

Operon Promoter Landscape

| Operon | Strand | Operon start | Operon end |
|--------|--------|--------------|------------|
| gadY   | +      | 3662887      | 3662991    |

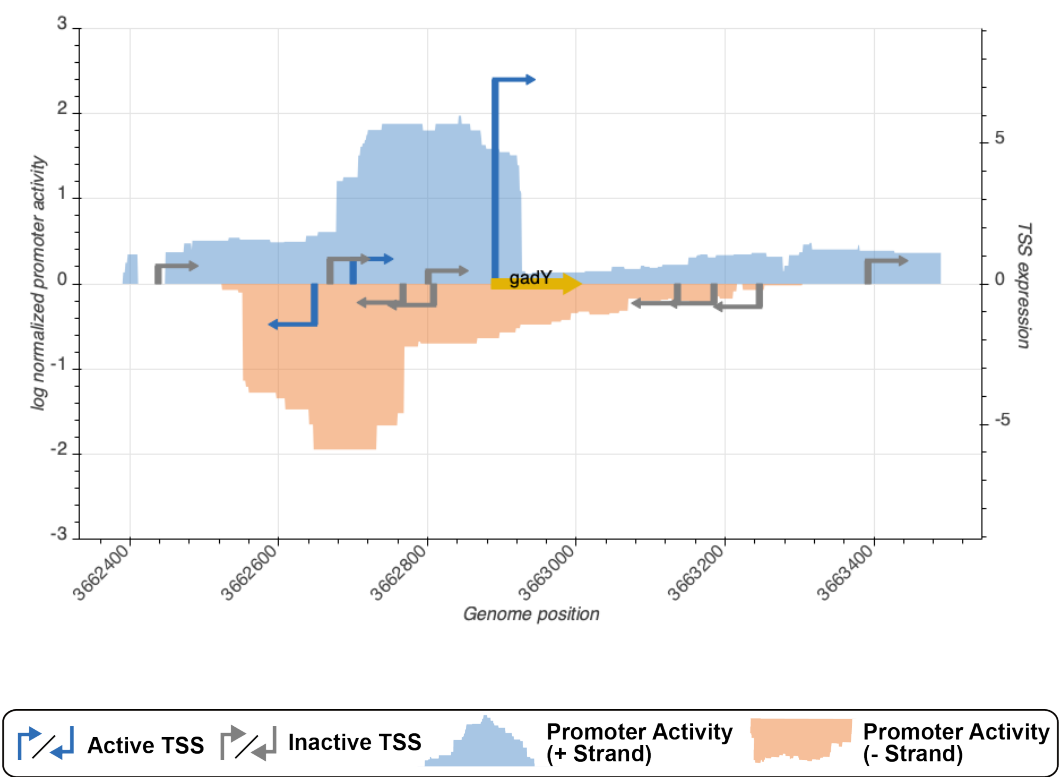


Figure 1: **Promoter activity in rich media (LB) surrounding query operon.** 17,767 previously reported TSSs were evaluated by measuring the promoter activity (right Y-axis) of the 150 bp surrounding the TSS (-120 to +30) to determine which were active or inactive. The genome-wide promoter activity (left Y-axis) was determined by measuring expression of over 300,000 genomic fragments spanning the *E. coli* genome and averaging promoter activity at all nucleotide positions in a strand-specific fashion. Genome coordinates corresponds to *E. coli* genome version U00096.2.

TSS Summary

| TSS name            | TSS position | Strand | TSS activity | Category |
|---------------------|--------------|--------|--------------|----------|
| TSS_14204_storz     | 3662696      | +      | 0.8855690    | active   |
| TSS_14200_storz     | 3662434      | +      | 0.6370569    | inactive |
| TSS_14202_storz     | 3662665      | +      | 0.8826286    | inactive |
| TSS_14207_regulondb | 3662804      | -      | 0.7536042    | inactive |
| TSS_14205_storz     | 3662763      | -      | 0.6672163    | inactive |
| TSS_14201_wanner    | 3662644      | -      | 1.4434763    | active   |
| TSS_14206_storz     | 3662797      | +      | 0.4640673    | inactive |

TSS Scanning Mutagenesis

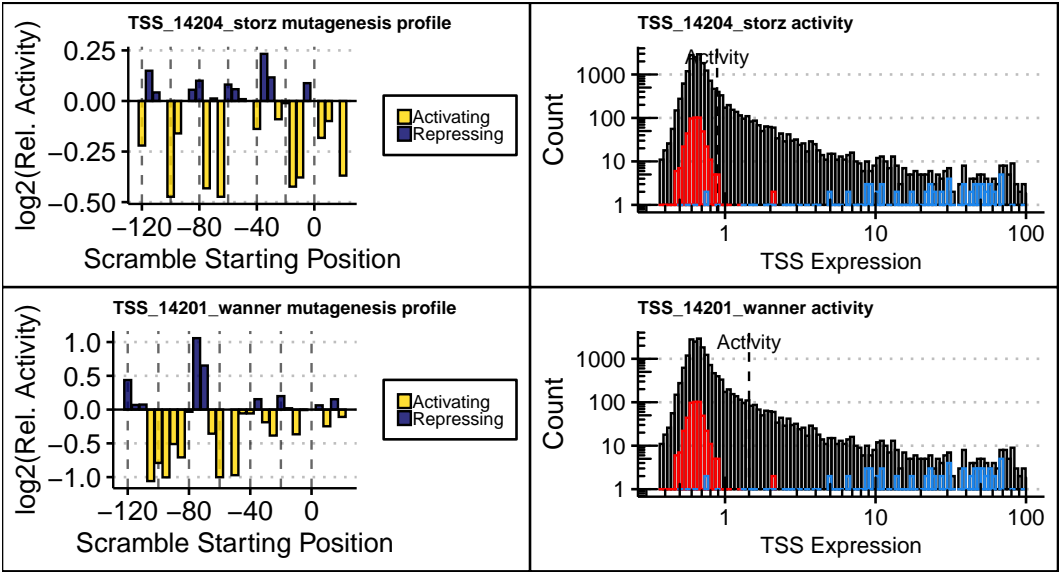


Figure 2: **(Left)** Mutagenesis profile(s) of active TSS(s). Sequences surrounding active TSSs were systematically mutated to identify regions controlling expression. Bar height indicates the relative change in promoter activity as a result of scrambling nucleotides within 10 bp regions at 5 bp intervals spanning the promoter. Bar color identifies the region as a putative activator (yellow) or repressor (purple). **(Right)** Dashed line indicates the expression of the indicated TSS relative to all tested TSS sequences. The distributions of expression is shown for all tested TSSs (black), 500 negative controls (red), and a set of constitutive promoters from the BioBrick registry (blue).