

Supplementary material

Bacterial biosynthesis of abietane-type diterpene ferruginol from glucose

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Table S1. DNA sequences used in this study.

Gene	DNA coding sequences
<i>GGPPS</i>	ATG TTCGATTTCAACGAATACATGAAGTCCAAGGCAGTGGCAGTGGATGCAGCACTGGATAAAGGCAATCCCCTG GAATACCCAGAAAAGATCCACGAATCCATGCGCTACTCCCTGCTGGCAGGCGGCAAGCGCGTGCGCCAGCACTG TGCATCGCAGCATGCGAACTGGTGGGCGGCTCCCAGGATCTGGCAATGCCAACCAGCATGCGCAATGGAAATGATC CACACCATGTCCCTGATCCACGATGATCTGCCATGCATGGATAACGATGATTTCCGCGCGGCAAGCCAACCAAC CACAAGGTGTTCCGGCGAAGATACCGCAGTGTCTGGCAGGCGATGCACTGCTGTCTTCGCATTTCGAACACATCGCA GTGGCAACCTCCAAGACCGTGCCATCCGATCGCACCTGCGCGTGATCTCCGAACCTGGGCAAGACCATCGGCTCC CAGGCGCTGGTGGGCGGCCAGGTGGTGGATATCACCTCCGAAGGCATGCAAACGTGGATCTGAAGACCTGGAA TGGATTACATCCACAAGACCGCAGTGTCTGCTGGAATGCTCCGTGGTGTCCGGCGGCATCCTGGGCGGCGCAACC GAAGATGAAATCGCACGCATCCGCCGCTACGCACGCTGCGTGGGCTGCTGTTCCAGGTGGTGGATGATATCCTG GATGTGACCAAGTCTCCGAAGAACTGGGCAAGACCGCAGGCAAGGATCTGCTGACCGATAAAGGCAACCTACCCA AAGCTGATGGGCTGGAAAAGGCAAGGAGTTTCGCAGCAGAACTGGCAACCCGCGCAAAGGAAGAACTGTCTCTCC TTCGATCAGATCAAGGCAGCACCACTGTCTGGGCTGGCAGATTACATCGCATTCGCCAGAACTAA
<i>idsA</i>	TTG AGCAGTTTCGATGCCCATGACCTTGACCTCGACAAATTTCCGGAGGTCTGTCGAGATCGTTTGACGAGTTT CTCGATGCTCAAGAGCTAACAATTGCTGATATCGCGCTCCTGTGCACAGATGCTGTGCCCCATCTTCGAGTTT GTGCTCAATGGAGGAAAGCGAATCCGTCCTCTTTATGCGTGGGCTGGTTTCTGGCGGCGCAAGGCCATAAAAAAT TCTTCTGAAAAACTTGAGTCCGTCCTTGACGCCGACGCGAGTCTCGAATTTATCCAGGCTTGTGCCTTGATTTCAT GACGATATTATCGATTCTTCTGATACCCGGCGCGGAGCCCCACAGTTCACCGGGCTGTGGAAGCTGATCACCGC GCCAATAATTTCAAGGCGATCCCGAGCACTTTGGCGTTTCAGTCTCGATTTTGGCTGGCGATATGGCATTGGTG TGGGCAGAAGACATGCTGCAGGATTCGGTTTGGTGCAGAGGCATTGGCCCGCAGAGGGAATGCTTGGCGTGGC ATGCGTACTGAGGTATTGGCGGCGCAGCTGCTTGATTTATCTAGAGTGCACGCCAACGAGTCGGTGGAGCTT GCGGATTCTGTCAACCGCTTCAAAAACGGCCGCTTACACGATTGCGCGCCCATTCACCTGGGCGCCTCCATTGCT GGCGGTTTCGCCGACGCTTATCGACGCGCTCCTCCACTACGGCCACGACATCGGCATTGCATTCCAGTTGAGGGAT GATCTGCTTGGTGTGTTTGGTGTATCCCGCTATCACCGGCAACACAGCTGGAGACGATATCCGTGAAGCAAGCGC ACTGTTCTTCTTGGCTCGCTCTACAACGCGCTGATAAGCAATCTCCTGAAGCTGCACGGCCATTTCGCGCAGGT GTTGGAAAGGTGACTTCACCAGAAGATATTGCTGTCTTACAGAGCATATTGAGCTACTGGTGTGAAGAAGAA GTTGAGCAGCGAATTTCCAGCTGACTGAATCCGGTTTGGCTCACCTCGATGATGTAGACATCCCCGATGAGGTG CGCGCACAGTTGCGGCGACTGGCTATCCGCTCAACCGAACGTCGGATGTAG
