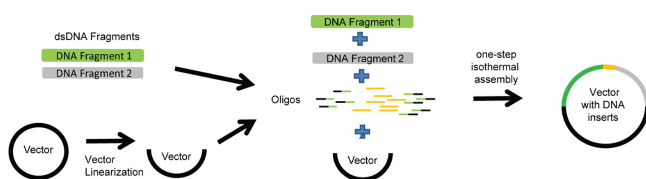
ACS Synthetic Biology
In This Issue
pubs.acs.org/synthbio

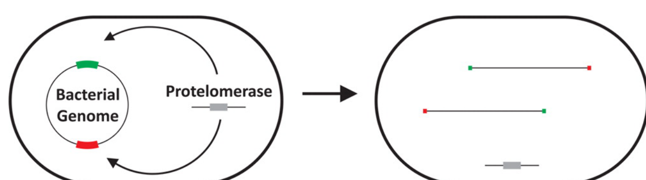
IN SITU OVERLAP AND SEQUENCE SYNTHESIS DURING DNA ASSEMBLY



While cloning methods and DNA synthesis have rapidly evolved in recent years, there remain two drawbacks with current techniques: almost all cloning techniques require overhangs to be introduced during a PCR reaction and the introduction of medium-sized *de novo* DNA sequences (90–300 base pairs) during cloning is either expensive or requires multiple steps. In this Technical Note, Paetzold *et al.* (DOI: 10.1021/sb400067v) report a novel DNA assembly strategy based on the previously described one-step isothermal assembly.

The authors describe a strategy that allows the *in situ* synthesis of overhangs from single stranded DNA oligonucleotides during DNA assembly, bypassing PCR. It further allows the one step, simultaneous synthesis of medium sized DNA stretches during double stranded DNA assembly. This new strategy promises to be useful both for individual researchers needing to introduce medium sized *de novo* DNA stretches in complex DNA assemblies such as promoters, tags, fusions, or customized RBS sites and for laboratories doing high-throughput cloning.

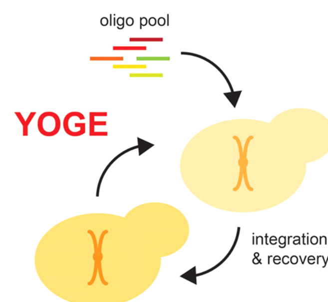
E. COLI WITH TWO LINEAR CHROMOSOMES



A major limitation in the field of synthetic biology is the DNA maximum assembly size imposed by the currently available technologies. This constraint complicates the *de novo* synthesis of most bacterial genomes, including that of *E. coli*. Here, Liang *et al.* (DOI: 10.1021/sb400079u) present a proof of concept, setting the basis for a genome engineering process that would allow the assembly and propagation of bacterial genomes composed of multiple individual smaller chromosomes.

The authors used a bacteriophage pro-telomerase that site-specifically cleaved the genome at 2 edited chromosome regions, and report the *in vivo* fragmentation of the *E. coli* circular genome. This resulted in a viable bacterial strain harboring 2 linear chromosomes. These findings provide a new tool to study and understand the replication and segregation of bacterial genomes.

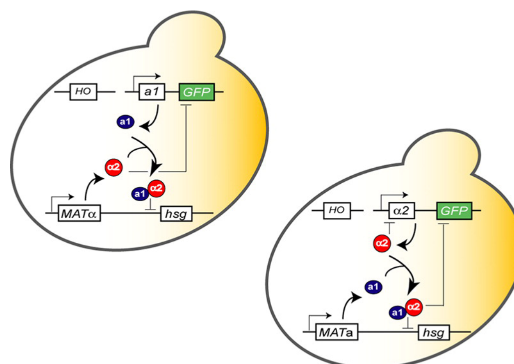
YEAST OLIGO-MEDIATED GENOME ENGINEERING



High-frequency oligonucleotide-directed recombination engineering, or recombineering, has enabled the rapid modification of several prokaryotic genomes to date. Here, DiCarlo *et al.* (DOI: 10.1021/sb400117c) describe the development of an efficient recombination engineering technique in *Saccharomyces cerevisiae*.

