Design, delivery and expression of synthetic genetic elements in diverse microorganisms

Jaymin Patel, Laura Quinto, Shenqi Wang, Katie Mageeney, Joe Schoeniger, Jason Crawford, Farren Isaacs

The overall goal for the Intrinsic Control for Genome and Transcriptome Editing in Communities (InCoGenTEC) project funded under the Secure Biosystems Design initiative is to expand our mechanistic and practical understanding of horizontal gene transfer mechanisms in bacterial communities, and to harness mobile elements to create and deliver constructs to transform, control and detect the genetic and biochemical state of bacteria. Improved ability to engineer genomes of both isolatable and non-isolatable species will enable better scientific understanding of bacterial communities, and facilitate biotechnology applications that promote the growth of the bioeconomy.

A major challenge is in mobilizing, stabilizing and selectively activating functional genetic programs such as biosynthetic pathways in undomesticated environmental strains or into specific organisms *in situ* in intact microbiomes. Here, we describe an integrated computational—experimental technology to decouple biosynthetic capacity from host-range constraints that enabled pathway-targeted metabolite analyses in a diverse set of prokaryotic and eukaryotic hosts to harness their innate metabolic adaptability and activate silenced pathways. By placing these pathways under transcriptional control mechanisms orthogonal to the bacterial host, we can standardize design, maximize reuse of components, minimize unexpected behavior, and ensure that genetic subfragments of our heterologous synthetic pathways are non-functional, thereby improving both versatility of use and biocontainment.

Specifically, we developed a computational algorithm to redesign genes and their regulatory regions to adopt hybrid elements for cross-species expression of synthetic genetic elements (SGEs) in gram- and gram+ bacteria and eukaryotes. These algorithms have been converted to a web interface to enable impact in the broader scientific community. To transfer SGEs into diverse hosts, we also developed a mobilization strategy by engineering conjugation, transposition, and site-specific recombination, establishing feasibility on a validated violacein pathway. Further, we are developing redesigned pathways for intrinsic bioluminescence, which can enable facile labeling of individual strains within a complex community. We are currently expanding the capabilities of SGE mobilization to broaden host range, enable intracellular and environmental biosensing, introduce mechanisms for strain targeting, and multiplexing of genetic cargo. Jointly with other parts of the InCoGenTEC project, we are also exploring the incorporation of our SGEs into transforming bacteriophage vectors as an alternative, strain-specific delivery and transformation mechanism. These technologies establish a general strategy for highly-controlled expression and mobilization of genetic elements in diverse organisms and communities.