Promoter-Based Engineering of Noisy Gene Expression

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***Short Abstract* — We developed a "combinatorial promoter design" strategy to characterize how the position and multiplicity of $tetO_2$ operator sites within the *GAL1* promoter affect gene expression levels and gene expression noise in *Saccharomyces cerevisiae*. We find a strong dependence of gene expression level and gene expression noise on operator site positioning and multiplicity. We were able to capture the experimentally observed differences for seven engineered promoters by computational modeling. Our results suggest that independent binding of single repressors is not sufficient to explain the behavior of the multiple operator-containing promoters.**

***Keywords* — gene expression noise, combinatorial design, predictability, engineered promoter, stochastic modeling.**

I. Introduction

Understanding the behavior of basic bio-molecular components as parts of larger systems is one of the goals of the developing field of synthetic biology [1,2]. A multidisciplinary approach, involving mathematical and computational modeling in parallel with experimentation, is often crucial for gaining such insights and improving the efficiency of artificial gene network design.

II. Combinatorial promoter design

As a basis for combinatorial promoter design, we first constructed a set of three promoters (S1, S2 and S3), each containing a single operator inserted at a different position between the TATA box and transcription start site of the yeast *GAL1* promoter [3,4]. Next, we designed and constructed a set of double operator-containing promoters (D12, D13 and D23), combining the operator of S1 with that of S2, S1 with S3, and S2 with S3, respectively. Finally, we designed and constructed a triple operator-containing promoter (T123), combining all operators of S1, S2 and S3.

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III. The effect of operator site location and multiplicity

We observed stronger transcriptional repression and higher gene expression noise as a single operator site was moved closer to the TATA box, while for multiple operator-containing promoters we found that the position and number of repressor sites together determined the dose response curve and gene expression noise.

IV. Computational modeling

We developed a generic computational model based on a chemical reaction scheme that included transitions between three promoter states, as well as mRNA and protein synthesis and degradation. This generic model successfully captured the experimentally observed differences for each of the promoters.

V. Predictability

We also developed more detailed models to successively predict the behavior of multiple operator-containing promoters from single operator-containing promoters. Our results suggest that the independent binding of single repressors is not sufficient to explain the behavior of the multiple operator-containing promoters.

VI. Conclusion

Taken together, this study [5] highlights the importance of joint experimental-computational efforts and some of the challenges of using a bottom-up approach to predict the behavior of a synthetic gene network based on its isolated bio-molecular components.

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