A Thousand Highly Non-Repetitive Promoters for Controlling Transcription Rates in Clostridia during Syngas Fermentation

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Project Goals:

New genetic tools are needed to engineer metabolic pathways and networks with many genes (enzymes, transporters, regulators), particularly in non-model organisms growing in conditions with industrial relevance. As a key challenge, it remains difficult to stably express many genes because designers are forced to re-use similar genetic parts, thereby introducing repetitive DNA sequences that trigger homologous recombination and genetic instability. To overcome this challenge, we applied our new algorithm, the Non-Repetitive Parts Calculator, to design over 30000 highly non-repetitive promoter sequences to control transcription rates in *Clostridia*. We experimentally characterized these non-repetitive promoters in *Clostridium autoethanogenum* during gas fermentation using a low-cost syngas feedstock, obtaining over 1000 transcription rate measurements simultaneously. To do this, we combined barcoded oligopool synthesis, library-based cloning into integrative vectors, high-throughput transformations, state-of-the-art gas fermentation facilities, and next-generation sequencing (DNA-Seq, RNA-Seq). With this toolbox, over 1000 genes can be simultaneously expressed in *Clostridia* with tunable transcriptional control across a 1,000,000-fold range, all without introducing more than a 15 bp repeat sequence. This non-repetitive toolbox of promoters enables a breadth of metabolic engineering applications in an important industrial organism.

References

Ayaan Hossain, Eriberto Lopez, Sean M. Halper, Daniel P. Cetnar, Alexander C. Reis, Devin Strickland, Eric Klavins, and Howard M. Salis. "Automated Design of Thousands of Highly Non-Repetitive Genetic Parts for Engineering Evolutionary Robust Genetic Systems", Nature Biotechnology, *in review*.

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