Introduction

As the field of synthetic biology grows, so should its toolkit. This year, the Lethbridge iGEM team worked to design a whole new class of parts for the iGEM community: programmable ribosomal frameshifting elements. To do this, we have characterized the PK401 pseudoknot, derived from infectious bronchitis virus, for use within the Bodurka system. Pseudoknots and RNA secondary structural elements that can cause the ribosome to switch between translational frames, giving an additional degree of freedom when engineering genetic circuits. We see this new class of parts as a tool that can be used for a variety of applications, such as variable tagging of translated proteins, regulated operon expression, and dual-coding of genes to minimize plasmid size. To facilitate the use of this part, we have developed a software program to assist in the design of overlapping coding sequences, so as to utilize both the 0 and –1 reading frame in an engineered construct. Additionally, we have explored the biosafety implications of this new regulatory element as it pertains to the DNA synthesis industry.

Engineering a Pseudoknot Library

Mutagenesis of PK401

Error-prone PCR was used to generate mutated primers with which to amplify the PK401 construct. This will result in a library of pseudoknot sequences that can be transformed into Escherichia coli cells and characterized for frameshifting efficiency. The final library will have pseudoknots that range in frameshifting efficiency from low to high that can be used for a variety of applications.

Software for Dual Coding

Designing DNA Sequences for Dual-Coded Proteins

One application we envision for frameshifting elements is the ability to dual-code parts in the same DNA sequence. To facilitate this application, we have developed a software program to help users find and design dual-coding DNA sequences for use with pseudoknots. This software has two main functions:

- The user specifies proteins of interest and the program attempts to design primers that allow translation in the frames specified by the pseudoknot.
- The system finds a list of proteins that satisfy the desired conditions and attempts to find useful primer pairs.

This method can provide solutions for the design of dual-coding parts and can be used to create custom libraries of codons that can be used with pseudoknots.

Results

Expression of PK401

PK401 was tested for expression in E. coli. We observed a 4:1 ratio of CFP to YFP in a 0.5 OD Urea AAG 2-6 pmole Lanes. The relative fluorescence intensity was measured at 537 nm. The fluorescence emission measured for the PK401 samples after excitation at 510 nm indicates that YFP was translated and that PK401 successfully induced ribosomal frameshifting.

Characterization of PK401-Induced Frameshifting

Fluorescence spectra of E. coli DH5α cell lysates from the overexpression of Bba_K1210000 were measured after excitation of CFP (430 nm) and YFP (510 nm). Fluorescence emission from YFP was only expected for samples that successfully frameshifted after translation of CFP and encountering the PK401 pseudoknot.

DNA Synthesis and Biosecurity

Synthetic companies implement screening procedures to ensure hazardous sequences do not end up in the wrong hands. To ensure our part did not allow individuals to bypass these screening protocols, we worked with major North American synthetic companies to see if a frameshifting element posed a threat to biosecurity. The screening methods currently in place appear to be effective at detecting attempts to obscure dangerous sequences with our part, but this is only one of the potential threats that synthetic DNA poses to biosecurity. The potential to develop novel proteins with a desired function from YFP that was only expected for samples that successfully frameshifted after translation of CFP and encountering the PK401 pseudoknot.

Achievements

- Designed a new class of gene regulatory parts that induce ribosomal frameshifting
- Constructed and characterized Bba_K1210000 by showing successful frameshifting by PK401 with an estimated frameshifting efficiency of 9% ± 2%.
- Instructed construction of a frameshifting part library by mutating the PK401 sequence
- Developed software that uses codon redundancy to assist in designing dual-coded DNA sequences that can be used with a frameshifting element.
- Assessed implications of frameshifting elements and new biotechnologies for biosecurity

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References