HIGH-THROUGHPUT DNA ASSEMBLY FOR THE GENETIC OPTIMIZATION OF A REFACTORED GENE CLUSTER

Michael J. Smanski, Dehua Zhao, and Christopher Voigt; Synthetic Biology Center and Department of Bioengineering, MIT, Cambridge, MA 02139; smanski@mit.edu

Synthetic biology, genetic refactoring, nitrogen fixation

Introduction. Prokaryotic gene clusters encode many useful functions with broad applications in medicine, energy, catalysis, and the production of multi-functional materials. The size and complexity of natural gene clusters has hindered engineering efforts aimed at optimizing or diversifying the encoded functions for biotechnological applications. Current work towards engineering complex genetic systems builds on previous efforts in the Voigt lab to refactor the nitrogen fixation gene cluster from *Klebsiella oxytoca*.[1] Genetic refactoring results in more modular genetic systems composed entirely of well-characterized genetic parts whose expression is de-coupled from native regulation. As predicted, the refactoring process reduces gene cluster performance compared to the native sequence.
The objective of this work is to streamline the genetic optimization of biological functions encoded in large refactored gene clusters by dramatically accelerating the Design-Build-Test cycle. The presented pipeline has been used to improve the performance of a refactored gene cluster 4-fold and to rapidly prototype new genetic architectures aimed at increasing the genetic modularity and enabling new analytical methods.

Methods. Genetic refactoring entails a systematic and rational replacement of native regulation with well-characterized orthogonal genetic elements.[1] In this project, we develop a DNA assembly pipeline composed of both new and previously described[2] methods to rapidly produce libraries of individually designed cluster permutations. Performance of the permuted nitrogen fixation gene clusters are measured via the acetylation reduction assay. Depending on the specific library design, screening results are probed to find motif-activity relationships or are interpreted by multifactorial analysis[3] to extract information that is used to design successive libraries.

Results. The most current results of this on-going optimization and redesign process will be presented at the conference and include:

- A genetic Plackett-Burman analysis[3] leading to the production of a gene cluster with 3-fold improved performance compared to the original refactored cluster and the identification of genes whose expression level has the largest effect on overall cluster performance.
- The diverse restructuring of the *nifUSVWZM* operon (Fig. 1) including library members with 4-fold improvement of activity. Genetic parts analysis of the library identified beneficial motifs that were used to guide future library design.
- Ongoing efforts to increase the functional modularity of the gene cluster by implementing a monocistronic design have resulted in fully monocistronic gene clusters with reduced performance and partially monocistronic gene clusters with identical performance as the original refactored cluster.

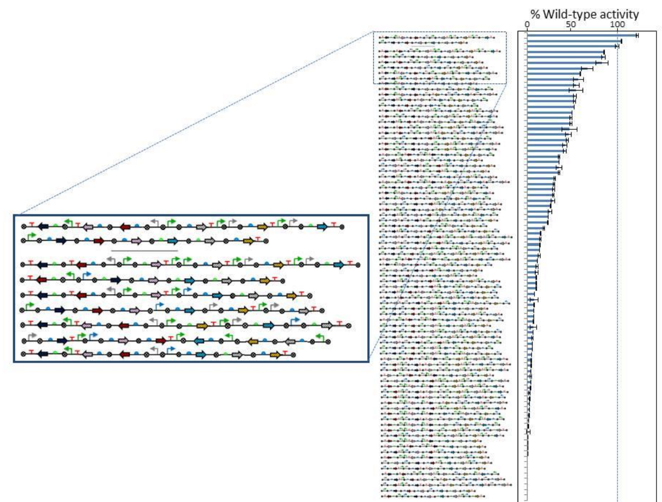

Fig.1 Diverse genetic architectures explored in a *nifUSVWZM* operon library led to full recovery of wild-type activity.

Conclusions. The genetic optimization pipeline presented here has allowed us to accelerate our Design-Build-Test cycle by ~100-fold and has resulted in 4-fold performance improvements in several suboperons of the nitrogen fixation gene cluster.

Acknowledgements. Portions of this project were supported by Defense Advanced Research Projects Agency Living Foundries (DARPA-BAA-11-60), the US Army Research office ICB program (W911NF-09-D-0001), and the NSF Synthetic Biology Engineering Research Center (SynBERC; SA5284-11210). MJS is a HHMI fellow of the Damon Runyon Cancer Research Foundation.

References.

1. Temme K, Zhao D, Voigt CA (2012) *Proc Natl Acad Sci USA.* 109:7085-7090.
2. Weber E, Engler C, Gruetzner R, Werner S, Marillonnet S (2011) *PLoS ONE* 6:e16765.
3. Plackett RL, Burman JP (1946) *Biometrika* 33:305-313