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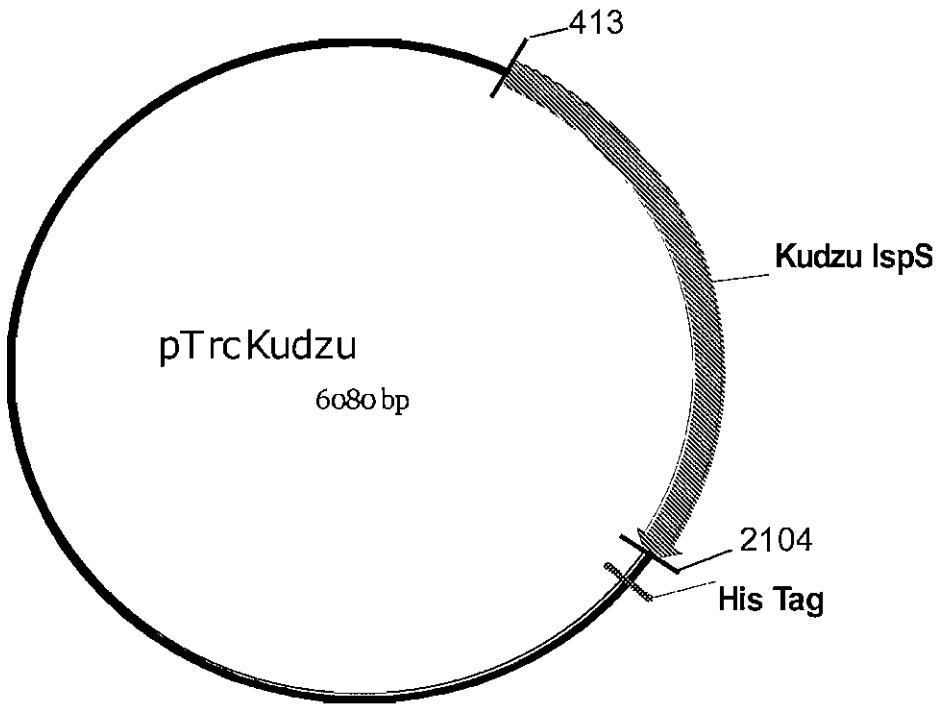

Figure 3A

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Figure 3B

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Figure 3C

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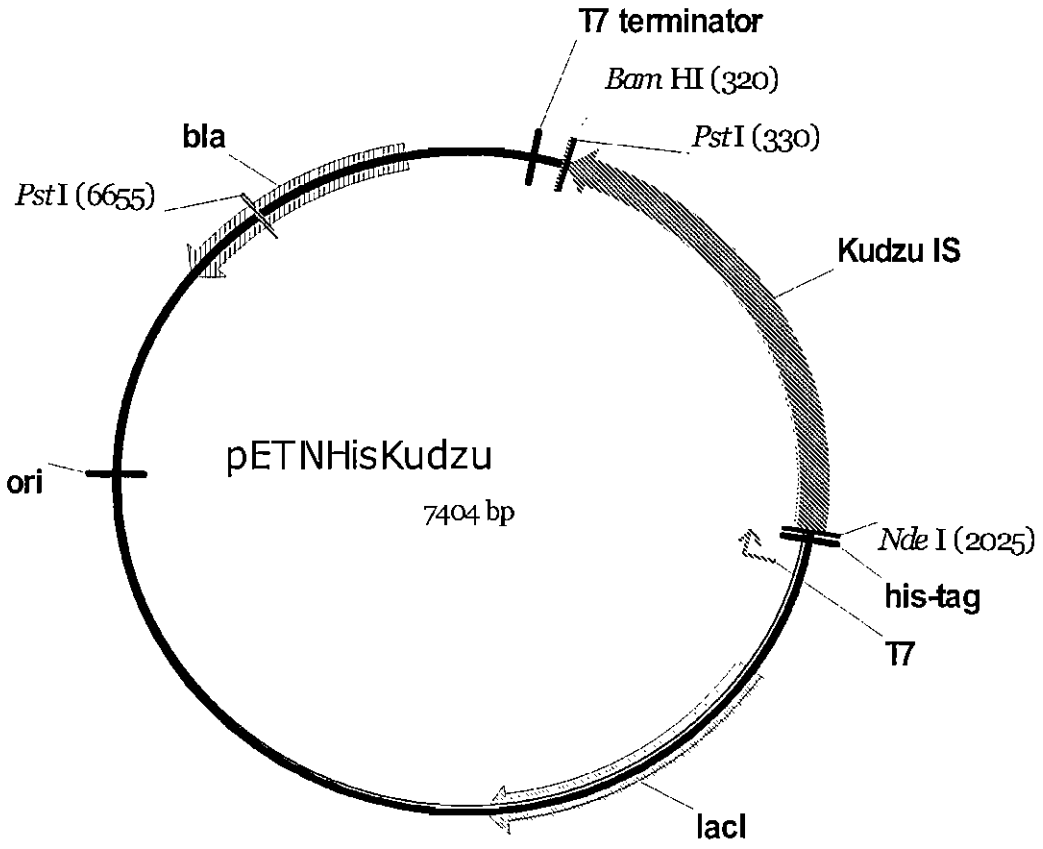
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Figure 5A

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Figure 5C

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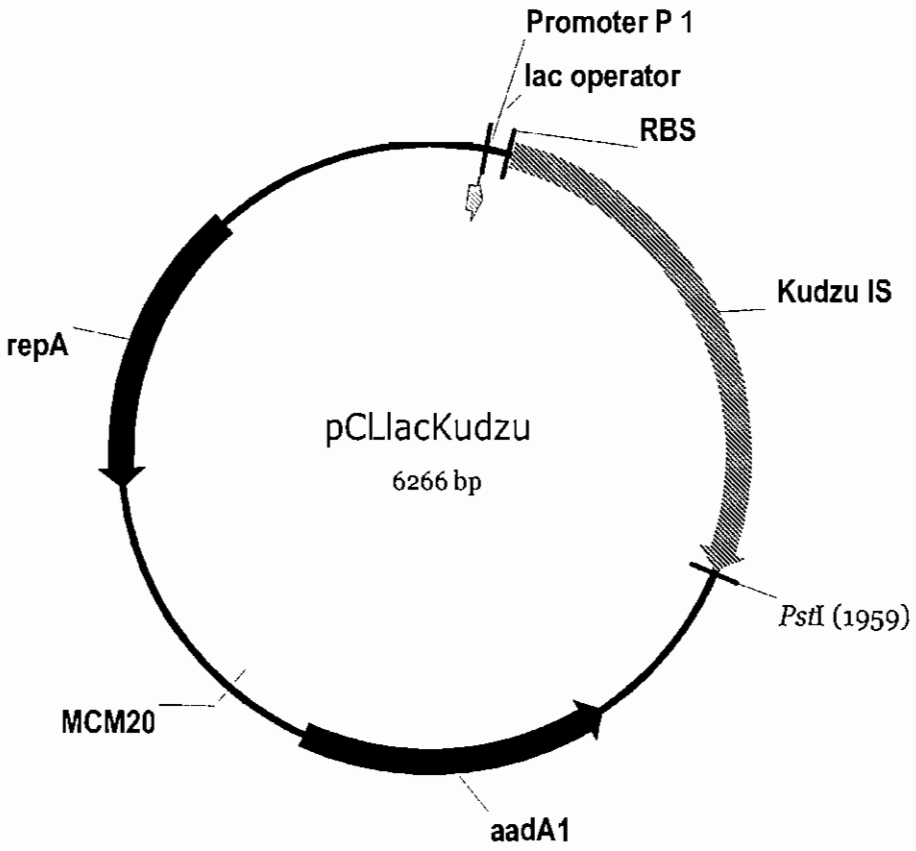
Figure 6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lac operator
RBS
Kudzu IS
repA
pCLlacKudzu
6266 bp
PstI (1959)
MCM20
aadA1

Figure 7A

1-
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Figure 7B

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Figure 7C

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Figure 8A

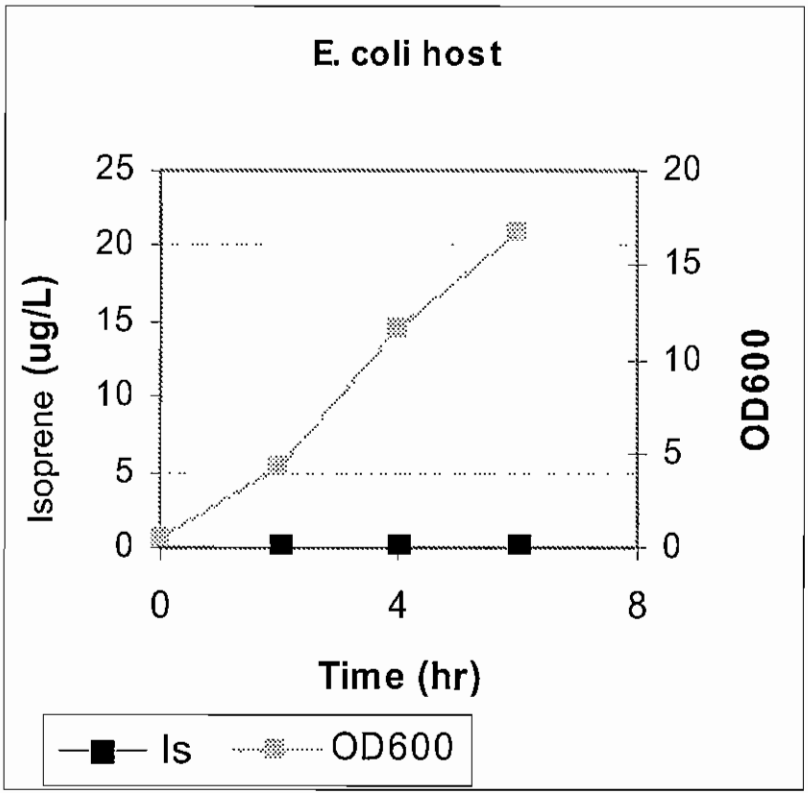

E. coli host
Isoprene (ug/L)
OD600
Time (hr)
Is
OD600
0
5
10
15
20
25
0
4
8

Figure 8B

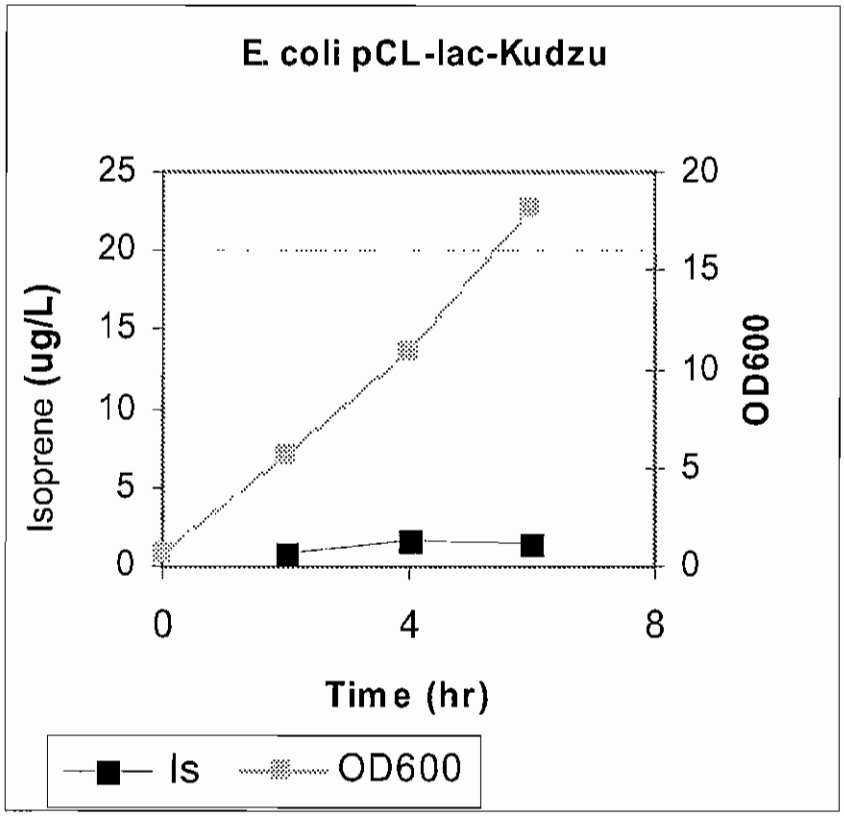

E. coli pCL-lac-Kudzu
Isoprene (ug/L)
OD600
Time (hr)
Is
OD600
0
5
10
15
20
25
0
4
8

Figure 8C

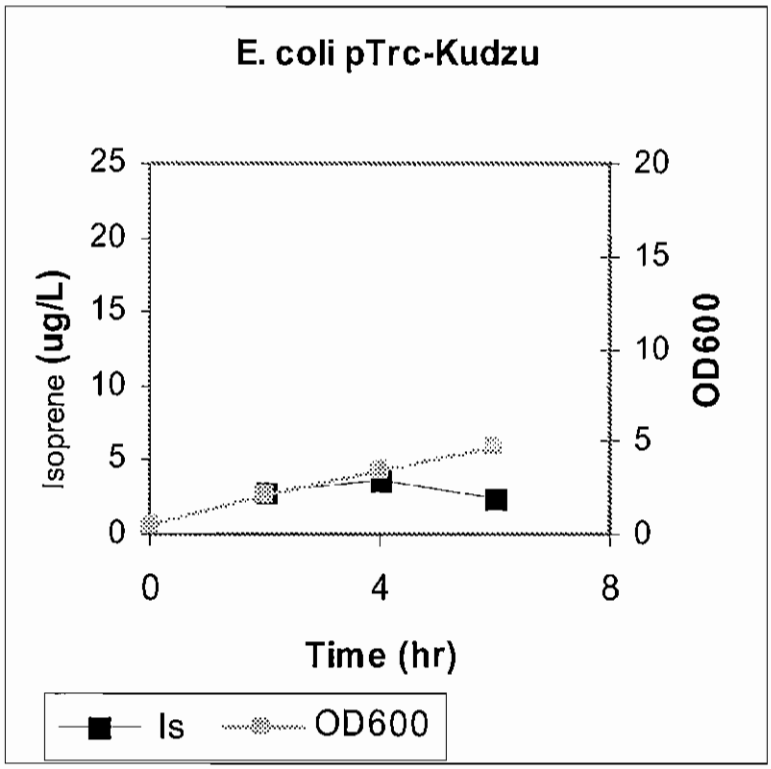

E. coli pTrc-Kudzu
Isoprene (ug/L)
OD600
25
20
15
10
5
0
20
15
10
5
0
0
4
8
Time (hr)
Is
OD600

Figure 8D

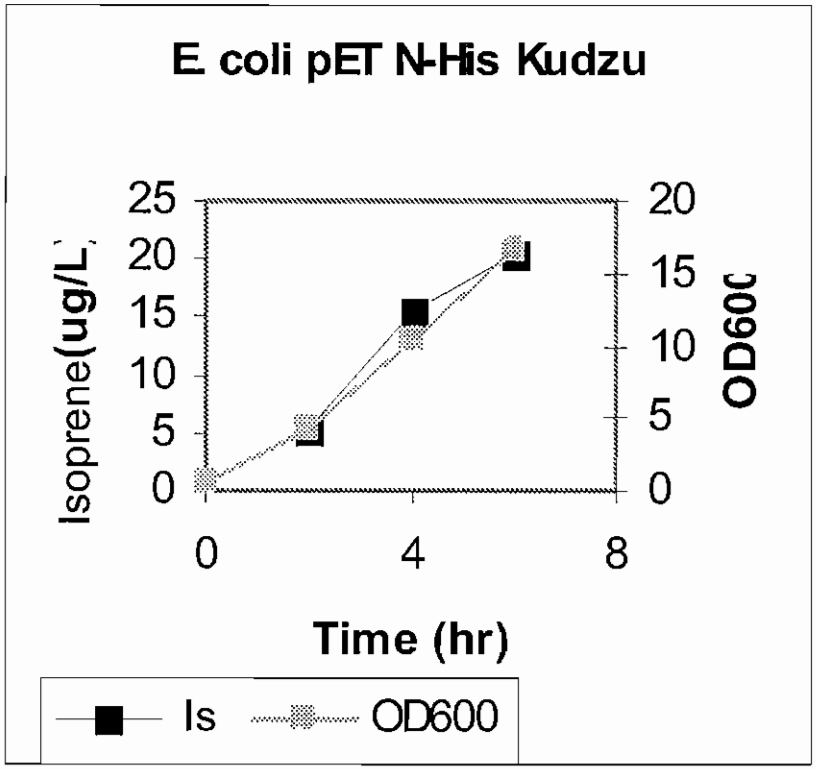
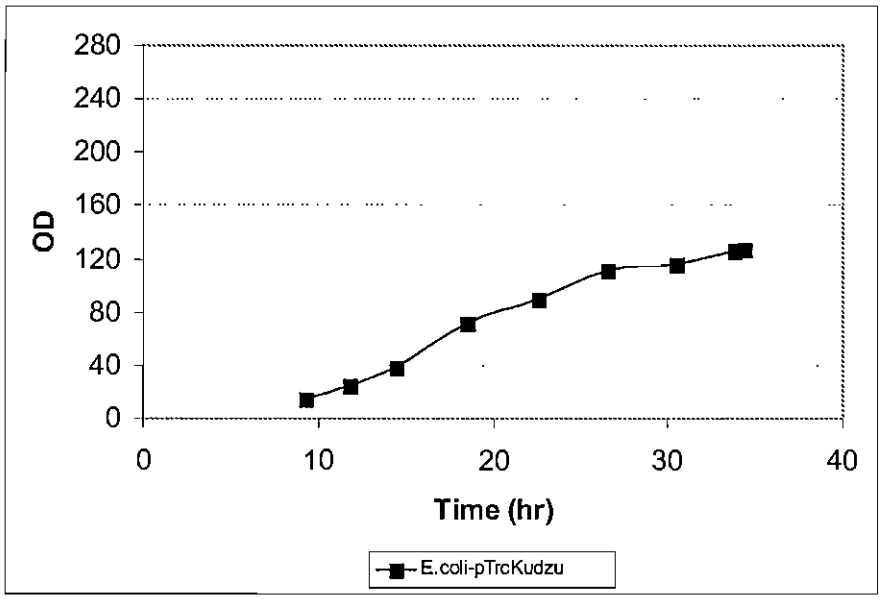

E. coli pET N-His Kudzu
Isoprene(ug/L)
OD600
25
20
15
10
5
0
20
15
10
5
0
0
4
8
Time (hr)
Is
OD600

Figure 9

A.


OD
280
240
200
160
120
80
40
0
0
10
20
30
40
Time (hr)
E.coli-pTrcKudzu

B.

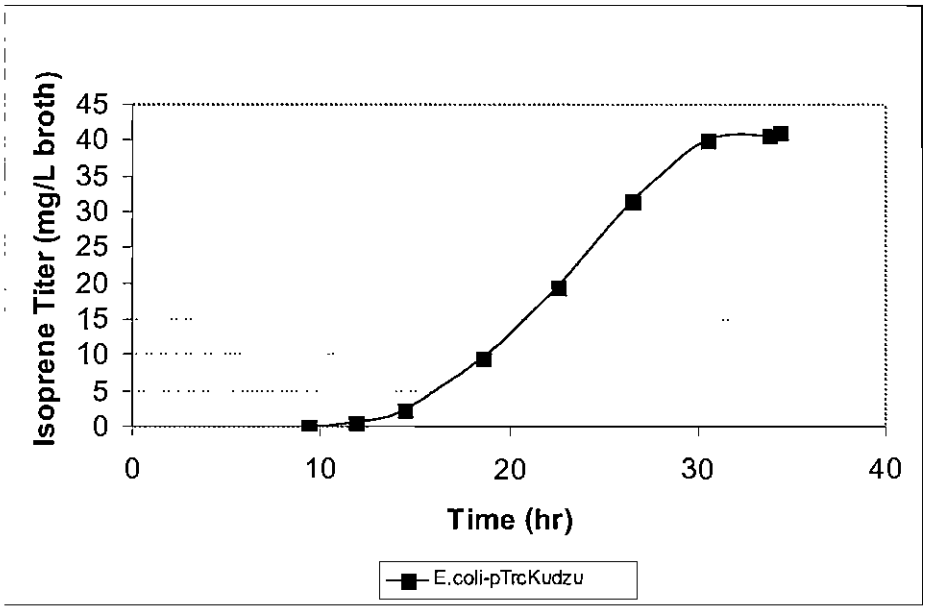

Isoprene Titer (mg/L broth)
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
Time (hr)
E.coli-pTrcKudzu

Figure 10A

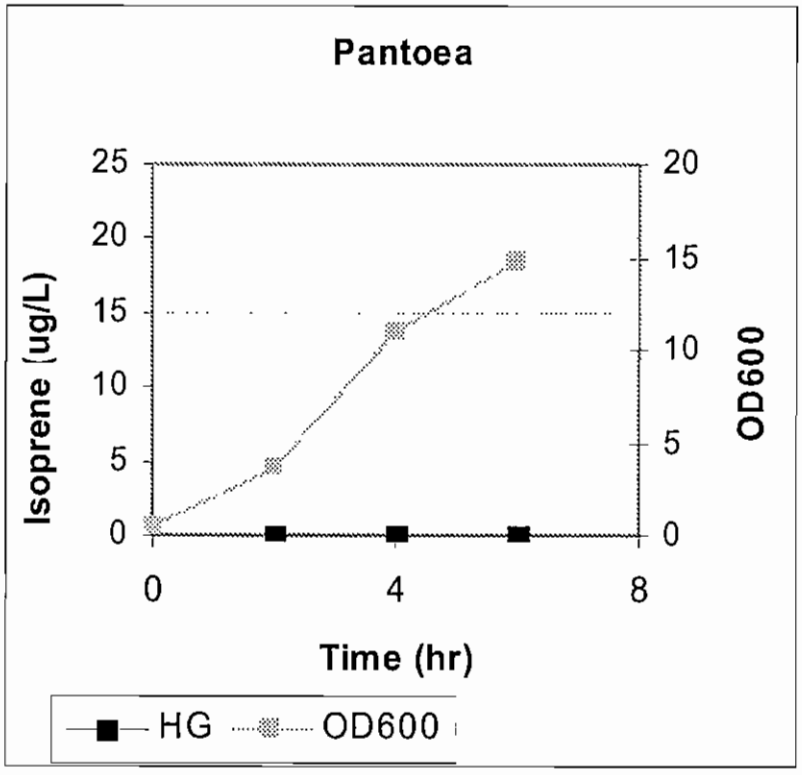

Pantoea
Isoprene (ug/L)
OD600
Time (hr)
HG
OD600
25
20
15
10
5
0
20
15
10
5
0
0
4
8

Figure 10B

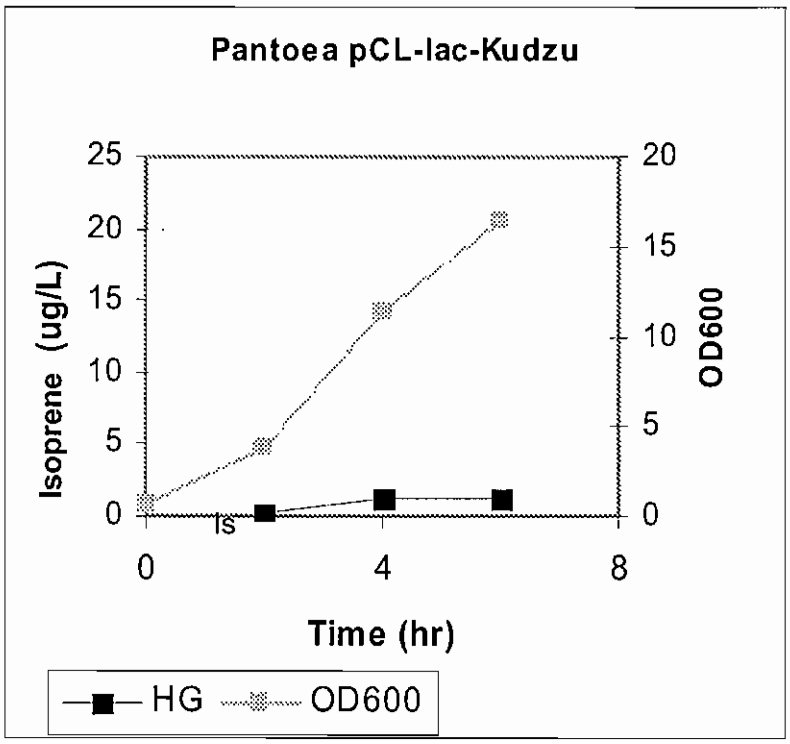

Pantoea pCL-lac-Kudzu
Isoprene (ug/L)
OD600
Time (hr)
HG
OD600
25
20
15
10
5
0
20
15
10
5
0
0
4
8
Is

Figure 10C

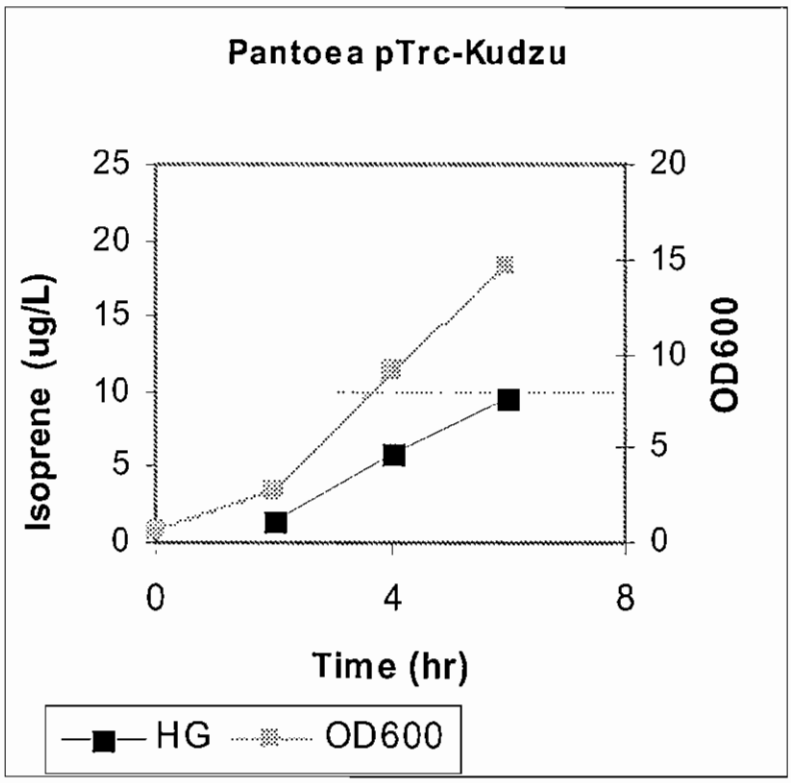

Pantoea pTrc-Kudzu
Isoprene (ug/L)
OD600
25
20
15
10
5
0
20
15
10
5
0
0
4
8
Time (hr)
HG
OD600

Figure 11

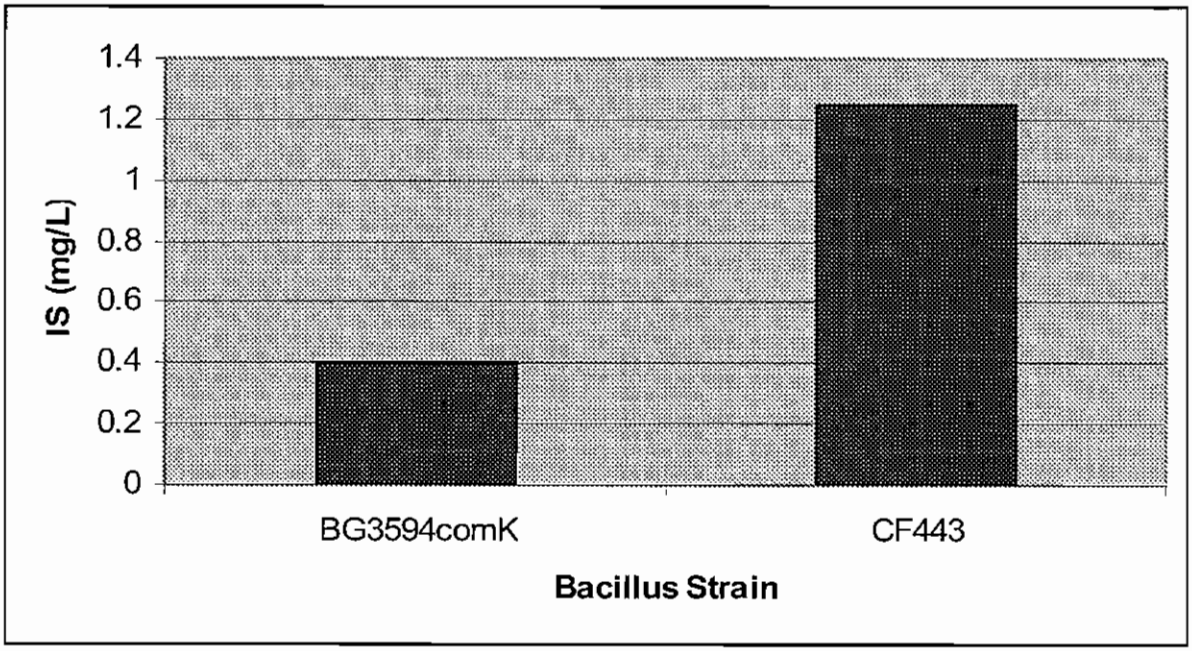


Figure 12A

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Figure 12B

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Figure 12C

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(SEQ ID NO:56)

Figure 13

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(SEQ ID NO:8)

Figure 14

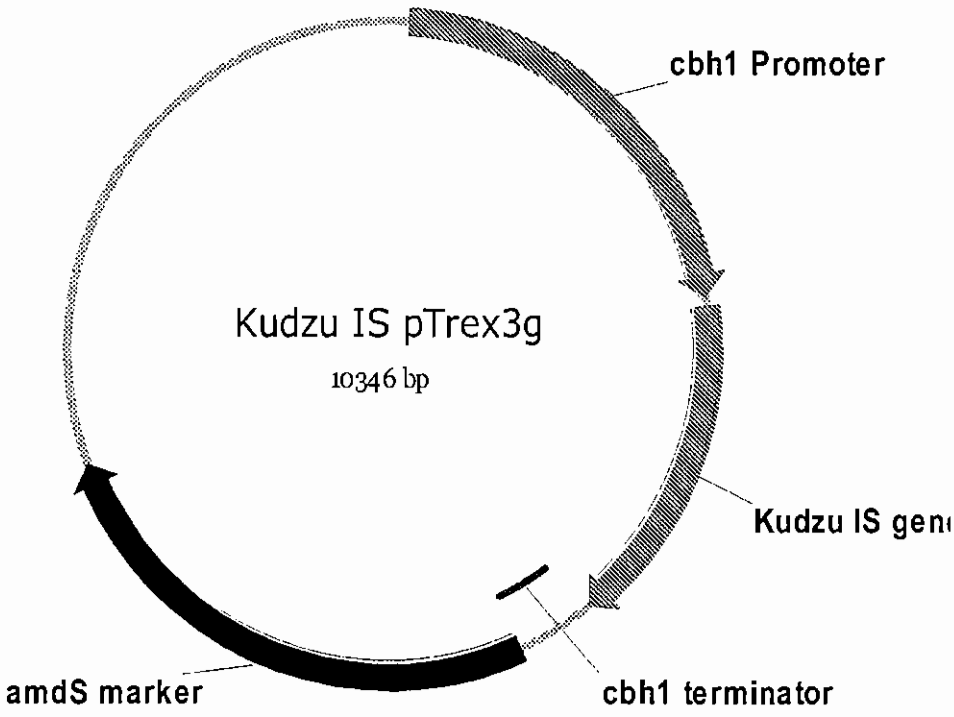
cbh1 Promoter
Kudzu IS pTrex3g
10346 bp
Kudzu IS gen
amdS marker
cbh1 terminator

Figure 15A

```
   1 TCGACCGGTG AGAAGAACAG CATCGGGACA AGGGAAGGAA GAACAAAGAC AAAGAAAACA
  61 AAAGAAAGCA ATTGAAAACA AAACAAAACA ATTTTCATTC CTTCTCTTAT CATTCCTTTT
 121 CTTTTCTTTT CTCTCATTCA ACGCACTCCA TCGTATCCGT ATTCCTCTTA TTTTTTCTCT
 181 TTCTCTATAT CCATTTCTTT CTCTCTAGGT GTGTCCTCTC TCTCTCTTCA ATTTCTCTAC
 241 TCCGCATTCC AACGCATCCT TCCCCCAACC TCCCATTTCC TCCTTACGGC CCGATAGCGA
 301 TCGTCTTTCC CTCGCTATCA CTCGCTACCG GCCCCTCCTC TGCACCGTAA CCTCCTACGT
 361 ATTTACCATA TCATAAAGTT TTTTCCGACG CTTATCGCTG ACCCCCTGTC GCCCTCCTAT
 421 TGGCTTCCGG ATTATCTTCT TGTCCATAAG GTGATCCATG CTTCCTGAAG ATTCCCGAAA
 481 TGTGTCCACT TTGGCGGGGA ATCATTCCAT CCACTTCTTT CTCTCTCGCT TTCCTCATTC
 541 GGCGCTCCCC TTCCGCGTCT CATTGGTCTT CCGCTCCGTT TTTGCTTTGC CGATGTTACT
 601 TGGGGAGAGG TGCGATAATC CTTTCGCAAA AACTCGGTTT GACGCCTCCC ATGGTATAAA
 661 TAGTGGGTGG TGGACAGGTG CCTTCGCTTT TCTTTAAGCA AGAGAATCCC ATTGTCTTGA
 721 CTATCACGAA TTCACATACA TTATGAAGAT CACCGCTGTC ATTGCCCTTT TATTCTCACT
 781 TGCTGCTGCC TCACCTATTC CAGTTGCCGA TCCTGGTGTG GTTTCAGTTA GCAAGTCATA
 841 TGCTGATTTC CTTCGTGTTT ACCAAAGTTG GAACACTTTT GCTAATCCTG ATAGACCCAA
 901 CCTTAAGAAG AGAAATGATA CACCTGCAAG TGGATATCAA GTTGAAAAAG TCGTAATTTT
 961 GTCACGTCAC GGTGTTAGGG CCCCTACAAA AATGACTCAA ACCATGCGTG ATGTCACTCC
1021 TAATACATGG CCAGAATGGC CCGTTAAATT AGGATATATT ACACCAAGAG GTGAACACTT
1081 GATATCACTT ATGGGCGGTT TTTACCGTCA AAAATTCCAG CAACAAGGAA TCCTTTCTCA
1141 GGGCTCCTGT CCTACTCCTA ACTCCATATA TGTCTGGGCT GACGTCGATC AGCGTACTTT
1201 AAAAACTGGT GAAGCATTCC TTGCTGGTTT GGCACCACAA TGTGGCTTGA CAATTCATCA
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1741 AGCTATATCA AATGCCCTTA ATCCCAACGC CACTGAATCA AAACTTCCAG ATATTTCACC
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1921 AGTATTTGAA CGTCTTGCTG ATAAAAGTGG AAAACAATAT GTTTCTGTAT CTATGGTTTA
1981 TCAAACACTA GAACAACTTC GATCACAGAC TCCCCTTTCT CTAAATCAGC CTGCCGGATC
2041 TGTTCAACTT AAAATTCCAG GTTGCAATGA TCAAACAGCC GAGGGTTACT GTCCTCTTTC
2101 CACTTTTACA AGAGTTGTTT CCCAATCTGT TGAACCTGGA TGCCAACTTC AATAATGAGG
2161 ATCCAAGTAA GGGAATGAGA ATGTGATCCA CTTTTAATTC CTAATGAATA CATGCCTATA
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2401 GAAAGAAAGT CTTGTTCTTT TATTTCCTTT TTTCCATCTT CAAGGCTTTT CTTTTCTTCC
2461 TCCTCCTCGT TCATCTTGAG GTTTGACGTG TCTGTTTAGA ATTTTGAGCT GTTGCAGCAT
2521 CTTATTTTTT GTTTTGCGAA AACGAAGCGC TTTACTCTCT TCATCAGTTG GACGATTGTA
2581 CCTTTGAAAA CCAACTACTT TTGCATGTTT TGTATAGAAA TCAATGATAT TAGAATCCCA
2641 TCCTTTAATT TCTTTCAAAG TAGTTGAGCT ATAGTTAAGT GTAAGGGCCC TACTGCGAAA
2701 GCATTTGCCA AGGATGTTTT CATTAATCAA GAACGAAAGT TAGGGGATCG AAGACGATCA
2761 GATACCGTCG TAGTCTTAAC CATAAACTAT GCCGACTAGG GATCGGGCAA TGTTTCATTT
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2881 CGCAAGGCTG AAACTTAAAG GAATTGACGG AAGGGCACCA CAATGGAGTG GAGCCTGCGG
2941 CTTAATTTGA CTCAACACGG GGAAACTCAC CAGGTCCAGA CATAGTAAGG ATTGACAGAT
3001 TGAGAGCTCT TTCTTGATTC TATGGGTGGT GGTGCATGGC CGTTCTTAGT TGGTGGAGTG
3061 ATTTGTCTGC TTAATTGCGA TAACGAACGA GACCTTAACC TGCTAAATAG CTGGATCAGC
3121 CATTTTGGCT GATCATTAGC TTCTTAGAGG GACTATTGGC ATAAAGCCAA TGGAAGTTTG
3181 AGGCAATAAC AGGTCTGTGA TGCCCTTAGA TGTTCTGGGC CGCACGCGCG CTACACTGAC
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3361 ATTCGATGTT GCAGATTTTA CAAGTTTTTA AAATGTATTT CATTATTACT TTTTATATGC
3421 CTAATAAAAA AGCCATAGTT TAATCTATAG ATAACTTTTT TTCCAGTGCA CTAACGGACG
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Figure 15B

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3661 GTTGACTTGC TTTATCGAAT AATTACCGTA CCTGTAATTG GCTTGCTGGA TATAGCGGTA
3721 GTCTAATATC TAGCAAAAAT CTTTTGGGTG AAAAGGCTTG CAATTTCACG ACACCGAACT
3781 ATTTGTCATT TTTTAATAAG GAAGTTTTCC ATAAATTCCT GTAATTCTCG GTTGATCTAA
3841 TTGAAAAGAG TAGTTTTGCA TCACGATGAG GAGGGCTTTT GTAGAAAGAA ATACGAACGA
3901 AACGAAAATC AGCGTTGCCA TCGCTTTGGA CAAAGCTCCC TTACCTGAAG AGTCGAATTT
3961 TATTGATGAA CTTATAACTT CCAAGCATGC AAACCAAAAG GGAGAACAAG TAATCCAAGT
4021 AGACACGGGA ATTGGATTCT TGGATCACAT GTATCATGCA CTGGCTAAAC ATGCAGGCTG
4081 GAGCTTACGA CTTTACTCAA GAGGTGATTT AATCATCGAT GATCATCACA CTGCAGAAGA
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4201 AAGATTTGGA CATGCTTATT GTCCACTTGA CGAAGCTCTT TCTAGAAGCG TAGTTGACTT
4261 GTCGGGACGG CCCTATGCTG TTATCGATTT GGGATTAAAG CGTGAAAAGG TTGGGGAATT
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4381 GCATGTTACC TGCTTATATG GTAGTAATGA CCATCATCGT GCTGAAAGCG CTTTTAAATC
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5761 CTTTGTCGAT ATCATGTAAT TAGTTATGTC ACGCTTACAT TCACGCCCTC CCCCCACATC
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6001 GTCTCTCTCT TGGCCTCCTT TCTAGGTCGG GCTGATTGCT CTTGAAGCTC TCTAGGGGGG
6061 CTCACACCAT AGGCAGATAA CGTTCCCCAC CGGCTCGCCT CGTAAGCGCA CAAGGACTGC
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6241 CAAGGGAAAA CGCAAGCGCA AAGAGAAAGC AGGTAGCTTG CAGTGGGCTT ACATGGCGAT
6301 AGCTAGACTG GGCGGTTTTA TGGACAGCAA GCGAACCGGA ATTGCCAGCT GGGGCGCCCT
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6601 GGCGCCCGGT TCTTTTTGTC AAGACCGACC TGTCCGGTGC CCTGAATGAA CTGCAGGACG
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6781 TGTCATCTCG CCTTGCTCCT GCCGAGAAAG TATCCATCAT GGCTGATGCA ATGCGGCGGC
6841 TGCATACGCT TGATCCGGCT ACCTGCCCAT TCGACCACCA AGCGAAACAT CGCATCGAGC
6901 GAGCACGTAC TCGGATGGAA GCCGGTCTTG TCGATCAGGA TGATCTGGAC GAAGAGCATC
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Figure 15C

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7861 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG
7921 GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG
7981 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG
8041 CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC
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8161 TCGCCGCAGC CGAACGACCG AGCGCAGCGA G
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(SEQ ID NO:11)

Figure 16

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 241 TGCAGCGGTT GGGTTTGACT TATAAATTCG AGAAGGACAT TATCAAGGCA CTGGAGAACA
 301 TTGTGCTCCT CGACGAGAAC AAGAAGAACA AGTCTGATCT TCACGCTACC GCTCTCTCTT
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 481 ACGAGGCGTC CTACCTGGGA TTCGAGGGAG AGAACCTCCT GGAGGAAGCT CGTACATTTT
 541 CCATCACTCA CCTTAAGAAT AACCTTAAGG AGGGAATTAA CACCAAGGTG GCCGAGCAGG
 601 TTTCTCACGC CCTGGAGCTC CCCTACCACC AACGGCTCCA TAGACTGGAG GCTCGTTGGT
 661 TCCTGGACAA ATATGAGCCA AAGGAGCCTC ATCATCAGTT GCTGTTGGAG TTGGCCAAGC
 721 TGGACTTCAA TATGGTTCAG ACGCTGCACC AAAAGGAGTT GCAGGACCTG TCTCGATGGT
 781 GGACCGAGAT GGGATTGGCC TCGAAGCTGG ATTTTGTCCG TGACCGACTT ATGGAGGTCT
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 961 ACGAGTTGCA GCTGTTCACT GACGCCGTCG AGCGATGGGA TGTGAACGCC ATTAATACTC
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1261 CCTACTTCTC CGTCTGCCAG CAGCAGGAGG ATATTTCCGA TCATGCTCTT AGATCGCTGA
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1561 TCATGGAGAT CGCCGTGAAC ATGGCTCGAG TTTCCCATTG TACTTACCAG TACGGTGACG
1621 GCCTGGGTCG TCCGGACTAC GCTACAGAGA ACCGAATCAA GCTGCTGCTC ATCGACCCCT
1681 TCCCTATCAA CCAATTGATG TACGTGTAAT AGTCTAGAGG ATCC
```

(SEQ ID NO:12)

Figure 17

```
   1 GAATTCAACA AAAATGTGCT CTGTTTCCAC TGAGAACGTG TCCTTTACTG AGACTGAGAC
  61 TGAAGCACGT AGAAGCGCCA ACTACGAACC CAACTCCTGG GATTATGACT TTCTGCTGTC
 121 TTCTGACACC GACGAGTCGA TCGAGGTTTA TAAGGATAAG GCCAAGAAAC TTGAGGCCGA
 181 GGTCAGACGA GAGATTAACA ACGAGAAGGC CGAGTTCCTG ACCCTTCTTG AGCTGATCGA
 241 CAACGTTCAA CGACTTGGTC TTGGTTACCG TTTCGAATCC GATATCCGAC GTGCATTGGA
 301 TCGATTTGTC TCGTCCGGAG GTTTCGATGG TGTGACTAAG ACGTCGCTGC ACGCCACAGC
 361 TCTTTCCTTC AGACTGTTGC GGCAGCATGG ATTTGAGGTT TCCCAGGAAG CCTTTTCTGG
 421 TTTCAAGGAT CAGAACGGAA ACTTTTTGGA GAATCTCAAG GAGGACACCA AGGCCATCCT
 481 GTCGTTGTAT GAGGCCTCGT TCCTGGCTCT TGAGGGCGAG AATATTCTGG ATGAGGCTCG
 541 GGTTTTCGCT ATTTCGCACC TGAAGGAGTT GTCGGAGGAA AAGATCGGAA AGGAACTGGC
 601 CGAGCAGGTC AACCATGCAC TTGAACTTCC CCTGCATCGA CGTACCCAGC GACTGGAGGC
 661 CGTGTGGAGC ATCGAGGCGT ACAGAAAAAA GGAGGATGCT AATCAGGTTC TGCTCGAACT
 721 CGCTATCCTC GACTATAACA TGATTCAGAG CGTGTACCAG CGTGACTTGC GAGAGACAAG
 781 CCGGTGGTGG CGACGGGTGG GACTGGCCAC GAAGCTCCAC TTTGCTAAAG ATCGATTGAT
 841 TGAGTCGTTC TACTGGGCAG TGGGTGTGGC CTTTGAGCCT CAGTACTCCG ACTGCCGAAA
 901 CTCCGTTGCA AAGATGTTTT CTTTTGTCAC TATCATCGAC GACATCTACG ATGTTTACGG
 961 CACTCTCGAT GAACTCGAAC TCTTCACGGA CGCTGTCGAG CGATGGGATG TGAATGCCAT
1021 TAATGATCTG CCAGATTATA TGAAGTTGTG TTTCTTGGCG CTCTACAACA CAATTAATGA
1081 AATTGCCTAC GACAACCTCA AGGACAAGGG AGAGAACATT CTGCCCTACC TTACTAAAGC
1141 CTGGGCCGAC CTGTGTAACG CCTTTTTGCA GGAAGCCAAG TGGCTCTATA ACAAATCTAC
1201 TCCTACATTT GATGACTACT TCGGCAACGC TTGGAAGTCT TCCAGCGGCC CTCTCCAGTT
1261 GATCTTCGCT TACTTTGCAG TGGTCCAGAA CATCAAGAAA GAGGAGATTG AGAACCTCCA
1321 GAAGTATCAC GACATCATCT CCCGACCTTC GCACATCTTT CGACTGTGCA ATGACCTTGC
1381 CTCCGCATCC GCTGAGATTG CCCGAGGAGA AACAGCCAAT TCTGTGTCGT GTTACATGCG
1441 TACAAAGGGC ATCTCCGAGG AGCTGGCTAC CGAGTCTGTG ATGAACCTGA TCGATGAAAC
1501 CTGTAAGAAG ATGAACAAAG AGAAACTGGG CGGTTCTCTG TTCGCCAAAC CATTTGTTGA
1561 AACCGCGATC AATCTGGCTC GTCAGTCTCA TTGTACTTAC CATAACGGTG ACGCGCACAC
1621 TTCGCCGGAC GAATTGACCC GTAAGCGTGT GCTTTCGGTG ATTACCGAGC CGATCCTGCC
1681 GTTCGAAAGA TAATAGGATC C
```

(SEQ ID NO:13)

Figure 18A1

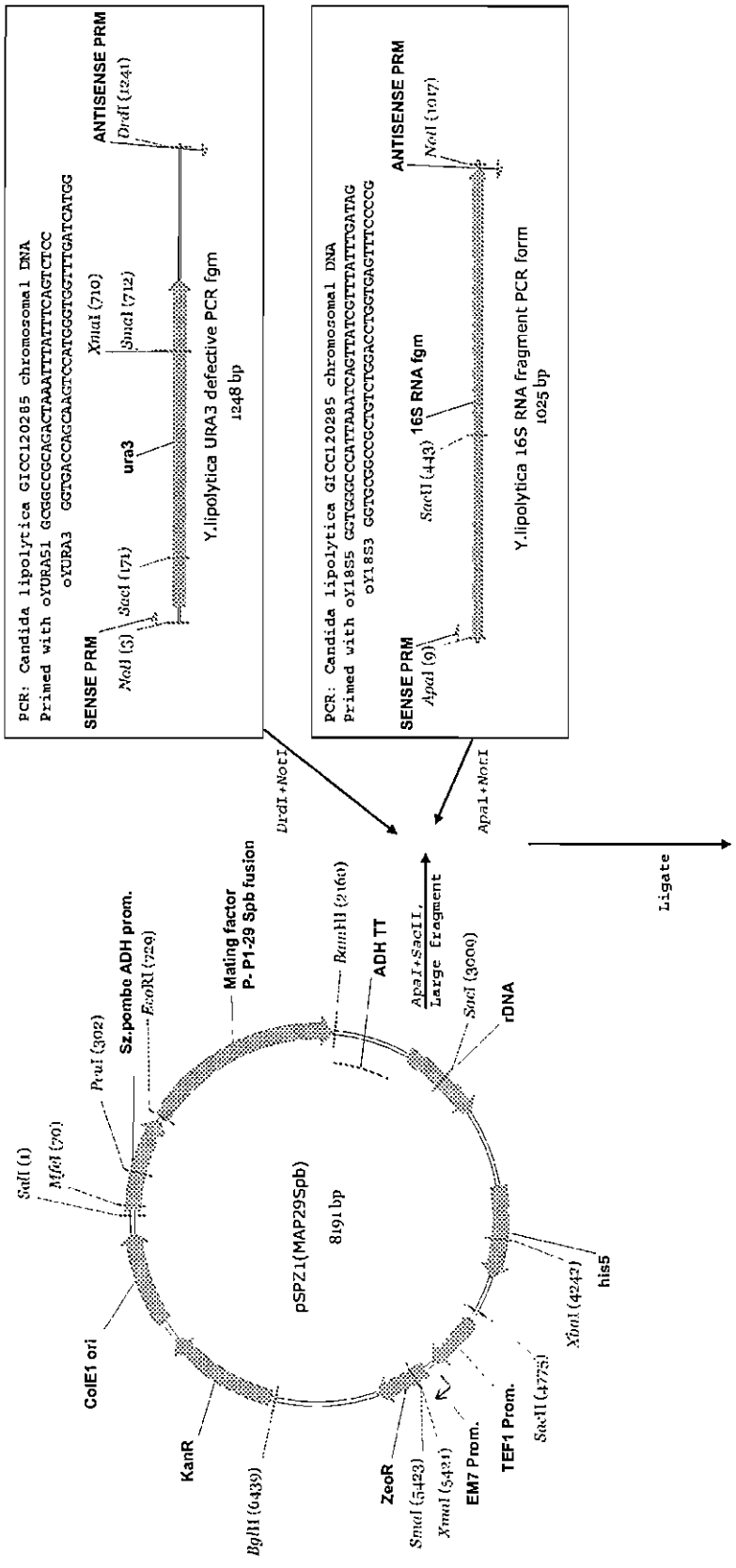
PCR: Candida lipolytica GICC120285 chromosomal DNA
Primed with oYURA51 GCGGCCGCAGACTAAATTTATTTCAGTCTCC
oYURA3 GGTGACCAGCAAGTCCATGGGTGGTTTGATCATGG
SENSE PRM
NotI (3)
SacI (171)
ura3
XmaI (710)
SmaI (712)
ANTISENSE PRM
DrdI (1241)
Y.lipolytica URA3 defective PCR fgm
1248 bp
PCR: Candida lipolytica GICC120285 chromosomal DNA
Primed with oY1855 GGTGGGCCCATTAAATCAGTTATCGTTTATTTGATAG
oY1853 GGTGGCGGCCGCTGTCTGGACCTGGTGAGTTTCCCCG
SENSE PRM
ApaI (9)
SacII (443)
16S RNA fgm
ANTISENSE PRM
NotI (1017)
Y.lipolytica 16S RNA fragment PCR form
1025 bp
DrdI+NotI
ApaI+NotI
Ligate
pSPZ1(MAP29Spb)
8191 bp
SalI (1)
MfeI (70)
PvuI (302)
Sz.pombe ADH prom.
EcoRI (729)
Mating factor
P-P1-29 Spb fusion
BamHI (2160)
ADH TT
ApaI+SacII,
Large fragment
SacI (3000)
rDNA
XbaI (4242)
his5
SacII (4775)
TEF1 Prom.
EM7 Prom.
XmaI (5421)
SmaI (5423)
ZeoR
BglII (6439)
KanR
ColE1 ori

