

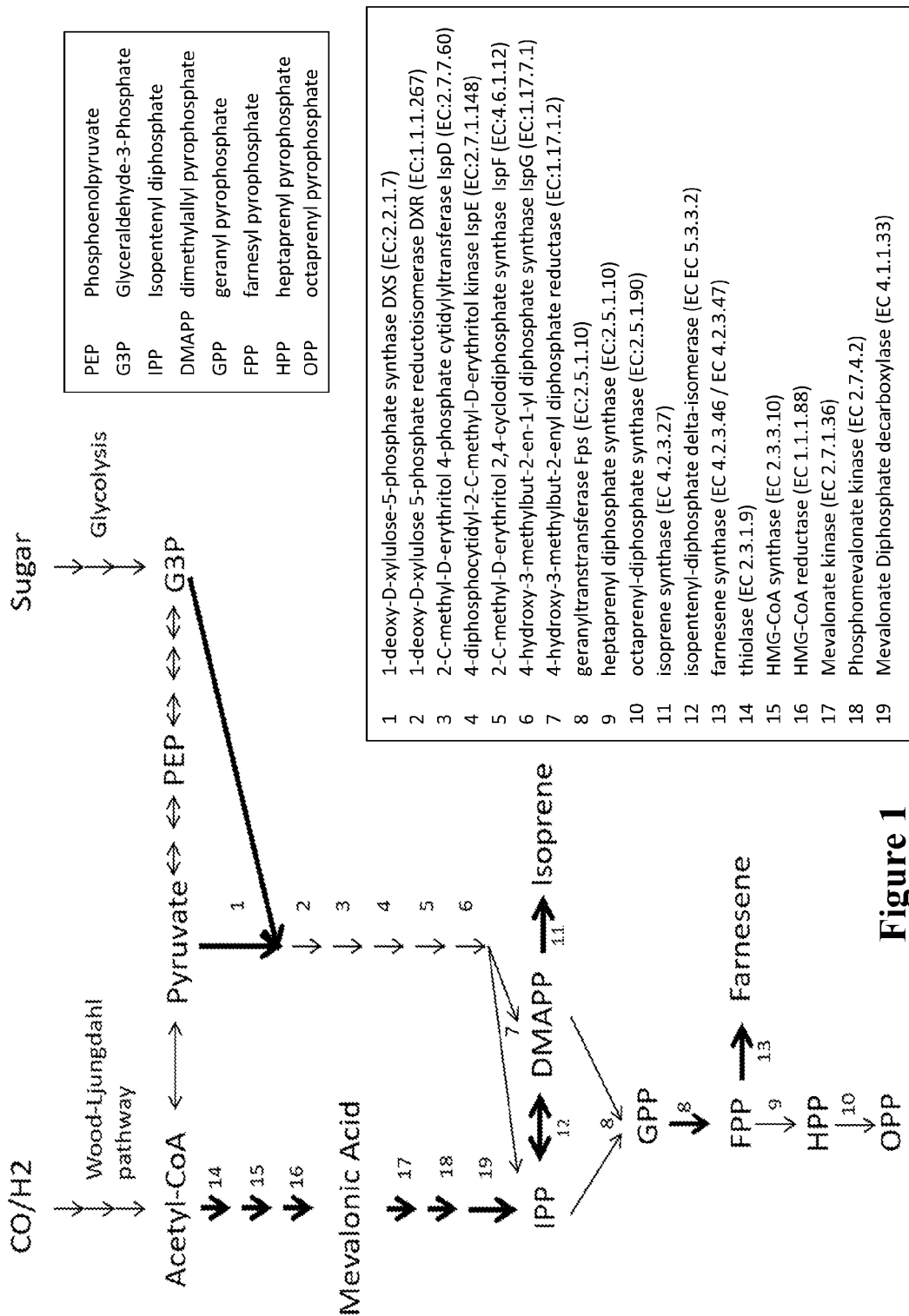


Figure 1

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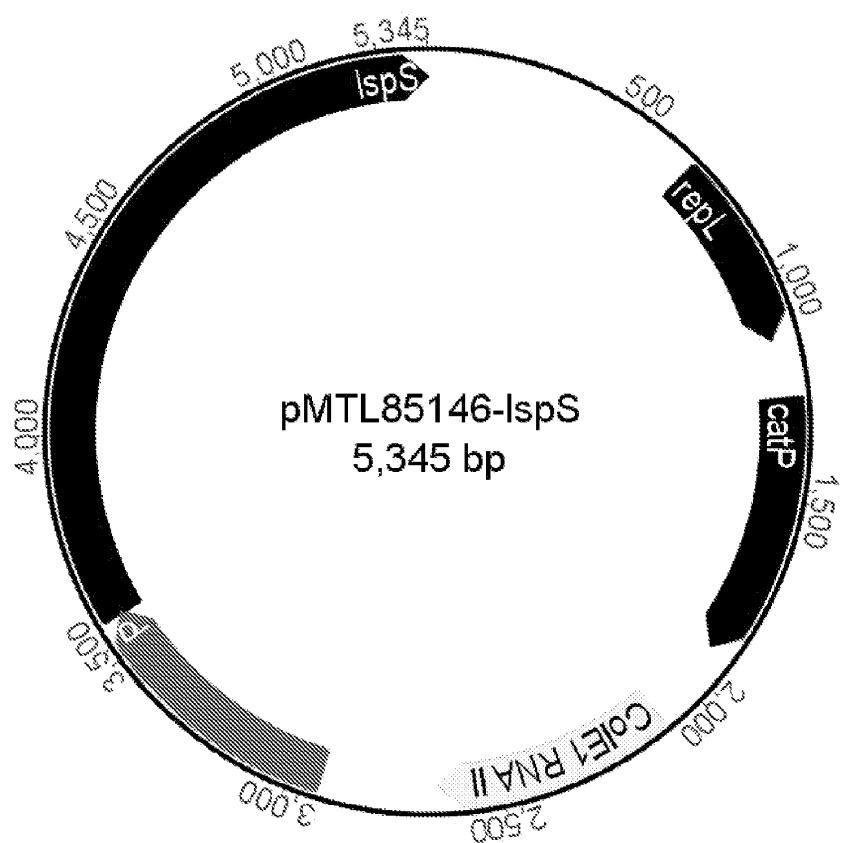
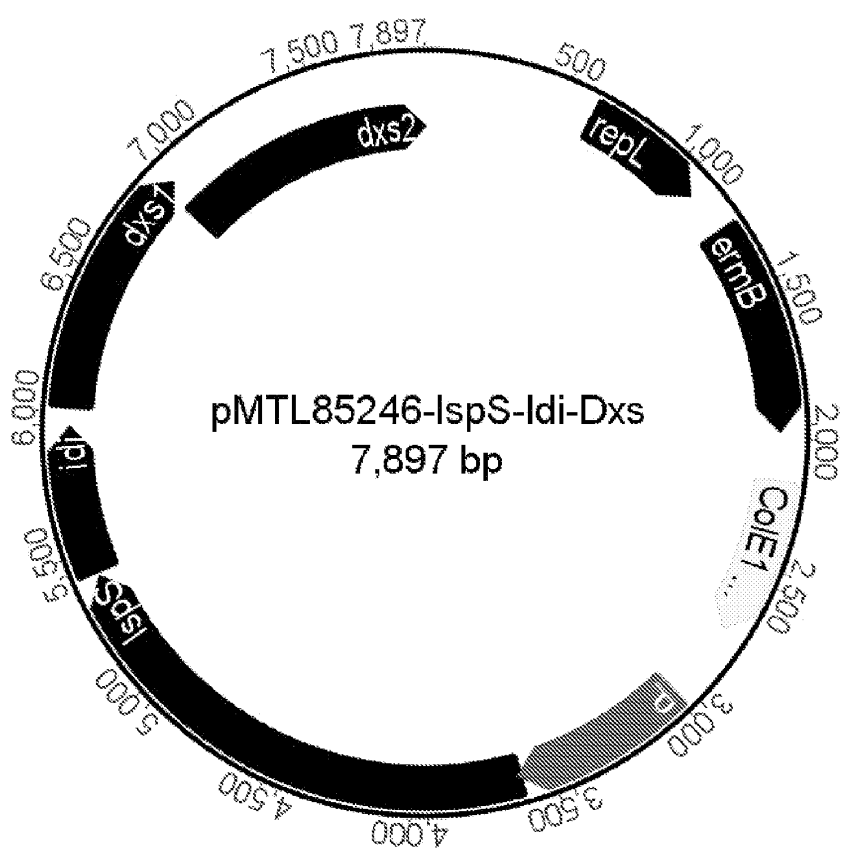
**Figure 2**



