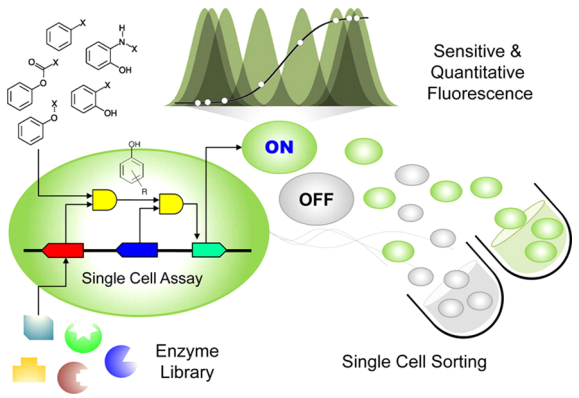
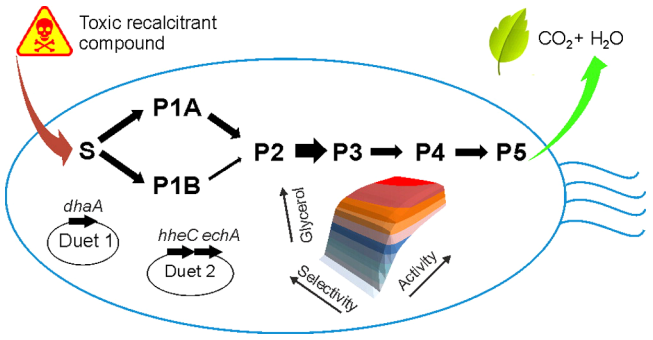
ARTIFICIAL GENETIC CIRCUIT BASED HIGH-THROUGHPUT ENZYME SCREENING SYSTEM



The development of cost-effective biological processes, for the production of sustainable biobased chemicals, is largely dependent on the large-scale screening of enzyme libraries. Here, Choi *et al.* (DOI: 10.1021/sb400112u) describe the highly efficient Genetic Enzyme Screening System (GESS). GESS is a versatile and sensitive strategy for enzyme screening from a metagenome using high throughput flow cytometry.

The significance of this approach is that this single system can be applied to isolate a variety of enzymes that may produce a phenol compound from their respective synthetic phenyl-substrates. Thus, GESS along with flow cytometry techniques allow for a widely applicable tool-kit for discovering and engineering novel enzymes in an individual cell level.

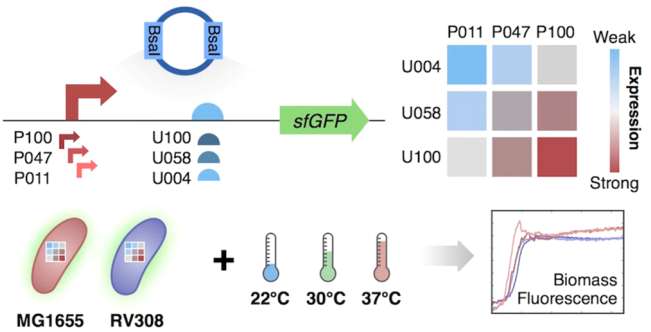
COMPUTER-ASSISTED ENGINEERING OF A SYNTHETIC PATHWAY FOR BIODEGRADATION



Halogenated hydrocarbons are widely used for agricultural, industrial, and military purposes. However, once introduced into the environment, they persist due to the lack of natural biodegradation pathways. Now, Kurumbang *et al.* (DOI: 10.1021/sb400147n) describe the engineering of a synthetic metabolic pathway for biodegradation of such toxic pollutants.

Using a mathematical model, the authors assembled a synthetic route for conversion of the highly toxic and recalcitrant 1,2,3-trichloropropane to glycerol in *Escherichia coli*. The resultant strains were characterized for their viability and degradation efficiency and excellent agreement between predicted and experimental data was observed. Thus, this study highlights the potential of forward engineering of micro-organisms for the degradation of toxic anthropogenic compounds.

EXPLORING THE PROTEIN EXPRESSION CHARACTERISTICS OF *E. COLI*



Synthetic biology has developed numerous parts for the precise control of protein expression, yet little is known about the effect these have upon a host, or the reliability of their performance to varying environmental conditions. To address this, Gorochowski *et al.* (DOI: 10.1021/sb4001245) used synthetic transcriptional and translational elements to create a library of constructs that modulated expression strength of a green fluorescent protein.

The authors combined this library with a microbioreactor platform and carried out a detailed, large-scale assessment of expression and growth characteristics of multiple *E. coli* strains across several temperatures. They report significant differences in the robustness of strains to differing types of protein expression and a complex response of transcriptional and translational elements to differing temperatures. Studies such as this one are essential to qualify the natural variability present during protein production and to ensure larger synthetic biological systems will work as expected across differing hosts and environmental contexts.

