ACS SyntheticBiology

In This Issue

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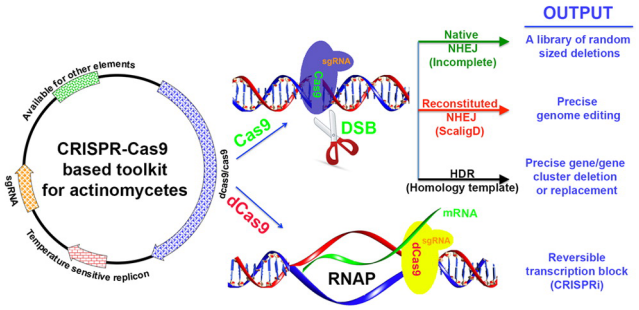
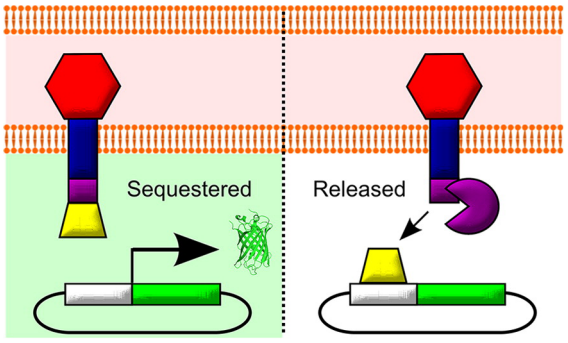
CRISPR-CAS9 BASED ENGINEERING OF ACTINOMYCETAL GENOMES



The increased occurrence of multidrug resistant pathogens has made the search for new antibiotics an urgent matter. Actinomycetes are one of the most important sources of clinically useful antibiotics and other bioactive molecules, but current approaches for metabolic engineering of these organisms to boost the yield of known antibiotics and/or to produce novel antibiotics are hampered by gene replacement and inactivation methods that are time-consuming and inefficient. Here, Tong *et al.* (DOI: 10.1021/acssynbio.5b00038) report an efficient, facile genetic manipulation system for actinomycetal genomes, based on the type II RNA-directed bacterial immune system, CRISPR-Cas9.

The authors demonstrate the successful use of this system by targeting two genes within actinorhodin biosynthesis in *Streptomyces coelicolor* for both gene deletion and reversible repression of expression. The CRISPR-Cas9 based system described here comprises a powerful and broadly applicable set of tools to manipulate actinomycetal genomes.

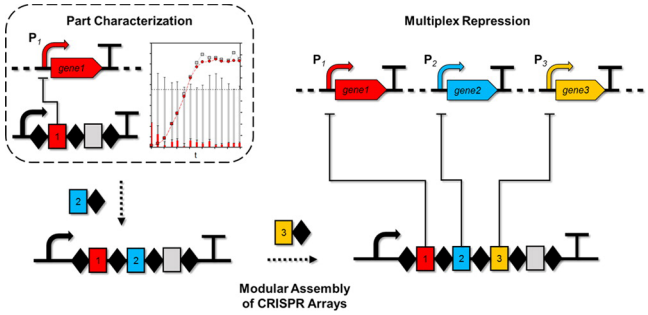
REGULATION OF BACTERIAL GENE EXPRESSION BY PROTEASE-ALLEVIATED SPATIAL SEQUESTRATION



In nature, the selective sequestration of regulators enables cells to respond rapidly to changes in their environment or internal state. This performance characteristic could be useful for synthetic biology applications ranging from biosensing to regulation of biosynthetic pathways. However, the literature contains contradictory observations as to whether engineering spatial sequestration prevents a transcriptional regulator from acting upon its cognate DNA sequence. Further, it remains unknown whether spatial sequestration may be harnessed for synthetic biology applications. Here, Pitner *et al.* (DOI: 10.1021/sb500302y) use a synthetic biology approach to investigate and answer the question, is the spatial sequestration of transcriptional regulators sufficient to confer conditional gene regulation?

The authors demonstrate that sequestration of both activators and repressors to the cytoplasmic membrane prevents their interaction with cognate promoters, and that protease-mediated release of these regulators from sequestration can thereby trigger either induction or repression of a target gene. The Protease-Alleviated Spatial Sequestration (PASS) platform developed here thus comprises a modular and easily adaptable strategy for regulating gene expression in *E. coli* and, potentially, other microbes. The observations reported here provide new insights into microbial gene regulation in nature and provide a robust strategy that may be harnessed for diverse applications in microbial synthetic biology.

