ACS SyntheticBiology

In This Issue

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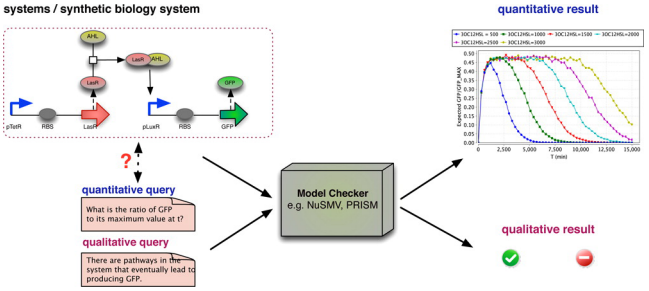
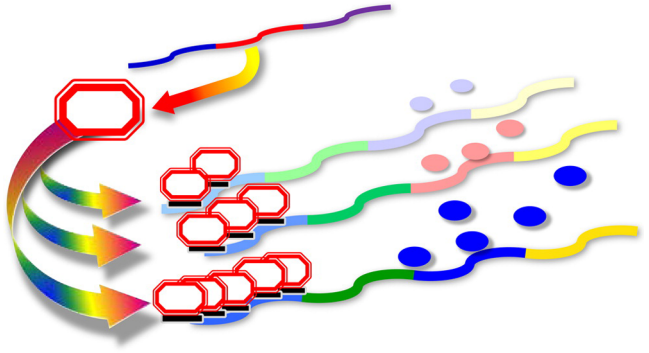
QUALITATIVE AND QUANTITATIVE ANALYSIS USING P SYSTEMS



In this tutorial, Konur *et al.* (DOI: 10.1021/sb500134w) use two well-known case studies, quorum sensing in *P. aeruginosas* and the pulse generator, to demonstrate how formal verification is utilized in systems and synthetic biology through qualitative vs quantitative analysis.

The authors use the Infobiotics Workbench tool, based on stochastic P systems, to model the systems and formally analyze them using model checkers integrated into the workbench.

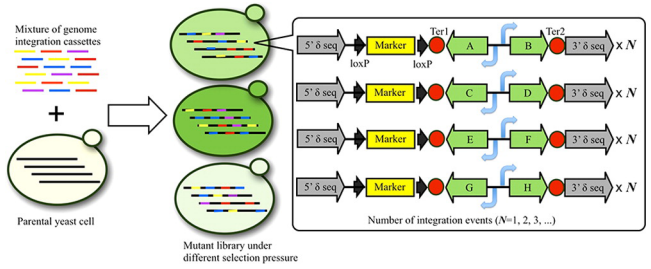
SIMULTANEOUS EXPRESSION OF MULTIPLE ENZYMES IN YEAST



There has been an increasing amount of research into bioprocesses that can produce valuable pharmaceutical and industrial products inexpensively. A key challenge in improving the production efficiency of these bioprocesses is the ability to control the expression levels of the individual enzymes in the metabolic flow. Here, Ito *et al.* (DOI: 10.1021/sb500096y) describe a novel strategy for widely controlling the expression level of multiple enzymes in the yeast *Saccharomyces cerevisiae*.

Using their previously described system, terminatome, the authors achieved an 8-fold increase in the level of protein expression, as well as the broadest dynamic range (30 000) reported to date in *Saccharomyces cerevisiae*. This system has the potential to accelerate the development of transgenic yeast for the efficient production of valuable, complex compounds.

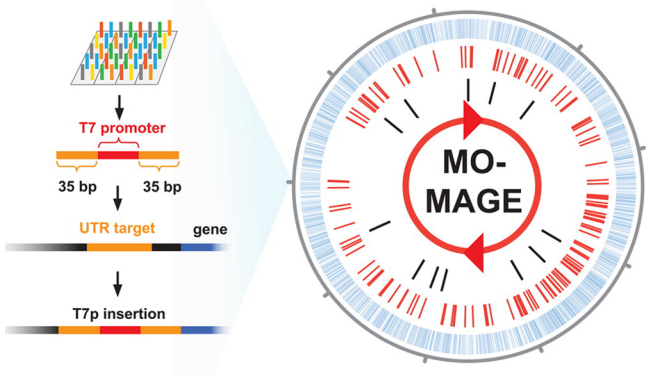
COMBINATORIAL ASSEMBLY OF LARGE BIOCHEMICAL PATHWAYS INTO YEAST CHROMOSOMES



Saccharomyces cerevisiae is often used as a microbial cell factory due to its ability to host diverse metabolite biosynthetic pathways. In this study, Yuan and Ching (DOI: 10.1021/sb500079f) describe a novel approach for the rapid construction of large biochemical pathways in yeast.

