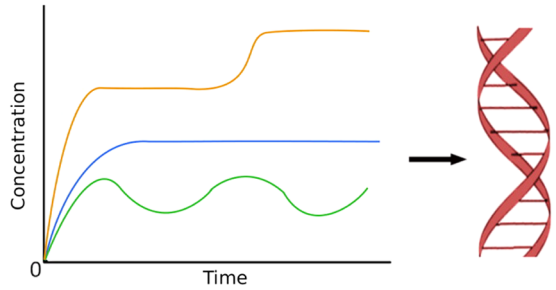
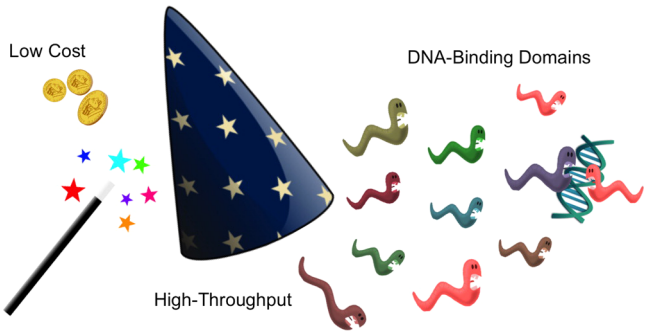
ANNOTATING SYSTEMS BIOLOGY MARKUP LANGUAGE MODELS



As the basic genetic technologies underlying synthetic biology continue to develop, one obstacle to the design and exchange of more complex synthetic genetic networks is the lack of standards that combine information on the structure and function of DNA. Now, Roehner and Myers (DOI: 10.1021/sb400066m) seek to address this need by integrating existing standards such as SBML, a well-established standard for biochemical models, and SBOL, a nascent standard for synthetic biology that currently describes DNA sequences and annotation of sequence features.

This methodology provides users the ability to construct hierarchical, modular libraries of SBML models annotated with SBOL DNA components in a semiautomated fashion. In addition, it can also be used to create modular libraries of genetic circuit models as useful inputs to genetic design automation tools that automate the process of composing DNA components to satisfy a behavioral specification.

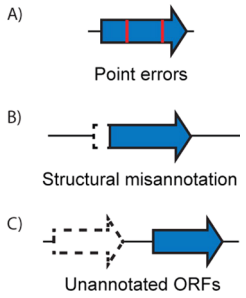
FAIRYTALE: A HIGH-THROUGHPUT TAL EFFECTOR SYNTHESIS PLATFORM



Due to their ability to target almost any almost any user-defined DNA sequence, recombinant transcription activator-like effectors (TALEs) are extremely useful for genome editing and gene regulation applications. Now, Liang *et al.* (DOI: 10.1021/sb400109p) introduce fairyTALE, a liquid phase high-throughput TALE synthesis platform.

FairyTALE can produce, with over 98% assembly efficiency, TALE-nucleases, activators, and repressors to recognize DNA sequences between 14 and 31 bp. As a proof of concept, the authors synthesized and tested 90 TALEs—96% of these were functional while 100% were correctly assembled.

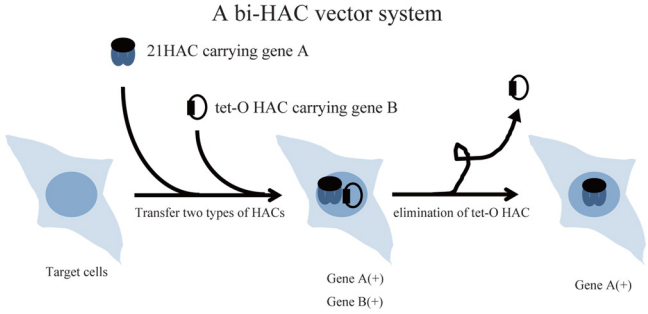
THE ENGINEERED DNA SEQUENCE SYNTAX INSPECTOR



Genetic engineering experiments can fail when synthesized DNAs contain design errors. One such design error is a syntax error in protein-coding sequences that disables the protein's desired function. Such errors come from incorrect database entries or improper annotation of genes. While experiment failures from syntax errors will likely be more prevalent in the future as more and more of the protein coding universe is explored, Hsiau and Anderson (DOI: 10.1021/sb400176e) now present a syntax error analysis pipeline.

