

# The Build-a-Genome Network: a Course-Based Undergraduate Research Experience for the Design and Modular Assembly of Bacteriophage Genomes

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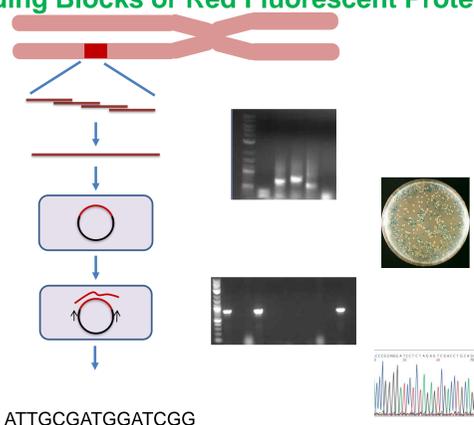
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## The Build-a-Genome Course

The Build-a-Genome course was initiated at Johns Hopkins University in 2007. It engages undergraduate students in the construction of synthetic DNA which is hierarchically assembled into larger pieces. These synthetic DNA fragments are incorporated into the Synthetic Yeast genome (Pennisi, *Science* 2014 343:1426). The Build-a-Genome network supports the “franchising” of the course to both research intensive and primarily undergraduate institutions. It supports three research workflows: Synthesis of yeast building blocks and Red Fluorescent Protein, SCRaMble (Synthetic Chromosome Recombination and Modification by LoxP-mediated Evolution), and Synthesis of phage genes or building blocks.

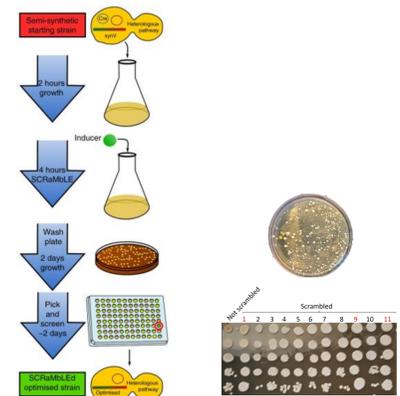
### Workflow 1: Yeast Building Blocks or Red Fluorescent Protein

- Oligo design**  
Break up desired sequence into overlapping oligos
- Building block synthesis**  
Assemble oligos into ~700 bp Building Block
- Ligation and transformation**  
Clone Building Block in bacteria. For yeast building blocks, blue-white screening is performed. For red fluorescent protein, transformation into an expression vector produces pink colonies.
- Colony screening PCR**  
Screen bacterial clones for size
- DNA sequence analysis**  
Screen bacterial clones for sequence



### Workflow 2: Synthetic Chromosome Recombination and Modification by LoxP-mediated Evolution (SCRaMble)

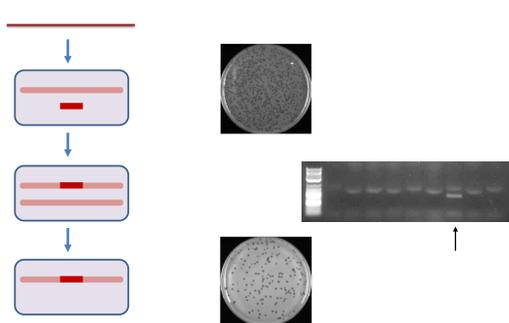
- SCRaMble uses a semi-synthetic strain  
In BAG, we use *Saccharomyces cerevisiae* 2.0 containing synthetic chromosomes V and X
- Cells are grown to log phase
- Induction of Cre recombinase  
A Cre-EBD fusion protein is induced in cells with 1 μM β-estradiol
- Cells are plated on selective media. Students have used 7% ethanol, 2 mg/ml caffeine, and varied concentrations of acidity, fluconazole and amphotericin. Many cells grow poorly, but some show enhanced growth under selective conditions.
- Enhanced growth is verified.  
Serial dilutions of equivalent numbers of yeast cells are performed and plated to verify enhanced growth under selective conditions.



### Workflow 3: Build-a-Phage (Phage Building Block synthesis)

We performed proof-of-principle experiments by designing, building, and testing a version of gene B8 from *Mycobacteriophage* Giles codon optimized for the host species, *Mycobacterium smegmatis*.