<i>BtsI</i>	ATG GGAAGCAAAGATCGATGAAGTATCAACAACGATCCAGTGTGGTCTCCAGAACGAATCCCTGATCTCCAAG CCATAACAACCACATCCTGCTGAAGCCAGGCAAGAACTTCCGCCTGAACCTGATCGTGCAGATCAACCGCGTGATG AACCTGCCAAAGGATCAGCTGGCAATCGTGTCCAGATCGTGGAACCTGCTGCACAACCTCCTCCCTGCTGATCGAT GATATCGAAGATAACGCACCACTGCGCCGCGGCCAGACCACCTCCCACCTGATCTTCGGCGTGCCATCCACCATC AACACCGCAAACATACATGTACTTCCGCGCAATGCAGCTGGTGTCCAGCTGACCACCAAGGAACCACTGTACCAC AACCTGATCACCATTCTCAACGAAGAACTGATCAACCTGCACCGCGCCAGGGCCTGGATATCTACTTGGCGGAT TTCTGTCAGAAATCATCCCAACCCAGGAAATGTACCTGCACATGGTGATGAACAAGACCGCGGCTGTTCGCG CTGACCTTGGCGCTGATGGAAGCACTGTCCCATCCTCCACCACGGCCACTCCCTGGTGCCATTTCATCAACCTG CTGGGCATCATCTACCAGATCCGCGATGATTACCTGAACCTGAAGGATTTCCAGATGTCTCCGAAAAGGGCTTC GCAGAAGATATCACCGAAGGCAAGCTGTCTTCCCAATCGTGCACGCACTGAACCTTACCAAGACCAAGGGCCAG ACCGAACAGCACAAAGAAATCCTGCGCATCCTGTGCTGCGCACCTCCGATAAGGATATCAAGCTGAAGCTGATC CAGATCCTGGAGTTCGATACCAACTCCCTGGCATAACCAAGAACTTCATCAACAGCTGGTGAACATGATCAAG AACGATAACGAAAACAAGTACCTGCCTGATCTGGCATCCCCTCCGATACCGCAACCAACCTGCACGATGAAGT CTGTACATCATCGATCACCTGTCCGAAGT
<i>ERG20 (F96C)</i>	ATG GCATCCGAAAAGGAAATCCGCCGCGAACGCTTCTGAACGTGTTCCCAAAGCTGGTGGAAGAACTGAACGCA TCCCTGCTGGCATAACGCGATGCCAAAGGAAGCATGCGATTGGTACGCACACTCCCTGAACATAACACCCAGGC GGCAAGCTGAACCGCGGCCTGTCCGTGGTGGATACCTACGCAATCCTGTCCAACAAGACCGTGGAACAGCTGGGC CAGGAAGAATACGAAAAGGTGGCAATCCTGGGCTGGTGCATCGAACTGCTGCAGGCATACTGCCTGGTGGCAGAT GATATGATGGATAAGTCCATCACCCGCCGCGGCTAGCCATGCTGGTACAAGGTGCCAAGTGGGCGAAATCGCA ATCAACGATGATTCATGCTGGAAGCAGCAATCTACAAGCTGCTGAAGTCCCCTTCCGCAAGCAAGAGTACTAC ATCGATATCACCGAACTGTTCCACGAAGTGACCTTCCAGACCGAACTGGGCCAGCTGATGGAATCTGATCACCGCA CCAGAAGATAAGGTGGATCTGTCCAAGTTCTCCCTGAAGAAGCACTCCTTCATCGTGACCTTCAAGACCGCATAC TACTCCTTCTACCTGCCAGTGGCACTGGCAATGTACGTGGCAGGCATCACCGATGAAAAGGATCTGAAGCAGGCA CGCGATGTGCTGATCCCACTGGGCGAATACTTCCAGATCCAGGATGATTACCTGGATTGCTTCGGCACCCAGAA CAGATCGGCAAGATCGGCACCGATATCCAGGATAACAAGTGCTCCTGGGTGATCAACAAGGCACTGGAAGTGGCA TCCGCAGAACAGCGCAAGACCTGGATGAAAACACGCGCAAGAGGATTCCTGGCGAGAAGCAAGTGAAGAAG ATTTTCAACGATCTGAAGATCGAACAGCTGTACCACGAATACGAAGAATCCATCGCAAGGATCTGAAGGCAAG ATTTCCAGGTGGATGAATCCCGCGGCTTCAAGGCAGATGTGCTGACCGCATTCCTGAACAAGGTGTACAAGCGC TCCAAGTAA
<i>dxs</i>	ATG GGCATTCTGAACAGTATTTCAACACCTGCTGACTTAAAGGCCCTTAATGATGAGGATTTGGACGCTCTTGCC AAAGAAATCCGAACCTTTCTGGTCGATAAAGTCGCAGCAACTGGTGGCCACTTAGGTCCAAATTTGGGCGTAGTG GAATTAACCATCGGTCTTCATCGAGTTTTCGATTGCGCTCAAGACCCGATCATCTTTGATACTTCTACCAAGTCC TATGTGCATAAAGATCCTGACGGTTCGCGCTAAAGATTTTGATTCTTTGCGTCAAAAAGATGGCCTTTCTGGTTAC ACCTGCCGTGCTGAAAGTGAGCACGATTGGACTGAGTCTTCGCATGCTTCGGCGGCCCTTGTCTTATGCGGATGGT TTGTCTAAAGCCAAGCAGTTGGATGGCGATACCACGCATAGTGTGGTTGCTGTCGTTGGTGGATGGCGCTCTAACT GGCGGCATGTGTTGGGAAGCACTGAACAATATTGCTGCTGGTAAAGACCGCAAGTGTGTGCTAGTCAATGAC AATGCGCGGAGTTATTCTCAACCATTTGGCGGATTTGCGGAAAACCTTGCGGGCCCTTCGCATGCAGCCTTTCTAT GATCGCTTCATGGAAGGGAAGACGTCCTGAAATCCATGGGGTGGGTAGGGGAGCGTACTTTTGAAGCGCTC