CRISPATHBRICK: MODULAR COMBINATORIAL ASSEMBLY OF TYPE II-A CRISPR ARRAYS FOR TRANSCRIPTIONAL REPRESSION IN *E. COLI*



Programmable control over an addressable global regulator would enable simultaneous repression of multiple genes and would have tremendous impact on the field of synthetic biology. In this paper, Cress *et al.* (DOI: 10.1021/acssynbio.5b00012) describe a combinatorial cloning method to construct type II-A CRISPR arrays from individual spacer-repeat "parts" to enable dCas9-mediated transcriptional repression of multiple genes simultaneously.

The authors demonstrate the assembly and functionality of these arrays in *E. coli* by repressing reporter genes, as well as endogenous virulence factors and biosynthetic genes, using a single plasmid in multiple divergent strains. They also developed a two-plasmid toolkit, with pCRISPathBrick and pCRISPReporter, for facile construction and characterization of CRISPR arrays. Finally, they show that CRISPathBrick can be used to repress endogenous metabolic targets, leading to improved production of a heterologous plant natural product in *E. coli*.

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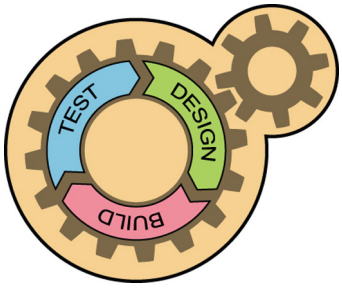
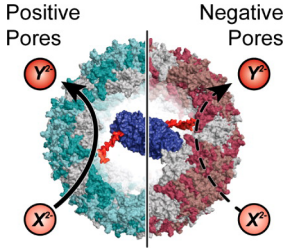
A HIGHLY CHARACTERIZED YEAST TOOLKIT FOR MODULAR, MULTIPART ASSEMBLY



Synthetic biology is at a major turning point as a field, with new tools and technologies catalyzing the leap from primarily using *E. coli* as the chassis to exploring eukaryotic hosts. Although model eukaryotic organisms and their biology have been well studied for decades, the tools for engineering them in a predictable manner have not been nearly as concisely defined or widely adopted as they have been for *E. coli*. In this work, Lee *et al.* (DOI: 10.1021/sb500366v) describe a framework for engineering *Saccharomyces cerevisiae*, an increasingly popular host due to its powerful genetic tools and long history of industrial applications.

The authors developed a standardized hierarchical assembly scheme, similar to MoClo, which enables robust and rapid cloning of DNA that can express several genes simultaneously. They also report the characterization of many essential parts including promoters, terminators, and origins of replication, to guide researchers in their design decisions. Finally, they implemented popular genome editing tools to assist the construction of stable strains and avoid the use of episomal plasmids.

INFLUENCE OF ELECTROSTATICS ON SMALL MOLECULE FLUX THROUGH A PROTEIN NANOREACTOR



Quantitative understanding of selectivity in protein nanoreactors will prove interesting to many areas of research in catalysis, biochemistry, and metabolic engineering. By influencing small molecule flux through the pores, one is able to create an altered local environment within the capsid relative to outside. This has implications *in vitro*, where porous viral capsids, liposomes, inorganic shells, and polymersomes are used to house catalyzed reactions. In this paper, Glasgow *et al.* (DOI: 10.1021/acssynbio.5b00037) use their previously developed technology to encapsulate the *E. coli* alkaline phosphatase in different mutants of the bacteriophage MS2 viral capsid.

Using site-directed mutagenesis, the authors generated capsids with various charged groups precisely positioned around the pore of the compartment. When the capsid pores had charges opposite that of the enzyme's substrate and product, the kinetics of the reaction were relatively unaffected. Conversely, when the pores and small molecules had the same charge, a substantial increase in the apparent Michaelis constant was observed. Kinetic modeling suggested that this was due to a buildup of product within the capsid which inhibits the enzymatic turnover. Thus, this study presents the first evidence of tailored pores in viral capsid nanoreactors.

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