Figure 18A2

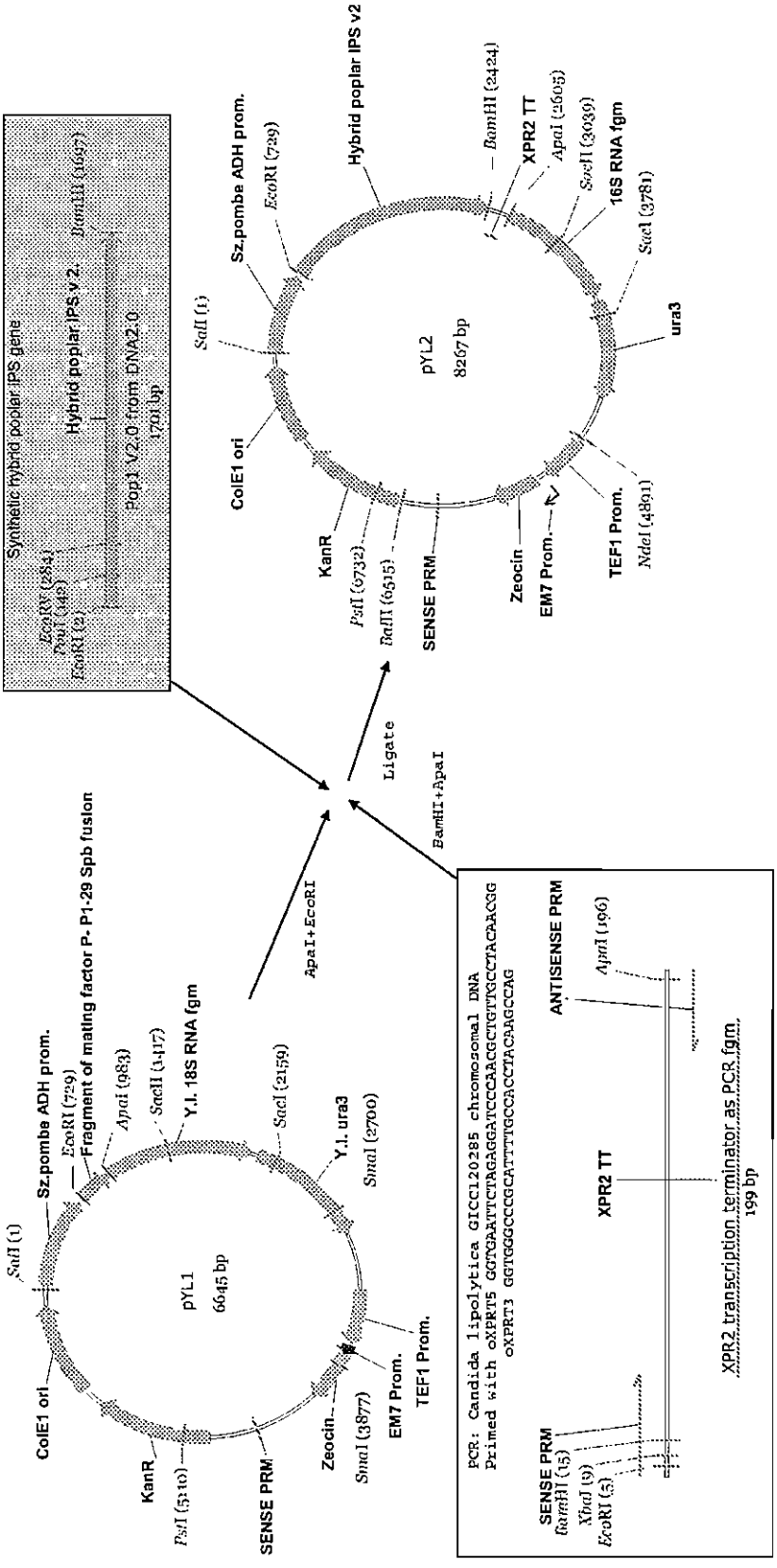
Synthetic hybrid poplar IPS gene
EcoRI (2841)
PstI (48)
EcoRI (2)
Hybrid poplar IPS v2
BamHI (1647)
Pop1 V2.0 from DNA2.0
1701 bp
SalI (1)
Sz.pombe ADH prom.
EcoRI (729)
Hybrid poplar IPS v2
BamHI (2424)
XPR2 TT
ApaI (2665)
SacII (3039)
16S RNA fgm
SacI (3781)
ura3
ColE1 ori
pYL2
8267 bp
KanR
PstI (6732)
BglII (6515)
SENSE PRM
Zeocin
EM7 Prom.
TEF1 Prom.
NdeI (4891)
ApaI+EcoRI
Ligate
BamHI+ApaI
SalI (1)
Sz.pombe ADH prom.
EcoRI (729)
Fragment of mating factor P-P1-29 Spb fusion
ApaI (983)
SacII (1417)
Y.l. 18S RNA fgm
SacI (2159)
Y.l. ura3
SmaI (2700)
ColE1 ori
pYL1
6645 bp
KanR
PstI (5310)
SENSE PRM
Zeocin
SmaI (3877)
EM7 Prom.
TEF1 Prom.
PCR: Candida lipolytica GICC120285 chromosomal DNA
Primed with oXPRT5 GGTGAATTCTAGAGGATCCCAACGCTGTTGCCTACAACGG
oXPRT3 GGTGGGCCCGCATTTTGCCACCTACAAGCCAG
ANTISENSE PRM
ApaI (196)
XPR2 TT
SENSE PRM
BamHI (15)
XbaI (9)
EcoRI (5)
XPR2 transcription terminator as PCR fgm
199 bp

Figure 18B

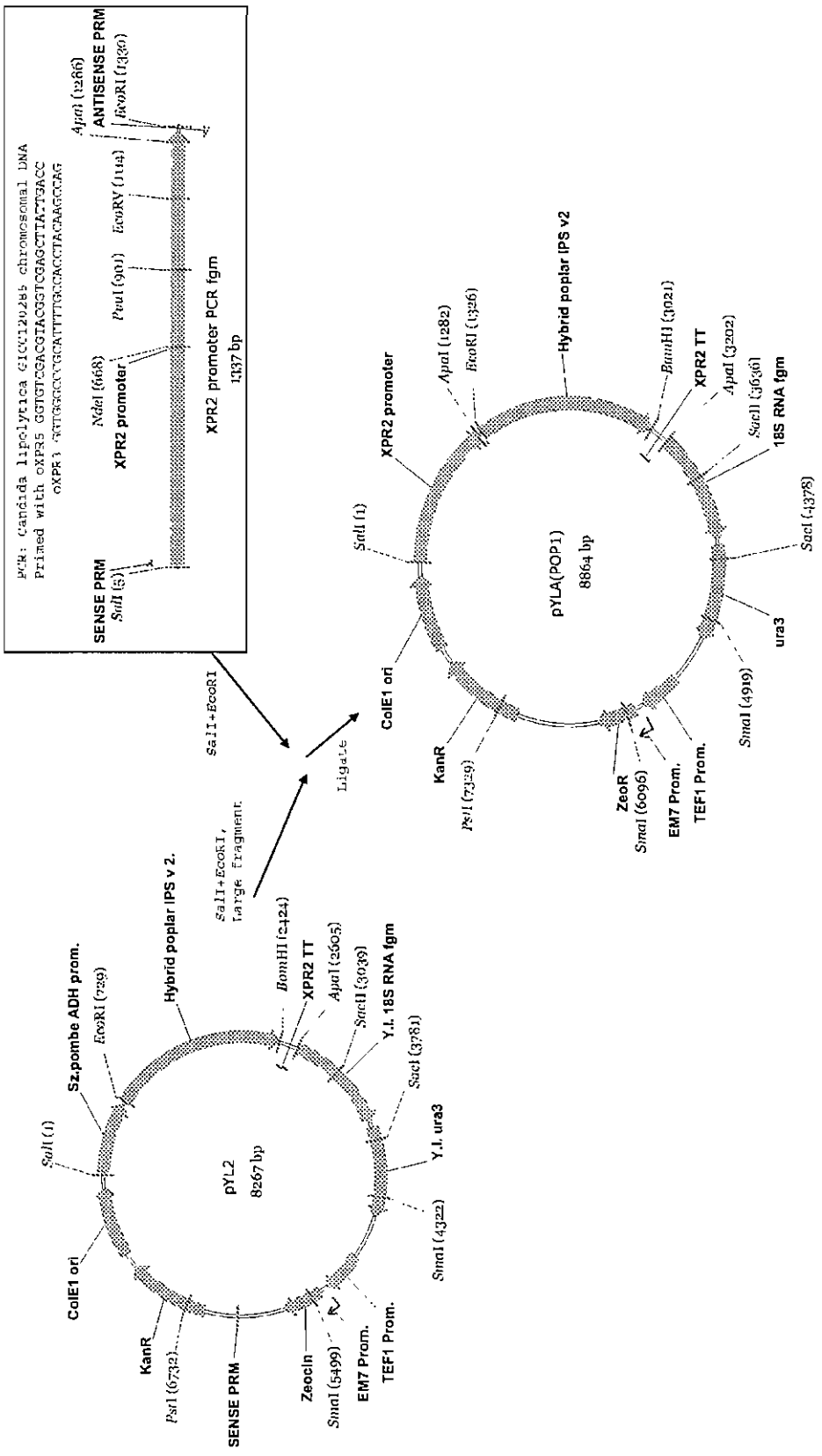
PCR: Candida lipolytica GICC120285 chromosomal DNA
Primed with oXPR5 GGTGTCGACGTACGGTCGAGCTTACTGACC
oXPR3 GGTGGGCCCGCATTTTGCCACCTACAAGCCAG
SENSE PRM
SalI (5)
XPR2 promoter
NdeI (668)
PvuI (901)
EcoRV (1114)
ApaI (1266)
ANTISENSE PRM
EcoRI (1330)
XPR2 promoter PCR fgm
1337 bp
SalI+EcoRI
Ligate
SalI+EcoRI,
Large fragment
pYL2
8267 bp
SalI (1)
Sz.pombe ADH prom.
EcoRI (729)
Hybrid poplar IPS v 2.
BamHI (2424)
XPR2 TT
ApaI (2605)
SacII (3039)
Y.l. 18S RNA fgm
SacI (3781)
Y.l. ura3
SmaI (4322)
TEF1 Prom.
EM7 Prom.
SmaI (5499)
ZeocIn
SENSE PRM
PstI (6732)
KanR
ColE1 ori
pYLA(POP1)
8864 bp
SalI (1)
XPR2 promoter
ApaI (1282)
EcoRI (1326)
Hybrid poplar IPS v2
BamHI (3021)
XPR2 TT
ApaI (3202)
SacII (3636)
18S RNA fgm
SacI (4378)
ura3
SmaI (4919)
TEF1 Prom.
EM7 Prom.
SmaI (6096)
ZeoR
PstI (7329)
KanR
ColE1 ori

Figure 18C

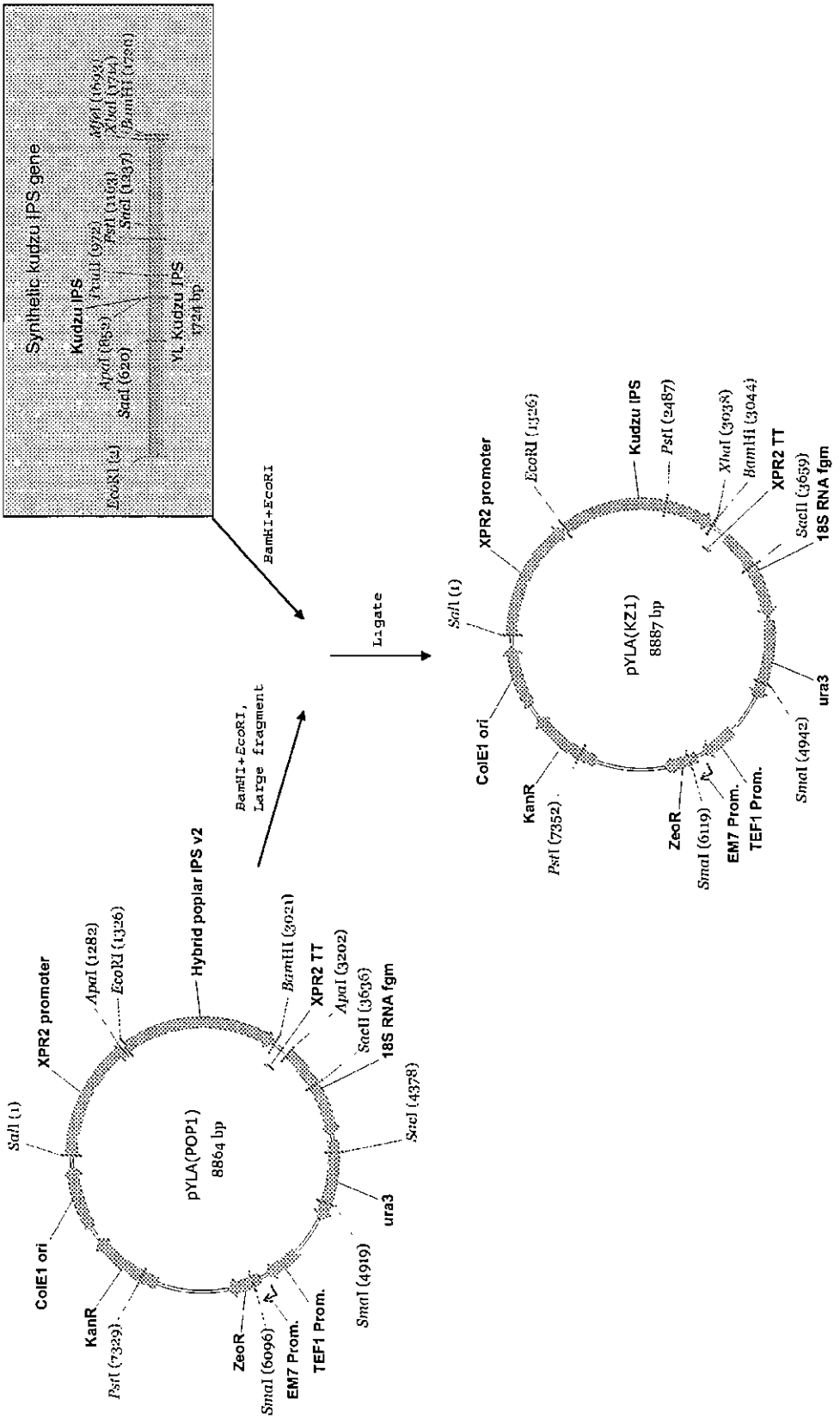
Synthetic kudzu IPS gene
Kudzu IPS
YL Kudzu IPS
1724 bp
BamHI+EcoRI
BamHI+EcoRI, Large fragment
Ligate
pYLA(POP1)
8864 bp
pYLA(KZ1)
8887 bp
XPR2 promoter
Hybrid poplar IPS v2
XPR2 TT
18S RNA fgm
ura3
ZeoR
EM7 Prom.
TEF1 Prom.
KanR
ColE1 ori
Sal1 (1)
ApaI (1282)
EcoRI (1326)
BamHI (3021)
ApaI (3202)
SacII (3636)
SacI (4378)
SmaI (4919)
SmaI (6096)
PstI (7329)
PstI (7352)
SmaI (6119)
SmaI (4942)
SacII (3659)
BamHI (3044)
XhaI (3038)
PstI (2487)
EcoRI (1326)
Kudzu IPS

Figure 18D

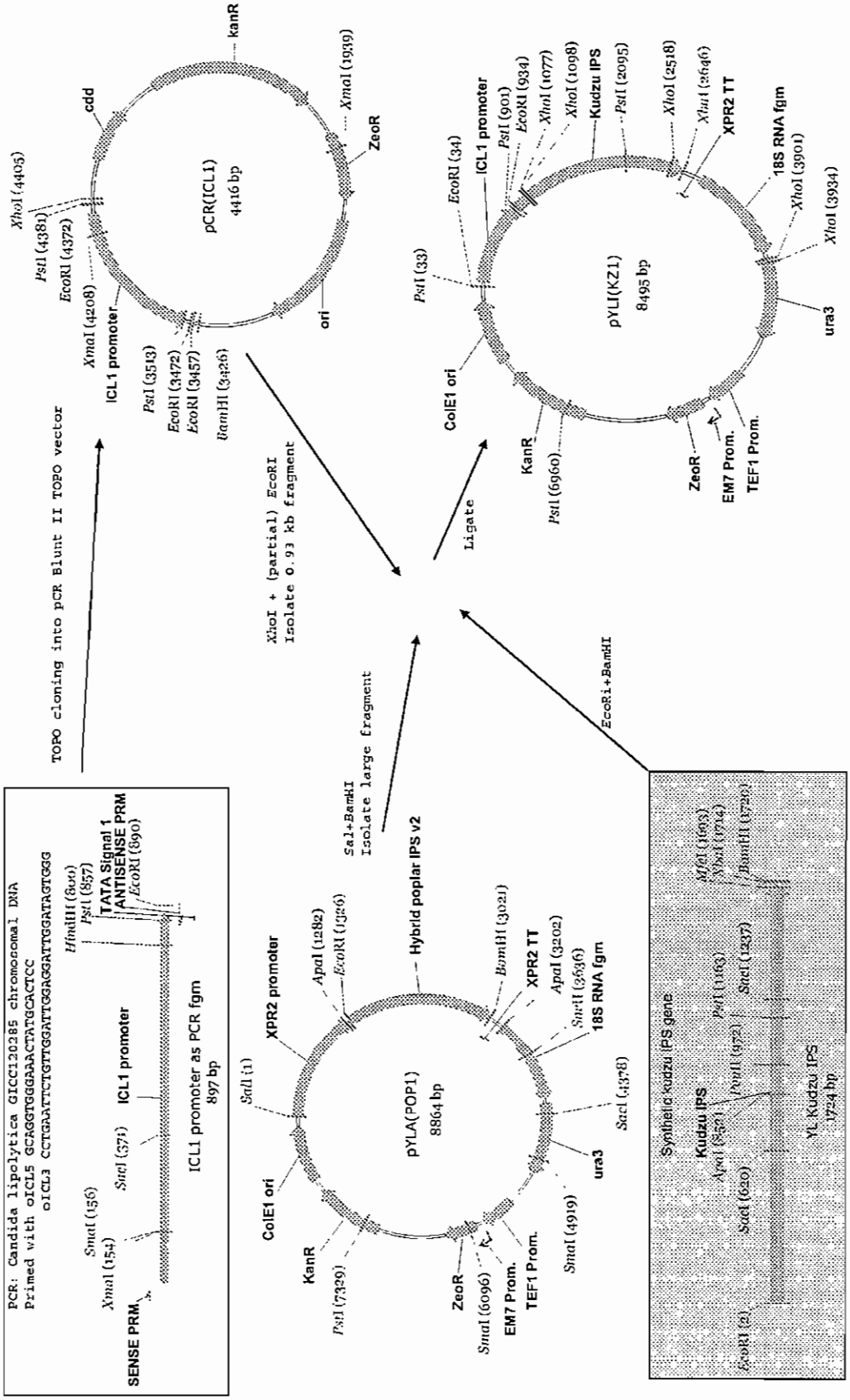
PCR: Candida lipolytica GICC120285 chromosomal DNA
Primed with oICL5 GCAGGTGGGAAACTATGCACTCC
oICL3 CCTGAATTCTGTTGGATTGGAGGATTGGATAGTGGG
HindIII (800)
PstI (857)
TATA Signal 1
ANTISENSE PRM
EcoRI (890)
SmaI (156)
XmaI (154)
SacI (371)
ICL1 promoter
SENSE PRM
ICL1 promoter as PCR fgm
897 bp
TOPO cloning into pCR Blunt II TOPO vector
pCR(ICL1)
4416 bp
kanR
cdd
ZeoR
ori
ICL1 promoter
XhoI (4405)
PstI (4381)
EcoRI (4372)
XmaI (4208)
PstI (3513)
EcoRI (3472)
EcoRI (3457)
BamHI (3426)
XmaI (1939)
XhoI + (partial) EcoRI
Isolate 0.93 kb fragment
Ligate
pYLI(KZ1)
8495 bp
ColE1 ori
KanR
ZeoR
EM7 Prom.
TEF1 Prom.
ICL1 promoter
Kudzu IPS
XPR2 TT
18S RNA fgm
ura3
PstI (33)
EcoRI (34)
PstI (901)
EcoRI (934)
XhoI (1077)
XhoI (1098)
PstI (2095)
XhoI (2518)
XhoI (2646)
XhoI (3901)
XhoI (3934)
PstI (6960)
SalI+BamHI
Isolate large fragment
Hybrid poplar IPS v2
pYLA(POP1)
8864 bp
SalI (1)
XPR2 promoter
ApaI (1282)
EcoRI (1326)
BamHI (3021)
XPR2 TT
ApaI (3202)
SmaI (3636)
18S RNA fgm
SacI (4378)
ura3
SmaI (4919)
TEF1 Prom.
EM7 Prom.
ZeoR
SmaI (6096)
PstI (7329)
ColE1 ori
KanR
EcoRi+BamHI
Synthetic kudzu IPS gene
Kudzu IPS
ApaI (8521)
EcoRI (2)
SacI (620)
PstI (1163)
PstII (972)
SmaI (1237)
MfeI (1693)
XhoI (1714)
BamHI (1726)
YL Kudzu IPS
1724 bp

Figure 18E

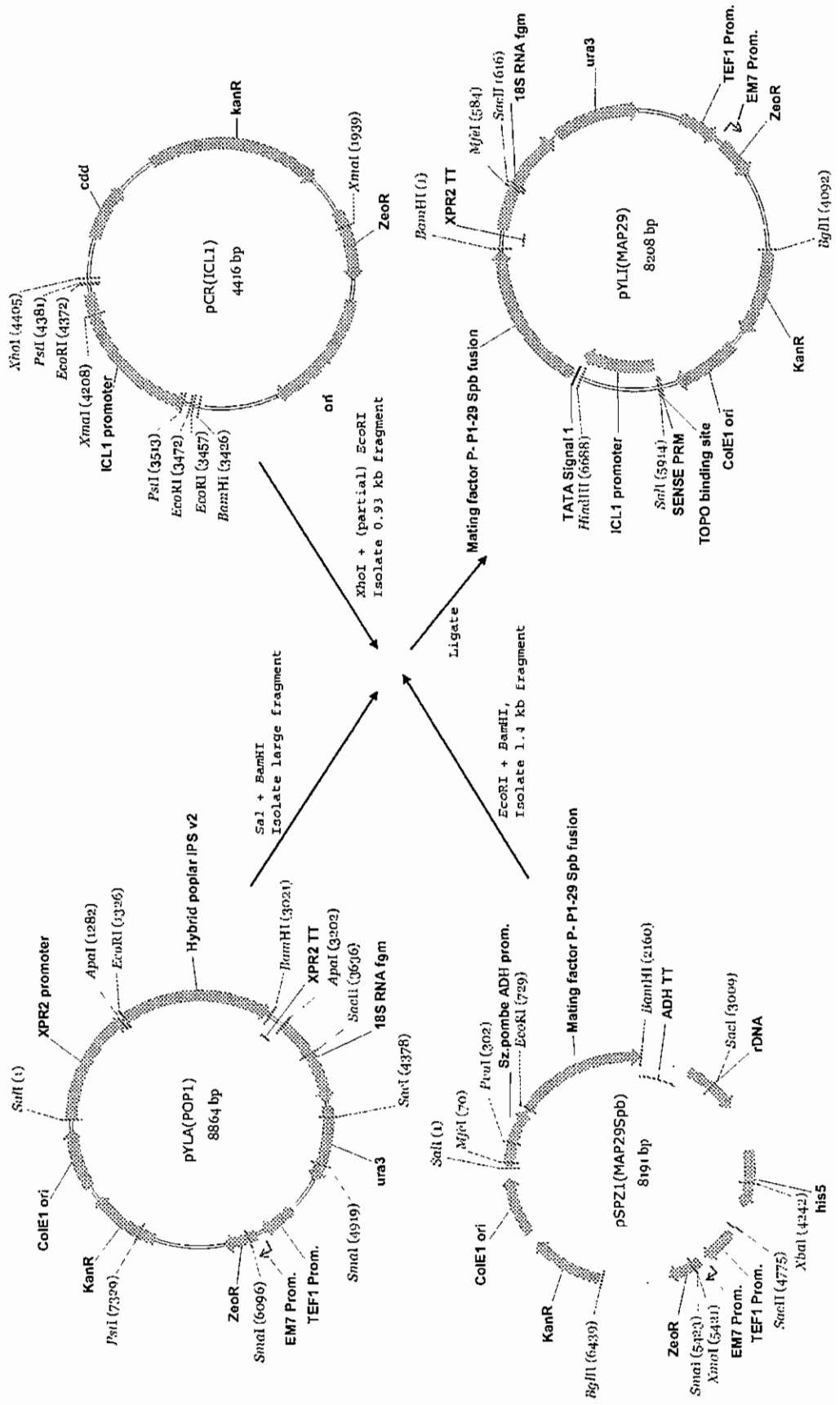
pCR(ICL1)
4416 bp
cdd
kanR
ZeoR
ori
ICL1 promoter
XmaI (1939)
XmaI (4208)
EcoRI (4372)
PstI (4381)
XhoI (4405)
BamHI (3426)
EcoRI (3457)
EcoRI (3472)
PstI (3513)
XhoI + (partial) EcoRI
Isolate 0.93 kb fragment
pYLA(POP1)
8864 bp
Hybrid poplar IPS v2
XPR2 promoter
ApaI (1282)
EcoRI (1326)
BamHI (3021)
XPR2 TT
ApaI (3202)
SacII (3636)
18S RNA fgm
SacI (4378)
ura3
SmaI (4919)
TEF1 Prom.
EM7 Prom.
ZeoR
SmaI (6096)
PstI (7320)
KanR
ColE1 ori
SalI (1)
Sal + BamHI
Isolate large fragment
pYLI(MAP29)
8208 bp
BamHI (1)
XPR2 TT
MfeI (584)
SacII (616)
18S RNA fgm
ura3
TEF1 Prom.
EM7 Prom.
ZeoR
BglII (4092)
KanR
ColE1 ori
TOPO binding site
SENSE PRM
SalI (5914)
ICL1 promoter
TATA Signal 1
HindIII (6688)
Mating factor P- P1-29 Spb fusion
Ligate
pSPZ1(MAP29Spb)
8191 bp
SalI (1)
MfeI (70)
PvuI (302)
Sz.pombe ADH prom.
EcoRI (729)
Mating factor P- P1-29 Spb fusion
BamHI (2160)
ADH TT
SacI (3009)
rDNA
his5
XbaI (4242)
SacII (4775)
TEF1 Prom.
EM7 Prom.
ZeoR
SmaI (5323)
XmaI (5431)
BglII (6439)
KanR
ColE1 ori
EcoRI + BamHI,
Isolate 1.4 kb fragment

Figure 18F

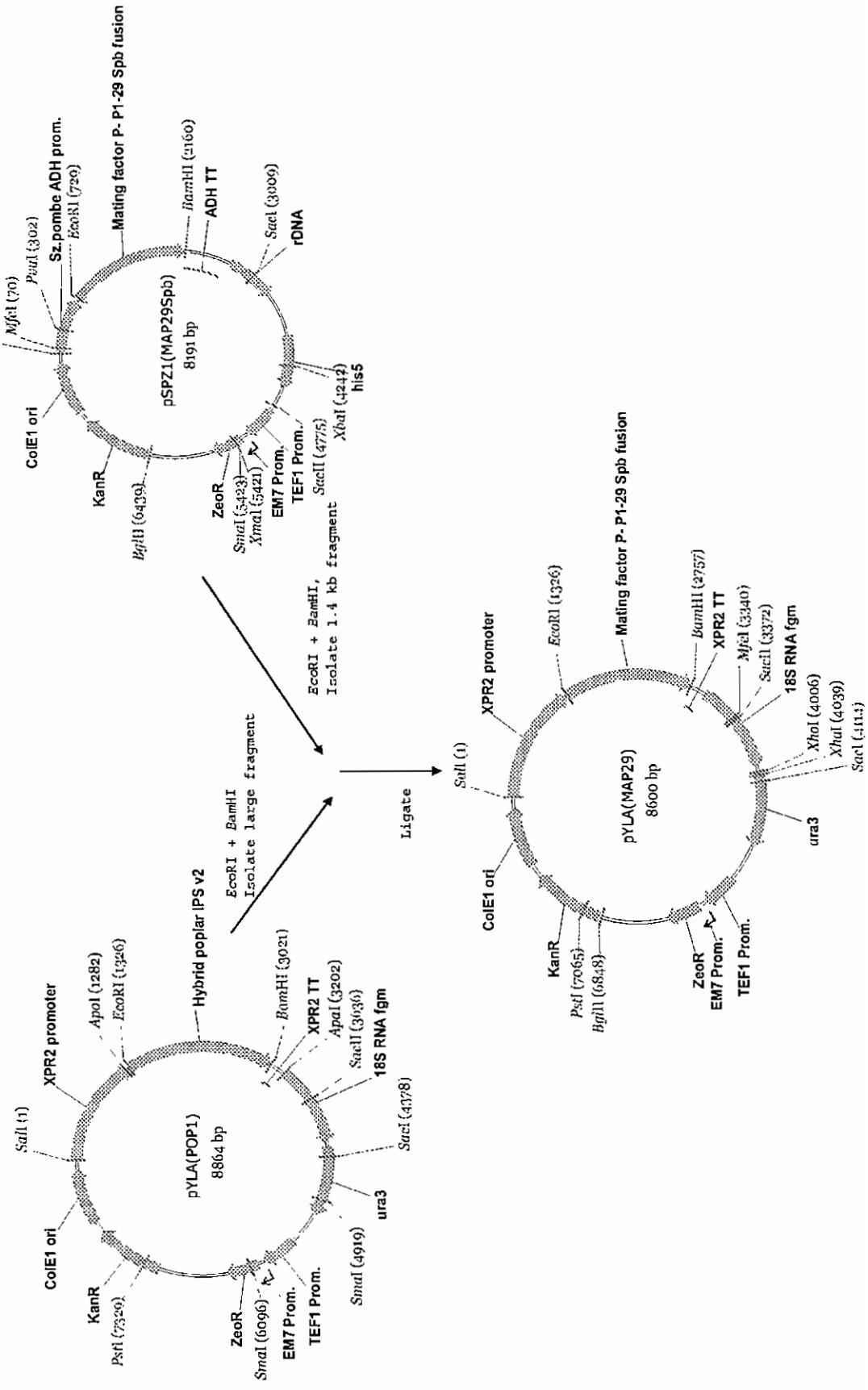
pSPZ1(MAP29Spb)
8191 bp
ColE1 ori
KanR
ZeoR
EM7 Prom.
TEF1 Prom.
Mating factor P- P1-29 Spb fusion
Sz.pombe ADH prom.
ADH TT
rDNA
his5
EcoRI + BamHI
Isolate 1.4 kb fragment
EcoRI + BamHI
Isolate large fragment
pYLA(POP1)
8864 bp
XPR2 promoter
Hybrid poplar IPS v2
XPR2 TT
18S RNA fgm
ura3
Ligate
pYLA(MAP29)
8600 bp
Mating factor P- P1-29 Spb fusion
XPR2 promoter
XPR2 TT
18S RNA fgm
ura3

Figure 19

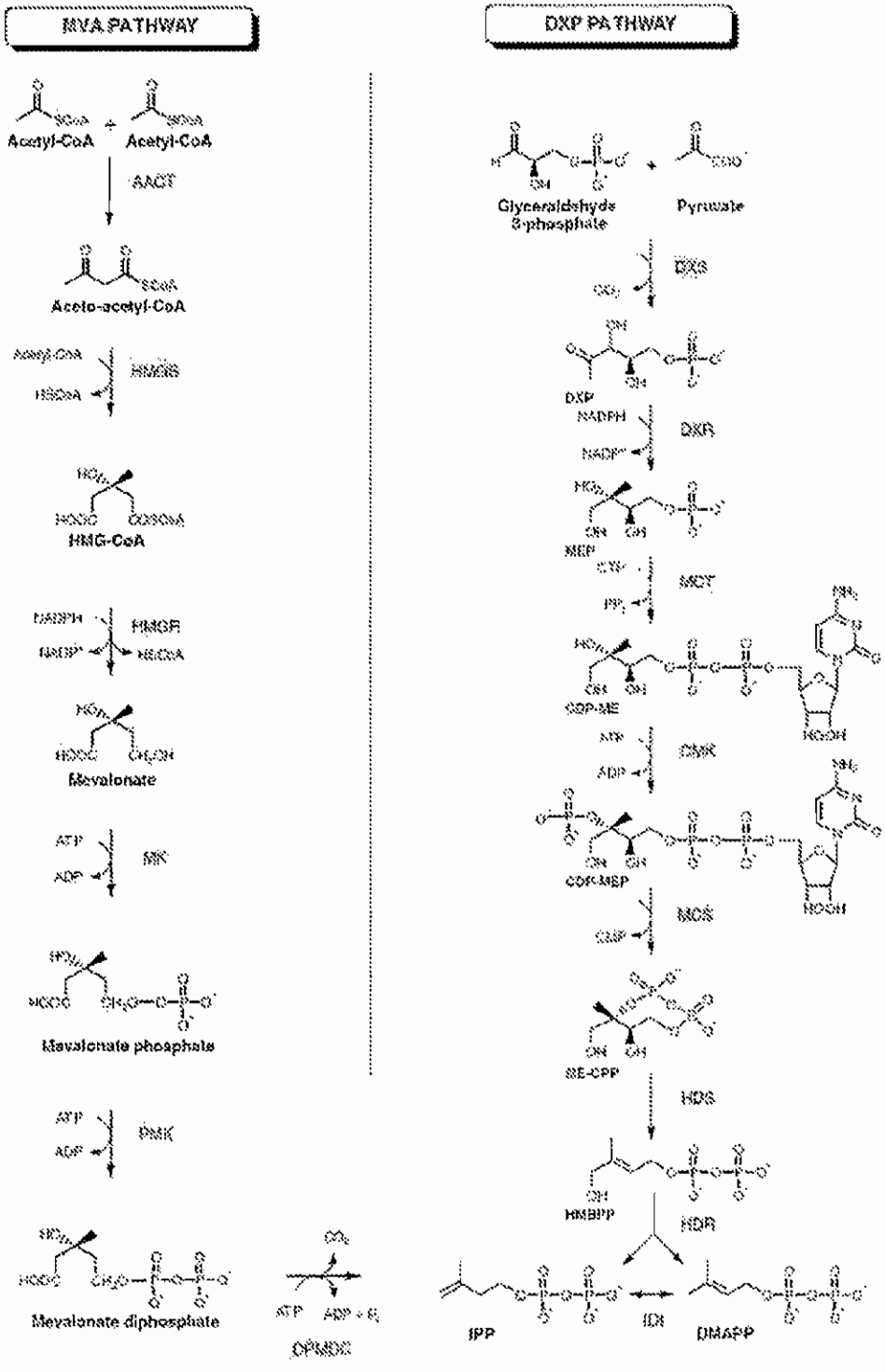
MVA PATHWAY
DXP PATHWAY
Acetyl-CoA
Acetyl-CoA
AACT
Aceto-acetyl-CoA
HMGS
HMG-CoA
NADPH
HMGR
Mevalonate
ATP
MK
Mevalonate phosphate
ATP
PMK
Mevalonate diphosphate
ATP
ADP + P_i
DPMDC
Glyceraldehyde 3-phosphate
Pyruvate
DXS
DXP
NADPH
DXR
MEP
CTP
MCT
CDP-ME
ATP
CMK
ADP
CDP-MEP
MDS
CMP
ME-CPP
HDS
HMBPP
HDR
IPP
IDI
DMAPP

Figure 20

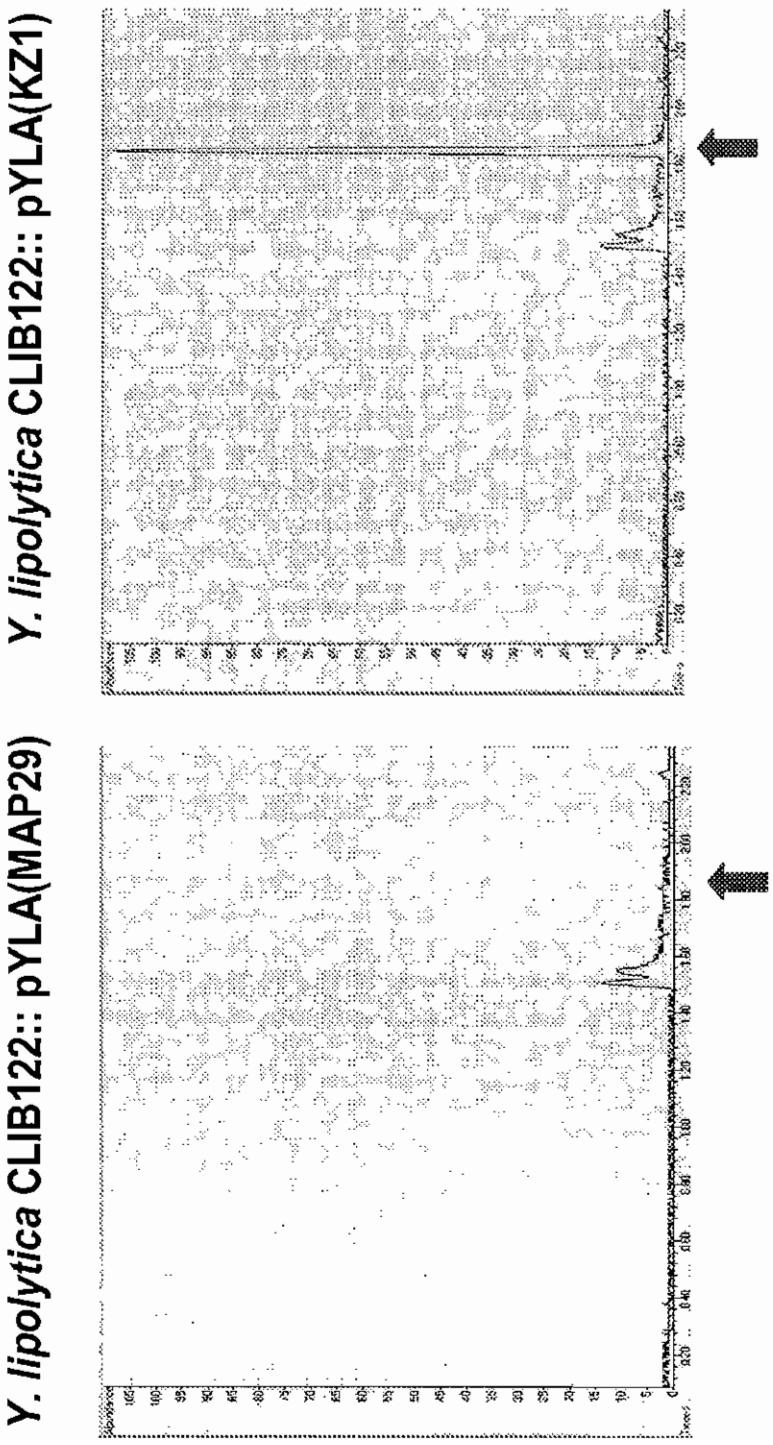
Y. lipolytica CLIB122:: pYLA(KZ1)
Y. lipolytica CLIB122:: pYLA(MAP29)

Figure 21

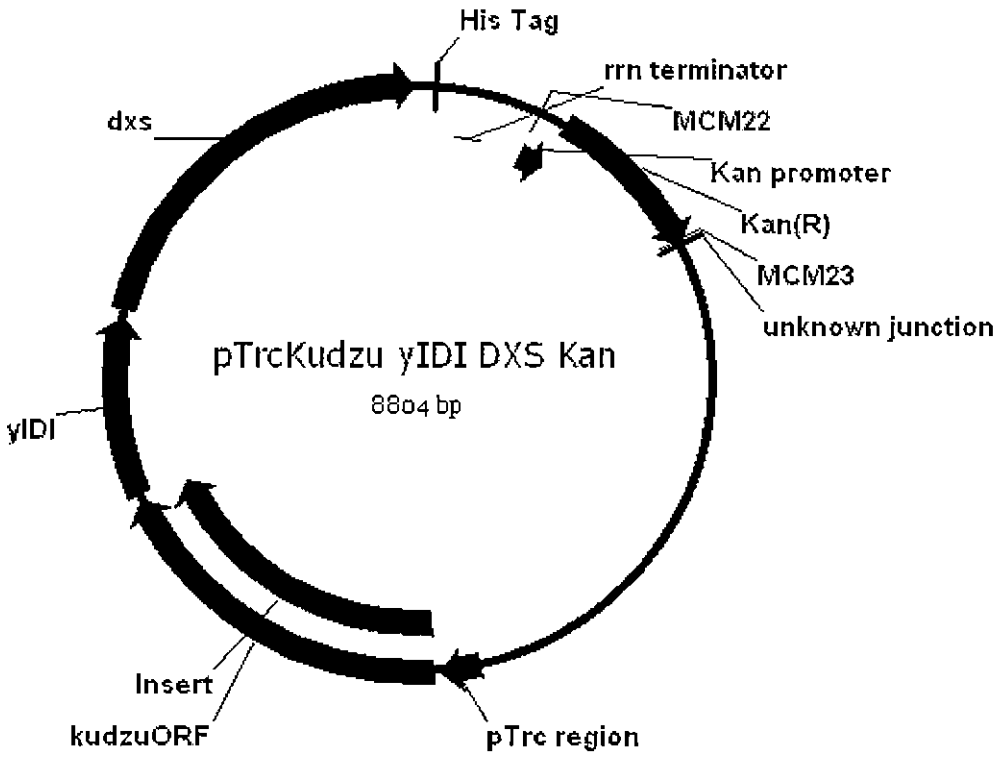
His Tag
rrn terminator
MCM22
Kan promoter
Kan(R)
MCM23
unknown junction
dxs
pTrcKudzu yIDI DXS Kan
8804 bp
yIDI
Insert
kudzuORF
pTrc region

Figure 22A

1-
gctggtaccatatgggaattcgaagctttctagaacaaaaactcatctcagaagaggatctgaa
tagcgccgtcgaccatcatcatcatcattgagtttaaacggtctccagcttggctgttttg
gcggatgagagaagattttcagcctgatacagattaaatcagaacgcagaagcggtctgataaa
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Figure 22B

gagtcaattcagggtggtgaatgtgaaaccagtaacgttatacgatgtcgcagagtatgccggt
gtctcttatcagaccgtttcccgcgtggtgaaccaggccagccacgtttctgcgaaaacgcggg
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Figure 22C

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Figure 22D

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gtgaacgaagtgctgatggcccatcgtaaaccagtacccgtgctgaacattggcctgccggact
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(SEQ ID NO:20)

Figure 23A

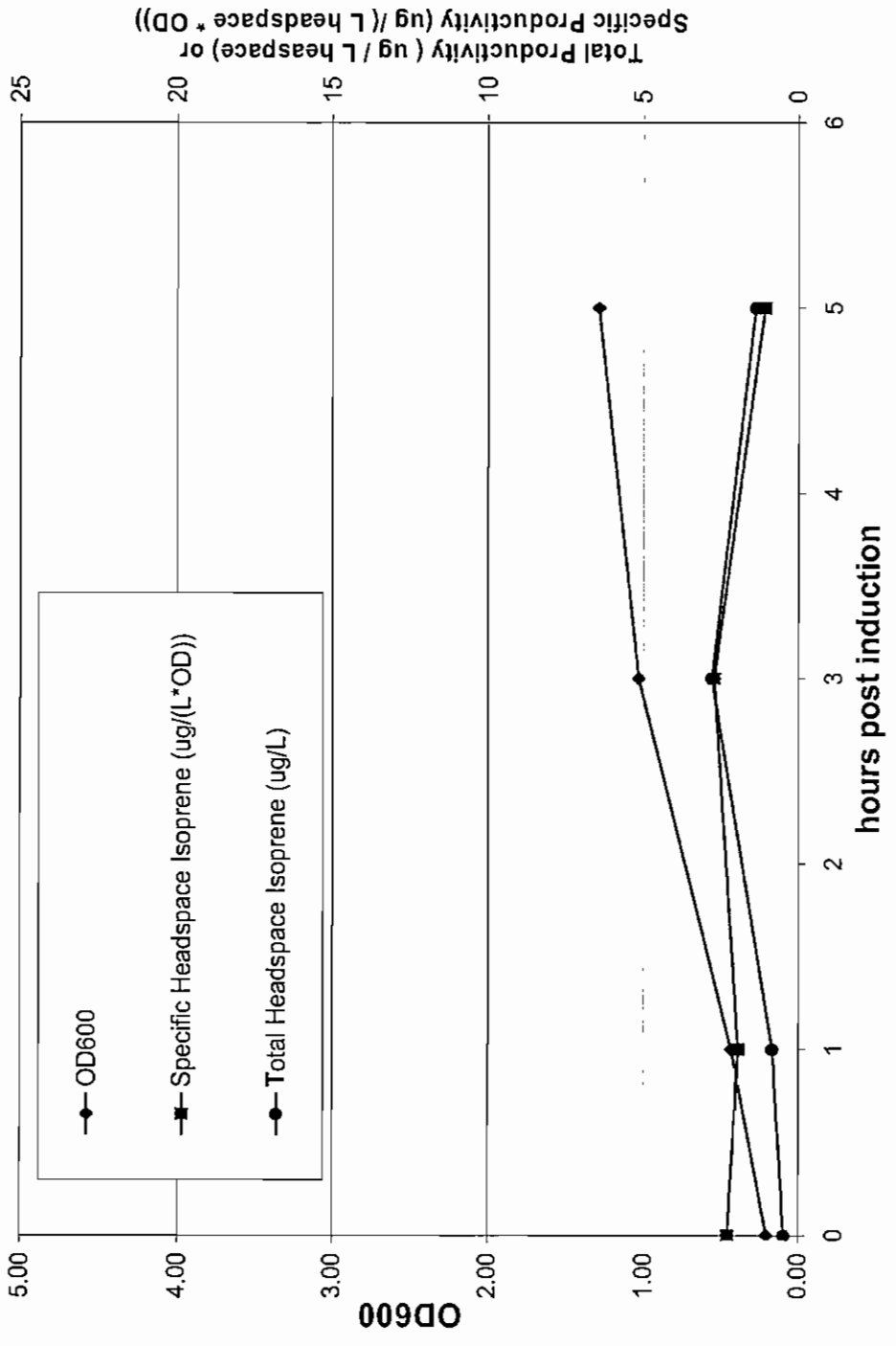

pTrcKudzu kan
OD600
Specific Headspace Isoprene (ug/(L*OD))
Total Headspace Isoprene (ug/L)
OD600
Total Productivity (ug / L headspace) or
Specific Productivity (ug /(L headspace * OD))
hours post induction
5.00
4.00
3.00
2.00
1.00
0.00
25
20
15
10
5
0
0
1
2
3
4
5
6

Figure 23B

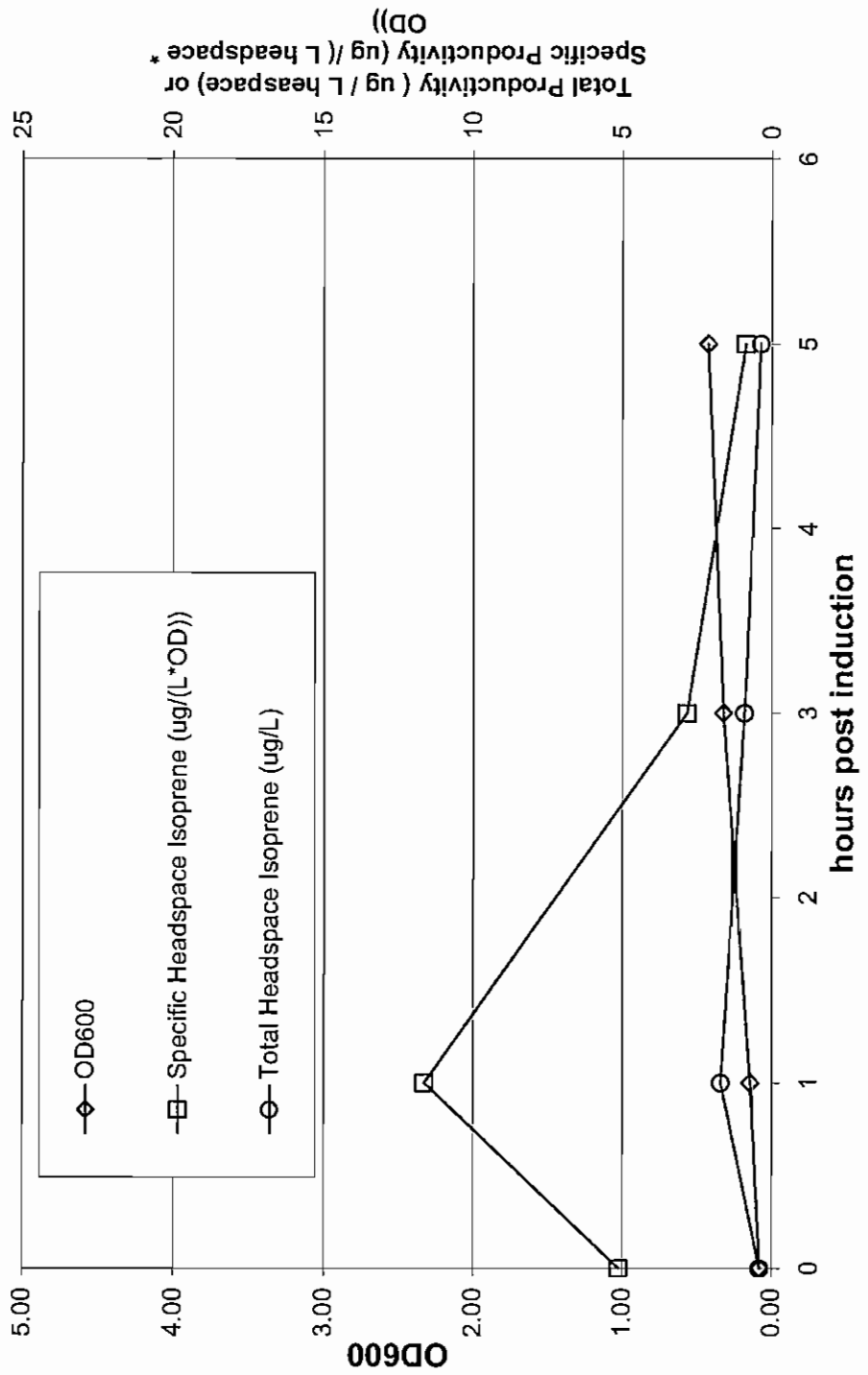

pTrcKudzu yIDI kan
OD600
Total Productivity (ug / L headspace) or Specific Productivity (ug /(L headspace * OD))
hours post induction
OD600
Specific Headspace Isoprene (ug/(L*OD))
Total Headspace Isoprene (ug/L)
0.00
1.00
2.00
3.00
4.00
5.00
0
5
10
15
20
25
0
1
2
3
4
5
6

Figure 23C

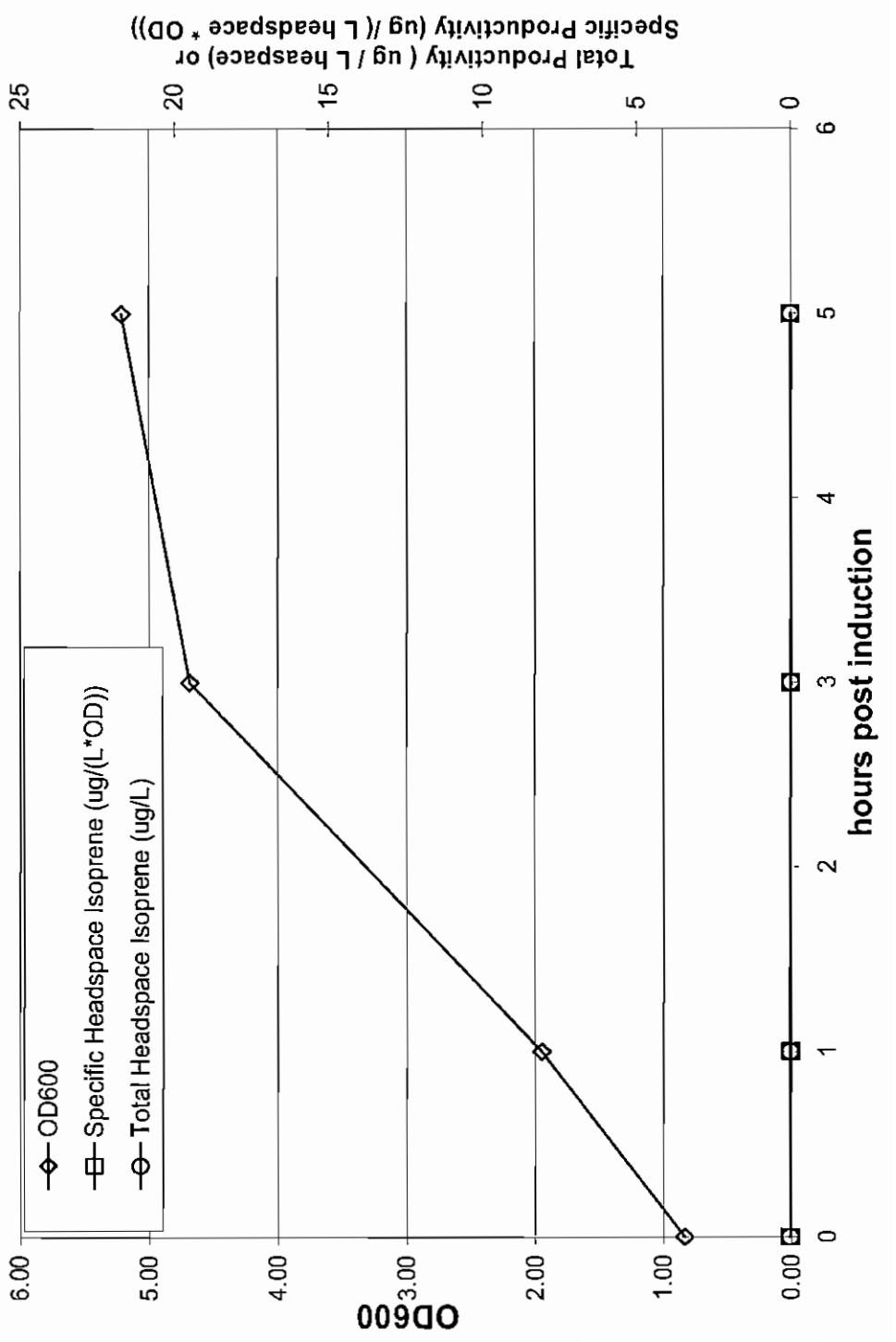

pTrcKudzu DXS kan
OD600
Specific Headspace Isoprene (ug/(L*OD))
Total Headspace Isoprene (ug/L)
OD600
Total Productivity (ug / L headspace) or
Specific Productivity (ug /(L headspace * OD))
hours post induction
0.00
1.00
2.00
3.00
4.00
5.00
6.00
0
5
10
15
20
25
0
1
2
3
4
5
6

