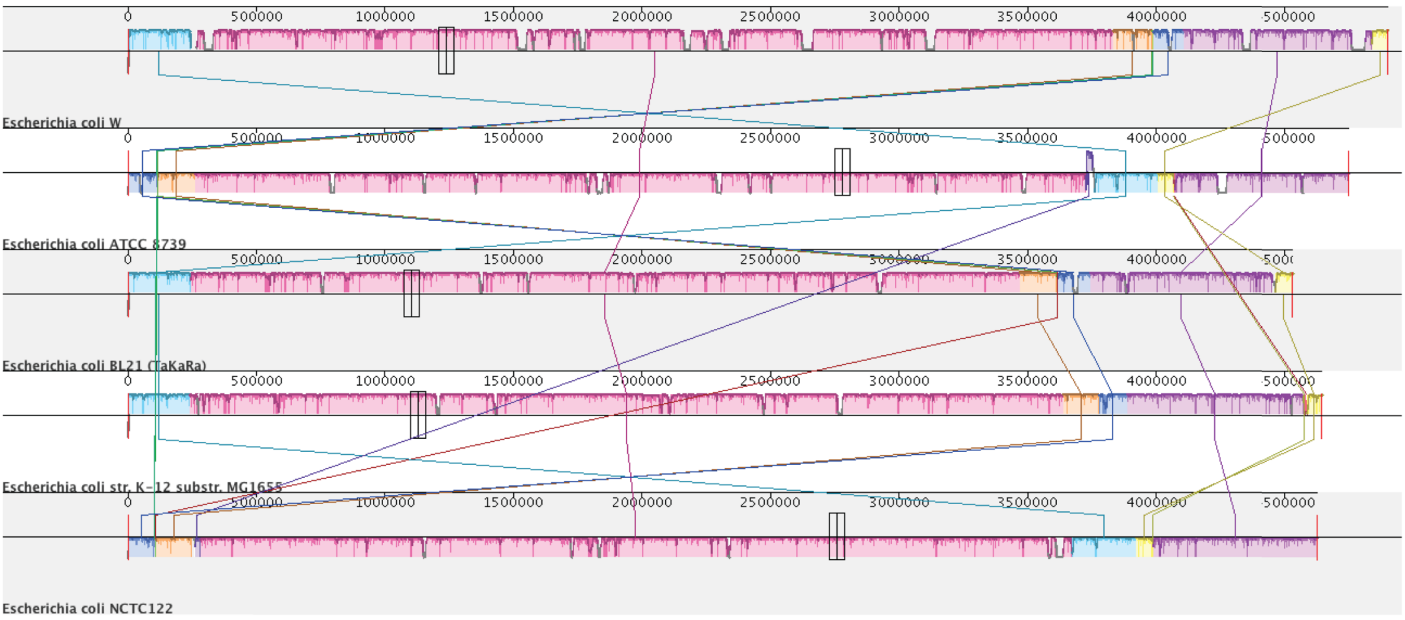

Figure S1. Mauve whole genome alignment of *E. coli* strains studied in this work. Nucleotide sequences from GenBank: K-12 substr. MG1655 (NC_000913.3), BL21 (NZ_CP010816.1), C strain C122 (NZ_LT906474.1), W strain ATCC9637 (NC_017635.1), and Crooks ATCC 8739 (NC_010468.1). Mauve alignment performed with default parameters. Each horizontal line represents one genome sequence. The coloured regions of each genome sequence represent parts that aligned to a part of another genome, and is presumably homologous with similarly coloured regions in other genomes. Vertical lines of the same colour connect the centre of each homologous region to that of the other homologous regions in the other genomes. The height of the bars in the coloured regions represent a similarity profile of the genome sequence that corresponds to the average level of conservation in that region of the genome sequence across the aligned genomes.

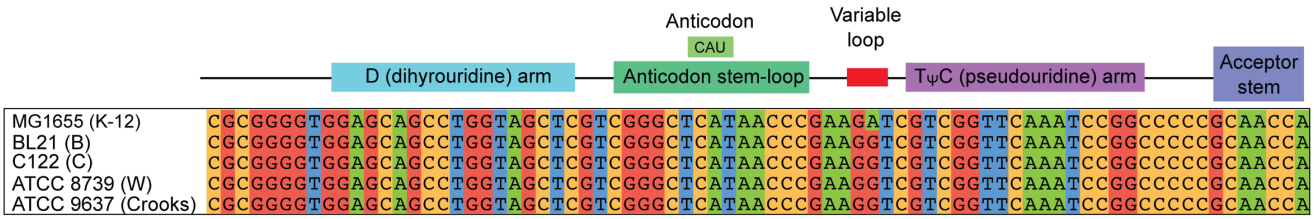

Figure S2. Alignment of *metY* gene sequences from *E. coli* strains studied in this work. Nucleotide difference in variable loop shown. *metY* gene sequences extracted from GenBank sequences: K-12 substr. MG1655 (NC_000913.3), BL21 (NZ_CP010816.1), C strain C122 (NZ_LT906474.1), W strain ATCC9637 (NC_017635.1), and Crooks ATCC 8739 (NC_010468.1).

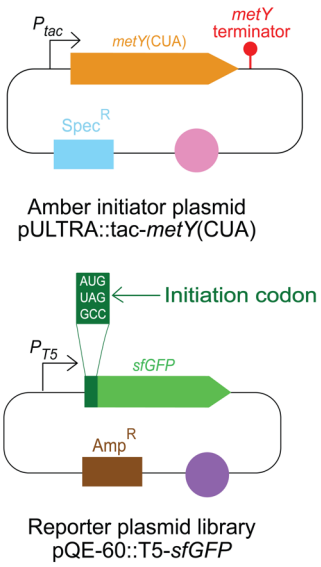

Figure S3. Maps for plasmids used in this work. Top, amber initiator plasmid pULTRA::*tac*-*metY*(CUA). The plasmid encodes the medium-copy ClodF13 origin, spectinomycin resistance (SpecR) marker, and inducible *tac* promoter. Bottom, pQE-60 reporter plasmids. The reporter plasmid set has a medium-copy ColE1 origin, T5 promoter and superfolder green fluorescent protein gene (sfGFP) with three different start codons (AUG, UAG, and GCC). All *metY*(CUA) and sfGFP gene variants have a Rho-independent terminator after individual gene sequences.