

Table S9: List of primers used for deleting methylotrophy-specific genes in *M. extorquens* PA1.

Primer	Primer Sequence	Primer Description
Δfae_us_f	ATGGATGCATATGCTGCAGCTCGAGCGGC CGCCGACGTGTCCCAAACCGATG	Gibson forward linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 695 bp
Δfae_us_r	GTCCAAGGAAGATCGAAGCCAGTCGCTG GA GGTCTCTCCCTGGATTCCTG	30 bp of ds region linker at 5'end. Product size = 695 bp
Δfae_ds_f	TCCAGCGACTGGCTTCGATC	Product size = 424 bp
Δfae_ds_r	GGTTAACACGCGTACGTAGGGCCCCGCGGC CGC CAGCGAGAGCCAGCTTGATG	Gibson reverse linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 424 bp
$\Delta ftfL_us_f$	GTC CCT ATG CTT CCG TGG TAG C	Product size = 617 bp
$\Delta ftfL_us_r$	GGTTAACACGCGTACGTAGGGCCCCGCGGC CGC CGC AGA ACG TGT GGG TGA AAC G	Gibson reverse linker with NotI and 24 bp of pCM433 backbone. Product size = 617 bp
$\Delta ftfL_ds_f$	ATGGATGCATATGCTGCAGCTCGAGCGGC CGCCGA CAT GAC GCT GCA TCT CTC C	30 bp of us region linker at 5'end. Product size = 451 bp
$\Delta ftfL_ds_r$	TTCCGTTTCGCTACCACGGAAGCATAGGGA CCCT GAC TTG GGC GGA TCG TTG	Gibson forward linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 451 bp
$\Delta mptG_us_f$	ATGGATGCATATGCTGCAGCTCGAGCGGC CGCGAT CTC GGC GAT CAG CTC ACC	Gibson forward linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 859 bp
$\Delta mptG_us_r$	GCC GTT GAG ATC GAG GAA GCC	Product size = 859 bp
$\Delta mptG_ds_f$	CTGCATTTCGGTTCTCGATCTCAACGGC CTC GCA GAA GGA GGC GGA	Gibson forward linker from 2049230 to 2049259 on PA1 genome. Product size = 723 bp
$\Delta mptG_ds_r$	GGTTAACACGCGTACGTAGGGCCCCGCGGC CGCGGT GAA GGC GAT CTT CGA GAC G	Gibson reverse linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size 723 bp
$\Delta glyA_us_f$	GAT CAG CTC GAT CTC GTG CTG CTG C	Product size = 497 bp
$\Delta glyA_us_r$	GGTTAACACGCGTACGTAGGGCCCCGCGGC CGCTGA CGC TTC CGA TCG CAC GTG	Gibson Reverse Linker with NotI and 24 bp of pCM433 backbone at 5'end. Product size = 497 bp
$\Delta glyA_ds_f$	ATGGATGCATATGCTGCAGCTCGAGCGGC CGCCGC GCA ACC TTC AGG TGA AGG ATG G	Gibson forward linker with NotI and 24 bp of pCM433 backbone at 5'end. Product size = 526 bp
$\Delta glyA_ds_r$	GGT ACC GAC AAC CAC CTG ATG CTG G	Product size = 526 bp
Δmxa_us_f	ATGGATGCATATGCTGCAGCTCGAGCGGC CGCGAT CGA GGT GCA ACT CGG CAG	Gibson forward linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 701bp
Δmxa_us_r	TGG TCC AGA TCG CGG TCA AC	Product size = 701bp
Δmxa_ds_f	CGTTGCGCCGGTTGACCGCGATCTGGACC ATCG TGA TGC TGA ACG CGC AC	Gibson linker with 4606355 through 4606384 on PA1 genome. Product size = 496bp
Δmxa_ds_r	GGTTAACACGCGTACGTAGGGCCCCGCGGC CGCAAC TCG ATG TCG AAG GCG TGC	Gibson reverse linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 496bp
$\Delta hprA_us_f$	ATGGATGCATATGCTGCAGCTCGAGCGGC CGCCTC ATC GAC AAC GGC GTG AAG G	Gibson forward linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 344bp
$\Delta hprA_us_r$	GGGCAATCGTGTCTCGCTCAC	Product size = 344bp
$\Delta hprA_ds_f$	GCAGGGGTTTTGTGAGCGACACGATTGCC CCTCGTGGACAACGTCGAAGC	30 bp from 2016078 to 2016107 on PA1 genome at 5' end. Product size = 447bp
$\Delta hprA_ds_r$	GGTTAACACGCGTACGTAGGGCCCCGCGGC CGCTCT CGA CCG CCT CGA ACA CC	Gibson reverse linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 447bp
Δfhc_us_f	ATGGATGCATATGCTGCAGCTCGAGCGGC CGCCCG AGA TGC TTG AGG CTC TG	Gibson forward linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 480bp
Δfhc_us_r	GCT CTC GCA AGG CTC GAT CC	Product size = 480bp
Δfhc_ds_f	CCCTCGACCCGGATCGAGCCTTGCGAGAG CTCC GGG TCG TAG ACG ATG AC	30 bp from 2044769 to 2044798 on PA1 genome at 5' end. Product size = 569bp
Δfhc_ds_r	GGTTAACACGCGTACGTAGGGCCCCGCGGC CGCTGA GGA AGC ATG GCA GCC TG	Gibson reverse linker with NotI and 24 bp of pCM433 backbone at 5' end. Product size = 569bp