	CATGCATTTAAAGAAGGTGTGAAGAGCACCGTCATTCCCACCGAAATGTTCCCTGAACTGGGCATGAAATACGTG GGTCCGGTTGATGGACATAACCAAAAAGCTGTGACAATGCGCTGAAATACGCTCATGATTATGATGCCCCATC ATCGTGACATGGTCAACGAAAAGGGTCGTGGTTACGCGCCTGCTGAGCAGGATTTGGACGAATTGATGCACTCC ACGGGCGTCAATCGATCCGCTCACAGGAGCTCCTAAATCTGCATCAAAGCCCCGGTTGGACCTCTGTGTTTCAGCGAT GAGCTGGTCAAGATTGGTGGCGAGAATGAAAACGTTGTTGCCATCACCGCCGCGATGCGAGGTCCACCGGTCTG TCCAAGTTTGAAGCCAATTTCCCCAACCGATTCTTTGATGTGGGCATTGCTGAGCAGCACGCGGTAACCTTCTGCC GCAGGCCCTCGCATTGGGTGGAAAACACCCTGTGGTGGCTATTTACTCCACGTTCTTTGAACCGCGCTTTTGATCAG CTGCTCATGGATGTGGGCATGCTCAACCAGCCTGTTACTTTGGTGGCTTGATCGCTCAGGTGTACGGGTTCCGAT GGAGCGAGCCACAATGGCGTCTGGGATATGGCGCTGACCTCGATCGTTCCAGGCGTGCAGGTGGCGGCACACAGT GATGAGGATTCCTTGCGTGAGCTGCTCAATGAGGCTATTTCCATCGATGATGGCCCCACAGTTGTGCGTTTCCCC AAGGGCGACTTGCCAACTCCAATTGTTGCTATCGACACCTTGAAGACGGCGTGGATGTCTCGCATATGAAGAC GCCACTGCAGTTGAATCAACCGACGATGCGCCATCAGTTCTCATCATTTGCGGTAGCGGAGCGCGCAACTGTTGCA CTTGACGTTGCTTCCAGGATTAAACAGCACGGCGTGAACGTCACGGTTGTTGACCCCCGCTGGATTGTCCCCATC CCGCAGTCTTTGGTCGCGCTGTCTGATGATCATGACCTCGTGATCACCATCGAAGACGGCGTCAATCCACGGCGGC GTGGGCTCCTTGCTCTCTGATGCGCTTAACGCCTCTGAGGTGGATACCCCTCGCCGACAAATCGCCGTGCCCCAG AAGTACCTGGATCACGCGTCCCGAATGAAGTGCTCGCCGATTATGGCCTCGACGCCGACGGCATTGAAACCACT GTTGTTGGATGGCTGGATTCCCTGTTCCGGGAATAA
<i>idi</i>	GTGTCTAAGCTTAGGGGCATGACTACTGAGGTTGAACCTGGTTGTTTTAGCTGATTCCGAGGGCAATCCTATTGGT ACTGCGCCGAAGCTACGGTGCACATCAAGACACGCTCTGCATTTGCGGTTTTCCACCTATATTTTGAACCCG CGTGGGGAGCTGTTGGTGACGCGTCGTGCATTGTGGAAGAAGACATGGCCTGGTGTGTGGACGAACCTATGTGT GGGACCCCTGGTCCGATGAGACAAACGCGGATGCGATTGCTGCGAGGGGTGTCGATGAGTTGGGGCTGGAGGTA GATTCTTTCTTGGATATTCAAGAGATTCTGCCTGATTACCAGTACCGTGCTGTGACGCGCTCCGGCATTTGTGGAG TGGGAGTTGTGCCCCGTCCACCTCGTGCGTTTAGCGGTGGGGGAATTTGTGGAGCCACTGGATGATGAGGTGGAG GAGTTCGAGTGGGCGGAACCGCAGAAGCTTTTCGACGCTGTTGATGCCACACCATTGTGTTTTCTCCATGGCTA GTGGATCAGCTTAGCGCCCTGAGCTGCGCCAAGCATCTCTGGAAGCGTTTGACGCAGAGTAA
<i>KSL</i>	ATGAGCCTTGCGTTTAAACCCTGCTGCAACCGCATTTAGCGGCAACGCGCGCGATCCCGCAGAGAGAACTTCCCC GTCAAGCATGTACCCGTCCGCGGATTCCCAATGATCACCAACAAGAGTTCATTTGCTGTAAAGTGCAACTTGACT ACCACCGACCTCATGGGAAGATTGCGGAGAAGTTCAAGGGTGAGGATTCCAATTTCCCGCGCGCCGACGTGTG CAGCCAGCAGCTGACATGCCATCTAACCTTTGTATCATTTGACACCTTGCAGAGGCTCGGCGTCGACCGCTACTTC CGCTCAGAAATAGACACAATTCTTGAAGACACCTACCGTCTGTGGCAGCGAAAAGAACGCGCAATTTTCTCCGAC ACGGCCATCCACGCGATGGCTTTTCGACTGCTTCGGGTGAAGGGCTACGAAGTTAGCAGTGAAGAGCTTGCTCCC TATGCTGATCAGGAGCACGTGGATCTTCAGACCATTGAAGTAGCCACCGTGATTGAACATATA TC GCGCAGGCCAA GAACGTACGGGAGAGGATGAGAGCTCGCTGAAGAAGCTGCACGCGTGGACCACCACATTCTTGAAGCAGAAGCTC CTGACGAACCTCAATCCAGACAAGAACTCCACAAGCTGGTTGAATATTACCTCAAAAATATCACGGCATTTTG