The approach detailed here uses antibiotic selection to evolve yeast chromosomes with multiple integrations at δ-sites of retrotransposons (Ty) elements. As proof-of-principle, a five-gene isobutanol pathway and an eight-gene mevalonate pathway were successfully assembled into yeast chromosomes in a one-step fashion. This novel approach could serve as a generalized technique for large pathway construction in yeast.

DIRECT MUTAGENESIS OF THOUSANDS OF GENOMIC TARGETS



Large-scale targeted mutagenesis requires hundreds to thousands of unique oligos, which are costly to synthesize and impossible to scale-up by traditional phosphoramidite column-based approaches. In this manuscript, Bonde *et al.* (DOI: 10.1021/sb5001565) describe a novel method to amplify oligos from microarray chips for direct use in MAGE to perturb thousands of genomic sites simultaneously.

The authors demonstrated the feasibility of large-scale mutagenesis to insert T7 promoters upstream of 2587 operons in *E. coli* using this method, which they termed Microarray

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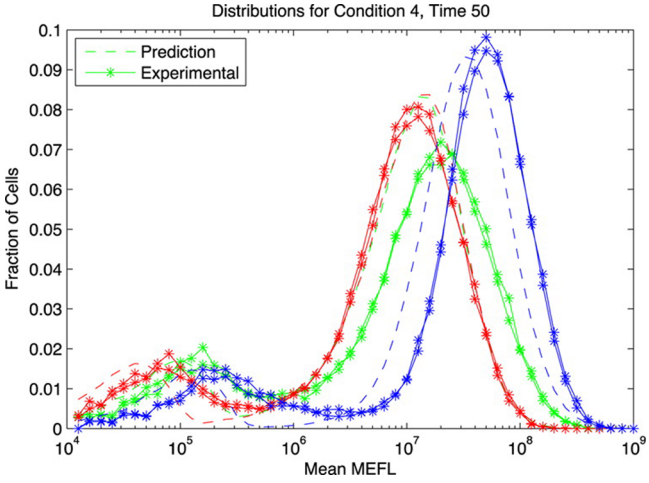
Oligonucleotide (MO)-MAGE. They further characterized this approach using deep frequency to profile mutagenesis across the population. This work exemplifies an emerging approach to combine large-scale DNA synthesis, whole-genome targeted mutagenesis, and deep-sequencing to interrogate genomic function and to engineer improved genomes for bioproduction. The described methods and protocol will enable other groups to generate large mutagenesis libraries in a variety of applications.

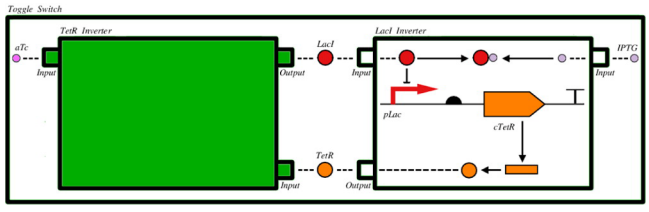
MODEL-DRIVEN ENGINEERING OF GENE EXPRESSION FROM RNA REPLICONS



RNA replicons are a platform of growing interest, with high potential for therapeutic application, particularly in the domain of vaccines and immunization. In this manuscript, Beal *et al.* (DOI: 10.1021/sb500173f) present a quantitative framework for precision engineering of gene expression in these RNA replicons.

The authors develop cotransfection of multiple replicons as a method to enable this precision engineering of protein expression by constructing a predictive quantitative model of replicon expression dynamics. They studied fluorescent protein expression in baby hamster kidney (BHK-21) cells using a replicon derived from Sindbis virus (SINV) and characterized expression dynamics for this platform, based on the dose–response of a single species of replicon over 50 h and on a titration of two cotransfected replicons expressing different fluorescent proteins. The work presented in this manuscript represents a major advance in the engineerability of these systems, as demonstrated by the application of this predictive framework to design a number of multireplicon systems with expression patterns closely in alignment with predictions.

