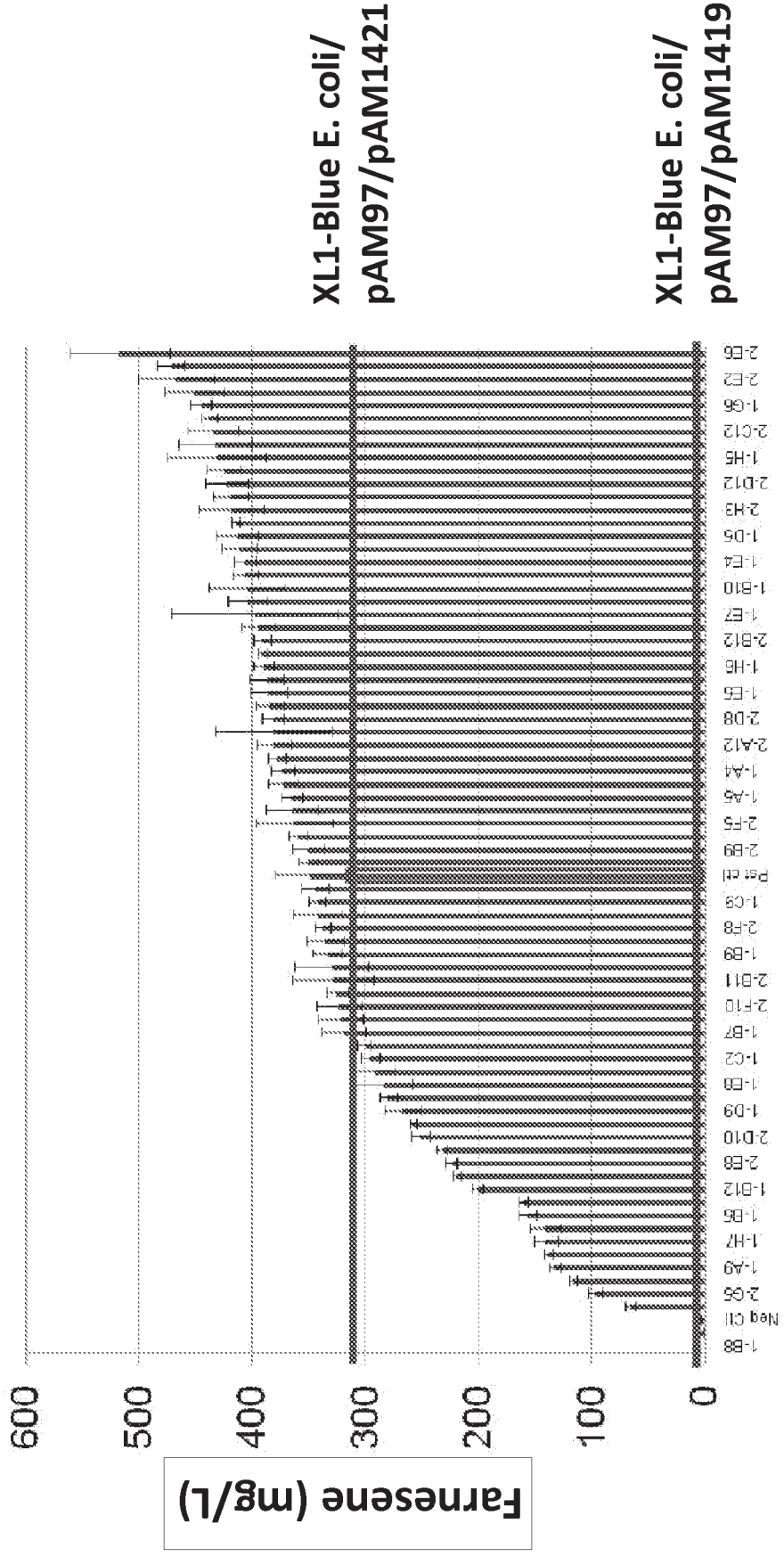

XL1-Blue E. coli/ pAM97/pAM1421
XL1-Blue E. coli/ pAM97/pAM1419
Farnesene (mg/L)
600
500
400
300
200
100
0
1-B8
Neg Ctl
2-G5
1-A9
1-H7
1-B5
1-B12
2-E8
2-D10
1-D9
1-E8
1-C2
1-B7
2-F10
2-B11
1-B9
2-F8
1-C9
Pst ctl
2-B9
2-F5
1-A5
1-A4
2-A12
2-D8
1-E5
1-H5
2-B12
1-E7
1-B10
1-E4
1-D5
2-H3
2-D12
1-H5
2-C12
1-G6
2-E2
2-E6

