Introduction
The flow of genetic information from DNA to RNA to protein is the commonly accepted dogma of molecular biology. Experimental results, however, are not always consistent with the central dogma. The goal of this project is to test the validity of several aspects of the central dogma, including bidirectional promoters, multiple start codons, and codon optimization.

Bidirectional Promoters

**Objective**
- What direction does a promoter promote in?
- How biased is a promoter in each direction?
- How do palindromic promoters affect directionality?

**Design**

**Method**
- Circuit was generated using Gibson Assembly
- Promoters were ligated between reporters
- Promoter strength and directionality correspond to measured fluorescence

**Results**

- All fluorescence was measured using a TECAN1000
- Fluorescence data was approximately normalized using the K constant calculated for bidirectional promoters
- The downstream start codon coding for sfGFP is preferred
- The downstream start codon was also recognized and translated at a lower rate

Multiple Start Codons

**Objective**
- What happens if there are multiple start codons on a single mRNA strand?
- Which start codon is selected for the initiation of protein synthesis?

**Design**

**Method**
- Circuit was generated using Gibson and PCR assembly
- Design allows for uninhibited protein translation through both ORFs
- Each start codon corresponds to a fluorescent protein in its reading frame

**Results**

- All fluorescence was measured using a TECAN1000
- Fluorescence data was approximately normalized using the K constant calculated for bidirectional promoters
- The downstream start codon coding for sfGFP is preferred
- The downstream start codon was also recognized and translated at a lower rate

Codon Optimization

**Objective**
- How does codon bias affect protein translation rate?
- What is the optimized codon for threonine?

**Design**

**Method**
- Circuit was generated using PCR assembly
- Repeated sequences were designed and inserted
- mCherry is the repeat-sequence reporter
- GFP is the control reporter

**Results**

- RFP variations correspond to level of codon optimization
- ACG is the optimized codon for threonine in DH10B E.coli
- Constant levels of GFP demonstrate that enough charged tRNA is available to continue protein synthesis in the cell
- Designed and implemented a genetic circuit which tests codon optimization, using threonine as an example

Human Practices

Penn State iGEM visited a local high school and presented the background, design, data, and results from this year’s project. Students were taught the basic terminology and techniques of synthetic biology.

A publicly accessible animation created by a team member was uploaded to YouTube, in addition to each project description presentation shown to the students.