- Steps 1-5. Synthesis of phage building block**  
Same steps as above for synthesis, cloning, screening, and sequence analysis of yeast building blocks.
- BRED (Bacteriophage Recombination)**  
Co-electroporate synthetic Building Block and WT phage DNA into host cells where recombination occurs
- Screen plaques**  
Use PCR Tags to identify phage that contain synthetic DNA
- 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



## Abstract

The Build-a-Genome network seeks to integrate the teaching of synthetic genomics into undergraduate curricula. The network provides professional development, access to shared resources, and standardized workflows for the synthesis of designer yeast chromosomes, yeast neochromosomes containing metabolic pathways, and bacteriophage genes.

We now describe expansion of this last workflow to include synthesis and cloning of a semi-synthetic phage genome. *Mycobacteriophage* Giles is a 53kb temperate phage for which half of its proteins remain uncharacterized. To enable understanding of gene structure and function, we have chosen to clone the Giles genome so that it can serve as a template for students to create semi-synthetic phages. Transformation-associated recombination (TAR) was used to clone the genome as 11 individual fragments in yeast, and these are joined into a complete genome using a second round of TAR.

By cloning the 55kb wild-type phage genome as 11 modular fragments, we now enable assembly of wild-type and synthetic fragments in various combinations. Undergraduate students can therefore design and construct synthetic fragments with targeted genomic changes and combine those fragments with the remaining wild-type sequences by TAR cloning in yeast to generate full phage genomes whose infectivity and properties can be assayed. We expect that this workflow would be especially attractive to schools in the HHMI SEA-PHAGES phage hunting program.

## The Build-a-Genome Network

Given the success of the Build-a-Genome course at Johns Hopkins, we wanted to expand the number of students who are able to participate in Build-a-Genome by forming a network of diverse institutions which would offer the course.

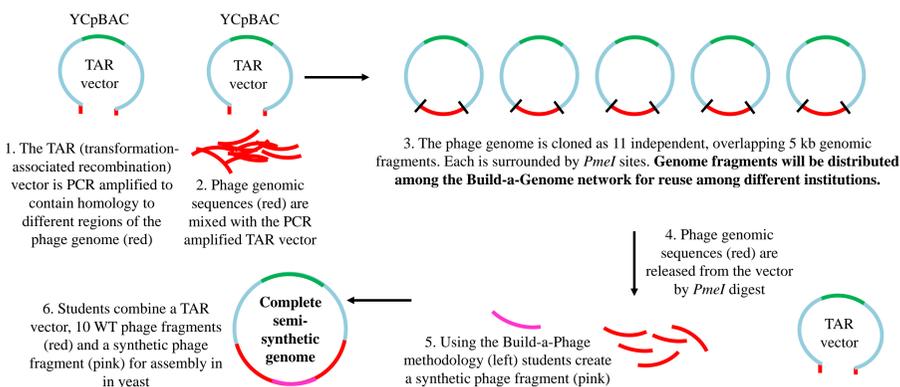
### Goals of the network

To expand the opportunities for undergraduates to engage in synthetic biology and synthetic genomics research and education

## Expansion of Build-a-Phage to include cloning of complete phage genomes

### Benefits of synthetic genomics using phages:

- Medical relevance is inherently interesting to students.** *Mycobacteriophages* are a diverse group of viruses. While we are working with *Mycobacterium smegmatis*, a harmless soil bacterium, the genus includes the human pathogen *Mycobacterium tuberculosis*.
- Students can participate in all aspects of the synthetic biology process (Design, Build, Test).** In Build-a-Phage can engage in hypothesis-driven design of phage genomes and then both build and test the altered sequences.
- There are many sequenced genomes that can serve as the basis for genome synthesis.** *Mycobacteriophages* have been isolated and sequenced through the University of Pittsburgh's PHIRE and HHMI's SEA-Phages programs. Little is known about the function of many genes or the genome organization.



## Acknowledgements and Contact Information

- The authors gratefully acknowledge the support and contributions of Jef Boeke, Joel Bader, Karen Zeller, Zhuwei Xu, and Graham Hatfull.
- This work was supported by a 5-year NSF RCN-UBE grant #1827204
- For more information about the Build-a-Genome Network please contact Lisa Scheifele [lscheifele@loyola.edu](mailto:lscheifele@loyola.edu)



To attend a workshop about Build-a-Genome, to obtain teaching materials about synthetic biology and synthetic genomics, and to obtain laboratory protocols and resources, please visit the Build-a-Genome Network site at <https://qubeshub.org/community/groups/bag> (login to see all resources)