Figure 3

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**Figure 4**

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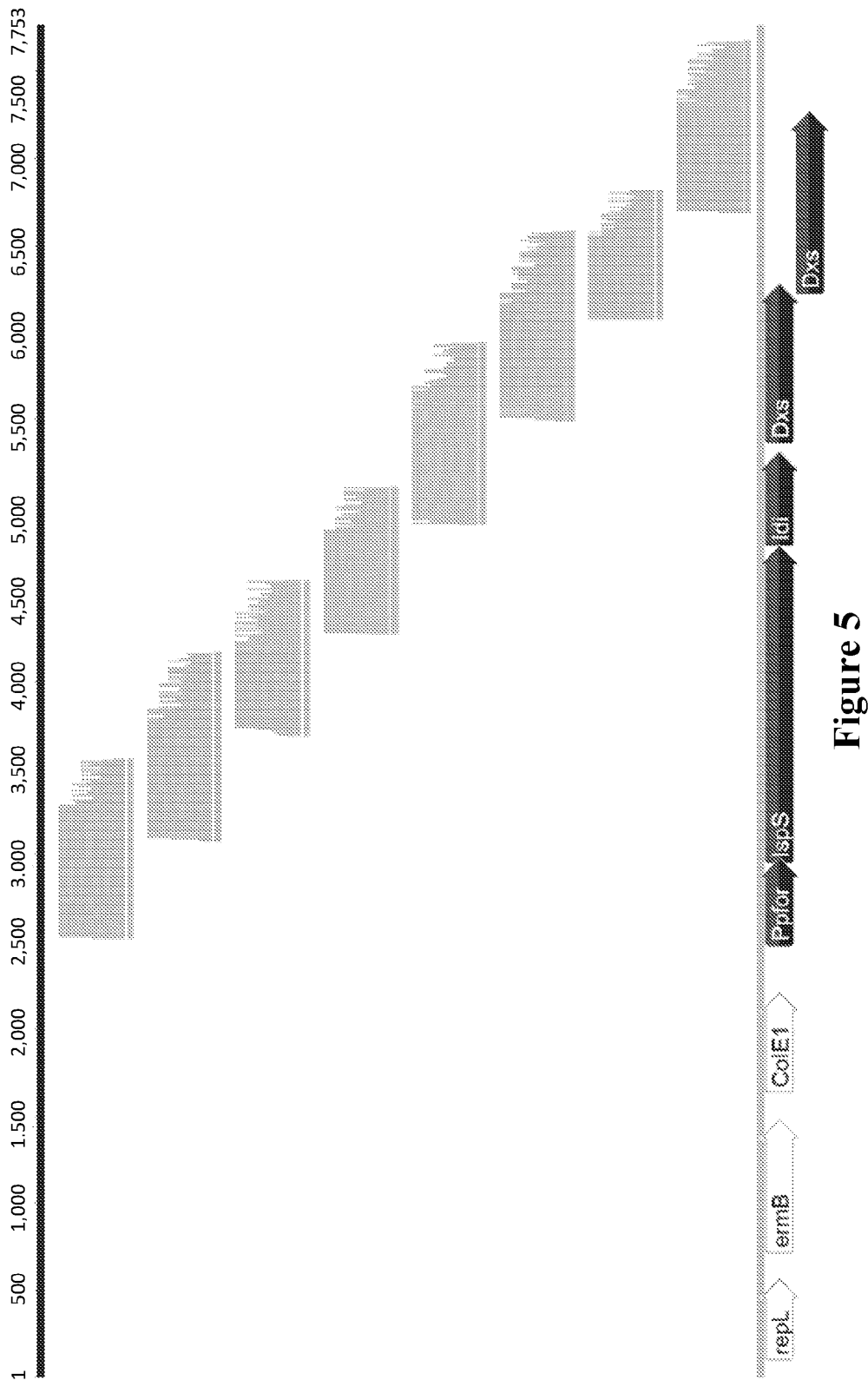


Figure 5

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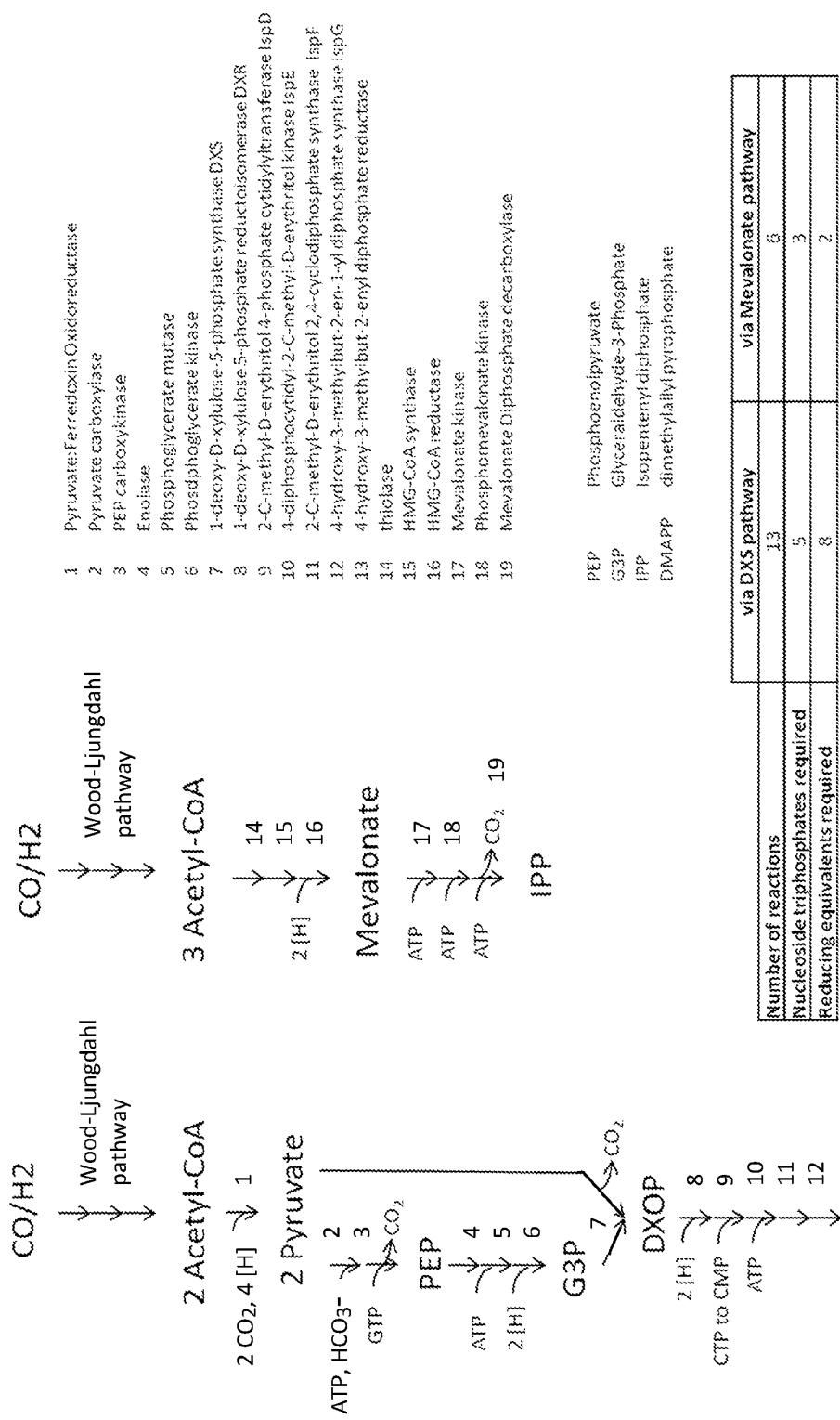