Figure 11

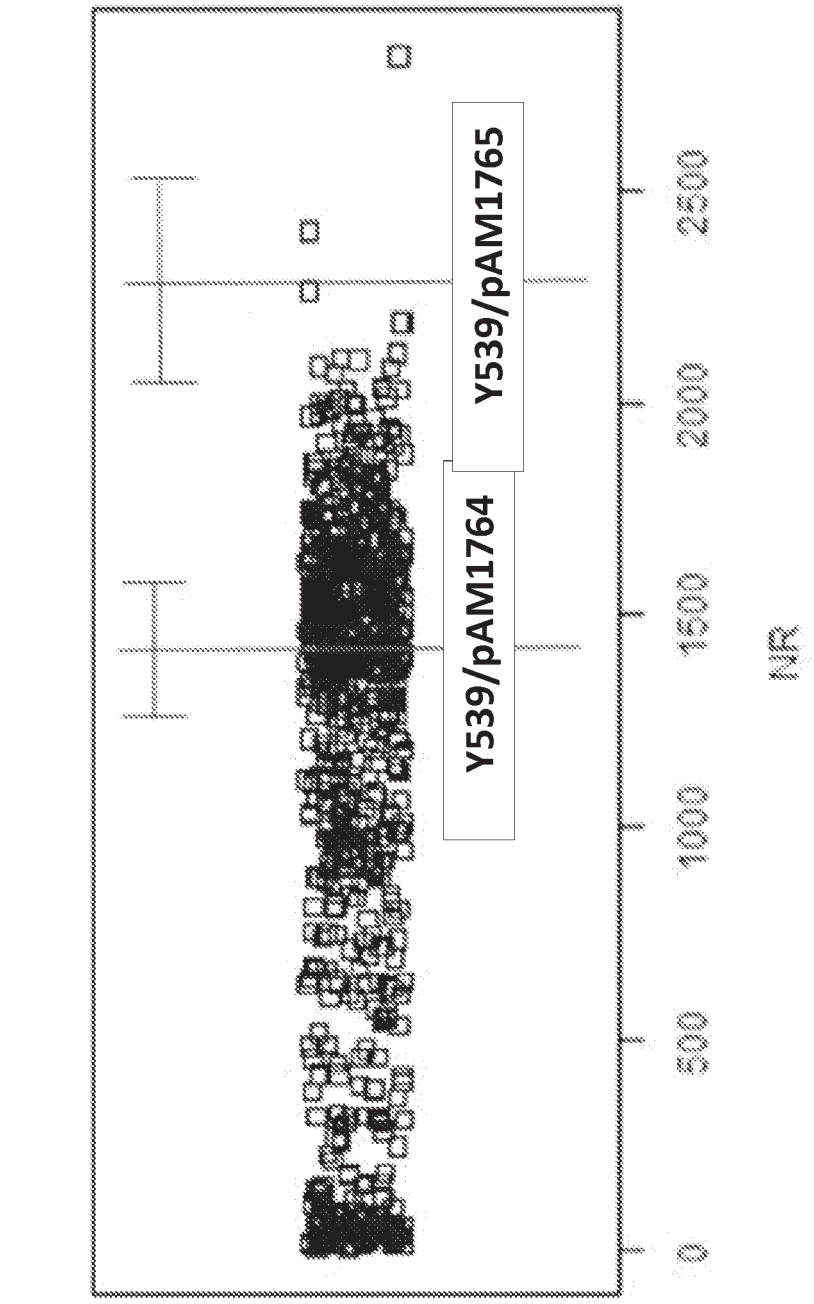


Figure 11 (continued)

