



Figure S4 Homology directed mut-*aphVIII* repair frequencies upon CRISPR/Cas9-induced double-strand breaks through oligonucleotides and a plasmid DNA in the parental CC3403-D5 strain. Shown is the number of PmR colonies obtained with the mut-*aphVIII* repair assay using *EMX1* mut-*aphVIII* SpCas/gRNA RNP and a single-stranded oligonucleotide template that matched either the protospacer strand (P) or the spacer strand (S) of the *APHVIII* gene, 40 pmol each, or their mixture (S+P), 20 pmol+20 pmol, or the similar mixture after annealing resulted in double-stranded oligonucleotide (dsODN), 20 pmol, or the 2 μ g (0.8 pmol) pHDR-*APHVIII* ^{Δ 120} plasmid (pl). In preliminary experiments it was found that variation of the amount of ssODNs in the range from 20 pmol to 40 pmol did not affect the number of targeted mutants within the experimental error. In all experiments, the number of cells was counted before plating, and the same number of cells was plated on TAP medium supplemented with Pm, 10 μ g/ml. Error bars represent SEM ($n = 6$ separate experiments for each category). HDR events from individual single-stranded repair templates were statistically different from HDR events from their mixture, dsODN or pHDR-*APHVIII* ^{Δ 120} ($p < 0.01$). HDR events from spacer ssODN were statistically different from HDR events from protospacer ssODN ($p < 0.05$).