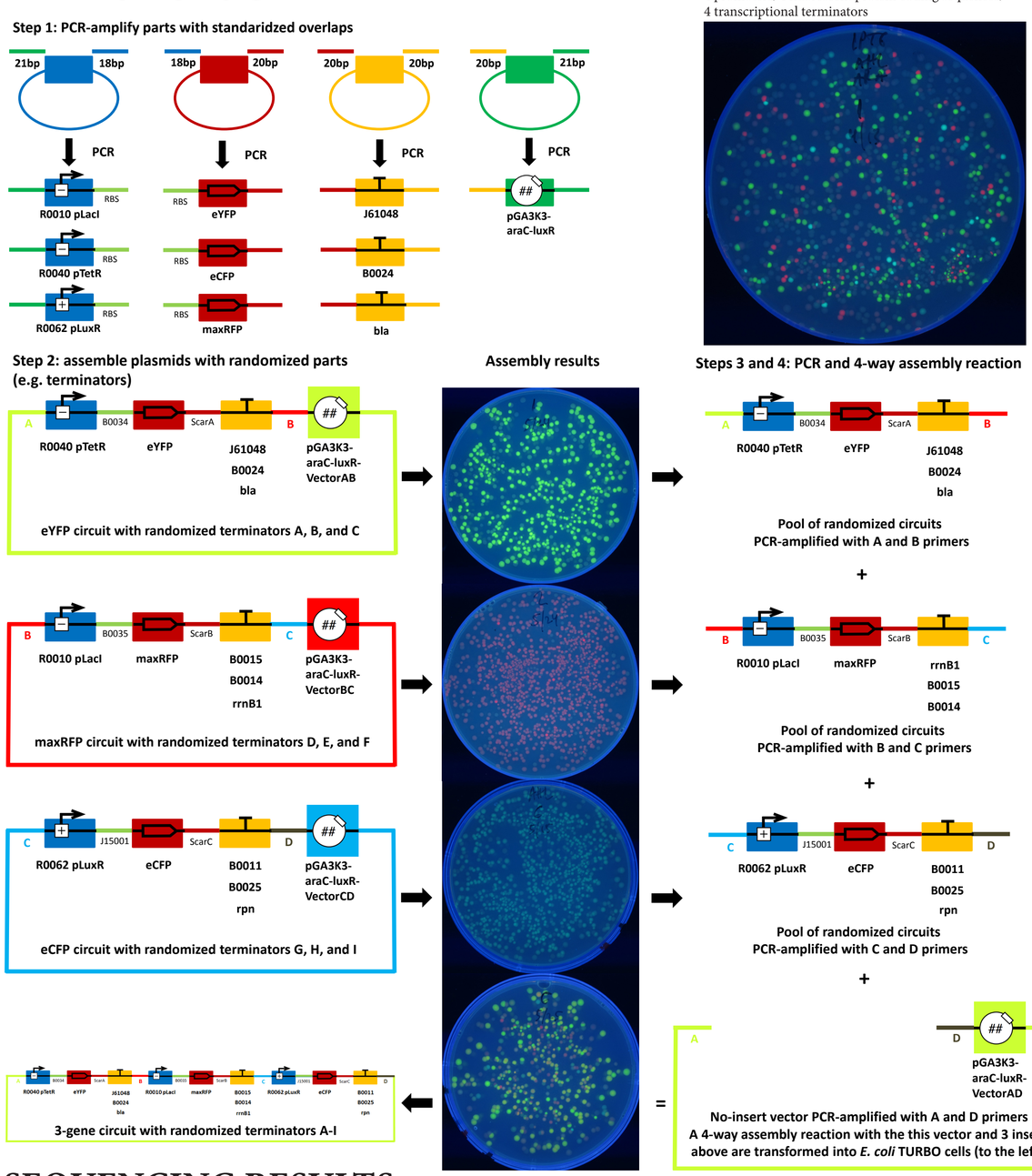
Randomized BioBrick Assembly: A novel DNA assembly method for randomizing and optimizing multi-gene circuits and metabolic pathways

Sean C. Sleight* and Herbert M. Sauro
University of Washington, Dept. of Bioengineering, Seattle, WA 98195, USA
*sleight@u.washington.edu