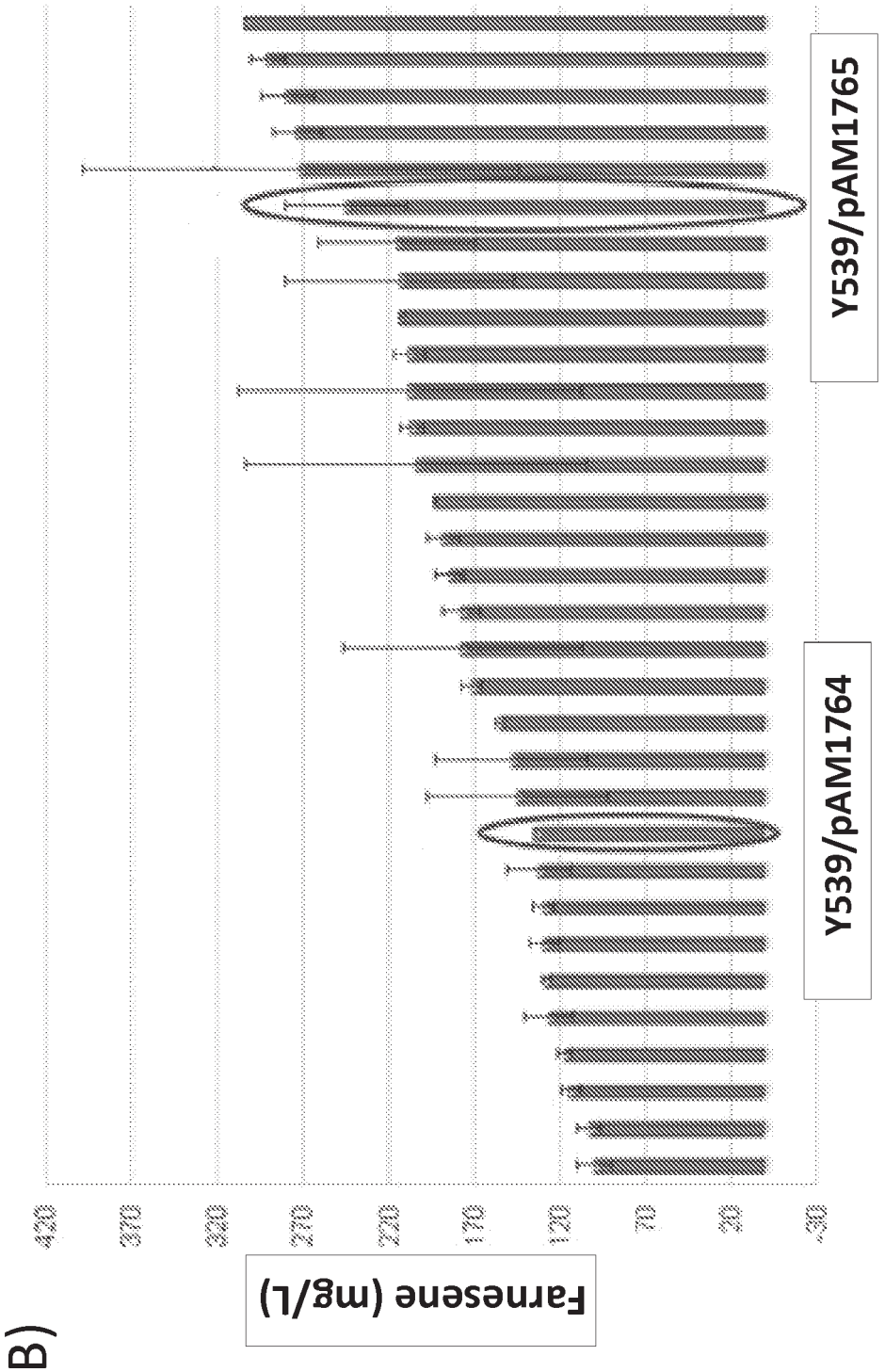


Figure 12

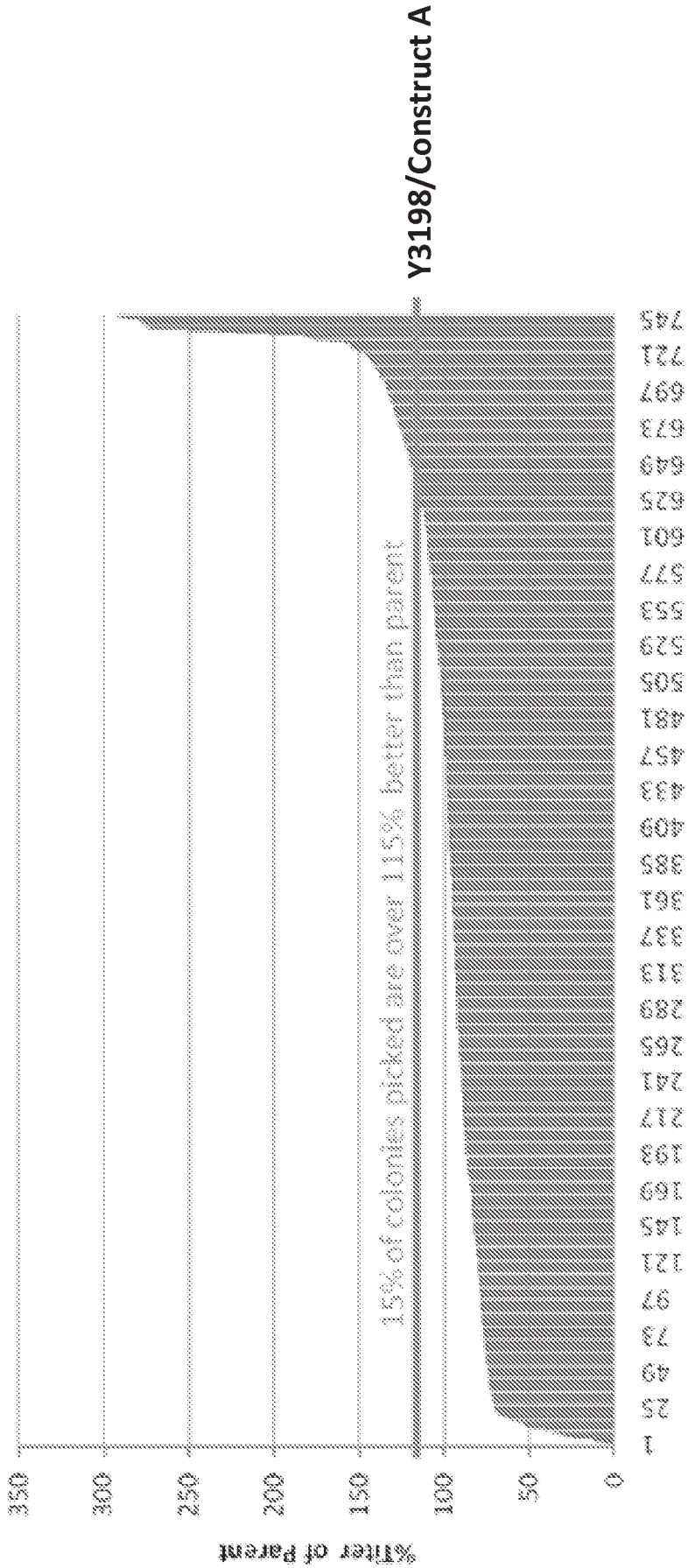
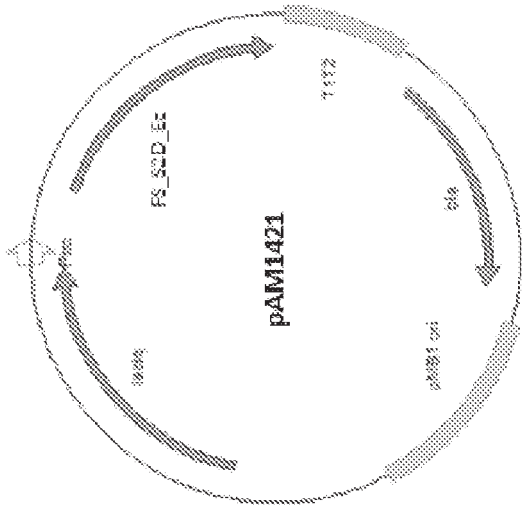
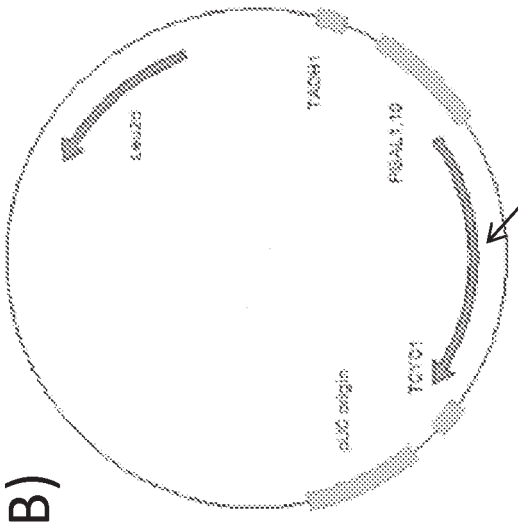
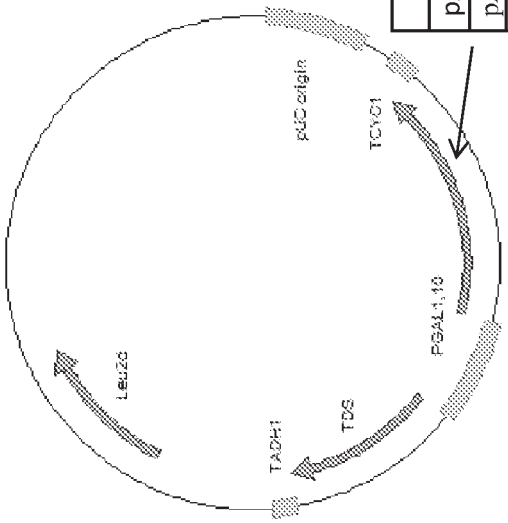

Y3198/Construct A
15% of colonies picked are over 115% better than parent
%Titer of Parent
350
300
250
200
150
100
50
0
1
25
49
73
97
121
145
169
193
217
241
265
289
313
337
361
385
409
433
457
481
505
529
553
577
601
625
649
673
697
721
745

Figure 13





Plasmid	Coding Sequence
pAM404	FS_Aa_SC
pAM1765	FS_S2D_Ec
pAM1795	TDS_Fs_Sc
pAM1549	IS_Pn_Sc



Plasmid	Coding Sequence
pAM1812	FS_Aa_Sc
pAM1895	FS_S2D_Ec
pAM1896	FS_Ad_Sc
pAM1948	IS_Pn_Sc