Figure 6

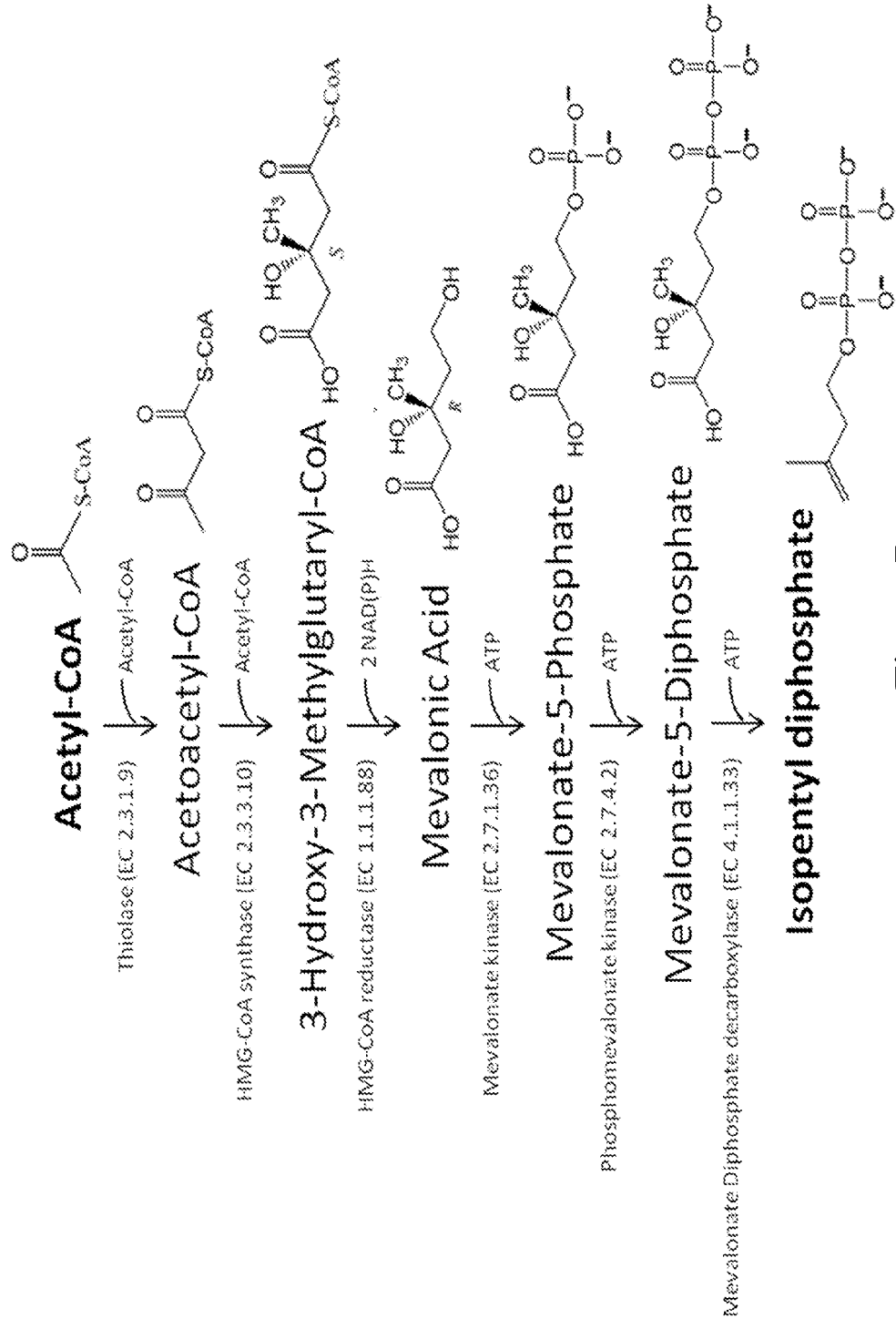


Figure 7

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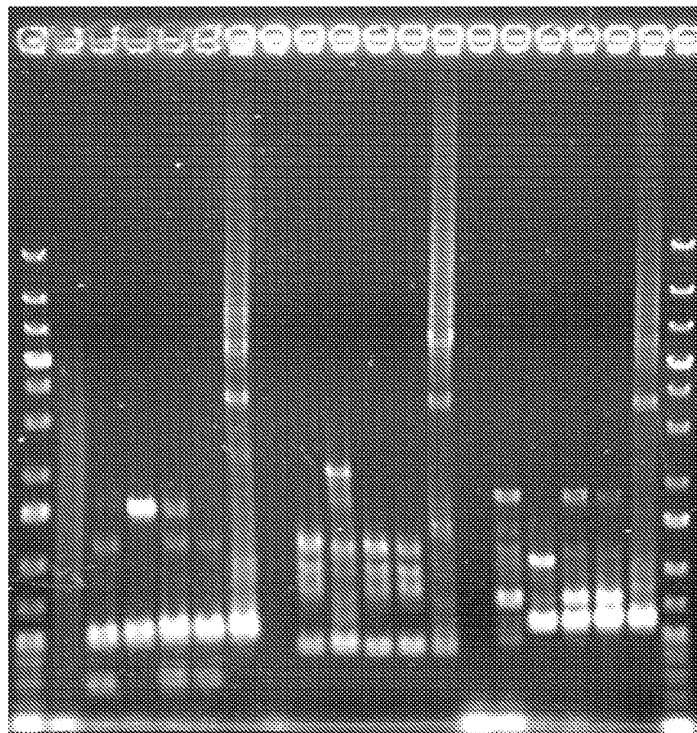
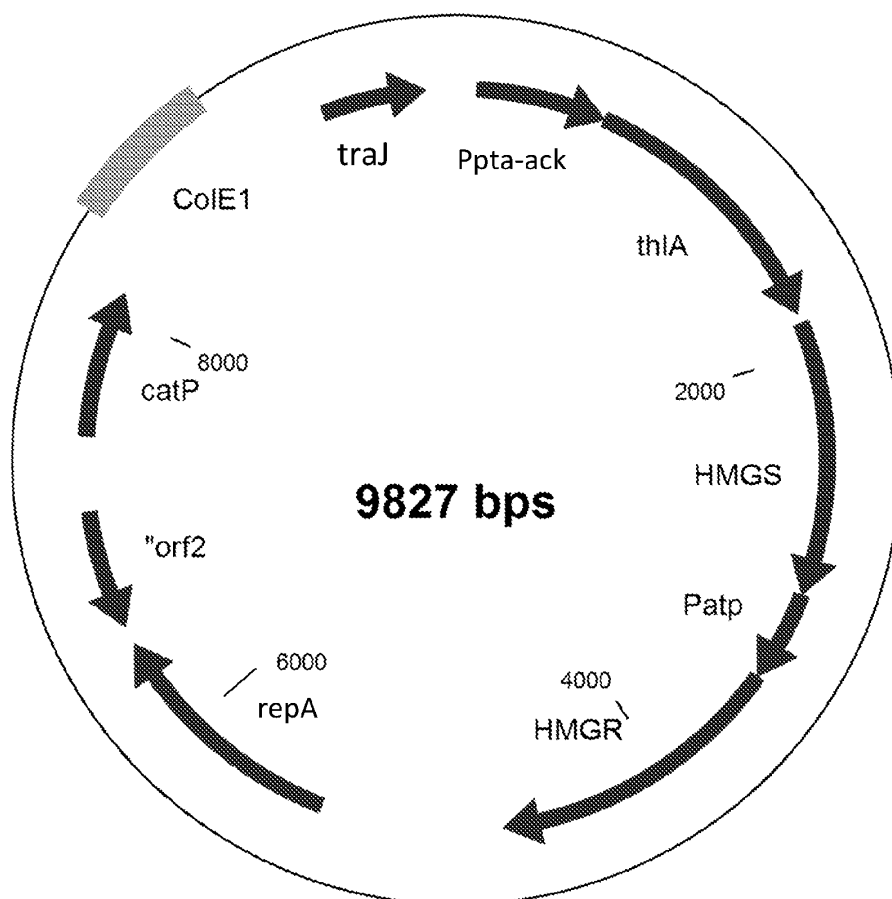


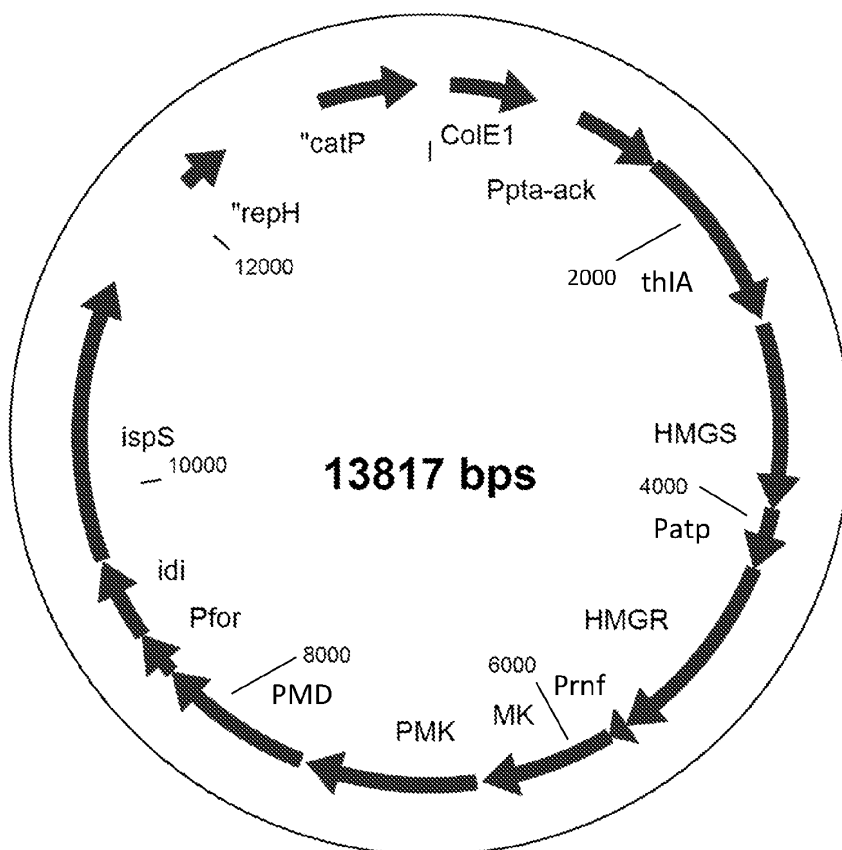
Figure 8

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pMTL8215-Pptaack-thlA-HMGS-Patp-HMGR**Figure 9**

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pMTL83145-thIA-HMGS-Patp-HMGR-Prnf-MK-PMK-PMD-Pfor-idi-ispS