ABSTRACT

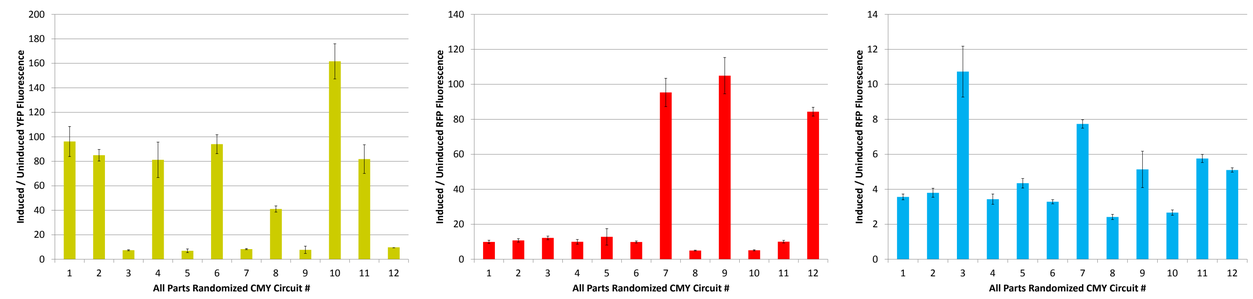
Synthetic biology requires DNA synthesis or the assembly of genetic parts into functional genetic circuits and metabolic pathways. The optimization of circuits and pathways often requires constructing various iterations of the same construct, or directed evolution to achieve the desired function. Alternatively, a method that randomizes individual parts in the same assembly reaction could be used for optimization by allowing for the ability to screen large numbers of individual clones expressing randomized circuits or pathways for optimal function. Here we describe a new assembly method to randomize genetic circuits and metabolic pathways from modular DNA fragments derived from PCR-amplified BioBricks. Each fragment of a particular part type (e.g. promoters, coding sequences, and transcriptional terminators) has the same standardized overlap on either side of the functional DNA, allowing for independent assembly with other fragments having the complementary overlap. When multiple fragments of a particular type are used in the same assembly reaction, there is competition between fragments, allowing for randomized assembly. As a proof-of-principle for this method, we first assembled eCFP, maxRFP, and eYFP gene expression cassettes with independently randomized promoters, ribosome binding sites, transcriptional terminators, and all parts randomized at once. These randomized expression cassettes were then combined to make fully functional and randomized three-gene circuits that express CFP, RFP, and YFP, producing colors close to Cyan, Magenta, and Yellow (CMY) under UV light. Sequencing results from 12 CMY circuits with nine randomized terminators show that 9/12 circuits are distinct and at least one circuit contains each of the nine terminators. When all parts are shuffled at once, 11/12 circuits are distinct and expression ranges from about 2 to 160-fold above background levels depending on the fluorescent protein. We then adapted this method to randomize the same promoters, ribosome binding sites, and terminators with the enzyme coding sequences (*crtE*, *crtB*, and *crtI*) from the lycopene biosynthesis pathway instead of fluorescent proteins, designed to allow each enzyme in the pathway to be independentally controlled from a different promoter. Sequencing results show that 7/8 pathways with all parts shuffled at once are distinct and lycopene production can be controlled with different inducer concentrations. Although the sample size is low, the randomized lycopene pathways have a strikingly different frequency of promoters and RBSs relative to the CMY circuit. These results demonstrate the ability to generate multiple unique three-gene circuits and pathways in the same assembly reaction, allowing for the construction of large libraries that can be subjected to high-throughput selections and screens. We expect that this randomization method will be useful for increasing DNA assembly efficiency, optimizing metabolic flux to maximize products of interest (e.g. biofuels), and is likely adaptable to other circuits and pathways.