GATCGCATGGGTGTGCGCCAAAACCTGGATCTCTACGACATCTCCTACTACCGCACCAGCAAGGCGGTAACCGT TTTTCTAATTTGTGTTCCGAAGATTTCCTTGCAATTTGCCCCACAAGATTTCAACATCTGCCAAGCTCAGCATCAA AAGAAGCTGCAGCAACTGCAACGCTGGTACGCCGACTGCAATTTGGATACTCTGAAATATGGTCGTGATGTGCTG CGGGTAGCAAAATTTCTCACTTCTGCTATCATCGGTGATCCTGAGTTGTGCGATGTTGCGATTGTTTTCGCCCAA CACATCGTGCTGGTCACAAGAATCGACGACTTCTTGATACCCGCGCAGTCTGTAAGAAAGTACGAGATCCTG GAGCTGATTAAAGAGTGGAAGGAAAAGCCAGCCGCCGAATACGGTTCAGAGGAAGTCGAGATCCTGTTTACGGCC GTGTACAACACCGTTAATGAGCTAGCAGAACGCGCCCATGTGGAGCAGGGGCGCTCGGTGAAAGATTTTCTGATC AAGTTATGGGTGCAATCTTGCTCTATCTTTAAGCGCGAAGTTGATACCTGGAGCGATGATACAGCACTTACCCTC GACGATTACCTGTGCGCGTCTTGGGTCTCCATCGGCTGCCGCATTTGCATTCTGATGTCCATGCAGTTTCATCGGC ATTAAACTCTGTGACGAAATGCTGCTCTCCGAAGAATGATATCGATCTGTGTAGGCACGTTTCAATGGTTGACCGT CTACTGAACGATGTTCAAACCTTCGAGAAAGAGCTAAGTGAAGAAAACACCGGAAACTCCGTGACTCTGTTGTGCT GCAAAACAAAGACGACTCCTCCTTCACTGAGGAGGAAGCAATCCGCATCGCGAAGGAGATGGCGGAGTGAATCGG CGACAGCTCATGCAGATCGTCTACAAAACCGGTACGATCTTCCCGCGGCAGTGCAAGGACATGTTCTTGAAGGTG TGTCGTATAGGATGCTACCTCTATGCATCGGGCGATGAGTTACCTCCCCGAGCAGATGATGGAAGATATGAAA TCTCTTGCTATGAACCTTTAACTATTCAATCCGCTTGTTGCCAACAATGTTTCGTGGTAAATAA
<i>CPS</i>	ATGGCCTCACTGAGCTCCACCATCTTGTGCGCTCACACGAGCCCGCCGCCGAATCACCCCTGCGAGTGCTAAG CTGCACAGGCCAGAGTGCTTTGCCACACGCGCTGGATGGGATCATCATCCAAAAATCTTTCCCTATCATATCAA CTCAACCACAAGAAGATCAGTGTGCTGCTACCGTCGACGCGCCACAGGTCCACGATCACGACGGCACCCACTGTTTCA CAAGGGCATGACGCGGTGAAGAACATTGAAGATCCAATTGAATACATTTCGGACACTGTTGCGCACCACCGGTGAT GGCCGTATCAGCGTATCTCCTTATGACACCGCGTGGGTTGCGATGATCAAGGATGTGCAAGGACGCGATGGGCCG CAGTTCCTCATCTTCTTGAATGGATCGTGCAAAATCAGCTGGAAGACGGCTCCTGGGGCGACCAGAAGTTGTTT TGCGTCTATGATCGTCTGGTGAACACCATTGCGTGTGTGGTTGCTCTGCGAAGCTGGAACGTGCACGCACATAAA GTCAAGCGGGGTGTAACGTACATCAAGGAAAATGTGGACAAACTCATGGAAGGAAATGAGGAACACATGACCTGT GGCTTCGAGGTAGTGTTCGCGGCTTGCTTCAGAAGGCTAAATCACTGGGTATTGAGGACCTCCCTACGACTCC CCTGCAGTTCAAGAAGTCTACCACGTGAGAGAACGAAATGAAGCGTATCCACTGGAGATCATGCAATAAGATT CCGACGTGCTCCTGTTTTCACTTGAAGGCCTGGAAAATTTGGATTGGGATAAATGCTGAAGCTTCAGAGTGCA GACGGATCTTTCTCACTTCTCCAGTTTCGACCGCCTTCGCATTATGCAGACAAAAGATGAAAAGTGCTACCAA TTCAATTAAGAACATATCGATACCTTCAACGGCGGAGCACCACACACCTACCCAGTTGATGTATTGCGCCGGCTG TGGGCTATCGACCGTTTGACGCGCCTTGAATTTCCCGATTCTTTGAGCCCGAAATTCGGGATTGCCTCTCTCAC ATCCACAAATCTTGACGGATAAGGGCGTTTTTTCCGGCCGCGAATCTGAATTTTTCGACATTGATGACACCTCC ATGGGCATGCGCCTGATGCGTATGCACGGCTACGAGCTTGATCCCAACGTCTACGTAACTTCAAGCAGAAAGAT GGCAAGTTTTTCGTGCTATGGTGGTCAAATGATCGAGTCCCCATCCCCATATACAACCTTTATCGTGCTCGCAG CTACGCTTTCCGGGCGAGGAGATTTTGAAGACGCCAAGCGTTTTCGCCTACGATTTCTGAAAGAGAACTAGCT AACAACCAGATCCTGGACAAGTGGGTCAATTTCCAAGCATCTTCTGATGAGATTAAGTTGGGTCTGGAGATGCCG