**Figure 10**

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pMTL83145-thIA-HMGS-Patp-HMGR-Prnf-MK-PMK-PMD-Pfor-idi-ispA-FS

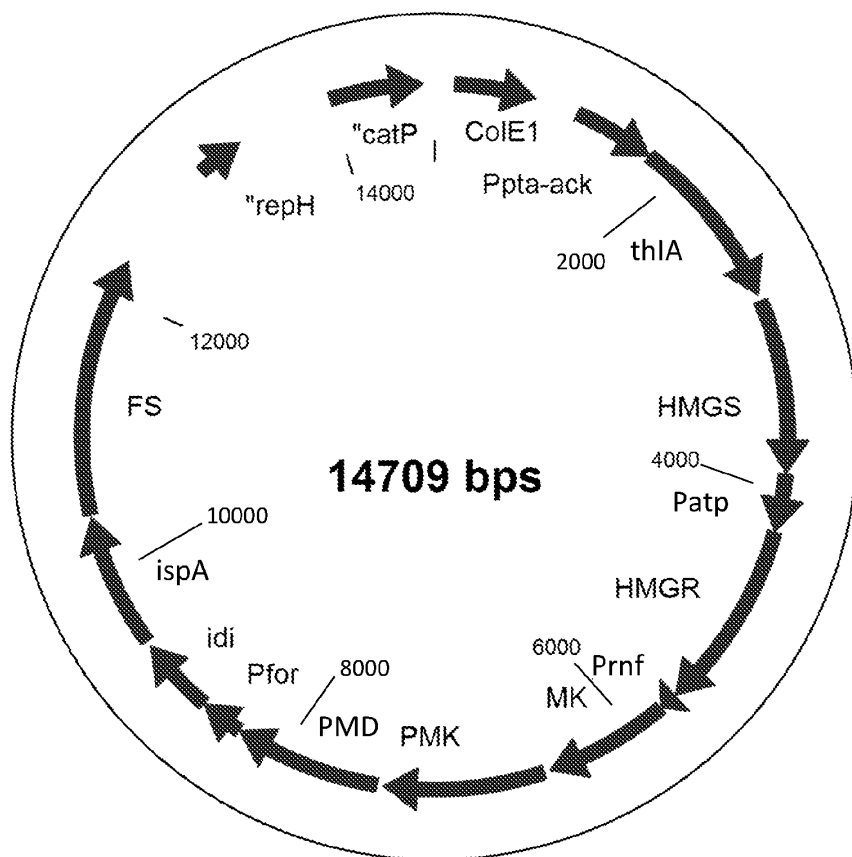
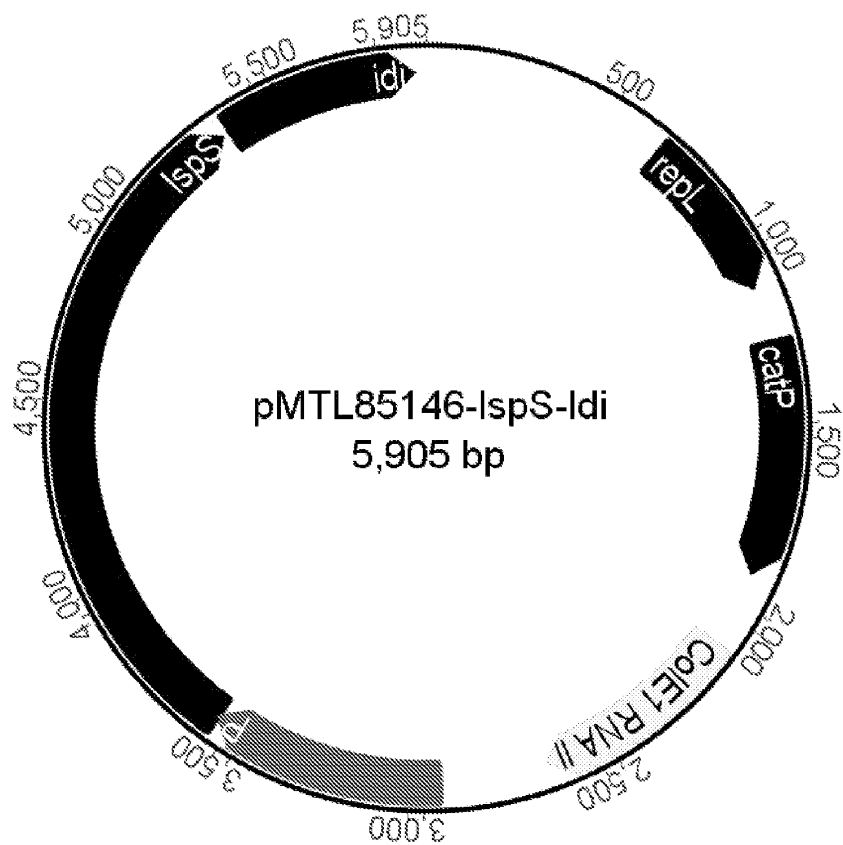


