

Supporting Information

S1 File: Sequences of artificial DNA fragments. Artificial fragments are 492 bp gene fragments synthesized by Integrated DNA Technologies (Coralville, Iowa). The fragments are derived from the sequence of the small subunit (SSU or 16S) ribosomal RNA (rRNA) gene of *Rhodanobacter denitrificans* 2APBS1, a denitrifying Gammaproteobacteria (GenBank accession number: NR_102497.1). The sequences shown represent one strand (5'-3') of the double-stranded molecules. The sequences highlighted in green represent the target position of the 515F primer, while the light blue sequences represent the target position of 806R primer (Caporaso et al. 2011). Positions highlighted in red indicate introduced mismatches relative to the primer pool, introduced into the synthesized DNA fragments to allow for testing of effects of mismatches between template and primer. The region highlighted in purple is a 10-bp region which is diagnostic for each of the four sequences. The 10 bp are re-arranged in each region to allow for identification of the template, while maintaining overall similarity of the gene fragment. The last two gene fragments contain mismatches that are not targeted by any of the primer variants found in the standard degenerate 515F and 806R primers. The forward primer (515F) primer site is highlighted in bright green, and the reverse primer site is highlighted in cyan when matching the lowest T_m reverse primer or olive green when matching the highest T_m reverse primer.

>**Mock A** (FP Tm = Low / RP Tm = Low)

TAAAGCACTTTTATCAGGAGCGAAATACCACGGGTTAATACCCTATGGGGCTGACG
GTACCTGAGGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAA TACGAAG
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGTAGGCCGGTACTTAA
GTCTGTCGTGAAATCCCCGGGCTCAACCTGGGAATGCGCATGGATACTGGGTGGCTA
GAGTGTGTCAGAGGATGGTGGAAATCCCCGGTGTAGCGGTGAAATGCGTAGAGATCG
GGAGGAACATCAGTGGCGAAGGCGGCCATCTGGGACAACACTGACGCTGAAGCAC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTAGTAGTCCACGCCCTAAACGAT
GCCAACTGGATGTTGGTCTCAACTCGGAGATCAGTGTCTGAAGCTAACGCGTTAAGTT
CGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA

>**Mock B** (FP Tm = Low / RP Tm = High)

TAAAGCACTTTTATCAGGAGCGAAATACCACGGGTTAATACCCTATGGGGCTGACG
GTACCTGAGGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAA TACGAAG
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGTAGGCCGGTACTTAA
GTCTGTCGTGAAATCCCCGGGCTCAACCTGGGAATGATAGGTAGCGCTGGGTGGCTA
GAGTGTGTCAGAGGATGGTGGAAATCCCCGGTGTAGCGGTGAAATGCGTAGAGATCG
GGAGGAACATCAGTGGCGAAGGCGGCCATCTGGGACAACACTGACGCTGAAGCAC
GAAAGCGTGGGGAGCAAACAGGATTAGAAACCCGGGTAGTCCACGCCCTAAACGAT
GCCAACTGGATGTTGGTCTCAACTCGGAGATCAGTGTCTGAAGCTAACGCGTTAAGTT
CGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA

>**Mock C** (FP Tm = Low + mismatch / RP Tm = Low)

TAAAGCACTTTTATCAGGAGCGAAATACCACGGGTTAATACCCTATGGGGCTGACG
GTACCTGAGGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTCA TACGAAG
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGTAGGCCGGTACTTAA
GTCTGTCGTGAAATCCCCGGGCTCAACCTGGGAATGACTGAAGTGCTGGGTGGCTA
GAGTGTGTCAGAGGATGGTGGAAATCCCCGGTGTAGCGGTGAAATGCGTAGAGATCG
GGAGGAACATCAGTGGCGAAGGCGGCCATCTGGGACAACACTGACGCTGAAGCAC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTAGTAGTCCACGCCCTAAACGAT
GCCAACTGGATGTTGGTCTCAACTCGGAGATCAGTGTCTGAAGCTAACGCGTTAAGTT
CGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA

>**Mock D** (FP Tm = Low + mismatch / RP Tm = Low + mismatch)

TAAAGCACTTTTATCAGGAGCGAAATACCACGGGTTAATACCCTATGGGGCTGACG
GTACCTGAGGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTCA TACGAAG
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGGTGCGTAGGCCGGTACTTAA
GTCTGTCGTGAAATCCCCGGGCTCAACCTGGGAATGGATAGCGATCTGGGTGGCTA
GAGTGTGTCAGAGGATGGTGGAAATCCCCGGTGTAGCGGTGAAATGCGTAGAGATCG
GGAGGAACATCAGTGGCGAAGGCGGCCATCTGGGACAACACTGACGCTGAAGCAC
GAAAGCGTGGGGAGCAAACAGGATAGATACCCTAGTAGTCCACGCCCTAAACGAT
GCCAACTGGATGTTGGTCTCAACTCGGAGATCAGTGTCTGAAGCTAACGCGTTAAGTT
CGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA