**Supplementary Material**

# Substrate promiscuity of polyketide synthase enables production of tsetse fly attractants 3-ethylphenol and 3-propylphenol by engineering precursor supply in yeast

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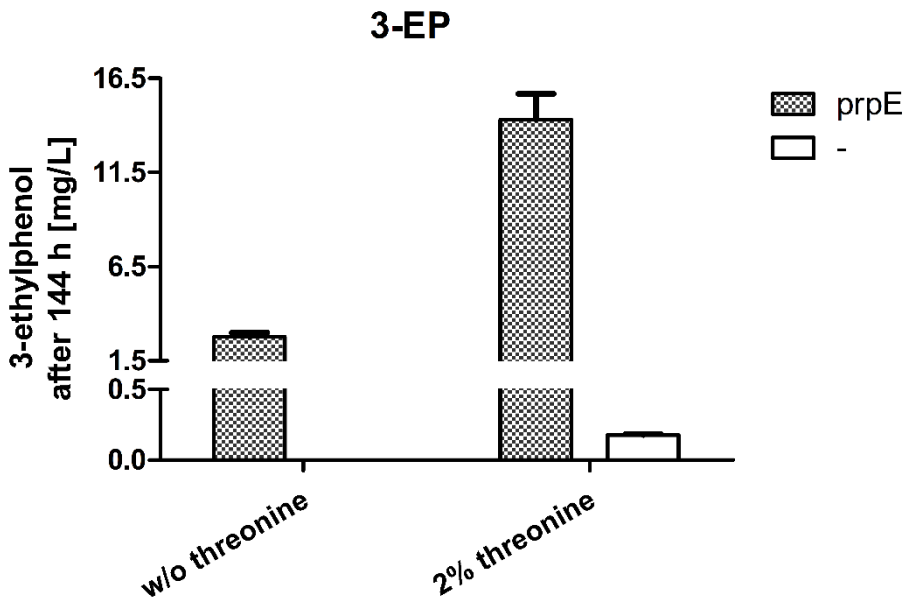


*Figure S1. Influence of overexpression of a propionyl-CoA synthetase on 3-ethylphenol formation with and without supplementation of external threonine.* Yeast strain CEN.PK2-1C with the *Δcit2Δcit3* double deletion expressing the 3-methylphenol pathway (*$^{Ppopt}$MSAS*, *$^{opt}$npgA* and *$^{opt}$patG*[14]) and with or without the propionyl-CoA synthase *$^{opt}$prpE* (strains JHY185 and JHY197, respectively), were inoculated at an OD of 5 and cultivated for 144 h in $KP_i$ buffered YPD medium (pH 6.5) with or without supplementation of 2 % threonine. Culture supernatants were analysed via HPLC for 3-alkylphenol production. Error bars represent standard deviations of biological duplicates.The y-axis was truncated to visualize also small values.

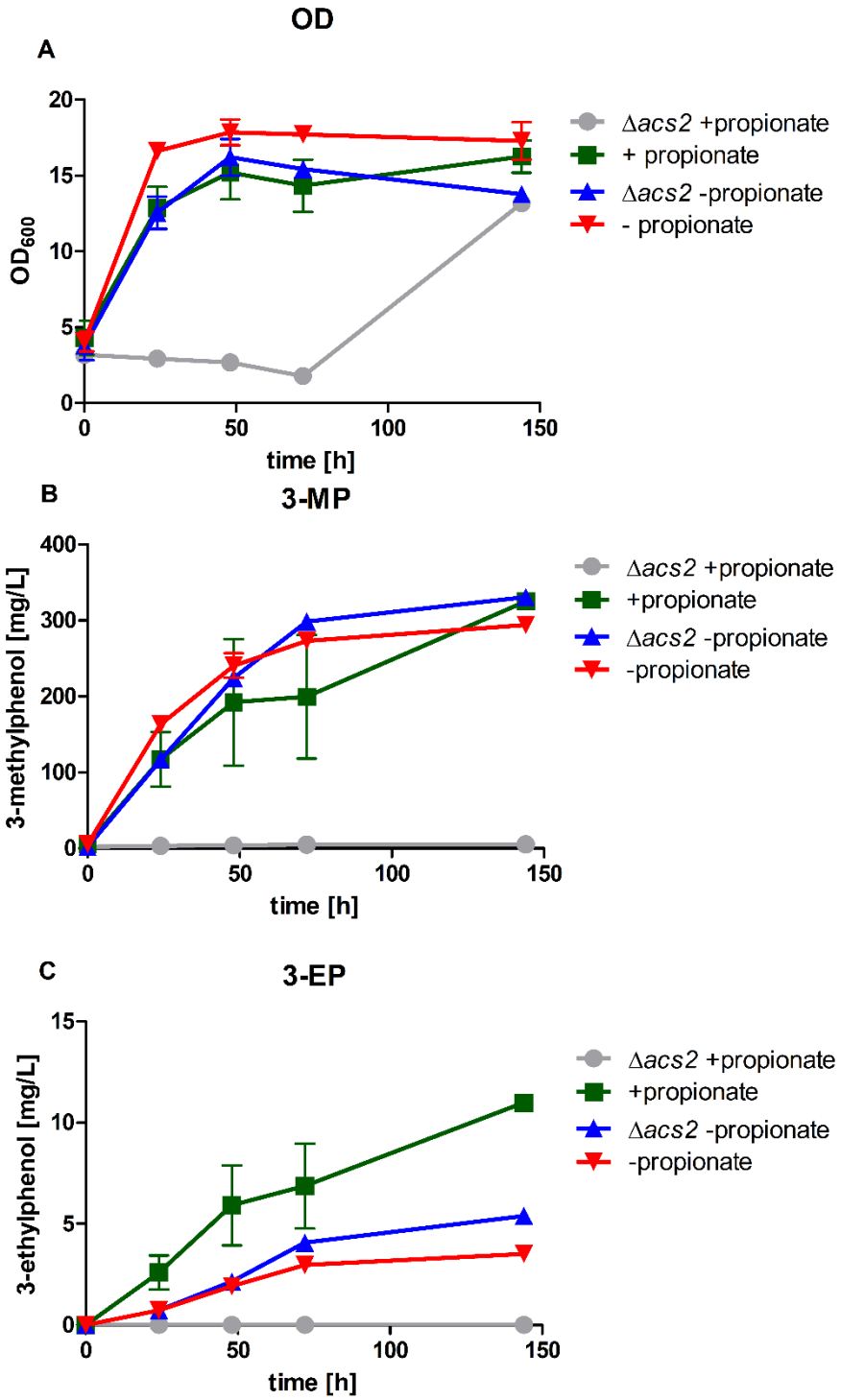


*Figure S2. Influence of acs2 deletion on growth (A), 3-methylphenol (B) and 3-ethylphenol (C) formation with or without supplementation of external propionate.* Yeast strains CEN.PK2-1C expressing the 3-methylphenol pathway (*$^{Ppopt}$MSAS*, *$^{opt}$npgA* and *$^{opt}$patG;* Hitschler and Boles, 2019), the propionyl-CoA synthase *$^{opt}$prpE*, *Δcit2Δcit3* double deletion and with or without *Δacs2* were inoculated at an OD of 3.5 and cultivated for 144 h in $KP_i$ buffered YPD medium (pH 6.5) optionally supplemented with 10 mM propionate. Culture supernatants were analysed via HPLC for 3-alkylphenol production. Error bars represent standard deviations of biological duplicates.

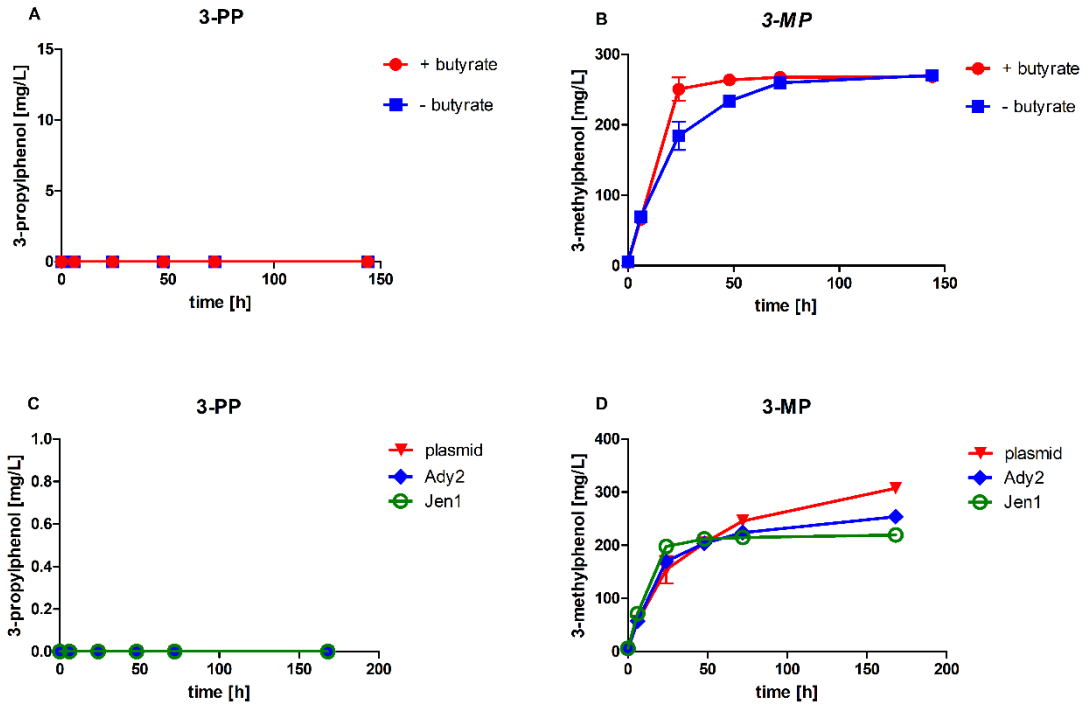


*Figure S3. 3-alkylphenol production in different yeast strains with or without butyrate supplementation.* 3-propylphenol (A) *and* 3-methylphenol (B) production of CEN.PK2-1C expressing the 3-methylphenol pathway (*$^{Ppopt}$MSAS*, *$^{opt}$npgA* and *$^{opt}$patG;* strain JHY162*;* Hitschler and Boles, 2019*)* with or without butyrate supplementation*.* 3-propylphenol (C) *and* 3-methylphenol (D) production of strain JHY211 (*Δpox1*) and expressing monocarboxylic acid transporters *JEN1* or *ADY2* from multi-copy plasmids pRS72N_JEN1 or pRS72N_ADY2 (empty plasmid pRS72N as control) in the presence of 10 mM butyrate. High-OD fermentations (starting OD = 4-5) were performed in biological duplicates at 30°C in $KP_i$ buffered YPD medium (pH 6.5) supplemented with (10 mM) or without butyrate. Culture supernatants were analysed via HPLC for 3-alkylphenol concentrations. Error bars represent standard deviations.

*Table S1. Primers for plasmid or strain construction used in the present work*

| **Primer name** | **5´-3´ sequence** | **Application** |
|---|---|---|
| **Deletion of *CIT2*** | | |
| ABP047_ProCIT2_fw | CATTTATCCGGTGGTCATCG | amplification of *CIT2*, forward |
| ABP048_terCIT2_rev | GCTAGCCAAGGCAGTAAGG | amplification of *CIT2*, reverse |
| ABP049_CIT2_rev | GCTTTCCAAGGCAGTTACAG | sequencing of *CIT2*, reverse |
| ABP054_CIT2_Del40_ Oligo | CTCAAAACTTTTTGTTTTAATAA TACTAGTAACAAGAAAATTGGAT | Donor-DNA for deletion of *CIT2* binding in *CIT2p* with overhang to *CIT2t*, forward |

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|---|---|---|
| | TACATCCTACTTTTACACCCCTC TGCATATTTTT | |
| ABP055_CIT2_Del40_ Oligo_Comp | AAAAATATGCAGAGGGGTGTAA AAGTAGGATGTAATCCAATTTTC TTGTTACTAGTATTATTAAAACA AAAAGTTTTGAG | Donor-DNA for deletion of *CIT2* binding in *CIT2t* with overhang to *CIT2p*, reverse |
| **Cloning of pJHV54** | | |
| MGP126_CrCASseq.fw | GGGAAACGCCTGGTATC | sequencing of gRNA, forward |
| WGP243_S-Cas9-1_Rv | TCTTCTTGAAGTAGTCTTCC | amplification of pRCC, reverse |
| WGP245_S-Cas9-3_Fw | GGCTATTGTTGACTTGTTG | amplification of pRCC, forward |
| JHP233_gRNA_CIT3_f | cttctgcatatatgtgcgccgTTTTAGAGC TAGAAATAGCAAGTTAAAATAAG G | amplification of pRCC with gRNA for *CIT3*, forward |
| JHP234_gRNA_CIT3_r | cggcgcacatatatgcagaagGATCATT TATCTTTCACTGCGGAGA | amplification of pRCC with gRNA for *CIT3*, reverse |
| **Deletion of *CIT3*** | | |
| JHP237_CIT3p_f | CCATGGTAGCGGTTCTAAAG | amplification of *CIT3*, forward |
| JHP238_CIT3t_r | TTTGTAAACGGCCCGAGG | amplification of *CIT3*, reverse |
| JHP239_CIT3seq_f | GGGATTAGCGGGTCCTTTG | seqencing of *CIT3*, forward |
| JHP240_CIT3_del40_O ligo | AGAATTTATACATAGACGCCGC TAAATAATTGAATACAAACGCAG TTCCAATTTACAAGAATGCTTCG TTTGCTATTACAA | Donor-DNA for deletion of *CIT3* binding in *CIT3p* with overhang to *CIT3t*, forward |
| JHP241_CIT3_del40_O ligo_comp | TTGTAATAGCAAACGAAGCATT CTTGTAAATTGGAACTGCGTTT GTATTCAATTATTTAGCGGCGT CTATGTATAAATTCT | Donor-DNA for deletion of *CIT3* binding in *CIT3t* with overhang to *CIT3p*, reverse |
| **Cloning of pJHV19** | | |
| JHP108_CC_sfa1#2_r | cacagtcccgcagccaaatacGATCATT TATCTTTCACTGCGGAG | amplification of pRCC with gRNA for *SFA1*, reverse |