	TGGTTGGCGACACTGCCTCGTGTGTAAGCGAAATACTACATTTCAGTACTATGCAGGTTCTGGCGATGTGTGGATT GGCAAAACTCTGTACCGCATGCCTGAAATCTCTAATGATACTTATCATGATCTTGCCAAAACCGACTTTAAACGC TGCCAAGCGAAGCACCAGTTTGAGTGGTTATACATGCAAGAATGGTACGAGTCTGTGGAATCGAAGAGTTCGGG ATCAGCAGGAAAGACTTACTCCTCTCTACTTCTCGCCACAGCTTCAATCTTCGAACTTGAGCGCACGAATGAA CGTATTGCATGGGCAAAATCCCAAAATCATCGCTAAGATGATTACTTCTTCAATAAAGAAACCGCTCTGAA GAAGATAAGCGCGCTTTATTGAACGAATTGGGCAACATCAACGGTCTGAACGACACCAATGGAGCAGGTCTGAG GGTGGCGCAGGTTCTATTGCACTTGCTACTCTCACCAGTTTCTTGAAGGTTTTGATCGATACCCGCCACCAG CTTAAGAACGCTTGAGCGTGTGGCTGACCAACTGCAGCAGGTGAAGCCGATGATGCTGAGCTTCTACCAAT ACTCTCAACATCTGTGCAGGTCATATCGCATTCCGCGAGGAAATTCTGGCACACAACGAGTACAAGGCTCTGAGC AACCTCACTTCCAAAATCTGCCGCCAGTTGAGCTTTATCCAATCCGAAAAGGAAATGGGTGTGGAGGGCGAAATT GCGGCAAAAAGCTCCATCAAGAACAAGGAGTTGGAAGAGGACATGCAGATGCTGGTTAAGCTCGTGTGGAGAAG TACGCGCGCATGCGACCGCAACATCAAAAAGGCATTTTGGCTGTGGCCAAGACCTATTATACCGCGGTATCAC GCTGCCGACACGATCGATACCCATATGTTCAAAGTGCTATTTGAGCCAGTGGCATAA
<i>CYP76A HI</i>	ATGGATTCTTTCCGTTACTTGCTGCGCTGTTCTTCATAGCAGCAACGATCACCTTCTGTCTTCCGACAGCA CGCAACCTTCCGCCGGGTCCCTTCCCATATCCCATTGTTGGCAACATGCTGCAACTGGGTGCTAATCCACACCAG GTTTTCGCCAAGCTCTCTAAGCGCTACGGCCATTGATGAGCATTCTCTCGGATCACTCTACACCGTCATCGTA TCAAGCCCGGAAATGGCAAAAGAAATCTTCACCGCCACGGCCAGGTGTTAGCGGACGCACCATTCGCGAAGCG GTGCATGCCCTGCGACCACGATAAGATCAGCATGGGTTTTTGGCTGTGGCCAGTGAATGGCGCGCATGCGCTAAA ATCTGCAAGGAGCAGATGTTTTCCAAACAGTCCATGGAAGCATCAAAAGGCTTGAGGCGCCAAAACTGCGACAG CTGCTCGATCATGTGCAGAAGTGTTCTGACTCTGGCGTGCAGTGGATATCCGCGAAGCCGCGTTTATTACGACA CTCAACCTCATGTCTGCCACCTCTTTCTAGCCAAGCAACTGAGTTTGATTCAAAGGCTACCATGGAGTTCAAG GAAATCATCGAAGGTGTGCAACCATCGTTGGTGTCCCAAATTTTGTGACTACTTCCCCATTTTGCACCTTTTC GATCCGCGAGGTTGTGAAGCGTCGAGCAGATGTGTTCTTGGCAAGTTGCTTGCCAAAATGAAGGCTACCTGAAT GAGCGTCTGGAATCCAAACGGGCCAATCCCAATGCGCCAAAGAAAGACGACTTCTTGAGATCGTAGTCGACATC ATCCAAGCCAACGAGTTCAAATTTGAAAACACACCACTTCACTACCTGATGCTGGACTTATTTGTTGGAGGTTCC GACACCAACACCACCTCCATCGAGTGGGCGATGTGCGAGCTGGTGTGAACCCAGACAAGATGGCTCGTCTCAAG GCCGAGTTGAAGTCGTTGCTGGCGATGAAAAGATCGTTGATGAGTCTGCAATGCCAAAATCTCCTTATCTACAG GCAGTTATCAAGGAGTTCATGCGCATCCACCCACCAGGACCCCTACTTCTACCTCGCAAAGCTGAGTCCGATCAG GAAGTCAACGGATACCTCATTCCCAAAGGCACCCAGATCCTCATCAACGCTTACGCAATCGGCCGCGATCCGTCG ATTTGGACCGATCCTGAAACCTTTGACCCTGAGCGCTTCTTCGACAACAAGATTGATTTCAAGGGCCAAGATTAT GAGCTGCTGCCATTCGGAAGTGGCCGACGCGTATGCCAGGGATGCCCTTGCAACACGTATTCTGCACATGGCG ACTGCGACGTTGGTCCACAATTTGCACTGGAAGCTTGAAGATGATTCCACTGCCGCCGCTGATCATGTGGTGAA TTATTGCGCGTTGCCGTGCGTAGGGCGGTTCTTTGCGGATCATCCCGATTGTGAATCGTAA
