Non-Repetitive Promoters and Ribosome Binding Sites to Control *Clostridium autoethanogenum* Gene Expression Levels during Syngas Fermentation

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Project Goals: *Clostridium autoethanogenum* is an emerging platform organism that can convert C1 gas feedstock (e.g. waste gas) into valuable products, including acrylates and polymers. New genetic tools are needed to tune gene expression levels in *C. auto* for metabolic pathway and network engineering. For tunable transcriptional control, we constructed over 1000 highly non-repetitive promoters with *C. auto* specifications, characterized their transcription rates during syngas fermentation, and validated these measurements as compared to natural *C. auto* promoters. For tunable translational control, we constructed a toolbox of designed, synthetic ribosome binding sites and characterized their expression levels. Altogether, we demonstrate the ability to tune the expression levels of many proteins across over a 100,000-fold range in *C. auto* without introducing repetitive DNA sequences, facilitating metabolic pathway and network optimization.

C. autoethanogenum is a non-model organism that has become an emerging platform for industrial-scale bioconversion of C1 gas feedstock into valuable chemicals. Successes using *C. auto* include operation of a waste gas to ethanol commercial plant at a scale of 16 million gallon/year and successful demonstration of a pilot plant that utilizes gasified waste streams from lignocellulosic biomass and municipal solid waste (MSW) (1-2). As new pathways are introduced into *C. auto* to produce valuable products, its gene expression levels must be tuned to maximize production titers and growth rates. New genetic parts are needed that have highly non-repetitive sequences (to avoid homologous recombination) as well as characterized transcription and translation rates to tune gene expression levels.

Expanding on our previous work, we designed, constructed and characterized over 1000 highly non-repetitive promoters that varied *C. auto* transcription rates by over 100,000-fold during syngas fermentation conditions. We applied our new algorithm, the Non-Repetitive Parts Calculator, to carry out this rational design (3). We selected a representative subset of these non-repetitive promoters as well as a set of commonly used *C. auto* promoters and additionally measured their transcription rates. Separately, we designed, constructed, and characterized a set of synthetic ribosome binding sites, designed by the RBS Calculator v2.1 model, and characterized their translation rates using GusA and NanoLuc reporter assays in both *E. coli* and *C. auto*.

Altogether, these non-repetitive genetic parts will be utilized to tune enzymes expression levels in multi-enzyme pathways in *C. auto* as well to construct Extra-long sgRNA Arrays (ELSAs) to carry out many-gene CRISPRi for rewiring of metabolic fluxes (4, 5). We are also utilizing these datasets to test and improve biophysical models of gene expression in AT-rich genomes, such as *C. auto*.

References/Publications:

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