ACS SyntheticBiology

In This Issue

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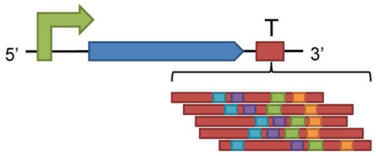
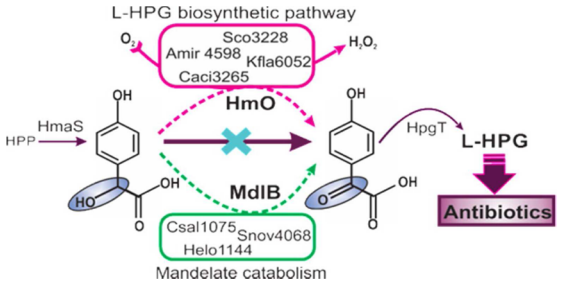
SHORT SYNTHETIC TERMINATORS FOR IMPROVED HETEROLOGOUS GENE EXPRESSION IN YEAST



Terminators are key players in the transcription process and influence net protein output by controlling mRNA half-life. Thus, terminators are important synthetic components considered in applications such as heterologous gene expression and metabolic engineering. Here, Curran *et al.* (DOI: 10.1021/sb5003357) describe a panel of terminators (ranging from 35 to 70bp) that can modulate gene expression in yeast.

The best of these synthetic terminators enabled a 3.7-fold higher net gene expression and 4.4-fold increase in transcript level compared to the common 240bp *CYC1* terminator, demonstrating the ability to create highly functional, yet short, terminator sequences. The authors also show that these terminators are transferrable between diverse yeast species by testing them in both *Saccharomyces cerevisiae* and *Yarrowia lipolytica*. This work expands the synthetic toolset for yeast and presents a movement toward short, minimal synthetic parts.

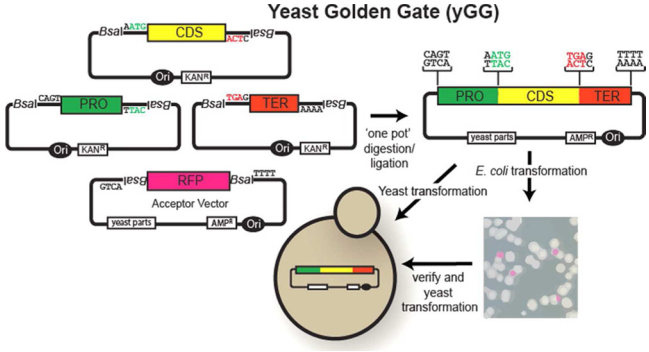
FUNCTIONAL EXCHANGEABILITY OF OXIDASE AND DEHYDROGENASE REACTIONS IN THE BIOSYNTHESIS OF HYDROXYPHENYLGLYCINE



The limited catalytic and expression traits of available enzymes required for synthesizing pharmaceutical compounds is a major bottleneck for the rational engineering of new biosynthetic pathways. Using enzymes from unrelated physiological pathways can be a promising strategy to diversify the toolbox of catalytic mechanisms, cofactors and structural features. In this study, Diez *et al.* (DOI: 10.1021/sb500368w) effectively use this approach to re-engineer the biosynthesis of a nonribosomal peptide, hydroxyphenylglycine, a building block for the calcium-dependent antibiotic of *Streptomyces coelicolor*.

The authors replaced the native hydroxymandelate oxidase (HmO), whose byproduct is the potentially harmful H_2O_2, with heterologous oxidases and catabolic mandelate dehydrogenases involved in mandelate utilization. The biosynthesis of the calcium-dependent antibiotics was effectively restored in the engineered strains, and the kinetic characterization of the isolated enzymes confirmed their divergent roles and mechanisms. The authors demonstrate that heterologous enzymes from different physiological contexts can be used to provide an expanded library of building blocks for the effective engineering of antibiotic production.

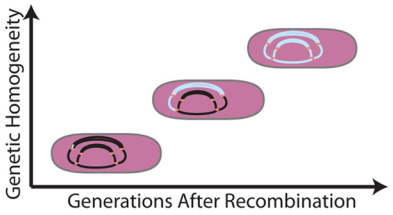
YEAST GOLDEN GATE FOR THE EFFICIENT ASSEMBLY OF YEAST TRANSCRIPTION UNITS



Saccharomyces cerevisiae, or budding yeast, is an important model organism in research laboratories and is also widely used for many industrial applications. The ease with which yeast can be engineered to modulate the expression of native and non-native pathways has important applications in both arenas. Here, Agmon *et al.* (DOI: 10.1021/sb500372z) describe a method called yeast Golden Gate (yGG) to assemble yeast genes, or transcription units (TUs), from three basic subparts: promoters, coding sequences, and terminators.

These TUs may be assembled in a single step by yGG, in a designer acceptor vector intended for direct expression into yeast. To track protein expression, the authors also describe a variant of yGG that allows C-terminal tagging of a TU. yGG provides a new and efficient means by which to engineer *S. cerevisiae* pathways.

QUANTIFYING IMPACT OF CHROMOSOME COPY NUMBER ON RECOMBINATION



Genome engineering requires the precise and efficient recombineering of synthetic DNA, in a quantitative manner, into organisms of interest. Despite considerable efforts toward characterization of recombination in *E. coli*, significant variability in recombination efficiencies persist. In this study, Reynolds and Gill (DOI: 10.1021/sb500338g) describe a mechanism explaining how genetic polymorphism would impact recombination efficiency when measured using a screenable phenotype.

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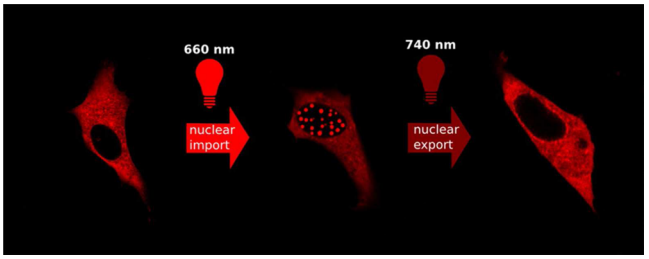
The authors describe the confounding effects multiple chromosomes can have on engineering *E. coli*, and present a model that shows how extended recovery time after recombination procedures can improve the stability of genetic mutations in a cell line. Predictions from the model are also supported with experimental evidence.

RED LIGHT-REGULATED REVERSIBLE NUCLEAR LOCALIZATION OF PROTEINS



Optogenetic strategies for the light-dependent manipulation of cells that employ photoreceptors associating (or dissociating) upon light exposure are widely studied. In addition to several blue-light-sensitive systems, the red-light-responsive plant phytochrome B (PhyB) is often used to control diverse cellular processes. Here, Beyer *et al.* (DOI: 10.1021/acssynbio.5b00004) report the development of a red light-inducible, and far-red light-reversible, synthetic system for controlling nuclear localization of proteins in mammalian cells and zebrafish.

The authors functionally reconstitute the *Arabidopsis* PhyB:phytochrome-interacting factor 3 (PhyB:PIF3) pair in mammalian cell culture and zebrafish to effect red-light-induced nuclear translocation of PhyB and associated cargo. By attaching a nuclear-export signal to PhyB, the authors convert the originally nonreversible system in to a reversible one, such that red light promotes nuclear import and far-red light promotes nuclear export. The authors further demonstrate this PhyB:PIF3 nuclear-import/gene-expression setup in zebrafish, where a luciferase reporter is shown to be regulated by red/far-red light.

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