Demonstrating the Orthogonality of CRISPRa Expression Devices in a Multi-Gene System

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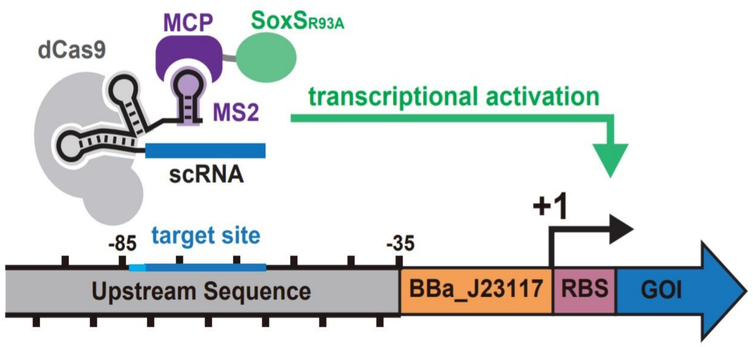
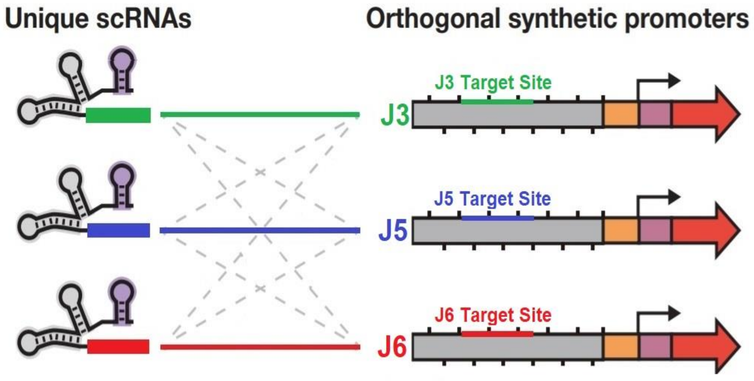
Abstract

The CRISPR-Cas9 system has been modified to become a widely used tool for regulating gene expression in engineered cells. CRISPR-Cas activation (CRISPRa) has allowed for sequence-specific activation of gene expression in eukaryotes such as yeast (Zalatan *et al.*, 2015). This technique has since been integrated into work with prokaryote systems such as *Escherichia coli* (Dong *et al.*, 2018). Recently, we have developed a series of CRISPRa-responsive promoters, providing a novel toolkit of expression devices that have been verified to function in isolation. To investigate the modular and orthogonal nature of these tools, we will quantify CRISPRa activity when multiple genes are activated simultaneously. If these devices can be independently regulated, then this technology could be used to optimize a heterologous, three-gene pathway in *E. coli* that will produce complex oligosaccharides (Sprenger *et al.*, 2017). These chemically complex oligosaccharides have applications that range from integration in nutritional supplements to incorporation into biotherapeutic methods. However, they are difficult to produce through traditional organic chemistry and through isolation from natural, physiological sources. Through the introduction of a CRISPRa-regulated oligosaccharide production pathway in *E. coli*, these sugars can be produced cheaply and efficiently.

