Tuning Gene Networks with Simple Sequence Repeats

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***Short Abstract* — Building synthetic gene circuits in the lab often requires several rounds of labor-intensive bench work to meet design specifications. Typically, we adjust RNA and protein production/degradation rates by cloning and screening new genetic variants. We have developed a mechanism to explore, in parallel, the parameter space reachable by altering translation rates in bacterial gene networks. This mechanism uses simple sequence repeats inserted in the ribosome binding site (rbSSR). With the rbSSR as a 'tuning knob,' we characterized the range of translation rates in a GFP expression library cloned into *Escherichia coli*. We also generated and are characterizing a library of bistable switch circuits. Finally, we are starting to couple the rbSSR with directed evolution as an *in vivo* optimization tool.**

***Keywords* — Tuning gene networks, bistable switch, stochastic state switching, directed evolution.**

I. Introduction

Complex synthetic gene circuits are difficult to implement. The internal complexity of cells increases uncertainty in how individual circuit components might behave, especially with changing environmental and physiological conditions. In addition, novel circuits are often constructed in the wrong parameter regime (e.g. plasmid copy number, promoter and ribosome binding site strengths) with respect to the desired behavior, resulting in poor circuit performance. Iteratively searching the parameter space (e.g. multiple cloning and screening steps) can be difficult, while randomly searching the parameter space (e.g. mutagenesis) can be inefficient – both strategies are time-consuming. What is needed is a way to increase the efficiency of matching gene circuit performance to design specifications with as few cloning and screening cycles as possible.

For this purpose, we introduce the ribosome binding site simple sequence repeat (rbSSR): a 'tuning knob' for bacterial gene networks. Variation in tandem repeats of short nucleotide sequences inserted into the RBS spacer region modulates gene expression by altering the translation initiation rate. Our specific contributions are: (1) methods for generating rbSSR variation in synthetic gene circuits; (2) an experimental characterization of the rbSSR system with fluorescent protein assays; (3) a demonstration of the mechanism, applied to a bistable switch.

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II. Results

To characterize the range and resolution of translation rates achievable with rbSSRs, we generated GFP expression libraries via PCR using multiple constitutive promoters. We observed a strong bias for SSR insertion and deletion mutations over other mutations. Each rbSSR library exhibits monotonically decreasing GFP levels from peak expression to autofluorescence as the number of repeats increases, and a dynamic range of up to three orders of magnitude with evenly spaced steps.

Synthetic toggle switches based on mutual repressor proteins have been developed to hold a system state [2, 3], but the operational richness of the architecture has not been systematically explored. Using rbSSRs in the RBS of each repressor gene, we built a new variant using polycistronic *lacI-gfp* and *tetR-RFP* operons. We generated expression libraries (Figure 1) with PCR and oligo assembly [4] methods to explore switching behavior locally and uniformly, respectively.

With control over large dynamic ranges and high tuning resolutions, the rbSSR is an effective tool both for prototyping and optimizing gene networks, encoded on plasmids or the genome. We are exploring the use of rbSSRs coupled with selective pressure to rapidly evolve optimal gene circuits.

References

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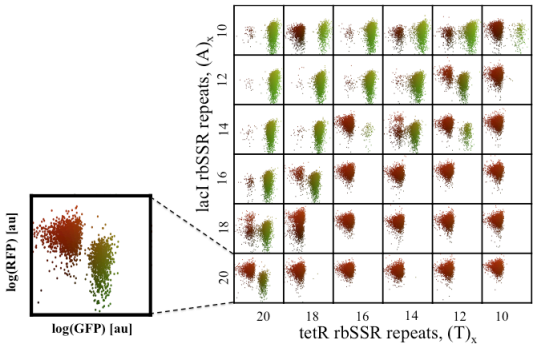

Figure 1: Compilation of cytometry scatter plots showing fluorescence distributions for an rbSSR bistable switch library. Each variant exhibits either unimodal or bimodal distributions with unique switching frequencies. The members of the library are being evaluated as long-term memory and coin-flipping devices.