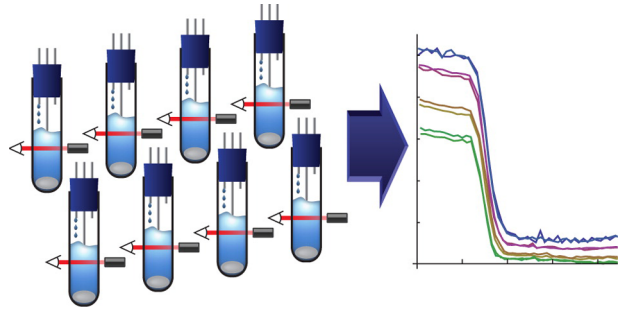
PROPOSED DATA MODEL FOR THE NEXT VERSION OF THE SYNTHETIC BIOLOGY OPEN LANGUAGE



The Synthetic Biology Open Language (SBOL) is an emerging computational standard for synthetic biology that has growing support among software tools that aid in the design and construction of synthetic biological circuits. While the current version of SBOL provides for the hierarchical, modular annotation of DNA components at the sequence level, the data model proposed here by Roehner *et al.* (DOI: 10.1021/sb500176h) provides a roadmap for extending SBOL to describe a broader range of genetic structure and function.

In particular, the proposed data model may represent both genetic and nongenetic components and combine them into modules that describe their regulatory interactions and environmental context. It could also provide a starting point to engage the synthetic biology community on the vital yet often overlooked topic of standards.

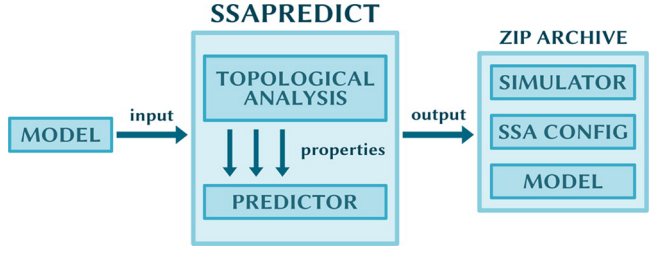
A LOW COST, CUSTOMIZABLE TURBIDOSTAT FOR SYNTHETIC CIRCUIT CHARACTERIZATION



A key aspect of parts characterization in synthetic biology is the ability to measure how strongly a gene in a network is being expressed as a function of time. However, gene networks are easily affected by environmental factors such as nutrient concentration, toxin accumulation, and cell state, which regularly skew the results of characterization, especially when cells are grown in batch culture. The turbidostat, a continuous culture device that holds cell density and chemical environment constant, is potentially ideal for characterization. However, turbidostats are not commercially available in configurations that are relevant to synthetic biologists and are difficult to design and construct from first principles. Now, Takahashi *et al.* (DOI: 10.1021/sb500165g) describe the design of a low cost, open source, eight-chamber turbidostat that can be manufactured following the author-provided online guide and used with minimal experience in electrical or software engineering.

This paper presents the design and characterization of the device as well as an application to parts characterization in yeast.

META-STOCHASTIC SIMULATION OF BIOCHEMICAL MODELS



There are many variants of stochastic simulation algorithms (SSA) described in the literature which are used for biochemical simulation. However, it is not clear which particular variant performs well with any given model as there can be orders of magnitude difference in computational time between algorithms. In this paper, Sanassy *et al.* (DOI: 10.1021/sb5001406) introduce a novel web based tool (*ssapredict*) for synthetic biology that allows scientists to predict the best performing SSA for their particular biochemical model.

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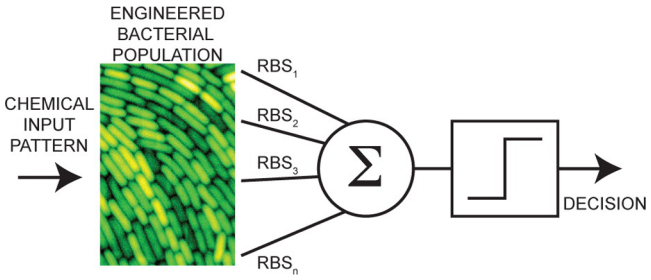
Using *ssapredict*, scientists can also simulate their query model with the predicted fastest algorithm using a preconfigured version of a high performance simulator (*ngss*) on Windows, Linux, or Mac. *ssapredict* is a free software, and its source code is distributed under the terms of the GNU Affero General Public License.

DISTRIBUTED CLASSIFIER BASED ON GENETICALLY ENGINEERED BACTERIAL CELL CULTURES



Pattern recognition and classification is an important statistical discipline with applications in computer vision, medical diagnosis, natural language processing, speech recognition, *etc*. Several pattern recognition algorithms are biologically motivated, with biological organisms making decisions based on the classification of external environmental cues. In this manuscript, Didovyk *et al*. (DOI: 10.1021/sb500235p) propose and characterize a novel concept of distributed classifiers based on genetically engineered bacterial cell cultures.

The authors engineered a "master population" of bacterial cells with synthetic sensing circuits and randomized sensitivities to incoming signals and then, using principles of machine learning, trained the classifier by shaping the population via a sequence of positive and negative examples.

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