Figure 23D

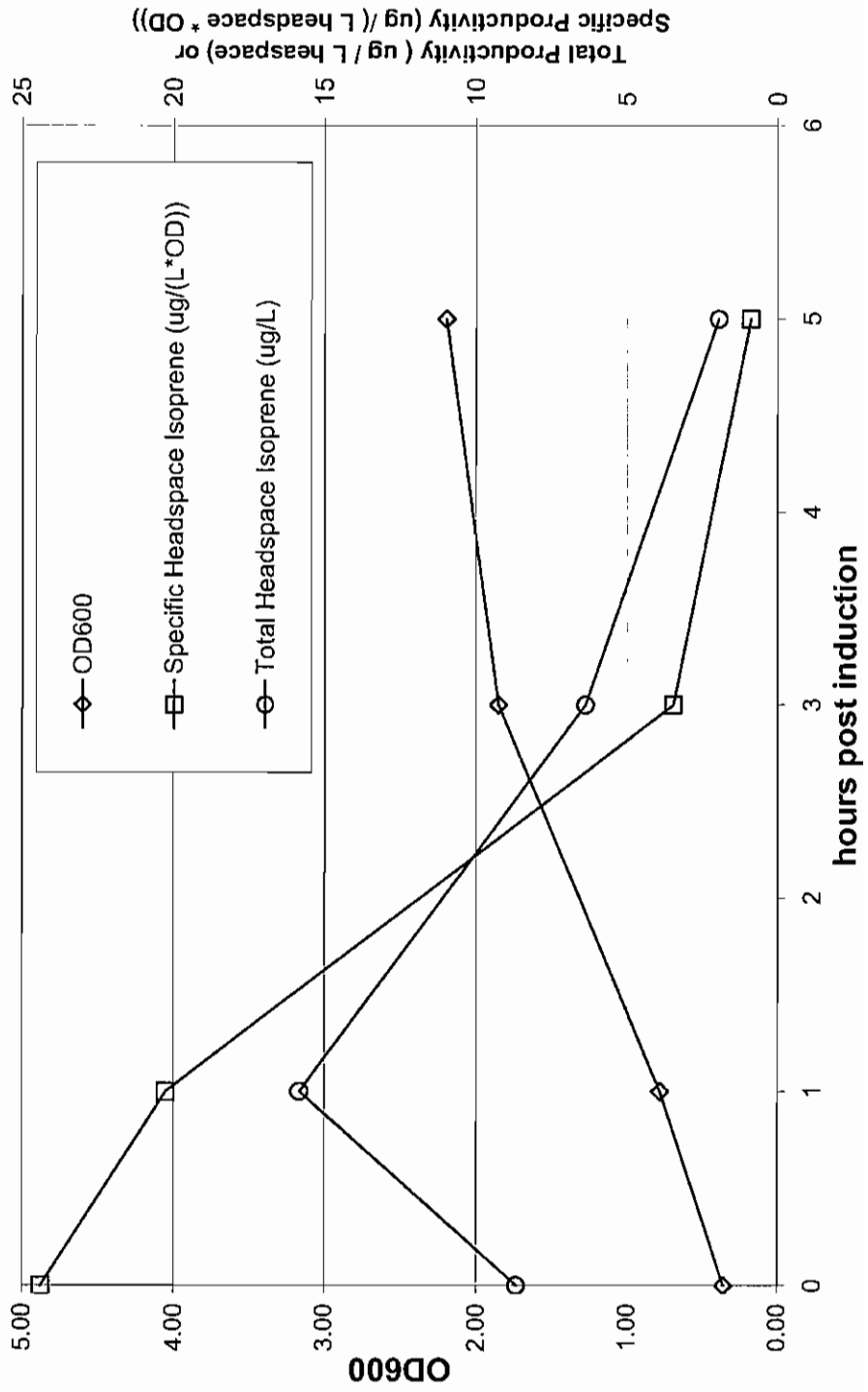

pTrcKudzu yIDI DXS kan
OD600
Specific Headspace Isoprene (ug/(L*OD))
Total Headspace Isoprene (ug/L)
OD600
Total Productivity (ug / L headspace) or
Specific Productivity (ug /(L headspace * OD))
hours post induction
0.00
1.00
2.00
3.00
4.00
5.00
0
5
10
15
20
25
0
1
2
3
4
5
6

Figure 23E

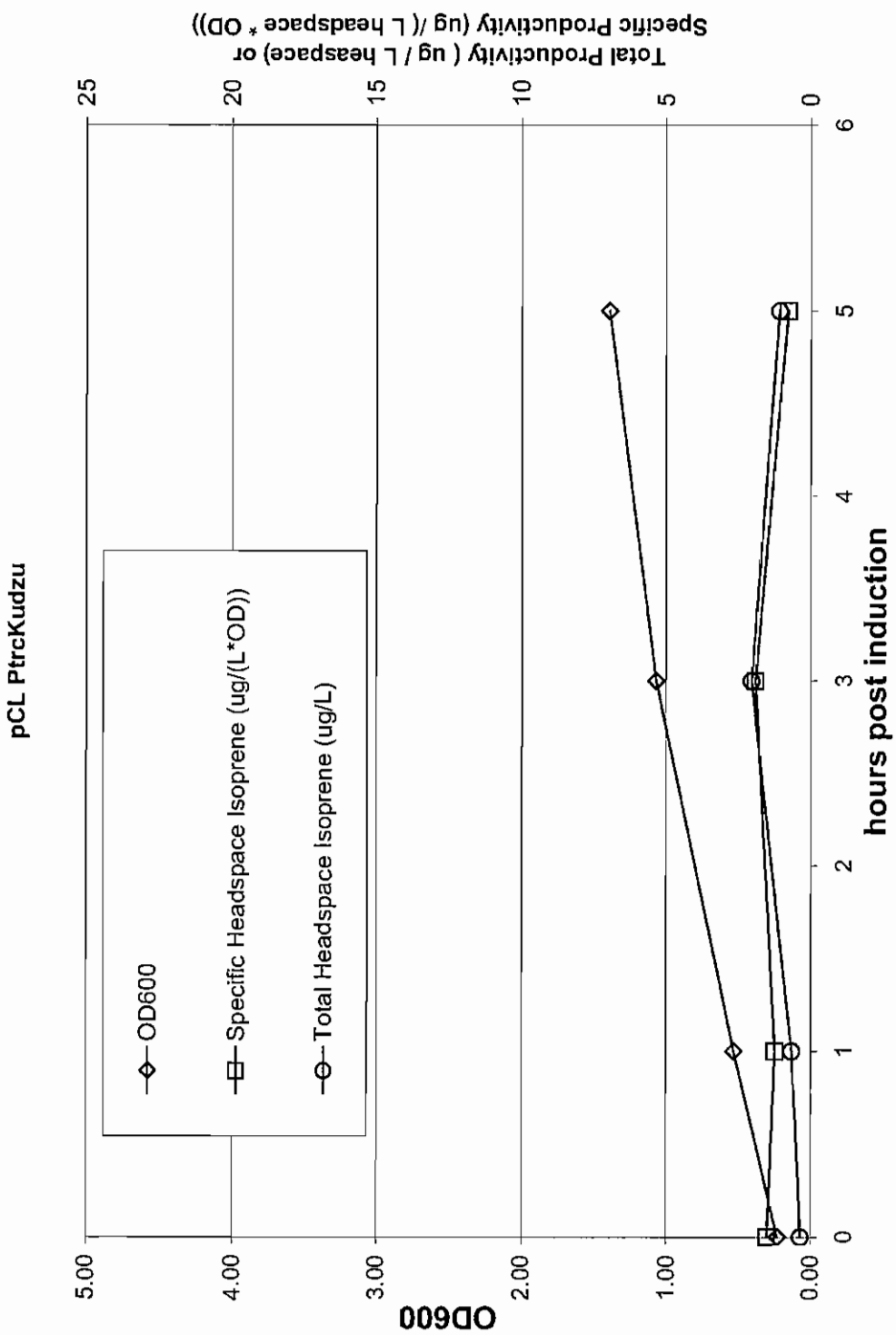

pCL PtrcKudzu
OD600
Total Productivity (ug / L headspace) or
Specific Productivity (ug /(L headspace * OD))
hours post induction
OD600
Specific Headspace Isoprene (ug/(L*OD))
Total Headspace Isoprene (ug/L)
0.00
1.00
2.00
3.00
4.00
5.00
0
5
10
15
20
25
0
1
2
3
4
5
6

Figure 23F

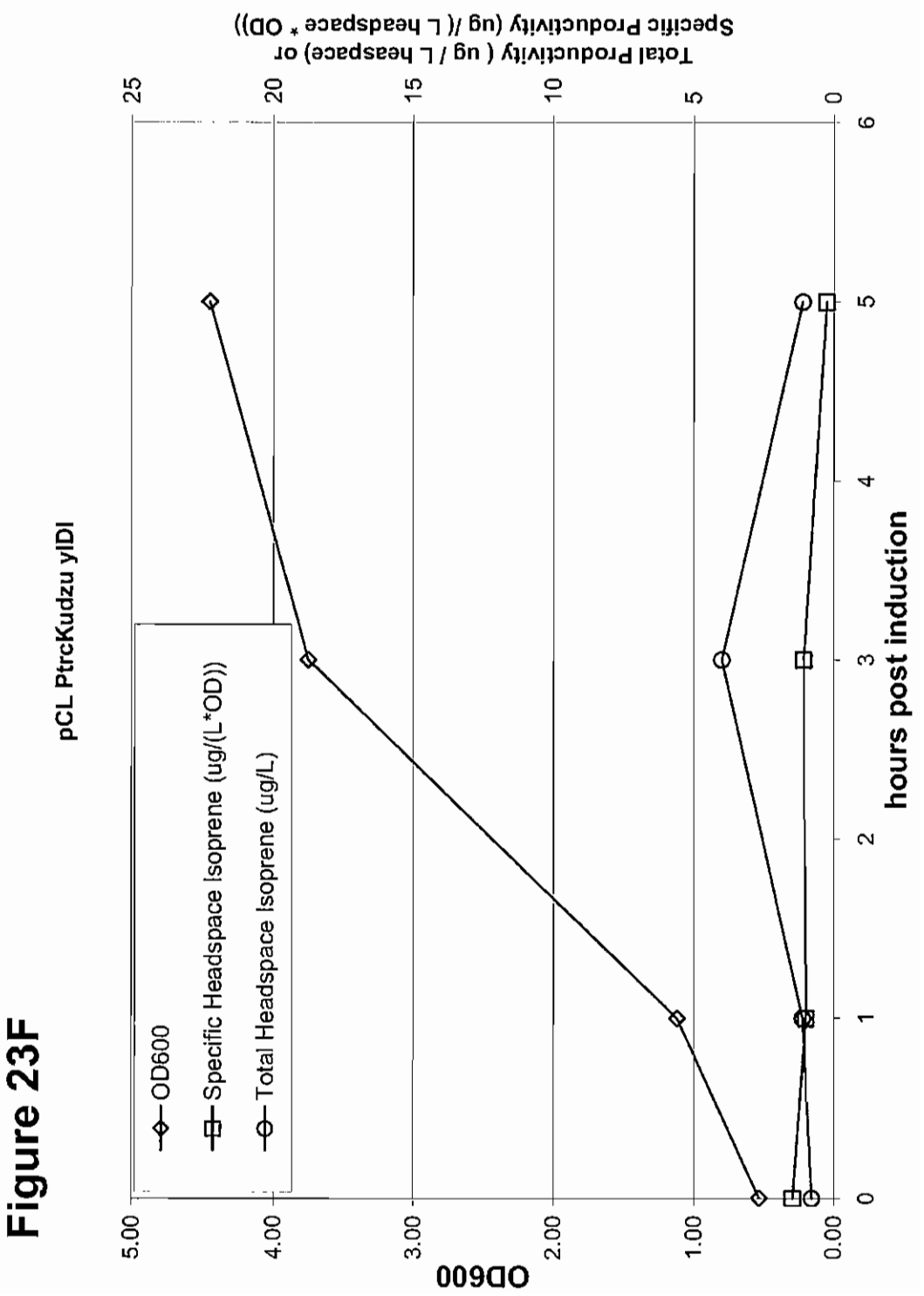
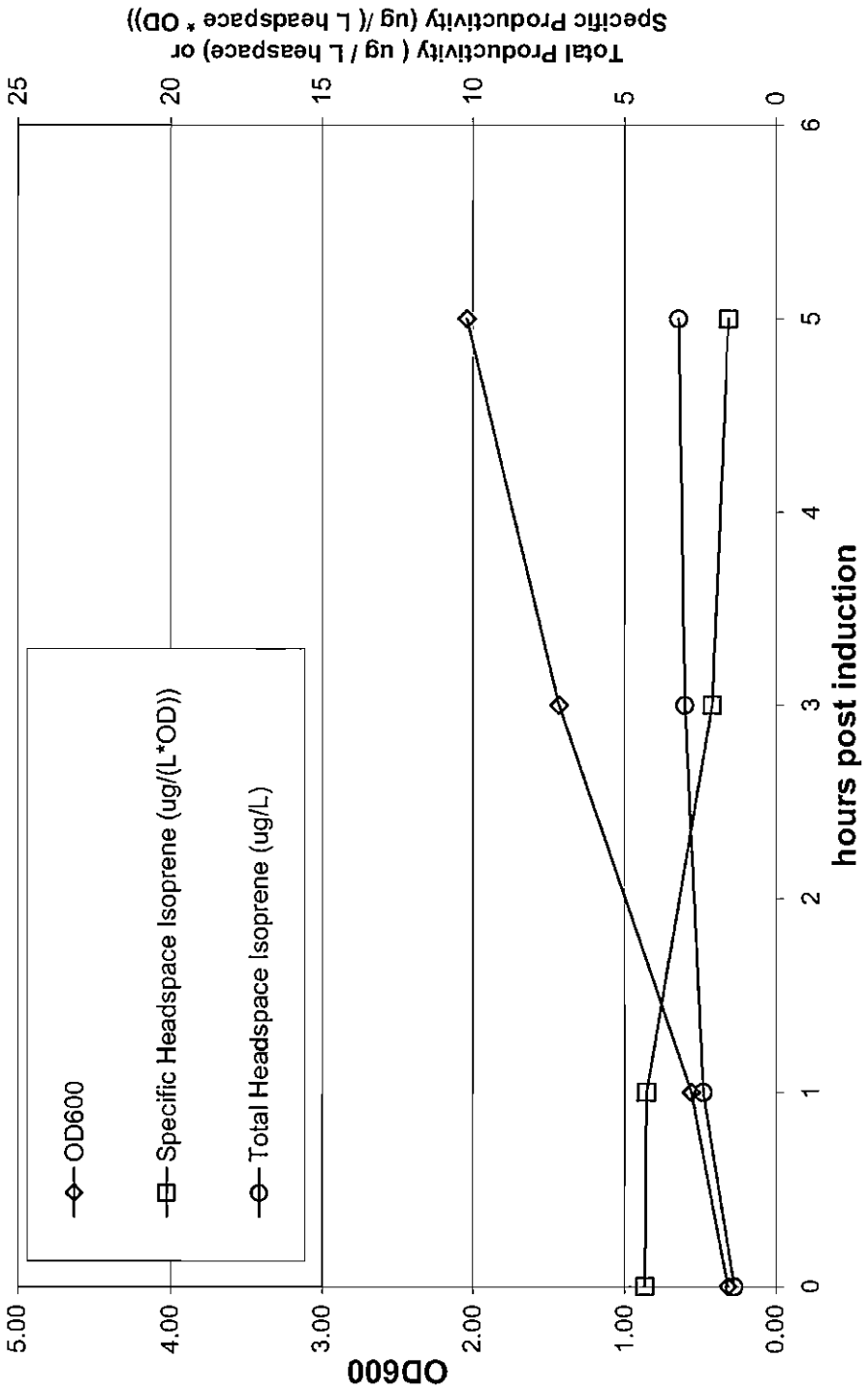

pCL PtrcKudzu yIDI
OD600
Specific Headspace Isoprene (ug/(L*OD))
Total Headspace Isoprene (ug/L)
OD600
Total Productivity (ug / L headspace) or
Specific Productivity (ug /(L headspace * OD))
hours post induction
5.00
4.00
3.00
2.00
1.00
0.00
25
20
15
10
5
0
0
1
2
3
4
5
6

Figure 23G


pCL PtrcKudzu DXS
OD600
Total Productivity (ug / L headspace) or
Specific Productivity (ug /(L headspace * OD))
hours post induction
OD600
Specific Headspace Isoprene (ug/(L*OD))
Total Headspace Isoprene (ug/L)
5.00
4.00
3.00
2.00
1.00
0.00
25
20
15
10
5
0
0
1
2
3
4
5
6

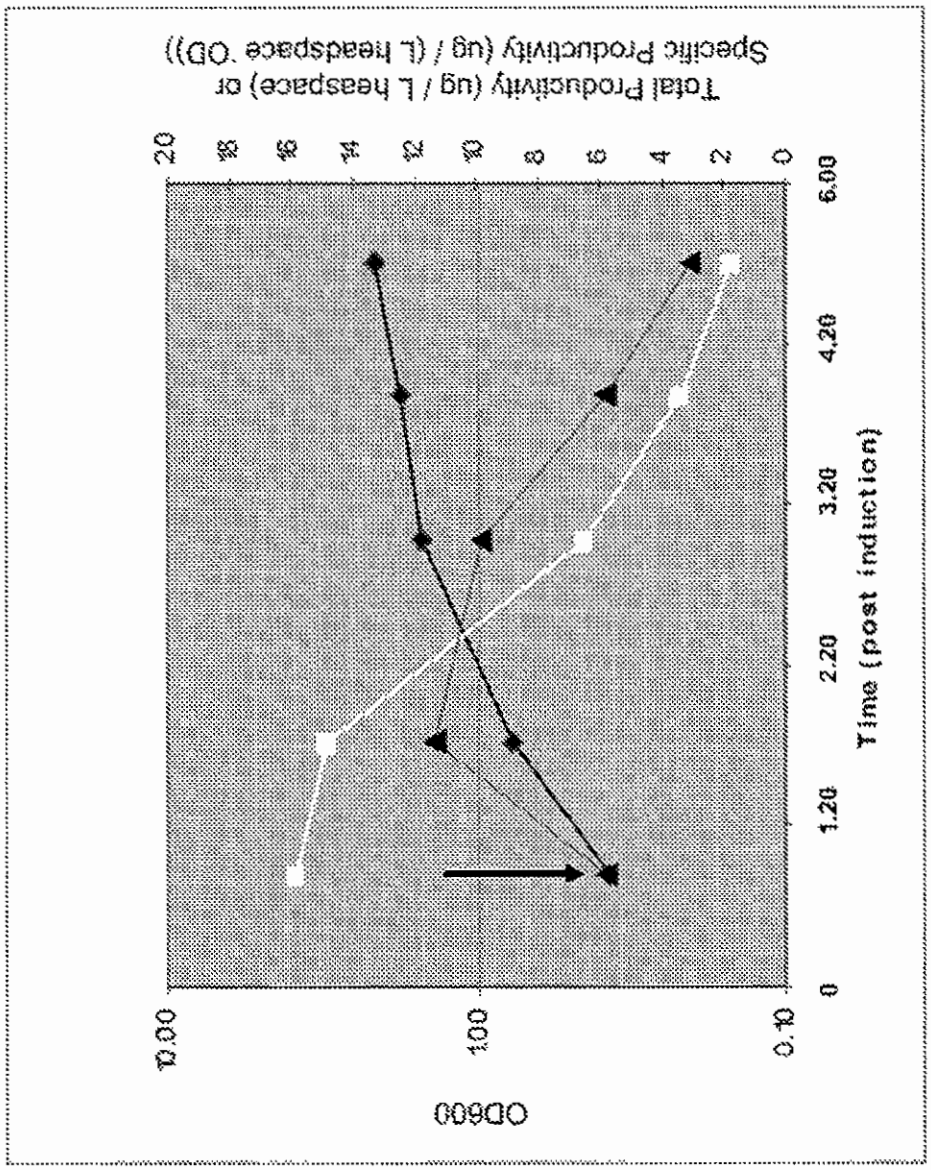

Total Productivity (ug / L headspace) or
Specific Productivity (ug / (L headspace *OD))
20
18
16
14
12
10
8
6
4
2
0
6.00
4.20
3.20
2.20
1.20
0
Time (post induction)
10.00
1.00
0.10
OD600

Figure 23H

Figure 24

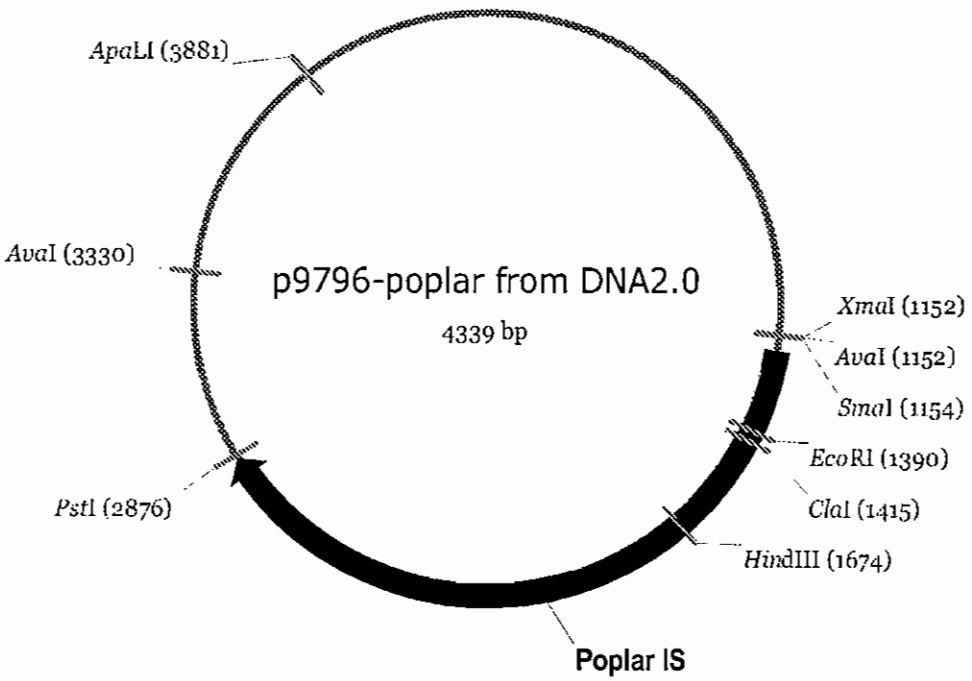

ApaLI (3881)
AvaI (3330)
p9796-poplar from DNA2.0
4339 bp
XmaI (1152)
AvaI (1152)
SmaI (1154)
EcoRI (1390)
ClaI (1415)
HindIII (1674)
PstI (2876)
Poplar IS

Figure 25A

5'-
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Figure 25B

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Figure 26

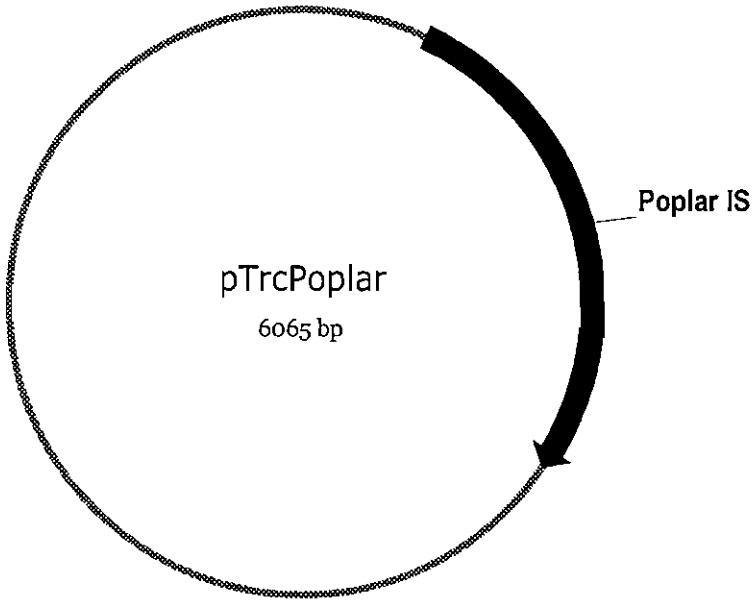

Figure 27A

5'-
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Figure 27B

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Figure 27C

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Figure 28

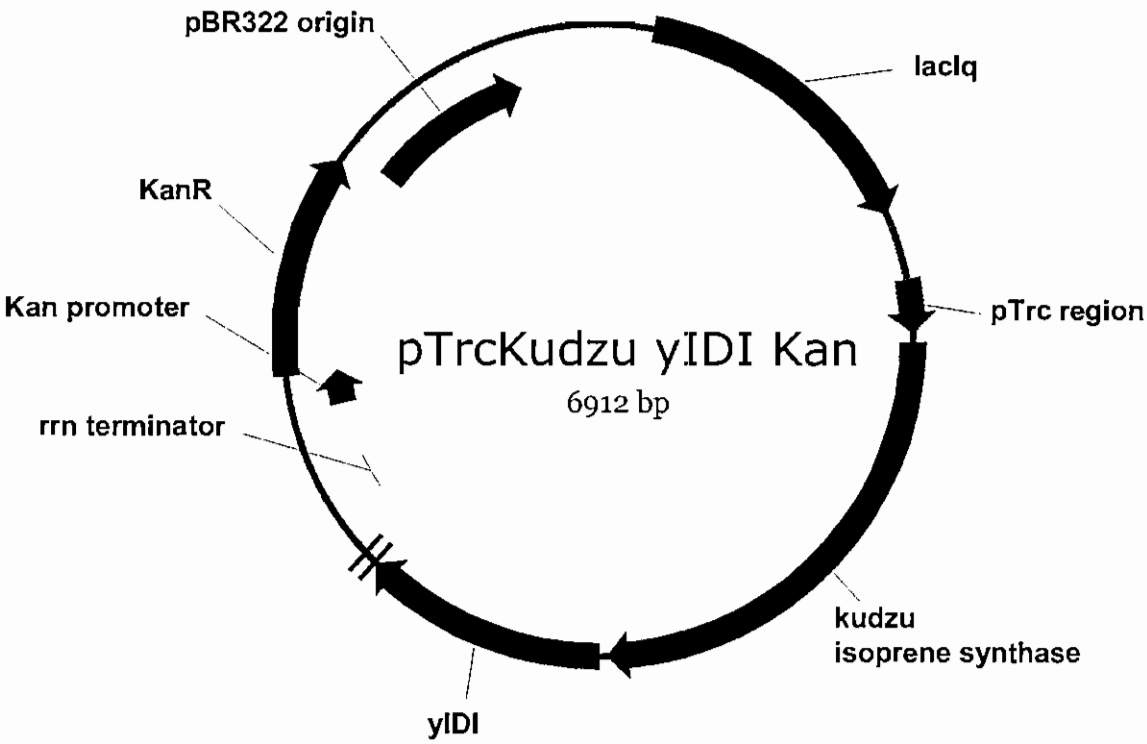
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Figure 29A

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Figure 29B

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Figure 29C

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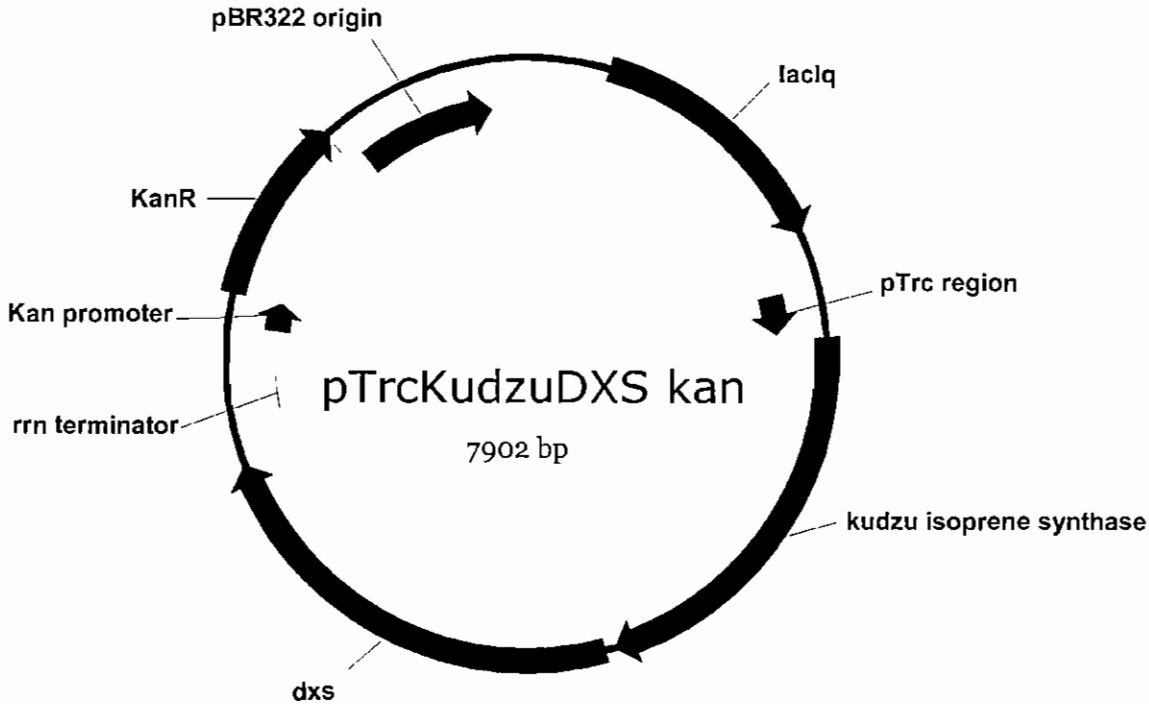
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Figure 31A

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Figure 31B

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Figure 31C

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Figure 32

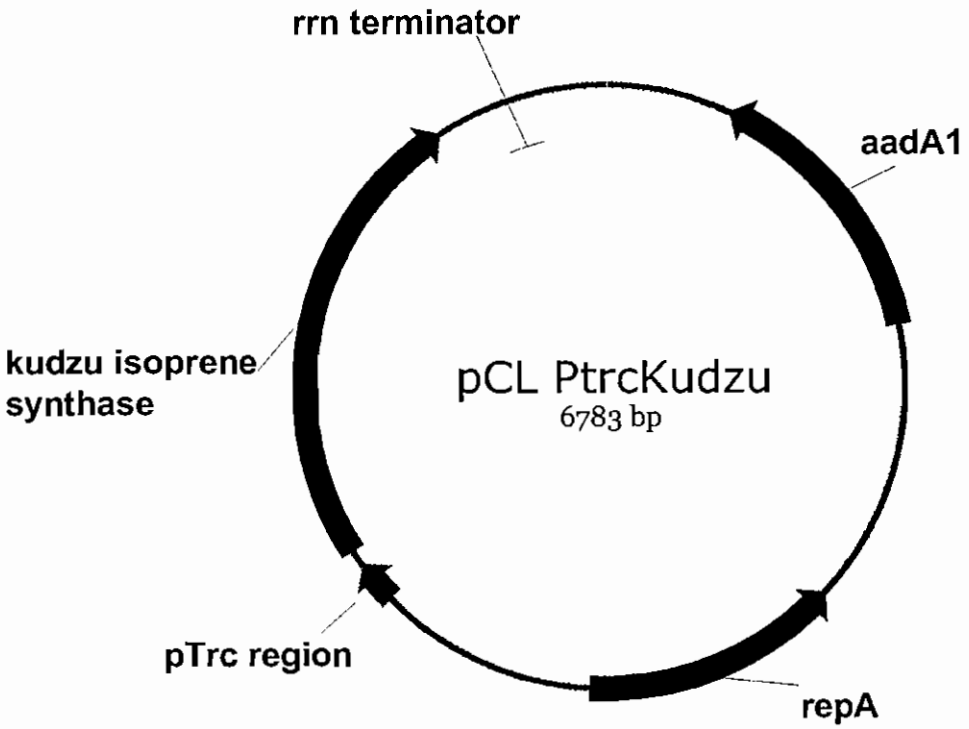
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Figure 33A

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Figure 33B

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Figure 33C

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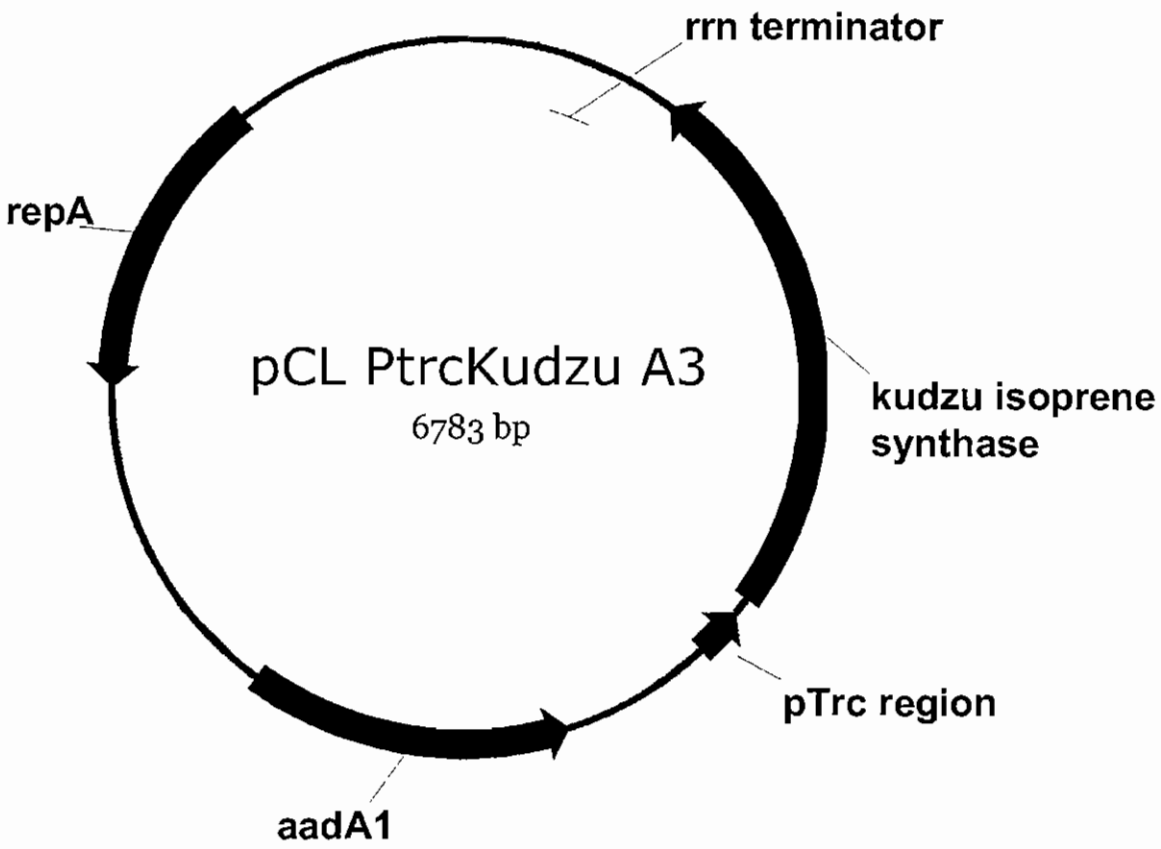
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Figure 35A

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Figure 35B

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Figure 35C

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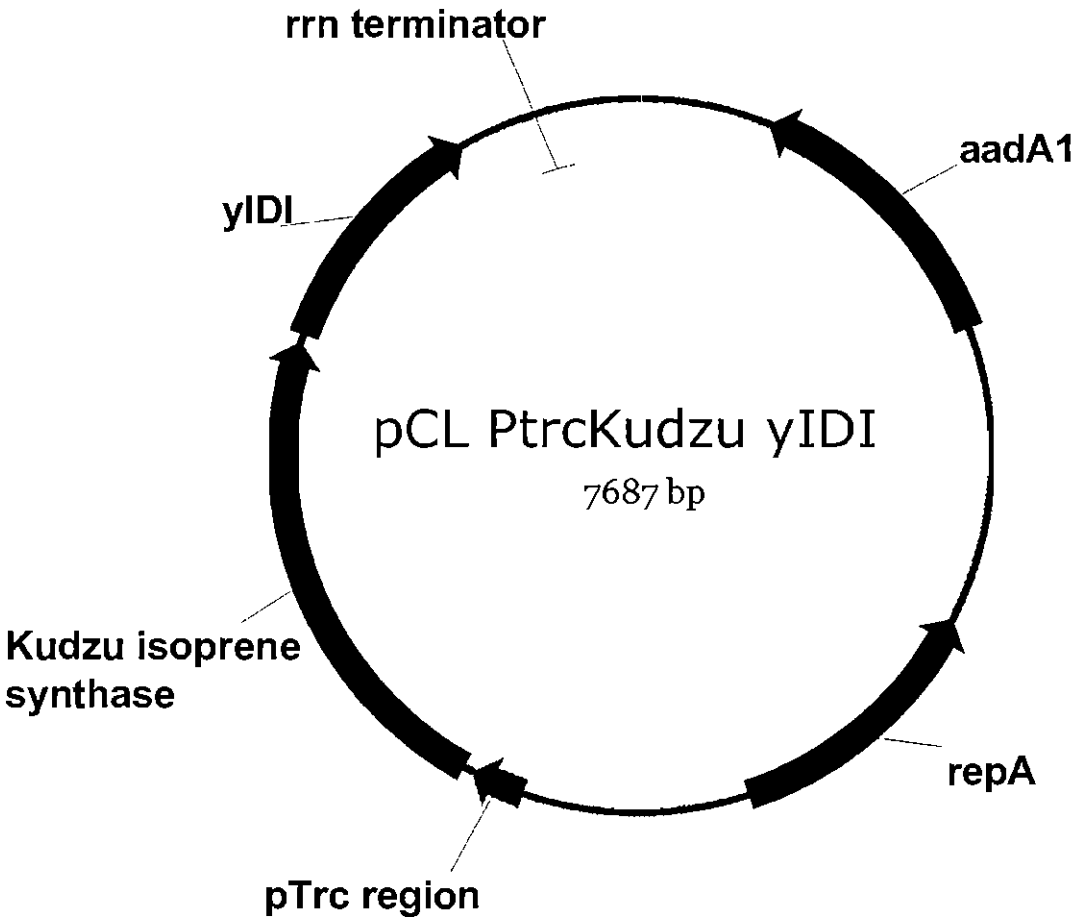
Figure 36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repA
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Figure 37A

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Figure 37B

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Figure 37C

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Figure 38

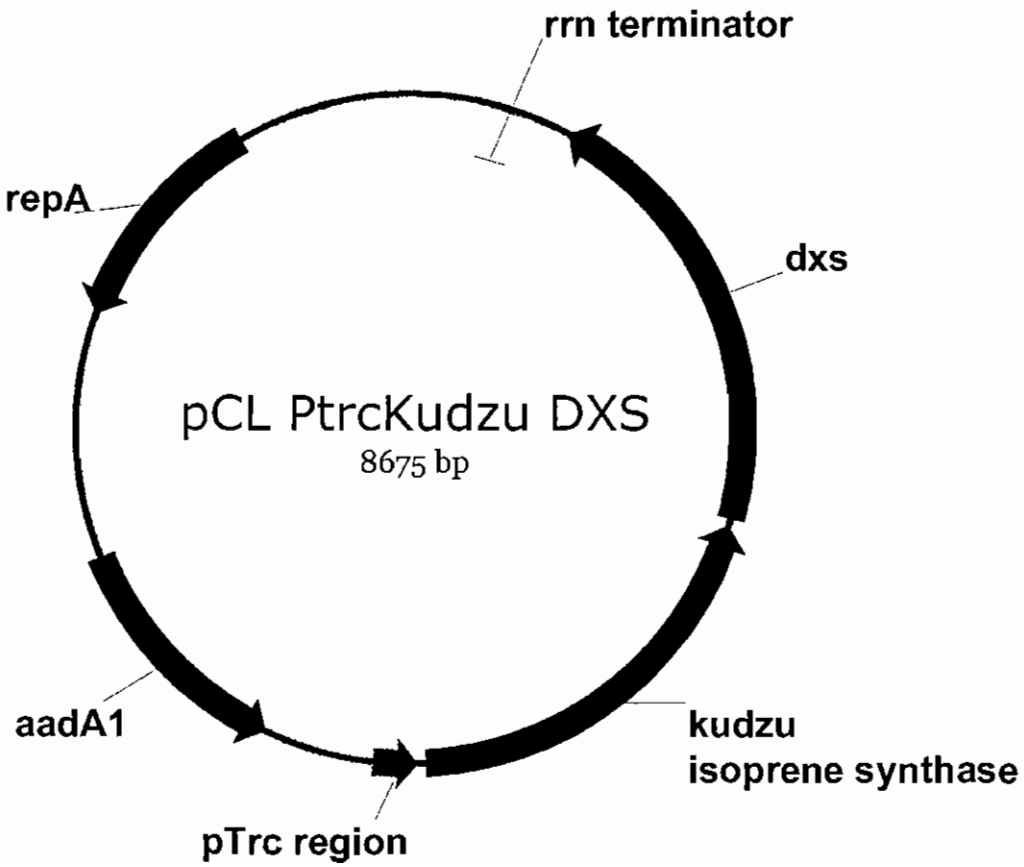
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Figure 39A

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Figure 39B

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Figure 39C

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Figure 39D

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(SEQ ID NO:28)

Figure 40A

A.

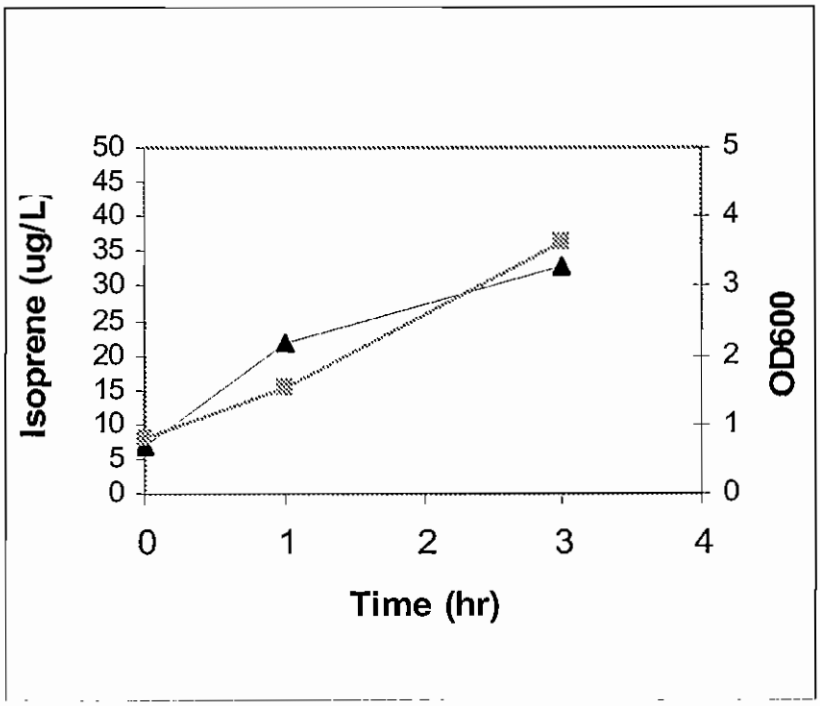

Isoprene (ug/L)
OD600
Time (hr)
0
5
10
15
20
25
30
35
40
45
50
0
1
2
3
4
5
0
1
2
3
4

Figure 40B

B.

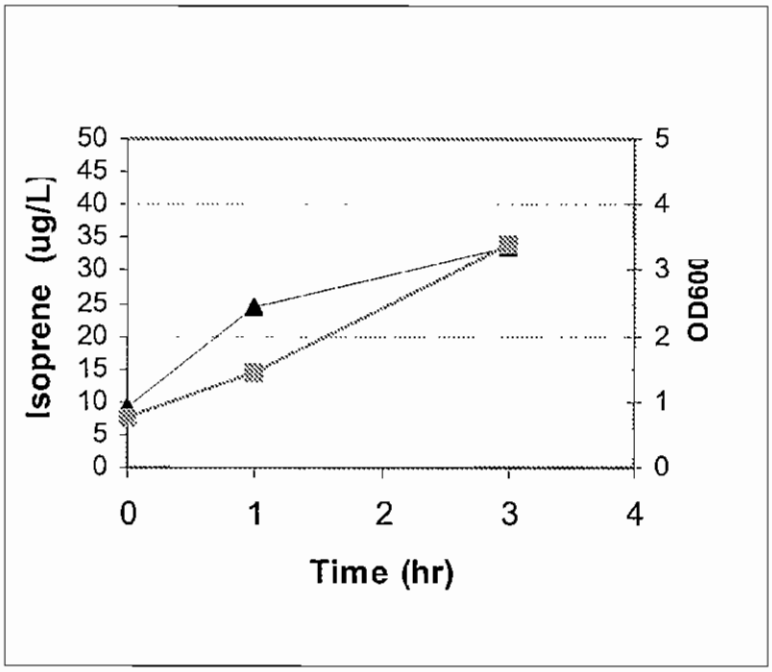

Isoprene (ug/L)
50
45
40
35
30
25
20
15
10
5
0
5
4
3
2
1
0
OD600
0
1
2
3
4
Time (hr)

Figure 40C

C.

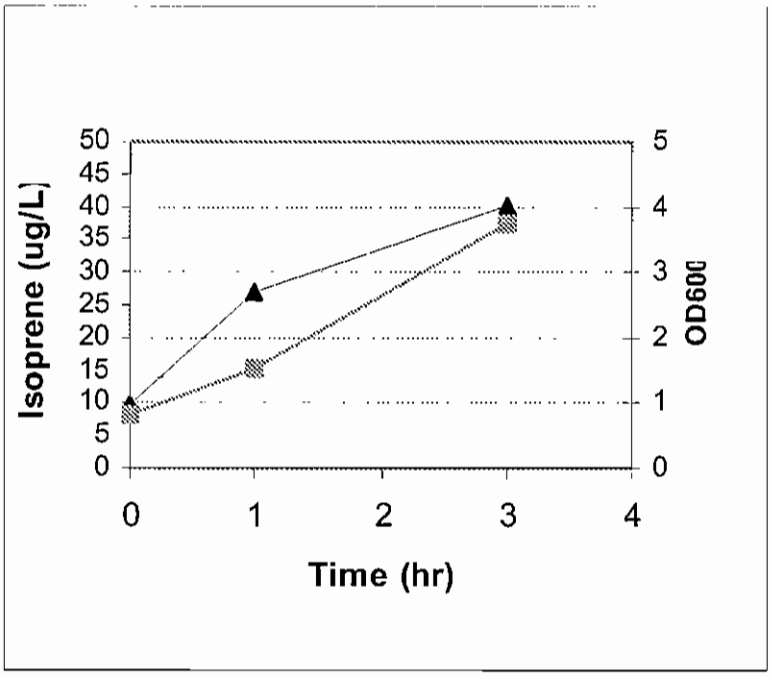

Isoprene (ug/L)
50
45
40
35
30
25
20
15
10
5
0
OD600
5
4
3
2
1
0
0
1
2
3
4
Time (hr)

Figure 40D

D.

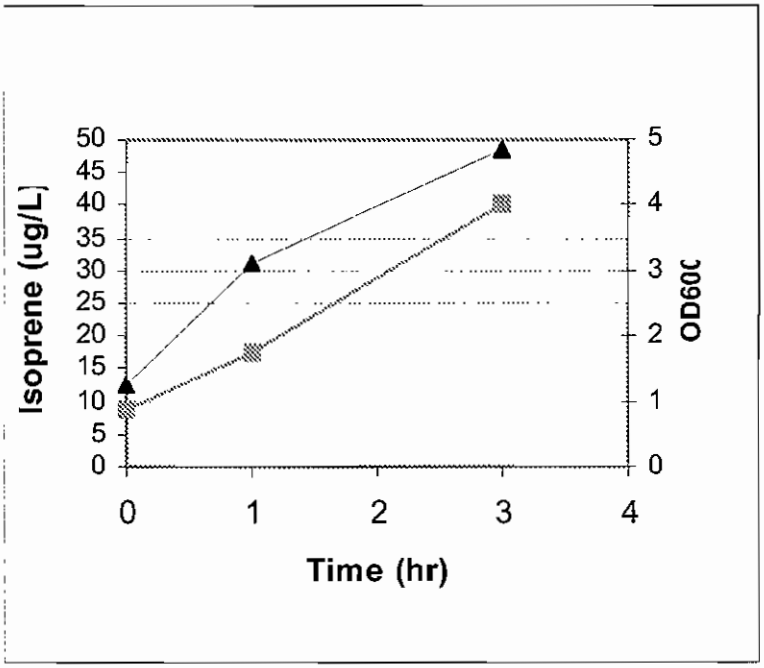

Isoprene (ug/L)
50
45
40
35
30
25
20
15
10
5
0
OD60C
5
4
3
2
1
0
0
1
2
3
4
Time (hr)

Figure 40E

E.