<i>CPR</i>	ATGGAGCCGTCGTCGAAGAAGCTTAGCCCCCTGGATTTTATCACTGCTATCCTCAAAGGTGACATCGAGGGCGTG GCGCCGCGCGGTGTGCGCCGCGATGTGATGGAAGAACCGGGATTTGGCAATGGTCTTACCCTAGCGTTGCAGTG CTGATCGGCTGCGTGGTAGTCTCGCTGGCGCAGAACGGCAGGTAGTGCGGGAAAAAAGCAGCTGCAGCCTCCC AAACTCGTGGTGCCCAAGCCTGCCGCGGAGCCAGAGGAAGCTGAAGATGAAAAGACCAAGGTGTCTGTTTTTTC GGTACCCAAACCGGCACCGCAGAGGGCTTCGCAAAAGCATTTGCGGAAGAAGCAAAAGCGAGGTACCCGAAAGCC AAGTTCAAGGTGATCGATCTCGACGATTACGCCGCTGATCAGCAGCAATATGAAGAACGATGAAGAAGGATCG CTGGCATTTTTTCTTCTTGGCCTCCTATGGCGACGGCGAACCAGCTGACAATGCGGCTCGTTTTCTACAAGTGGTTC ACCGAGGGTAAGGATCGCGAGGACTGGCTAAAAAACCTTCAGTACGGCGTGTGTTGGCCTCGGGAACCGTCAGTAC GAGCACTTCAACAAGATAGCCATTGTTGGTGGATGACTTGATCACAGAGCAGGGCGGTAAGAACTTGTTCAGTT GGCCTGGGTGATGATGATCAGTGCAATTGAAGATGACTTCTCTGCATGGCGTGAGCTGGTCTGGCCAGAGTTAGAC AAGCTCCTGAGGAACGAGGATGATGCTACTGTAGCCACCCCTTACACCGCTGTGGTTCTGCAATACCGCGTTGTT CTGCACGATCAGACAGATGGTCTGATCACCAGAAATGGGTGCGCTAACGGCCACGCCAATGGCAACACCATTTAT GACGCTCAGCACCCCTGCCGTGCTAATGTGGCGCTCCGTCGAGAACTCCACACGCCCGCTCCGACCCTCTTGC ACCCATCTGGAGTTCGACACCTCCGGCACTGGACTTGTCTACGAAACCGGCGATCACGTCGGCGTTTACTGCGAA AACCTCCTAGAAAAATGTCGAGGAAGCAGAAAAATTGCTTAACTTGAGTCCACAGACCTACTTCTCCGTCCATACC GACAACGAAGATGGAACCCCTTGTCTGGCAGCTCGTTGCCTCCGCCATTCCCGCCGTGACTCTTCGTACGGCC CTGACGAAGTACGCTGATCTGATTTCCATGCCAAAAAATCAGTGCTGGTTGCGTTGGCAGAGTATGCATCTAAC CAGTCTGAGGCGGACCGCTGCGCTACCTCGCTAGCCGTGACGGTAAAGAGGAGTATGCCCAATACATCGTCTGCT TCCCAGCGTAGCTTGCTGGAAGTAATGGCAGAAATTTCCAAAGCGCAAAGCCACCACTGGGAGTCTTTTTTGTCTGCT ATTGCACCGCGGTTGCAGCCGCGCTTCTACTCCATTTCTCTCGCCGAAGATCGCGCTACACGTGTTTCATGTT ACTTGTGCACTTGTATGATAAGACCCCAACAGGACGCATCCACAAGGGAATCTGCTCCACCTGGATCAAGAAC GCGGTGCCACTTGAAGAATCCTCAGACTGCTCTTGGGCACCAATTTTCATCCGCAACTCCAACCTTTAAATTGCCT GCGGACCCCAAGGTGCCTATCATCATGGTGGGGCCAGGAAGTGGCCTTGACCCCTCCCGTGGATTCTTGAGGAG CGCTTAGCTCTCAAAGAATCCGGTGGGAGCTCGGACCAAGCCATTTTGTCTTTGGCTGTGGAATCGCAAAATG GATTTCAAGCTCAGAGAGCTCACTCTTTTTGTAAGGTTGGAGCGATTTCAAGAACTCATGTGGCGTTAGT CGCGAAGGCCCTGCTAAGGAATACGTCCAACACAAAATGTCCAGCGCGCATCCGATATTTGGAAGATGATCTCA GACGGCGGTTACATGTATGTATGTGGCGATGCTAAGGGTATGGCCCGAGATGTGCATAGAACCTTGACACCATC GCGCAAGAACAAGGCTCACTCTCGTCAAGCGAAGCCGAAGGTATGGTTAAAAATTTACAAACGACAGGTCGCTAT CTACGGGACGTGTGGTAA