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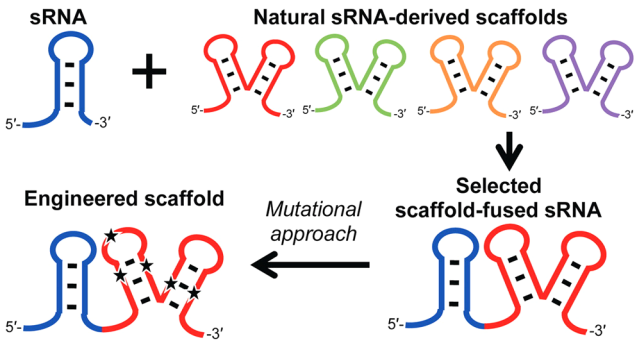
MULTIPLE AMINO ACID-EXCLUDED GENETIC CODES

UUU	Phe	UCU	Ser	UAU	Ser	UGU	Ser
UUC		UCC		UAC		UGC	
UUA	Leu	UCA		UAA	Stop	UGA	Stop
UUG		UCG		UAG		UGG	Ser
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC		CCC		CAC		CGC	
CUA		CCA		CAA	Gln	CGA	
CUG		CCG		CAG		CGG	
AUU	Ile	ACU	Thr	AAU	Ser	AGU	Ser
AUC		ACC		AAC		AGC	
AUA		ACA		AAA	Lys	AGA	Arg
AUG	Met	ACG		AAG		AGG	
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC		GCC		GAC		GGC	
GUA		GCA		GAA	Glu	GGA	
GUG		GCG		GAG		GGG	

A simplified genetic code is useful as an engineering tool for the improvement of industrial enzymes and pharmaceuticals, and also provides new insights into the assessment of protein evolution. While a recently developed simplified genetic code assigned just 19 amino acids to the sense codons, Amikura *et al.* (DOI: 10.1021/sb400144h) now report the construction of a multiple amino acid-excluded genetic code in which only 16 amino acids are assigned to the sense codons.

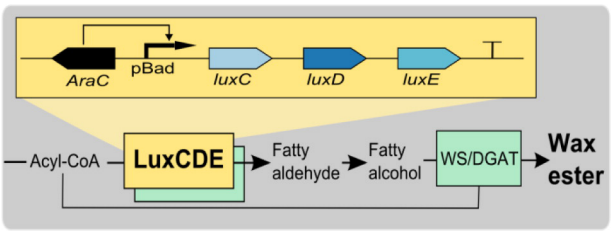
IMPROVING THE GENE-REGULATION ABILITY OF SMALL RNAS



Artificial small RNAs have been described as valuable genetic tools in the application of synthetic biology. To date, these artificial small RNAs have been engineered by mainly altering the antisense region that hybridizes with the target mRNA. Now, Sakai *et al.* (DOI: 10.1021/sb4000959) describe an alternative engineering method to improve the gene regulation ability without altering the antisense region.

The authors directly fused RNA scaffolds derived from naturally occurring small RNAs and tested them in *E. coli*. The scaffold-fused small RNAs were further improved by introducing rational mutations predicted to stabilize their secondary structure. This fusion of scaffold and introduction of mutations resulted in an increase in the small RNA gene regulation ability. This strategy is shown to be effective against small RNAs that regulate target gene expression by different mechanisms thus having the potential to be valuable in the engineering of artificial small RNAs for use in synthetic biology.

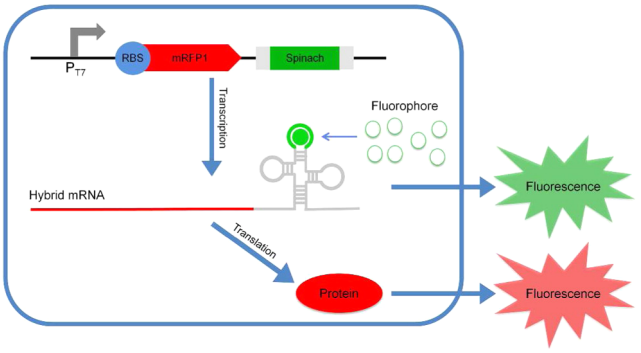
REWIRING THE WAX ESTER PRODUCTION PATHWAY



Wax esters are industrially relevant molecules exploited in several applications of oleochemistry; bacterial production platforms are suggested to replace these expensive chemical processes. Here, Santala *et al.* (DOI: 10.1021/sb4000788) use *Acinetobacter baylyi* ADP1, a natural producer of wax esters, as a tunable production platform for obtaining broader range of customized wax esters.

In order to modify the natural wax esters of *A. baylyi* ADP1, the natural fatty acyl-CoA reductase was replaced with a well-known fatty acid reductase complex LuxCDE. The engineered strain was able to produce wax esters and the produced wax esters were shorter and more saturated compared to the wild type strain. Thus, the authors demonstrate the potentiality for regulated production of a customized bioproduct.

