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Build-a-Phage Lab Manual
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Phage Building Block (500-800 bp) synthesis
We performed proof-of-principle experiments by designing, building, and testing a version of gene 68 from Mycobacteriophage Giles which is codon optimized for the host species, *Mycobacterium smegmatis*.

1. Redesign synthetic genome
   Codon optimize the gene for host species; redesign regions with high GC content; introduce PCR Tags (watermarks) to identify synthetic DNA

2. Oligo design
   Break up desired sequence into overlapping oligos

3. Building block synthesis
   Assemble oligos into 500-800 bp Building Blocks

4. Ligation and transformation
   Clone Building Block in bacteria and perform blue-white screening

5. Colony screening PCR
   Screen bacterial Building Block clones for size

6. DNA sequence analysis
   Screen bacterial clones for sequence

7. BRED (Bacteriophage Recombination)
   Co-electroporate synthetic Building Block and WT phage DNA into host cells where recombination occurs

8. Screen plaques
   Use PCR Tags to identify plaques that contain synthetic DNA

9. Determine if synthetic phage is viable
   Plaques containing synthetic DNA are diluted and replated. If synthetic phage are viable, can obtain pure plaques containing only synthetic DNA
Module Description:

This resource is a lab protocols manual for the "Build-a-Phage" workflow. This is one of the three workflows for Build-a-Genome, a course-based undergraduate research experience (CURE) in which students edit and manipulate genomes to learn about genome structure and function. This course, which was designed to align with many of the goals of Vision and Change, allows students to conduct authentic, interdisciplinary research in the cutting edge field of synthetic biology by designing, building, and testing the function of a selected genome. The manual includes protocols for an initial boot-camp phase (“Building Block assembly”) in which students learn the techniques by assembling a model gene such as green or red fluorescent protein. Students then proceed to use the same techniques for the assembly of semi-synthetic phage genomes. Student assignments and a materials supply list are also included.

Teaching Setting:

The "Build-a-Phage" course includes topics and lab techniques from genomics, bioinformatics, molecular biology, and synthetic biology and would therefore be appropriate for use in a variety of undergraduate biology laboratory courses.

Citation:

Related Materials and Opportunities:

This resource was created by the Build-a-Genome (BAG) Network. The BAG Network is a Research Coordination Network in Undergraduate Biology Education (RCN-UBE) that evolved from the Build-a-Genome (BAG) course initiated at Johns Hopkins University by Dr. Jef Boeke. The BAG course integrated synthetic biology into the undergraduate curriculum and contributed significantly to the Synthetic Yeast Project, an international effort to create the first synthetic eukaryotic chromosome. The BAG Network has expanded beyond yeast chromosome synthesis to develop workflows for bacteriophage genome synthesis (featured in this ROW), yeast neochromosome synthesis and programmed genome rearrangement in yeast.

The BAG Network now seeks to expand synthetic biology education and faculty training to new and diverse institutions. The BAG Network is offering their annual workshop from August 15-18, 2019 at Loyola University Maryland, Baltimore, MD. The goals of the workshop are:

- To introduce faculty to the field of synthetic biology (and specifically synthetic genomics)
- To provide faculty with the resources and tools to be able to implement the Build-a-Genome course and workflow at their home institutions
- To bring together diverse experts to share their knowledge of the field and their experiences with the course and its implementation.

A draft conference program is available. Applications will be accepted until workshop fills.

If you are interested in learning about additional upcoming BAG Network activities and opportunities, please join the BAG Network group.
If you adopt and adapt this module, you are highly encouraged to share your adaptation back with the QUBES community using the QUBES Resources System for sharing Open Education Resources.