

cFhRaAnMgEer

Programmed Ribosomal Frameshifts as Synthetic Biology Tools

University of Lethbridge

Lethbridge, AB, Canada

November 2, 2013

FRAMEchanger



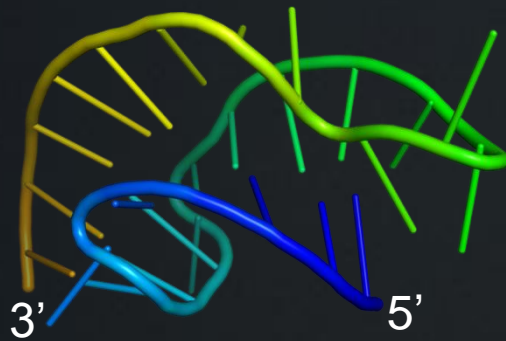
Project Goals

- Design a new class of regulatory parts
- Design software for the iGEM community
- Investigate biosecurity implications of our new part

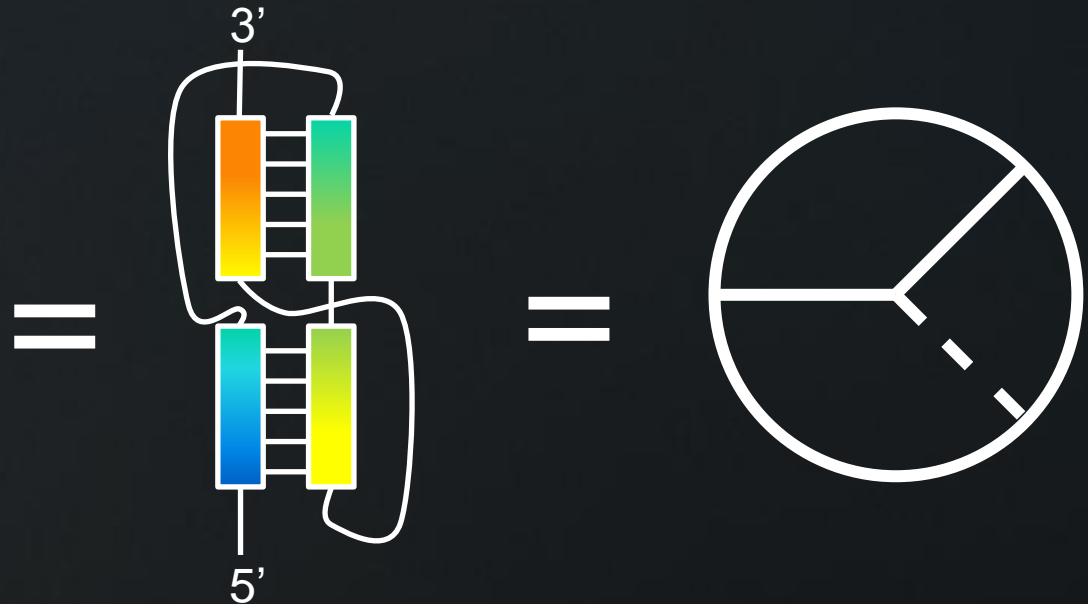


PROJECT

Pseudoknot



PDB ID
2A43

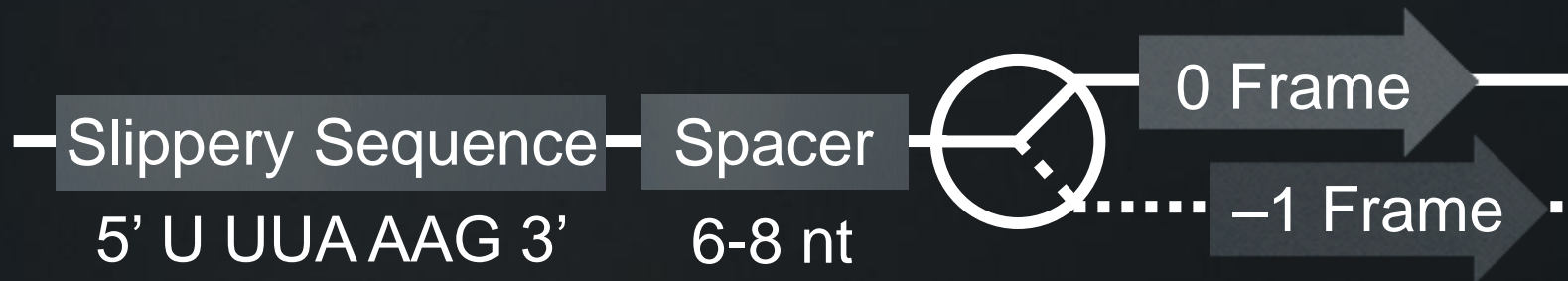


Pallan *et al.* *Biochemistry* (2007).



PROJECT

Pseudoknot Elements



PROJECT

0 Frame Protein Produced



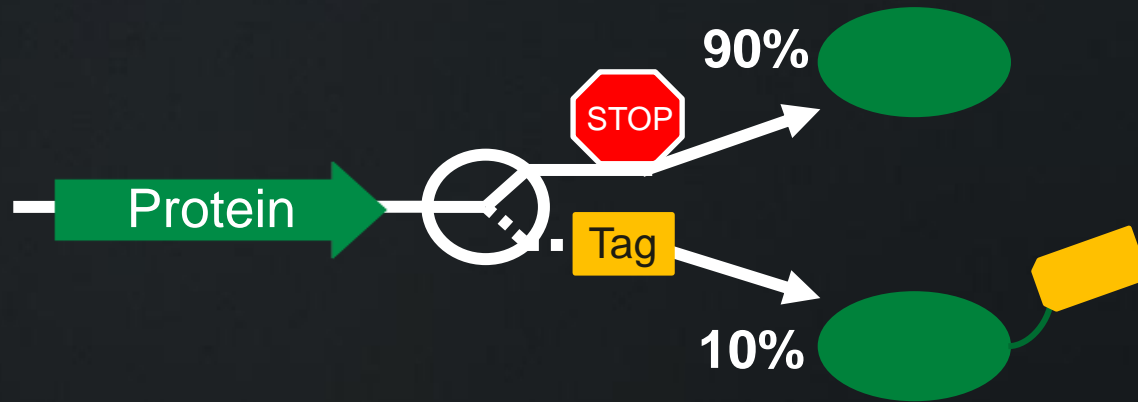
PROJECT

-1 Frame Protein Produced



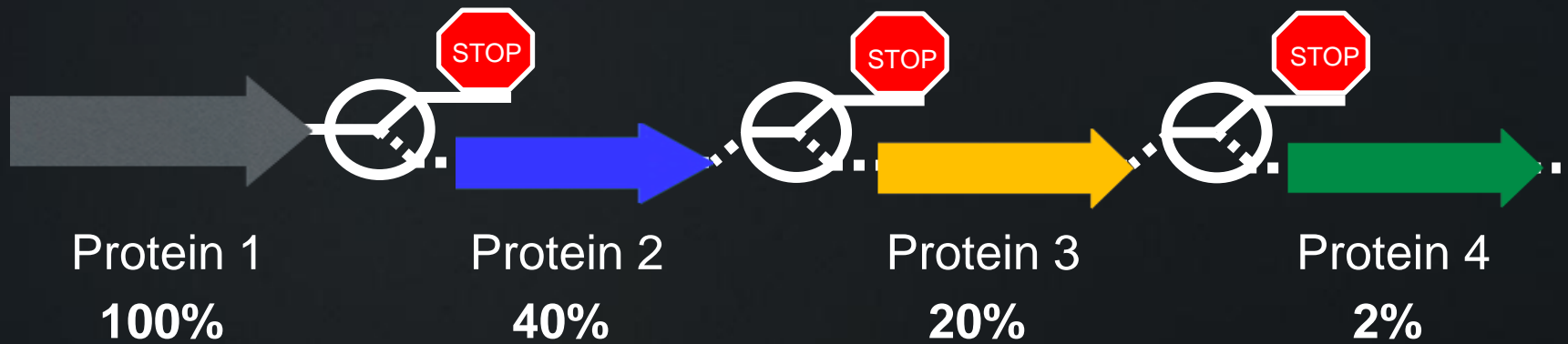
PROJECT

Variable Tagging and Fusion of Proteins



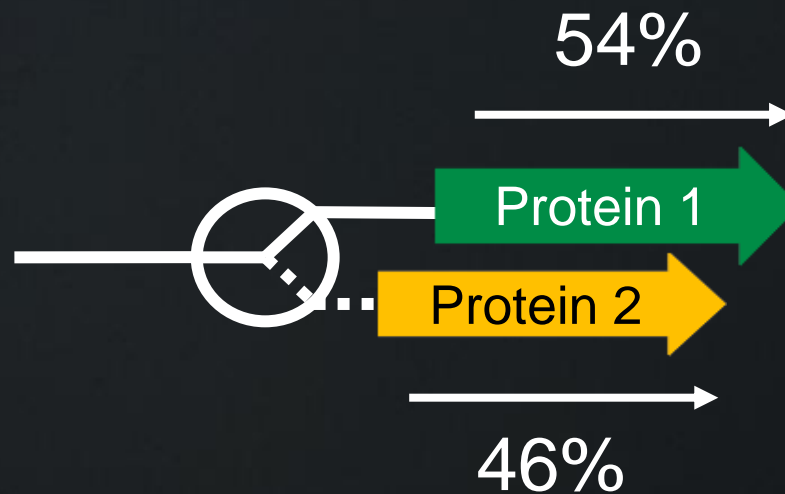
PROJECT

Operon Regulation



PROJECT

Dual Coding Proteins in One Nucleotide Sequence



Napthine *et al.* *J Mol Biol* (1999).



PROJECT

Dual Coding Sequences

M N V P N I → AUGAAUGUACCGAAUAUU
 M Y R I F E → AUGUAUCGGAUUAUUCGAA

AUGAAUGUACCGAAUAUU
 ✓ X X ✓
 AUGUAUCGGAUUAUUCGAA

M N V P N I ← AUGAAUGUACCGAAUAUU
 M Y R I F E ← AUGUAUCGGAUUAUUCGAA



PROJECT

Software Development

- Software currently utilizes
 - Codon redundancy
 - Variable overlap position
- Future software changes
 - Utilize multiple sequence alignments
 - Database searching

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

Cannot find a zipper overlap with Starting position: 0
Found a zipper overlap with Starting position: 1
0: Frame: -2 Sequence: CAAAGUACCAUAAAUUAACCAACGAAACCAUCC
1: Frame: -2 Sequence: CAAAGUACCAUAAAUUAACCAACGAAACCAUCC
2: Frame: -2 Sequence: CAAAGUACCAUAAAUUAACCAACGAAACCAUCC
3: Frame: -2 Sequence: CAAAGUACCAUAAAUUAACCAACGAAACCAUCC
Cannot find a zipper overlap with Starting position: 2
Cannot find a zipper overlap with Starting position: 3
Cannot find a zipper overlap with Starting position: 4
Cannot find a zipper overlap with Starting position: 5