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|---|---|---|
| JHP107_CC_sfa1#2_f | gtatttggctgcgggactgtgTTTTAGAG CTAGAAATAGCAAGTTAAAATAA GG | amplification of pRCC with gRNA for *SFA1*, reverse |
| **Genomic integration of *TDH3p-$^{Stopt}$prpE-sfa1t* into *sfa1* locus** | | |
| JHP018_ovTDH3p_r | *ttgatagatctttggtagaattcagagaaagac atTTTGTTTGTTTATGTGTGTTTA TTC* | amplification of *TDH3p* with overhang to *prpE* and pJHV1, reverse |
| JHP019_ovPGK1t_f | aacaaatcagacaagctatcgaagaataac cgcggATTGAATTGAATTGAAATC GATAG | amplification of *PGK1t* with overhang to *prpE* and pJHV1, forward |
| JHP058_prpE_ovTDH3 p | tttaaaacacCAAGAACTTAGTTTCG AATAAACACACATA | amplification of *prpE* with overhang to *TDH3p* and *PGK1t,* forward |
| JHP059_prpE_ovPGK1 t | gagaaaagaaAAAAATTGATCTATC GATTTCAATTCAATTC | amplification of *prpE* with overhang to *TDH3p* and *PGK1t,* reverse |
| JHP060_ovTDH3p_Sac I_f | tttaatttgcggccggtacccaattcgcccgag ctcACAGTTTATTCCTGGCATCCA CTA | amplification of *TDH3p* with overhang to *prpE*, forward |
| JHP061_ovPGK1t_Ba mHI_r | agcgcgcaattaaccctcactaaagggaac aaggatccAAATAATATCCTTCTCG AAAGC | amplification of *PGK1t* with overhang to *prpE*, reverse |
| JHP075_prpE_d->c_1r | cgaagatagtgtccatagaagtAGCCAAA GCAACAGCGTAAC | correct deletion at 783 bp in *prpE*, reverse |
| JHP076_prpE_d->c_2f | agacgttggtggttacgctgTTGCTTTGG CTACTTCTATG | correct deletion at 783 bp in *prpE*, forward |
| JHP079_TDH3p_prpE_ ovsfa1up_f | ataatacttacggggctagttttattttctaccac aaataaaaataaagcatcttttaaACAGTT TATTCCTGGCATCCACTA | amplification of *prpE* and *TDH3p* with overhang to *SFA1* upstream region, forward |
| JHP080_prpE_ovsfa1t_ r | ttgattttcaaagtattccagaaaatttgagtcat gcttacttagtttaattaagtactcTTATTCT TCGATAGCTTGTCTGATT | amplification of *prpE* and *TDH3p* with overhang to *SFA1t*, reverse |
| vsp328_pSFA1_fw | GAATAAGTCCTGGTTCCAGGCA AAACC | primer binding upstream of *SFA1* locus to check integration, forward |

| | | |
|---|---|---|
| MGP146_SFA1down-rev | GTTAGGAACAGGCGAGGTC | primer binding downstream of *SFA1* locus to check integration, reverse |
| JHP021_prpEseq1 | CGCTGTTGACAGATGGAGAG | sequencing of *prpE* |
| JHP022_prpEseq2 | CCCAGACTGTGGTGTTTG | sequencing of *prpE* |
| JHP083_sfa1_seq1_f | GTGACAACCGAAAGTCAG | sequencing primer binding upstream of *SFA1* locus, forward |
| JHP084_sfa1_seq2_r | TTTCTTCAGGTCTAACTGATTG | sequencing primer binding downstream of *SFA1* locus, reverse |
| **Deletion of *ACS2*** | | |
| JHP288_p-HYG-t_ovACS2_f | catatgcgtttcccggggccgaagcgttattgccgatattTTCGTACGCTGCAGGTCGAC | amplification of TEFp-hphNT1-CYC1t with overhang to upstream region of *ACS2*, forward |
| JHP289_p-HYG-t_ovACS2_r | cttttaccctatcccgggcgaagaaccccgtcacagtgGCATAGGCCACTAGTGGATCTG | amplification of *TEFp- hphNT1-CYC1t* with overhang to downstream region of *ACS2*, reverse |
| Vsp333_tACS2_rev | AACAAGGCAAAATAGCGTTAACAACC | primer binding downstream of *ACS2* locus to check deletion, reverse |
| Vsp334_pACS2_fw | TTTCCTGTGAGAAGTTTAAATCCACTAAGG | primer binding upstream of *ACS2* locus to check deletion, forward |
| Vsp338_ACS2_fw | TTGGCTGTGGCTCGTATTGGTGC | primer binding in *ACS2* locus to check deletion, forward |
| MGP122_hphNT1_fw | TCACTGGCAAACTGTGATGG | primer binding in *hphNT1* to check integration, forward |
| **Deletion of *POX1*** | | |
| ABP017*_POX1_Del40_Oligo | TCACAGAAAAAAAGAAAATATAATAAATTAGTATTGCGATGTAGAGGTTTCCTGTTTTCCTTCGAACCCTCTGTTTTGCG | Donor-DNA for deletion of *POX1* binding in *POX1p* with overhang to *POX1t*, forward |
| ABP025_POX1_Del40_Oligo_comp | CGCAAAACAGAGGGTTCGAAGGAAAACAGGAAACCTCTACATCGCAATACTAATTTATTATATTTTCTTTTTTTCTGTGA | Donor-DNA for deletion of *POX1* binding in *POX1t* with overhang to *POX1p*, reverse |