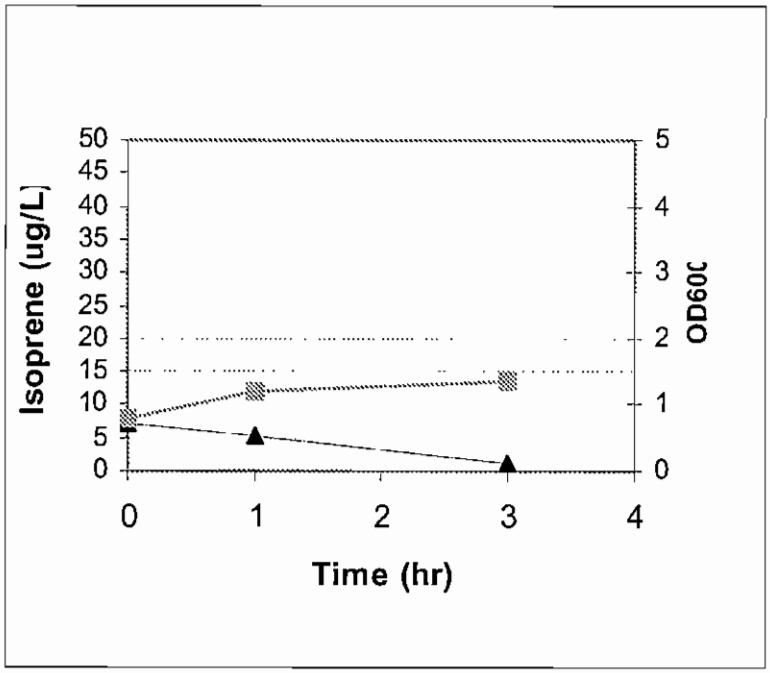

Isoprene (ug/L)
50
45
40
35
30
25
20
15
10
5
0
5
4
3
2
1
0
OD600
0
1
2
3
4
Time (hr)

Figure 41A

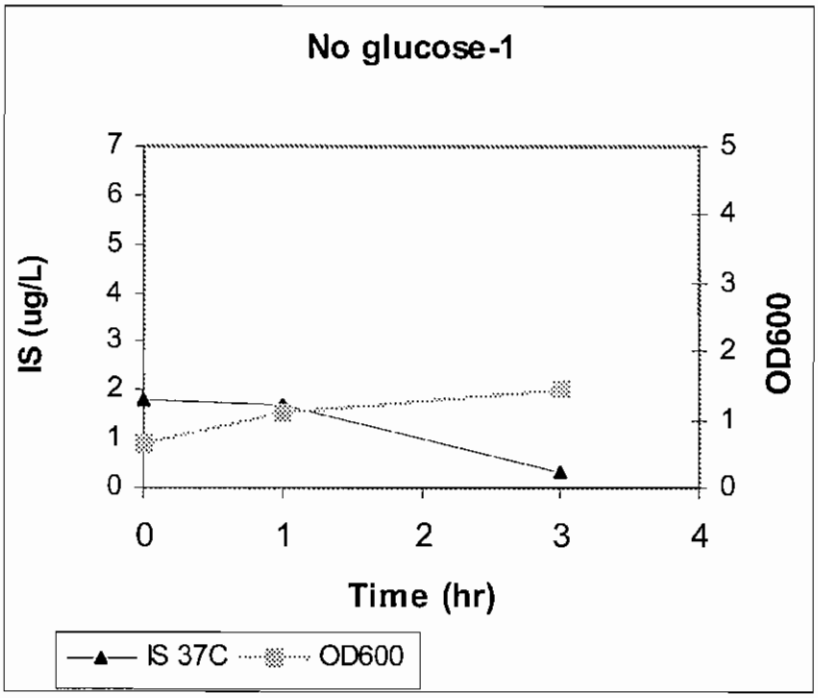


Figure 41B

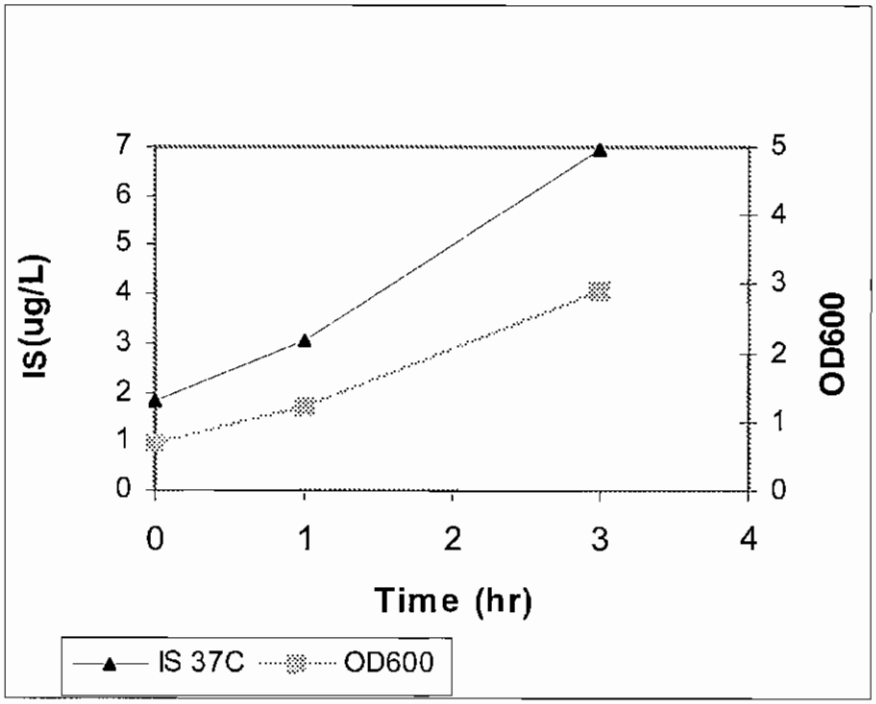


Figure 41C

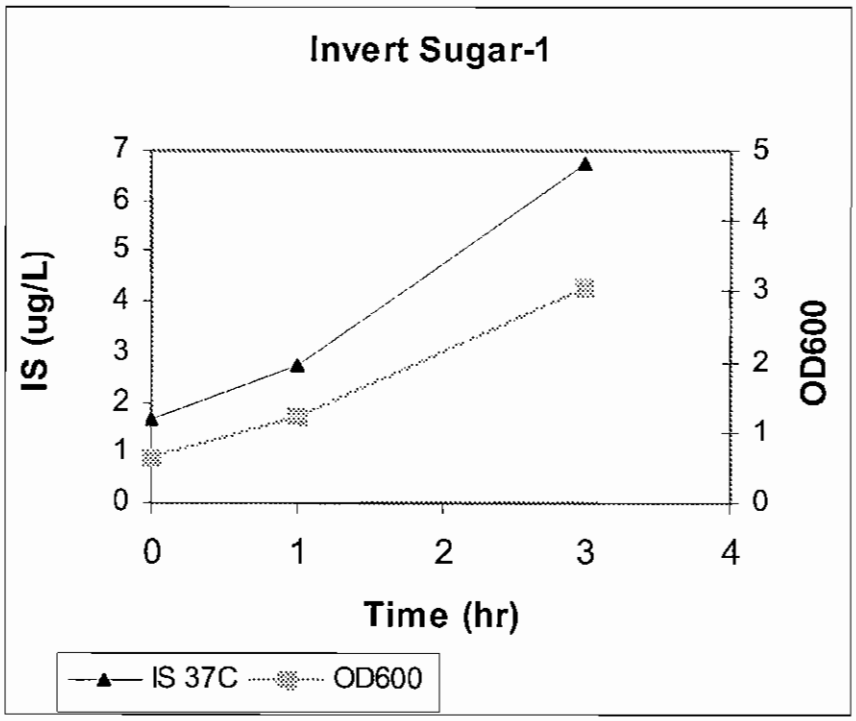


Figure 41D

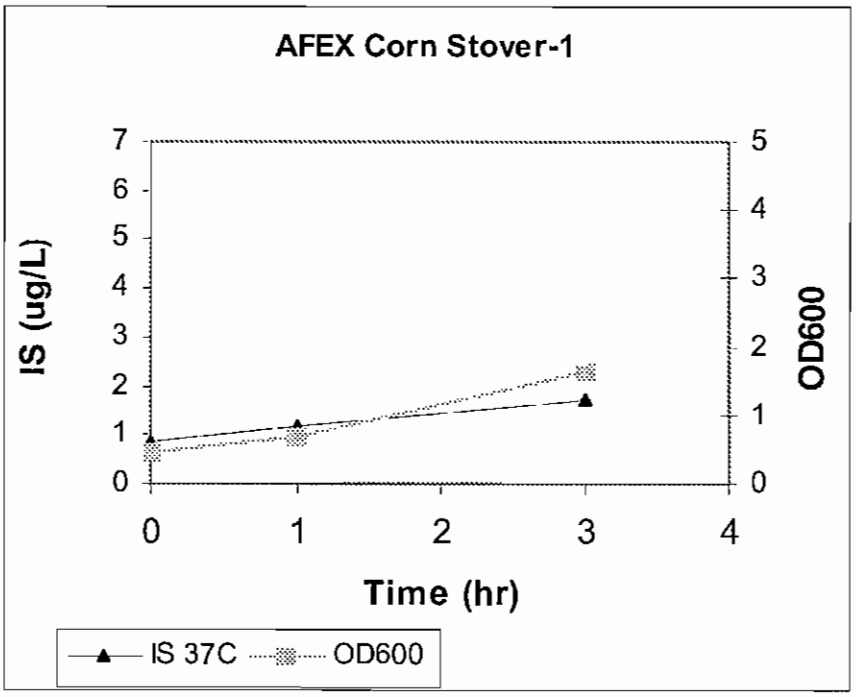


Figure 42A

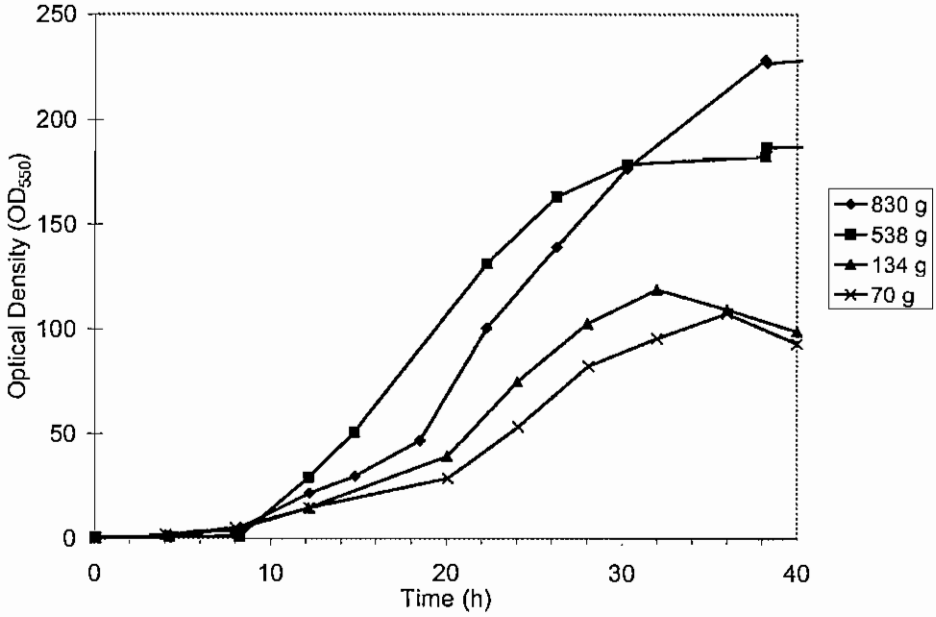


Figure 42B

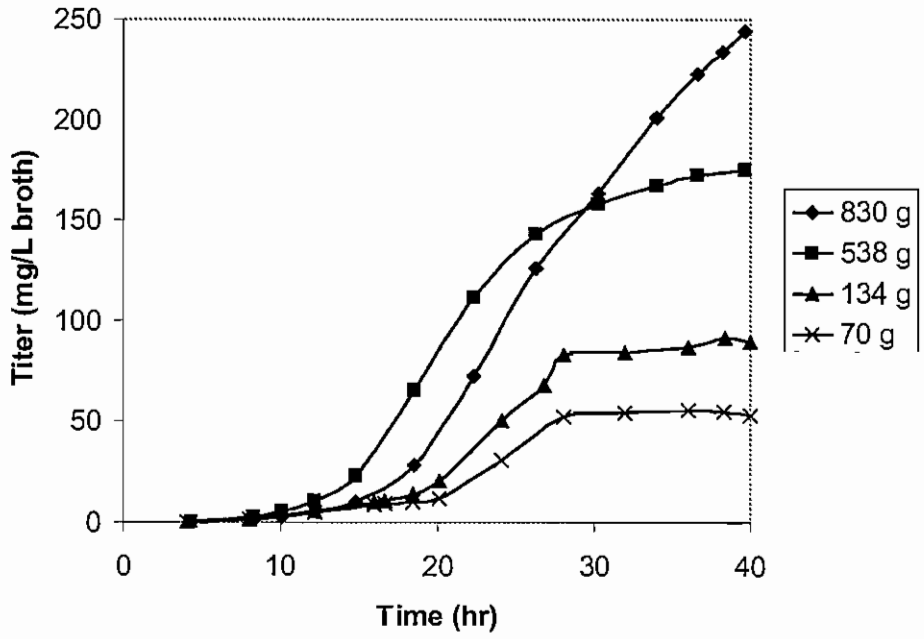


Figure 42C

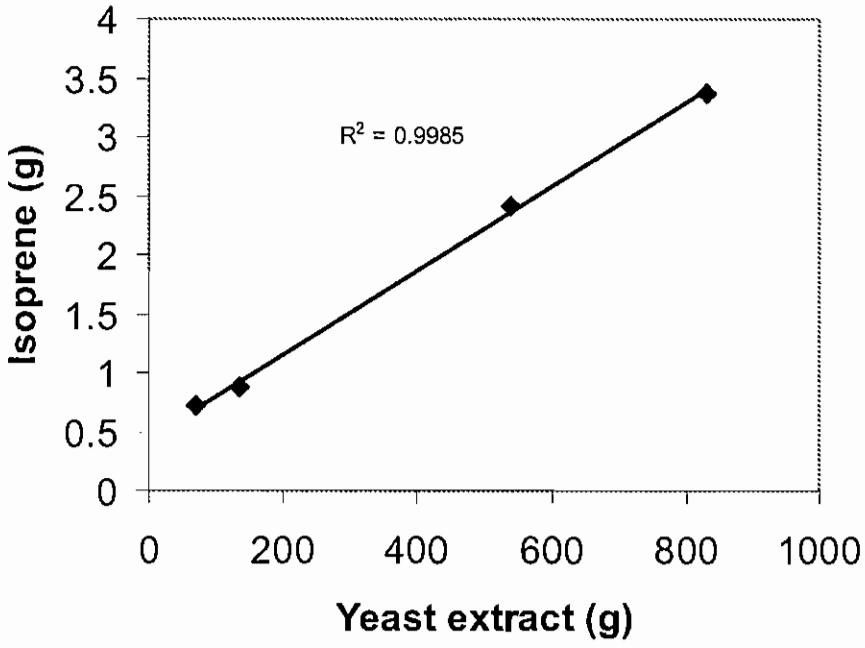

4
3.5
3
2.5
2
1.5
1
0.5
0
Isoprene (g)
R^2 = 0.9985
0
200
400
600
800
1000
Yeast extract (g)

Figure 43A

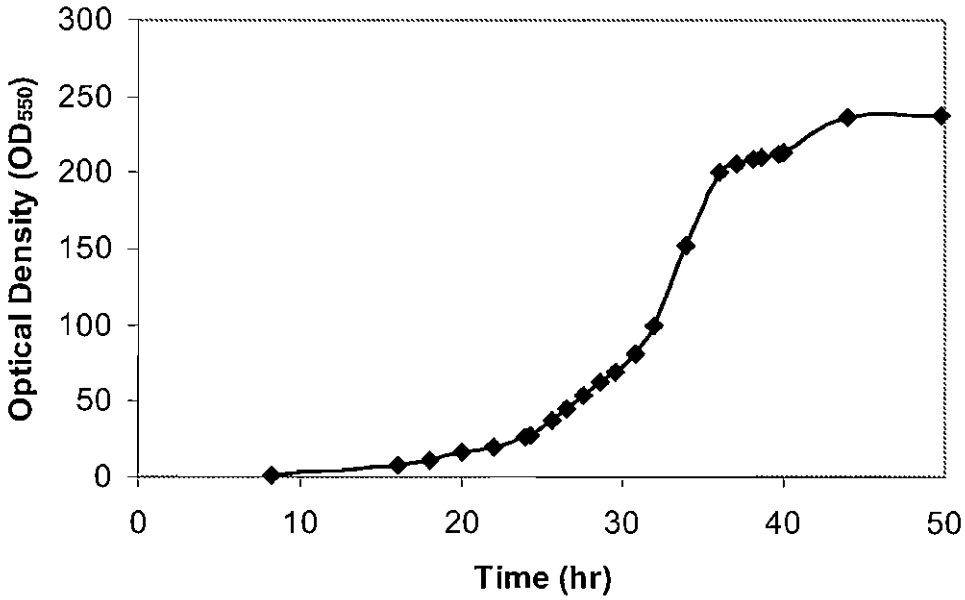


Figure 43B

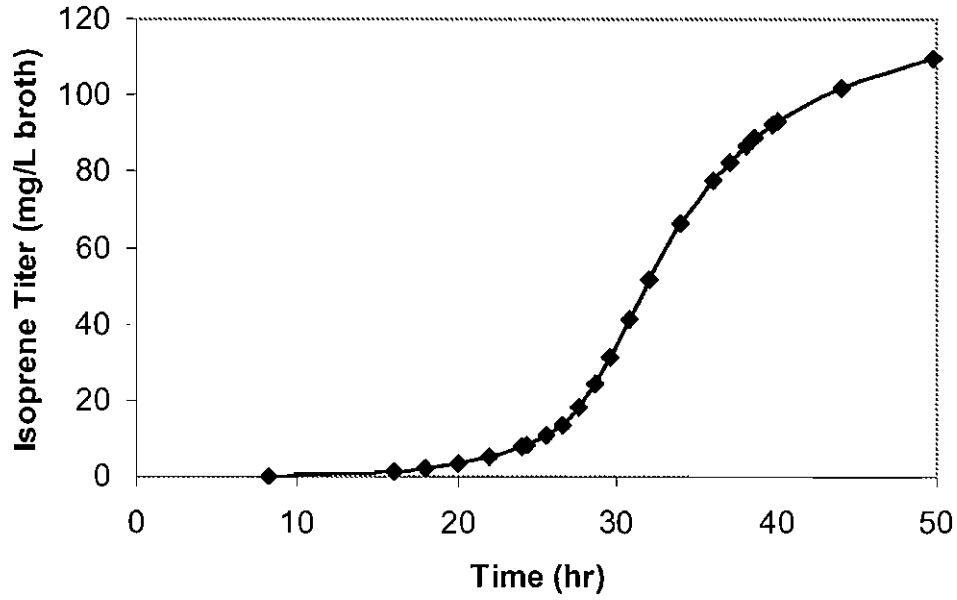


Figure 43C

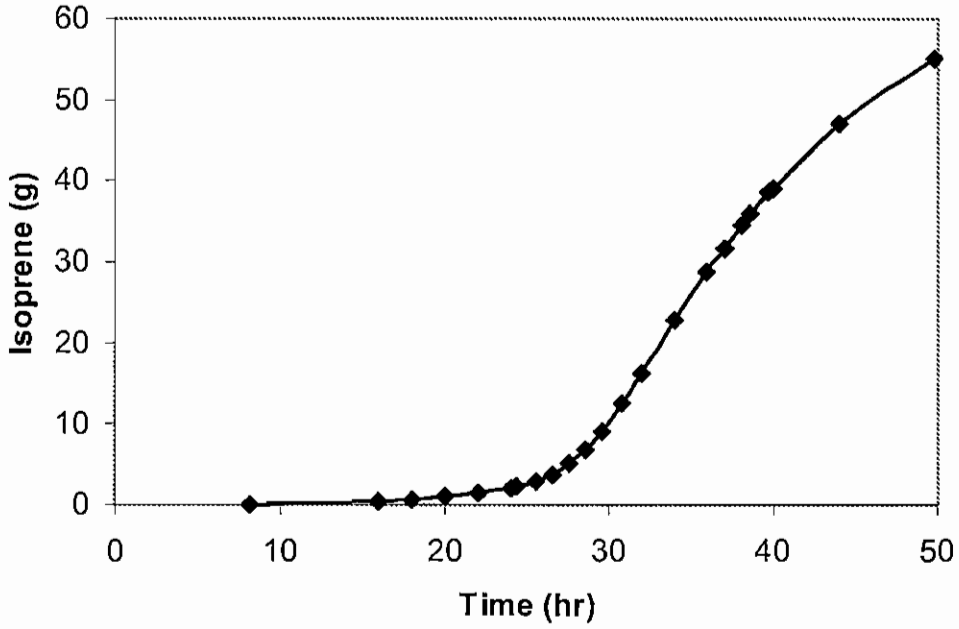


Figure 44

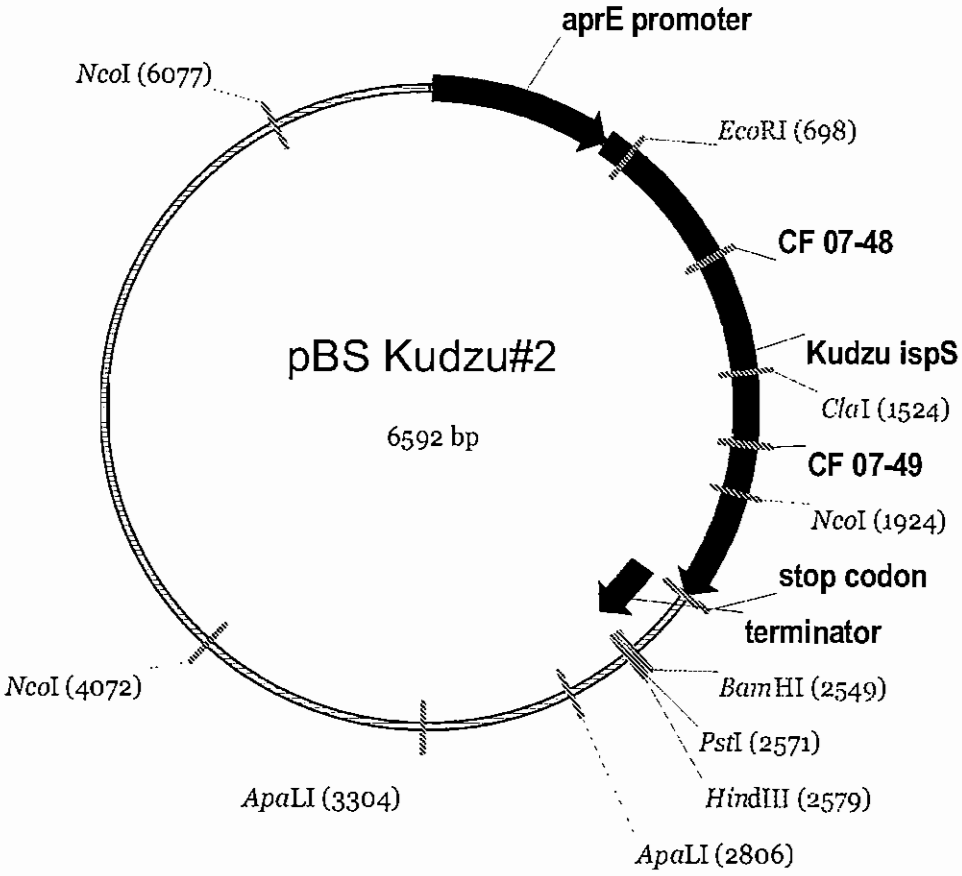

aprE promoter
NcoI (6077)
EcoRI (698)
CF 07-48
pBS Kudzu#2
6592 bp
Kudzu ispS
ClaI (1524)
CF 07-49
NcoI (1924)
stop codon
terminator
NcoI (4072)
BamHI (2549)
PstI (2571)
ApaLI (3304)
HindIII (2579)
ApaLI (2806)

Figure 45A

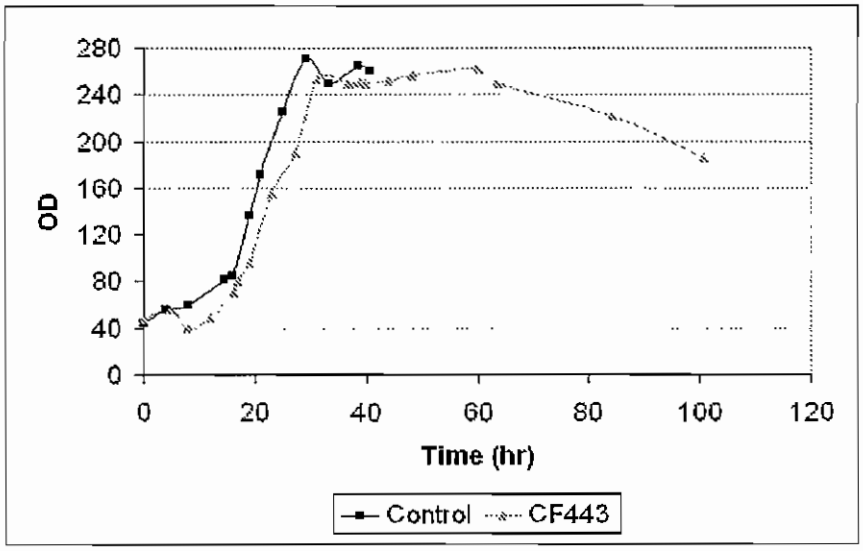
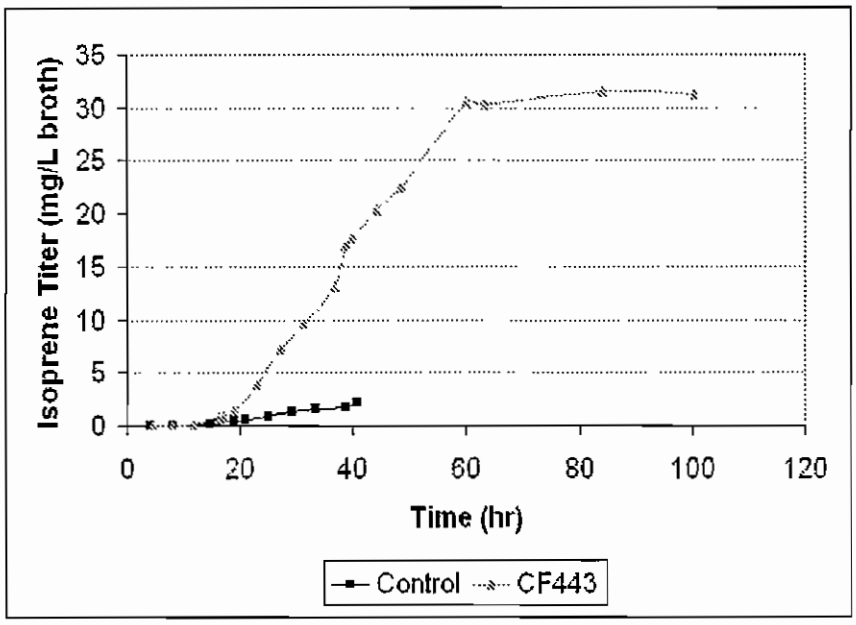
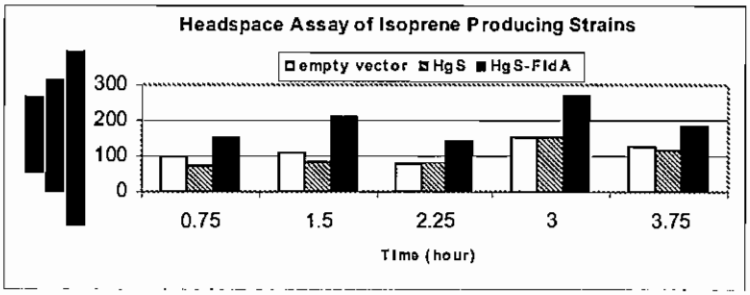


Figure 45B

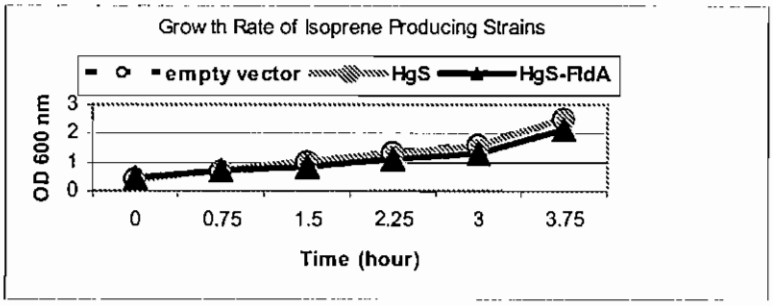


Figures 46A-46D

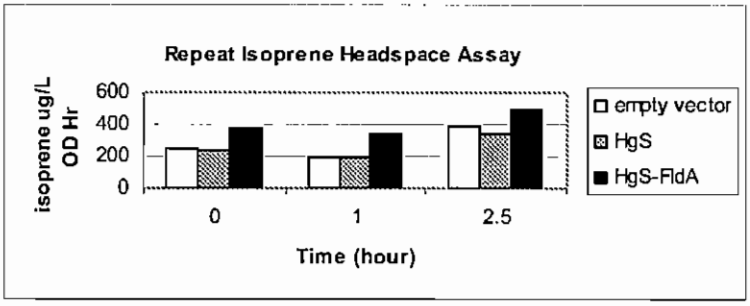
A



B



C



D

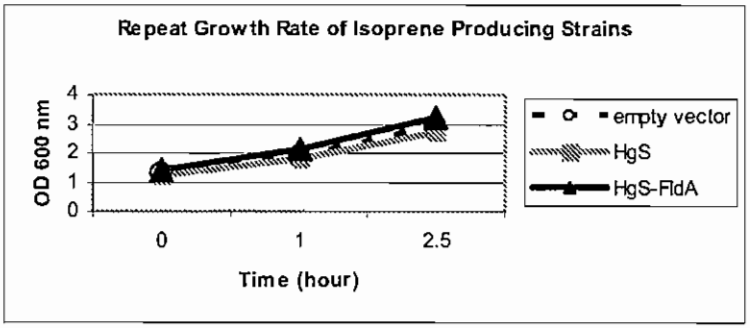
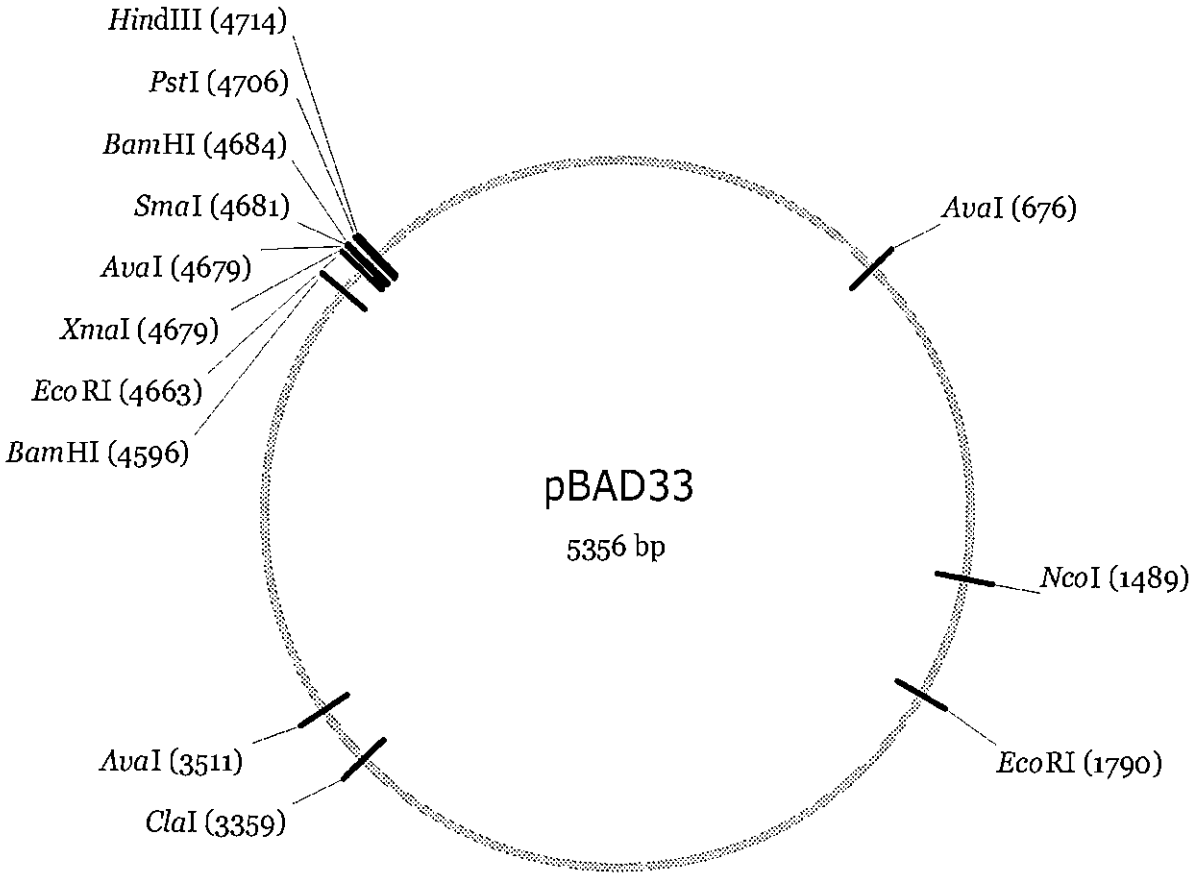


Figure 46E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PstI (4706)
BamHI (4684)
SmaI (4681)
AvaI (4679)
XmaI (4679)
EcoRI (4663)
BamHI (4596)
AvaI (676)
NcoI (1489)
EcoRI (1790)
AvaI (3511)
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Figure 46F

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Figure 46G

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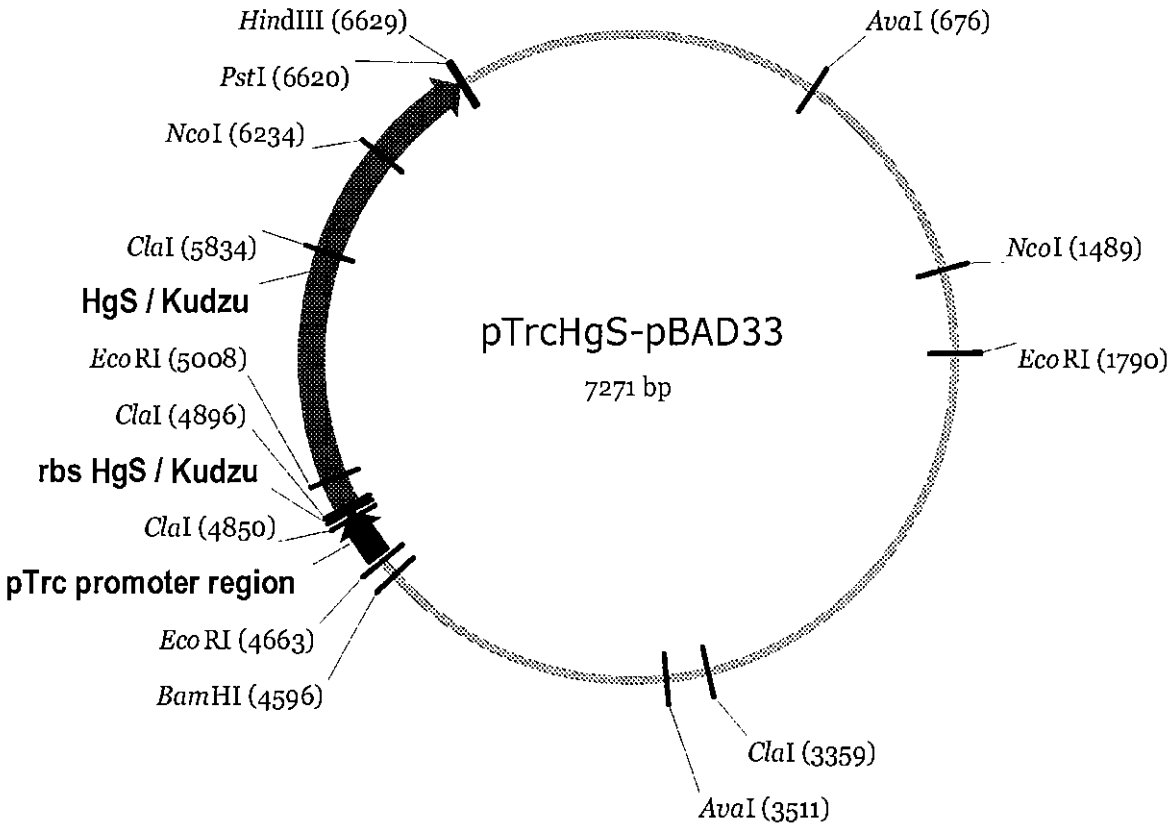
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Figure 46I

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Figure 46J

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Figure 46K

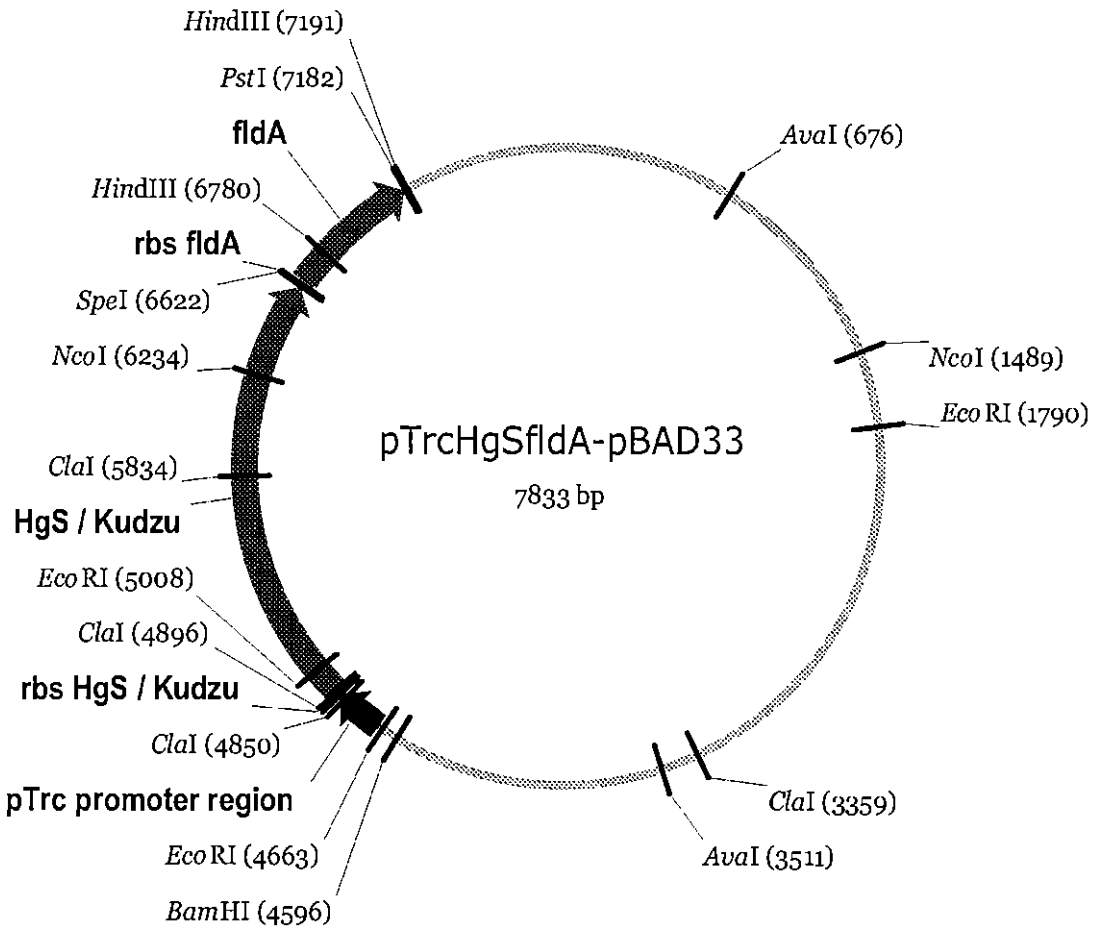
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Figure 46L

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Figure 46M

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Figure 47

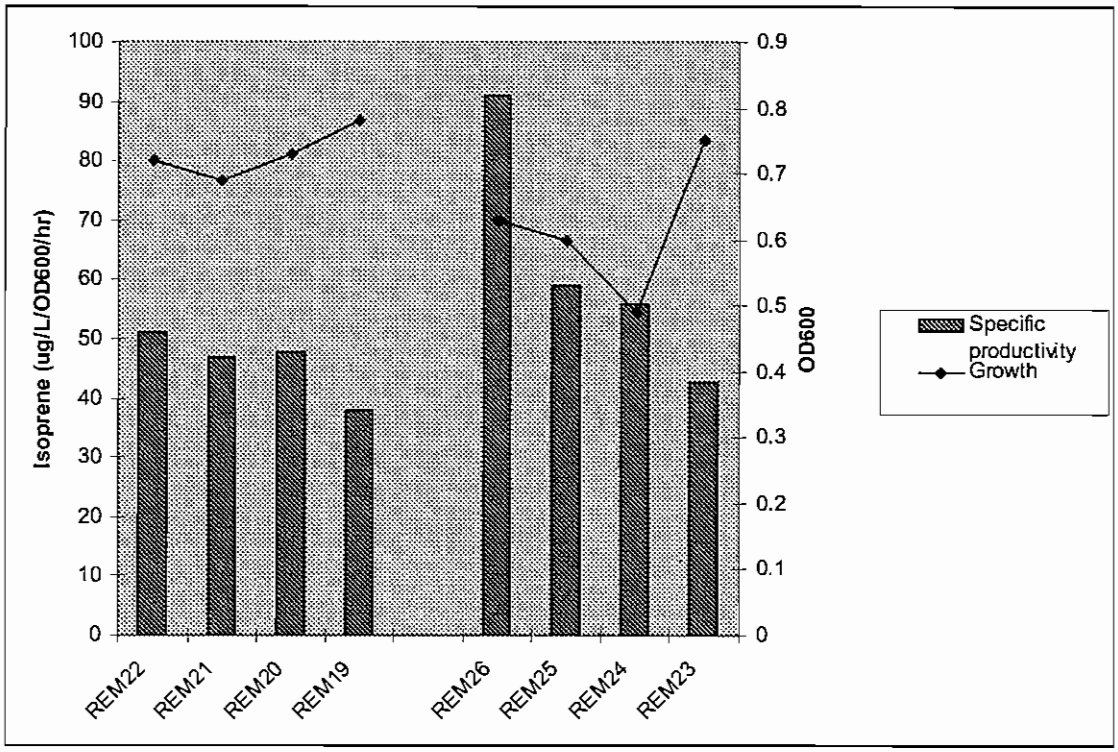

Isoprene (ug/L/OD600/hr)
OD600
100
90
80
70
60
50
40
30
20
10
0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
REM22
REM21
REM20
REM19
REM26
REM25
REM24
REM23
Specific productivity
Growth

Figure 48

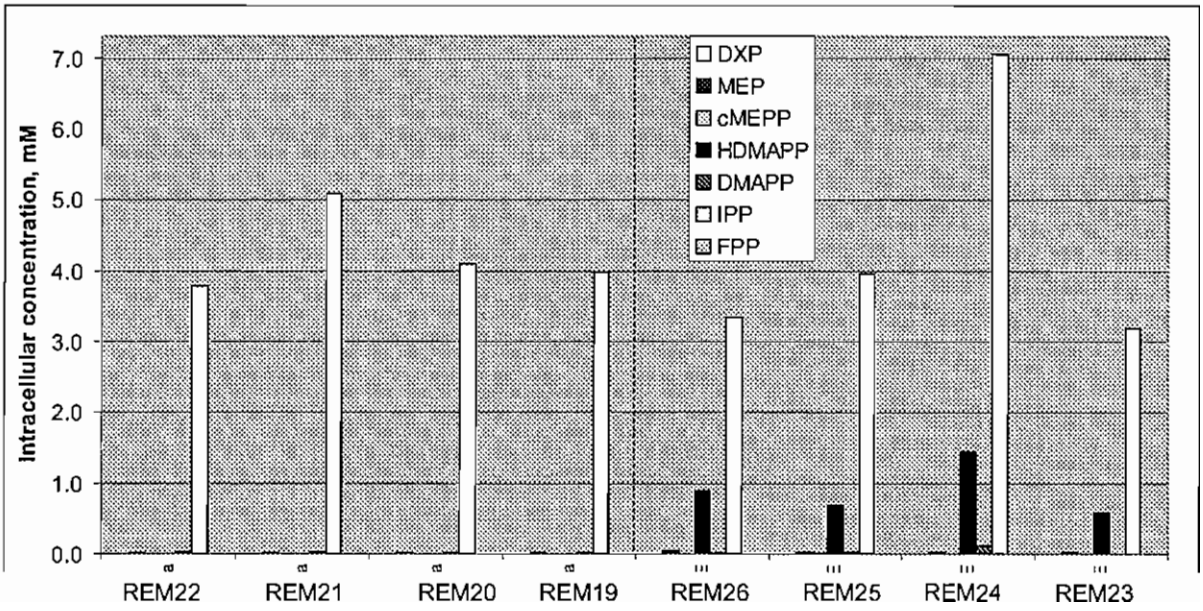

Intracellular concentration, mM
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
DXP
MEP
cMEPP
HDMAPP
DMAPP
IPP
FPP
a
a
a
a
III
III
III
III
REM22
REM21
REM20
REM19
REM26
REM25
REM24
REM23

Figure 49

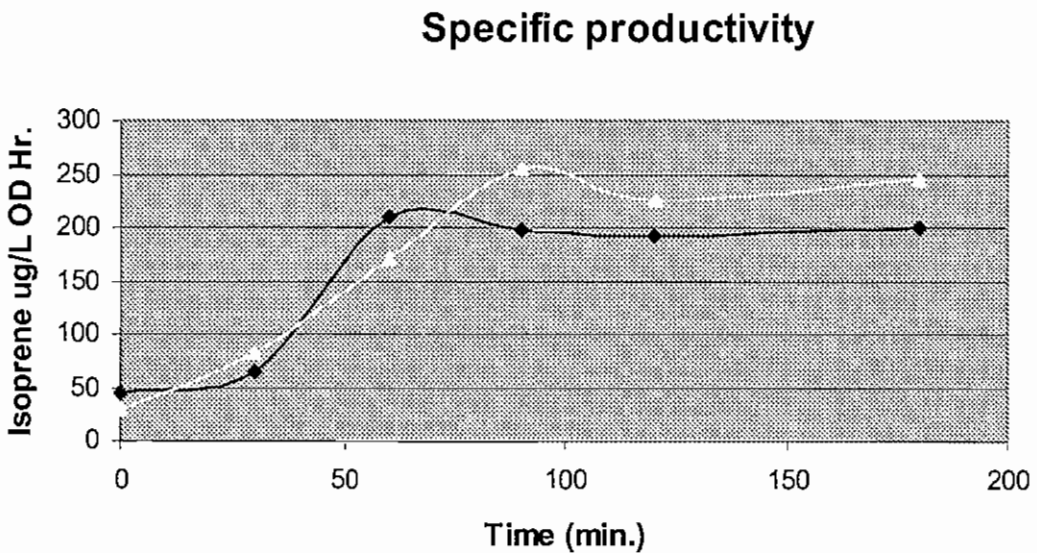
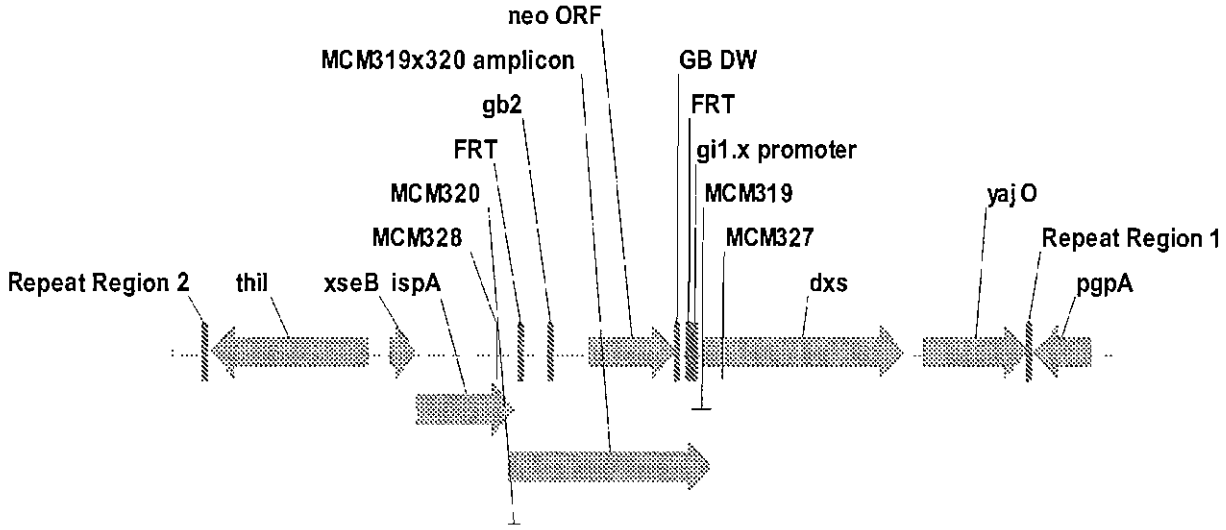

Specific productivity
Isoprene ug/L OD Hr.
300
250
200
150
100
50
0
0
50
100
150
200
Time (min.)

Figure 50



MCM617-625 - gi1.x integrated at dxs

9013 bp

Figure 51

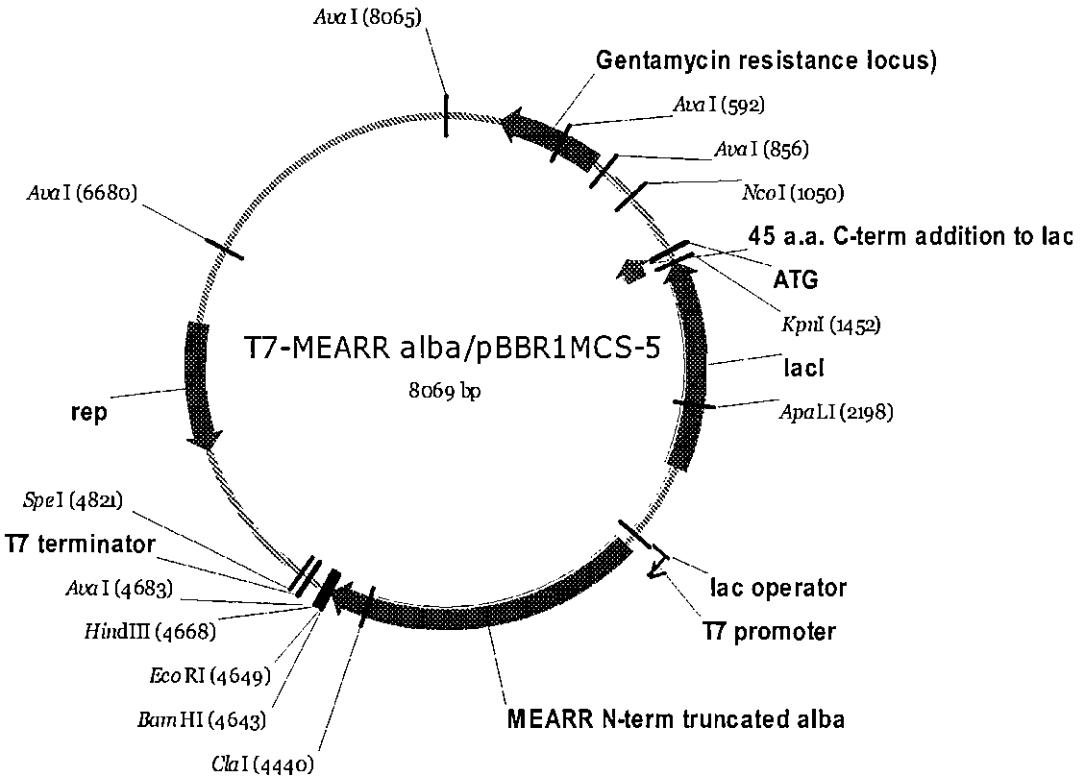

AvaI (8065)
Gentamycin resistance locus)
AvaI (592)
AvaI (856)
NcoI (1050)
45 a.a. C-term addition to lac
ATG
KpnI (1452)
lacI
ApaLI (2198)
AvaI (6680)
T7-MEARR alba/pBBR1MCS-5
8069 bp
rep
SpeI (4821)
T7 terminator
AvaI (4683)
HindIII (4668)
EcoRI (4649)
BamHI (4643)
ClaI (4440)
lac operator
T7 promoter
MEARR N-term truncated alba

Figure 52

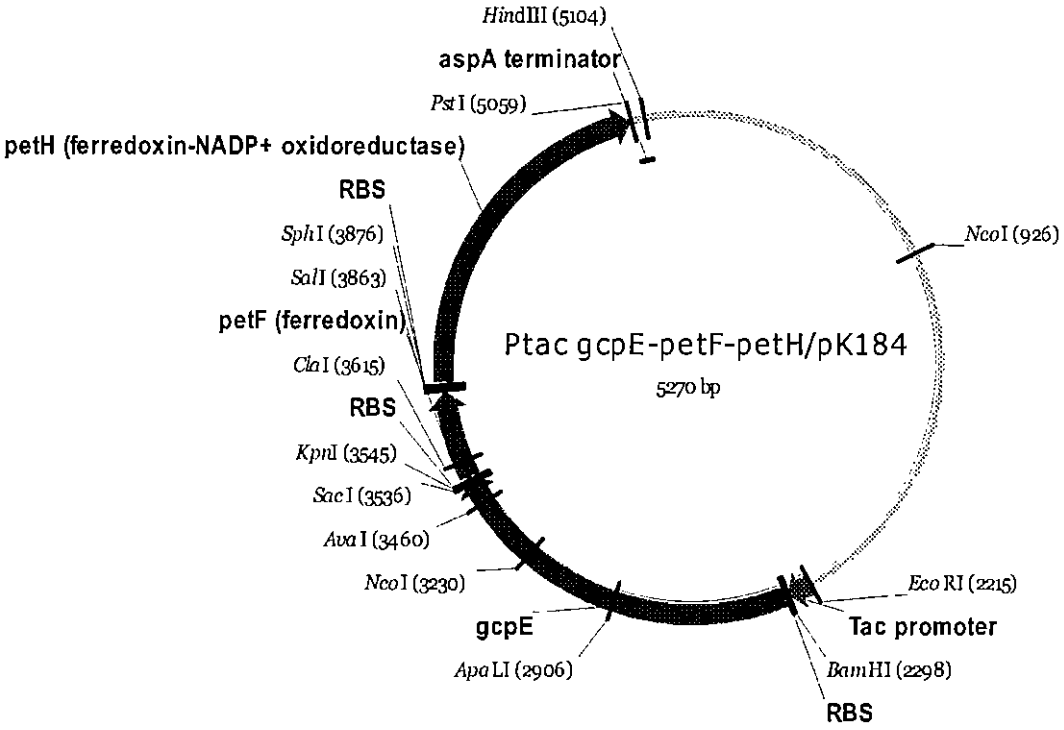

HindIII (5104)
aspA terminator
PstI (5059)
petH (ferredoxin-NADP+ oxidoreductase)
RBS
SphI (3876)
SalI (3863)
petF (ferredoxin)
ClaI (3615)
RBS
KpnI (3545)
SacI (3536)
AvaI (3460)
NcoI (3230)
gcpE
ApaLI (2906)
NcoI (926)
Ptac gcpE-petF-petH/pK184
5270 bp
EcoRI (2215)
Tac promoter
BamHI (2298)
RBS

Figure 53A

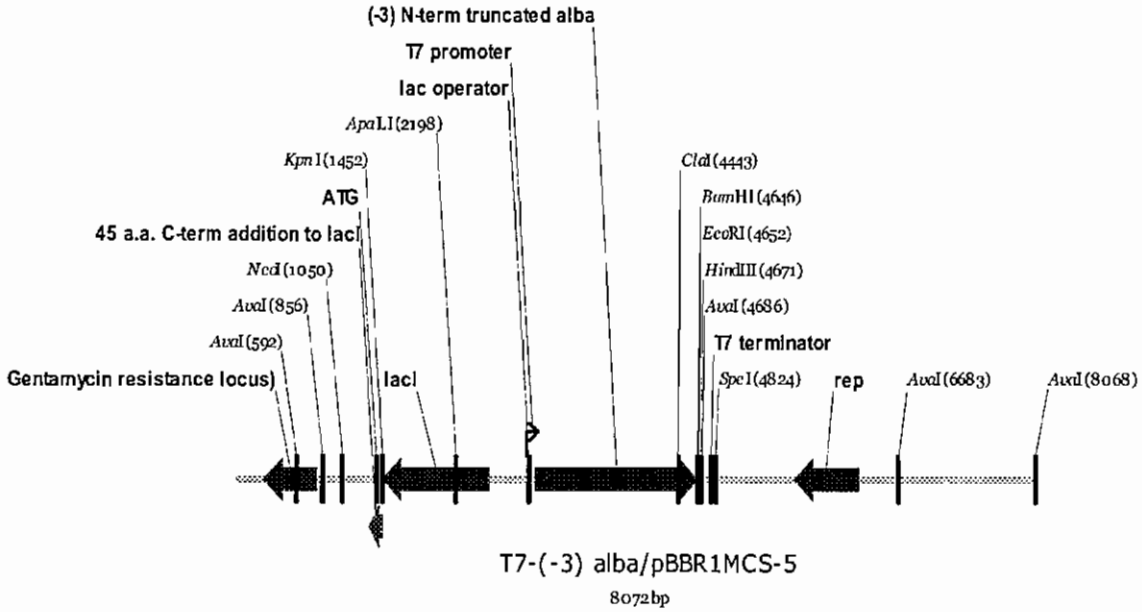


Figure 53B

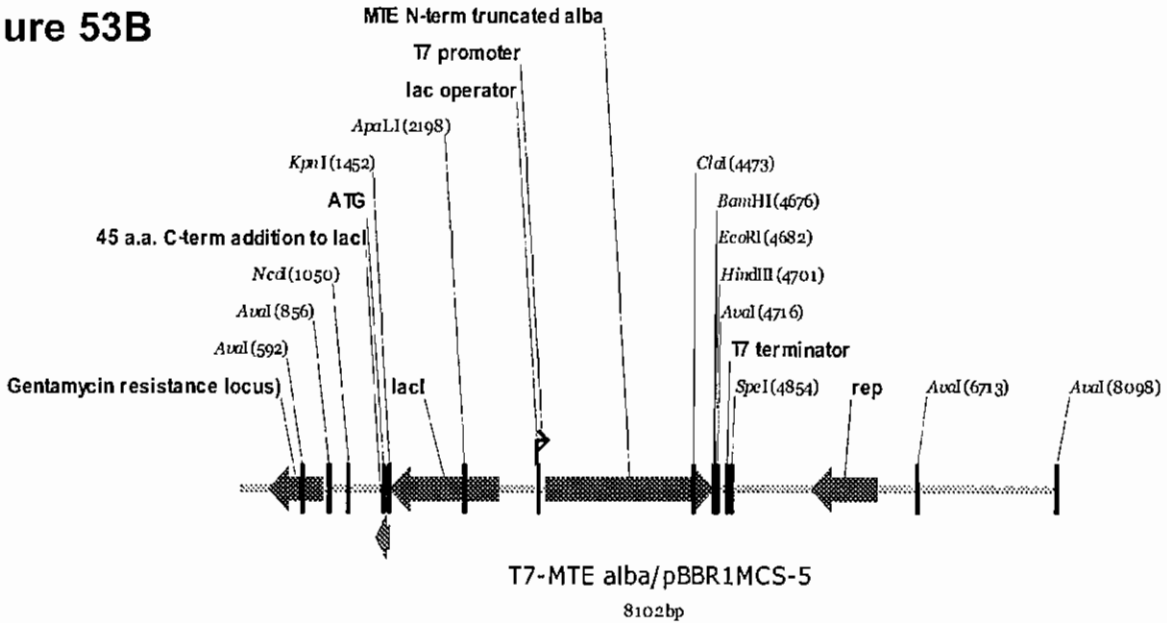


Figure 54

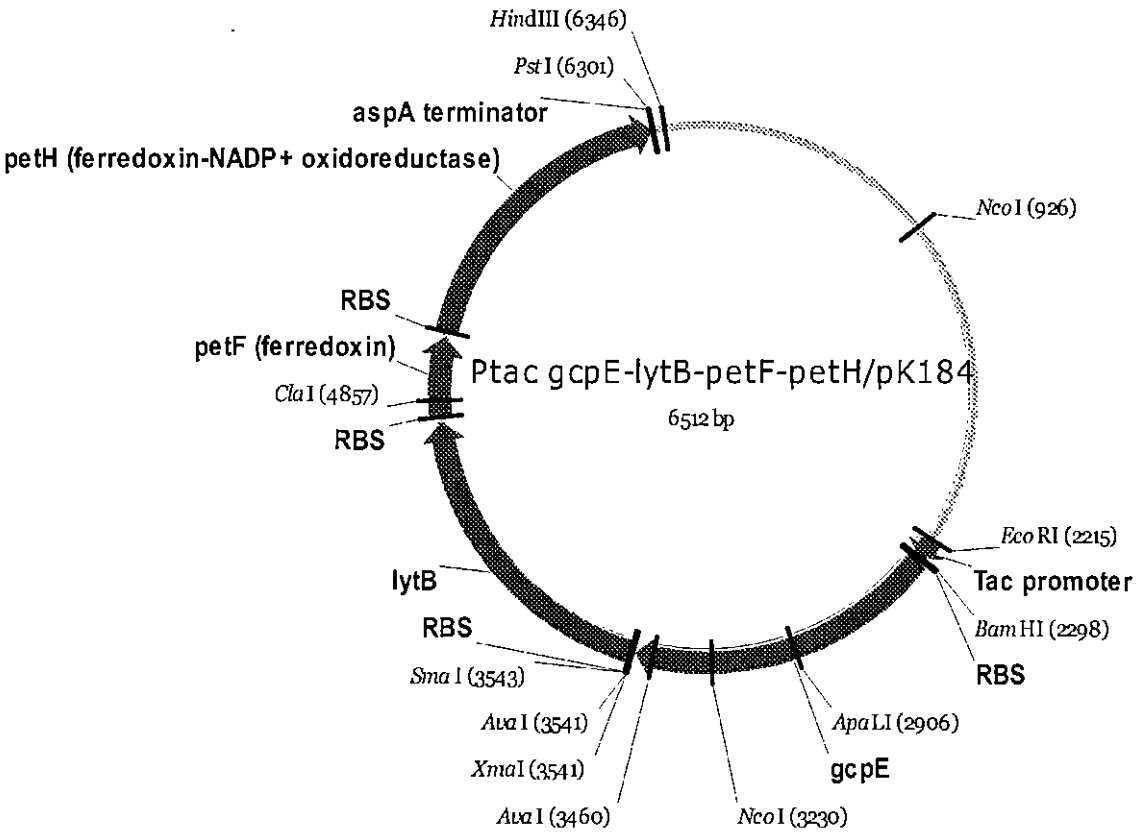

HindIII (6346)
PstI (6301)
aspA terminator
petH (ferredoxin-NADP+ oxidoreductase)
NcoI (926)
RBS
petF (ferredoxin)
ClaI (4857)
RBS
Ptac gcpE-lytB-petF-petH/pK184
6512 bp
EcoRI (2215)
Tac promoter
BamHI (2298)
RBS
lytB
RBS
SmaI (3543)
AvaI (3541)
XmaI (3541)
AvaI (3460)
NcoI (3230)
ApaLI (2906)
gcpE

