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In This Issue

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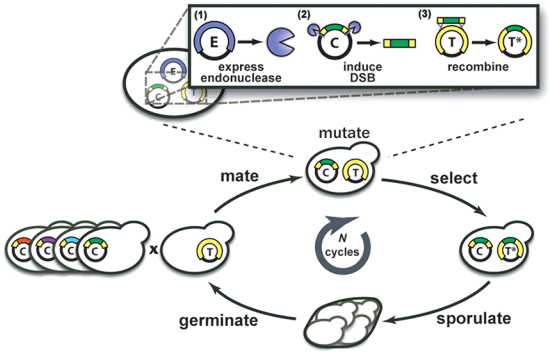
A HERITABLE RECOMBINATION SYSTEM IN YEAST

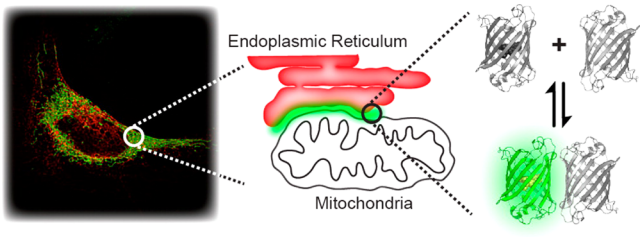
Recent advances in synthetic biology have led to intense interest in reprogramming genes and genomes within the context of the living cell. Recently, the first reports of cutting-edge technologies for mutating genes directly in the cell have appeared in high profile journals. Previous work has demonstrated sequential directed evolution of a target gene in the environment of a living cell; here Romanini et al. (DOI: 10.1021/sb3000904) describe a method that provides a way to combine beneficial mutations that arise in separate cells without resorting to costly and time-consuming sequencing and dedicated manual manipulation of the DNA.



The Heritable Recombination system the authors report here shares with previously described technologies the goal of mutating genes directly in the cell but goes an important step further by building into the mutagenesis technology the ability to readily cross beneficial mutations by exploiting sexual reproduction in yeast. This technology has the potential to be immediately adopted across a range of fields including antibody engineering, metabolic engineering, and systems biology.

DIMERIZATION-DEPENDENT GREEN AND YELLOW FLUORESCENT PROTEINS

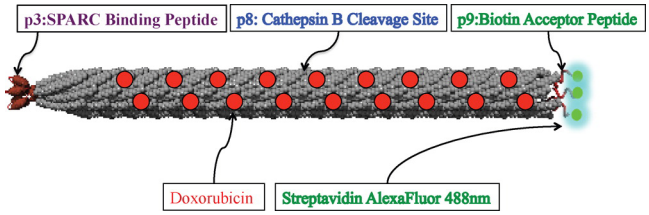
Detection of protein−protein interactions in the intracellular milieu is critical to helping researchers understand, and ultimately manipulate, the genetic circuits of single cells. Here Alford et al. (DOI: 10.1021/sb300050j) use protein engineering to develop fluorescent proteins (FPs) that are dimly fluorescent in their monomeric states and brightly fluorescent when dimerized.



In this work, the authors describe the development of green and yellow dimerization-dependent FPs (ddFPs) that expand the palette of colors beyond their previously reported red ddFP. To demonstrate the usefulness of the new ddFPs, the authors also demonstrate the interaction between two different membrane bound organelles in single cells.

A NEW PLATFORM FOR TUMOR-CELL IMAGING AND DRUG DELIVERY

M13 bacteriophage is a well-characterized tool for peptide display. Peptides are fused directly to phage coat proteins via modification of the phage genome. However, the phage genome contains a number of genetic element overlaps that prevent orthogonal modification of phage gene products. With the goal of producing a phage capable of p9 display and independent manipulation of genes, Ghosh and Kohli et al. (DOI: 10.1021/sb300052u) redesigned the genome of M13 bacteriophage at the gene VII/gene IX junction by removing overlapping gene elements and inserting useful restriction endonuclease sites.



This process, known as genome refactoring, allows for orthogonal modification of gene VII and gene IX while maintaining the viability of the phage. The authors demonstrate the p9 display capabilities of the refactored phage by displaying a biotin acceptor peptide and also show the first application of genome refactoring by recasting the phage as a vector for cancer cell imaging and drug delivery.

ORGANIZATION OF A SYNTHETIC ALPHOID DNA ARRAY IN A HUMAN ARTIFICIAL CHROMOSOME

Despite several advances, mammalian synthetic biology still faces significant challenges, many of which reside in the genome integration of synthetic networks due to the absence of an efficient episomal replication system in mammals. Human artificial chromosomes (HAC s) represent a novel promising episomal system for functional genomics, gene therapy and synthetic biology. Here, Kouprina et al. (DOI: 10.1021/sb3000436) describe, for the first time, the detailed characterization of HAC engineering from alphoid DNA arrays.

In this work, the authors describe organization of a megabase-size synthetic alphoid DNA array in the HAC and also show that the structure and functional domains of the HAC remains unchanged after several rounds of its transfer into different host cells. The knowledge of the HAC structure provides a tool to control HAC integrity during different manipulations. These results also shed light on a mechanism for *de novo* HAC formation in human cells.

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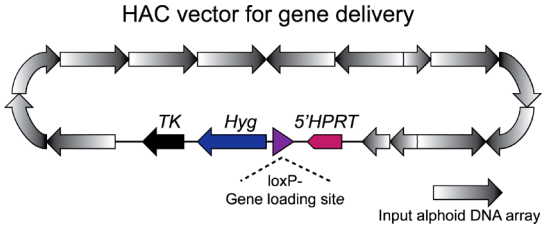
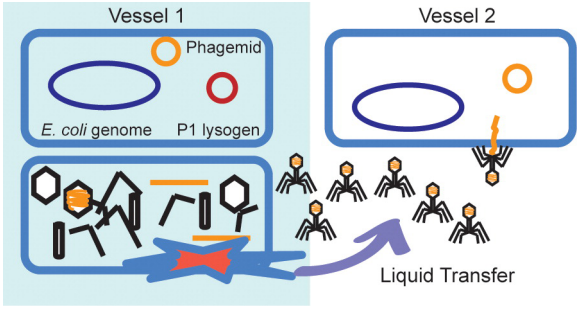


SCALABLE PLASMID TRANSFER USING ENGINEERED PHAGEMIDS

Genetic engineers draw from a diverse toolkit to navigate the engineering design cycle. One important class of tools is biological entities or their derivatives, including both fundamental tools such as restriction enzymes and powerful methods such as phage display and yeast two-hybrid assays. Here, Kittleson et al. (DOI: 10.1021/sb300054p) describe the development of a novel biological tool based on the bacteriophage P1.



This tool operates purely through liquid handling procedures to transfer DNA between *E. coli* cells, eliminating the traditional requirement for vacuum, centrifugation, or temperature shift steps. Importantly, the P1 derived system can transfer large DNAs at low volumes, making it amenable to use with high throughput platforms such as microfluidic devices and acoustic liquid handlers. This new biological tool has the potential for immediate application to the evolution of biosynthetic pathways and may ultimately enable rapid, cheap DNA fabrication.

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