Figure 13 (continued)

D)

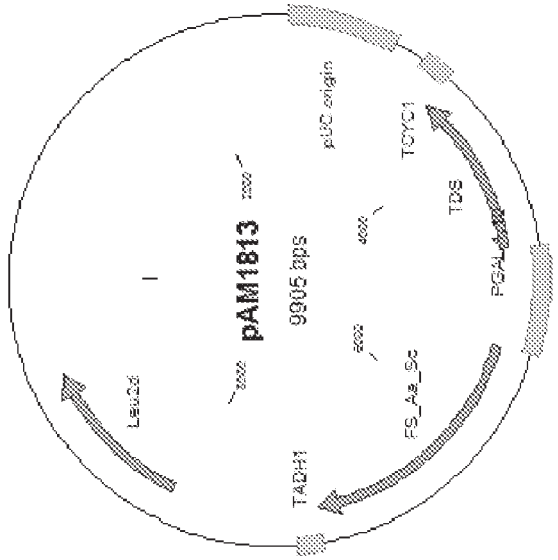


Figure 14

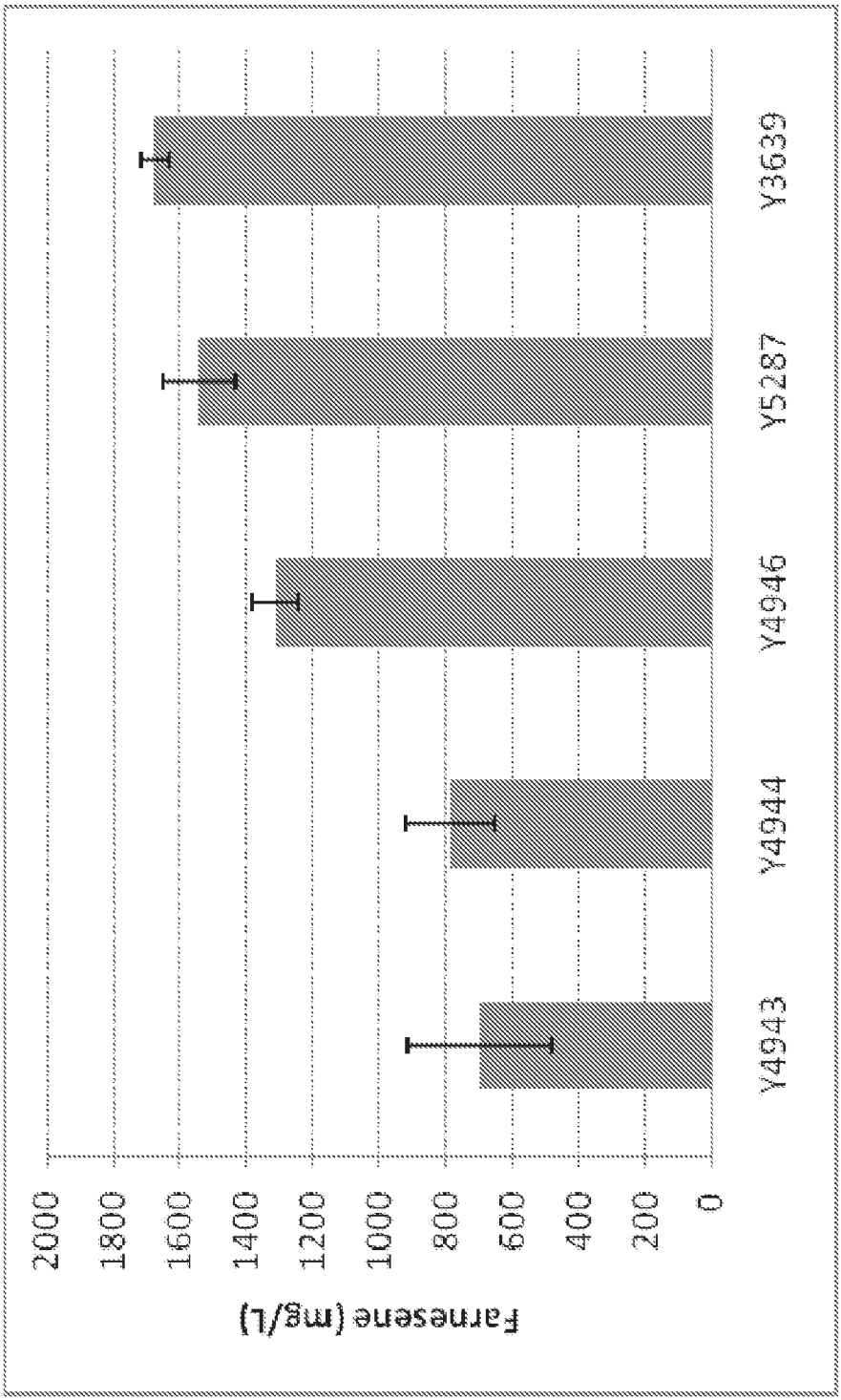

Farnesene (mg/L)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Y4943
Y4944
Y4946
Y5287
Y3639