Figure 55A

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Figure 55B

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Figure 55C

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Figure 56A

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Figure 56B

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Figure 57A

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Figure 57B

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Figure 57C

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Figure 58A

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Figure 58B

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Figure 58C

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Figure 59A

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Figure 59B

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Figure 60A

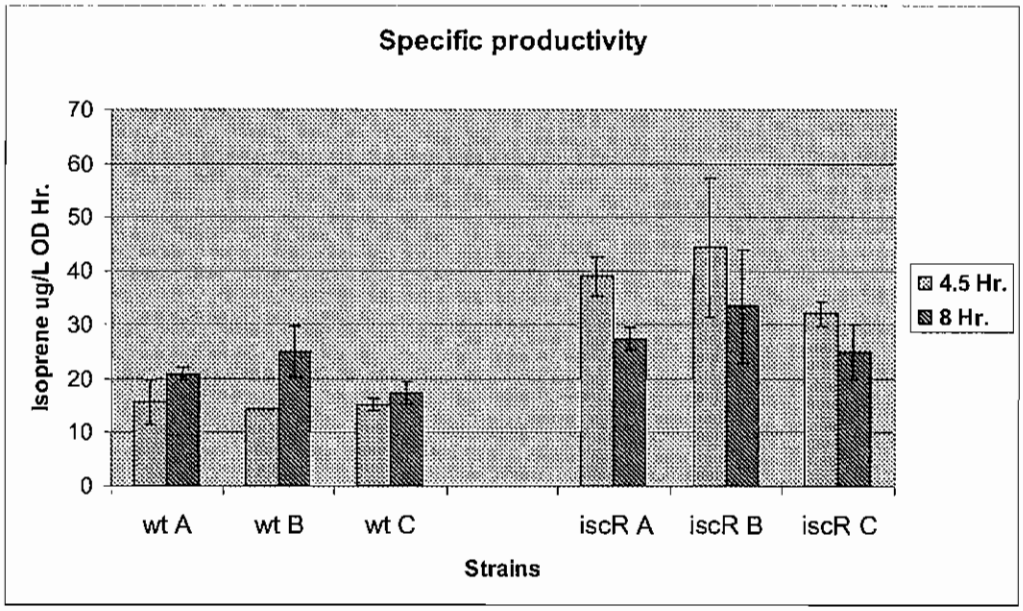


Figure 60B

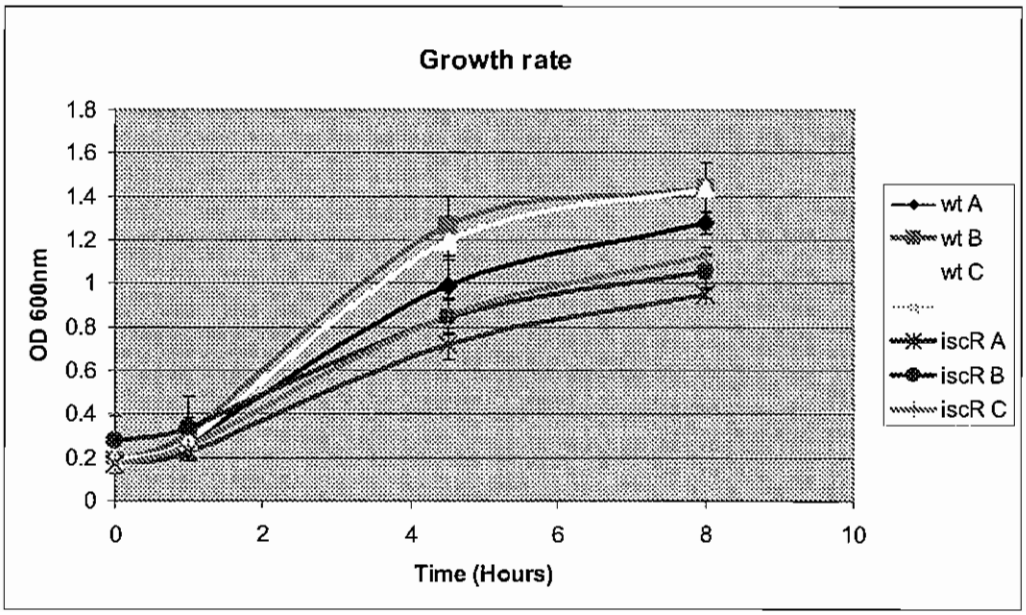
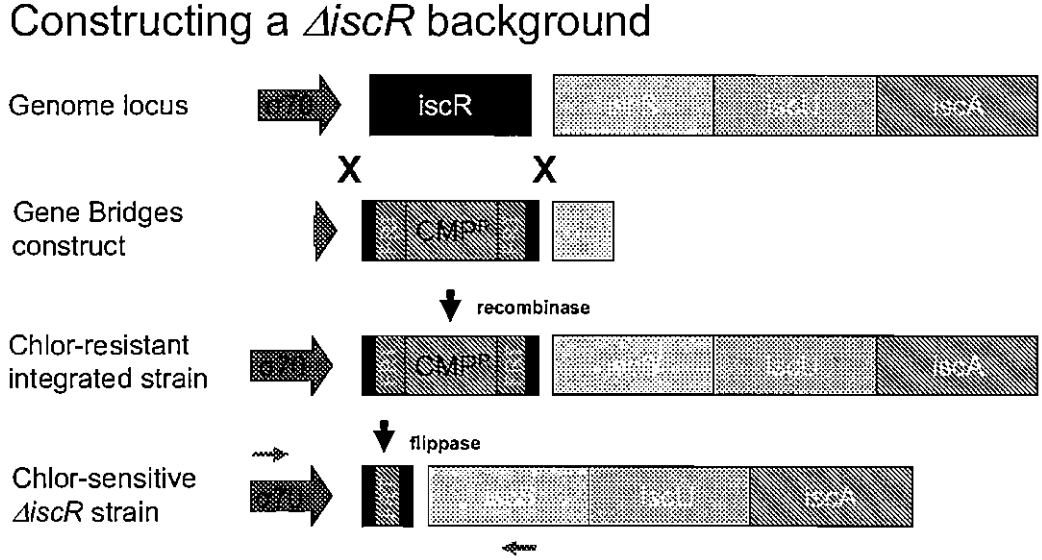


Figure 61



Diagnostic primers used to verify the removal of *iscR* from the BL21(DE3) genome

Figure 62

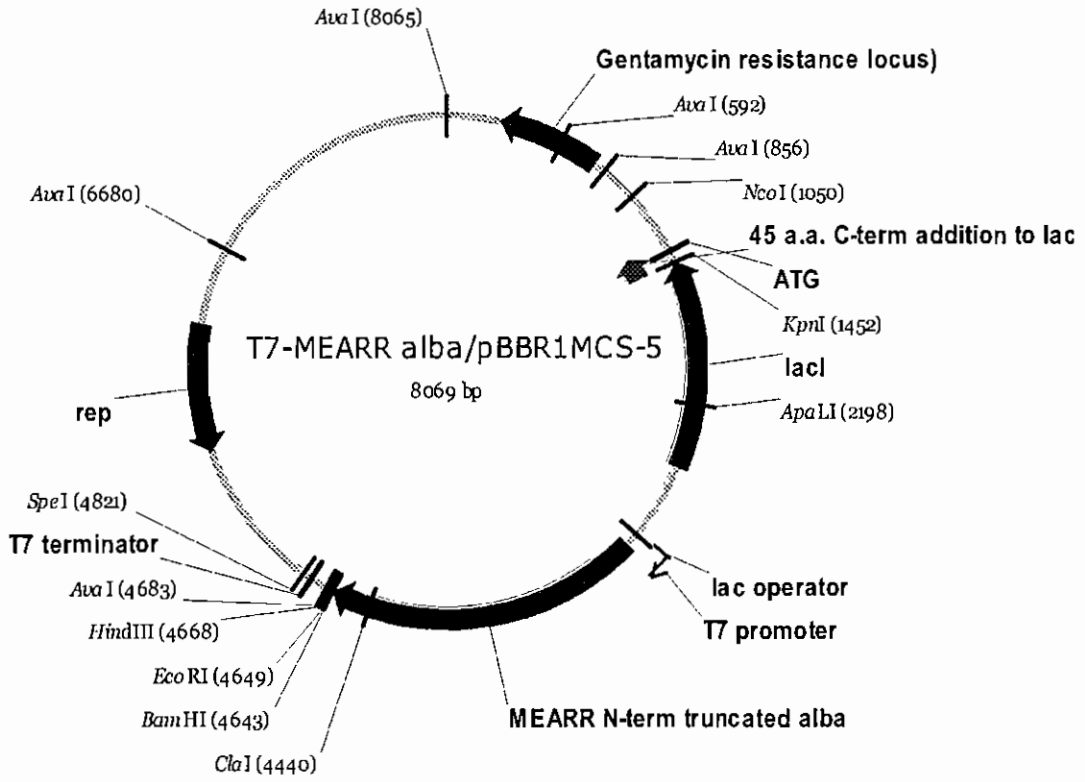

AvaI (8065)
Gentamycin resistance locus)
AvaI (592)
AvaI (856)
NcoI (1050)
45 a.a. C-term addition to lac
ATG
KpnI (1452)
lacI
ApaLI (2198)
AvaI (6680)
T7-MEARR alba/pBBR1MCS-5
8069 bp
rep
SpeI (4821)
T7 terminator
AvaI (4683)
HindIII (4668)
EcoRI (4649)
BamHI (4643)
ClaI (4440)
lac operator
T7 promoter
MEARR N-term truncated alba

Figure 63

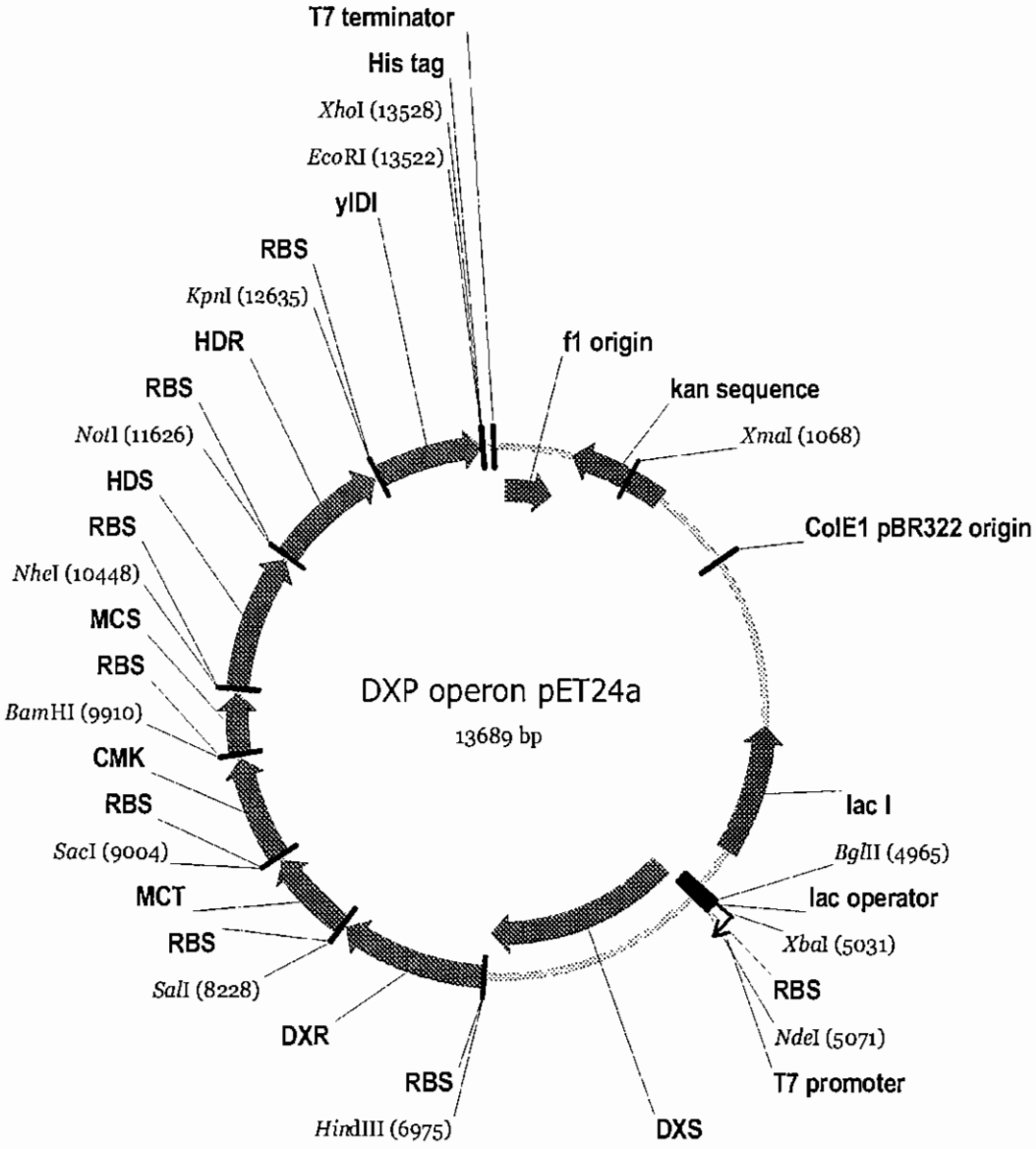

T7 terminator
His tag
XhoI (13528)
EcoRI (13522)
yIDI
RBS
KpnI (12635)
HDR
RBS
NotI (11626)
HDS
RBS
NheI (10448)
MCS
RBS
BamHI (9910)
CMK
RBS
SacI (9004)
MCT
RBS
SalI (8228)
DXR
RBS
HindIII (6975)
DXS
RBS
NdeI (5071)
T7 promoter
lac operator
XbaI (5031)
BglII (4965)
lac I
ColE1 pBR322 origin
XmaI (1068)
kan sequence
f1 origin
DXP operon pET24a
13689 bp

Figure 64A

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Figure 64B

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Figure 64C

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Figure 65A

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Figure 65B

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Figure 65C

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Figure 65D

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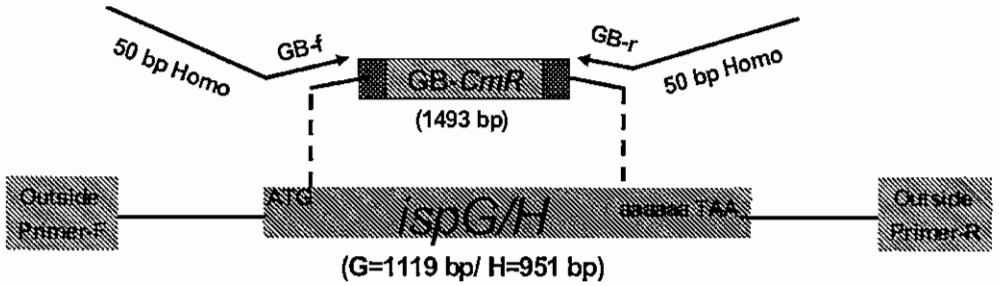
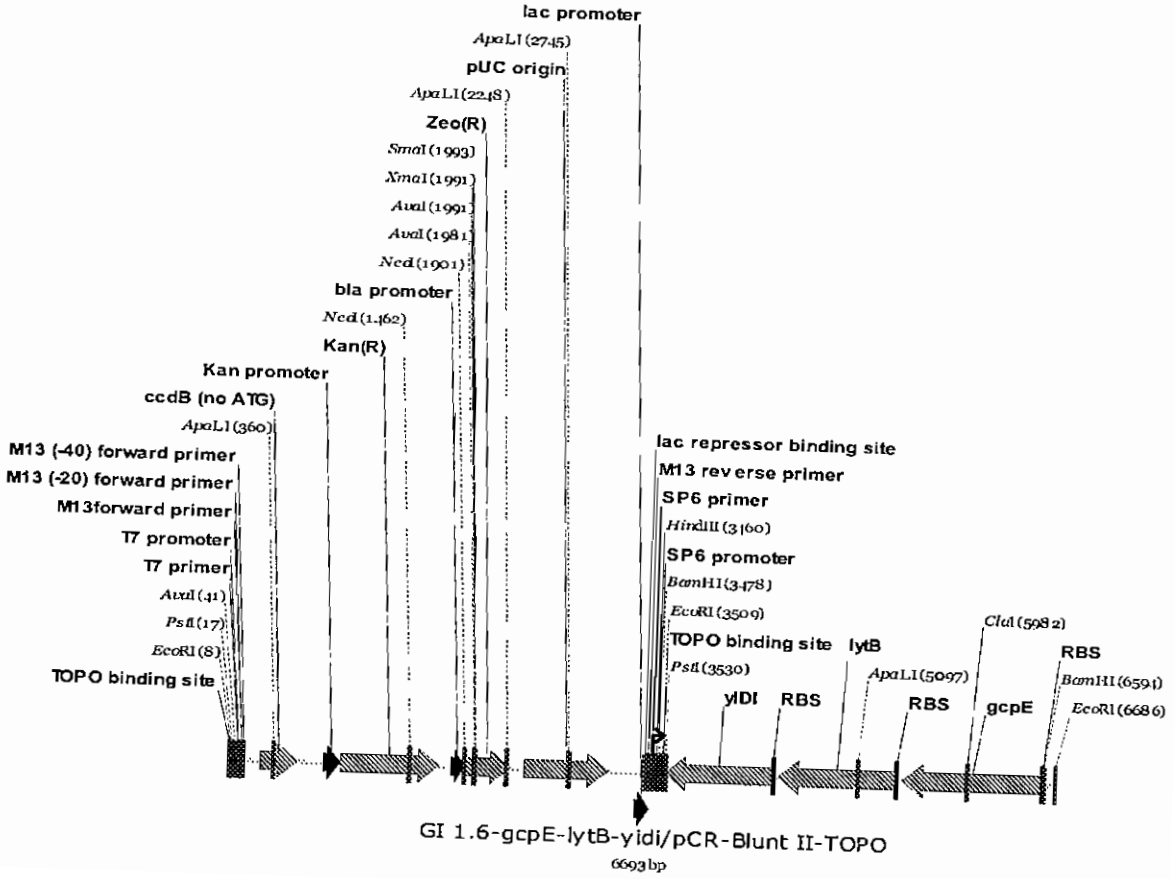
Figure 66



(G=1119 bp/ H=951 bp)

Figure 67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pUC origin
ApaLI (2248)
Zeo(R)
SmaI (1993)
XmaI (1991)
AvaI (1991)
AvaI (1981)
NcoI (1901)
bla promoter
NcoI (1462)
Kan(R)
Kan promoter
ccdB (no ATG)
ApaLI (360)
M13 (-40) forward primer
M13 (-20) forward primer
M13forward primer
T7 promoter
T7 primer
AvaI (41)
PstI (17)
EcoRI (8)
TOPO binding site
lac repressor binding site
M13 reverse primer
SP6 primer
HindIII (3160)
SP6 promoter
BamHI (3478)
EcoRI (3509)
TOPO binding site
PstI (3530)
ClaI (5982)
lytB
ApaLI (5097)
RBS
BamHI (6591)
EcoRI (6686)
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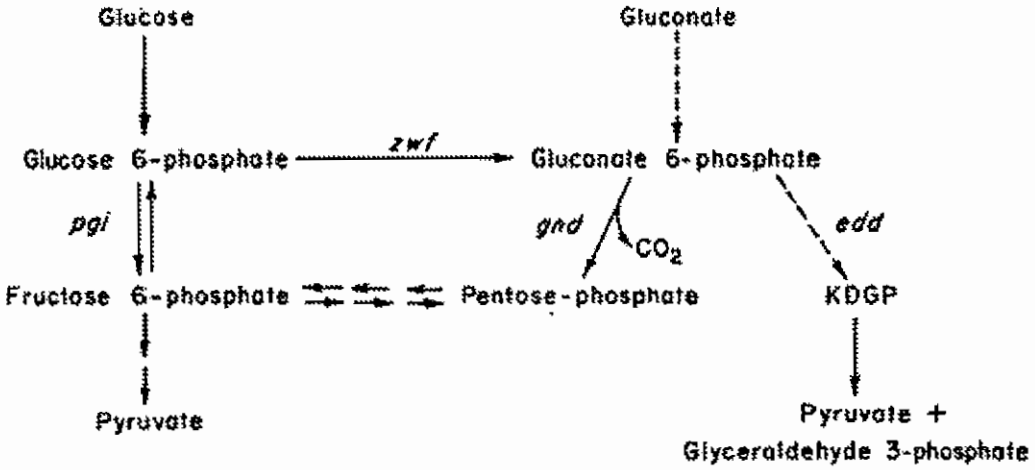
GI 1.6-gcpE-lytB-yidi/pCR-Blunt II-TOPO
6693 bp

Figure 68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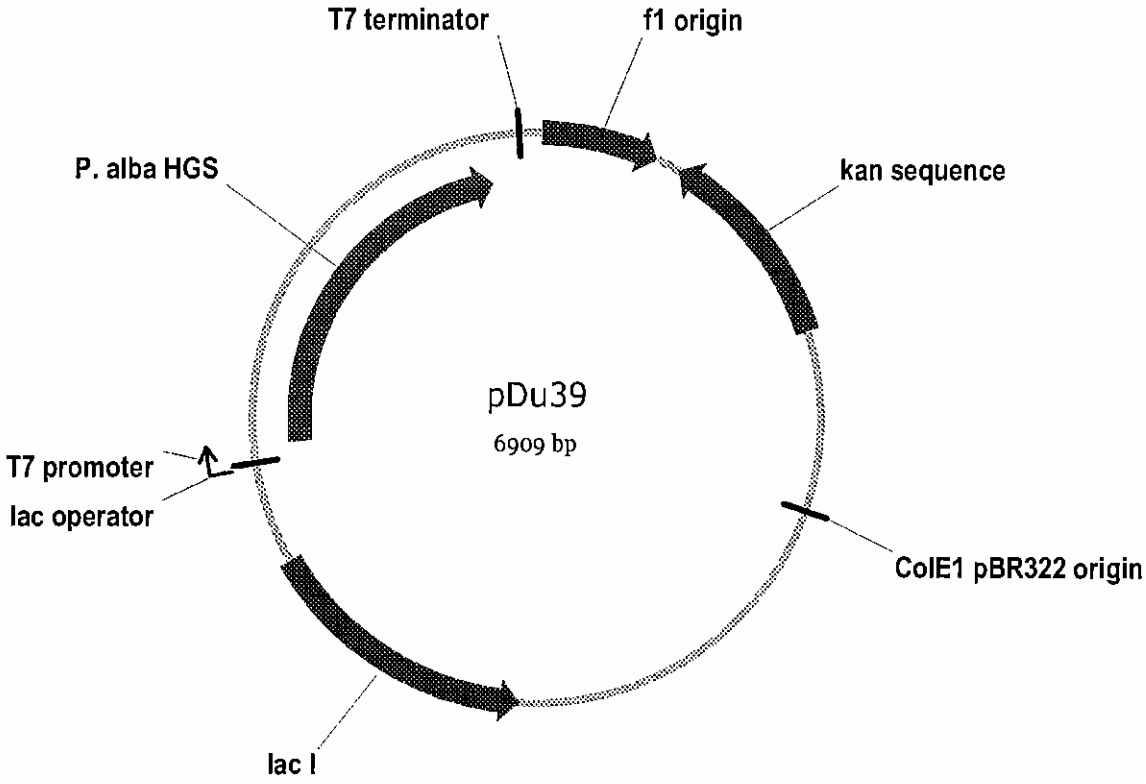
Gluconate
Glucose 6-phosphate
zwf
Gluconate 6-phosphate
pgi
gnd
CO2
edd
Fructose 6-phosphate
Pentose-phosphate
KDGP
Pyruvate
Pyruvate +
Glyceraldehyde 3-phosphate

Figure 69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6909 bp
T7 promoter
lac operator
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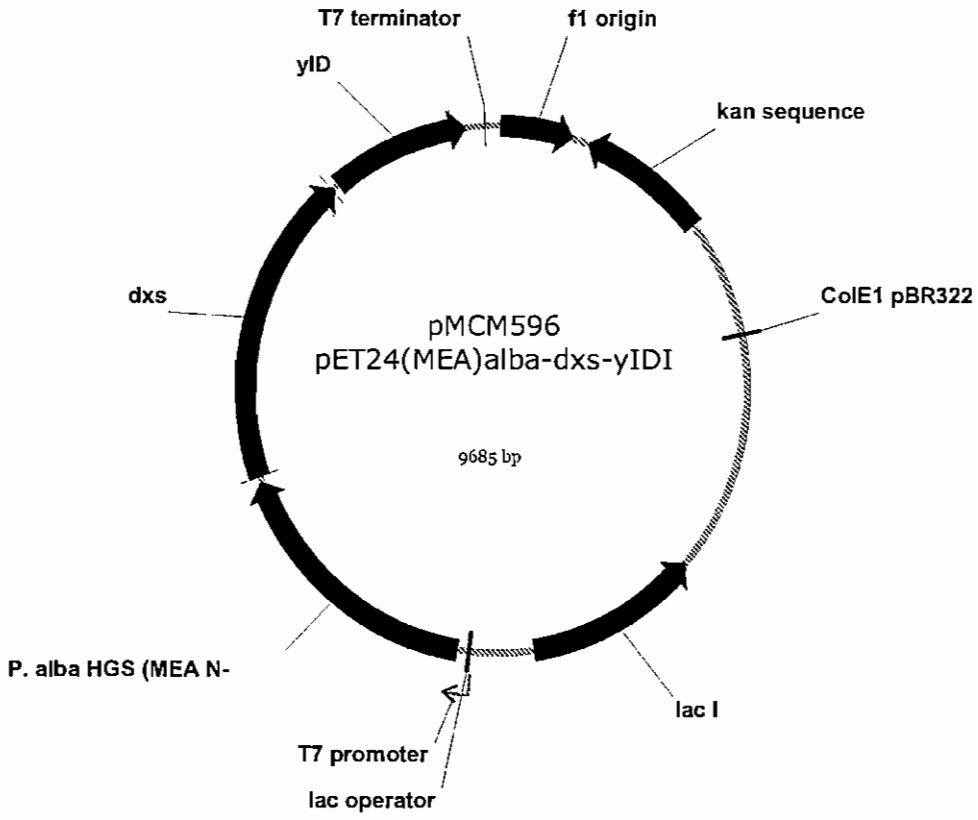
lac I

Figure 70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yID
kan sequence
dxs
ColE1 pBR322
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9685 bp
P. alba HGS (MEA N-
lac I
T7 promoter
lac operator

Figure 71A

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Figure 71B

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Figure 72A

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Figure 72B

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Figure 72C

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Figure 73A

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Figure 73B

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Figure 73C

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Figure 74

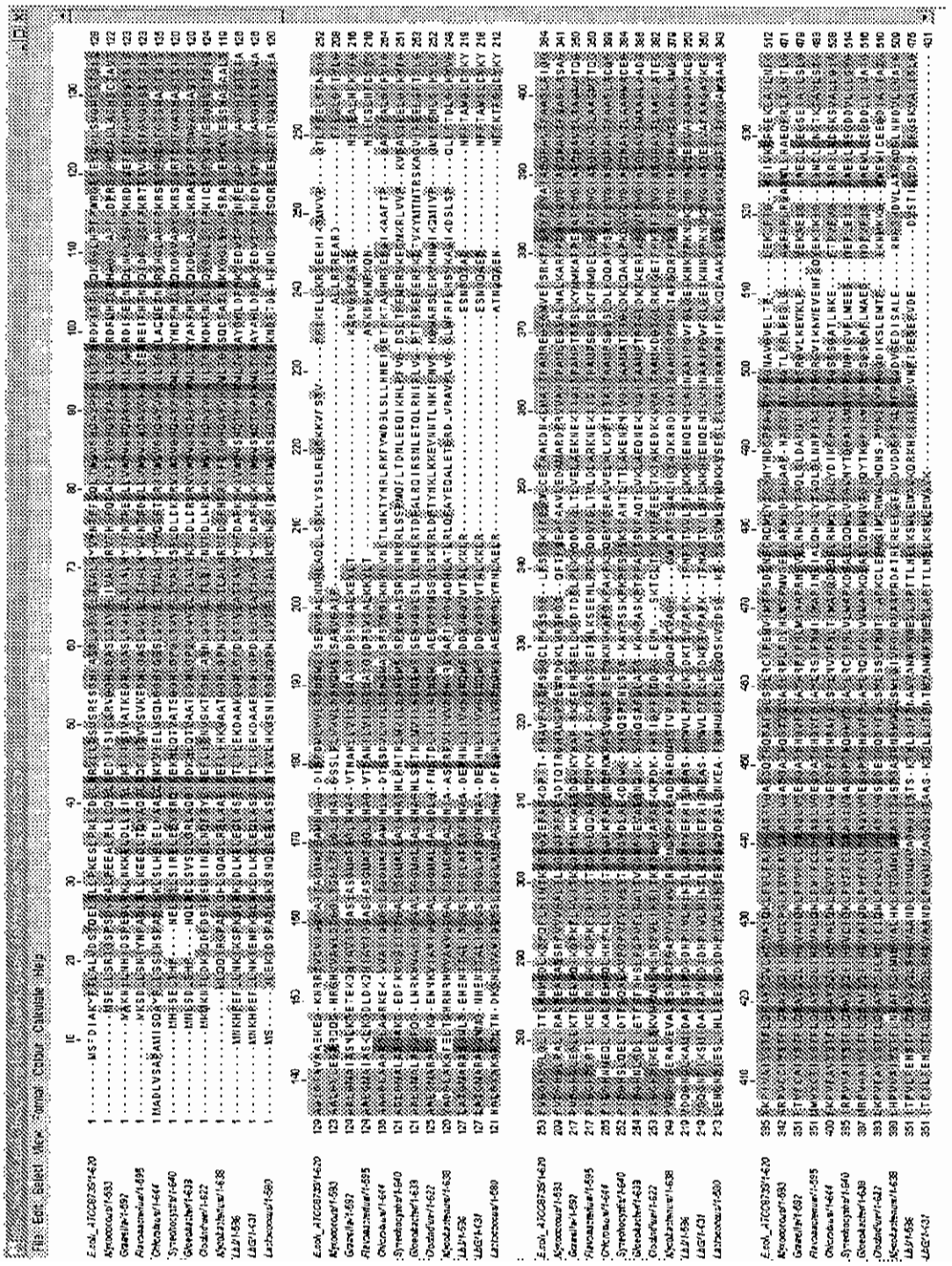

Figure 75A

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Figure 75B

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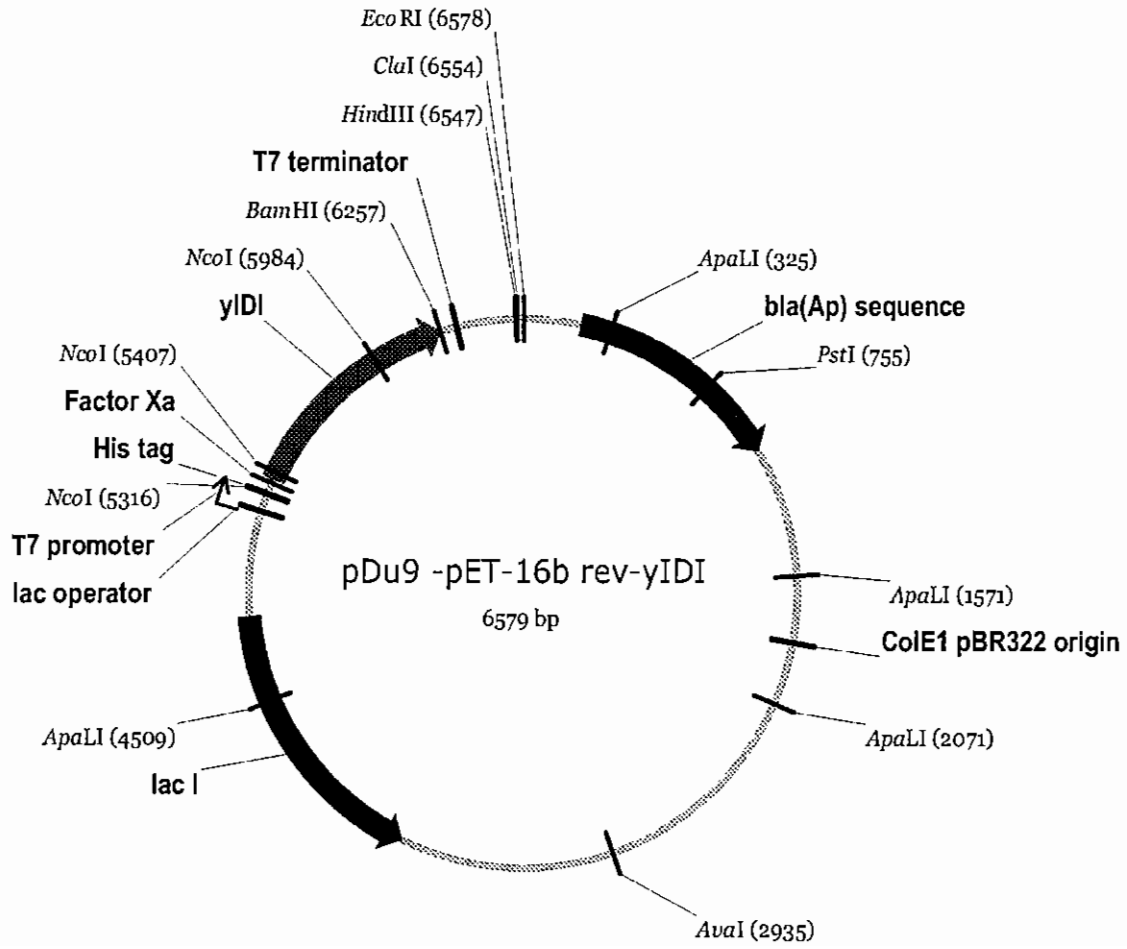
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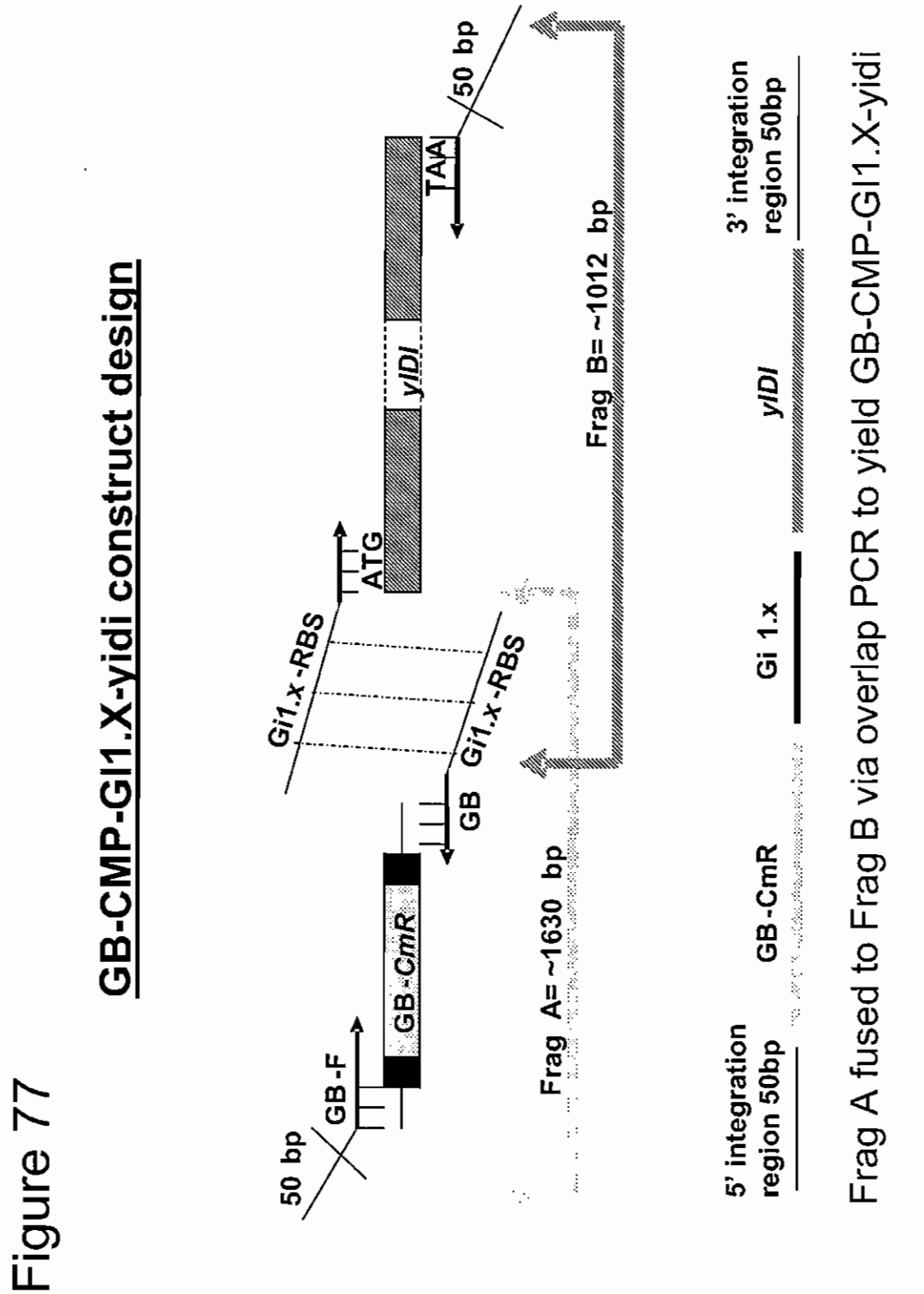
AvaI (2935)

Figure 77



Frag A fused to Frag B via overlap PCR to yield GB-CMP-GI1.X-yidi

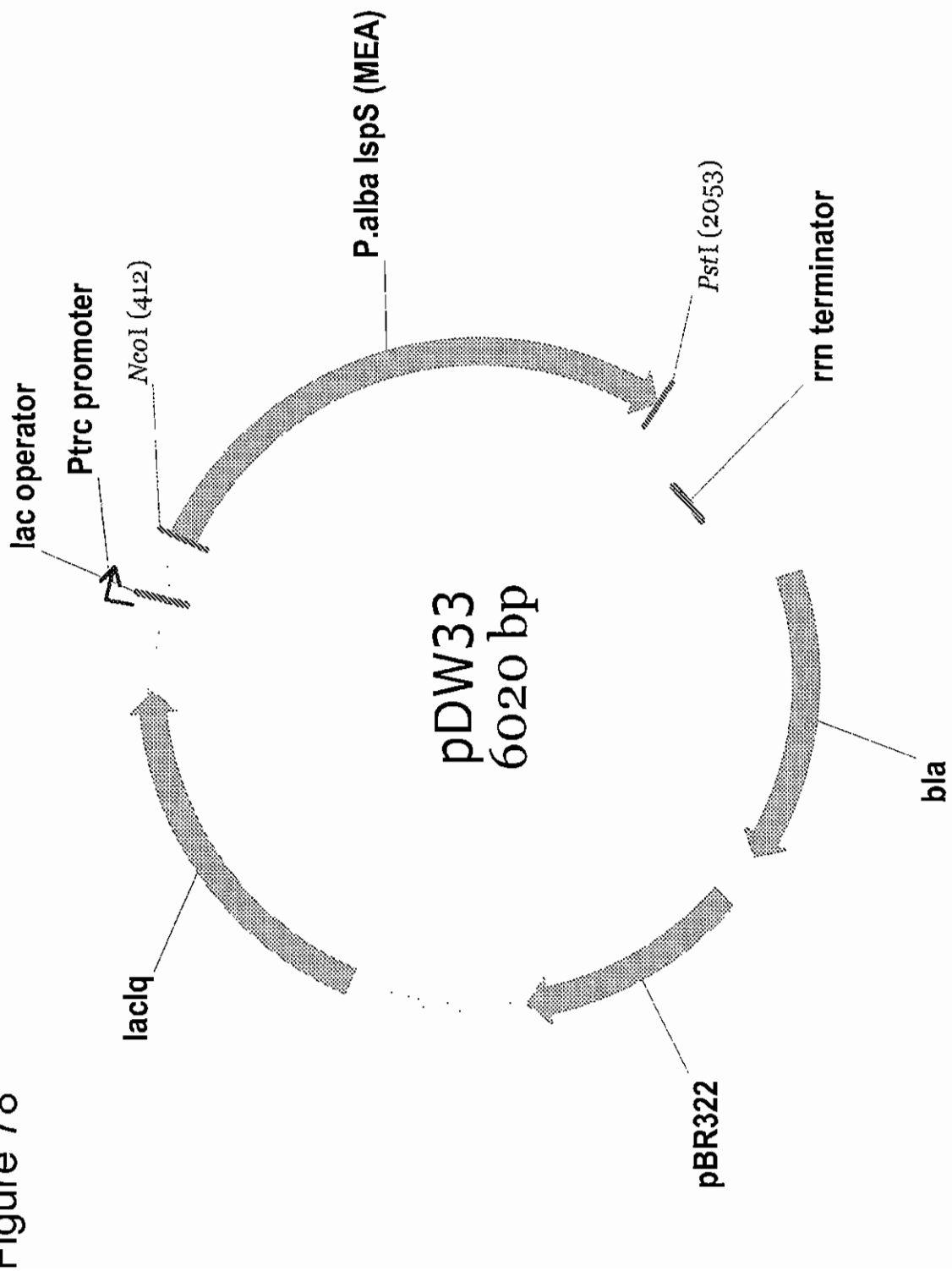

lac operator
Ptrc promoter
NcoI (412)
P.alba IspS (MEA)
PstI (2053)
rrn terminator
pDW33
6020 bp
bla
pBR322
laclq

Figure 78

Figure 79A

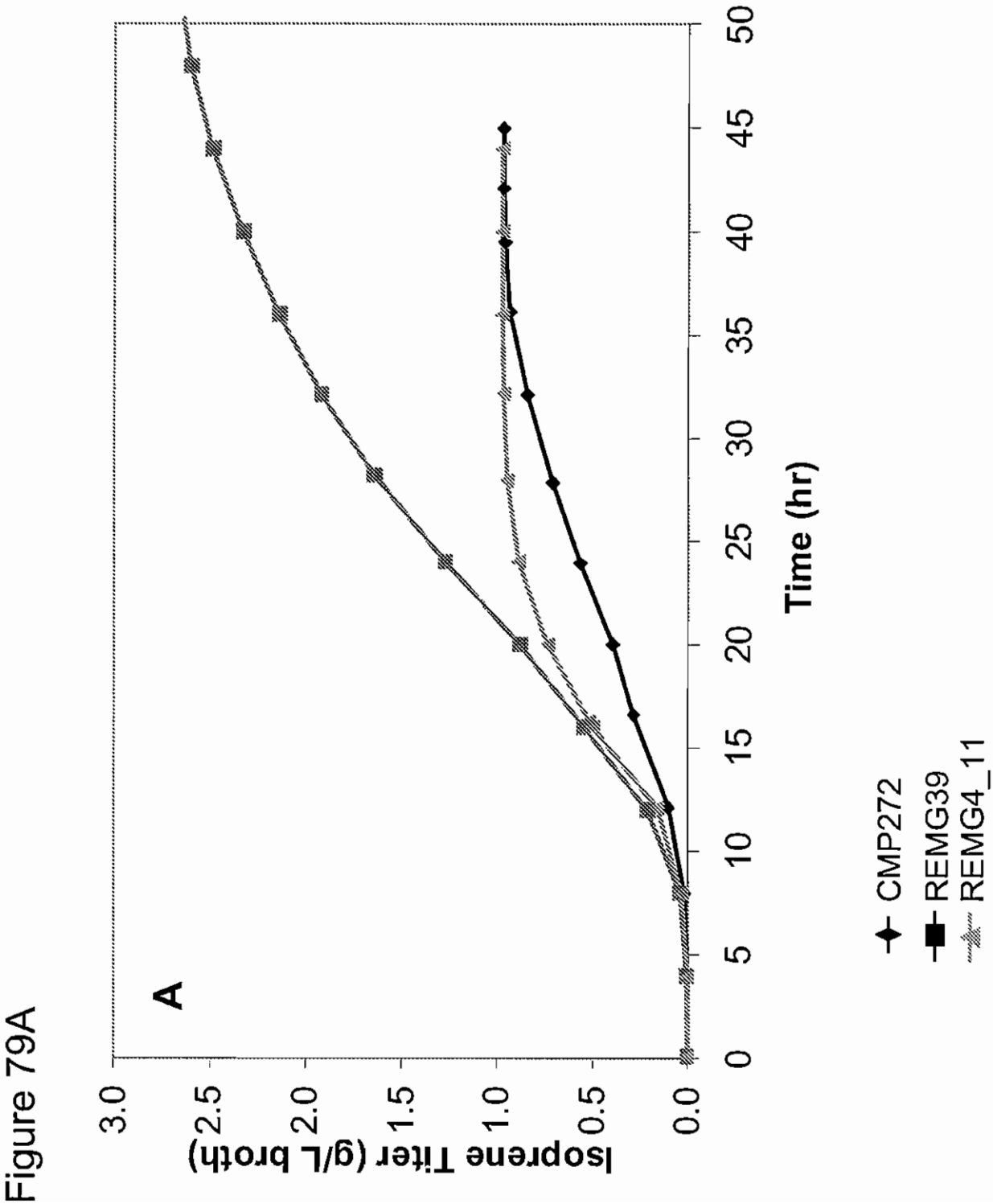

A
Isoprene Titer (g/L broth)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
40
45
50
Time (hr)
CMP272
REMG39
REMG4_11

Figure 79B

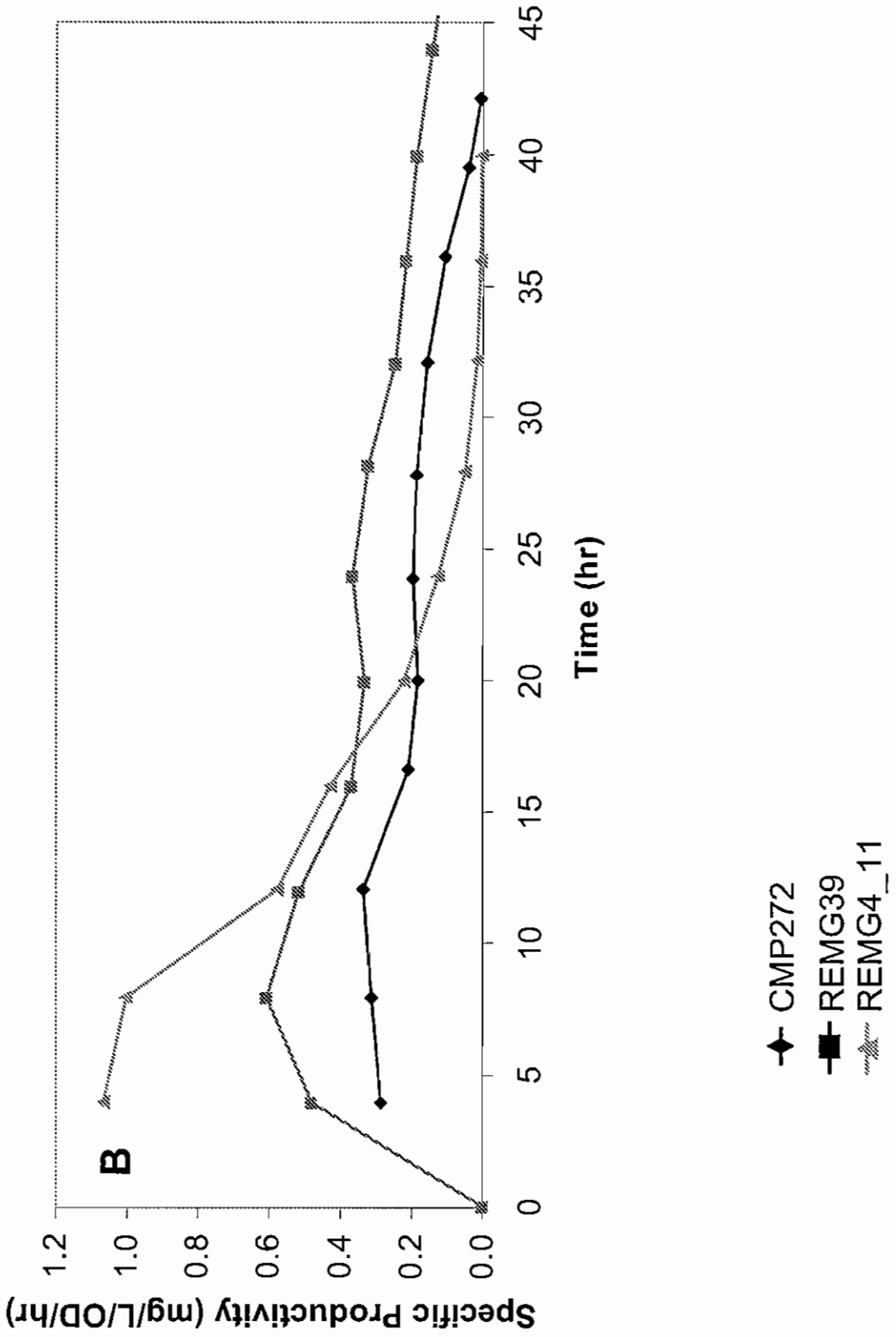

B
Specific Productivity (mg/L/OD/hr)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45
Time (hr)
CMP272
REMG39
REMG4_11

Figure 79C

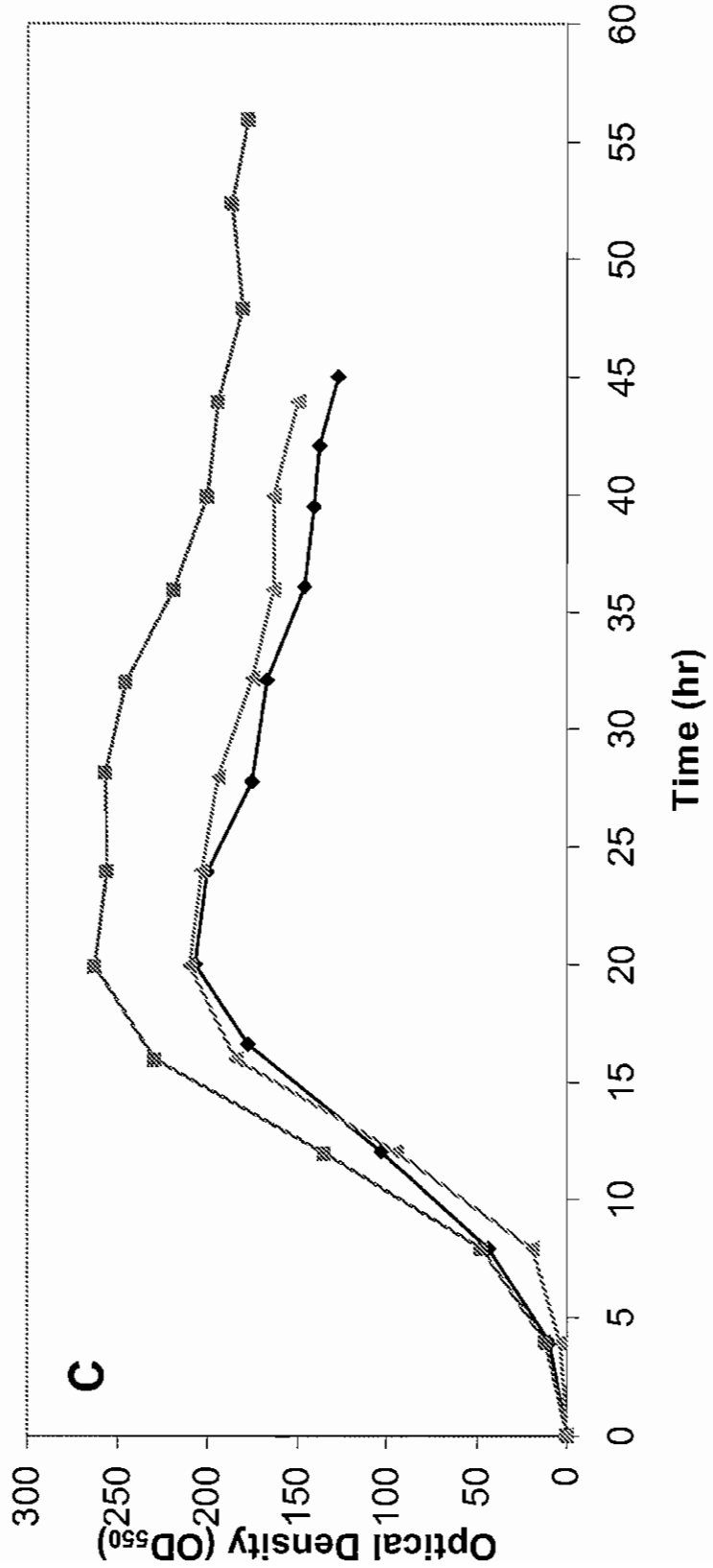

C
Optical Density (OD550)
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (hr)
CMP272
REMG39
REMG4_11