| **Assembly via part plasmids and sequencing of pJHV62 and pJHV65** | | |
|---|---|---|
| GDP253 Rv TER_ACP1 GG as 3 | CGTCTCAGGTCGGTCTCAGGATTTAAATTCTGTCGAATCTTTCAACTTC | amplification of *Ter* with overhangs for Golden Gate part 3, reverse |
| GDP261 Fw tdTER GG as 3 | CGTCTCGTCGGTCTCATATGATTGTTAAGCCAATGGTTAGAAACAAC | amplification of *Ter* with overhangs for Golden Gate part 3, forward |
| GDP255 Rv Crt_mtMDH GG as 3 | CGTCTCAGGTCGGTCTCAGGATTTATCTGTTCTTGAAACCTTCAATC | amplification of *Crt* with overhangs for Golden Gate part 3, reverse |
| GDP263 Fw Crt GG as 3 | CGTCTCGTCGGTCTCATATGGAATTGAACAACGTCATC | amplification of *Crt* with overhangs for Golden Gate part 3, forward |
| GDP259 Rv ERG10_mtNC GG as 3 | CGTCTCAGGTCGGTCTCAGGATTTAAATCTTTTCAATGACAATAGAGGAAGC | amplification of *ERG10* with overhangs for Golden Gate part 3, reverse |
| GDP262 Fw ERG10 GG as 3 | CGTCTCGTCGGTCTCATATGTCTCAAAACGTTTACATTG | amplification of *ERG10* with overhangs for Golden Gate part 3, forward |
| GDP265 Rv Hbd GG as 3 part 1 | CTGGGTTGAAGAAGTGCATACCAATAACCTTATCTGGCCTCTTAGTAGCAGAAG | amplification of *Hbd* part 1 without *Bsa*I cutsite and with overhangs for Golden Gate part 3, reverse |
| GDP266 Fw Hbd GG as 3 part 2 | TCTGCTACTAAGAGGCCAGATAAGGTTATTGGTATGCACTTCTTCAAC | amplification of *Hbd* part 2 without *Bsa*I cutsite and with overhangs for Golden Gate part 3, forward |
| GDP267 Rv Hbd GG as 3 part 2 | CGTCTCAGGTCGGTCTCAGGATTTACTTAGAGTAATCGTAGAAACCC | amplification of *Hbd* part 2 without *Bsa*I cutsite and with overhangs for Golden Gate part 3, reverse |
| GDP285 Fw Hbd GG as 3 part 1 | CTGATTCTGTGGATAACCGTAGTCGGTCTCATATGAAGAAGGTTTGTGTTATTG | amplification of *Hbd* part 1 without *Bsa*I cutsite and with overhangs for Golden Gate part 3, forward |
| hdp073 | GGTTGCATCACTCCATTG | sequencing primer binding in *TDH3p,* reverse |
| Hdp446 | TCCTTTACGCTAAAATAATAGTTTATTT | sequencing primer binding in *PGK1t,* forward |

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|---|---|---|
| JTP302 | AAGGCATTAAAAGAGGAGCG | sequencing primer binding in *PGK1t,* reverse |
| SZ069seq1EcPPC_for | GTTATCCCCTGATTCTGTG | primer binding in SiHV110 backone, forward |
| vsp84_Hbd_ovpPGK1_fw | TCTACTTTTTACAACAAATATAAAACAATGAAGAAGGTTTGTGTTATTGG | sequencing primer binding in *Hbd,* forward |
| vsp156_seq3_tVMA16 | CATACACATGTATCTCAGATATCTC | sequencing primer binding in *VMA16t,* reverse |
| Vsp157_seq4_ERG10 | TTTCGTTGTCGAACTTACC | sequencing primer binding in *ERG10,* reverse |
| Vsp160_seq7_hbd | TATTGCTATTGGTAAGGATCC | sequencing primer binding in *Hbd,* forward |
| Vsp162_seq9_crt | CACCGAAACCTGGGG | sequencing primer binding in *Crt,* reverse |
| vsp313_seq55_tdTer | CCGTCTTGAAGCCATTCGG | sequencing primer binding in *Ter,* forward |
| vsp314_seq56_tdTer | TTACAGACACGACTTCTTGGC | sequencing primer binding in *Ter,* forward |
| vsp315_seq57_pTDH3 | CAACTACAGAGAACAGGGGC | sequencing primer binding in *TDH3p,* forward |

*Table S2. Genes used in the study with their source organism and sequence.* Sequences codon-optimized (opt) for *S. cerevisiae* are indicated by prefixes in superscript.

| **Gene** | **Sequence** | **Source organism** |
|---|---|---|
| $^{Ppopt}$MSAS | ATGCACTCTGCTGCTACTTCTACTTACCCATCTGGTAAGACTTC<br>TCCAGCTCCAGTTGGTACTCCAGGTACTGAATACTCTGAATAC<br>GAATTCTCTAACGACGTTGCTGTTGTTGGTATGGCTTGTAGAG<br>TTGCTGGTGGTAACCACAACCCAGAATTGTTGTGGCAATCTTT<br>GTTGTCTCAAAAGTCTGCTATGGGTGAAATCCCACCAATGAGA<br>TGGGAACCATACTACAGAAGAGACGCTAGAAACGAAAAGTTCT<br>TGAAGAACACTACTTCTAGAGGTTACTTCTTGGACAGATTGGA<br>AGACTTCGACTGTCAATTCTTCGGTATCTCTCCAAAGGAAGCT<br>GAACAAATGGACCCACAACAAAGAGTTTCTTTGGAAGTTGCTT<br>CTGAAGCTTTGGAAGACGCTGGTATCCCAGCTAAGTCTTTGTC<br>TGGTTCTGACACTGCTGTTTTCTGGGGTGTTAACTCTGACGAC<br>TACTCTAAGTTGGTTTTGGAAGACTTGCCAAACGTTGAAGCTT<br>GGATGGGTATCGGTACTGCTTACTGTGGTGTTCCAAACAGAAT<br>CTCTTACCACTTGAACTTGATGGGTCCATCTACTGCTGTTGAC<br>GCTGCTTGTGCTTCTTCTTTGGTTGCTATCCACCACGGTGTTC<br>AAGCTATCAGATTGGGTGAATCTAAGGTTGCTATCGTTGGTGG<br>TGTTAACGCTTTGTGTGGTCCAGGTTTGACTAGAGTTTTGGAC | *Penicillium patulum* |

