



Figure S1. Creation of mutants. A. Schematic strategy for replacement mutagenesis by PCR. PCRs are performed using F1/R1, F2/R2 and F3/R3 primers. The three amplicons are purified and mixed in equal amounts. A final PCR amplicon is obtained using F1/R3. P1 and P2 are sequencing primers. T1 is the internal gene-specific primer. **B. Flowchart of essential and non-essential gene identification.** The numbers of genes at each phase of the study are shown. “Non-promoter” and “promoter” refer to genes mutagenized with the promoterless and promoter-containing constructs, respectively. “Single-band” and “double-band” refer to the number of locus-specific amplicons detected by PCR.