

## Supplementary Data – Martin et al.

Supplementary Table 1. Pairwise amino acid identities (%) of group 3 SDIMOs.

	<b>SmoX</b>	<b>SmoY</b>	<b>SmoB1</b>	<b>SmoC1</b>	<b>SmoZ</b>
<b>Mmo<sup>1.</sup></b>	52	37	39	38	42
<b>Bmo<sup>2.</sup></b>	55	34	41	34	34

	<b>MmoX<sup>1.</sup></b>	<b>MmoY</b>	<b>MmoB</b>	<b>MmoC</b>	<b>MmoZ</b>	<b>MmoD</b>
<b>Bmo<sup>2.</sup></b>	65	43	53	39	38	13

1. Mmo subunits from *M.capsulatus* Bath.
2. Bmo subunits from *Thaëura butanivorans*.

>pSmo insert sequence

GAGAAAGGGAGTCCACATCTGTAAGAATTCTTAATTACTAGCGCCACCGTTTATGAAGTTGGTCTCAAATAGTCCAATGCCCCGAAACGAA  
CGAGTTTCTGTGTATGACTTCCCACTGCCGGGAAAGGTGTCCGGACACCCGTGGCGCCGCACGCGAGCGTACGCGGACAGGAGGTGCATTCC  
TGGTGATGGACCTGGGCTGGGACTCGGACACGATTCCGGGAAGTATCCGACGAAGTACAAATTCGACCCCAATGCCCGTGGAGGTTCAAGC  
TTGTGCCCGCGATTACGGCCGGATGGAAGGGGAGAAGGACGACCCGAGTACGGCAGCTTGTGGATTCCCTGGCCGGCTGAAGCCACCGAC  
CGAGTCCAGCCCGTGGCCGAGGTCATGAAGCTGCTCGCGGGCAGTGGAGTTGGCGAGTACAACGCGATCGCCGGTTCCGGCTCTCTG  
GCCGACACGACCCGATCTCCGAGCTTCGCAACGGCTACCTCATGCAGGTGGAAGACGAGGTGCCACACCACGACGACGATTACCTCGCCA  
AGTACTACGCCGCTAGTACTACGACCCCGCGGTTCCAGGACATGCGCAAAATGGCGTTACATCAACCCGCTGTTCCCGCCACGATGCAGGC  
ATTTGGCGAGAATTCTCGCGGGGCGACCCGGTGTTCGCTCGCTGAACCTGCAGCTGGTGGCCGAGGCGTGTTCACCAACCCGTTGATCGTG  
GCGATGACCGAGTGGAGCGCGGGAACGGAGATGAAATCACGCCGACGATCTTCTTGTGATTGATCCGACGAGATGCGGCACATGCCAACG  
GGTACCAGACCATCGTGTCTGGCCACGACGCCGACAACATCGACTTCCGACCCGACCTGGAGAATGCATTCCTGGTGCAGCATCGCTT  
CGCTACGCCCATCGTGGAGCCGGCTTTGAGTATGGCGCGGTGAACAAGCTGGAGCCGTGGGCGAAGGTGTGGGACCCGTTGGGTGATGAGGAC  
TGGGCGGCATTGGCTGGCCGGTGGAGAAGTTCGGGTGAAGTCCGGCGGCAACCTCGCGGATGCTAAGCGGCAGGCGATTGGGGTCATC  
ACTACACCTACGCGGTGGCTATCGGATATGGCTTTGTGGCTTCCGATGGACCCACCAACGCGCGGACATGGAGTGGTTCGAGAACAA  
CTACCCCGTTGGCACTCGAAGTCGGCCATATGTTGGATTCTGGCGGGAGATGGCCCTGGCCGACCCCGCAATCACACGTTGCCCGCCAG  
CTGGTACGCGACGGAAGTGGCGATCTATTTCTGCCGTGTGTCAATTTCCGGTTCATCATCCCCACGCTCACCGGTGCGTTGGACGATGTGC  
GGATCTTGGAGCTCAACGTCGAAGCATCCGCTGTGCTCGAGTTGGTGGCAGGATGTTCCTGAAGGAGCCGGAACGGTACCAGGGCGAGAA  
CCTGTGGAGAAGTTCGACGGCTGGAACATCGCTGACGTTGATGCTGCGCGCGGTGCGCAGCAGCGCAAGACGCTGCTTGCACGCGC  
CATCTGAACAGTGGAGGATGGGACGCTGGACGACCTGCGCGCTTGCACGCGGTGATCCGCGATCCGCTCAAGACCGGGGCGTCTGGCTGG  
AAACGGTTAACGCCGTTGCGCGCGACACCCCGAAACCCCGGGCCGTTGGCCCGCGGGAACGCTGACGCACAACCCGCCCAGTTGAGGCG  
GACCAAAATCAAATTTAGGACCTAGCGAGGACACATGACTATTACCGAGGGCGATGTGCCTGCCCGCGGAGATGGCAACGCGCGAACATTT  
TCAACCCCGCTACGACATCGAGCGCGAGCCGCTGGAACCCGACGCACTCGCATGGTACGCCAAACCCATAGACGTCGGATTTCCGAGTA  
GAACTCGCTGTTGCTTACGCGCAGTCCGCCCCGACTGGATTCGCCGCTCGTACTGGCGGCTCTGCAACAAGTTTCCGGCGCCGCT  
CGCGCGTGGGAACTACGACGAGGAGGCAAGTCCCTCGGACTGGTTTGTGTTCCGGATCCCTCCGGTCCGCGACAGCCAGTATGTAGCGC  
AGAAAGCAGAGGAGAACGAAGAGGCGGGCGGATGTTCCGCGCTACGCGGACGAGGCGATGTACCGGGACCTGGATCGGGAATGGACCTTTGA  
CGTGTGCTACTGGGTTAGTGGCTTCGCGCACACGAGTACGGGCTTTTCGAGCTCTCGTAGGGGCGCCGGCCGCGACGTTTCCCGC  
GTGTTGCCGCGAGGCGGTGATCACCGAGCGATCGACCATCTCGACAACGCGAAATGATCCAAGCTGAAAAGGTGTTCCGTCCAAGAAATGTCG  
ACGGCTTCCGAAAGCTCGCGCCGCGCCGTAATTCGGCTGTGGACCCGGTGTACAGGACCCCGCGGCGATTGTGAGCGGCTGTGGGG  
CGAGGTGATGACCACATCGAGATCATTTTCGCGACGTTATGTTGACGAGCCCTCTCTTCGGGCGTTTTCGCGCCAGCAGCTTCTTCCCGG  
CTGGCTCCGATGACCGGTGATCAGCTCACCCGCGCATCATGTGGCCGACGATCACTGCCGCCAAATCGACGCGAAATGGGCGTTTCGAGCTGT  
TTGGCCACGCTTTGGCCAGGACCCGAAATTCGAGAACACAACCGGGCGTGTGCGGTTGTGGGCACGGGATGGATGCTGCAGTCTGTGGC  
CGGATGGCCGAGTTCCGCCGATGTTTCCCGAACAGCACTTCCGCTCTCAGGCGTCCAGCGAGACGGTTAACGAGCCGCGTTCGATCGTG  
ATCGGCGACTGGGCGCGCGTTATGCGCCCATCTTCGGGCTGGAGGTCAACGTGACGCAACTGGTCAAAAGCGTACAGCAGCGTACACGGGCG  
CGGTCCGCTGCATCGCCACCGAAGCGCTTGACCCGACCGAACAATGACAGCACATCTCCAGTCCGCTTTACGGAGCGCGCAATGACCT  
TAGATGACCCGAGGTGACAGCTTCGACAGGCGACAAGAGTCTGGGCTGCGAGACCATACCACGCGCAACCGGCTCCGAGAGACTTCAACCC  
TCAGGCGTTGCAAGACCGCATGTATGGCGAGGCGCGCGGCTCCTGTTGCTGAATCCCGTCCGCTCGTGTGCTTGTGATGAAAAGCAGCGAA  
GTCGAAATCGTCTGACGAGCTGATGGAGGATACGCCGAGGACGATTTTTCATCGTCAAGACGCGGGCACCTTCTATCGGCTGGAAAACG  
ACGAGGGCTTCGAGATTGACCTCGACGTCATCGAGCCGTTGATCGGGCACCATACGAGCTTTTCGACTTCATGTTGAAGCTACGACGACCAT  
CGGTCGCGGTATAACGACGGAACAAGTTCTGTGATGACCACGAAAATGATGGGCTGGAGGAGGAGTCCCAAGGTTCCAACGCGGTGGCGAC  
GACGAATCCGACGACCTCAATCACATCGGGCGCCGGAGGGAGCGGTAGCGCGGGGCTTGGGATCCCTCCGGCTTCCAGTCCACTGAGATGG  