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|---|---|---|
| | AAGGCTGGTGCTATCTCTTCTGACGGTTCTTGTAAGTCTTTCG<br>ACGACGACGCTCACGGTTACGCTAGAGGTGAAGGTGCTGGTG<br>CTTTGGTTTTGAAGTCTTTGCACAGAGCTTTGTTGGACCACGA<br>CAACGTTTTGGCTGTTATCAAGGGTTCTGCTGTTTGTCAAGAC<br>GGTAAGACTAACGGTATCATGGCTCCAAACTCTGTTGCTCAAC<br>AATTGGCTGCTAACAACGCTTTGTCTGCTGCTAACATCGACCC<br>ACACACTGTTAGATACGTTGAAGCTCACGCTACTTCTACTCCAT<br>TGGGTGACCCAACTGAAATCTCTGCTATCGCTTCTGTTTACGG<br>TGCTGACAGACCAGCTGACGACCCATGTTACATCGGTTCTATC<br>AAGCCAAACATCGGTCACTTGGAAGCTGGTGCTGGTGTTATGG<br>GTTTCATCAAGGCTGTTTTGGCTATCCAAAAGGGTGTTTTGCC<br>ACCACAAGCTAACTTGACTAAGTTGAACTCTAGAATCGACTGG<br>AAGACTGCTGGTGTTAAGGTTGTTCAAGAAGCTACTCCATGGC<br>CAGAATCTGACCCAATCAGAAGAGCTGGTGTTTGTTCTTACGG<br>TTACGGTGGTACTGTTTCTCACGCTGTTATCGAAGAATTCTCTC<br>CAATCTTGCAACCAGACCCATTGGGTAACGGTGCTGTTTCTGG<br>TCCAGGTTTGTTGTTGTTGTCTGGTCCACAAGAAAAGAGATTG<br>GCTTTGCAAGCTAAGACTTTGAGAGACTGGATGACTGCTGAAG<br>GTAAGGACCACAACTTGTCTGACATCTTGACTACTTTGGCTACT<br>AGAAGAGACCACCACGACTACAGAGCTGCTTTGGTTGTTGACG<br>ACTACAGAGACGCTGAACAAGTTTTGCAATCTTTGGCTAACGG<br>TGTTGACCACACTTTCACTACTCAATCTAGAGTTTTGGGTTCTG<br>ACATCTCTAAGGACGTTGTTTGGGTTTTCTCTGGTCACGGTGC<br>TCAATGGCCAGACATGGGTAAGCAATTGATCCACAACCCAGTT<br>TTCTTCGCTGCTATCCAACCATTGGACGAATTGATCCAAGCTG<br>AAATCGGTTTGTCTCCAATCGAATTGTTGAGAACTGGTGACTTC<br>GAATCTTCTGACAGAGTTCAAATCTTGACTTACGTTATGCAAAT<br>CGGTTTGTCTGCTTTGTTGCAATCTAACGGTATCACTCCACAA<br>GCTGTTATCGGTCACTCTGTTGGTGAAATCGCTGCTTCCGTTG<br>TTGCTGGTGCTTTGTCTCCAGCTGAAGGTGCTTTGATCGTTAC<br>TAGAAGAGCTTTGTTGTACAGACAAGTTATGGGTAAGGGTGGT<br>ATGATCTTGGTTAACTTGCCATCTGCTGAAACTGAAGAAATCTT<br>GGGTTCTAGATCTGACTTGGTTGTTGCTATCGACTCTTCTCCAT<br>CTTCTTGTGTTGTTGCTGGTGACAAGGAATTGGTTGCTGAAAC<br>TGCTGAAGCTTTGAAGGCTAGAGGTGTTAAGACTTTCACTGTT<br>AAGTCTGACATCGCTTTCCACTCTCCAACTTTGAACGGTTTGGT<br>TGACCCATTGAGAGACGTTTTGGCTGAAACTTTGTCTCCAGTTT<br>CTCCAAACGTTAAGTTGTACTCTACTGCTTTGGCTGACCCAAG<br>AGGTCAAGACTTGAGAGACGTTGAATACTGGGCTGGTAACATG<br>GTTAACAGAGTTAGATTGACTTCTGCTGTTAAGGCTGCTGTTG<br>AAGACGGTTACAGATTGTTCTTGGAAGTTTCTACTCACCCAGTT<br>GTTTCTCACTCTATCAACGAAACTTTGATGGACGCTGGTATGG<br>AAGACTTCGCTGTTATCCCAACTTTGTTGAGAAAGAAGCCAAC<br>TGAAAAGCACATCTTGCACTCTATCGCTCAATTGCACTGTAGA<br>GGTGCTGAAGTTAACTGGGCTGCTCAAATGCCAGGTAGATGG<br>GCTACTGGTGTTCCAACTACTACTTGGATGCACAAGCCAATCT<br>GGAGAAAGATCGAAACTGCTCCATTGCACACTGGTTTGACTCA<br>CGACGTTGAAAAGCACACTTTGTTGGGTCAAAGAATCCCAGTT<br>CCAGGTACTGACACTTACGTTTACACTACTAGATTGGACAACG<br>ACACTAAGCCATTCCCAGGTTCTCACCCATTGCACGGTACTGA<br>AATCGTTCCAGCTGCTGGTTTGATCAACACTTTCTTGAAGGGT<br>ACTGGTGGTCAAATGTTGCAAAACGTTGTTTTGAGAGTTCCAG<br>TTGCTATCAACGCTCCAAGATCTGTTCAAGTTGTTGTTCAACAA<br>GACCAAGTTAAGGTTGTTTCTAGATTGATCCCATCTGAACCATC<br>TCAATTGGACGACGACGCTTCTTGGGTTACTCACACTACTGCT<br>TACTGGGACAGAAAGGTTGCTGGTTCTGAAGACAGAATCGACT | |

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|---|---|---|
| | TCGCTGCTGTTAAGTCTAGATTGGTTACTAAGTTGGCTGACAA<br>CTTCTCTATCGACTACTTGGACAAGGTTGGTGTTTCTGCTATG<br>GGTTTCCCATGGGCTGTTACTGAACACTACAGAAACGACAAGG<br>AAATGTTGGCTAGAGTTGACGTTAACCCAGCTATCTCTGGTGA<br>CGCTCCATTGCCATGGGACTCTTCTTCTTGGGCTCCAGTTTTG<br>GACGCTGCTACTTCTGTTGGTTCTACTATCTTCCCAACTCCAG<br>CTTTGAGAATGCCAGCTCAAATCGAAAGAGTTGAAGTTTTCACT<br>TCTCAAGACCCACCAAAGATCTCTTGGTTGTACGTTCAAGAAG<br>CTTCTGACTCTGTTCCAACTTCTCACGTTTCTGTTGTTTCTGAA<br>GCTGGTGAAGTTTTGGCTAAGTTCACTGCTATGAGATTCTCTG<br>AAATCGAAGGTACTCCAGGTGTTTCTGGTTCTATGGAATCTTTG<br>GTTCACCAAATCGCTTGGCCACCAGCTACTCCAGCTGAAGAAC<br>CATTGTCTATCGAAACTGTTATCTTGGTTTCTCCAGACGCTACT<br>ACTAGAGCTTTGTACGCTGCTTCTTTGCCAACTAGAGTTAACTC<br>TTTCCAATTCTCTTCTACTCAAGAATTCTTCTCTAACGCTTCTTC<br>TTTGCCATTGGAAAAGGGTACTGTTGTTACTTACATCCCAGGT<br>GAAGTTGCTTCTTTGGCTGAAGTTCCAGCTGCTTCTGAATCTTT<br>CACTTGGAACTTGTTGGAATTGATCAAGTTCACTGTTAACGGTT<br>CTTTGCCAATCAAGGTTTTCACTTTGACTGCTAACATCGGTGAA<br>GGTCAAACTCCAACTGCTTTGGCTCAATCTCCATTGTACGGTTT<br>GGCTAGAGTTATCGCTTCTGAACACCCAGACTTGGGTACTTTG<br>ATCGACGTTGAAGAACCAGTTATCCCATTGTCTACTATGAGATA<br>CATCCAAGGTGCTGACATCATCAGAATCAACGACGGTATCGCT<br>AGAACTTCTAGATTCAGATCTTTGCCAAGAAACAAGTTGTTGCC<br>AGCTTCTGAAGGTCCAAGATTGTTGCCAAGACCAGAAGGTACT<br>TACTTGATCACTGGTGGTTTGGGTGTTTTGGGTTTGGAAGTTG<br>CTGACTTCTTGGTTGAAAAGGGTGCTAGAAGATTGTTGTTGAT<br>CTCTAGAAGAGCTTTGCCACCAAGAAGAACTTGGGACCAAGTT<br>TCTGAAGACTTGCAACCAACTATCGCTAAGATCAGATTGTTGG<br>AATCTAGAGGTGCTTCTGTTCACGTTTTGCCATTGGACATCACT<br>AAGCCAGACGCTGTTGAACAATTGACTACTGCTTTGGACAGAT<br>TGTCTTTGCCATCTGTTCAAGGTGTTGTTCACGCTGCTGGTGT<br>TTTGGACAACGAATTGGTTATGCAAACTACTAGAGACGCTTTCA<br>ACAGAGTTTTGGCTCCAAAGATCGCTGGTGCTTTGGCTTTGCA<br>CGAAGTTTTCCCACCAAAGTCTGTTGACTTCTTCGTTATGTTCT<br>CTTCTTGTGGTAACTTGGTTGGTTTCACTGGTCAAGCTTCTTAC<br>GGTTCTGGTAACGCTTTCTTGGACACTTTGGCTACTCACAGAG<br>CTAGATTGGGTGACGCTGCTGTTTCTTTCCAATGGACTTCTTG<br>GAGAGGTTTGGGTATGGGTGCTTCTACTGACTTCATCAACGCT<br>GAATTGGAATCTAAGGGTATCACTGACGTTACTAGAGACGAAG<br>CTTTCGCTGCTTGGCAACACTTGGCTAAGTACGACATGGACCA<br>CGGTGTTGTTTTGAGATCTAGAGCTTTCGAAGACGGTGAACCA<br>ATCCCAGTTTCTATCTTGAACGACATCGCTGTTAGAAGAGTTG<br>GTACTGTTTCTAACACTTCTCCAGCTGCTGCTGGTTCTTCTGAC<br>GCTGTTCCAACTTCTGGTCCAGAATTGAAGGCTTACTTGGACG<br>AAAAGATCAGAGGTTGTGTTGCTAAGGTTTTGCAAATGACTGC<br>TGAAGACGTTGACTCTAAGGCTGCTTTGGCTGACTTGGGTGTT<br>GACTCTGTTATGACTGTTACTTTGAGAAGACAATTGCAACTTAC<br>TTTGAAGATCGCTGTTCCACCAACTTTGACTTGGTCTCACCCAA<br>CTGTTTCTCACTTGGCTGTTTGGTTCGCTGAAAAGTTGGCTAA<br>GTAA | |

