Abstract
The Johns Hopkins team presents the work of a Build-a-Genome course that fabricates synthetic yeast genome S2C2 and provides students tools to edit and assemble arrays of DNA into redesigned synthetic chromosomes. This was done through:
- Removal of introns, transposons and other repeats
- INRA gene replication (hot spots for instability)
- loxP site introduction to allow reshuffling
- We are part of a larger effort to develop new technologies and standards for synthetic genomic construction allowing for production of longer, more complicated DNA sequences without certain constraints of current BioBrick standards. We used the following assembly strategy to create pieces of chromosomes and finally full chromosomes, efficiently and cheaply:
- Two-stage overlap assembly PCR: 1-PCR-2-PCR
- Unial Specific Excision Reaction (USER)
- Homologous recombination
Here we present improved methodology for building block synthesis, the software created to aid in our synthesis, and applications of the yeast genome redesign, focusing on the implications the Build-a-Genome course has on future genomic technologies that both rely on and teach students.

Why Synthetic Yeast?
Yeast
- Yeast is an aneukaryote, with fast reproduction, and few introns, it’s very safe.
- It has a completely mapped and very well annotated genome.
- Many homologous proteins are found in humans.
- Therefore, it is a simple model for human biology.

Synthetic Yeast
- We will be the first fully synthetic aneukaryotic genome through removal of junk DNA and incorporation of in vitro expressed genes.
- Long-term projects include genome scrambling/shuffling.
- We address a fundamental question: how do genomes function?
- We have created controllable units that can easily map gene relationships and reveal hidden roles of genomic structure.
- We will create BioBrick chassis for future yeast engineering solutions to the problems of today’s world.

Overall Accomplishments
- We have submitted 87 Building Blocks and have ~300 more that can be submitted. They have been sequenced and have been characterized appropriately. For example, many have been assembled into longer segments and we now have a ~50 kb-3L assembly “in yeast”.
- We have designed/implemented 4 programs that can aid in geometrically complex assemblies.
- We have proposed a new standard that will create long seamless DNA sequence that do not exhibit scarring. We obtained an rLEM variance for the new standard.

Project 2: Build a Genome Standard - RFC38
The physical assembly of needed parts is currently a non-automated process, which can either come from direct genome PCR with restriction enzyme sites incorporated into the PCR primers, or overlap assembly PCR. Current BioBrick construction standards require an extensive enzyme based method, which can be time consuming and expensive.
Our method has the following advantages:
- In vitro compatible and non-automated format, based on the desired use of ORFs
- No restriction enzymes which many of these processes are sensitive to and work with any sequence
- Multi-fragment assembly of large DNA strands in one shot

Project 3: Genome Stabilization
- INRA genes relocated into INRA array, orome chromosome, and reduplicated.
- Reduce genomic changes caused by DNA breaks due to “collisions” between RNA pol III and DNA polymerase in replication (stalled forks)
- Reduce background homologous recombination (repetitive removals)

Project 4: Genome Shuffling
Cre – LoxPyrm System
- Cre recombinase is used to recombine pairs of LoxPyrm sites.
- Successfully synthesized and mutated synthetic chromosome arm “ASH” into yeast – IT WORKS!!!
- Modified as to be regulated by SEX hormone estradiol (Cre-EBD)
- Use this system to create permutations.
- Genes of surviving colonies can be analyzed.
- Cre - loxPyrm system works adding extra kills -90% of cells and survivors show phenotype diversity.
- We are testing the Cre - loxPyrm system using the FLO8 gene which results in a “fuzzy” yeast phenotype.

Safety of our Work (Answers to questions)
1. Aflatoxin is strong – 0/5.0 (mostly logisitic or safe). No serious safety concerns.
2. No JHU Institutional Review Board Committee.
3. State yeast is "Novel" from mammalian DNA regulation, no serious safety concerns. T.E.C.
4. No
Safety Officers: James & Tidyard

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For further information, visit the Synthetic Yeast wiki:
www.syntheticyeast.org

Build A Genome
Making a Synthetic Organism
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