ChromoBricks: A Platform for Chromosome Engineering with BioBricks

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Objective

- Why engineer chromosomes?
  - Stable maintenance
  - Precise copy number
  - Unlimited size
Current Methods of Chromosome Engineering

Homologous recombination

Diagram showing before and after chromosome recombination.
Currently no standard system in iGEM for integrating DNA into the chromosome

Our goal: Stable and efficient integration system

Solution: φC31 integrase enzyme
Design

Landing Pad Strain:
- Landing pad
  - Target for integration of BioBrick construct
- Integrase expression plasmid
  - Facilitates recombination

Donor Strain:
- BioBrick donor plasmid
  - BBa construct exchanged with landing pad
Integrases recognizes attachment-B and attachment-P sites and exchanges DNA between them, maintaining orientation.
- *attP* sites for integrase recognition

- Selection markers
  - *sacB* – sucrase sensitivity
  - kanamycin resistance
  - *rfp*
- $\phi C_{31}$ integrase
  - Cloned behind constitutive promoter
  - Chloramphenicol resistant

- Introduced into the landing pad strain (LPS)
Intermediate vector for target BBa
  - Flanked by attB sites and two OriT sites
  - OriT sites ensure the BBa construct and surrounding attB sites are transferred
Integrate Mechanism

- Core dinucleonutide overhangs are created at recognized \textit{att} sites
- Marker gene segment and BioBrick construct are exchanged
- \textit{attB} and \textit{attP} sites become \textit{attR} and \textit{attL} sites, not reused
Multiple ChromoBricks

- $attP$ and $attB$ sites with six different core dinucleotides
- $attP$ not cross-reactive when embedded within a BBa construct on a donor plasmid with $attB$ sites
- allows continual integration of constructs and devices into the chromosome
- effect of $attL$ and $attR$ sites’ accumulation on Integrase activity can be modelled
To eliminate garbage, one first has to simulate the system.

Therefore, the primary objective of our software was to model integrase ($\Phi C_{31}$) mediated DNA rearrangement.
Introduction

- Programmed in Python
- Assumed $10^7$ DNA strands at steady state
- Difficult to simulate steady state
- Insufficient resources to simulate to steady state
- Indeterminate whether it reaches steady state at all
The Process in a Picture

DNA “Bag”
- Strands are taken from the DNA bag to be reacted.
- The new products are added to the DNA bag.

Strands are tokenized.
- Each attP site is reacted against each attB site.
- *This eliminates duplicates hidden by DNA circularity.

Tokenized strings are converted to canonical* form.
- Sequence of Interest
- att sites are located.
Comprised of Strings

- DNA is easily represented by strings
- ASCII encoding has 256 states per character
- Reverse compliments halve that: $256/2=128$ states
- Reserve states for the origin, att sites, and nucleotides (ATCG)
Canonicalization

- Canonicalize:
  - Convert multiple equivalent representations into a single one
- Look for the best starting element
- Compare the adjacent elements to determine “correct” direction
Canonicalization

- BTTDYOPTTQX
- xqAApgydAAb

- “O” is the greatest element in both representations, so use first representation.
- Next to “O” is “Y” and “P”
- “P” is greater than “Y”, so we move in that direction

BTTDYOPTTQX
OPTTQXBTTTDY
Filters remove DNA molecules that would be selected against
- e.g., loss of plasmid origin, lack of markers
- Different categories
  - Frequent
  - Moderate
  - Infrequent
**Runtime**

- DNA to react with itself and every other DNA molecule in the bag-o-DNA.
- Runtime of $n^n$... so it’s $\Omega(n^n)$
  - This lower bound is optimistic.
- Hoped that filtering would remove enough DNA so that the system would reach a steady state.
Modeling Lessons

- As proof on concept, it works
  - Not possible to simulate to steady state
- A non-brute force algorithm to solve the problem may exist
  - We don’t know it
Chromosome Re-engineering

- Use ChromoBricks to build new chromosome in native E.coli genome
- Specific att-sites facilitate separation of two genomes
- Nucleases used to degrade native genome
- Synthetic genome remains undigested & induced
Nuclease Construct:

- Reverse promoter: anti-sense transcript for repression
- CI repressor protein: ensures high concentration of CI
- Remains repressed until IPTG removed from media
Assembly of interchangeable parts on chromosomal scale

- Apply BioBrick paradigm for chromosomal re-engineering

- Ability to create designer cells with specific features
  - Minimal/simple cell, makes product efficiently
Results

- Chromosome degradation construct completed and added to the registry
- Landing pad construct completed
- Five variable dinucleotide *att*P sites added to the registry
Those who are excited about synthetic biology research are those who are aware of it.

More of those who are aware of synthetic biology believe that benefits outweigh risks.

Nine out of ten adults feel that more should be done to inform the public about synthetic biology research.

In a study of 1,001 adults:
Our Approach

- Our goal is to educate and excite the public about synthetic biology such that our efforts will contribute to the development of a community that is scientifically literate.

- We will achieve this goal by engaging in outreach and fostering a regional synthetic biology community.
Goal: increase science communication between synthetic biologists and iGEM teams

These groups are important for fostering development and education with stakeholders

- Industry
- Students
- General public
Outreach

Goals

- Inform the public about synthetic biology
- Highlight exciting opportunities in science education & research
- Broaden the influence of iGEM
- Showcase opportunities in the field of science
- Create an enriched science experience

Audiences

- Young students (elementary school)
- Science students (high school & undergraduate)
- General Public (non-science background)
- General Public (science background)

Engineering Science Quest
Conclusions & Future Work

What we accomplished:

- Excited young minds about science
- Developed interactive activities and displays
- Gave multiple audiences the foundational tools to understand future scientific developments
- Gained insight about what the public believes about modern science
- Increased communication between local synthetic biologists
- Communicated the work of iGEM projects
- Strengthened our regional synthetic biology community
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