Methods for controlling gene expression level of bacterial populations

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***Short Abstract* — Synthetic biology has seen remarkable innovations but is still limited by the lack of predictable and versatile biological parts for controlling gene expression level. Inspired by the robustness of the human adaptive immune system, we engineered a generalizable control system for regulating population-level gene expression profile in synthetic microbial consortia. Combining CRISPR/Cas9-mediated plasmid copy number control, horizontal gene transfer (HGT), and antibiotic-mediated selection pressure, we demonstrate that our technology can orthogonally regulate multiple genes and amplify gene expression dynamic range by >10-fold compared to the typical regulation methods (e.g., inducible promoters).**

***Keywords* — gene expression control, microbial consortia, synthetic biology, CRISPR/Cas9, horizontal gene transfer**

I. Introduction

DESPITE rapid advancement in synthetic biology, only a few synthetic circuit systems can perform in a quantitative, predictable manner. Much of this shortcoming is attributed to the lack of predictable tuning capacity and limited dynamic range of gene expression regulatory elements [1]. To address this gap and achieve a greater degree of dynamic control, previous efforts have focused mostly on inventing or optimizing new regulatory elements, such as promoters [1] and ribosome-binding sites [2]. However, such strategies are cost-inefficient, requiring arduous characterization procedures to comprehend their intrinsic properties [3]. Consequently, building larger networks via logical combination of components is still challenging [4].

To circumvent this obstacle, we borrow the design principle of the human adaptive immune system, in which immune cells clonally expand in response to antigen invasion, while staying in small number under normal condition. We hypothesize that population-level gene expression control can serve as a generalizable strategy to program microbial consortia with an amplified dynamic range and reduced basal level expression. Our platform combines three control layers: (i) in each cell, inducible SpCas9 cuts the plasmids encoding the gene of interest to regulate average copy number per cell; (ii) HGT tunes the population-wide relative frequency of the plasmid; and (iii) antibiotic-mediated selection modulates the population fraction carrying the gene of interest. In this work, we use mathematical modeling and experimental validation to demonstrate our system's responsiveness when regulating expression of a fluorescence reporter.

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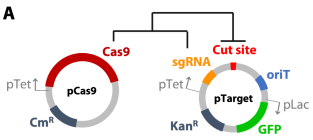
II. Results and Discussion

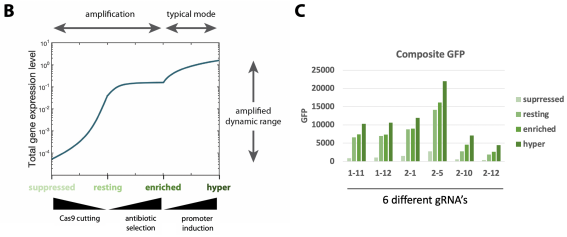
A. Circuit design

We built a two-plasmid circuit system that can tune the copy number of a target plasmid (pTarget) encoding the gene of interest (GFP) using CRISPR/Cas9 machineries (pCas9) (**Fig. A**). Because the sgRNA is located on pTarget, the cleavage activity diminishes as the pTarget copy number is reduced, creating a negative feedback loop to prevent a complete elimination of pTarget. Since pTarget encodes an origin of transfer (oriT), pTarget can persist in population via conjugation [5].



B. Modeling analysis & Experimental validation

Our mathematical analysis predicted that our control architecture can significantly enhance the dynamic range of total gene expression level by tuning Cas9 activity level and antibiotic selection. Our platform can complement the typical mode of control by using inducible promoters to further enhance the dynamic window (**Fig. B**). The model prediction was also validated experimentally in *E. coli* (**Fig. C**).



III. Conclusion

Our results demonstrate that our control system can improve both the generalizability and the dynamic range of population-level total gene expression control.

References

[1] Lutz R, Bujard H (1997) Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res*, **25**, 1203-1210.

[2] Salis HM, Mirsky EA, Voigt CA (2009) Automated design of synthetic ribosome binding sites to control protein expression. *Nat Biotechnol*, **27**, 946-950.

[3] Mutalik VK *et al.* (2013) Precise and reliable gene expression via standard transcription and translation initiation elements. *Nat Methods*, **10**, 354-360.

[4] Cameron DE, Bashor CJ, Collins JJ (2014) A brief history of synthetic biology. *Nat Rev Microbiol*, **12**, 381-390.

[5] Lopatkin AJ *et al.* (2017) Persistence and reversal of plasmid-mediated antibiotic resistance. *Nat Commun*, **8**, 1689.