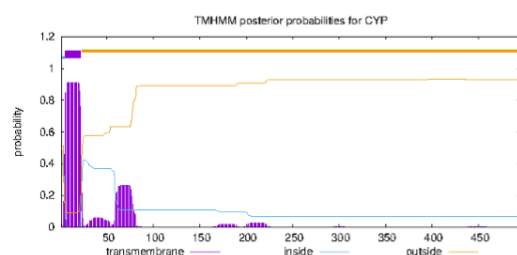
Note: The start codon is indicated in bold, while the underlined regions represent the DNA segments corresponding to the truncation. The gene sequences provided were codon-optimized for *Corynebacterium glutamicum*, with the exception of the *idi* and *idsA* genes.

Figure S1. Transmembrane topology plot of CYP (A) and CPR (B) and their truncation versions using a web service TMHMM 2.0. The amino acid (AA) sequence shown in purple is predicted to form a transmembrane helix motif. The underline sequence was truncated for soluble expression. A start codon (ATG) was introduced to ensure proper translation of the truncated versions. (<https://services.healthtech.dtu.dk/services/TMHMM-2.0/>).

A

TMHMM result

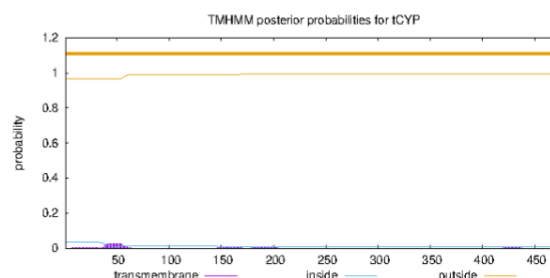
```
# CYP Length: 495
# CYP Number of predicted TMHs: 1
# CYP Exp number of AAs in TMHs: 24.13161
# CYP Exp number, first 60 AAs: 18.57722
# CYP Total prob of N-in: 0.40759
# CYP POSSIBLE N-term signal sequence
CYP TMHMM2.0 inside 1 4
CYP TMHMM2.0 TMhelix 5 22
CYP TMHMM2.0 outside 23 495
```



CYP76AH1

TMHMM result

```
# tCYP Length: 476
# tCYP Number of predicted TMHs: 0
# tCYP Exp number of AAs in TMHs: 0.6173799999999999
# tCYP Exp number, first 60 AAs: 0.53395
# tCYP Total prob of N-in: 0.03602
tCYP TMHMM2.0 outside 1 476
```



Truncated CYP76AH1 (M1-F22)

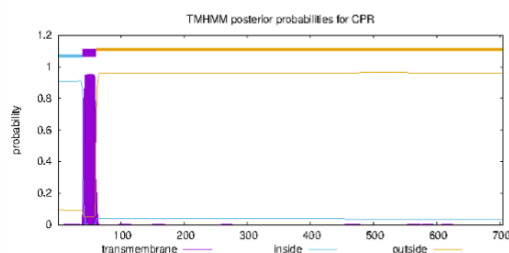
Amino acid sequence of CYP76AH1

MDSFPLLAALFFIAATITFLSRRRRNLPPGPFPIVGNMLQLGANPHQVFAKLKSKRYGPLMSIHLGSLYTVIVSSPEMAKEILHRHGQVFSGRGTIAQAV
HACDHDKISMGFLPVASEWDRMRKICEQMFSNQSMESQGLRRQKLQQLLDHVKQCDSDGRAVDIREAAFITTLNLSATLFSSQATEFDSKATMEFKEI
IEGVATIVGVNPFADYFPILRPFDPQGVKRRADVFFGKLLAKIEGYLNERLESKRANPNAPKKDDFLEIVVDIIQANEFKLKTHHFTHLMLDLFVGGSDTN
TTSIEWAMSELVMNPDKMARLKAELKSVAGDEKIVDESAMPKLPYLQAVIKEVMRIHPPGPLLPRKAESDQEVNGYLIPKGTQILINAYAGRDPISIWD
PETFDPERFLDNKIDFGQDYELLPFGSGRRVCPGMPPLATRI LHMATATLVHNFWDKLEDDSTAADHAGELFGVAVRRRAVLRIIPIVKS