CRISPR-Cas Activation

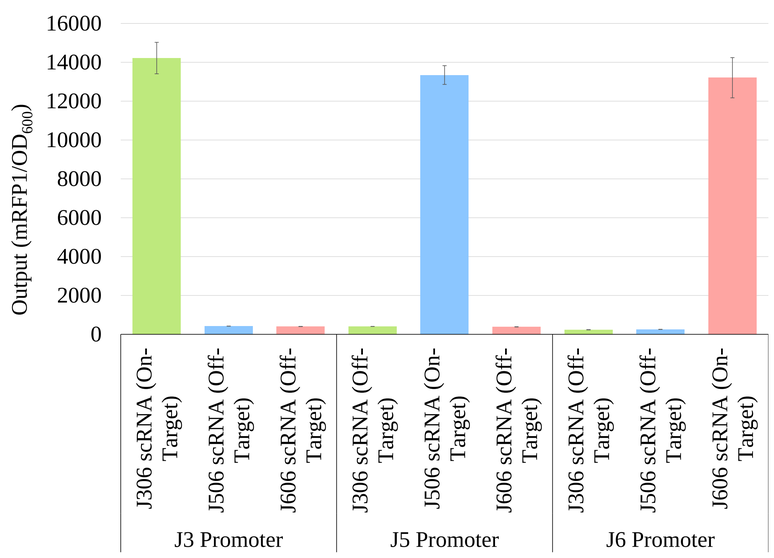


The transcription activator is recruited to the CRISPR-Cas9 complex by catalytically inactive Cas9 (dCas9) paired with a programmable RNA scaffold (scRNA) (Fontana, 2019). Once indirectly bound to the promoter sequence, the activator can initiate transcription of the downstream region.



Development of orthogonal synthetic promoters followed several design rules: no cross-activation, no binding sites for transcription factors, no homology with each other or the genome (Fontana, 2019). These promoters could be used in combination to selectively express multiple genes within a single pathway.

Orthogonal Activation of Promoters



The orthogonality of the J3, J5, and J6 promoters was demonstrated by comparing the expression of mRFP1 in both on-target and off-target promoter/gRNA pairings. In each of the combinations, promoter activation increased by 33-fold or more when paired with its on-target gRNA as compared to off-target. This demonstrates that cross activation is minimal between off-target promoter/gRNA within a single pairwise combination. If we can establish that these promoters are selectively inducible when simultaneously present, then they could be used as expression controllers in a multigene pathway.

References

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Sprenger, G. A., Baumgärtner, F., & Albermann, C. (2017). Production of human milk oligosaccharides by enzymatic and whole-cell microbial biotransformations. Journal of Biotechnology, 258, 79-91.

Tomosugi, W., Matsuda, T., Tani, T., Nemoto, T., Kotera, I., Saito, K., ... & Nagai, T. (2009). An ultramarine fluorescent protein with increased photostability and pH insensitivity. Nature methods, 6(5), 351.

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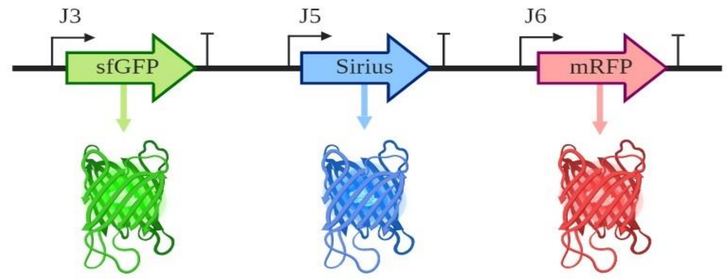
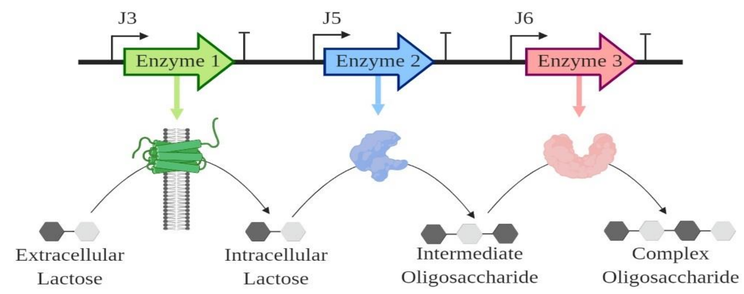
Designing a Multi-Gene Pathway



Within a plasmid, expression of visible proteins (sfGFP, Sirius, and mRFP1) will be controlled by three separate synthetic promoters; J3, J5, and J6. Using this system, the levels of cross-activation will be determined by quantifying the expression of each of the proteins after introduction of one of the gRNAs. If activation is specific to on-target pairings, then this independent, orthogonal, and tunable control of enzyme expression could have metabolic engineering applications.



Using a similar design to the fluorescent protein multigene plasmid, a plasmid will be constructed containing a three-enzyme metabolic pathway to convert lactose into a complex oligosaccharide within engineered *E. coli*. Within this system, tunable activation will be used to optimize output production.

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