The authors identify these syntax errors by comparing sequences of similar proteins and looking for statistically unlikely changes in the protein sequence. This is the first report detailing the types of syntax errors encountered when designing DNAs encoding for proteins and a pipeline for remedying such errors.

BI-HAC VECTOR SYSTEM TOWARD GENE AND CELL THERAPY



The use of conventional gene delivery vectors is restricted by their limited cloning capacity and the risk of insertional mutagenesis. However, human artificial chromosome (HAC)-based vectors now provide an alternate mechanism of gene delivery and expression with unlimited cloning capacity. In this paper, Iida *et al.* (DOI: 10.1021/sb400166j) describe a novel gene expression system consisting of two differently marked HAC vectors containing unique gene loading sites.

The authors established a bi-HAC vector system for gene delivery, using the 21HAC and the tet-O HAC. The 21HAC is stably maintained during cell division and provides stable gene expression while the tet-O-HAC can be eliminated and is useful

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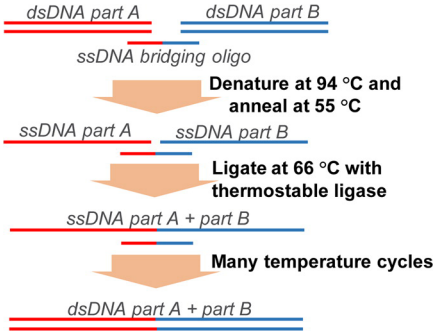
when transient gene expression is required. Based on proof of principle experiments, the authors conclude that the combination of two HAC vectors could prove to be a powerful tool for gene and cell therapy.

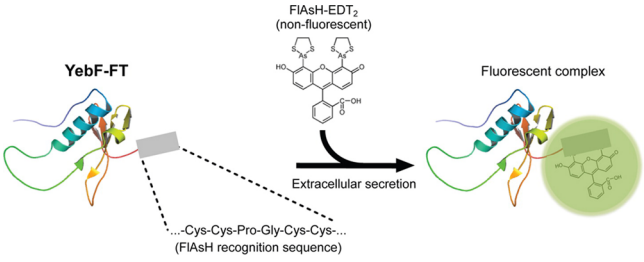
RAPID AND RELIABLE DNA ASSEMBLY



Assembly of DNA parts into DNA constructs is a foundational technology in the emerging field of synthetic biology. An efficient DNA assembly method is essential for high-throughput, automated DNA assembly in biofabrication facilities. Now, de Kok *et al.* (DOI: 10.1021/sb4001992) describe a one-step DNA assembly method via ligase cycling reaction (LCR).

LCR assembly uses single-stranded bridging oligos complementary to the ends of neighboring DNA parts, a thermostable ligase to join DNA backbones, and multiple denaturation-annealing-ligation temperature cycles to assemble complex DNA constructs without leaving any scar sequences. When compared to other DNA assembly methods, LCR assembly outperformed them on the number of DNA parts that can be reliably assembled as well as on the assembly time. LCR assembly is expected to become the DNA assembly method of choice for both manual and automated assembly of DNA parts into DNA constructs.

UNIVERSAL ASSAY FOR ENGINEERING EXTRACELLULAR PROTEIN EXPRESSION



The bacterium *Escherichia coli* remains one of the most widely used hosts for synthetic biology and metabolic engineering applications due to its well-established genetics and robust systems for making heterologous proteins in the cytoplasmic and periplasmic compartments. A limitation of *E. coli* is the generally accepted paradigm that laboratory strains lack dedicated secretion systems and do not secrete proteins into the extracellular medium under normal growth conditions. Since current methods to overcome this limitation offer relatively low yields, new methods are needed for engineering microbial hosts with enhanced secretion capacity. Now, Haitjema *et al.* (DOI: 10.1021/sb400142b) have developed a universal genetic approach for studying and engineering protein-secretion pathways.

In this assay, modification of a secreted protein of interest with a C-terminal tetracysteine motif permits the specific labeling of the secreted protein with biarsenical fluorescent compounds. The outer membrane of *E. coli* is naturally impermeable to these dyes and thus inhibits the labeling of intracellular proteins, resulting in an assay highly selective for secreted proteins. Finally, the authors demonstrated that this strategy was easily adapted to other protein secretion pathways, confirming the universality of the approach.

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