Figure 79 D

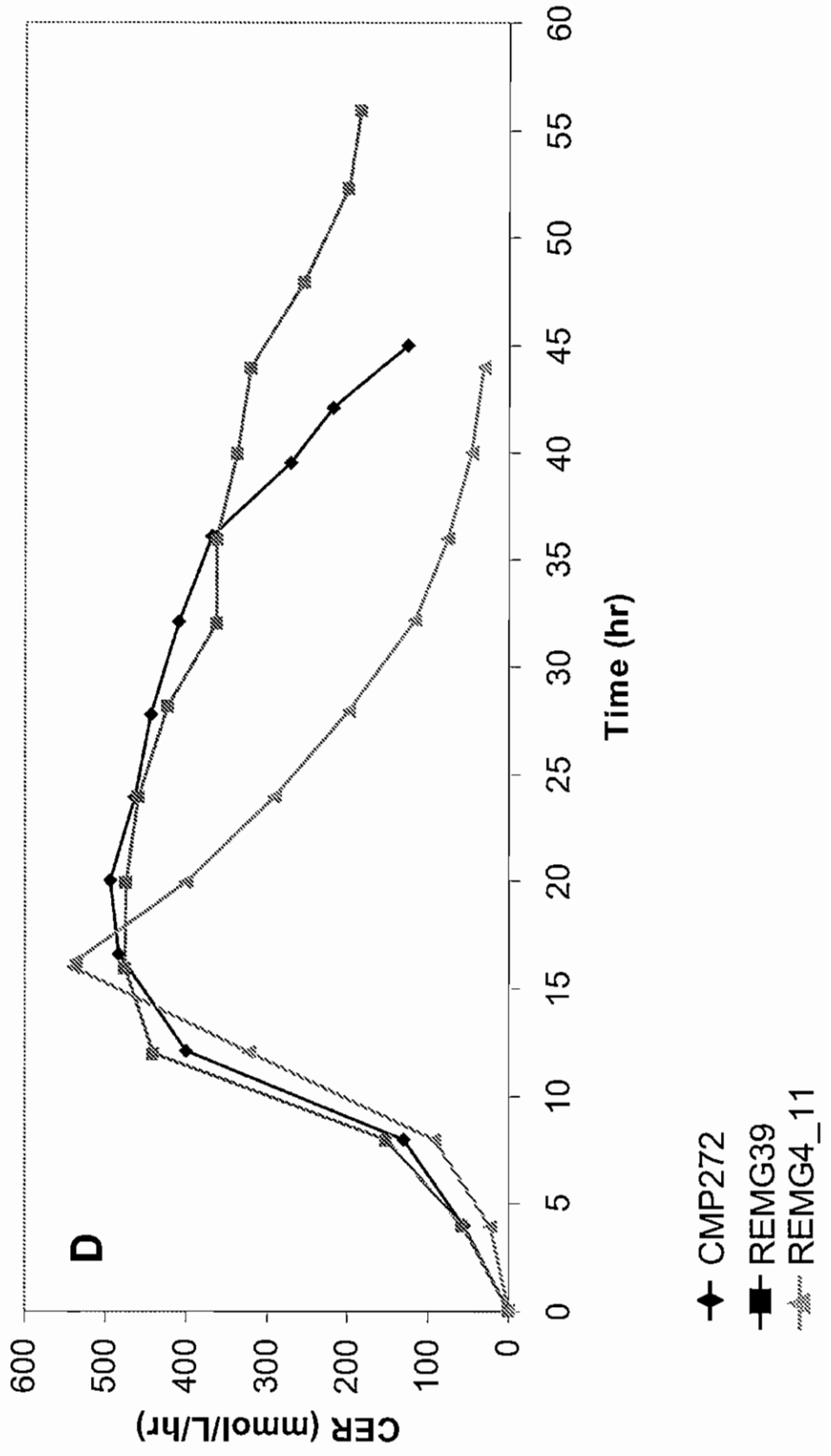

D
CER (mmol/L/hr)
0
100
200
300
400
500
600
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (hr)
CMP272
REMG39
REMG4_11

Figure 79 E

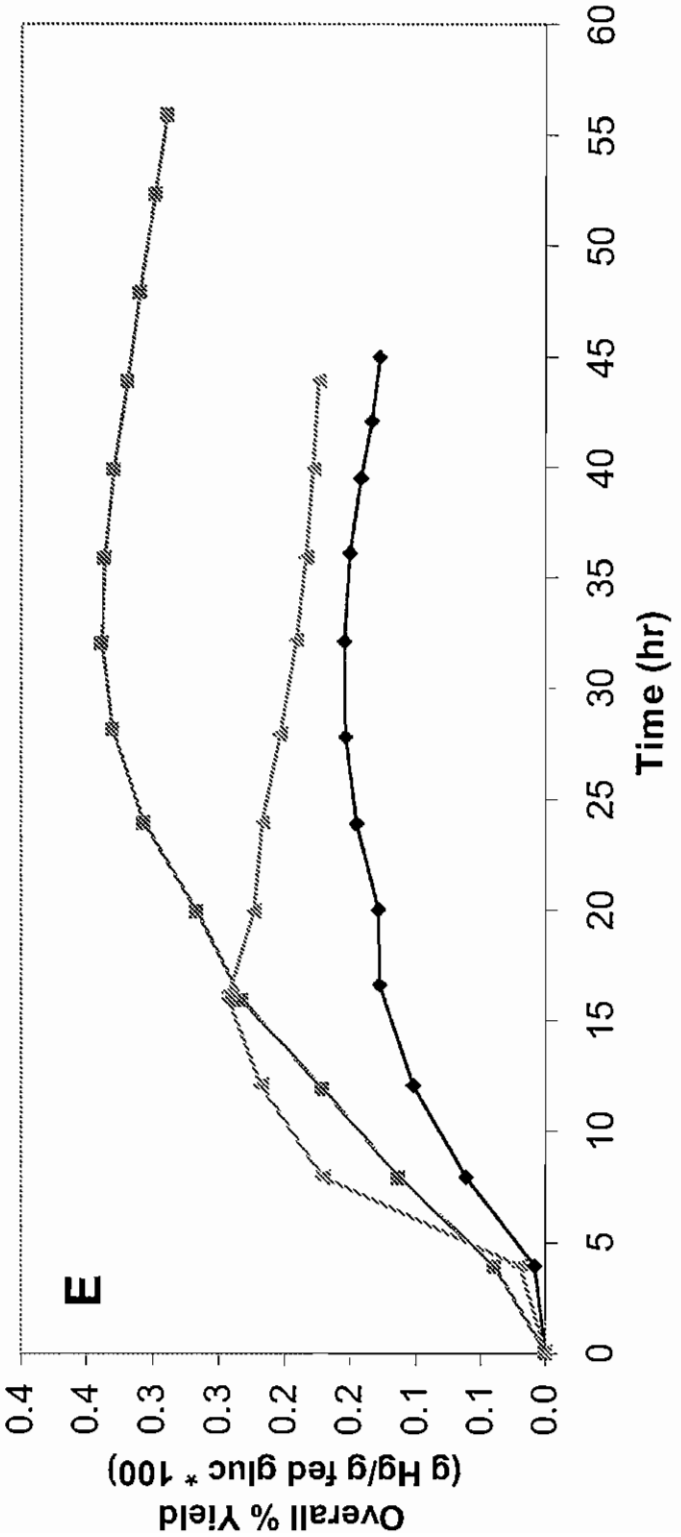

E
Overall % Yield
(g Hg/g fed gluc * 100)
0.0
0.1
0.1
0.2
0.2
0.3
0.3
0.4
0.4
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (hr)
CMP272
REMG39
REMG4_11

Figure 80A

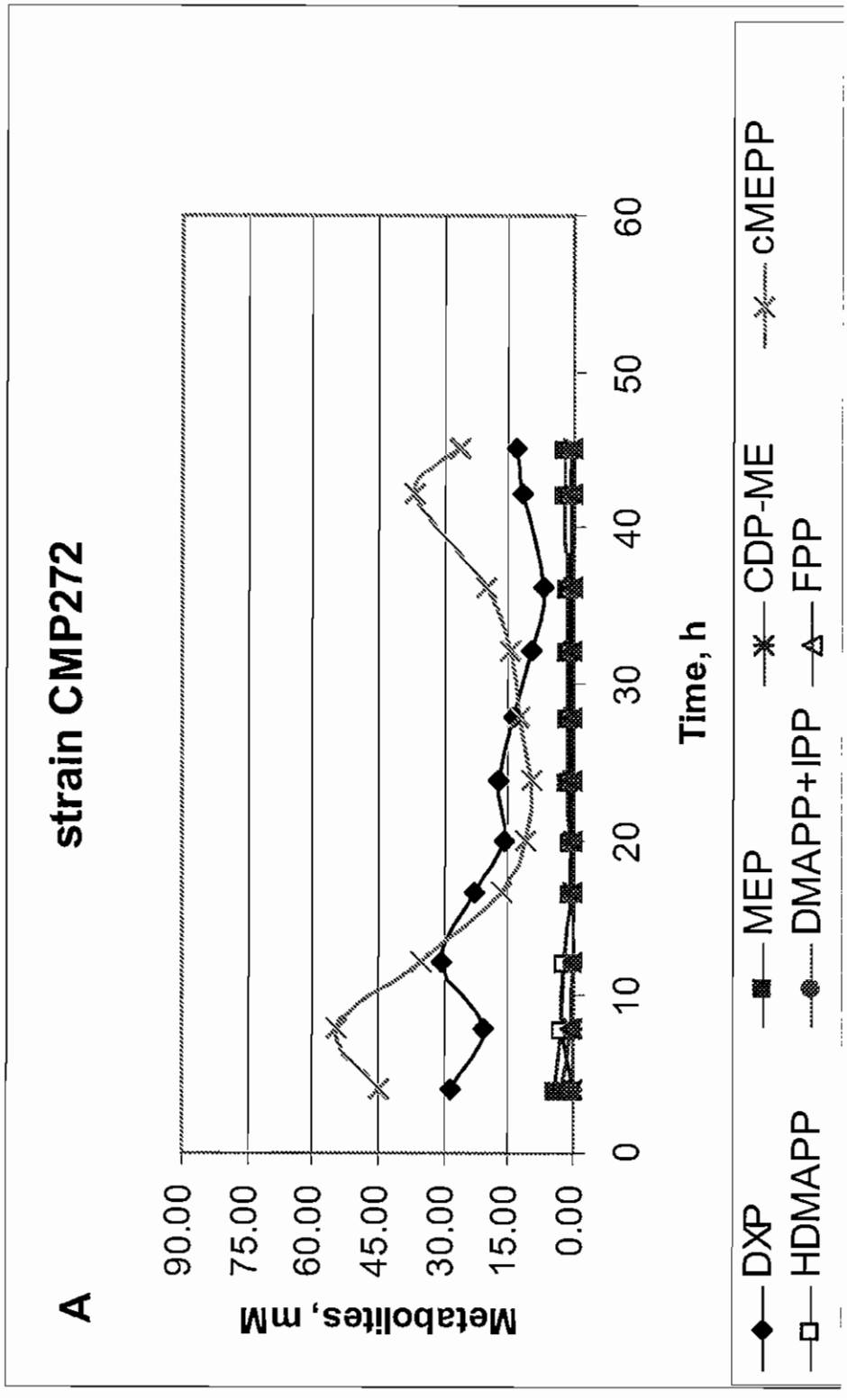

A
strain CMP272
Metabolites, mM
90.00
75.00
60.00
45.00
30.00
15.00
0.00
0
10
20
30
40
50
60
Time, h
DXP
MEP
CDP-ME
cMEPP
HDMAPP
DMAPP+IPP
FPP

Figure 80B

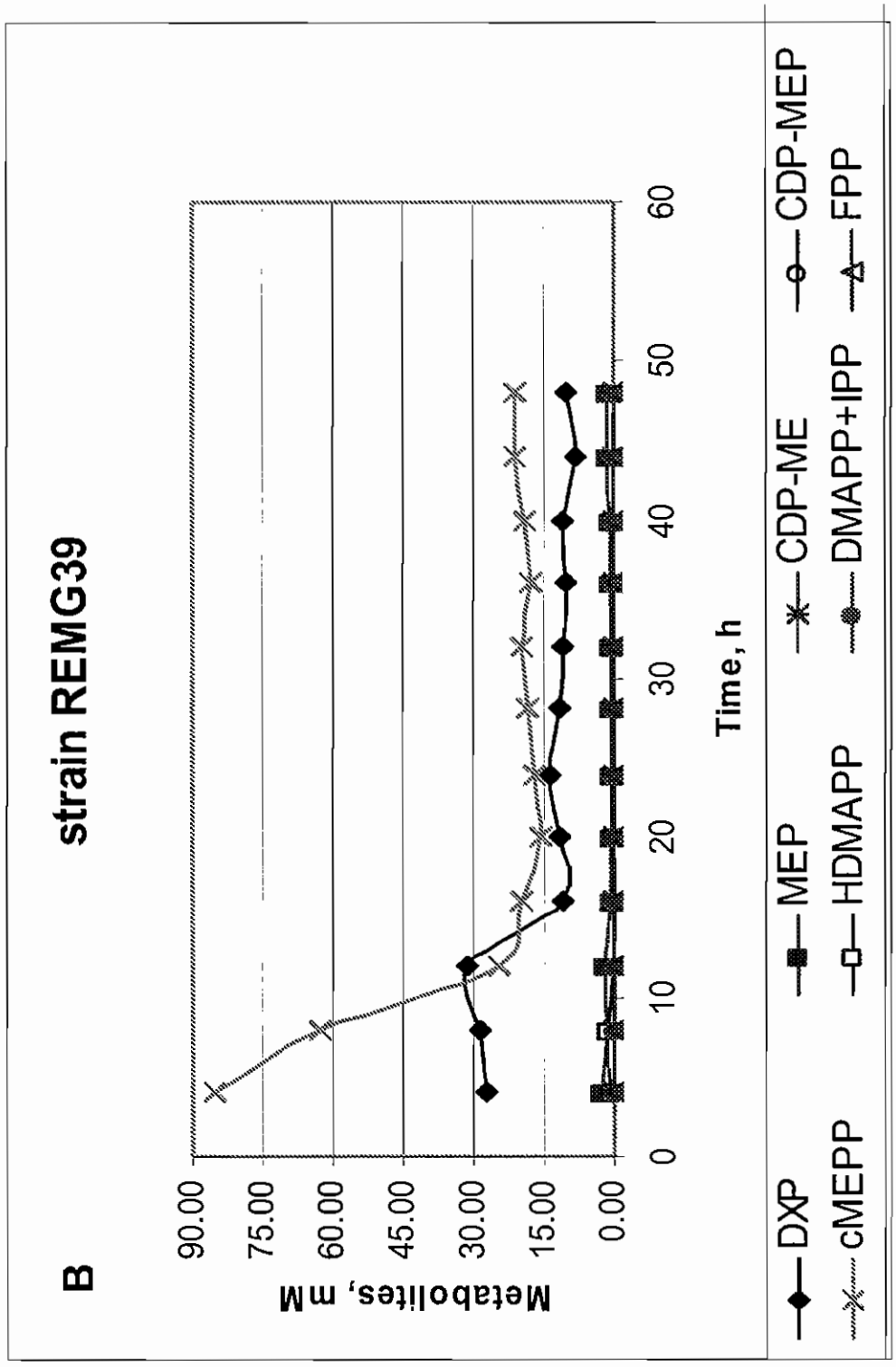

B
strain REMG39
Metabolites, mM
90.00
75.00
60.00
45.00
30.00
15.00
0.00
0
10
20
30
40
50
60
Time, h
DXP
MEP
CDP-ME
CDP-MEP
cMEPP
HDMAPP
DMAPP+IPP
FPP

Figure 80C

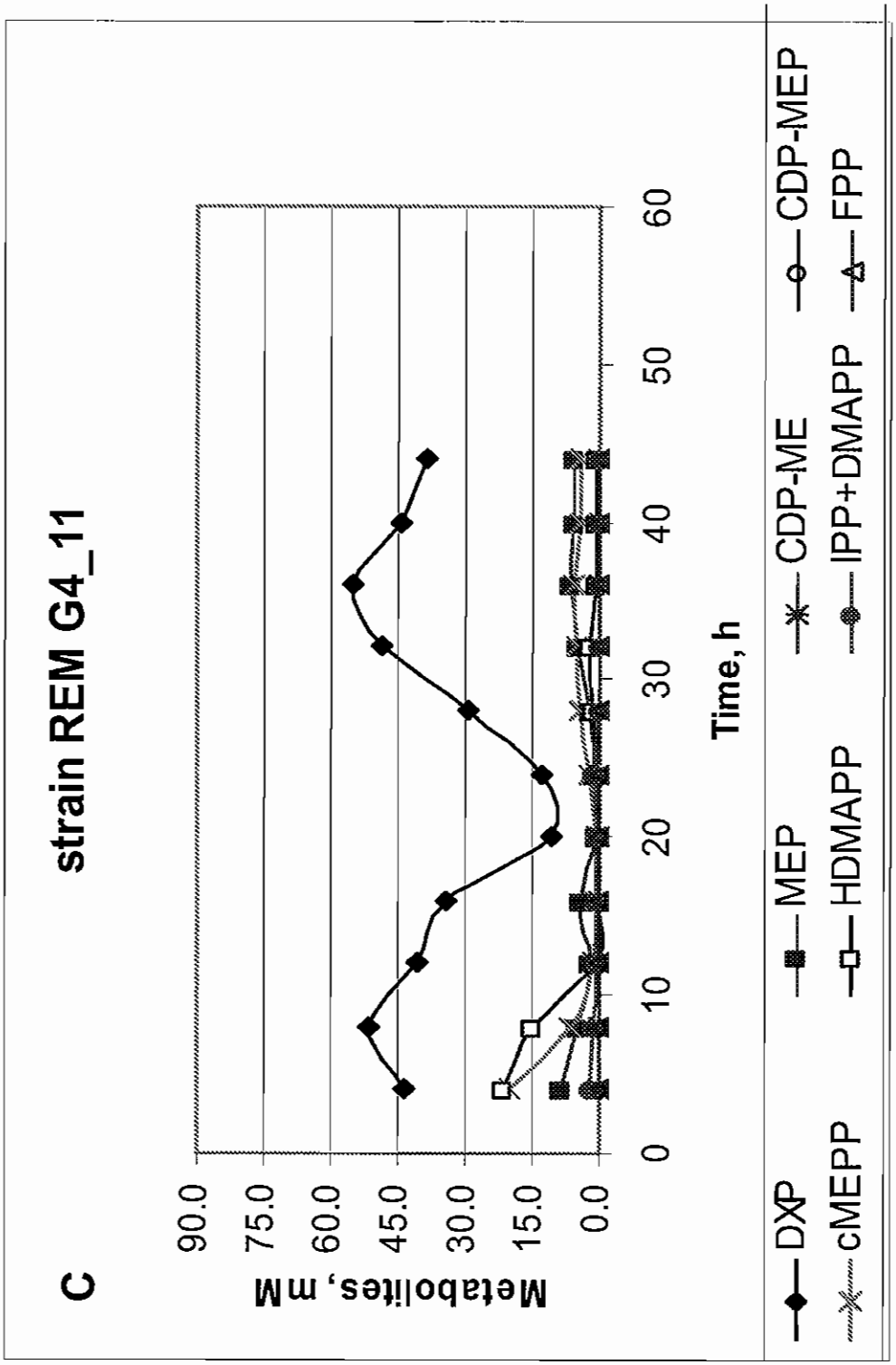
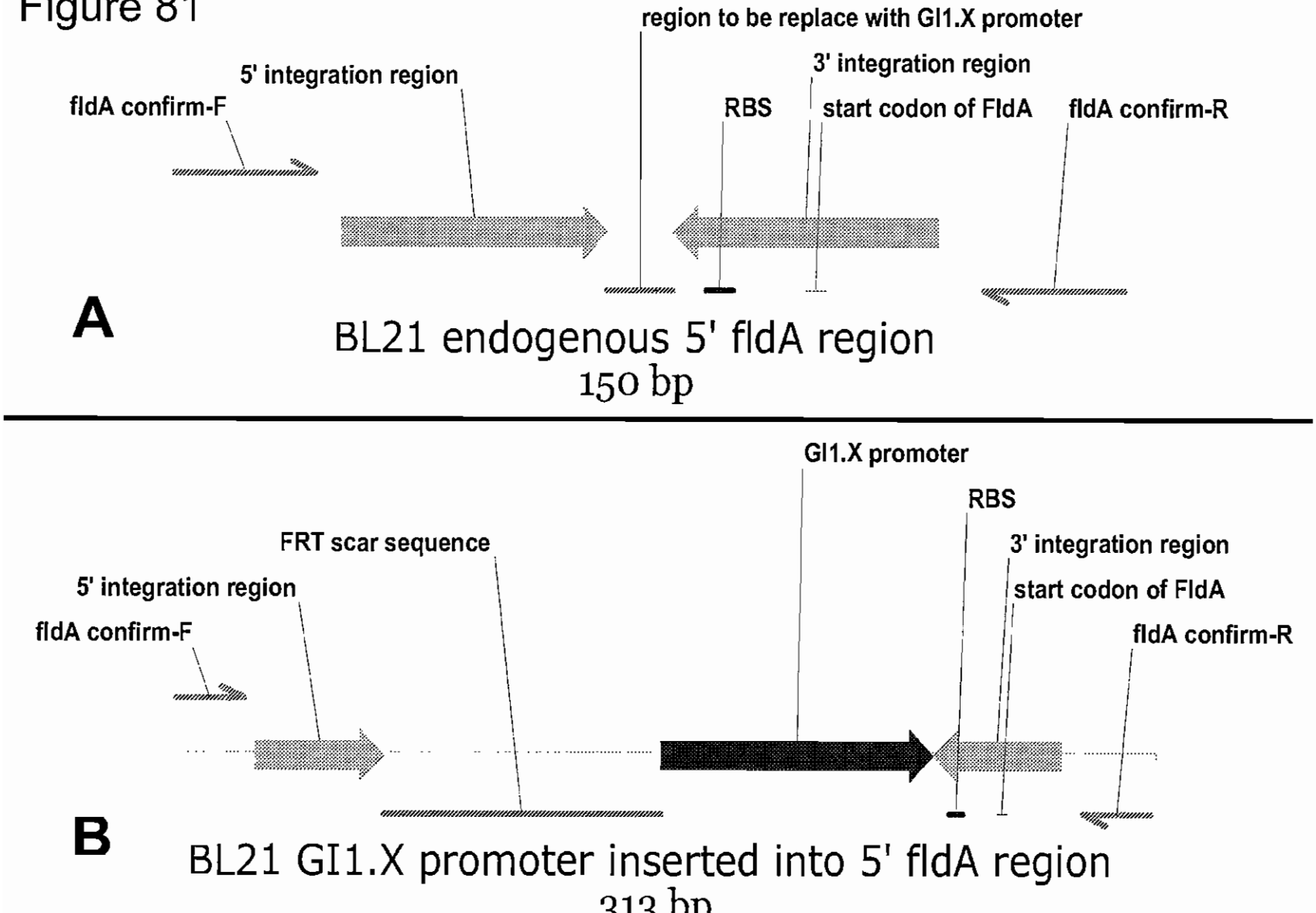

C
strain REM G4_11
Metabolites, mM
90.0
75.0
60.0
45.0
30.0
15.0
0.0
0
10
20
30
40
50
60
Time, h
DXP
MEP
CDP-ME
CDP-MEP
cMEPP
HDMAPP
IPP+DMAPP
FPP

Figure 81


region to be replace with GI1.X promoter
5' integration region
3' integration region
fldA confirm-F
RBS
start codon of FldA
fldA confirm-R
A
BL21 endogenous 5' fldA region
150 bp
GI1.X promoter
RBS
FRT scar sequence
3' integration region
5' integration region
start codon of FldA
fldA confirm-F
fldA confirm-R
B
BL21 GI1.X promoter inserted into 5' fldA region
313 bp

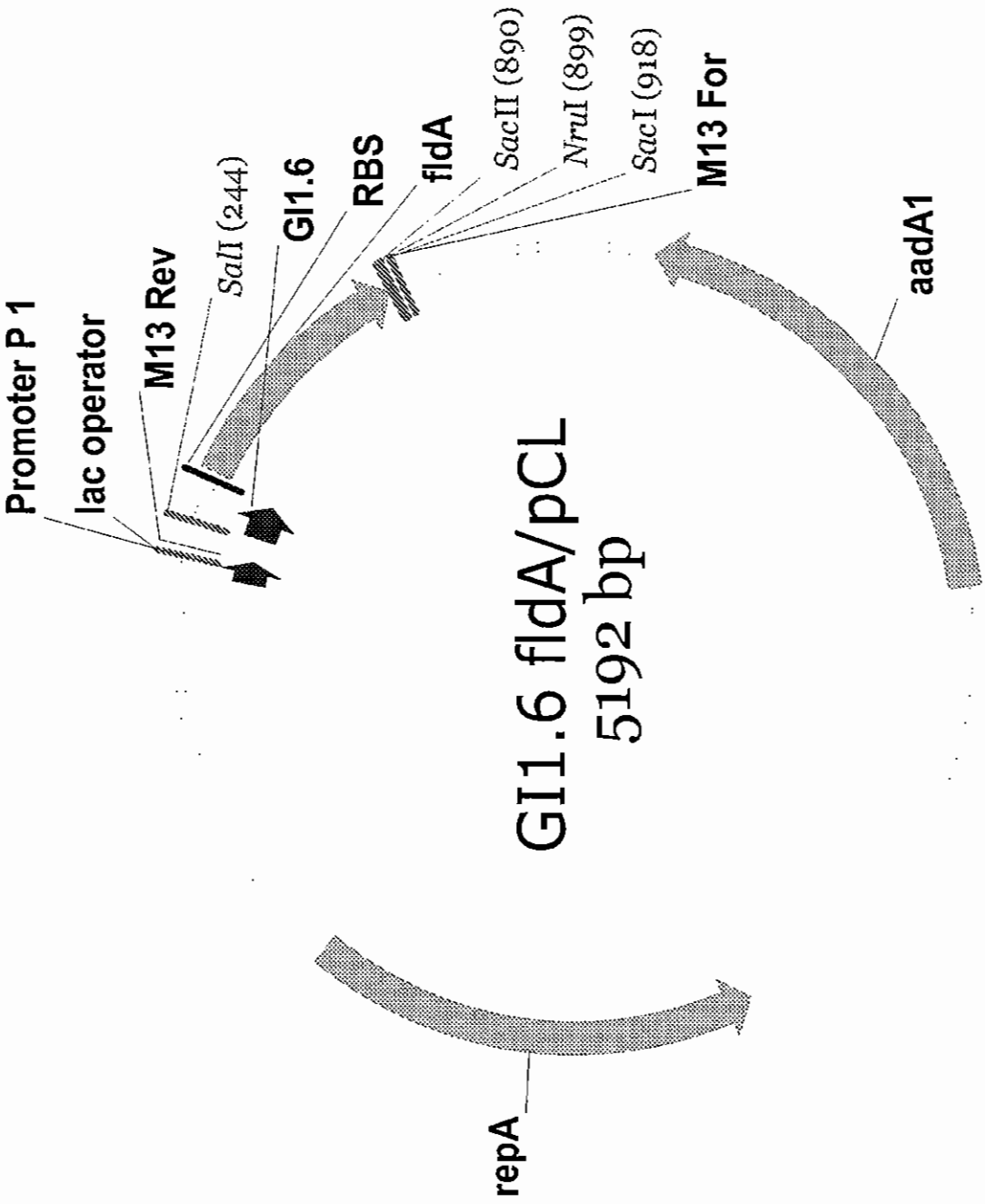

Promoter P 1
lac operator
M13 Rev
SalI (244)
GI1.6
RBS
fldA
SacII (890)
NruI (899)
SacI (918)
M13 For
aadA1
repA
GI1.6 fldA/pCL
5192 bp

Figure 82

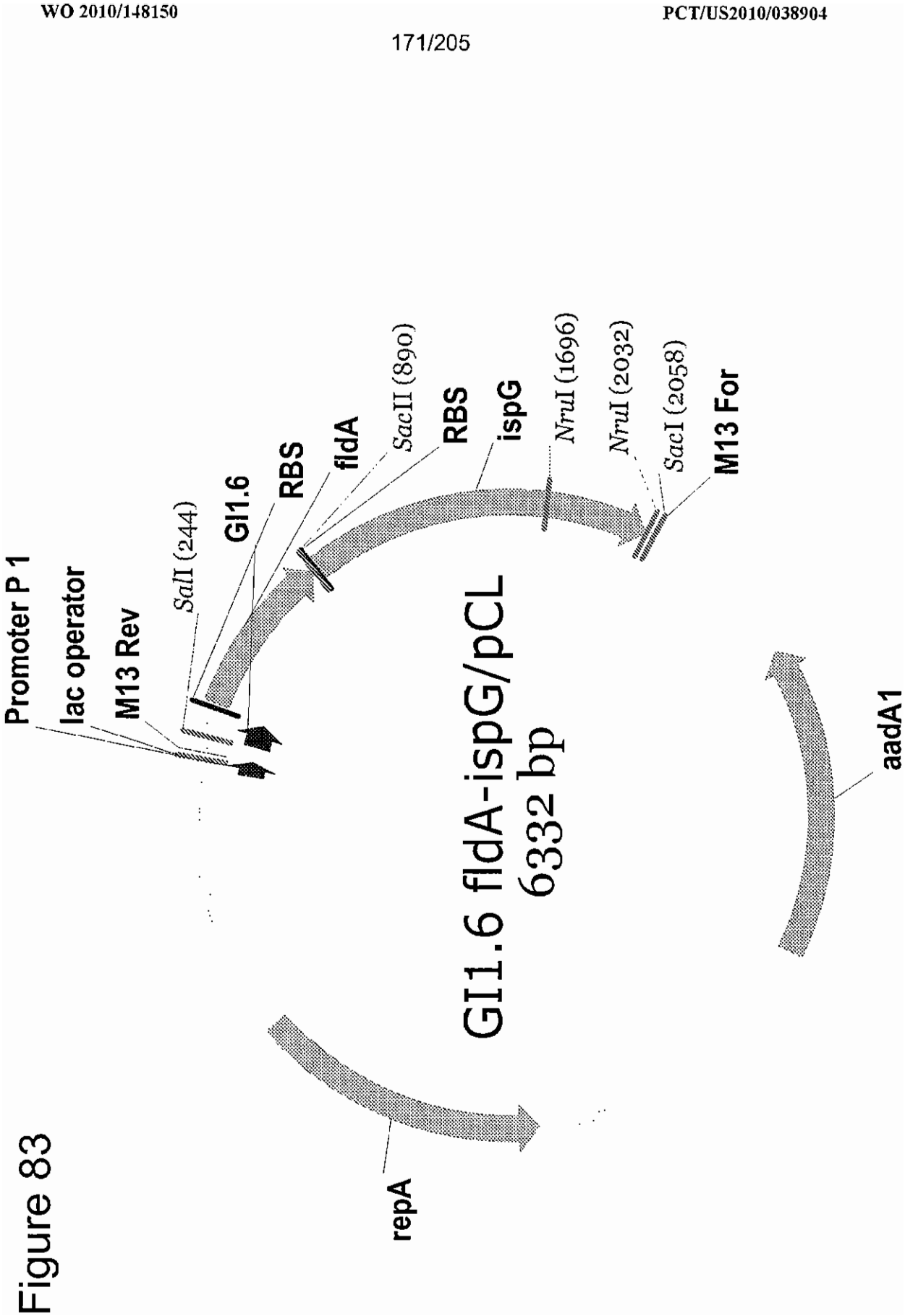

GI1.6 fldA-ispG/pCL
6332 bp
Promoter P 1
lac operator
M13 Rev
SalI (244)
GI1.6
RBS
fldA
SacII (890)
RBS
ispG
NruI (1696)
NruI (2032)
SacI (2058)
M13 For
aadA1
repA

Figure 83

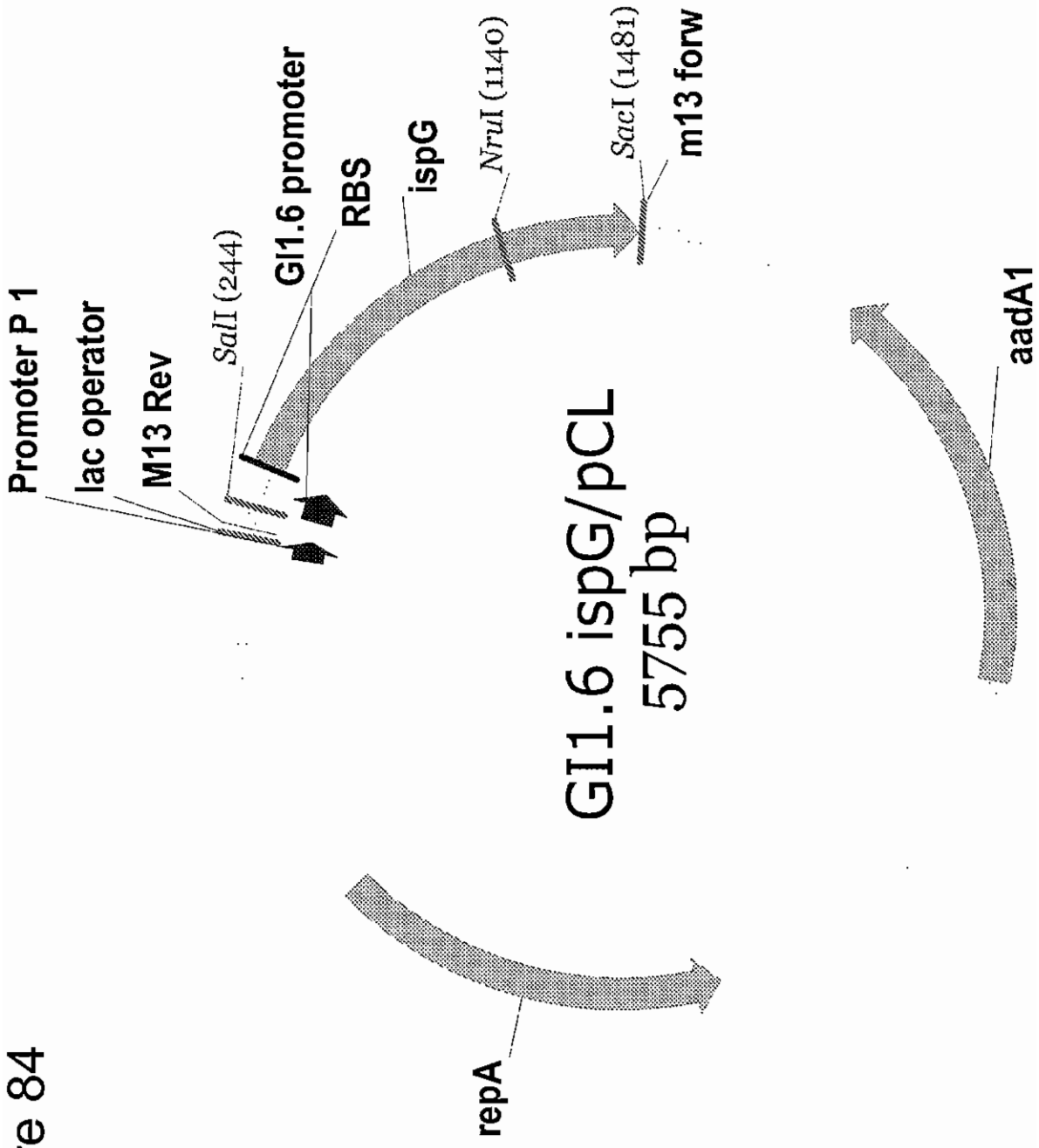

Promoter P 1
lac operator
M13 Rev
SalI (244)
GI1.6 promoter
RBS
ispG
NruI (1140)
SacI (1481)
m13 forw
aadA1
repA
GI1.6 ispG/pCL
5755 bp

Figure 84

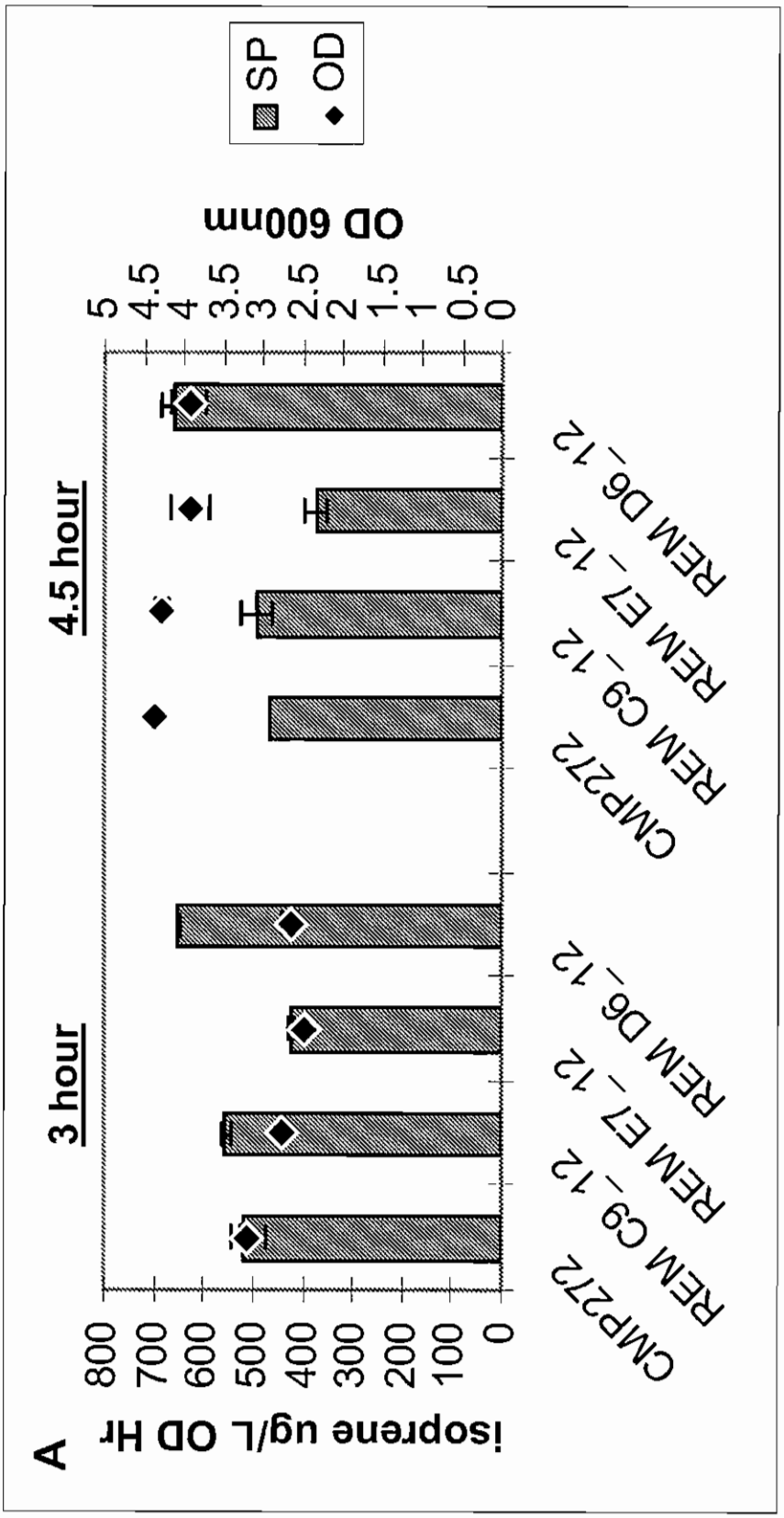

A
isoprene ug/L OD Hr
800
700
600
500
400
300
200
100
0
3 hour
4.5 hour
OD 600nm
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
SP
OD
CMP272
REM C9_12
REM E7_12
REM D6_12
CMP272
REM C9_12
REM E7_12
REM D6_12

Figure 85A

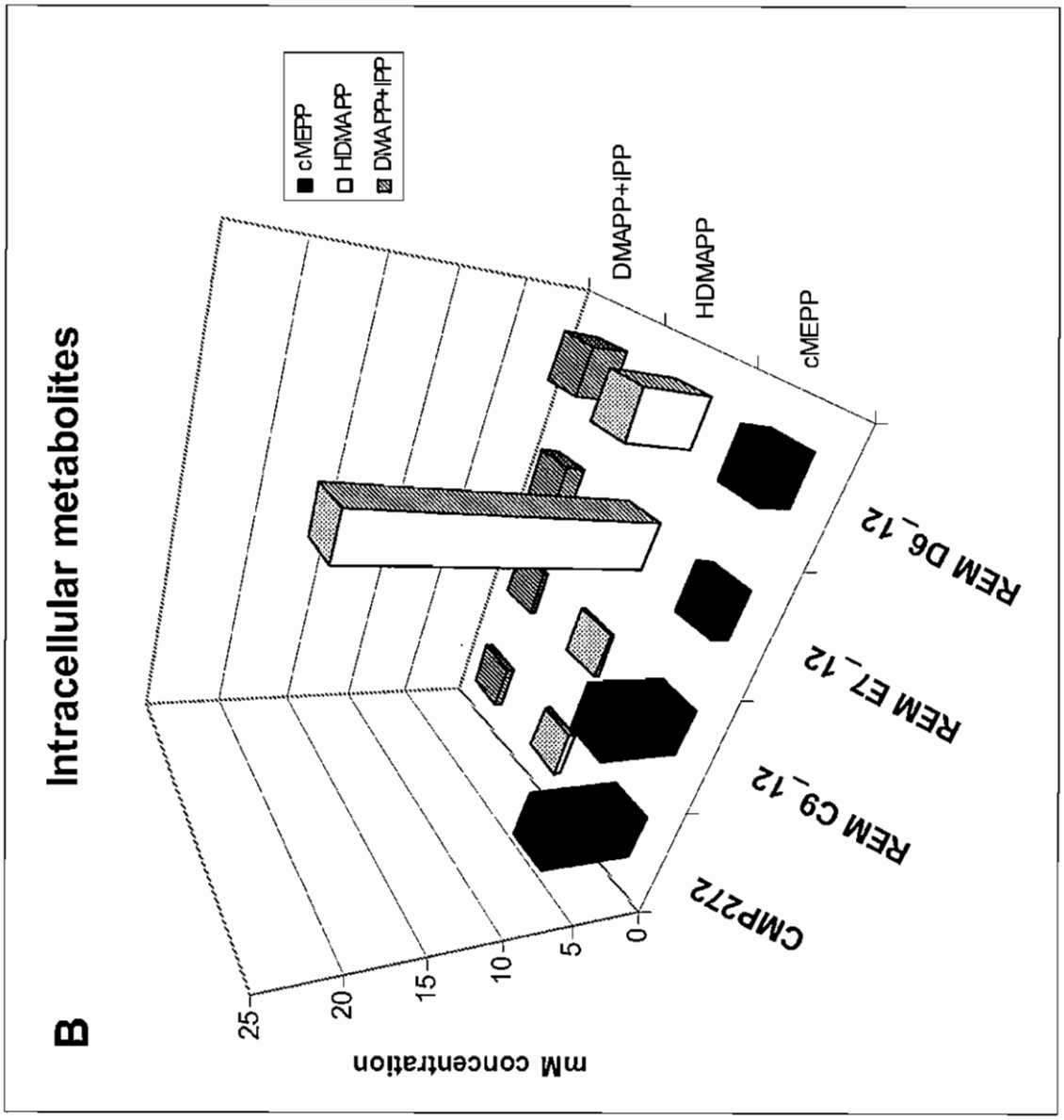

B
Intracellular metabolites
mM concentration
25
20
15
10
5
0
cMEPP
HDMAPP
DMAPP+IPP
CMP272
REM C9_12
REM E7_12
REM D6_12
cMEPP
HDMAPP
DMAPP+IPP

Figure 85B

Figure 86A

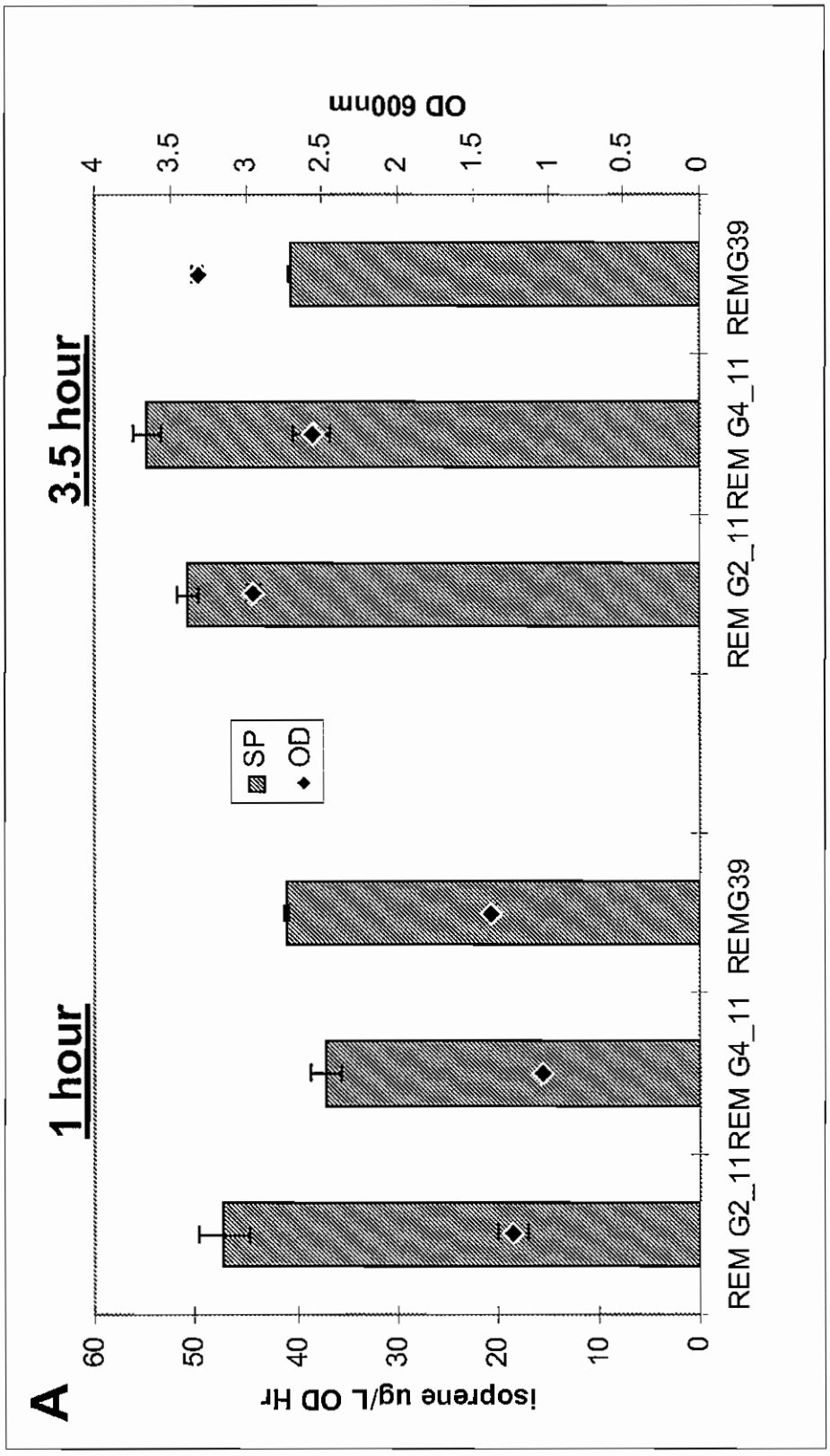

A
1 hour
3.5 hour
Isoprene ug/L OD Hr
OD 600nm
SP
OD
0
10
20
30
40
50
60
0
0.5
1
1.5
2
2.5
3
3.5
4
REM G2_11
REM G4_11
REMG39
REM G2_11
REM G4_11
REMG39

Figure 86B

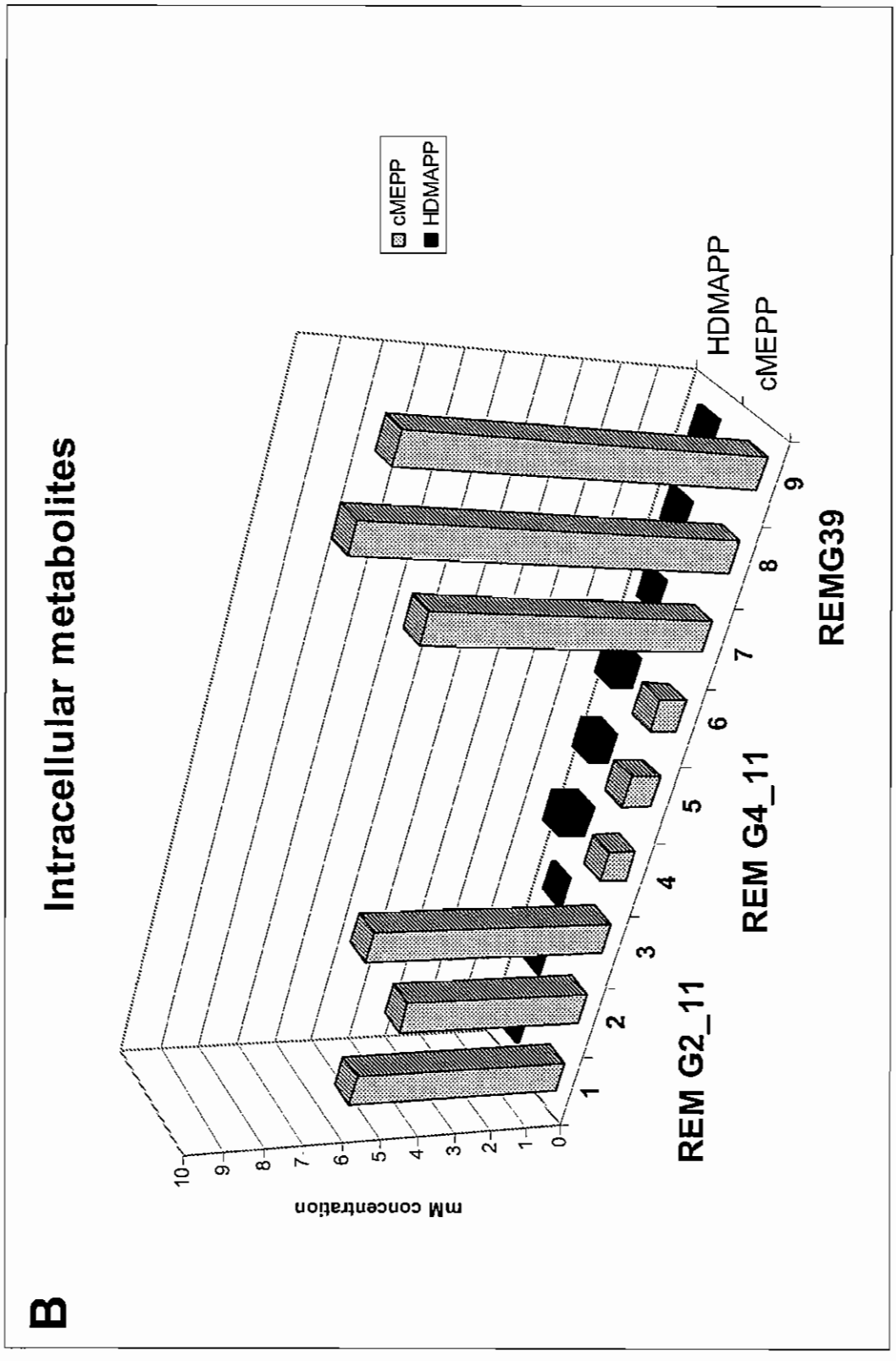
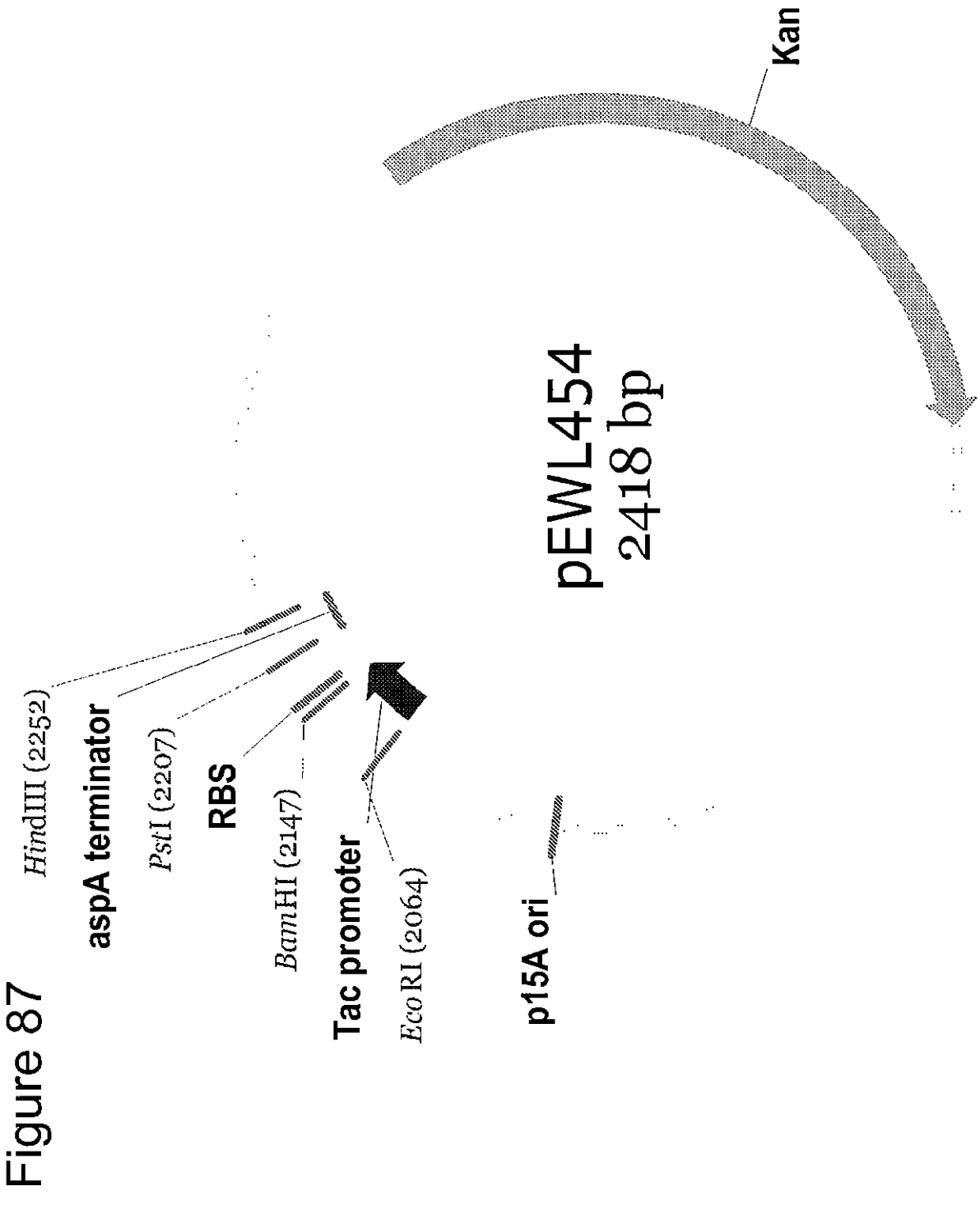