Figure 15

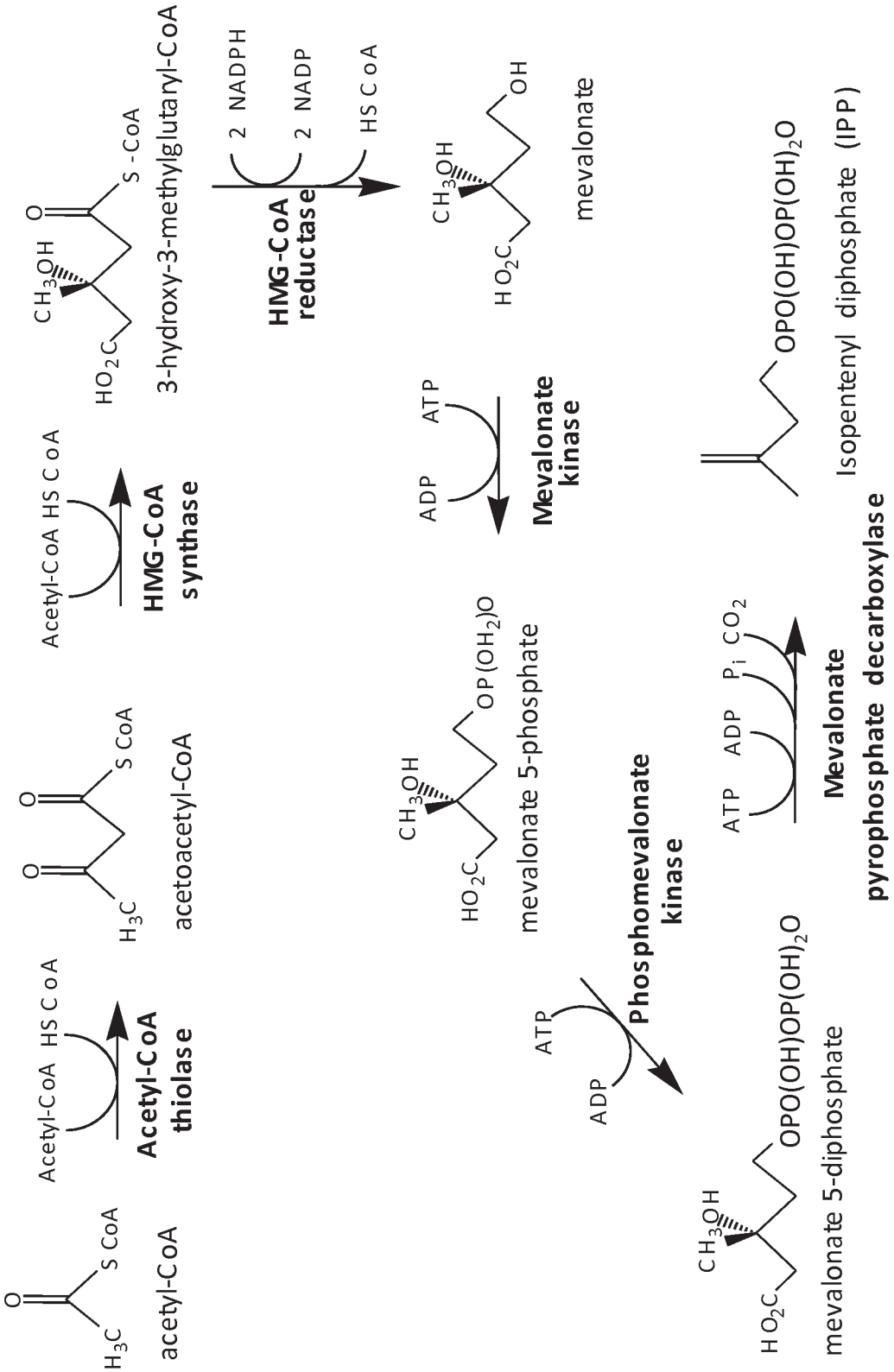

acetyl-CoA
Acetyl-CoA HS CoA
Acetyl-CoA thiolase
acetoacetyl-CoA
Acetyl-CoA HS CoA
HMG-CoA synthase
3-hydroxy-3-methylglutaryl-CoA
2 NADPH
2 NADP
HS CoA
HMG-CoA reductase
mevalonate
ADP ATP
Mevalonate kinase
mevalonate 5-phosphate
ATP
ADP
Phosphomevalonate kinase
mevalonate 5-diphosphate
ATP ADP Pi CO2
Mevalonate pyrophosphate decarboxylase
Isopentenyl diphosphate (IPP)