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| *opt*npgA | ATGGTTCAAGACACTTCTTCTGCTTCTACTTCTCCAATCTTGAC<br>TAGATGGTACATCGACACTAGACCATTGACTGCTTCTACTGCT<br>GCTTTGCCATTGTTGGAAACTTTGCAACCAGCTGACCAAATCT<br>CTGTTCAAAAGTACTACCACTTGAAGGACAAGCACATGTCTTT<br>GGCTTCTAACTTGTTGAAGTACTTGTTCGTTCACAGAAACTGTA<br>GAATCCCATGGTCTTCTATCGTTATCTCTAGAACTCCAGACCCA<br>CACAGAAGACCATGTTACATCCCACCATCTGGTTCTCAAGAAG<br>ACTCTTTCAAGGACGGTTACACTGGTATCAACGTTGAATTTAAC<br>GTTTCTCACCAAGCTTCTATGGTTGCTATCGCTGGTACTGCTTT<br>CACTCCAAACTCTGGTGGTGACTCTAAGTTGAAGCCAGAAGTT<br>GGTATCGACATCACTTGTGTTAACGAAAGACAAGGTAGAAACG<br>GTGAAGAAAGATCTTTGGAATCTTTGAGACAATACATCGACATC<br>TTCTCTGAAGTTTTCTCTACTGCTGAAATGGCTAACATCAGAAG<br>ATTGGACGGTGTTTCTTCTTCTTCTTTGTCTGCTGACAGATTGG<br>TTGACTACGGTTACAGATTGTTCTACACTTACTGGGCTTTGAAG<br>GAAGCTTACATCAAGATGACTGGTGAAGCTTTGTTGGCTCCAT<br>GGTTGAGAGAATTGGAATTCTCTAACGTTGTTGCTCCAGCTGC<br>TGTTGCTGAATCTGGTGACTCTGCTGGTGACTTCGGTGAACCA<br>TACACTGGTGTTAGAACTACTTTGTACAAGAACTTGGTTGAAGA<br>CGTTAGAATCGAAGTTGCTGCTTTGGGTGGTGACTACTTGTTC<br>GCTACTGCTGCTAGAGGTGGTGGTATCGGTGCTTCTTCTAGAC<br>CAGGTGGTGGTCCAGACGGTTCTGGTATCAGATCTCAAGACC<br>CATGGAGACCATTCAAGAAGTTGGACATCGAAAGAGACATCCA<br>ACCATGTGCTACTGGTGTTTGTAACTGTTTGTCTTAA | *Aspergillus nidulans* |
| *opt*patG | ATGGCTAAGATCGACGTTCACCACCACTTCTACCCACAAGCTA<br>TGAGAGAAGCTTTGGAAAGAGCTGGTGGTGACCCATCTGGTT<br>GGTACATCCCACCATGGACTTTGGACTTGGACAAGGAAATCTC<br>TAGAGTTTTGAAGGTTCAAACTACTATCTTGTCTGTTACTGCTC<br>CAGGTCCAGGTATCGAAACTGACCCAGGTAAGGCTGCTGCTTT<br>GGCTAGATTGTGTAACGAAGAAGCTGCTGCTATCAGAGACGCT<br>CACCCATTGCAATACGGTTTCTTCGCTTCTGTTCCATCTTTGTT<br>CGACACTGCTGCTGTTTTGGCTGAAATCGAACACGCTTTCACT<br>AACTTGCACGCTGACGGTGTTACTTTGTACACTAGATACGGTG<br>CTGGTCACTCTTACTTGGGTGACGAAAGATTCAGACCAGTTTG<br>GGCTGAATTGTCTAAGAGAAGAGCTGTTGTTTTCATCCACCCA<br>ACTCACGCTGTTGACACTCAATTGATCAACTCTTGGATGCCAC<br>AACCAATGTTCGACTACCCACACGAAACTGGTAGAACTGCTAT<br>GGACTTGTTGACTAGAGGTGTTATCAGAGACTACCCAGGTTGT<br>AAGATCATCTTGTCTCACGCTGGTGGTACTTTGCCATACTTGAT<br>CCACAGAGCTGCTACTATGTTGCCATTCATGCCAAGAAACTTG<br>GGTATGTCTAGAGAAGAAATCGTTGAAGCTGCTAGAACTTTGT<br>ACTTCGACACTGCTATCTCTGCTAACCCAGTTACTTTGAAGGCT<br>TTGTTGGAATTCGCTAAGCCAGGTCACGTTTTGTTCGGTTCTG<br>ACTTCCCAAACGCTCCAAGAGGTGCTATCACTCACTTCACTTC<br>TTTCTTGGAAGGTTACGACAACATGTCTGAAGAAACTAGAAGA<br>TTGGTTGAAAGAGAAGCTGCTTTGGAATTGTTCCCAAGATTGA<br>GAGGTCAATCTACTAGAGCTTGTTTGTAA | *Aspergillus clavatus* |
| *opt*prpE | ATGTCTTTCTCTGAATTCTACCAAAGATCTATCAACGAACCAGA<br>AGCTTTCTGGGCTGAACAAGCTAGAAGAATCGACTGGAGACAA<br>CCATTCACTCAAACTTTGGACCACTCTAGACCACCATTCGCTA<br>GATGGTTCTGTGGTGGTACTACTAACTTGTGTCACAACGCTGT | *Salmonella typhimurium* |

