Extension of Genetic Circuit Design Optimization to Industrially-relevant Organisms

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Project Goals:

- Extend genetic circuit design software (Cello) to species used in bio-production, including yeast (*S. cerevisiae*), *E. coli*, and fast-growing bacteria (*V. natriegens*)
- Develop gates that can be carried stably on the genome without antibiotic selection, as opposed to plasmids
- Design new gates that are more modular and easier to connect by mining small orthogonal repressors from bacteriophage
- Develop methods to integrate –omics data with simulations to identify and correct the causes of failures, predict the impact of carrying the circuit, and determine the impact on metabolic precursors

Abstract text:

Strains used in bio-production, such as for fuels and chemicals, typically are engineered to continuously express the introduced pathways. Genetic circuits have the potential to optimize production by timing when genes are turned on, differentiating cells to perform different tasks, implement feedback control, respond to stress and re-direct metabolic flux based on need. However, there are several problems with genetic circuits that limit their use: 1. they are usually built in laboratory strains, 2. they are carried on plasmids, 3. they lead to a growth defect, and 4. they are difficult to build and often break. Over the last year, we have developed new gates that can be carried in the genomes of industrially-relevant organisms, including *S. cerevisiae*, E. coli and *V. natriegens*. They are carried in "landing pads" that are designed to carry the circuits without interfering with host processes. These data were put into the design automation software package Cello 2.0[1], which includes updated capabilities to be able to work with these organisms. A user can now specify a new circuit design using Verilog and then automatically "re-compile" it for these new hosts simply by selecting them from a drop-down menu. We demonstrate that the genome-encoded circuits can be carried for many generations – over weeks – without breaking or imposing a growth defect.

We are still limited in the scale of the circuits that can be constructed (about 6 repressors) because larger circuits faced unpredictable breakage due to their burden on cellular resources. Several approaches are being taken to overcome this problem. First, new gates are being designed using repressor proteins identified from phage. This has led to the identification of 18 new orthogonal gates that produce near-identical and large responses when carried in the genome. We have also formulated computational models, based on transcriptomics data, to quantify cellular resource usage and predict breakage[2,3]. These tools can also simulate the dynamics of circuit transitions and elucidate transient malfunctions[4].

Collectively, these advances make it easier for a strain engineer to routinely incorporate genetic circuits into their designs. Genetic circuits with significantly more control units can now be automatically designed and assembled, with predictable dynamics and resource usage. More stable circuits can now be easily built with high fidelity on the genome and in new strains supporting a wider range of biosynthesis processes.

References/Publications

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