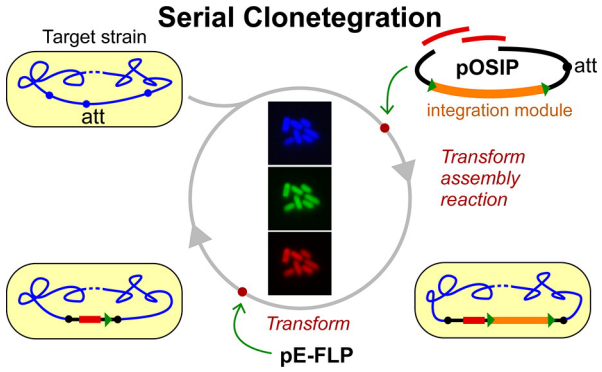
ACS SyntheticBiology

In This Issue

pubs.acs.org/synthbio

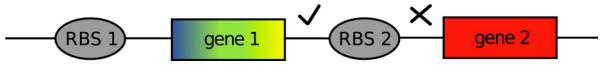
ONE-STEP CLONING AND CHROMOSOMAL INTEGRATION OF DNA



Integration of synthetic sequences onto bacterial genomes is an important procedure toward building genetically engineered cells in synthetic biology and metabolic engineering. While chromosomes can accommodate longer synthetic sequences than traditional multicopy plasmids, the current procedures to integrate DNA fragments into prokaryotic chromosomes are very laborious. Thus, plasmids remain the most popular expression vectors. Now, St-Pierre et al. (DOI: 10.1021/sb400021j) describe clonetegration, a powerful one-step method that combines the ease of plasmid cloning with the usefulness of chromosomal integration.

This method presents an important conceptual advance over traditional methodologies by enabling bacterial chromosomes to function as traditional cloning vectors.

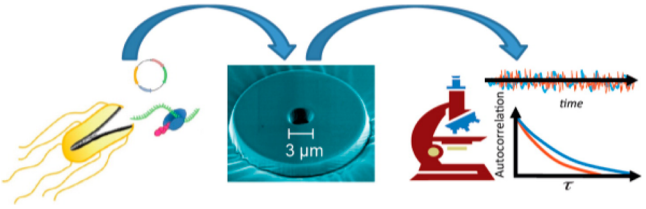
FLUORESCENT PROTEINS AND *IN VITRO* GENETIC ORGANIZATION FOR CELL-FREE SYNTHETIC BIOLOGY



The synthesis of a cell from scratch requires that the ability to encode relative protein levels so that the structural and metabolic needs of the cell are met. One difficulty in achieving desired protein concentrations is that the way in which synthetic genomes are assembled impacts how the genes perform. To better understand such influences, Lentini et al. (DOI: 10.1021/sb400003y) screened a series of fluorescent proteins to identify which proteins reliably express with minimal machinery.

The authors then assembled synthetic constructs in such a way as to probe the influences of spacing and sequence of noncoding regions on protein production. The data allowed them to formulate simple rules to follow when assembling genomes. The coupling of data with the use of standardized parts, such as transcriptional promoters and ribosome binding sites of known strength, should facilitate the construction of *in vitro* genetic devices that are built with fully defined components.

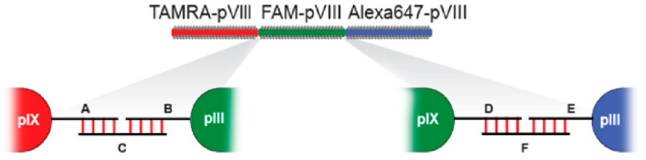
PROBING CELL-FREE GENE EXPRESSION NOISE IN FEMTOLITER VOLUMES



Noise in gene expression plays an essential role in the function of many natural and synthetic gene networks. However, studying noise in living cells is complicated by cell growth, division, mutation, and environmental variation. Here, Karig et al. (DOI: 10.1021/sb400028c) present an approach for studying gene expression noise in cell-relevant volumes but without the confounding factors.

The authors confine gene expression reactions in cell-sized containers and quantify the expression in each container using fluorescent microscopy. Significant well-to-well variation in expression was observed, suggesting that key protein expression machinery components are not well-mixed at the cell scale. The authors also analyzed gene expression noise and saw that expression dies out over time due to decreases in transcription rate. This method for probing gene expression variability will ultimately offer insights into the construction of more complex and reliable gene circuits in synthetic biology, both in living cells and in cell-free systems.