## Build-a-Genome affects students' skills and attitudes

### Affect on students' ability to conduct research

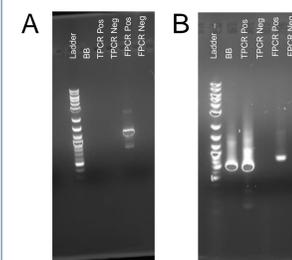


Figure 1. Example gel images of student troubleshooting. Electrophoresis gels (A and B) contain a complete templateless and finish PCR reaction for the building block (BB), positive (TPCR Pos) and negative (TPCR Neg) controls included with templateless PCR, and positive (TPCR Pos) and negative (TPCR Neg) controls included with finish PCR. The gel in panel A was completed during the first two weeks. Students then completed a “PCR Troubleshooting” worksheet and then repeated the assembly and ran another gel (B) using modified reaction conditions (in this case, properly keeping reagents on ice and using Phusion HotStart enzyme instead of Herculase).

### How did this lab reflect real research?

- “We tackled the problems one day at a time and had to think very hard every step of the way, constantly problem solving.”
- “We didn't know what to expect or if what we were doing would work. We used basic techniques to find something new.”
- “It's about what I expected...disappointment of failure affected me more, but it made the success that much better.”
- “Involves learning from mistakes”
- “Previously unaware how research is a large collaborative effort”
- “It did not go as smoothly/quickly as I thought research was”

### Student gains in understanding the research process

#### Student gains on pre-class and post-class survey of student attitudes towards science and research

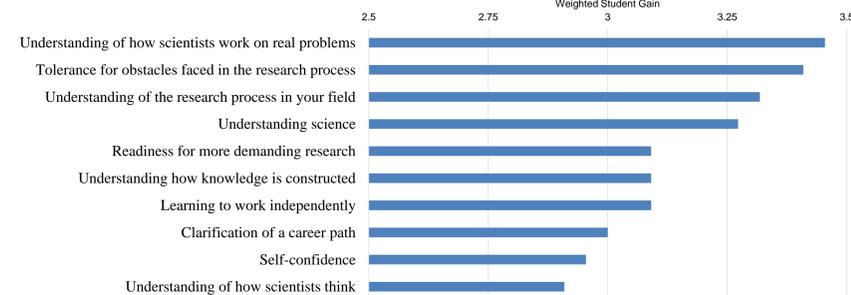


Figure 2. Student attitudes towards science careers and research was assessed using the CURE survey (Lopatto 2008). Pre-course and post-course scores were compared, and students showed the largest increases in understanding the process of research, understanding science, and preparation for more demanding research and tolerance to research obstacles.

### Student gains in attitudes towards science

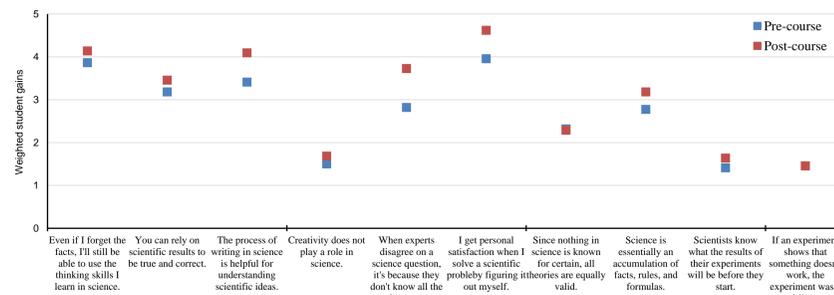


Figure 3. Student attitudes towards science and the process of science was assessed using the CURE survey (Lopatto 2008). Pre-course and post-course scores were compared, and students showed the largest increases in appreciating the thought processes learned in science, appreciating the role of creativity in science, and being able to discriminate between the validity of different theories.

### Effectiveness of the course

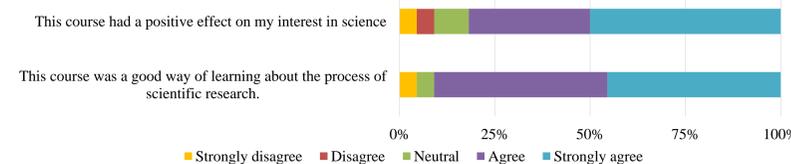


Figure 4. Student opinion about the effectiveness of the course was assessed using the CURE survey (Lopatto 2008). The post-course revealed that a strong majority agreed or strongly agreed that the course had a positive effect on their interest in science (82%) and was an effective way to learn about the process of scientific research (91%).