Using overexpression of endogenous genes and mismatch repair knockouts, the authors demonstrate a method that can be cycled for generations of targeted modifications at screenable frequencies. This method is of significant interest for yeast strain engineers aiming to make precise mutations for directed strain evolution, pathway tuning, genome functional element interrogation, and strain diversity generation.

ARTIFICIAL CONVERSION OF MATING-TYPE WITHOUT AUTOPOLYPLOIDIZATION



In conventional methods for yeast mating-type conversion, the two different yeast mating-types generated from a parental strain can mate with each other in a process referred to as autopolyploidization. This, however, makes it difficult to isolate target cells of the desired mating-type and impedes subsequent crossbreeding for yeast genetic engineering purposes. Here, Fukuda *et al.* (DOI: 10.1021/sb400016j) report that the artificial expression of mating-type-dependent transcriptional regulators prevents autopolyploidization.

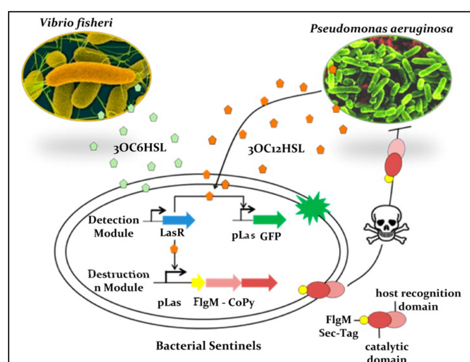
The authors successfully use MATa and MATα haploid cells, as the parental strains, in a mating-type conversion approach. This method holds the potential to generate yeast mating strains, with all the attractive traits from the parental strains,

Received: November 22, 2013
Published: December 20, 2013

ACS Publications © 2013 American Chemical Society

thus enabling production of favorable yeast strains useful for a wide range of applications.

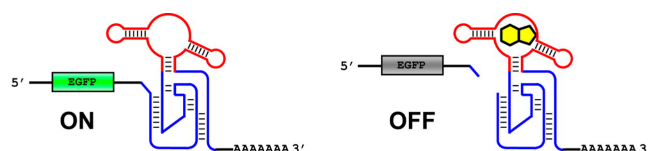
GENETICALLY PROGRAMMABLE PATHOGEN SENSE AND DESTROY



Since its inception, the field of synthetic biology has been rapidly growing, generating methodologies that have made possible the engineering of complex synthetic gene networks in bacteria. This new engineering framework has been harnessed to facilitate the creation of novel cellular functions and behaviors, in an effort to address real-world medical and environmental challenges. One such challenge is the emergence of drug resistant pathogenic bacteria. As a way to circumvent this, Gupta *et al.* (DOI: 10.1021/sb4000417), now describe an engineered bacterial system that specifically detects and destroys pathogenic bacteria in its environment.

The authors used engineered *E. coli* cells as sentinels that detect the quorum sensing molecule 3OC12HSL, produced exclusively by the pathogen *Pseudomonas aeruginosa*. Upon detection, these sentinel cells respond by secreting a potent and specific anti *P. aeruginosa* bacteriocin into their surroundings, leading to the complete eradication of *P. aeruginosa* cells. This new bacterial system provides a potential cell therapy approach against *P. aeruginosa* and has several advantages over previously reported synthetic biology-driven antimicrobial strategies.

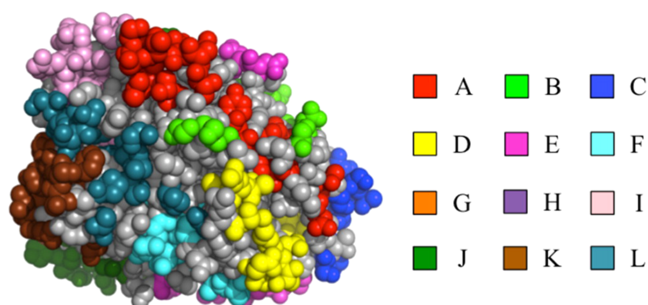
CONTROLLING MAMMALIAN GENE EXPRESSION



Self-cleaving ribozymes engineered to be responsive to various molecules are promising tools for controlling gene expression in living cells from bacteria to mammalian cells. However, the predominantly used ribozyme, the hammerhead ribozyme, provides only modest dynamic ranges of gene regulation. Now, Nomura *et al.* (DOI: 10.1021/sb400037a) report on engineered ribozymes that respond to two small molecules.