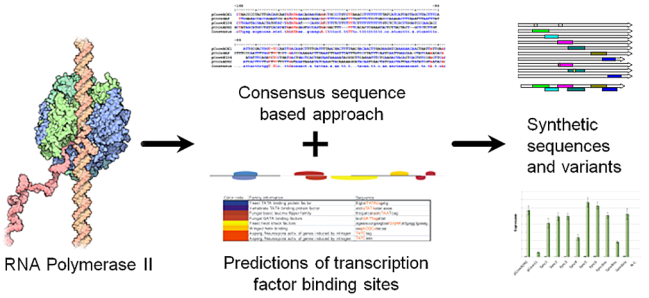
SPINACH RNA APTAMER AS A CHARACTERIZATION TOOL



In this concise technical note, Pothoulakis et al (DOI: 10.1021/sb400089c) describe their work developing and testing the Spinach RNA aptamer as a tool to characterize gene expression for synthetic biology in *E. coli*. Despite previous publications on the Spinach aptamer, there have been no reports on the utility of Spinach as a measurement tool. The authors now, for the first time, utilize Spinach for part characterization. They placed Spinach, an RNA mimic of GFP, into an mRNA actively translated in *E. coli* that encodes for the red fluorescent protein, mRFP1. Using fluorescence microscopy and a two-color flow cytometer they were able to simultaneously and separately measure transcription and translation of the same mRNA in live cells. The authors then used their setup as a system with which different promoters (T7, T5) and designed ribosome binding site sequences can be assessed. By measuring protein abundance independently from transcript abundance, they were able to highlight the fundamental temporal differences between transcription and translation.

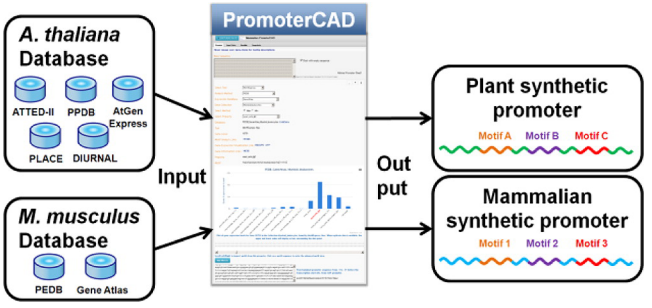
dx.doi.org/10.1021/sb500095n | *ACS Synth. Biol.* 2014, 3, 108–110

SYNTHETIC CORE PROMOTERS FOR *PICHIA PASTORIS*



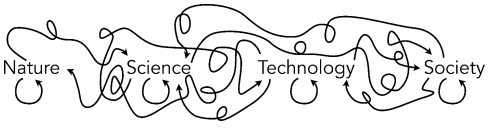
The expression of regulatory factors or enzymes in synthetic biology or metabolic engineering often requires the fine-tuning of the amounts of these proteins. The stage of transcription is a key factor for regulating and fine-tuning biological systems. Synthetic promoters, providing tailor-made regulation, are commonly used to achieve this goal. In eukaryotes, such as the yeast *Pichia pastoris*, the core promoter region is an important part of the entire promoter. Core promoters have variable sequences and are bound by general regulatory factors that provide efficient transcription. In prokaryotes, promoters are generally shorter and can be artificially designed by consensus sequence strategies. Here, Vogl *et al.* (DOI: 10.1021/sb400091p) describe the design of a synthetic core promoter for *P. pastoris* based on a consensus sequence and *in silico* strategy. The authors used this synthetic core promoter sequence to engineer a natural core promoter, thereby creating a set of core promoter variants providing a range of expression levels.

DATABASE CONSTRUCTION FOR PROMOTERCAD



The previously described web application, PromoterCAD, allows users to design synthetic promoter sequences that control gene expression in plants. PromoterCAD utilizes existing life science databases such as microarray expression data and has an easy-to-use interface that allows users without specialized computer programming knowledge, to design synthetic promoters to control where and when gene expression takes place in the model plant *Arabidopsis*. Now, Nishikata *et al.* (DOI: 10.1021/sb400178c) present step-by-step instructions for adding both regulatory motif and gene expression data to PromoterCAD, enabling the construction of databases for new organisms.

DESIGNING SYNTHETIC BIOLOGY



In this review, Agapakis (DOI: 10.1021/sb4001068) covers topics from the SB6.0 plenary session titled "Design and Synthetic Biology: Connecting People and Technology." She focuses on the ways that design might connect the spheres of science, technology, and society in the production of new synthetic biology applications.

This review details how learning and working with designers from other fields such as interaction design, product design, or industrial design can help synthetic biologists design for the context-dependent behavior of complex systems.

dx.doi.org/10.1021/sb500095n | *ACS Synth. Biol.* 2014, 3, 108–110