GTTGGAGCAATTTGGACGACACGGAACGGGTCAATTTGGAAGACGAAGAATTCACCGTAGTCTGATGAGGCAATCCCGTGTGGGAATGGT  
TTATCTTCCGTGGCGACGCTGAGATACAAAACGGCGTGTCAATTGACTGAGCGAGCGCATGTGAGGCGGCATGAAGATATTGGCTATTTTCA  
AGGACGGATAGGAAACGTTGCCAGGGGAGATACCGATTCCGCCGGAACGGGATGCGATGACCTTTAGTGTAAAGCTGTTTTTTGACGACGATC  
ACGAGCCGCGATCAGTTGCGAGCCGATGAGGATGTTATTTCCGCGCGCTGCGGCAAGGGCTCATTCTGATGAGCGAATGCCGGAGGGGGT  
GTCTCGACCTGCAAGTGTCTTTCGGCCGAGGGGGAATACAGCAGACTAATGTCGATTTCTGTTTACCGCTGTCCCGCCGCAAGAGGAAGAG  
GGTTTGGTGTGGCGTGGCGCTGCGTCCGGCTCGGATTTGGAGATCGAATTCGACTACCCGTTTCGATGGTCCAGCAGTACGAGGAGACCG  
AACGGCGGGGAGCCTCGACGAGATCGAGATGGTGTTCGACACGGTGTGCGGGTGGTGTGCGGACCTTGGGAGCGCAAGAGCCGCTCCGGTA  
TCTGCCAGGTGAGTTGTCGCGATCGCGTACCAACGGTGTGAGTTCGCGACTTCTCGATGGCAATGTTTCGGACGACTCGAGACGGCTCGAG  
TTTTTCATCCGGTGTATCCGACGCGCAATTCAGTACATCGCCCGACGCGAGTCCGGTGAACAGGTGACCTGCACGGACCCGAG  
GAAAATTCATCTCGTGACAATGAGCGACCCCTATGTTTATCGCCGCGCGCACCGGGCTGGCGCCGCTGCTGGCGATGCTGGCGAGCTGGC  
CACCGATCATGCTGATCGTCATCGGTTTTGATTTTCGAAAACCAACGTTGGCGACGCTTTCGGTGGCGAGGAATTAAGGCTTTGGGCGCG  
CGATTGCCTAACCTGCAAGTGAATTTCCAGTGTATCAACCCCGATGATTTGGACCGGCGTGTGGCACTGCGACAGCGGTAGCCGAAGAGT  
ACATATCGGGCAGGGGAGGGTCGGAGTTGCAATATTACTACTGCGGTCGCGGAAAAATGATCGAGGCAACAAACCAATGCTCGAAGTAGCAGG  
GGTGGGCGCGAATTCGCGCCACATGAAGACTTCGTGCCAGCAGAATGGAGGAAAGCAATGCGTCAAAACACGGTCCGGACGACGGCGGGC  
CCGCGCCAGGAATCGGTCCGCGCGAATTCGGTGGAGCCCTCGCCCTACGGCAATACGGCACTGCGGTCCGATTGGGCGGGCCGGCTGAA  
TCGCTGTGCGATTCGATGACCGGTGGCGACGCTGATGAATATCGGTGCGACCGATGGGCGAGCAAGACCAGGACAGCCTCGGATAGAGG  
CCCGGCTTGGAGAGCGGTCGCGGTGCTGCGCTGCAGTCGATGACCGACGAGGATTTTCGACCCGACGCTGACCGGTGAGACGCGCGCA  
TGTGTACCACGCTCGCGGACCGCTCGCCCGCTGGTACGATACGGGGAGATCGAGCGCATCAATGACGAGTTGAGTGCAGGATGAAATATAAG  
CCGCGGTCATGCCGCCAATCTCTTTCGCCACCGAGCGTCACTCAGTGAAGAGCTGATGAAAACCCGTACGGTTCAGTGGTACGGCAAT  
CCCTCGCGAGCTCGGGGAGGAGCGGGTGTGGTGGTCCACGCGACGCCACCCGGCAGTCAATGATGTTGACCATCCGGCGTCCGCGCATCGA  
AGCAGCACAAATGGAACGGTAGGCCCTAGCATTTAAATGGATCCGTTCTCAAATCTCTGATGTTACATTGCACAAGATAAAAATATATC  
ATCATGAACAATAAACTGTCTGCTTACATAAACAGTAATCAAGGGGTGTTATGAGCCATATTCACCGGA