METHODOLOGY

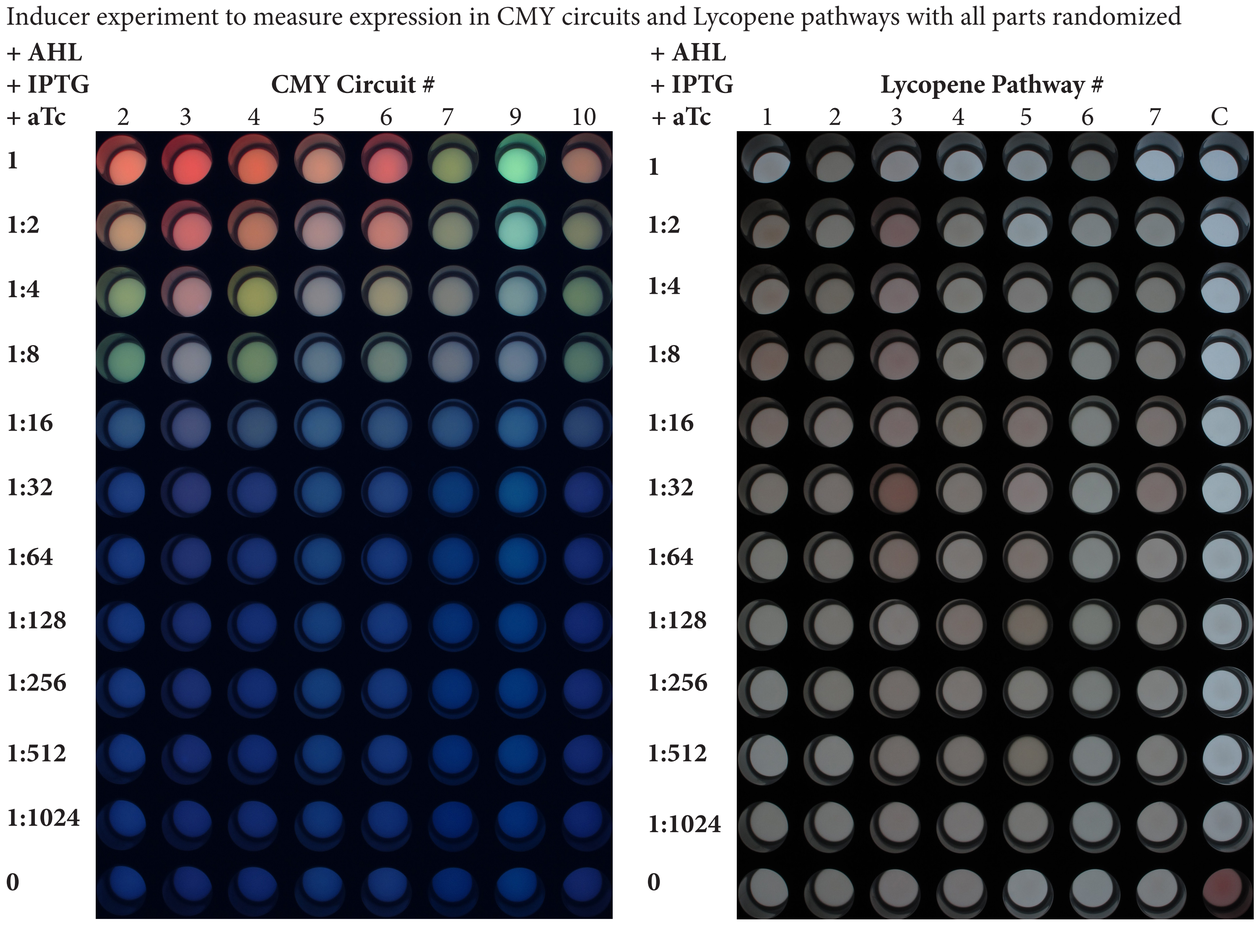


OPTIMIZATION AND CHARACTERIZATION

YFP, RFP, and CFP expression above background in CMY circuits with all parts randomized



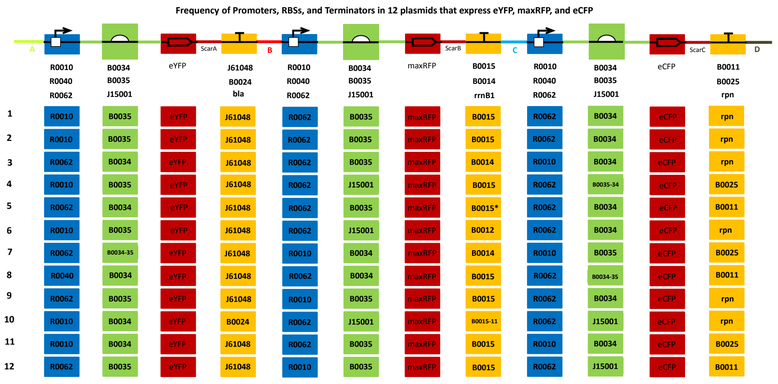
Inducer experiment to measure expression in CMY circuits and Lycopene pathways with all parts randomized



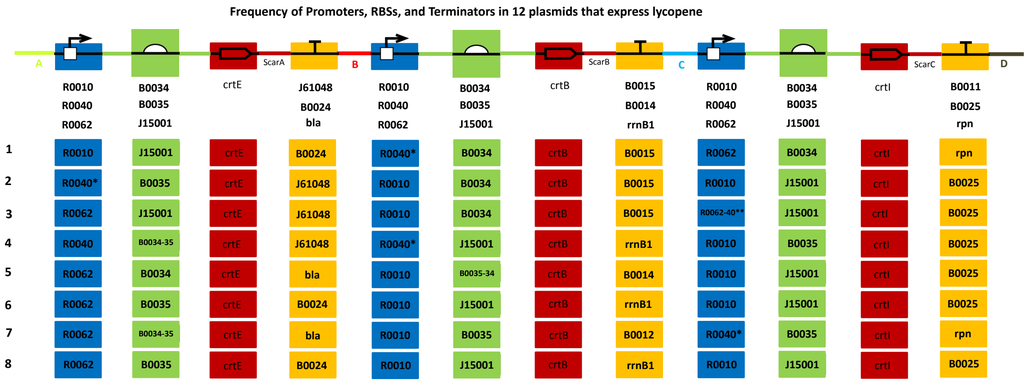
SEQUENCING RESULTS

Frequency of Terminators in 12 plasmids that express eYFP, maxRFP, and eCFP

Frequency of Promoters, RBSs, and Terminators in 12 plasmids that express eYFP, maxRFP, and eCFP



Frequency of Promoters, RBSs, and Terminators in 12 plasmids that express lycopene



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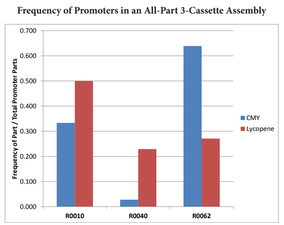
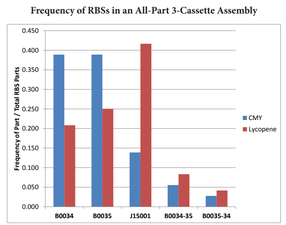
Registry of Standard Biological Parts

DNA 2.0

Frequency of Promoters in an All-Part 3-Cassette Assembly



Frequency of RBSs in an All-Part 3-Cassette Assembly



Frequency of Terminators in an All-Part 3-Cassette Assembly