Figure 16

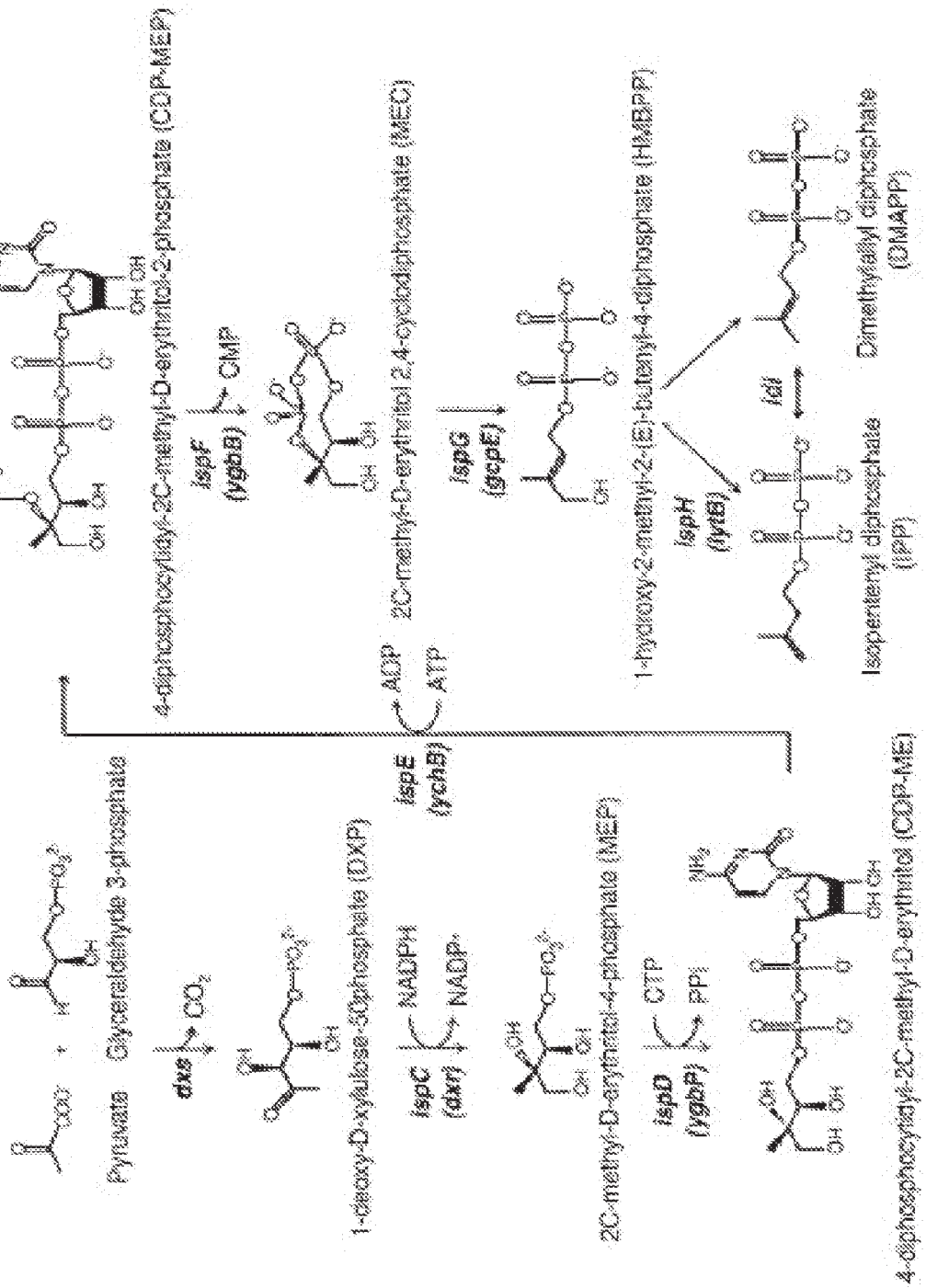

Pyruvate
Glyceraldehyde 3-phosphate
dxs
CO2
1-deoxy-D-xylulose-5phosphate (DXP)
ispC
(dxr)
NADPH
NADP+
2C-methyl-D-erythritol-4-phosphate (MEP)
ispD
(ygbP)
CTP
PPi
4-diphosphocytidyl-2C-methyl-D-erythritol (CDP-ME)
ispE
(ychB)
ADP
ATP
4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate (CDP-MEP)
ispF
(ygbB)
CMP
2C-methyl-D-erythritol 2,4-cyclodiphosphate (MEC)
ispG
(gcpE)
1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate (HMBPP)
ispH
(lytB)
Idi
Isopentenyl diphosphate
(IPP)
Dimethylallyl diphosphate
(DMAPP)