Focusing on a class of ribozymes that was first found in the hepatitis delta virus (HDV), the authors saw that one of the engineered ribozymes exhibited an impressive dynamic range of ~30-fold change in gene expression. They further demonstrated a NOR logic gate device based on the tandem fusion of two engineered ribozymes.

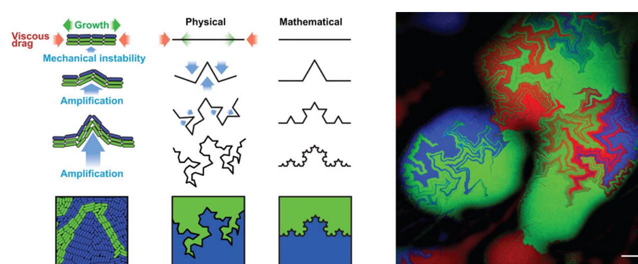
NONCONTIGUOUS RECOMBINATION TO IDENTIFY STABILIZING MUTATIONS



Noncontiguous recombination (NCR) is a method to identify structural elements that can be shuffled among homologous proteins to create new, chimeric proteins. Here, Smith *et al.* (DOI: 10.1021/sb400010m), using NCR, identify six stabilizing point mutations, including a previously undiscovered mutation that improves the thermostability of the industrially important *H. jecorina* cellobiohydrolase I (CBHI) by 3 °C.

The authors split the CBHI structure into a relatively large number of equally sized blocks and swapped these structural elements with stable homologues. They demonstrate that this is an efficient strategy to search for stabilizing point mutations, which also offers a new and useful approach to engineering stable proteins.

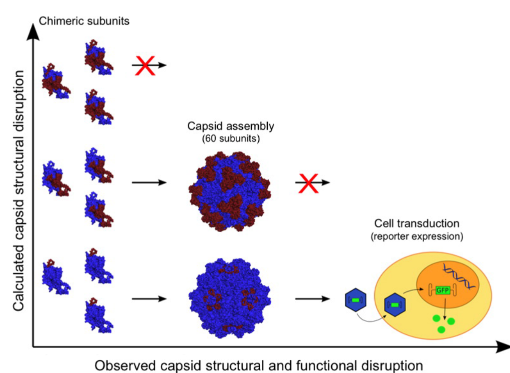
FRACTAL PATTERNING OF CELL LAYERS BY CELL POLARITY-DRIVEN INSTABILITY



In order to understand the formation of medically important biofilms as well as to engineer new biofilms in biotechnology, one has to understand the way cell populations interact during the early formation of these biofilms. Here, Rudge *et al.* (DOI: 10.1021/sb400030p) describe an encapsulated experimental system that can explicitly capture the physics, cellularity, and genetics of a simple system, and which allows a new type of "emergence in a test tube" approach.

Using high resolution imaging techniques, the authors saw that boundaries between growing bacterial cell populations have a fractal-like appearance. They then used large-scale computer simulations to confirm that these fractal-like patterns emerge from local physical interactions between cells and, finally, used a specialized microbial system to explore emergent behavior in large cell populations.

SCHEMA COMPUTATIONAL DESIGN OF VIRUS CAPSID CHIMERAS



Recombination is one mechanism by which genetic variability is introduced into viruses during evolution. Evolution, in turn, can be used to create viruses with properties that do not exist in nature. Adeno-associated virus (AAV) is a promising gene delivery vector for human gene therapy. In this paper, Ho *et al.* (DOI: 10.1021/sb400076r) examine the extent to which SCHEMA predictions correlate with structural disruption in AAV capsid chimeras created through recombination.

The authors provide the first experimental evidence that SCHEMA calculated disruption is inversely correlated with chimeric capsid transduction and nuclease protection of genomes, indicating that this algorithm will be useful for creating libraries enriched in chimeras that form viable capsids.