Figure 11

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**Figure 12**

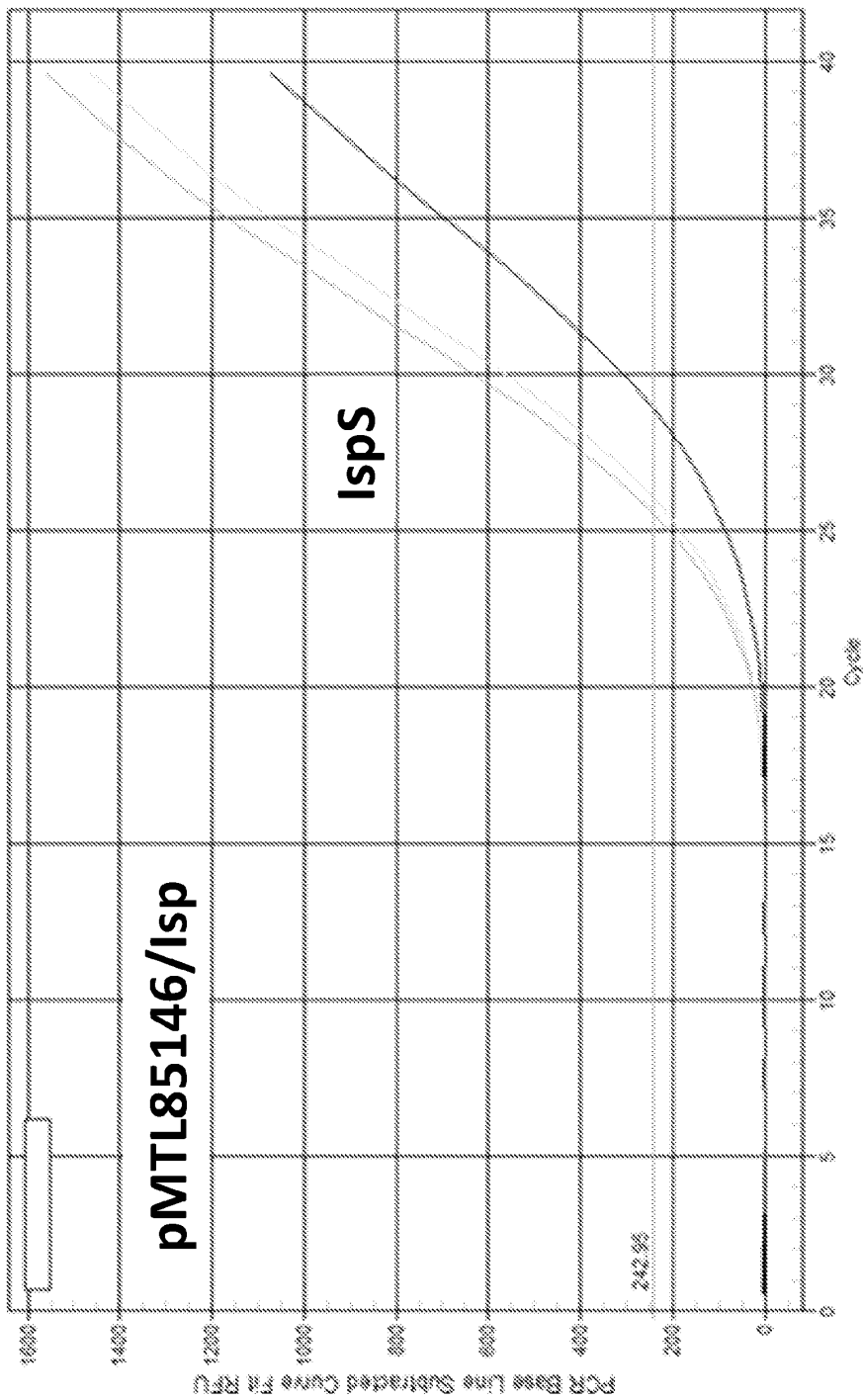


Figure 13

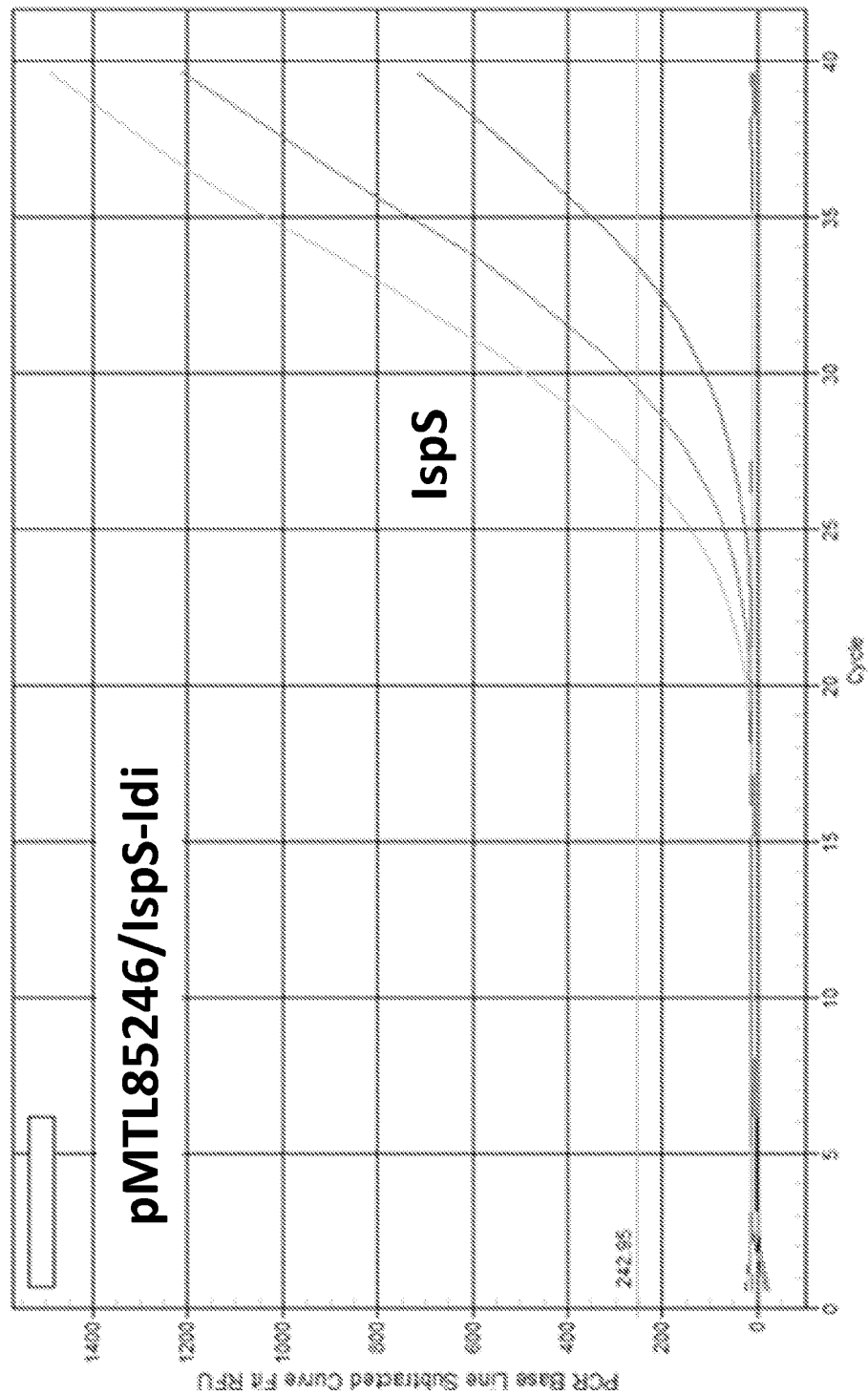


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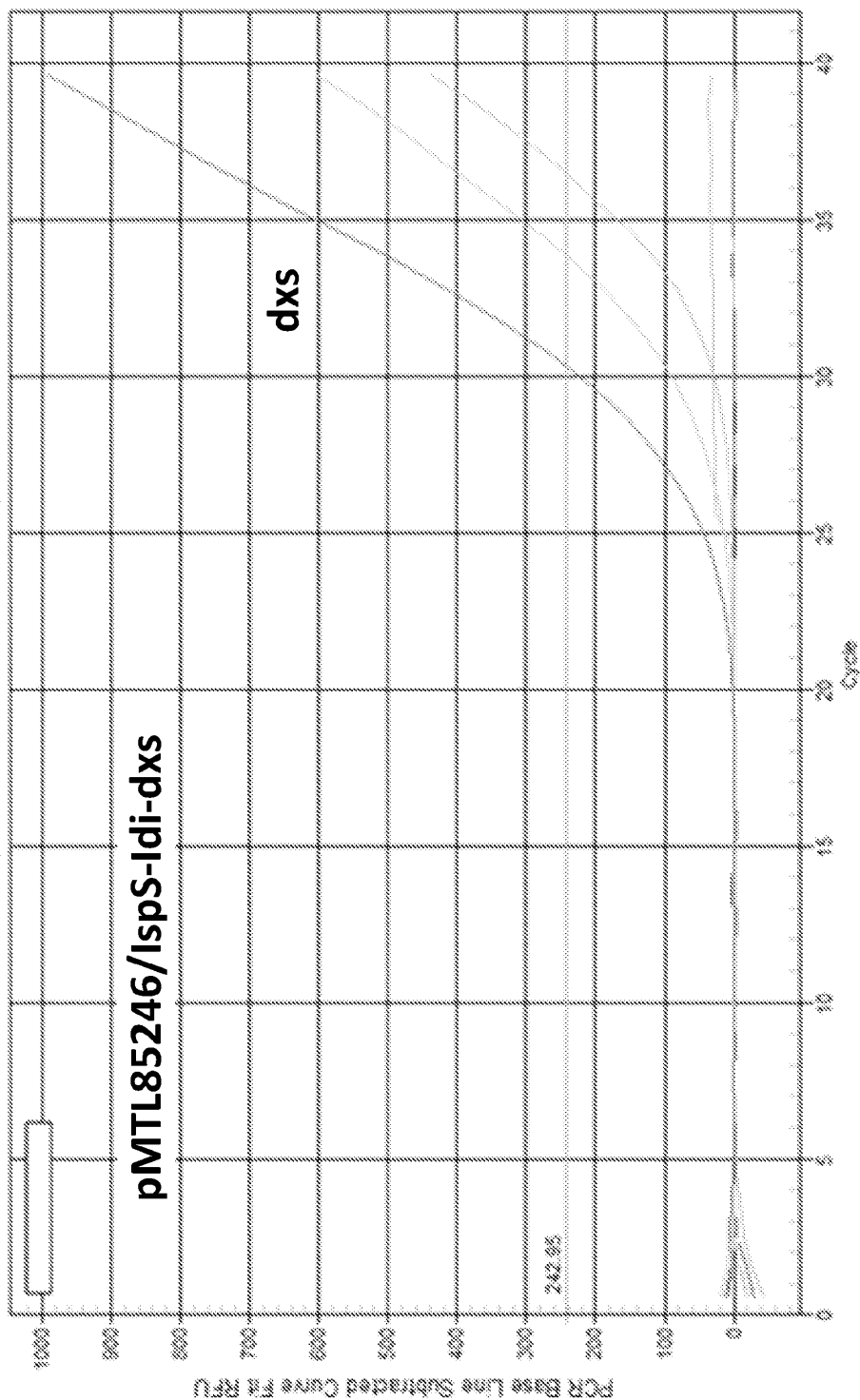


Figure 15

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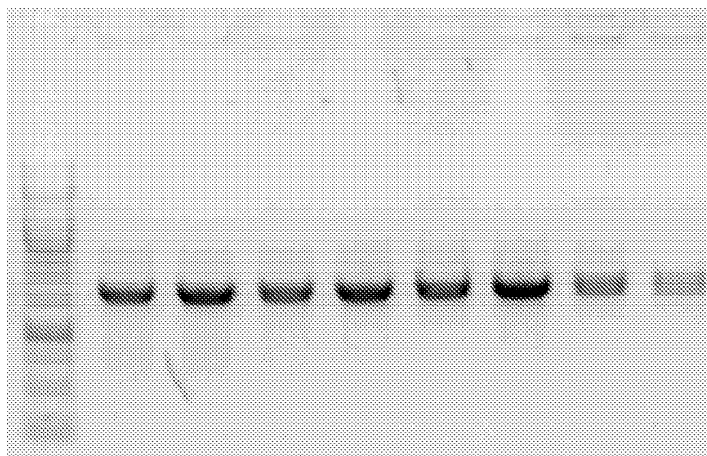