B
Intracellular metabolites
mM concentration
0
1
2
3
4
5
6
7
8
9
10
cMEPP
HDMAPP
REM G2_11
REM G4_11
REMG39
HDMAPP
cMEPP

Figure 87


pEWL454
2418 bp
Kan
HindIII (2252)
aspA terminator
PstI (2207)
RBS
BamHI (2147)
Tac promoter
EcoRI (2064)
p15A ori

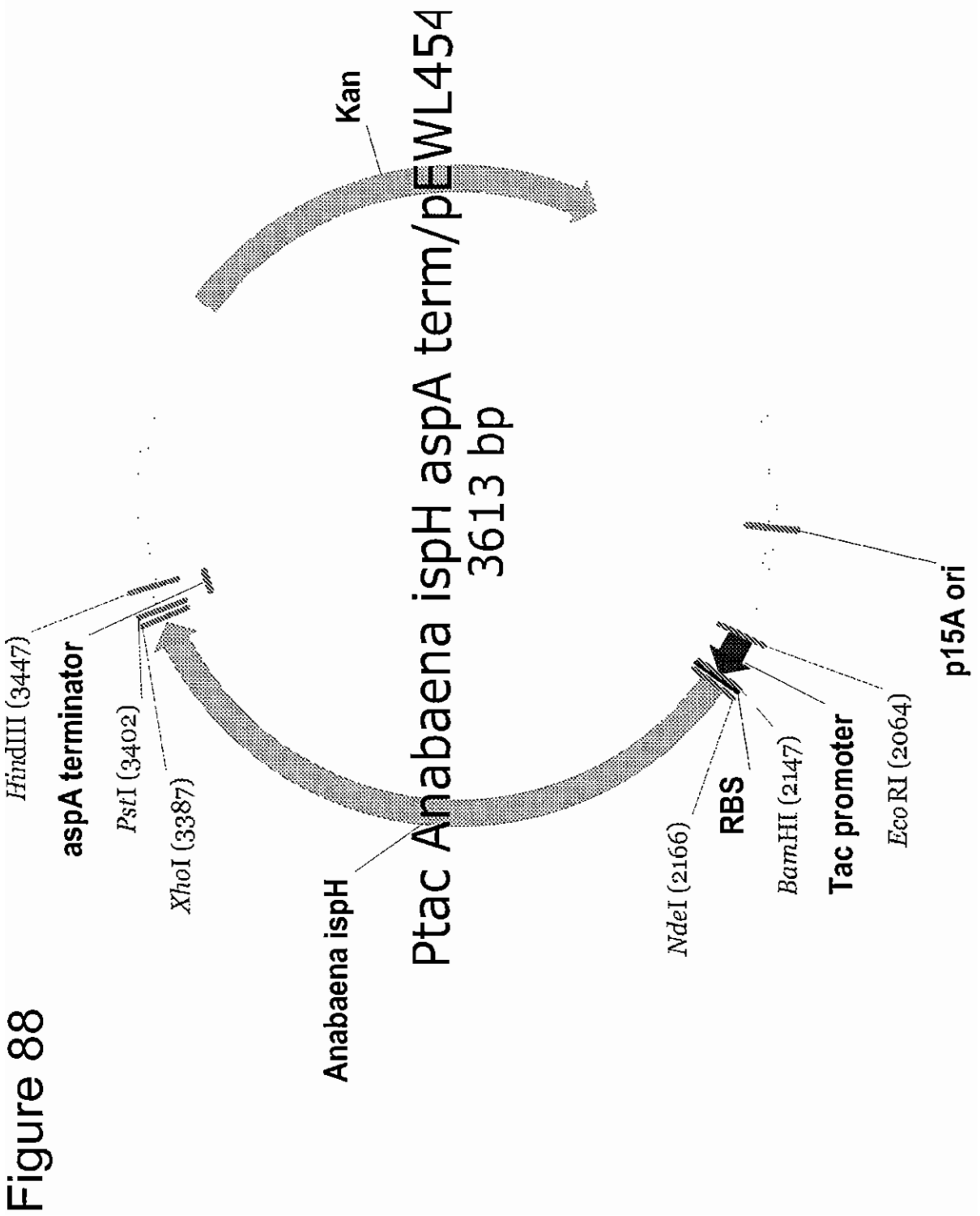

Ptac Anabaena ispH aspA term/pEWL454
3613 bp
Kan
p15A ori
Tac promoter
RBS
Anabaena ispH
aspA terminator
HindIII (3447)
PstI (3402)
XhoI (3387)
NdeI (2166)
BamHI (2147)
EcoRI (2064)

Figure 88

Figure 89

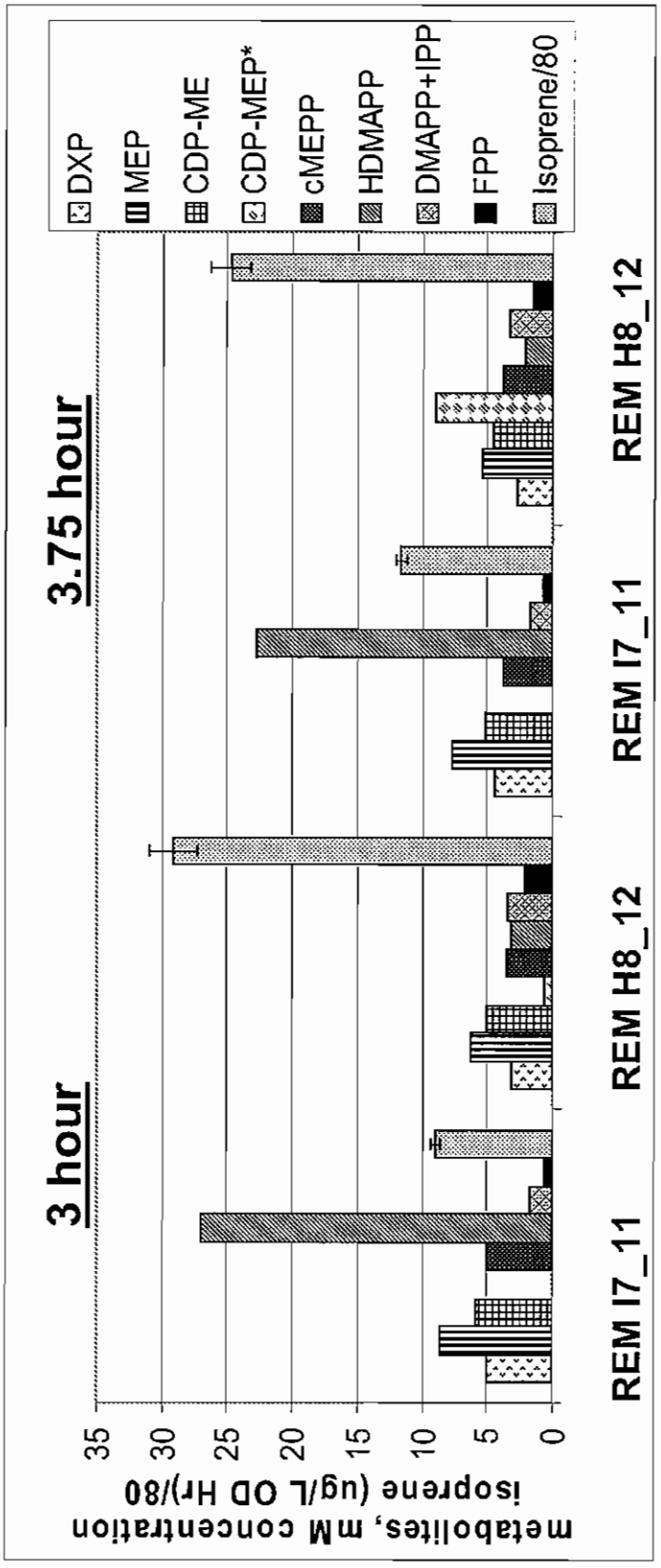

3 hour
3.75 hour
DXP
MEP
CDP-ME
CDP-MEP*
cMEPP
HDMAPP
DMAPP+IPP
FPP
Isoprene/80
metabolites, mM concentration
isoprene (ug/L OD Hr)/80
0
5
10
15
20
25
30
35
REM I7_11
REM H8_12
REM I7_11
REM H8_12

Figure 90A

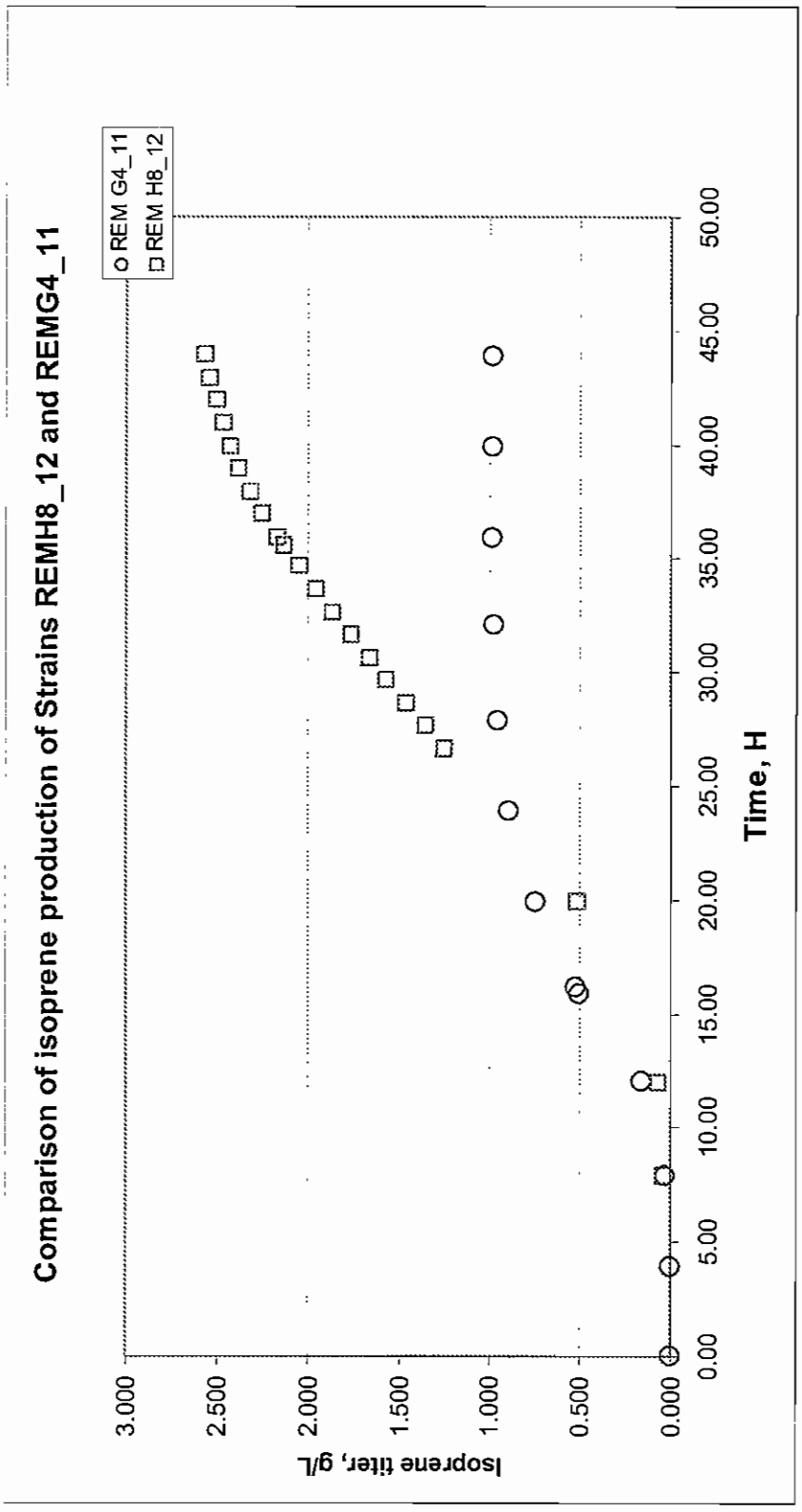

Comparison of isoprene production of Strains REMH8_12 and REMG4_11
REM G4_11
REM H8_12
Isoprene titer, g/L
Time, H
0.000
0.500
1.000
1.500
2.000
2.500
3.000
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00

Figure 90B

B

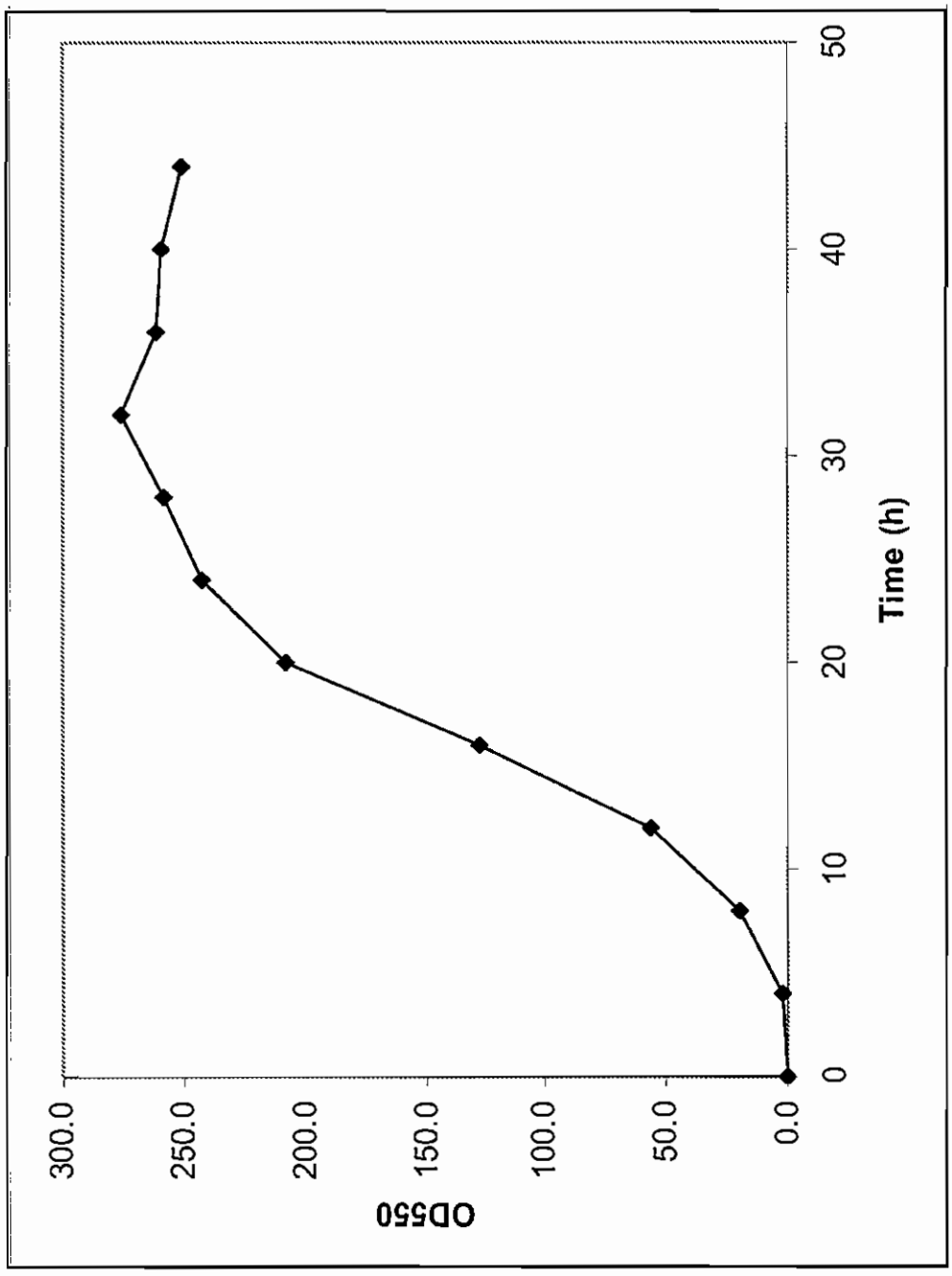
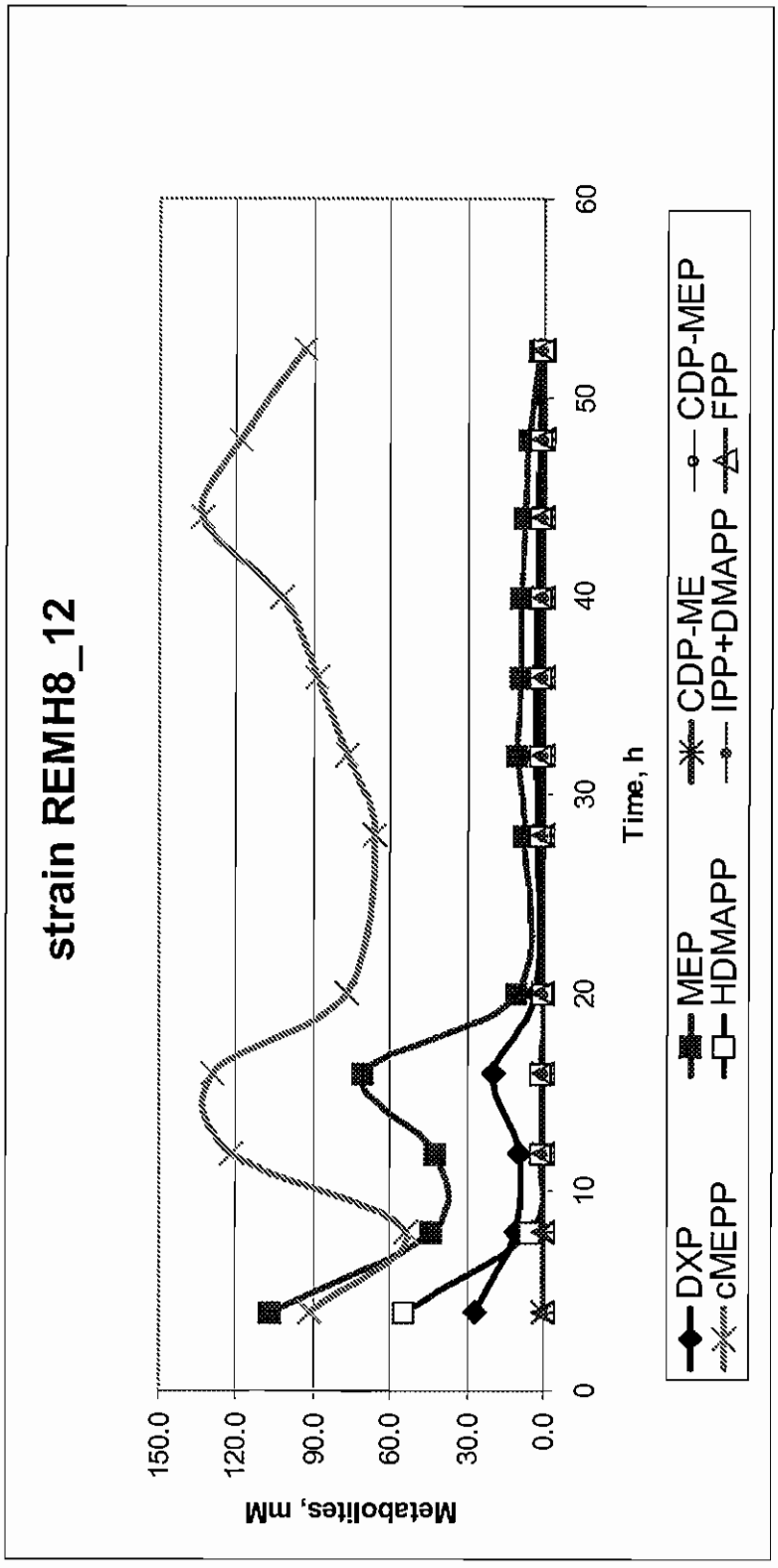


Figure 90C


strain REMH8_12
Metabolites, mM
150.0
120.0
90.0
60.0
30.0
0.0
0
10
20
30
40
50
60
Time, h
DXP
cMEPP
MEP
HDMAPP
CDP-ME
IPP+DMAPP
CDP-MEP
FPP

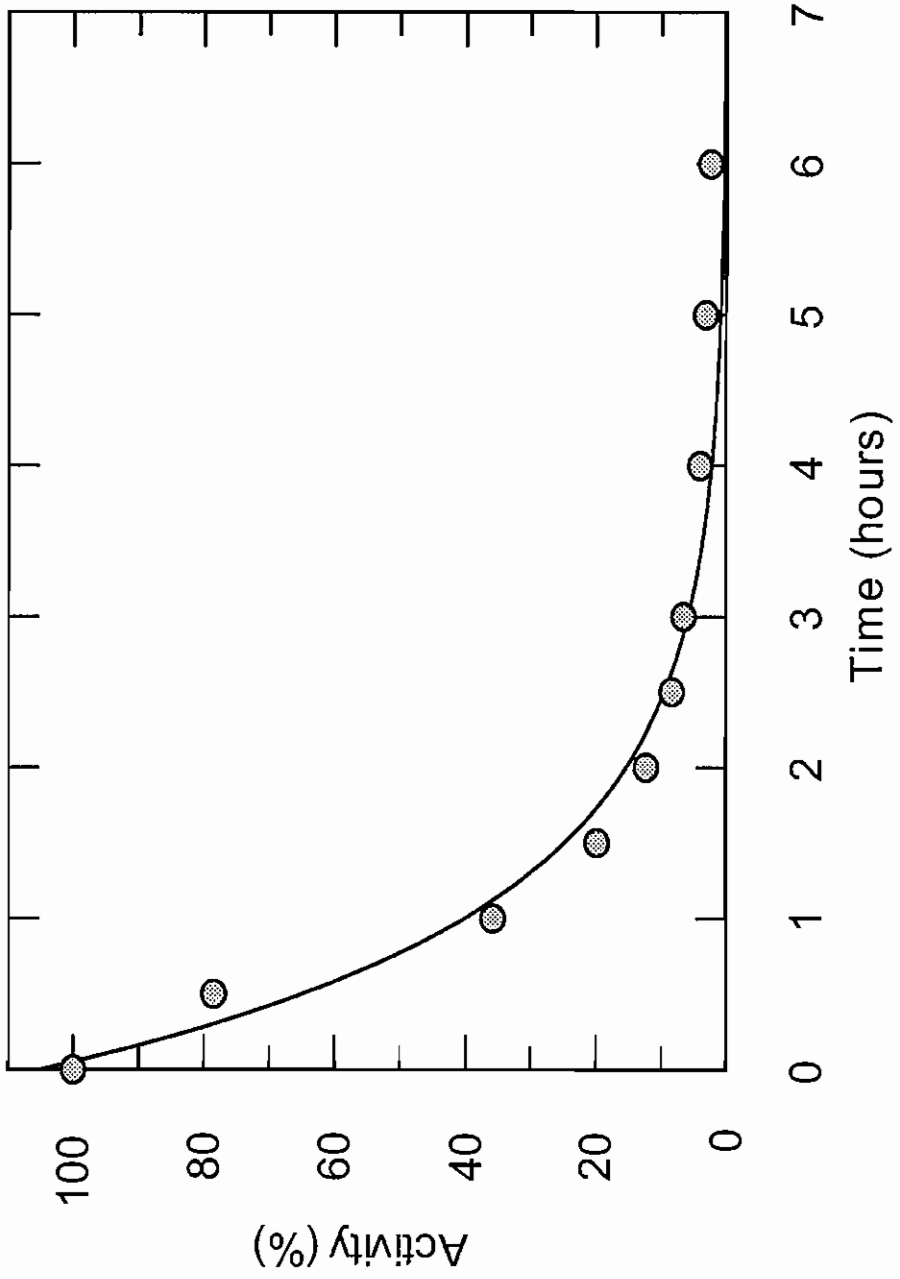


Figure 91

Figure 92A

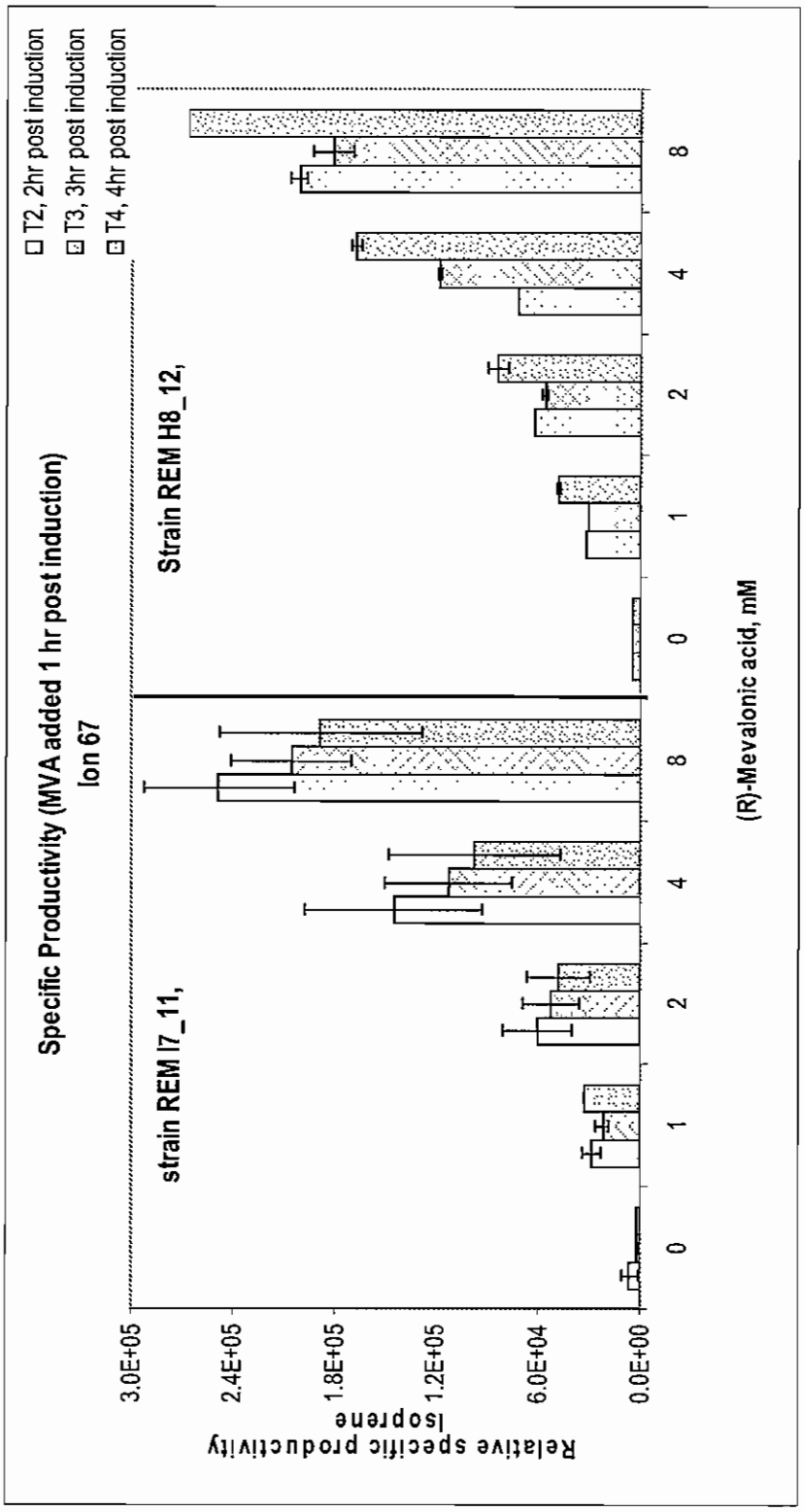
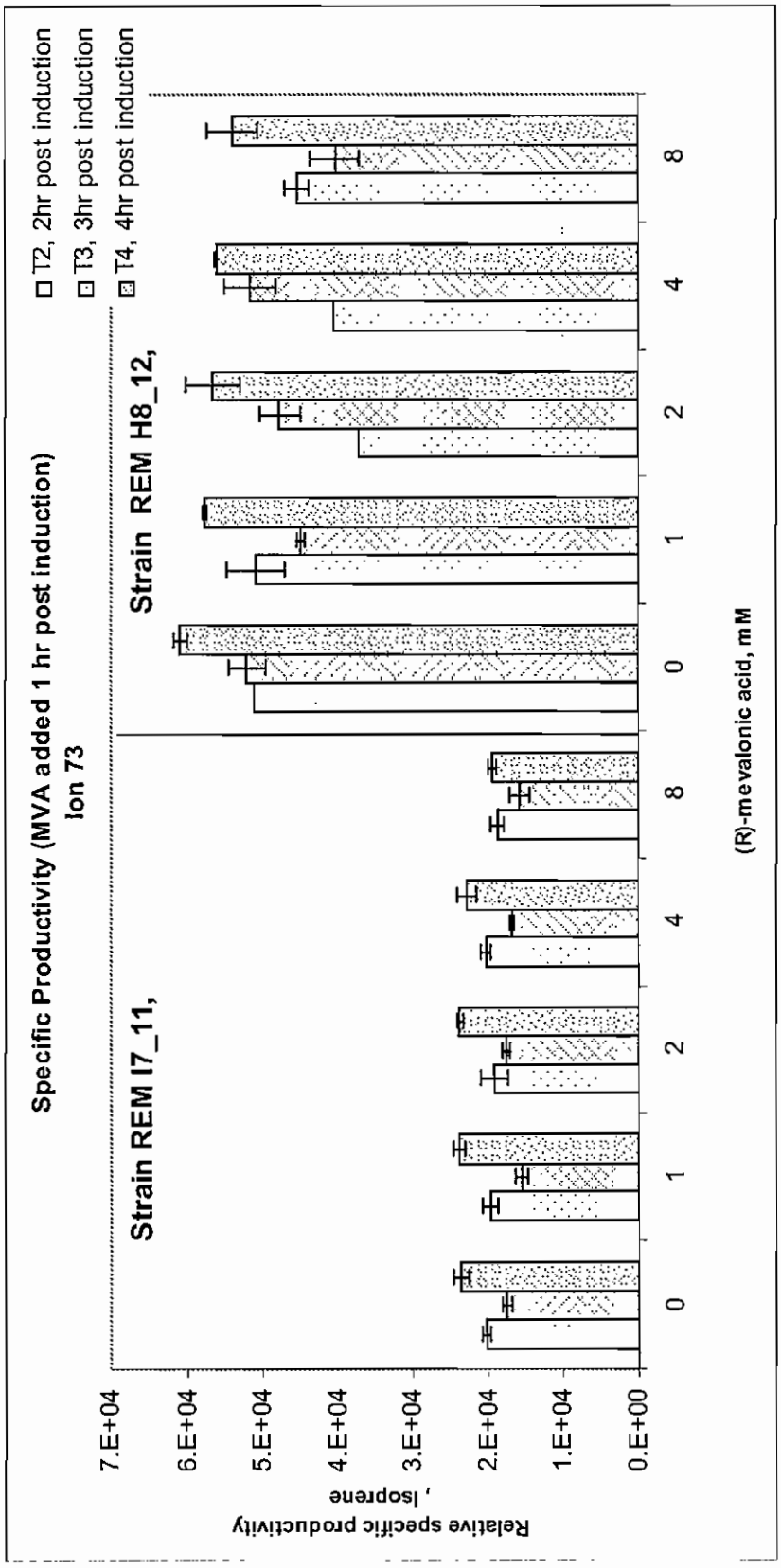

Specific Productivity (MVA added 1 hr post induction)
Ion 67
strain REM I7_11,
Strain REM H8_12,
T2, 2hr post induction
T3, 3hr post induction
T4, 4hr post induction
Relative specific productivity
Isoprene
3.0E+05
2.4E+05
1.8E+05
1.2E+05
6.0E+04
0.0E+00
0
1
2
4
8
0
1
2
4
8
(R)-Mevalonic acid, mM

Figure 92B


Specific Productivity (MVA added 1 hr post induction)
Ion 73
T2, 2hr post induction
T3, 3hr post induction
T4, 4hr post induction
Strain REM I7_11,
Strain REM H8_12,
Relative specific productivity, Isoprene
7.E+04
6.E+04
5.E+04
4.E+04
3.E+04
2.E+04
1.E+04
0.E+00
0
1
2
4
8
0
1
2
4
8
(R)-mevalonic acid, mM

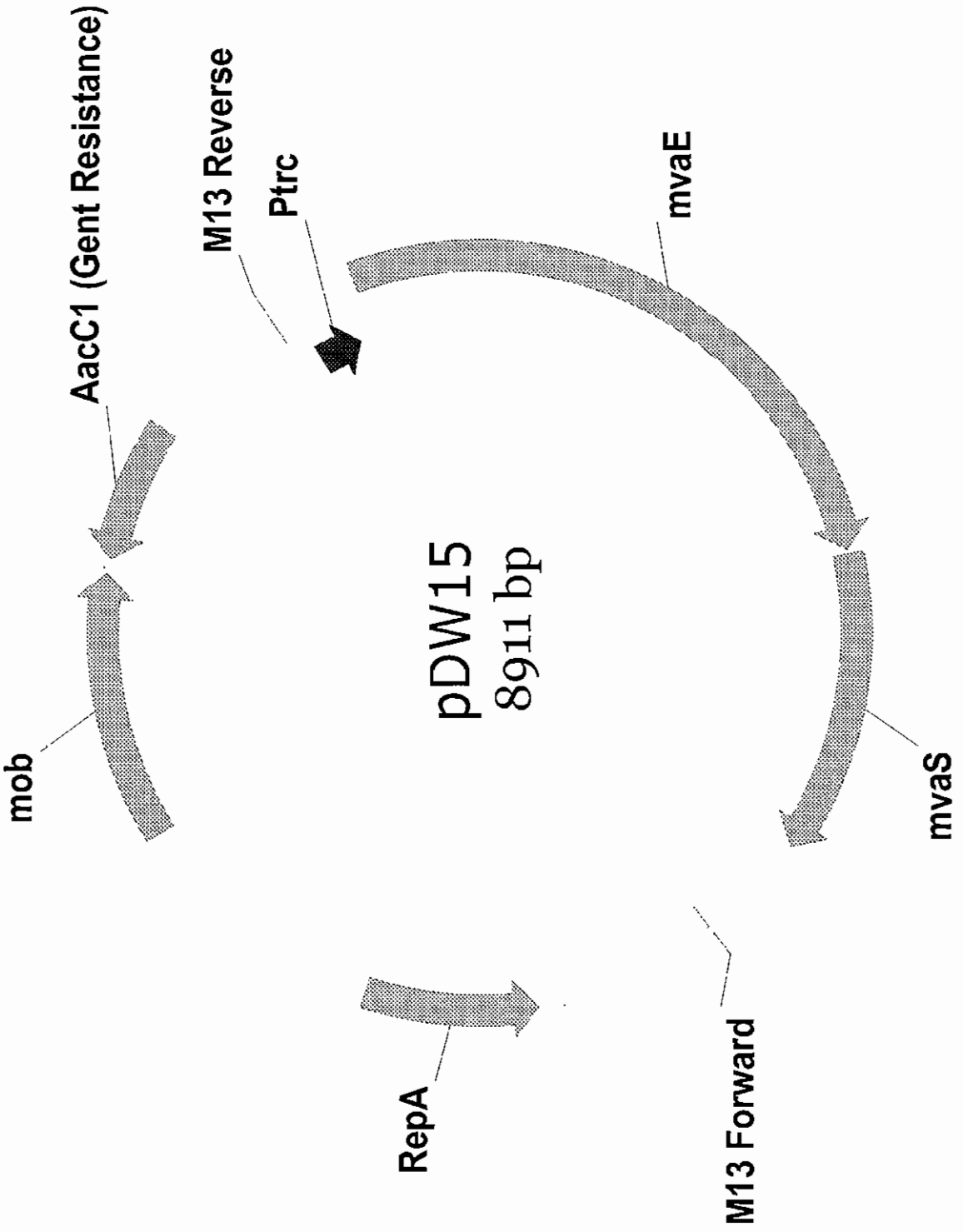

AacC1 (Gent Resistance)
M13 Reverse
Ptrc
mvaE
mob
pDW15
8911 bp
mvaS
RepA
M13 Forward

Figure 93

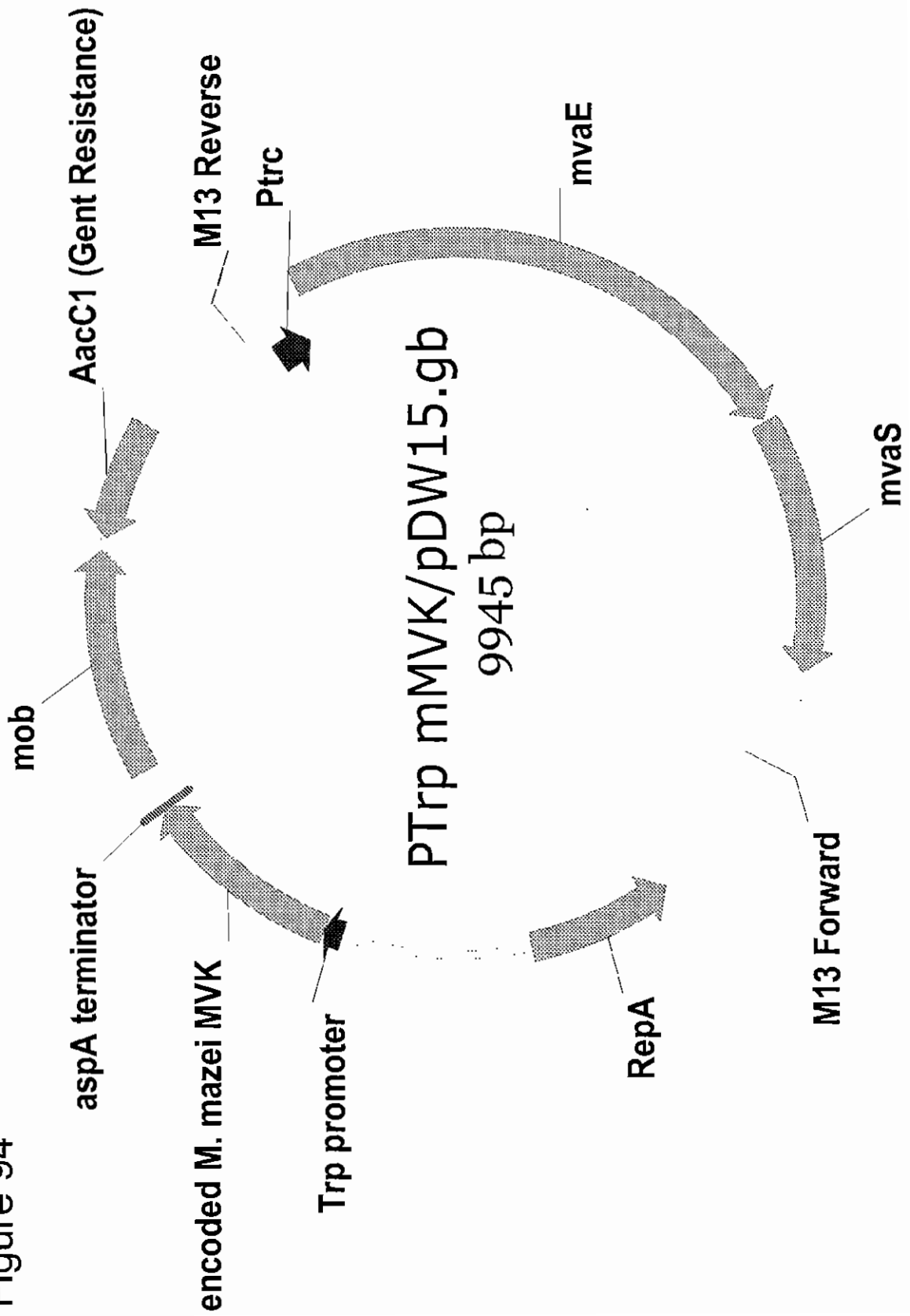

PTrp mMVK/pDW15.gb
9945 bp
AacC1 (Gent Resistance)
M13 Reverse
Ptrc
mvaE
mvaS
M13 Forward
RepA
Trp promoter
encoded M. mazei MVK
aspA terminator
mob

Figure 94

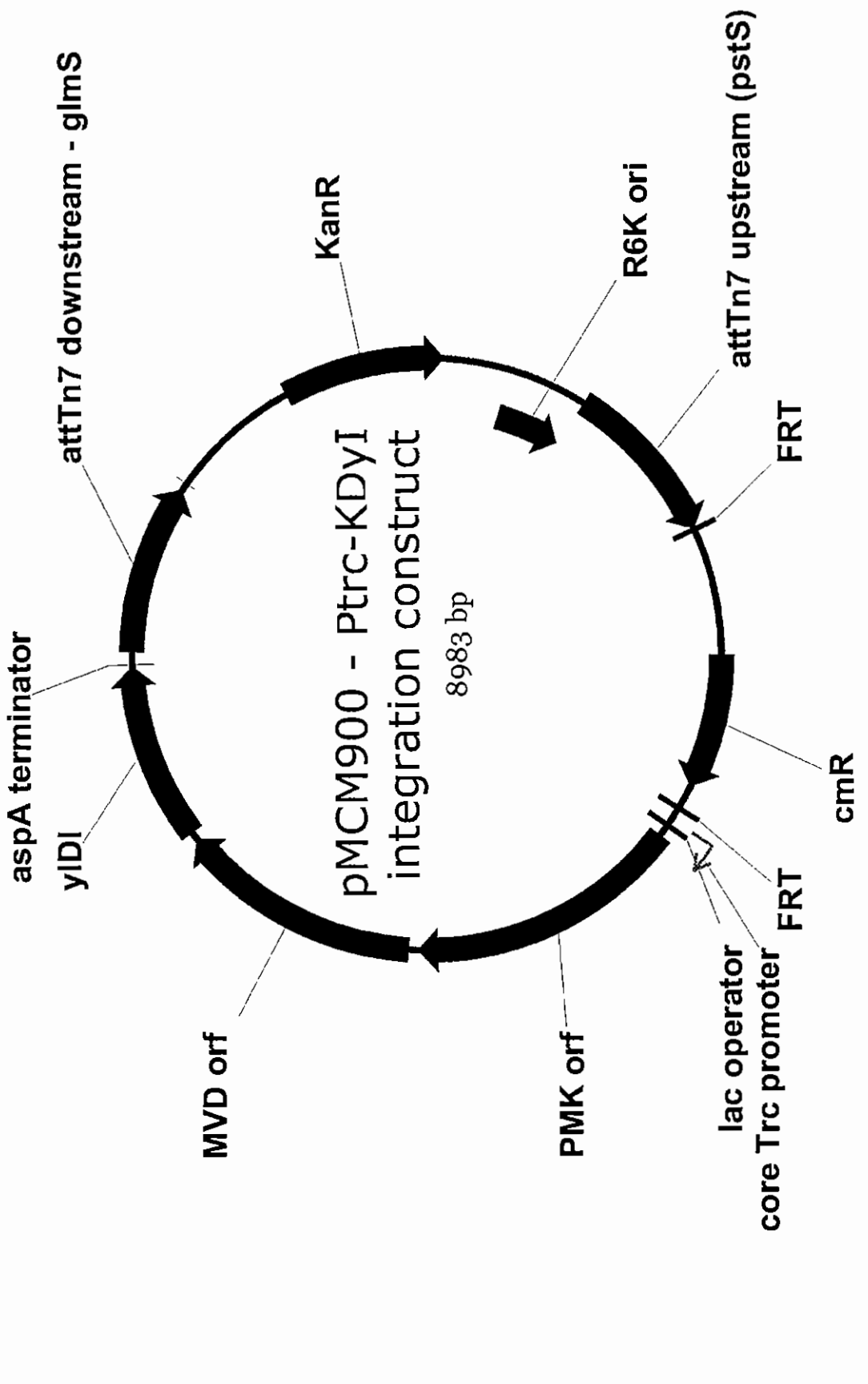

pMCM900 - Ptrc-KDyI
integration construct
8983 bp
attTn7 downstream - glmS
KanR
R6K ori
attTn7 upstream (pstS)
FRT
cmR
FRT
lac operator
core Trc promoter
PMK orf
MVD orf
yIDI
aspA terminator

Figure 95

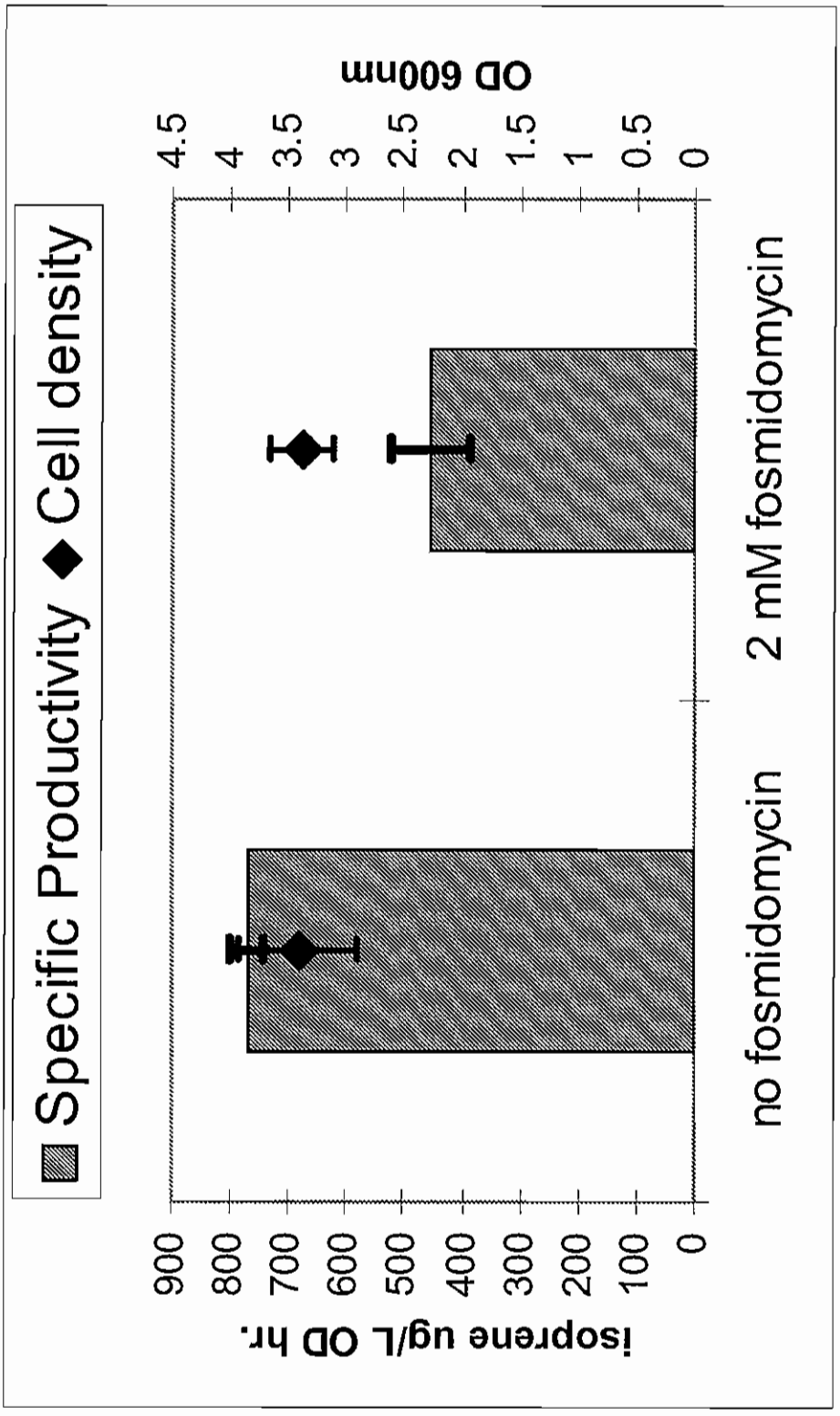

Specific Productivity
Cell density
isoprene ug/L OD hr.
OD 600nm
0
100
200
300
400
500
600
700
800
900
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
no fosmidomycin
2 mM fosmidomycin

Figure 96

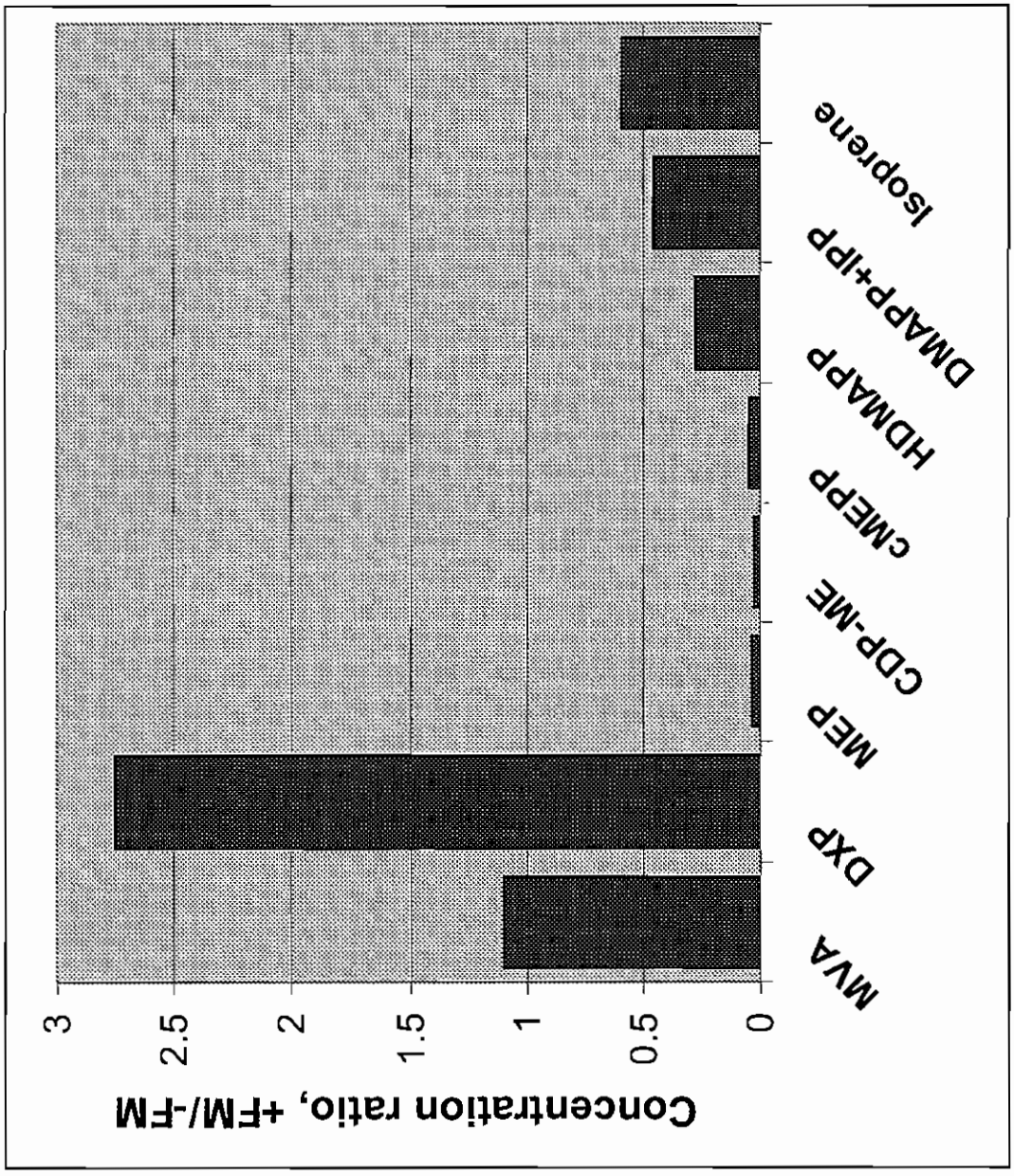

Concentration ratio, +FM/-FM
0
0.5
1
1.5
2
2.5
3
MVA
DXP
MEP
CDP-ME
cMEPP
HDMAPP
DMAPP+IPP
Isoprene

