**High throughput functional variant screens *via* in-vivo production of single-stranded DNA**

Index of supplemental materials:

**supplemental\_sequences.xlsx**: annotated list of selected oligonucleotides used in the study

**supplemental\_sequence\_maps**: generalized genbank plasmid maps for Retron Recombineering plasmids used in this study, and a sf.GFP-expressing control plasmid.

**Supplemental\_table\_1**: summarized data from Figure 2B, showing the edited fraction measured by different genotypes

**Supplemental\_table\_2**: summarized data from Figure 3C, showing the enrichment scores for all alleles

**Supplemental\_table\_3**: summarized data from Figure 3D, showing enrichment scores for rpoB alleles across all rifampicin concentrations.

**Supplemental\_table\_4**: regions of the RLR genomic Library for which Zero genomic coverage was observed. In all cases this was interpreted as artifacts due to the differences between the MG1655 reference genome and the BW25113::∆lacA ancestor of the evolved strain. The region spanning the termini of the linear reference sequence also displayed artifactually low coverage when aligning sequences from the circular genome.

**Supplemental\_table\_5**: regions of the RLR genomic Library for which very high coverage was observed. In all cases this was interpreted as artifacts due to mis-alignment of sequences present in multiple copies across the genome, such as insertion elements and ribosomal RNA.

All data and scripts necessary to reproduce figures and analysis can be found at <https://github.com/churchlab/rlr>