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| | TGACAGATGGAGAGACAAGCAACCAGAAGCTTTGGCTTTGATC<br>GCTGTTTCTTCTGAAACTGACGAAGAAAGAACTTTCACTTTCTC<br>TCAATTGCACGACGAAGTTAACATCGTTGCTGCTATGTTGTTGT<br>CTTTGGGTGTTCAAAGAGGTGACAGAGTTTTGGTTTACATGCC<br>AATGATCGCTGAAGCTCAAATCACTTTGTTGGCTTGTGCTAGA<br>ATCGGTGCTATCCACTCTGTTGTTTTCGGTGGTTTCGCTTCTCA<br>CTCTGTTGCTGCTAGAATCGACGACGCTAGACCAGCTTTGATC<br>GTTTCTGCTGACGCTGGTGCTAGAGGTGGTAAGATCTTGCCAT<br>ACAAGAAGTTGTTGGACGACGCTATCGCTCAAGCTCAACACCA<br>ACCAAAGCACGTTTTGTTGGTTGACAGAGGTTTGGCTAAGATG<br>GCTTGGGTTGACGGTAGAGACTTGGACTTCGCTACTTTGAGAC<br>AACAACACTTGGGTGCTTCTGTTCCAGTTGCTTGGTTGGAATC<br>TAACGAAACTTCTTGTATCTTGTACACTTCTGGTACTACTGGTA<br>AGCCAAAGGGTGTTCAAAGAGACGTTGGTGGTTACGCTGTTG<br>CTTTGGCTACTTCTATGGACACTATCTTCGGTGGTAAGGCTGG<br>TGGTGTTTTCTTCTGTGCTTCTGACATCGGTTGGGTTGTTGGT<br>CACTCTTACATCGTTTACGCTCCATTGTTGGCTGGTATGGCTA<br>CTATCGTTTACGAAGGTTTGCCAACTTACCCAGACTGTGGTGT<br>TTGGTGGAAGATCGTTGAAAAGTACCAAGTTAACAGAATGTTC<br>TCTGCTCCAACTGCTATCAGAGTTTTGAAGAAGTTCCCAACTG<br>CTCAAATCAGAAACCACGACTTGTCTTCTTTGGAAGCTTTGTAC<br>TTGGCTGGTGAACCATTGGACGAACCAACTGCTTCTTGGGTTA<br>CTGAAACTTTGGGTGTTCCAGTTATCGACAACTACTGGCAAAC<br>TGAATCTGGTTGGCCAATCATGGCTTTGGCTAGAGCTTTGGAC<br>GACAGACCATCTAGATTGGGTTCTCCAGGTGTTCCAATGTACG<br>GTTACAACGTTCAATTGTTGAACGAAGTTACTGGTGAACCATGT<br>GGTATCAACGAAAAGGGTATGTTGGTTATCGAAGGTCCATTGC<br>CACCAGGTTGTATCCAAACTATCTGGGGTGACGACGCTAGATT<br>CGTTAAGACTTACTGGTCTTTGTTCAACAGACAAGTTTACGCTA<br>CTTTCGACTGGGGTATCAGAGACGCTGAAGGTTACTACTTCAT<br>CTTGGGTAGAACTGACGACGTTATCAACATCGCTGGTCACAGA<br>TTGGGTACTAGAGAAATCGAAGAATCTATCTCTTCTTACCCAAA<br>CGTTGCTGAAGTTGCTGTTGTTGGTATCAAGGACGCTTTGAAG<br>GGTCAAGTTGCTGTTGCTTTCGTTATCCCAAAGCAATCTGACA<br>CTTTGGCTGACAGAGAAGCTGCTAGAGACGAAGAAAACGCTAT<br>CATGGCTTTGGTTGACAACCAAATCGGTCACTTCGGTAGACCA<br>GCTCACGTTTGGTTCGTTTCTCAATTGCCAAAGACTAGATCTG<br>GTAAGATGTTGAGAAGAACTATCCAAGCTATCTGTGAAGGTAG<br>AGACCCAGGTGACTTGACTACTATCGACGACCCAGCTTCTTTG<br>CAACAAATCAGACAAGCTATCGAAGAATAA | |
| *ERG10* | ATGTCTCAAAACGTTTACATTGTTTCTACTGCTAGAACCCCAAT<br>TGGTTCTTTCCAAGGTTCTTTGTCCTCCAAGACCGCTGTTGAAT<br>TGGGTGCTGTTGCTTTGAAGGGTGCTTTGGCTAAGGTTCCAGA<br>ATTGGATGCTTCCAAGGATTTCGACGAAATTATTTTCGGTAACG<br>TTTTGTCTGCTAACTTGGGTCAAGCTCCAGCTAGACAAGTTGC<br>TTTGGCTGCTGGTTTGTCTAACCACATCGTTGCTTCTACCGTTA<br>ACAAGGTCTGTGCTTCCGCTATGAAGGCTATCATTTTGGGTGC<br>TCAATCCATCAAGTGTGGTAACGCTGATGTTGTCGTTGCTGGT<br>GGTTGTGAATCTATGACTAACGCTCCATACTACATGCCAGCTG<br>CTAGAGCTGGTGCTAAGTTCGGTCAAACTGTTTTGGTTGATGG<br>TGTCGAAAGAGATGGTTTGAACGATGCTTACGATGGTTTGGCT<br>ATGGGTGTTCACGCTGAAAAGTGTGCTAGAGATTGGGATATTA<br>CTAGAGAACAACAAGACAACTTCGCTATCGAATCCTACCAAAA | *Saccharomyces cerevisiae* |