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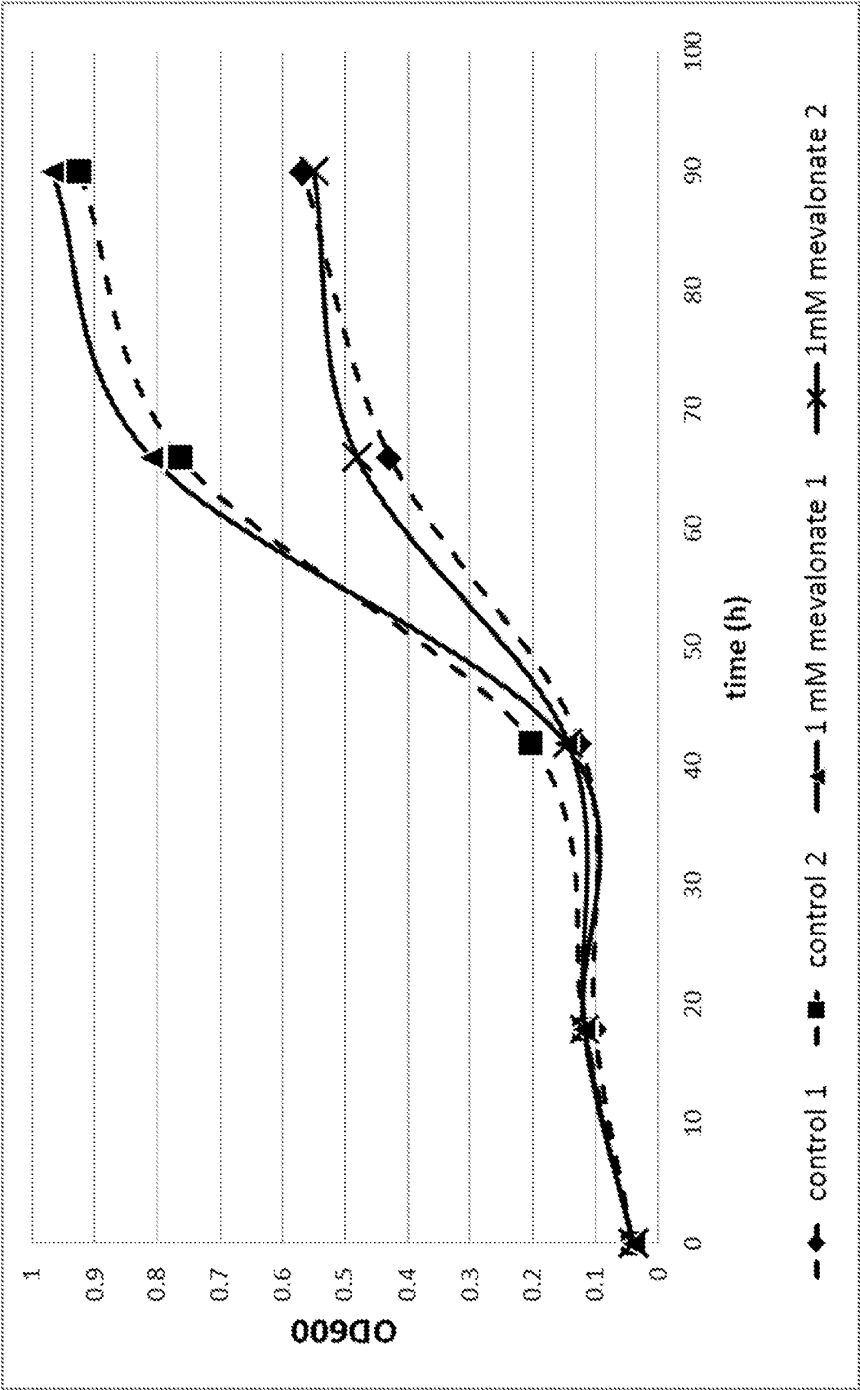


Figure 17

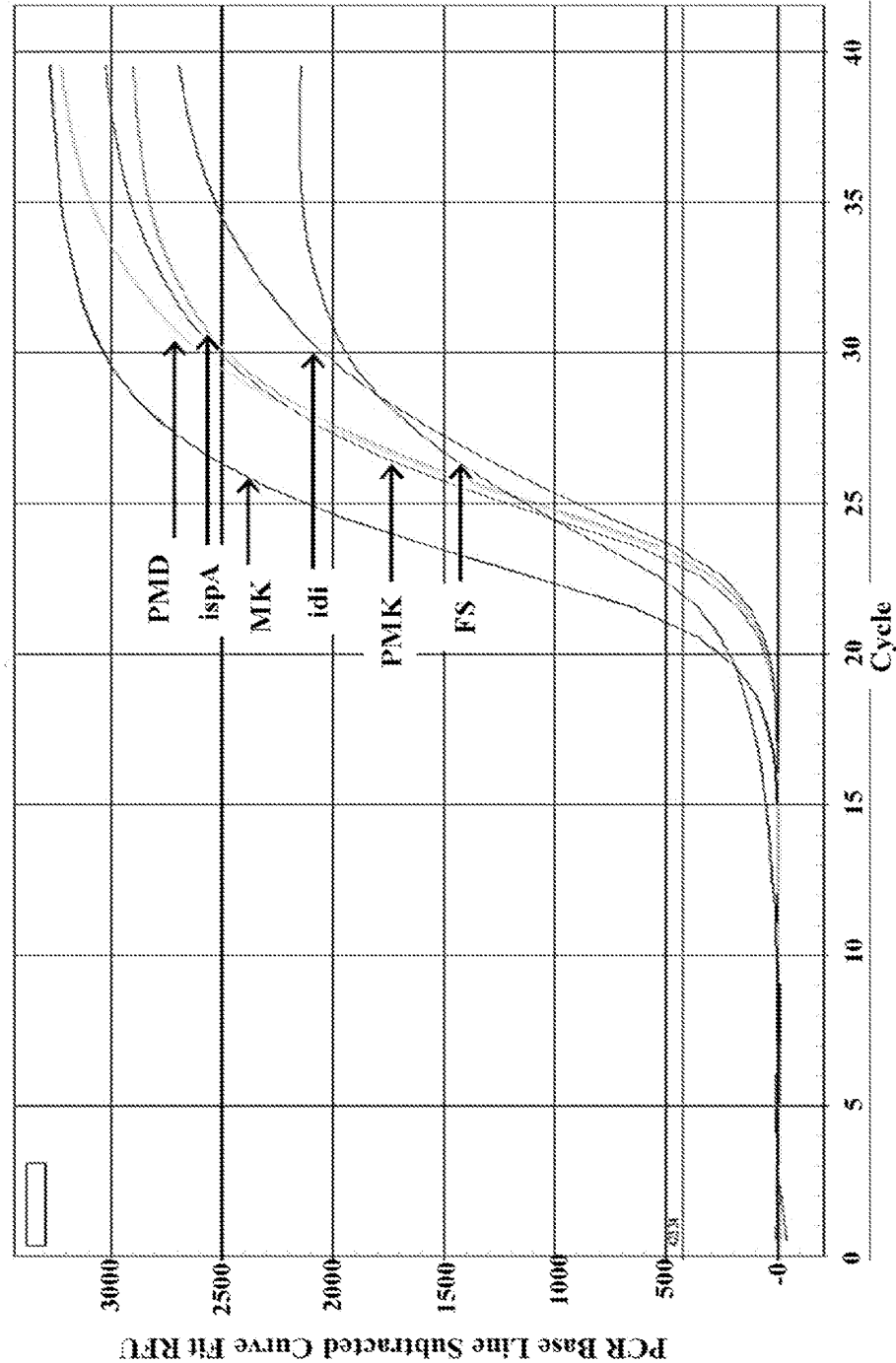


Figure 18

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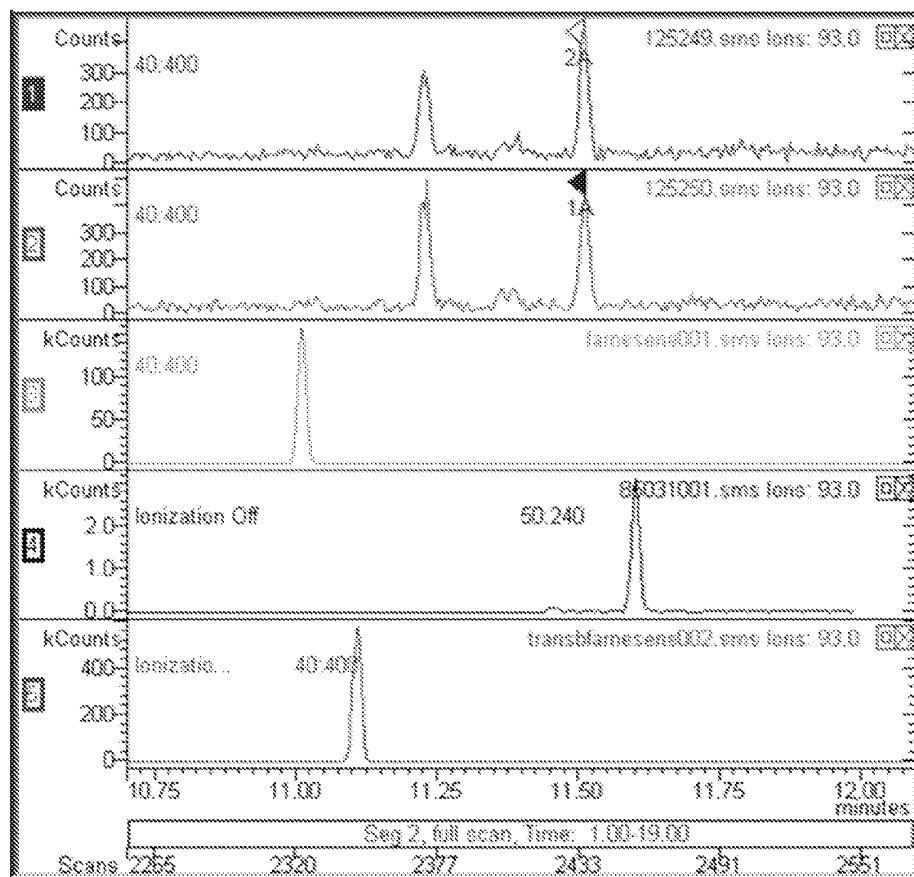