Figure 97

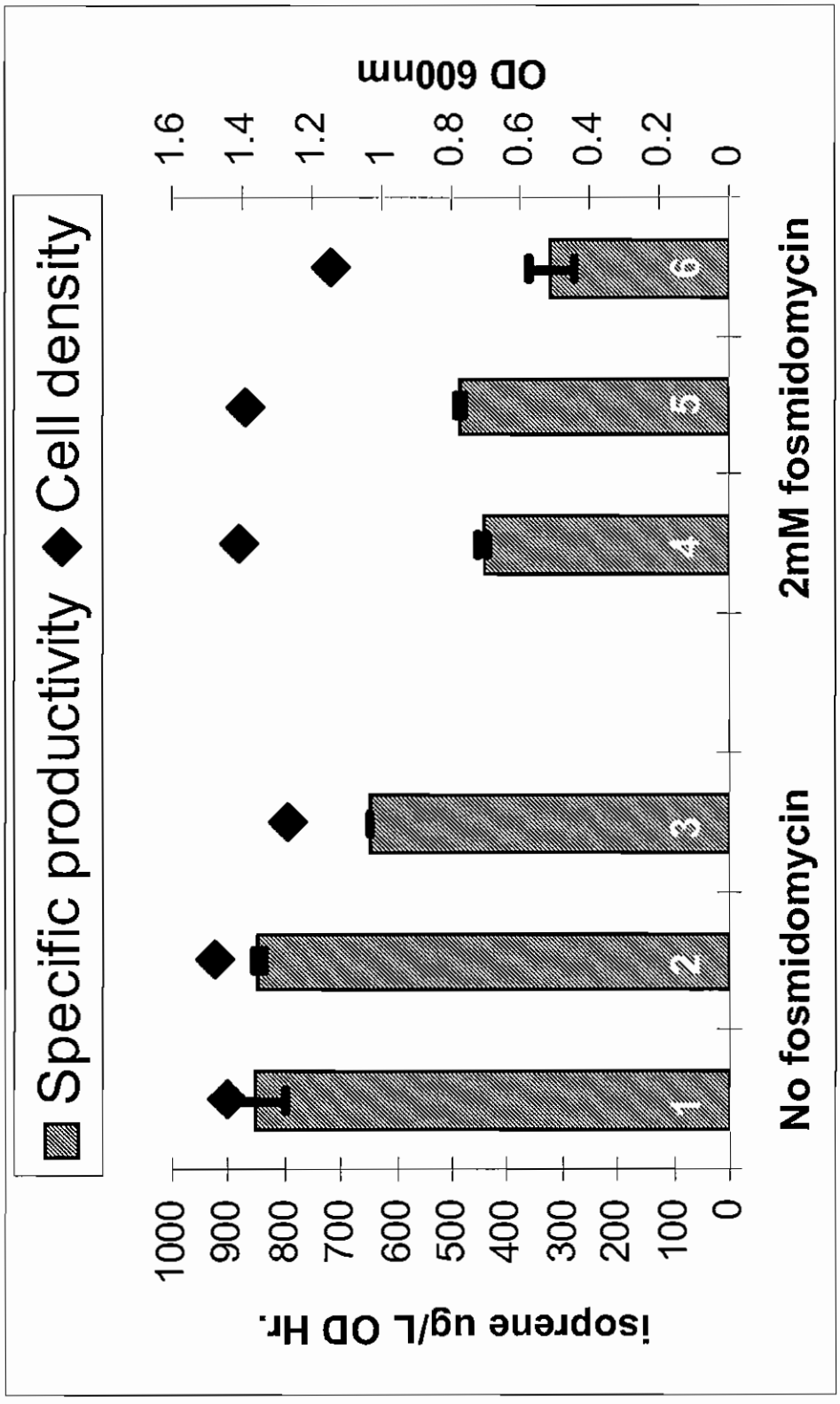

Specific productivity
Cell density
isoprene ug/L OD Hr.
OD 600nm
1000
900
800
700
600
500
400
300
200
100
0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
No fosmidomycin
2mM fosmidomycin

Figure 98

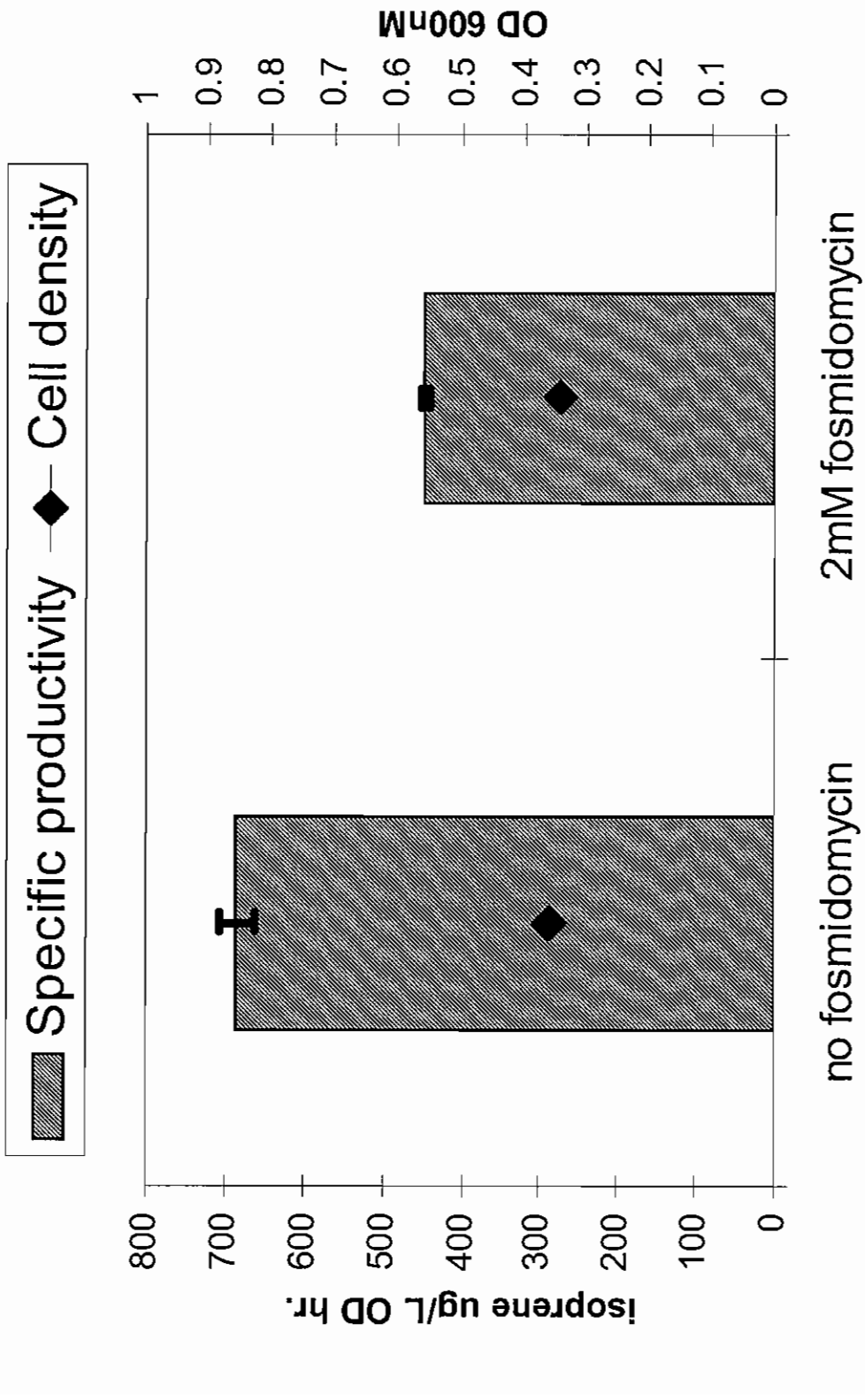

Specific productivity
Cell density
isoprene ug/L OD hr.
OD 600nM
0
100
200
300
400
500
600
700
800
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
no fosmidomycin
2mM fosmidomycin

Figure 99

Figure 100

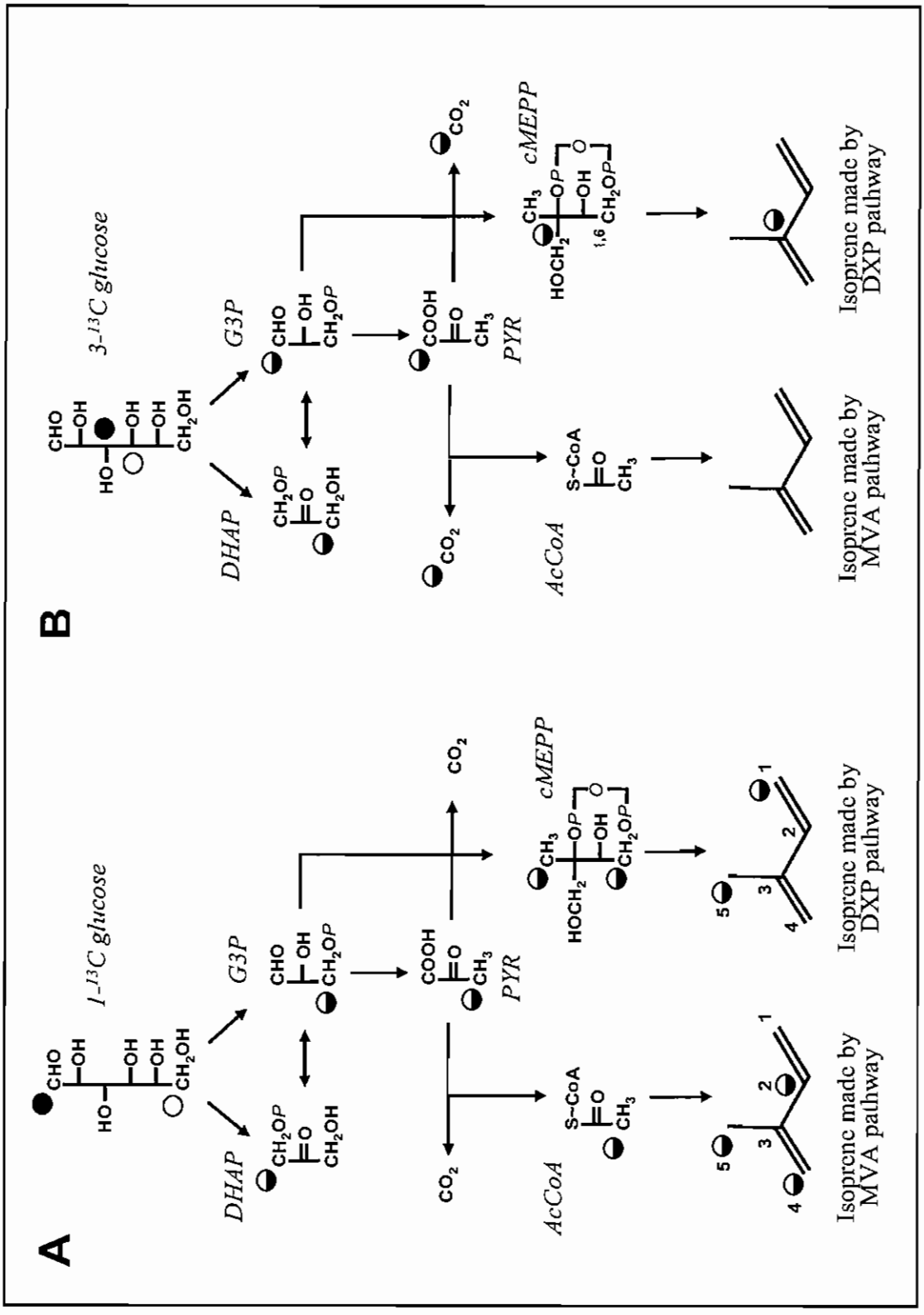

A
1-13C glucose
DHAP
G3P
PYR
AcCoA
cMEPP
CO2
Isoprene made by MVA pathway
Isoprene made by DXP pathway
B
3-13C glucose
DHAP
G3P
PYR
AcCoA
cMEPP
CO2
Isoprene made by MVA pathway
Isoprene made by DXP pathway

Figure 101 panel A

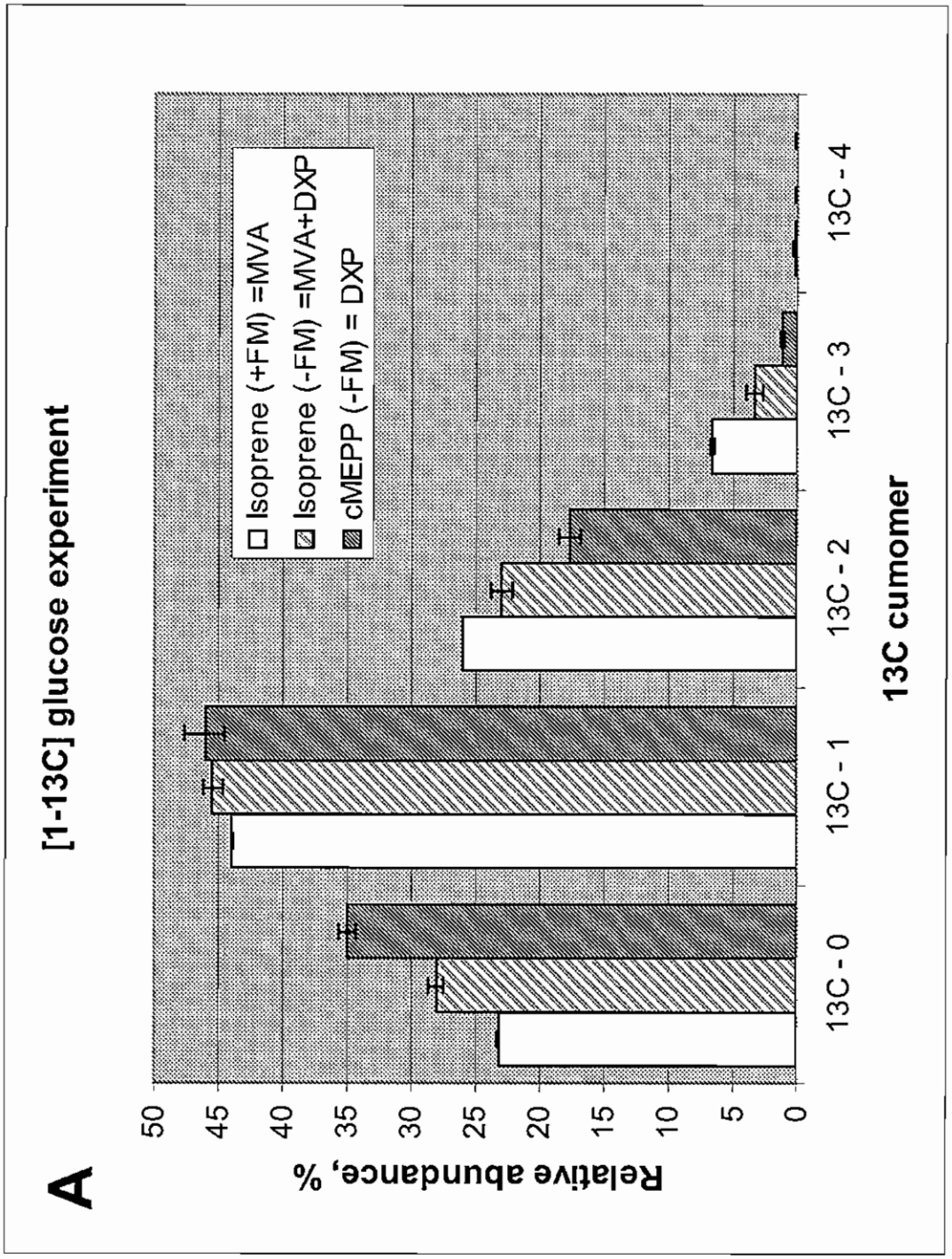


Figure 101 panel B

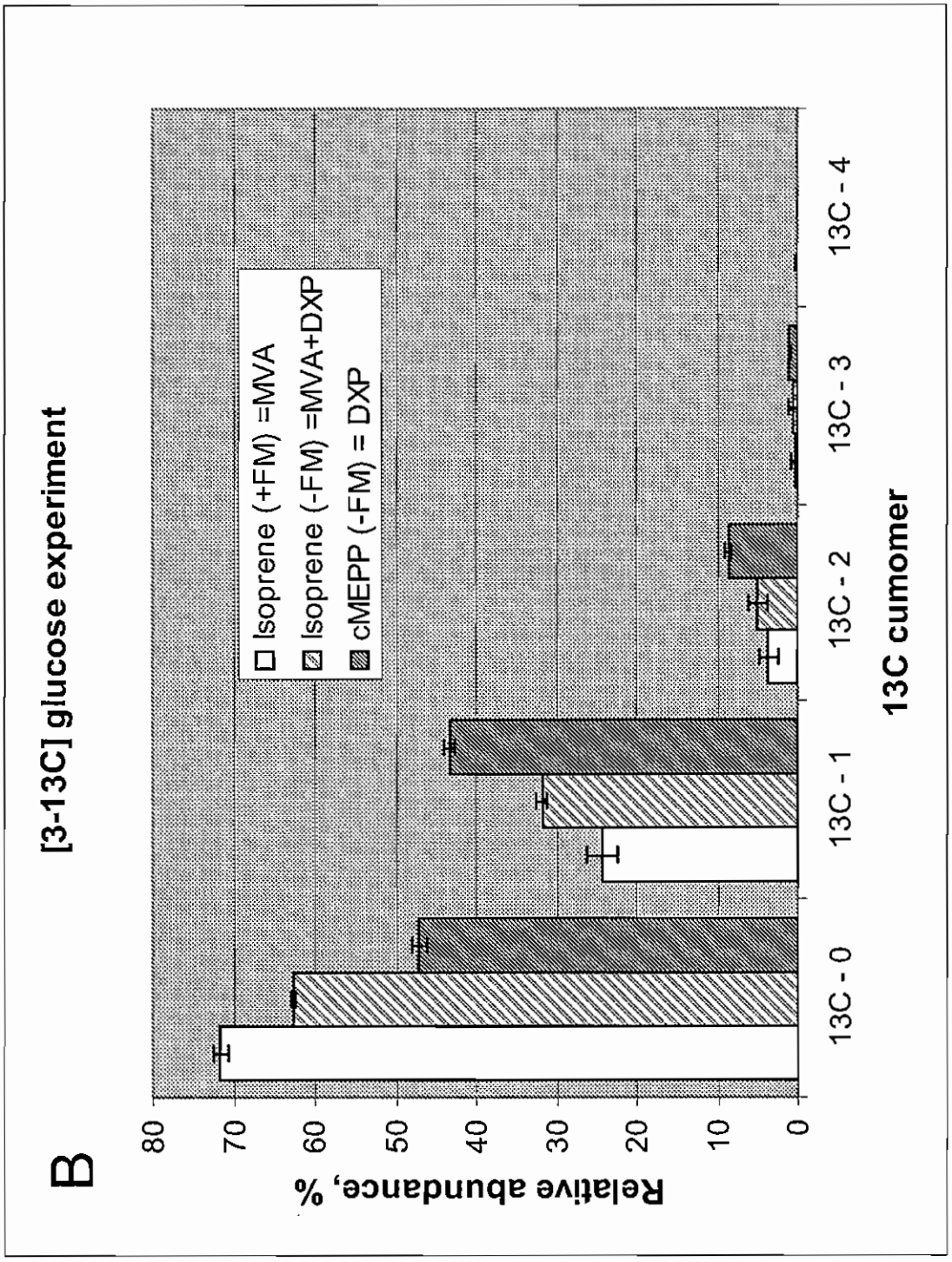


Figure 102

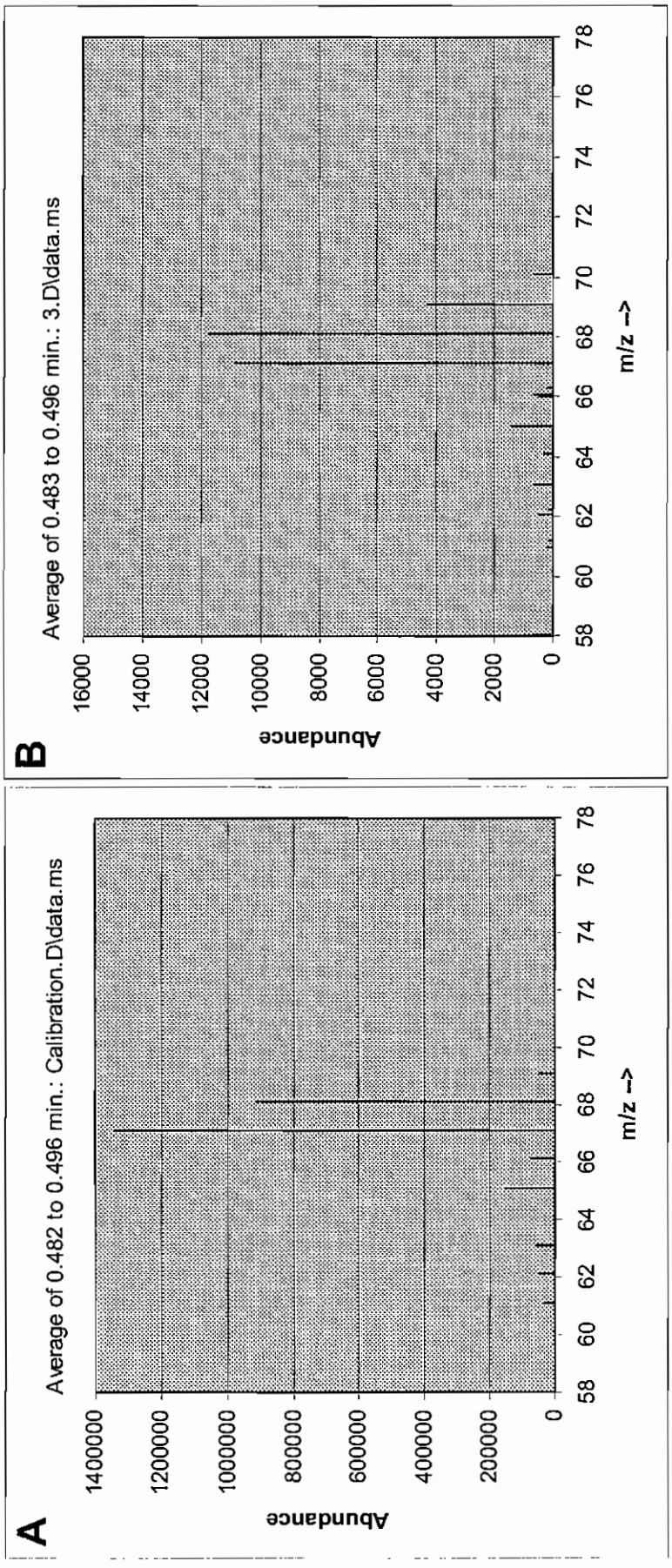

A
Average of 0.482 to 0.496 min.: Calibration.D\data.ms
Abundance
1400000
1200000
1000000
800000
600000
400000
200000
0
58 60 62 64 66 68 70 72 74 76 78
m/z -->
B
Average of 0.483 to 0.496 min.: 3.D\data.ms
Abundance
16000
14000
12000
10000
8000
6000
4000
2000
0
58 60 62 64 66 68 70 72 74 76 78
m/z -->

Figure 103

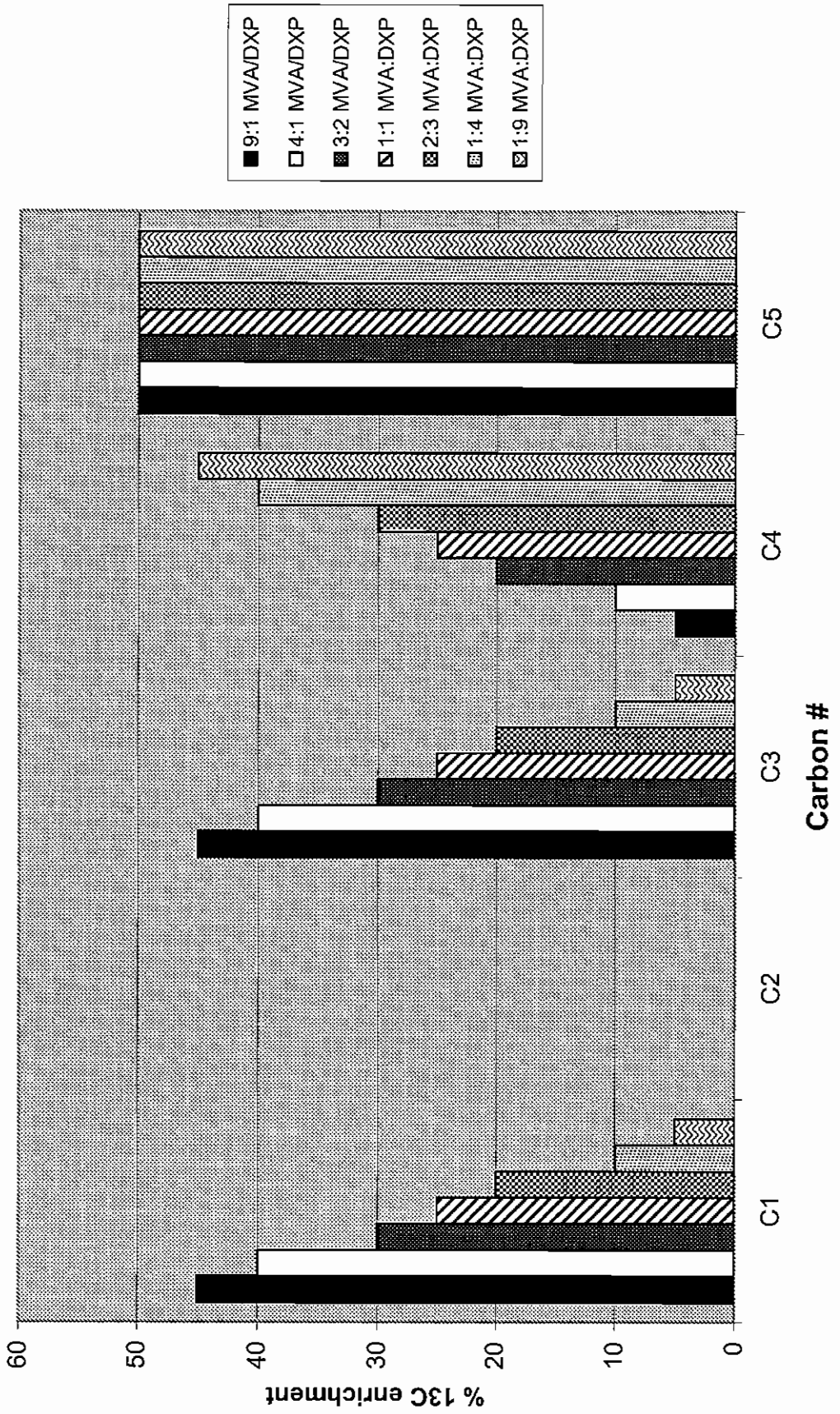

Isoprene ^{13}C isotope enrichment vs. MVA/DXP ratio
9:1 MVA/DXP
4:1 MVA/DXP
3:2 MVA/DXP
1:1 MVA:DXP
2:3 MVA:DXP
1:4 MVA:DXP
1:9 MVA:DXP
% 13C enrichment
0
10
20
30
40
50
60
C1
C2
C3
C4
C5
Carbon #

Figure 104

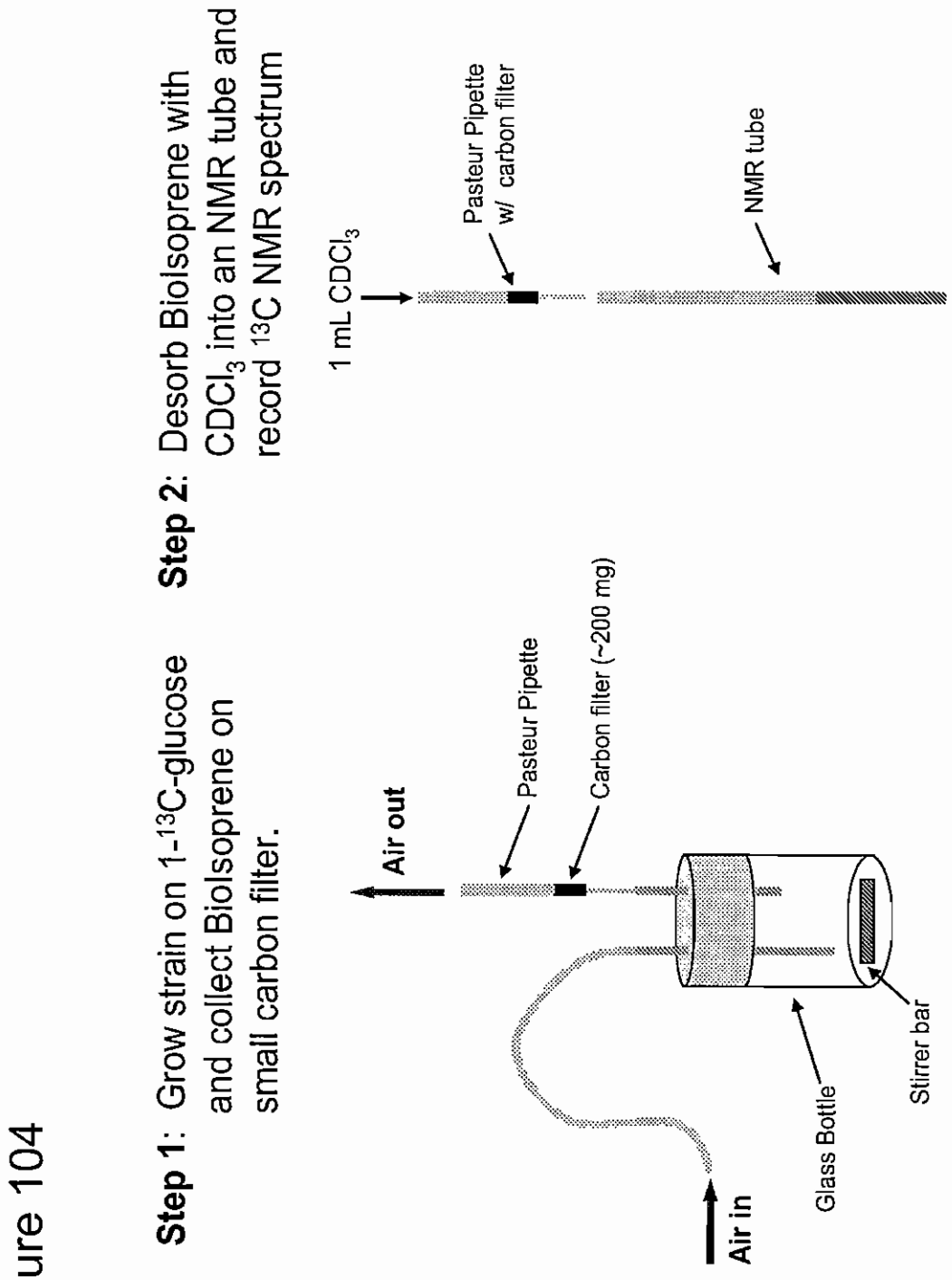

Step 1: Grow strain on 1-13C-glucose and collect Biolsoprene on small carbon filter.
Step 2: Desorb Biolsoprene with CDCl3 into an NMR tube and record 13C NMR spectrum
1 mL CDCl3
Pasteur Pipette w/ carbon filter
NMR tube
Air out
Pasteur Pipette
Carbon filter (~200 mg)
Air in
Glass Bottle
Stirrer bar

Figure 105

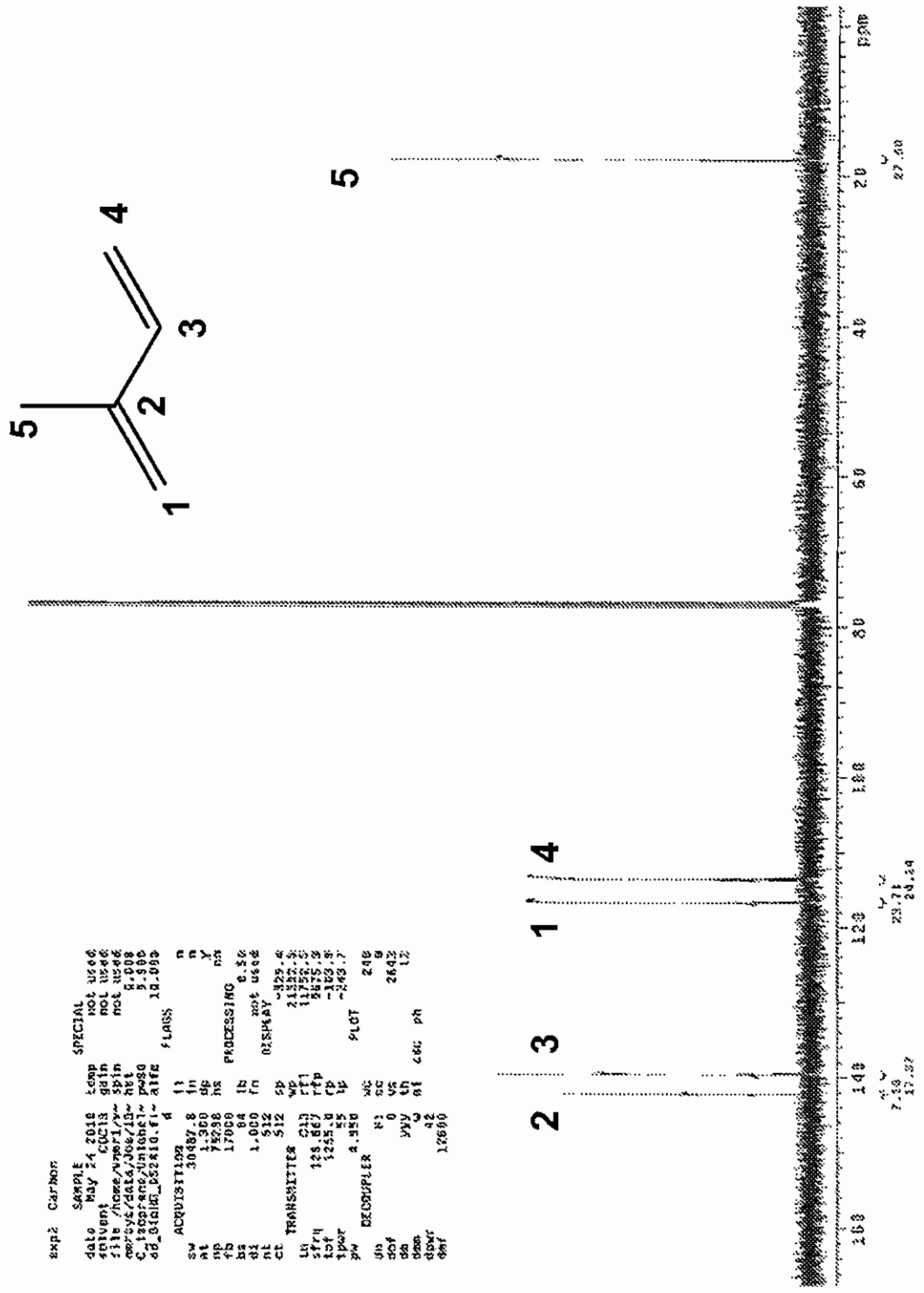


Figure 106

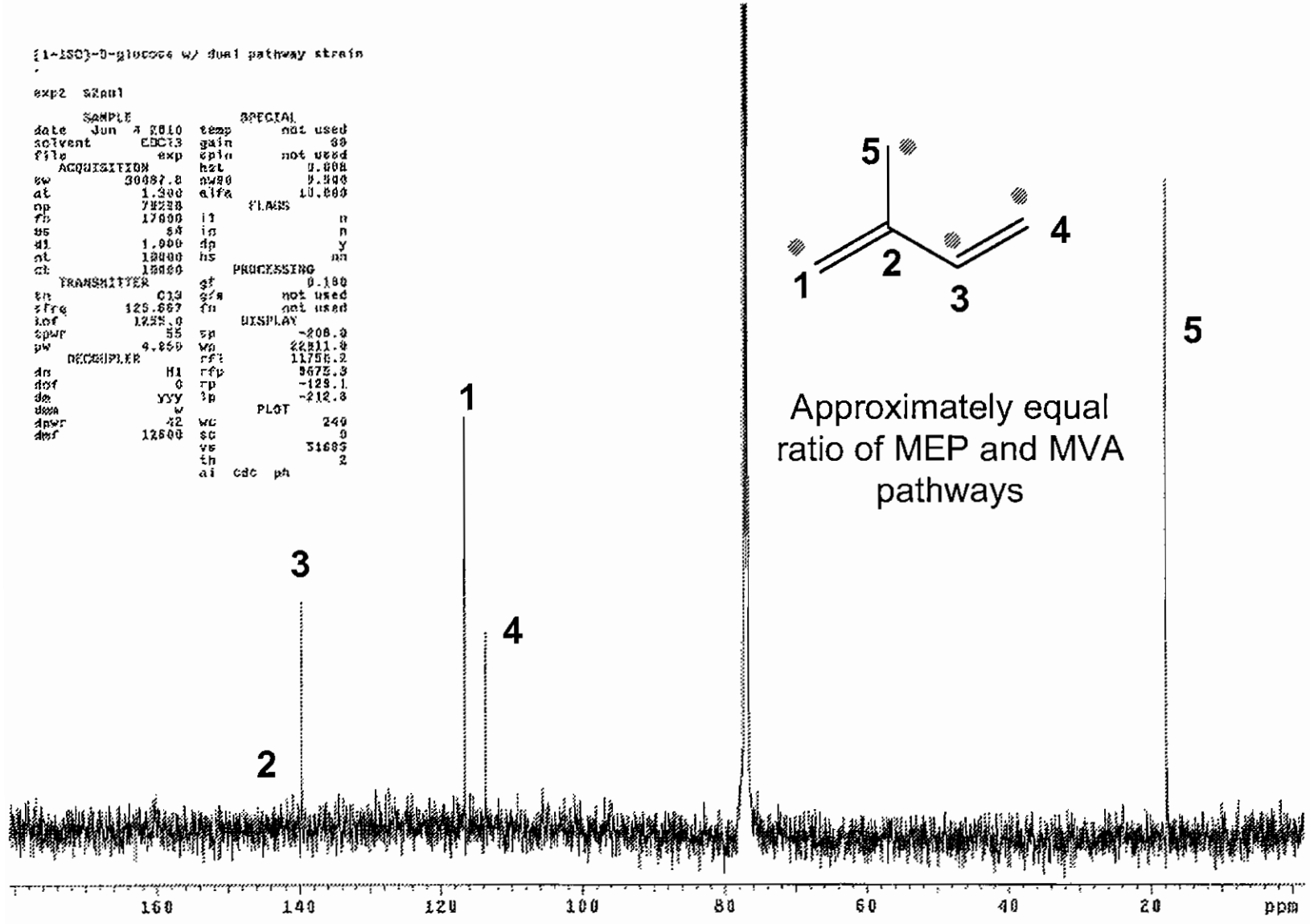
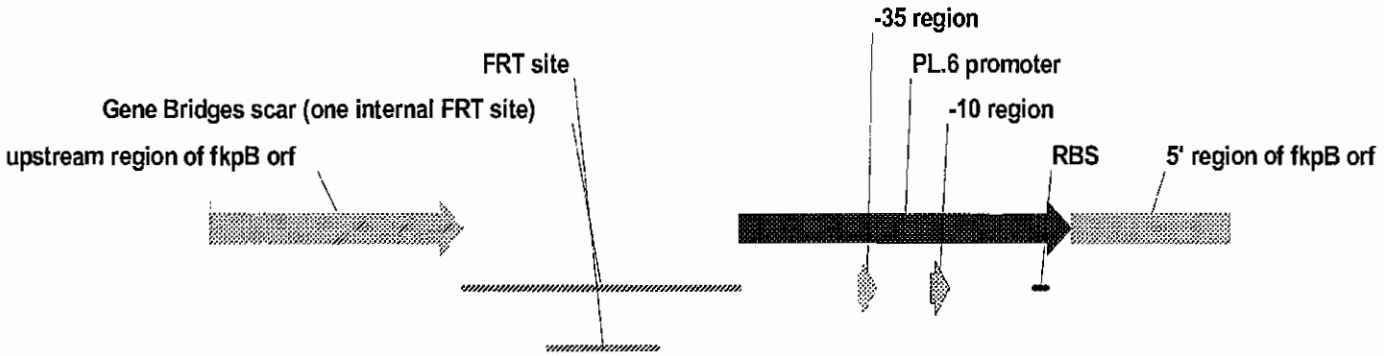

{1-13C}-D-glucose w/ dual pathway strain
exp2 s2pul
SAMPLE
date Jun 4 2010
solvent CDCl3
file exp
ACQUISITION
sw 30087.8
at 1.300
np 78288
fb 17000
ss 8
d1 1.000
nt 18000
ct 18060
TRANSMITTER
tn C13
sfrq 125.667
tof 1255.0
tpwr 55
pw 4.850
DECOUPLER
dn H1
dof 0
dm yyy
dmm w
dpwr 42
dmf 12500
SPECIAL
temp not used
gain 30
spin not used
hzt 0.008
nw90 9.500
alfa 10.000
FLAGS
il n
in n
dp y
hs nn
PROCESSING
gf 0.100
gfs not used
fn not used
DISPLAY
sp -208.9
wp 22811.9
rfl 11756.2
rfp 9673.3
rp -128.1
lp -212.8
PLOT
wc 249
sc 0
vs 3168
th 2
ai cdc ph
1
2
3
4
5
Approximately equal ratio of MEP and MVA pathways
160
140
120
100
80
60
40
20
ppm

Figure 107

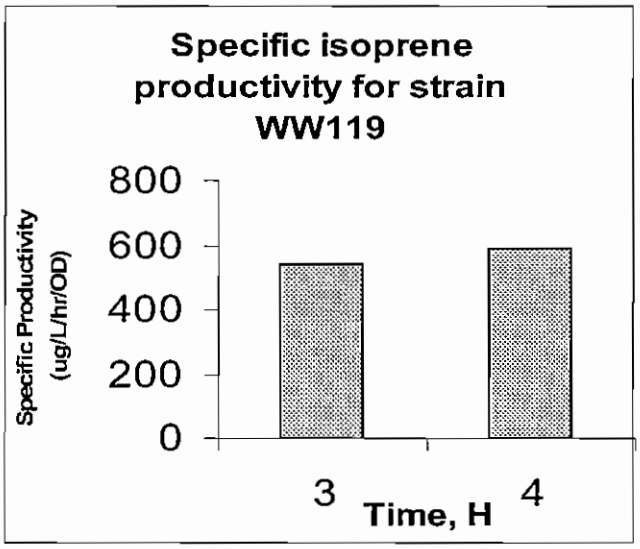


PL.6 fkpB locus

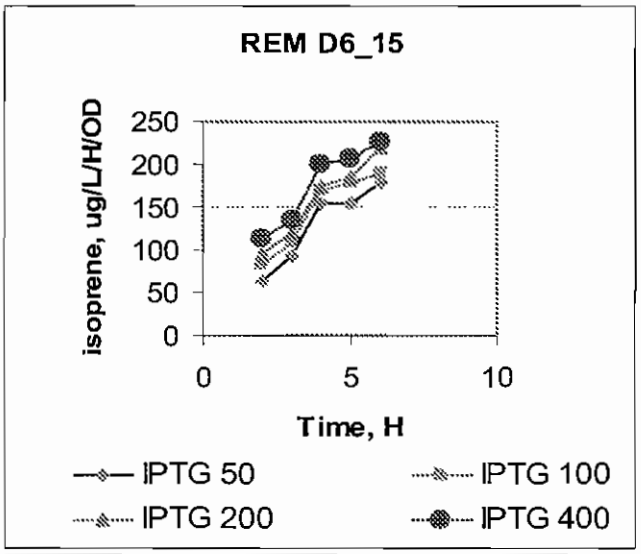
323 bp

agattgctgcggaaatcgtaggccggataaggcgttacgccgcatccggcaaaaaatcctaaaatataa
gagcaaacctgc**aattaaccctcactaaagggcggccgc**<u>**gaagttcctattctctagaaagtat**</u>
<u>**aggaacttc**</u>**ctcgagccctatagtgagtcgtatta**agataaccatctgcggtgataaattatctctgg
cggtgttgacataaataccactggcggtgatactgagcacatcagcaggacgcactgcaaaggagg
taaaaacatgtctgaatctgtacagagcaatagcgccgtcctggtgcacttcacgct

A



B



C

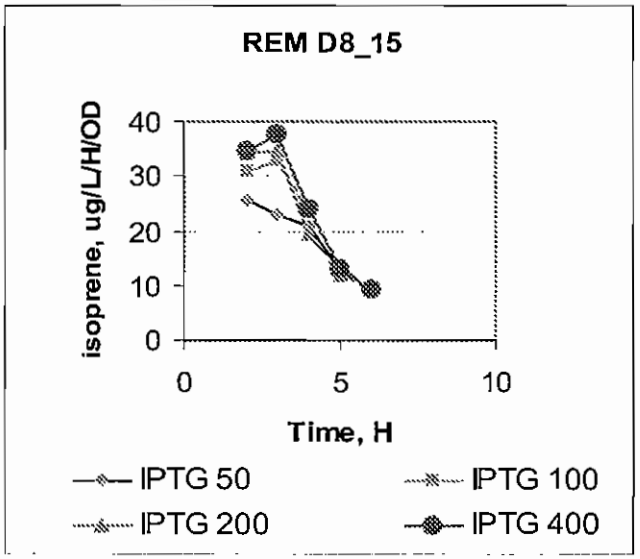


Figure 108

Figure 108 (con't)

D

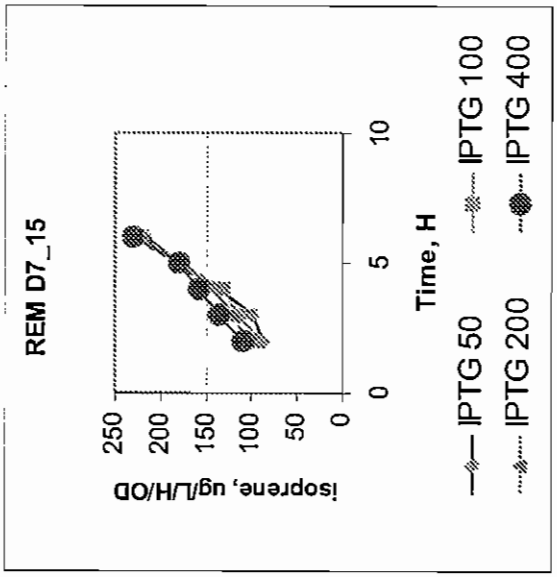


Figure 109

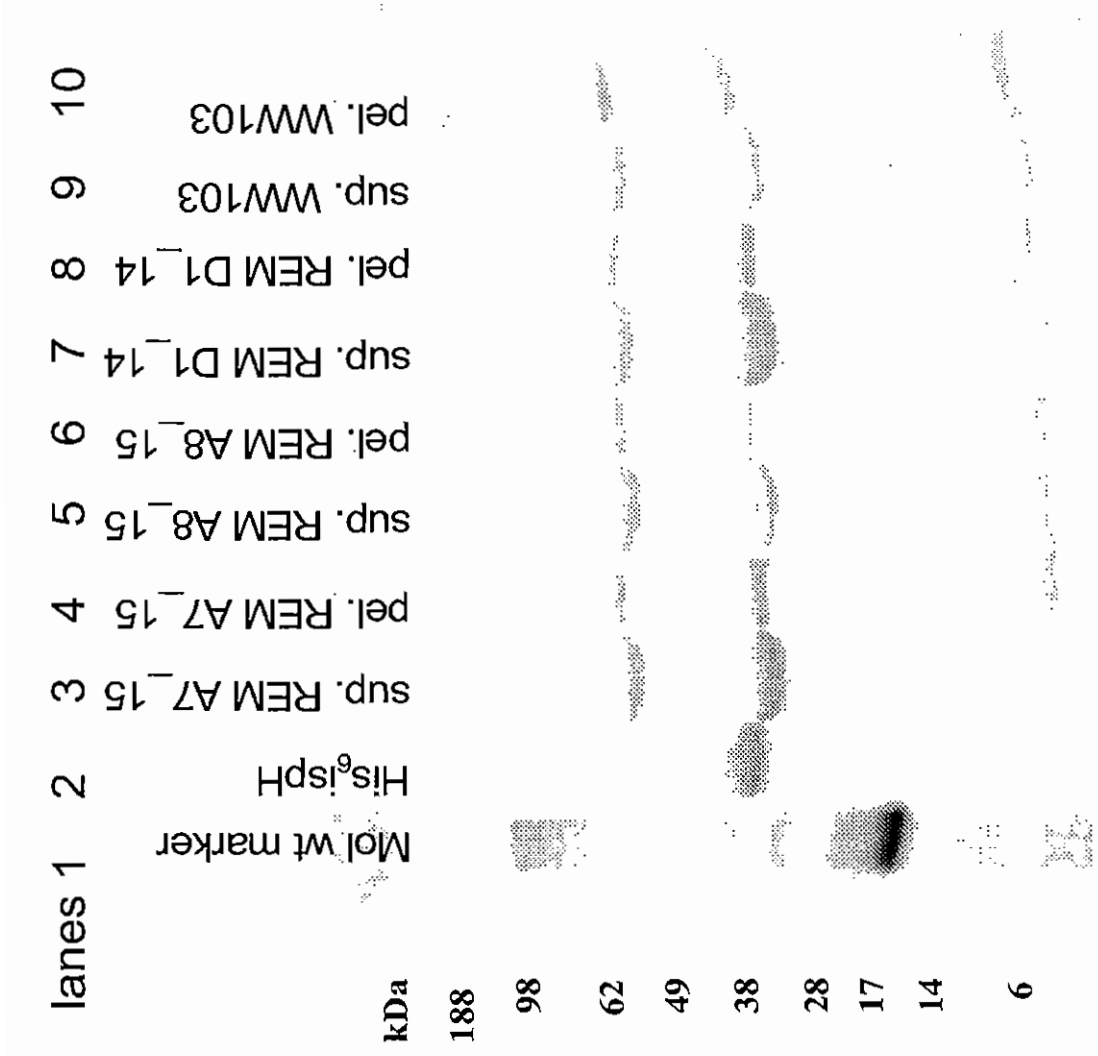
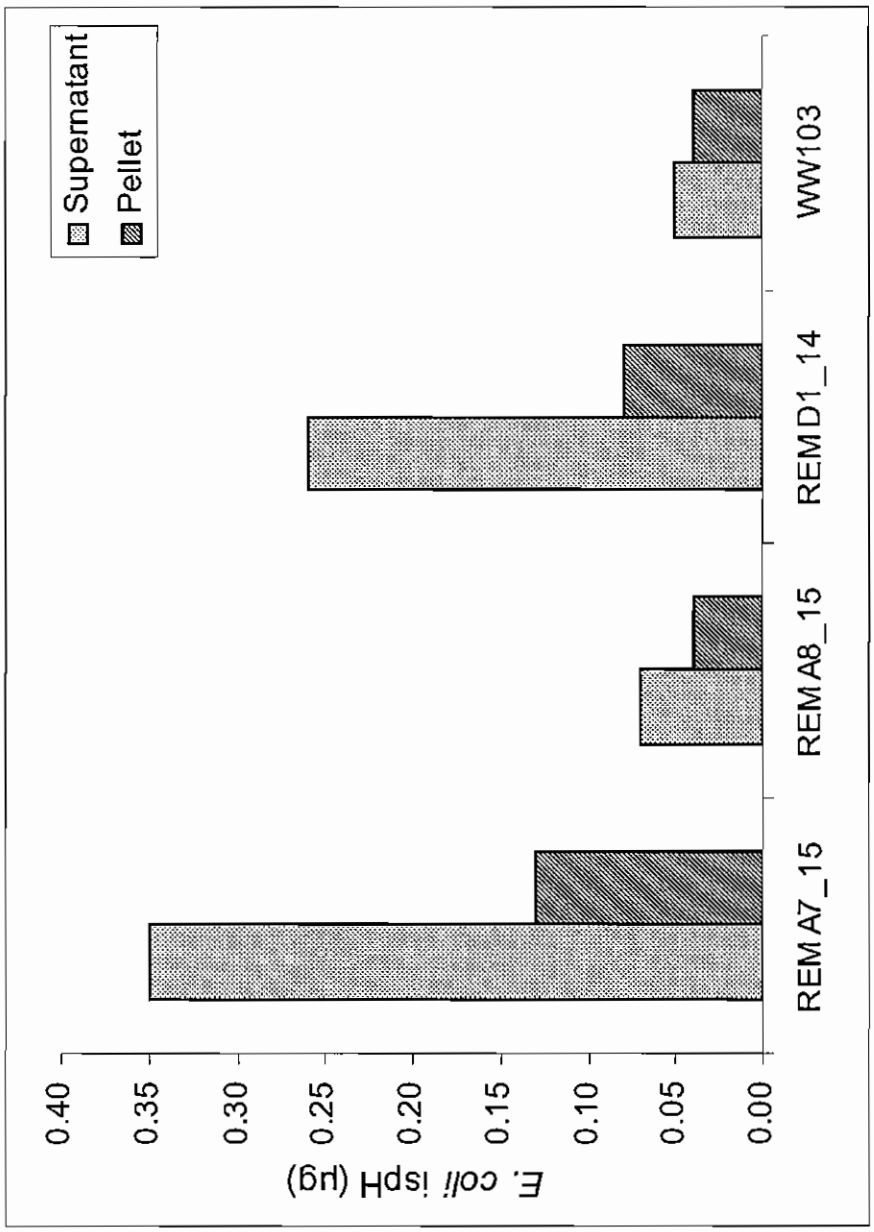

kDa
188
98
62
49
38
28
17
14
6
lanes 1
Mol. wt marker
2
His6ispH
3
sup. REM A7_15
4
pel. REM A7_15
5
sup. REM A8_15
6
pel. REM A8_15
7
sup. REM D1_14
8
pel. REM D1_14
9
sup. WW103
10
pel. WW103

Figure 110


Supernatant
Pellet
E. coli isph (µg)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
REMA7_15
REMA8_15
REMD1_14
WW103