**Abstract**
The aim of our project is to develop a fully featured platform for chromosome engineering, allowing the in vivo assembly of a synthetic chromosome from interchangeable parts, followed by selective degradation of the native chromosome. We have designed a proof-of-concept for chromosome building that will use the site-specific integrase of phage C31 to integrate a BioBrick into a defined locus of the E. coli genome.

**Goals**
- Move large BioBrick constructs from plasmids to the chromosome
- Allow repeatable insertion of BioBrick constructs into the chromosome
- Provide BioBrick constructs with stable copy number and selection-free maintenance
- Use tools that can be extended to use in other organisms

**Cassette Exchange**
Traditionally, DNA has been introduced into bacterial genomes using homologous recombination, or other recombination systems, like cre/lox, some of which are not stable. The C31 integrase from Streptomyces phage C31 is directional, stable, and patent-free, ideal for use in a standardized DNA integration system.

The goal of this aspect of the project was to develop a system capable of integrating a BioBrick into the chromosome of E. coli at a defined locus.

**landing pad strain**
The landing pad strain provides a chromosomal substrate for integrase-mediated cassette exchange. This strain need only be constructed once, and can then be used whenever integration into the chromosome is desired.
- The landing pad module consists of selectable markers (counter-selectable sacB and selectable kanamycin resistance) flanked by attP recombination sites.
- After cassette exchange, the markers will be lost, providing easy selection and screening.

**integration mechanism**
The ΦC31 integrase catalyzes a unidirectional strand-exchange between a 39 bp attP site and a 34 bp attB site.

**Chromosome Degradation**
The original aim of this part of the project was to engineer a genome-free, cell-based expression system capable of producing a desired protein or activating a pathway in response to an environmental signal.
- Genome degradation system uses Pmel exonuclease and T7 exonuclease.
- Coupled with chromosome engineering, this system could be used to build a new chromosome and degrade the old one.

**Modelling**
Our goal was to discover a sequence of att sites and markers that would allow predictable, repeatable insertion into a chromosome.
- Chromosome and plasmid loops are represented as strings of tokens where compression is achieved by only representing functional elements such as att sites and their operators.
- A brute force computational method was used to search for these sequences, with several filters and constraints to both reduce dimensionality and increase biological accuracy of the model.

**Human Practices**
Through educational outreach, we informed the public about synthetic biology, promoted education in science, and broadened the influence of iGEM.

**ontario team gathering**
Members from teams at the University of Toronto, Queen’s University, the University of Guelph, the University of Toronto at Mississauga and the University of Ottawa came to the University of Waterloo to exchange ideas and approaches to running an iGEM team. Andrew Hessel spoke about synthetic biology as a disruptive technology, and Mads Kæm equipped the talk on running an iGEM team.