Figure 19

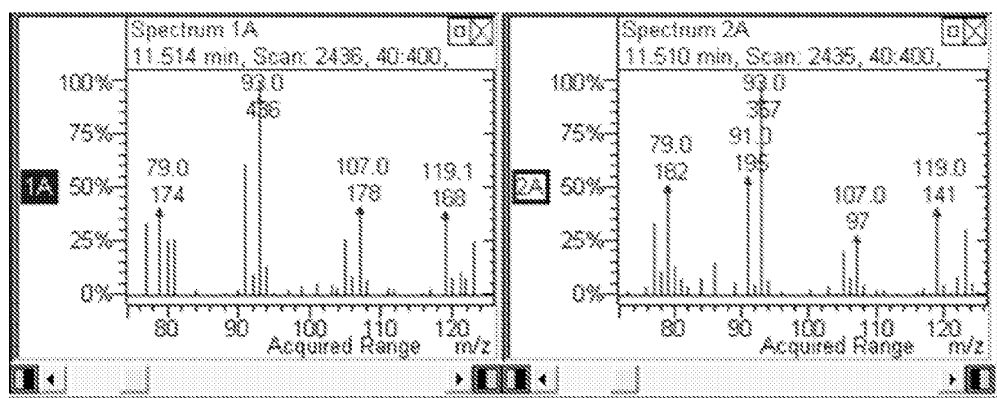
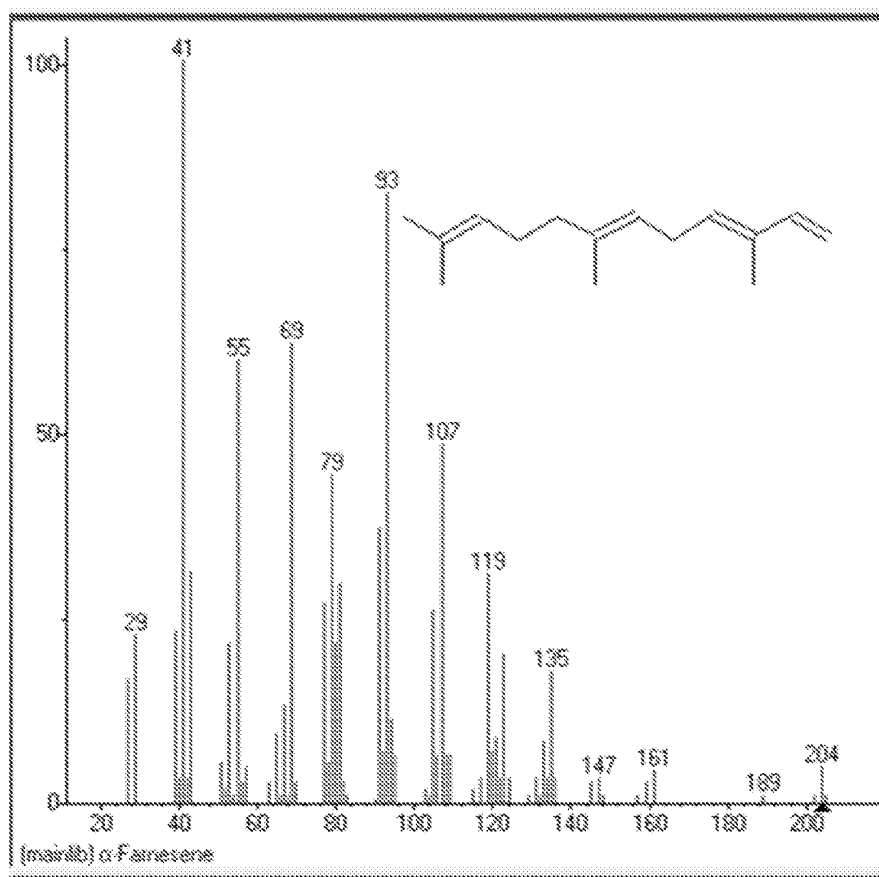


Figure 20

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**Figure 21**

Practical maximum isoprenol selectivity calculations

KEY RESULTS

- The highest practical maximum selectivity (PMS) across all pathways is 0.27 kg/Nm³ CO+H₂ reacted. This is equivalent to 98.5% substrate selectivity.
- PMS of pathways via acetone (3 & 4) are high and unaffected by gas-uptake ratio.
- Mevalonate pathways (5 & 6) achieve lower PMS and are more sensitive to gas-uptake ratio.
- 'IPA' pathways without acetone intermediate (1 & 2) reach higher PMS but are sensitive to gas-uptake ratio.

Model build date:
2021.08.13

Pathway:	Practical maximum selectivity [kg isoprenol/Nm ³ CO+H ₂ reacted]					
	1	2	3	4	5	6
SMOG	0.27	0.27	0.27	0.27	0.26	0.19
Syngas	0.27	0.27	0.27	0.27	0.20	0.15
High H ₂	0.19	0.24	0.27	0.27	0.13	0.10

