FRAMEchanger

Programmed Ribosomal Frameshifts as Synthetic Biology Tools

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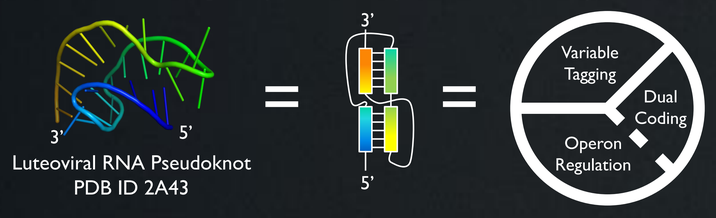
Introduction

As the field of synthetic biology grows, so should its toolset. This year, the Lethbridge iGEM team worked to design a whole new class of parts for the iGEM community: programmed ribosomal frameshifting elements. To do this, we have characterized the PK401 pseudoknot, derived from infectious bronchitis virus, for use within the BioBrick system. Pseudoknots are 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.

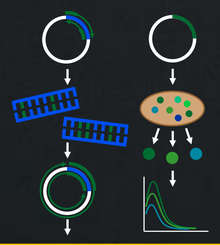


Luteoviral RNA Pseudoknot
PDB ID 2A43

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 efficiencies. 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 proteins in the same DNA sequence. To facilitate this application, we have developed a software program to help users find and design dual-coding DNA seqeunces for use with pseudoknots.

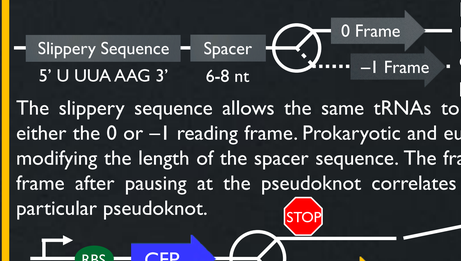
This software has two main functions:

- The user specifies proteins of interest and the program attempts to overlap them using variable start positions and codon changes
- The specifies a list of proteins they wish to find overlap partners for and 10,000,000 overlapping DNA sequences are generated to allow identity searching on databases such as NCBI

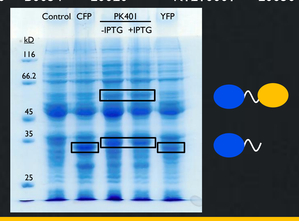
```
C:\>perl Zipper.pl -S YNVTIKPSNETIA -C QMLPLNLATKPSP
Zipper Run: Compare QMLPLNLATKPSP to YNVTIKPSNETIA

Cannot find a zipper overlap with Starting postion: 0
Found a zipper overlap with Starting postion: 1
        0: Frame: -2 Sequence: CAAAUGUUACCAUUAAAUUUACGCAACGAAACCAUCG
        1: Frame: -2 Sequence: CAAAUGUUACCAUUAAAUUUACGCAACGAAACCAUCG
        2: Frame: -2 Sequence: CAAAUGUUACCAUUAAAUUUACGCAACGAAACCAUCG
        3: Frame: -2 Sequence: CAAAUGUUACCAUUAAAUUUACGCAACGAAACCAUCG
Cannot find a zipper overlap with Starting postion: 2
Cannot find a zipper overlap with Starting postion: 3
Cannot find a zipper overlap with Starting postion: 4
Cannot find a zipper overlap with Starting postion: 5
Cannot find a zipper overlap with Starting postion: 6
Cannot find a zipper overlap with Starting postion: 7
Cannot find a zipper overlap with Starting postion: 8
Cannot find a zipper overlap with Starting postion: 9
Cannot find a zipper overlap with Starting postion: 10
Cannot find a zipper overlap with Starting postion: 11
Found a zipper overlap with Starting postion: 12
        0: Frame: -2 Sequence: CAG
        1: Frame: +2 Sequence: GCC
        2: Frame: -2 Sequence: CAG
        3: Frame: +2 Sequence: GCC
        4: Frame: +1 Sequence: GCA
        5: Frame: +1 Sequence: GCA
        6: Frame: -2 Sequence: CAG
        7: Frame: -2 Sequence: CAG

Success - an overlap was found
```