B

TMHMM result

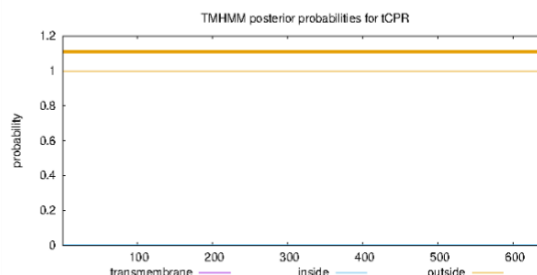
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# CPR Length: 705
# CPR Number of predicted TMHs: 1
# CPR Exp number of AAs in TMHs: 20.05256
# CPR Exp number, first 60 AAs: 19.35973
# CPR Total prob of N-in: 0.90753
# CPR POSSIBLE N-term signal sequence
CPR TMHMM2.0 inside 1 39
CPR TMHMM2.0 TMhelix 40 60
CPR TMHMM2.0 outside 61 705
```



SmCPR

TMHMM result

```
# tCPR Length: 646
# tCPR Number of predicted TMHs: 0
# tCPR Exp number of AAs in TMHs: 0.02266
# tCPR Exp number, first 60 AAs: 0.0004
# tCPR Total prob of N-in: 0.00159
tCPR TMHMM2.0 outside 1 646
```



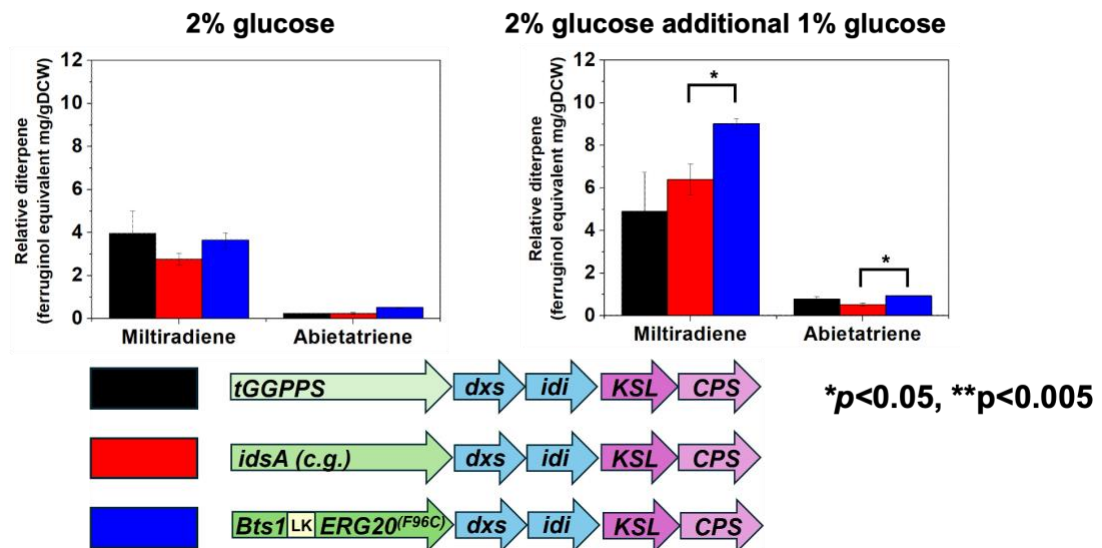
Truncated SmCPR (M1-W60)

CPR sequence:

MEPSSKKLSPLDFITAILKGDIEGVAPRGVAAMMENRDLAMVLTTSAVLIGCVVVLAWRRRTAGSAGKKQLQPPKLVVPKPAEPEEADEKTKVSF
FGTQTGTAEQFAKAFEEAKARYPAKFKVIDLDDYAADDDEYEEKLKKESLAFFFLASYGDGEPTDNAARFYKWFTEGKDREDWLKNLQYGVFGLGNR
QYEHFNKIAIVVDLITEQGGKKLVVGLGDDDDQCIEDDFSARELVWPELDKLLRNEDDATVATPYTAVVLQYRVVLDHQTDLITENGSPNGHANGN
TIYDAQHPCRANVAVRRELHTPASDRSCTHLEFDTSGTGLVYETGDHVGVCENLLENVEEAELNLSPQTYFSVHTDNEGTPLSGSSLPFPFPCT
LRTALTKYADLISMPKKSILVALAEYASNSQSEADRLRYLASPDGKEEYQYIVASQRSLELVMAEFPSAKPPLGVFFFAIAAPRLQPRFYSISSSPKIAP
TRVHTCALVYDKTPTGRIHKIGICSTWIKNAVPLESSDCSWAPIFIRNSNFKLPADPKVPIIMVGPGLTAPFRGFLQERLALKESGAEGLPAILFFG
CRNRKMDFIYDEELNSFVKVGAISELIVAFSREGPAKEYVQHKMSQASDIWKMSIDGGYMYVCGDAKGMDVHRTLHTIAEQGSLSSSEAGEMVKN
LQTTGRYLRDWW

Figure S2. Statistical analysis of relative miltiradiene and abietatriene in engineered *C. glutamicum*. A Student's t-test was conducted to identify the optimal gene expression design among three GGPPS modules for miltiradiene production. The varying arrangement of target genes in DiTPS (A and B) yielded the same conclusion.

A



B