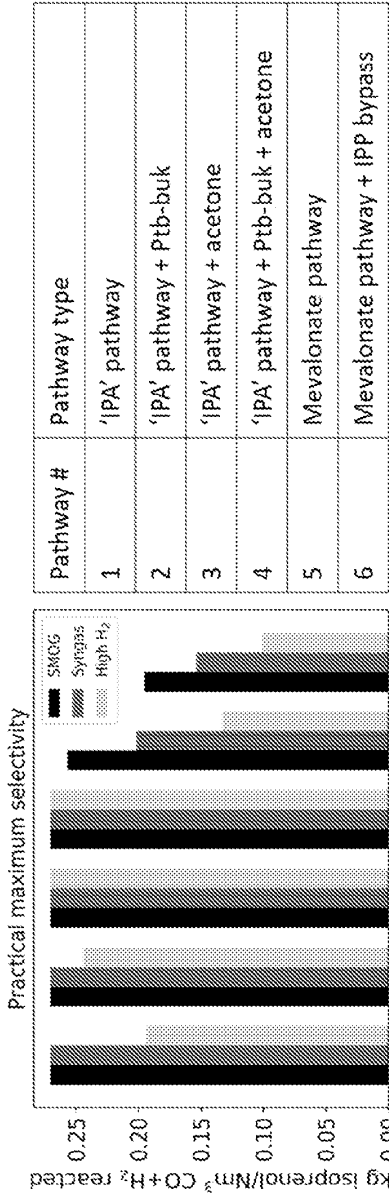


Figure 22

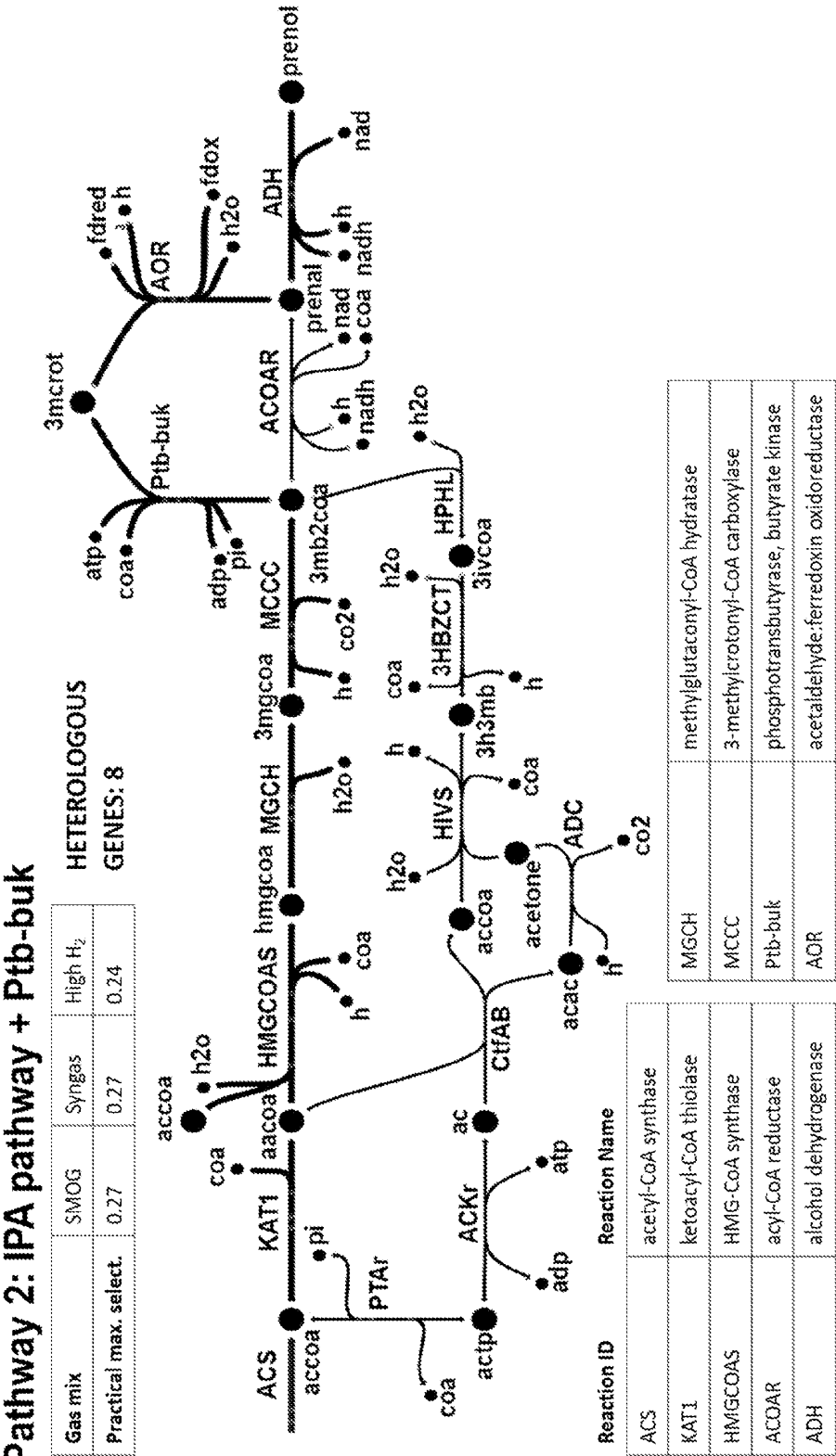
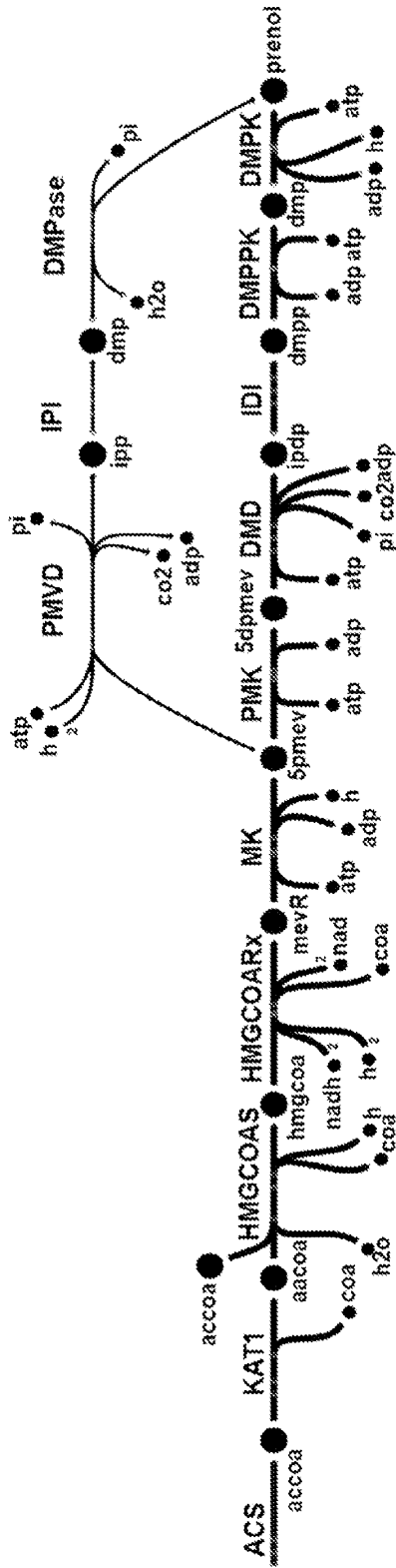


Figure 24

Pathway 5: Mevalonate pathway

Gas mix	SMOG	Syngas	High H ₂
Practical max. select.	0.26	0.20	0.13

HETEROLOGOUS
GENES: 9



Reaction ID	Reaction Name
ACS	acetyl-CoA synthase
KAT1	ketoacyl-CoA thiolase
HMGCoAS	HMG-CoA synthase
HMGCoARx	HMG-CoA reductase
MK	mevalonate kinase

PMK	phosphomevalonate kinase
DMD	diphosphomevalonate kinase
IDI	iso-pentenylidiphosphate isomerase
DMPK	dimethylallyldisphosphate kinase
DMPK	dimethylallylphosphate kinase

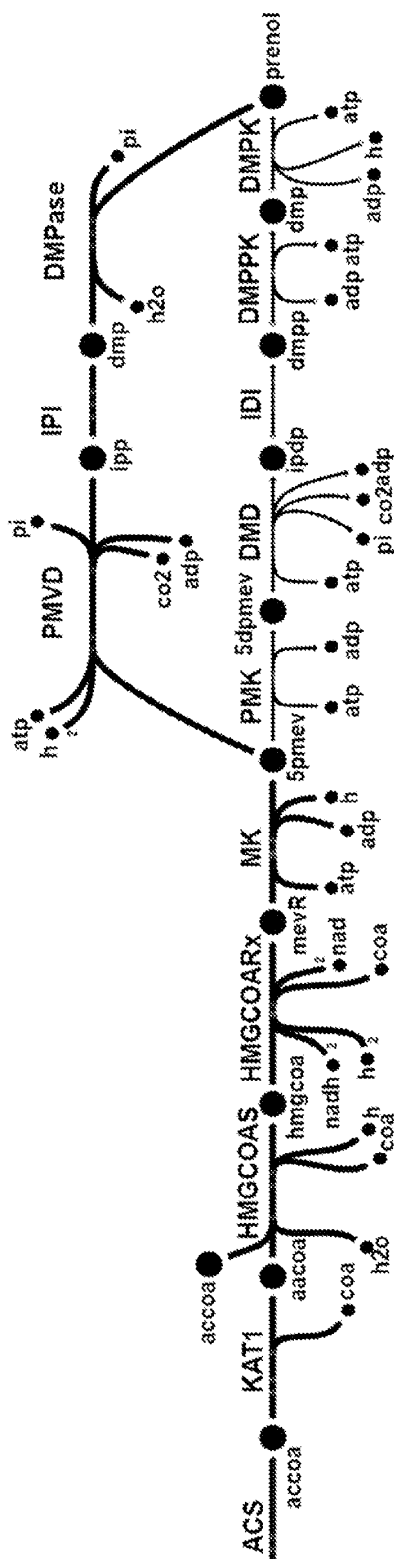
Figure 27

Pathway 6: Mevalonate pathway + IPP bypass

Gas mix	SMOG	Syngas	High H ₂
Practical max. select.	0.19	0.15	0.10

HETEROLOGOUS

GENES: 9



Reaction ID	Reaction Name
ACS	acetyl-CoA synthase
KAT1	ketoadyl-CoA thiolase
HMGCOAS	HMG-CoA synthase
HMGCOARx	HMG-CoA reductase
MK	mevalonate kinase

IPP BYPASS REACTIONS:	
PMVD	phosphomevalonate decarboxylase
IPI	iso-pentenylphosphate isomerase
DMPase	prenylphosphatase

Figure 28

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Metabolite ID	Full name	Note
<u>3h3mb</u>	3-hydroxy-3-methylbutanoate	
<u>3ivcoa</u>	3-hydroxyisovaleryl-CoA	
<u>3mb2coa</u>	3-methylbut-2-enoyl-CoA	Synonymous with 3-methylcrotonyl-CoA
<u>3mcrot</u>	3-methylcrotonate	
<u>3mgcoa</u>	3-methylglutaconyl-CoA	
<u>5dpmev</u>	5-diphosphomevalonate	
<u>5pmev</u>	5-phosphomevalonate	
<u>aacoa</u>	acetoacetyl-CoA	
<u>ac</u>	acetate	
<u>acac</u>	acetoacetate	
<u>accoa</u>	acetyl-CoA	
<u>acetone</u>		
<u>actp</u>	acetyl-phosphate	
<u>dmp</u>	dimethylallyl phosphate	i.e. "prenyl phosphate"
<u>dmp</u>	dimethylallyl diphosphate	Synonymous with prenyl diphosphate
<u>fdox</u>	oxidized ferredoxin	
<u>fdred</u>	reduced ferredoxin	
<u>hmgcoa</u>	3-hydroxy-3-methylglutaryl-CoA	
<u>ipdp</u>	isopentenyl diphosphate	This is "IPP." Not to be confused with isopentenyl phosphate.
<u>isopent-p</u>	isopentenyl phosphate	
<u>mevR</u>	mevalonate (R-form)	
<u>prenal</u>		Synonymous with 3-methylbut-2-enal
<u>prenol</u>		

Figure 29