Results

Expression of PK401 Construct, BBa_K1210000



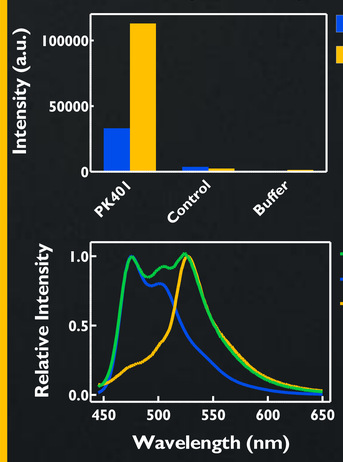
Programmed –1 Ribosomal Frameshifting. When the ribosome encounters the pseudoknot, it will pause and slip on the slippery sequence. The slippery sequence allows the same tRNAs to be used if the ribosome continues in either the 0 or –1 reading frame. Prokaryotic and eukaryotic systems can utilize this part by modifying the length of the spacer sequence. The fraction of ribosomes that change reading frame after pausing at the pseudoknot correlates to the frameshifting efficiency of that particular pseudoknot.



Overexpression of BBa_K1210000 in *E. coli* DH5α. Samples were taken 4 h after induction with IPTG (+IPTG) and analyzed by 12% SDS-PAGE. The relative band intensities of the frameshifted and non-frameshifted product indicate a frameshift efficiency of 21 ± 5%.

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.



Maximum fluorescence emission intensity after direct excitation of CFP and YFP. CFP emission was measured at 475 nm, and YFP emission was measured at 527 nm.

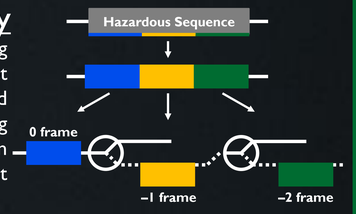
Normalized fluorescence emission after excitation of CFP.

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.

Human Practices

DNA Synthesis and Biosecurity

Synthesis 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 synthesis companies to see if a frameshifting element posed a threat to biosecurity.

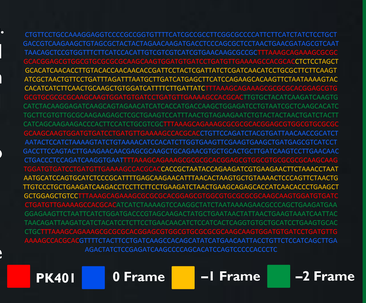


Methods

- Altered 17 sequences from organisms on U.S. Select Agents and toxins (11) and control sequences not on the list (6) by changing codon composition and distance between PK401 sequences
- Sent sequences to synthesis companies to screen as they would any DNA synthesis order

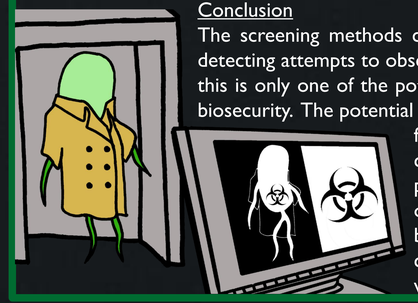
Results

- 17 of 17 sequences were correctly identified
- Indicates that our part does not have negative implications for DNA synthesis and biosecurity



Conclusion

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 has been demonstrated through computational design. It is also possible to purchase DNA synthesis machines without oversight. These new challenges facing biosecurity in synthetic biology must be considered today in order to address them when they become realities tomorrow.



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 frameshift efficiency of 21 ± 5%
- Initiated 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

Acknowledgements

Dr. Gaymon Bennett (Fred Hutchinson Cancer Research Center), Dr. Karmella Haynes (Arizona State University), and the Public Health Agency of Canada - Centre for Biosecurity.
Dallas Thomas (University of Lethbridge, Lethbridge Agriculture and Agri-Food Canada).
Sutherland Dubé, Dipankar Goyal, Kelsey Kristensen, Richard McLean, Fan Mo, Justin Vigar, and Anthony Vuong (University of Lethbridge).
The Department of Chemistry and Biochemistry, the Alberta RNA Research and Training Institute, the Kothe lab, Selinger lab, and Wieden lab at the University of Lethbridge.

Platinum Sponsor: Alberta Innovates Technology Futures
Silver Sponsor: GenomeAlberta
Bronze Sponsors: geneIOUS, IDT Integrated DNA Technologies, SARSTEDT

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