SELF-ASSEMBLY OF END-TO-END MULTIPHAGE STRUCTURES



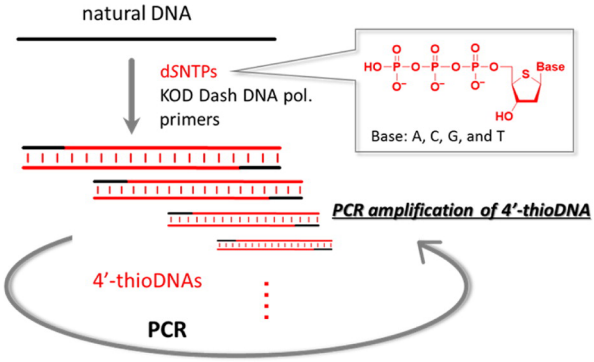
M13 bacteriophage has been used as a material scaffold in the construction of electronic and solar devices. The need to create more complicated multimaterial devices demands the development of new strategies to connect different phage particles in a precise manner. This requires labeling of specific M13 capsid proteins, which has been particularly difficult to achieve. To overcome these technical challenges, Hess et al. (DOI: 10.1021/sb400019s) now exploit sortase enzymes and DNA-conjugated peptides to form multimers of M13 in a predefined order arrangement.

The authors describe a method of conjugating DNA to the ends of the phage and demonstrate the formation of end-to-end multimers in a desired order. These constructs are a significant first step toward the self-assembly of patterned multimaterial nanowires with possible applications for transistors and diode devices.

Received: August 29, 2013
Published: September 20, 2013

dx.doi.org/10.1021/sb400121x | *ACS Synth. Biol.* 2013, 2, 478–479

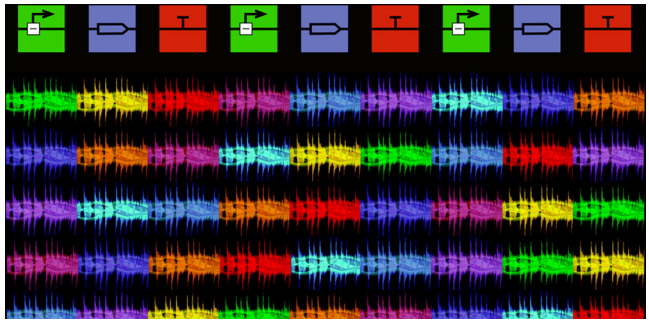
PCR AMPLIFICATION OF 4′-THIODNA



Recent research involves the incorporation of a large number of chemically modified nucleoside derivatives into oligonucleotides (ONs) for use in biological, bioengineering, and therapeutic applications. The most reliable methods, thus far, to ensure that a modified unit is inserted into the desired position in the ON sequence are chemical methods using the corresponding phosphoramidite units. However, it is unsuitable for preparing long chain sequences. Now, Kojima et al. (DOI: 10.1021/sb400074w) describe the comprehensive analysis of the enzymatic incorporation of 2′-deoxy-4′-thionucleoside 5′-triphosphates (dSNTPs) by DNA polymerases and the transcription from resulting 4′-thioDNA by an RNA polymerase.

This manuscript depicts the general and versatile utility of dSNTPs and 4′-thioDNA. The resulting 4′-thioDNA could be a good candidate for not only chemically modified aptamers but also synthetic genetic polymers.

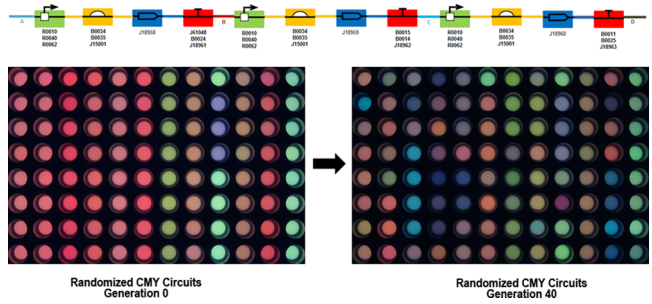
RANDOMIZED BIOBRICK ASSEMBLY



The optimization of genetic circuits and metabolic pathways often involves the construction of various iterations of the same construct or the use of directed evolution to achieve the desired function. An alternative approach, involving a method that randomizes individual parts in the same assembly reaction could be used for optimization by allowing for the ability to screen large numbers of individual clones expressing randomized circuits or pathways for optimal function. Here, Sleight and Sauro (DOI: 10.1021/sb4000542) describe a new assembly method to randomize genetic circuits and metabolic pathways from modular DNA fragments derived from PCR-amplified BioBricks.

The methodology described here can likely be adapted to several circuits and pathways to maximize products of interest.

EVOLUTIONARY STABILITY DYNAMICS AND COMPETITIVE FITNESS OF ENGINEERED *E. COLI*



Engineered strains for synthetic biology and metabolic engineering applications often express foreign proteins that reduce cellular fitness. Now, Sleight and Sauro (DOI: 10.1021/sb400055h) describe the construction of randomized cyan-magenta-yellow genetic circuits to quantify and visualize the evolutionary stability dynamics in engineered *E. coli*.

To do this, the authors use independently randomized promoters, ribosome binding sites and transcriptional terminators that express CFP, RFP, and YFP. The evolutionary stability dynamics of these randomized circuits can then provide ideas on robust circuit design.

dx.doi.org/10.1021/sb400121x | *ACS Synth. Biol.* 2013, 2, 478−479