Figure 17

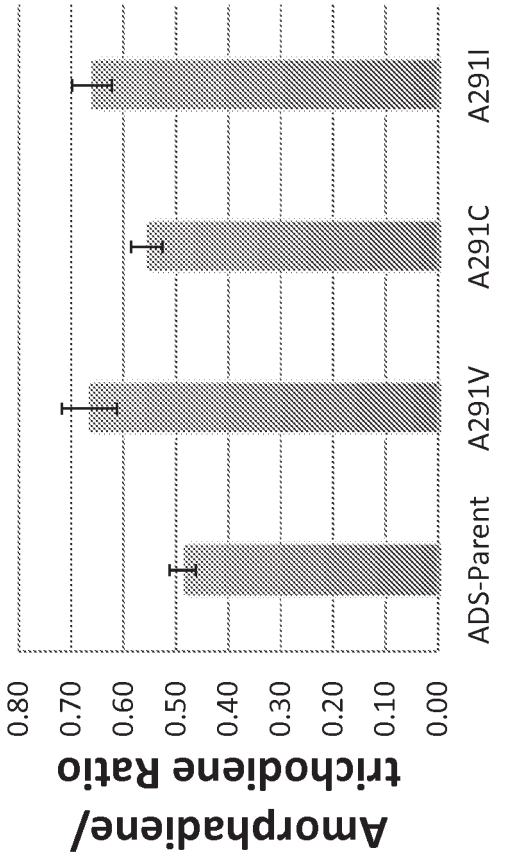
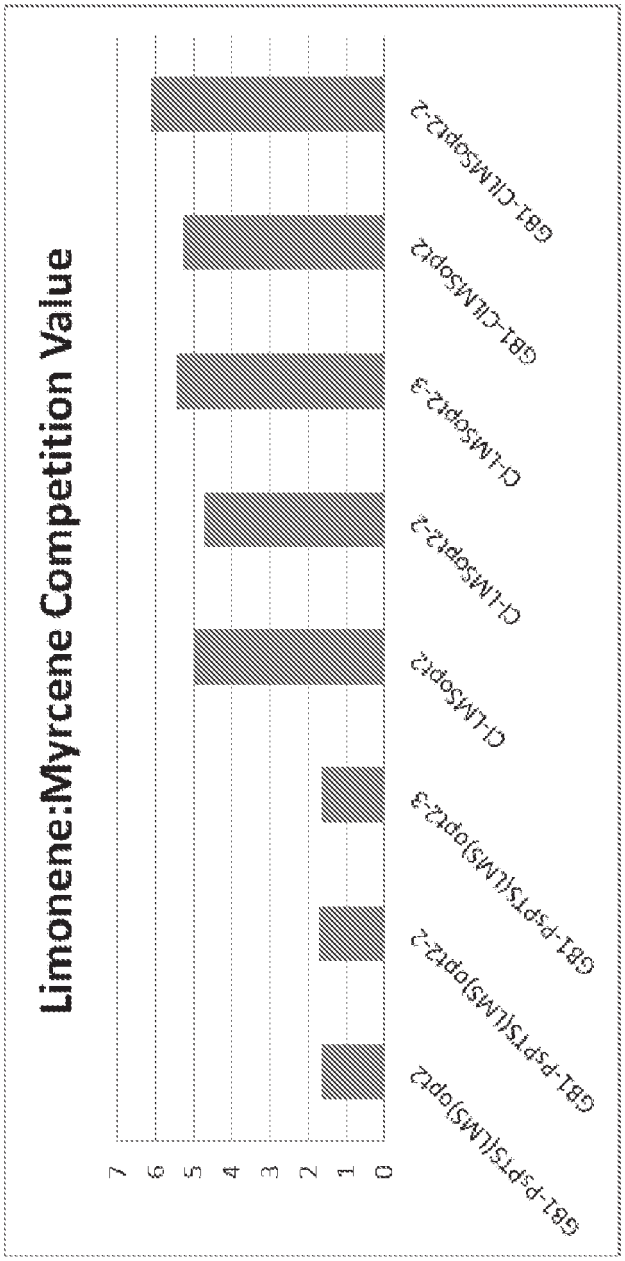

Amorphadiene/
trichodiene Ratio
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
ADS-Parent
A291V
A291C
A291I

Figure 18


Limonene:Myrcene Competition Value
0
1
2
3
4
5
6
7
GB1-PsPTS(LMS)opt2
GB1-PsPTS(LMS)opt2-2
GB1-PsPTS(LMS)opt2-3
Cr-LMSopt2
Cr-LMSopt2-2
Cr-LMSopt2-3
GB1-CrLMSopt2
GB1-CrLMSopt2-2