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| | GTCTCAAAAGTCTCAAAAGGAAGGTAAGTTCGACAACGAAATT<br>GTTCCAGTTACCATTAAGGGTTTCAGAGGTAAGCCAGATACTC<br>AAGTCACCAAGGACGAAGAACCAGCTAGATTGCACGTTGAAAA<br>GTTGAGATCTGCTAGAACTGTTTTCCAAAAGGAAAACGGTACT<br>GTTACTGCTGCTAACGCTTCTCCAATCAACGATGGTGCTGCTG<br>CTGTCATCTTGGTTTCCGAAAAGGTTTTGAAGGAAAAGAACTT<br>GAAGCCATTGGCTATTATCAAGGGTTGGGGTGAAGCTGCTCAC<br>CAACCAGCTGATTTCACCTGGGCTCCATCTTTGGCTGTTCCAA<br>AGGCTTTGAAGCACGCTGGTATCGAAGACATCAACTCTGTTGA<br>TTACTTCGAATTCAACGAAGCTTTCTCTGTTGTCGGTTTGGTTA<br>ACACTAAGATTTTGAAGTTGGACCCATCTAAGGTTAACGTTTAC<br>GGTGGTGCTGTTGCTTTGGGTCACCCATTGGGTTGTTCTGGTG<br>CTAGAGTTGTTGTTACCTTGTTGTCCATCTTGCAACAAGAAGGT<br>GGTAAGATCGGTGTTGCTGCTATTTGTAACGGTGGTGGTGGTG<br>CTTCCTCTATTGTCATTGAAAAGATTTAA | |
| *opt*Hbd | ATGAAGAAGGTTTGTGTTATTGGTGCTGGTACTATGGGTTCTG<br>GTATTGCTCAAGCTTTCGCTGCTAAGGGTTTCGAAGTTGTTTTG<br>AGAGATATTAAGGATGAATTCGTTGATAGAGGTTTGGATTTCAT<br>CAACAAGAACTTGTCTAAGTTGGTTAAGAAGGGTAAGATTGAA<br>GAAGCTACTAAGGTTGAAATCTTGACTAGAATTTCCGGTACCG<br>TTGACTTGAACATGGCTGCTGATTGTGATTTGGTTATTGAAGCT<br>GCTGTTGAAAGAATGGATATTAAGAAGCAAATTTTCGCTGACTT<br>GGACAACATTTGTAAGCCAGAAACCATTTTGGCTTCTAACACCT<br>CTTCTTTGTCTATTACCGAAGTTGCTTCTGCTACTAAGAGGCCA<br>GATAAGGTTATTGGTATGCACTTCTTCAACCCAGCTCCAGTTAT<br>GAAGTTGGTTGAAGTTATTAGAGGTATTGCTACCTCTCAAGAAA<br>CTTTCGATGCTGTTAAGGAAACCTCTATTGCTATTGGTAAGGAT<br>CCAGTTGAAGTTGCTGAAGCTCCAGGTTTCGTTGTTAACAGAA<br>TTTTGATTCCAATGATTAACGAAGCTGTTGGTATTTTGGCTGAA<br>GGTATTGCTTCTGTTGAAGACATTGATAAGGCTATGAAGTTGG<br>GTGCTAACCACCCAATGGGTCCATTGGAATTGGGTGATTTCAT<br>TGGTTTGGATATTTGTTTGGCTATTATGGATGTTTTGTACTCTG<br>AAACTGGTGATTCTAAGTACAGACCACACACCTTGTTGAAGAA<br>GTACGTTAGAGCTGGTTGGTTGGGTAGAAAGTCTGGTAAGGG<br>TTTCTACGATTACTCTAAGTAA | *Clostridium acetobutylicum* |
| *opt*Crt | ATGGAATTGAACAACGTCATCTTGGAAAAGGAAGGTAAGGTTG<br>CTGTTGTTACCATTAACAGACCAAAGGCTTTGAACGCTTTGAAC<br>TCTGATACCTTGAAGGAAATGGATTACGTTATTGGTGAAATTGA<br>AAACGATTCTGAAGTTTTGGCTGTTATTTTGACTGGTGCTGGTG<br>AAAAGTCTTTCGTTGCTGGTGCTGATATTTCTGAAATGAAGGAA<br>ATGAACACCATTGAAGGTAGAAAGTTCGGTATTTTGGGTAACA<br>AGGTTTTCAGAAGATTGGAATTGTTGGAAAAGCCAGTTATTGCT<br>GCTGTTAACGGTTTCGCTTTGGGTGGTGGTTGTGAAATTGCTA<br>TGTCTTGTGATATTAGAATTGCTTCTTCTAACGCTAGATTCGGT<br>CAACCAGAAGTTGGTTTGGGTATTACCCCAGGTTTCGGTGGTA<br>CCCAAAGATTGTCTAGATTGGTTGGTATGGGTATGGCTAAGCA<br>ATTGATTTTCACTGCTCAAAACATTAAGGCTGATGAAGCTTTGA<br>GAATCGGTTTGGTTAACAAGGTTGTTGAACCATCTGAATTGAT<br>GAACACCGCTAAGGAAATTGCTAACAAGATTGTTTCTAACGCT<br>CCAGTTGCTGTTAAGTTGTCTAAGCAAGCTATTAACAGAGGTAT | *Clostridium acetobutylicum* |

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| | GCAATGTGATATTGATACTGCTTTGGCTTTCGAATCTGAAGCTT<br>TCGGTGAATGTTTCTCTACCGAAGATCAAAAGGATGCTATGAC<br>CGCTTTCATTGAAAAGAGAAAGATTGAAGGTTTCAAGAACAGA<br>TAA | |
| *opt*Ter | ATGATTGTTAAGCCAATGGTTAGAAACAACATTTGTTTGAACGC<br>TCACCCACAAGGTTGTAAGAAGGGTGTTGAAGATCAAATTGAA<br>TACACCAAGAAGAGAATTACCGCTGAAGTTAAGGCTGGTGCTA<br>AGGCTCCAAAGAACGTTTTGGTTTTGGGTTGTTCTAACGGTTA<br>CGGTTTGGCTTCTAGAATTACCGCTGCTTTCGGTTACGGTGCT<br>GCTACTATCGGTGTTTCCTTCGAAAAGGCTGGTTCTGAAACCA<br>AGTACGGTACCCCAGGTTGGTACAACAACTTGGCTTTCGACGA<br>AGCTGCTAAGAGAGAAGGTTTGTACTCCGTTACTATTGACGGT<br>GACGCTTTCTCCGATGAAATCAAGGCTCAAGTTATCGAAGAAG<br>CTAAGAAGAAGGGTATTAAGTTCGATTTGATTGTTTACTCTTTG<br>GCTTCTCCAGTTAGAACCGATCCAGACACCGGTATTATGCACA<br>AGTCCGTCTTGAAGCCATTCGGTAAGACCTTCACCGGTAAGAC<br>CGTCGATCCATTCACCGGTGAATTGAAGGAAATCTCCGCTGAA<br>CCAGCTAACGATGAAGAAGCTGCTGCTACCGTTAAGGTTATGG<br>GTGGTGAAGACTGGGAAAGATGGATTAAGCAATTGTCTAAGGA<br>AGGTTTGTTGGAAGAAGGTTGTATTACCTTGGCTTACTCCTACA<br>TCGGTCCAGAAGCTACTCAAGCTTTGTACAGAAAGGGTACCAT<br>TGGTAAGGCTAAGGAACACTTGGAAGCTACTGCTCACAGATTG<br>AACAAGGAAAACCCATCTATTAGAGCTTTCGTTTCTGTTAACAA<br>GGGTTTGGTTACCAGAGCTTCTGCTGTTATCCCAGTTATTCCAT<br>TGTACTTGGCTTCCTTGTTCAAGGTTATGAAGGAAAAGGGTAA<br>CCACGAAGGTTGTATCGAACAAATTACCAGATTGTACGCTGAA<br>AGATTGTACAGAAAGGACGGTACCATCCCAGTCGATGAAGAAA<br>ACAGAATCAGAATCGACGACTGGGAATTGGAAGAAGACGTTCA<br>AAAGGCTGTTTCTGCTTTGATGGAAAAGGTTACCGGTGAAAAC<br>GCTGAATCTTTGACCGACTTGGCTGGTTACAGACACGACTTCT<br>TGGCTTCTAACGGTTTCGATGTTGAAGGTATCAACTACGAAGC<br>TGAAGTTGAAAGATTCGACAGAATTTAA | *Treponema denticola* |