*smoX*  
Hydroxylase  
alpha

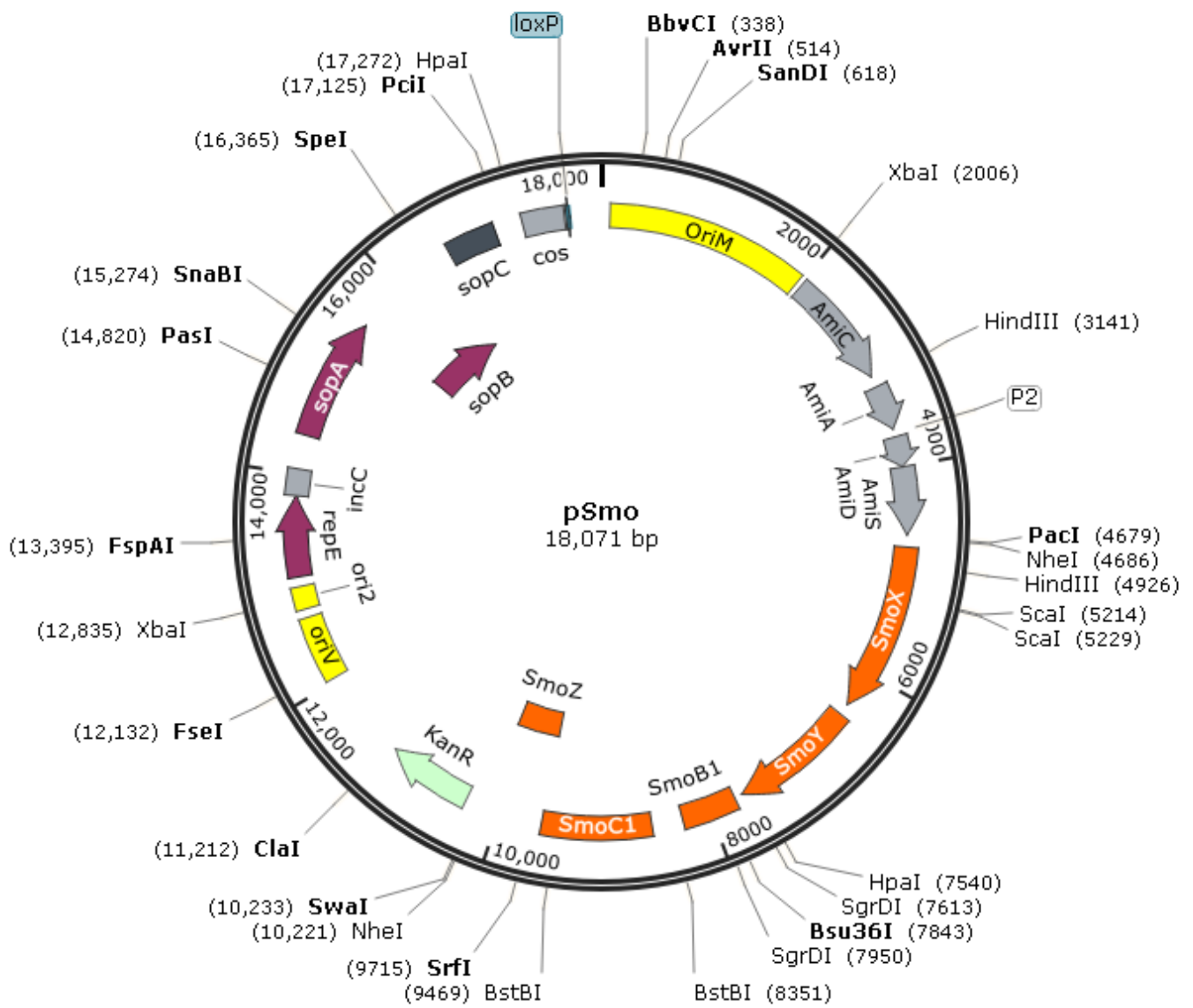
*smoY*  
Hydroxylase  
beta

*smoB1*  
Coupling/  
effector

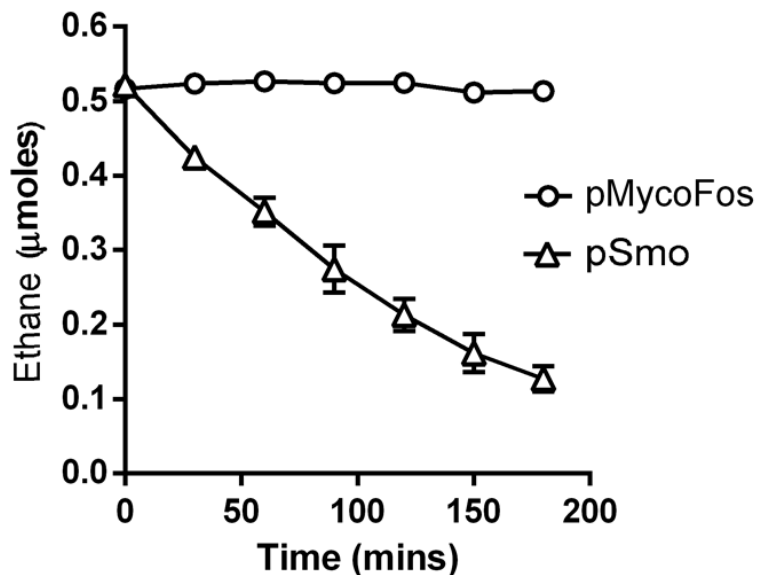
*smoC1*  
Hydroxylase  
gamma

*smoZ*  
Reductase

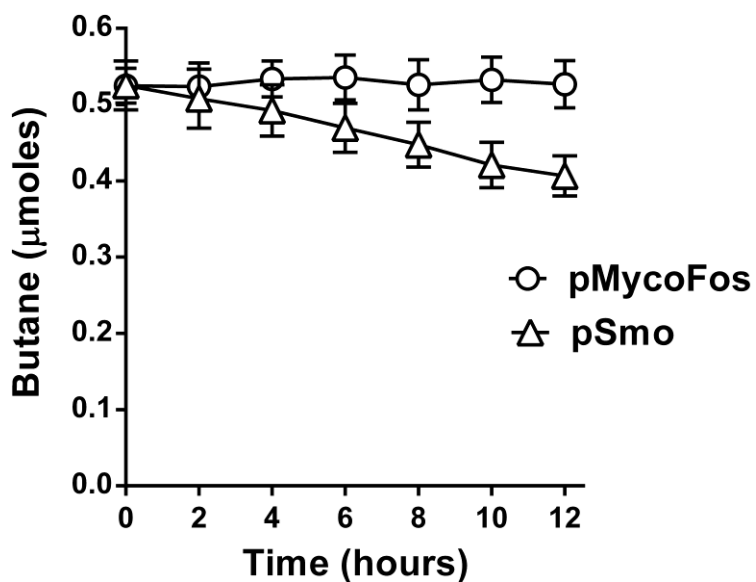
**Supplementary Figure 1.** Sequence of pSMO insert, confirming correct cloning of the *smoXYB1C1Z* genes. The *smo* genes sequence can be obtained from Genbank CP003054.1 or GU174751.2. Predicted start and stop codons and ribosome binding sites are underlined. The *NheI* sites used for cloning are underlined and highlighted in light blue.



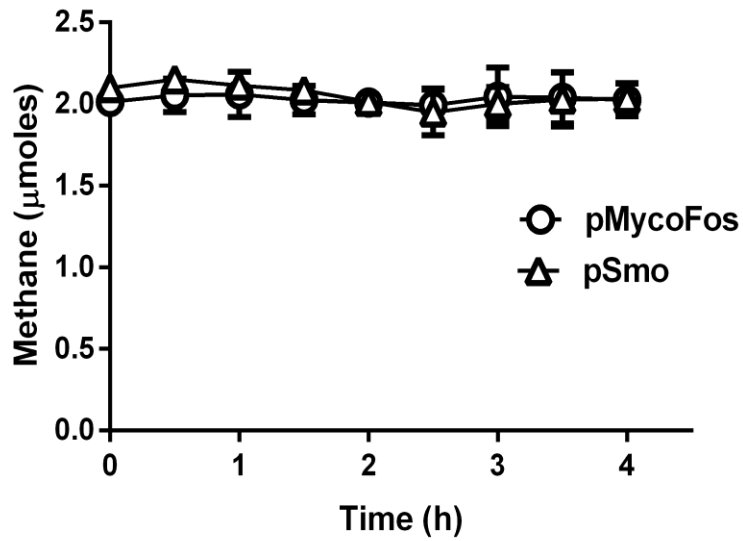
Supplementary Figure 2. Schematic of plasmid construct pSmo.



Supplementary Figure 3. Metabolism of ethane by cells of mc<sup>2</sup>-155(pMycoFos) and mc<sup>2</sup>-155(pSmo). Data are shown as the mean of three independent experiments, with error bars denoting the standard error of the mean (SEM).



Supplementary Figure 4. Metabolism of butane by cells of mc<sup>2</sup>-155(pMycoFos) and mc<sup>2</sup>-155(pSmo). Data are shown as the mean of three independent experiments, with error bars denoting the standard error of the mean (SEM).



Supplementary Figure 5. Test of methane metabolism with cells of  $mc^2$ -155(pMycoFos) and  $mc^2$ -155(pSmo). Data are shown as the mean of three independent experiments, with error bars denoting the standard error of the mean (SEM).