A part toolbox to tune genetic expression in Bacillus subtilis.

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Abstract

Libraries of well-characterised components regulating gene expression levels have become essential to many synthetic biology applications. While widely available for the Gram-negative model bacterium *Escherichia coli*, such collections are lacking for the Gram-positive model *Bacillus subtilis*, a key organism for basic research and biotechnological applications. Here we engineered a genetic toolbox comprising libraries of promoters, ribosome binding sites (RBS), and protein degradation tags to precisely tune gene expression in *B. subtilis.* We first designed a modular Expression Operating Unit (EOU), which facilitates parts assembly and modifications, and provides a standard genetic context for gene circuits implementation. We then selected native, constitutive promoters of *B. subtilis* and efficient RBSs from which we engineered three promoters and three RBSs libraries exhibiting _˜14,000-fold dynamic range in gene expression levels (protein concentration). We also designed a collection of SsrA proteolysis tags of variable strength. Finally, by using fluorescence fluctuation methods coupled with two-photon microscopy, we quantified the absolute concentration of GFP in a subset of strains from the library.

Our complete promoter and RBS library comprising over 150 constructs enables GFP concentration to be tuned over five orders of magnitude, from 0.05 μM to 900 μM. This toolbox of regulatory components will support many research and engineering applications in *B. subtilis.*

Keywords: Bacillus subtilis, gene expression, promoters, ribosome binding sites, degradation tags, standardisation

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