Cannot find a zipper overlap with Starting position: 6
Cannot find a zipper overlap with Starting position: 7
Cannot find a zipper overlap with Starting position: 8
Cannot find a zipper overlap with Starting position: 9
Cannot find a zipper overlap with Starting position: 10
Cannot find a zipper overlap with Starting position: 11
Found a zipper overlap with Starting position: 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: GCH
6: Frame: -2 Sequence: CAG
7: Frame: -2 Sequence: CAG

Success - an overlap was found

C:\>perl Zipper.pl -n
NAME
    Zipper.pl

SYNOPSIS
    Zipper.pl [-Options [-l [Arguments...]]]

DESCRIPTION
    Compares two Polypeptide or Protein Sequences to see if they overlap
    beginning with the first Amino Acid, all codons for each Amino Acid are gen
    erated and tested if they overlap. If they do - this sequence is stored and the
    tail is carried forward to the next iteration.

    Code is designed to check if two sequences will overlap in different frames
    .

    Switches in program call can be done in long or short form and are case sen
    sitive
    eg:
    Zipper.pl --infile
    Zipper.pl -l
    Zipper.pl -h (help)
    Zipper.pl -s (Sequence)

ARGUMENTS
    -infile <infile_name>
    -sequence <Protein/Polypeptide_Sequence>
    -comparison <Protein/Polypeptide_Sequence>
    -help : print Options and Arguments instead of fetching da
    ta
    -man : print complete man page instead of fetching data

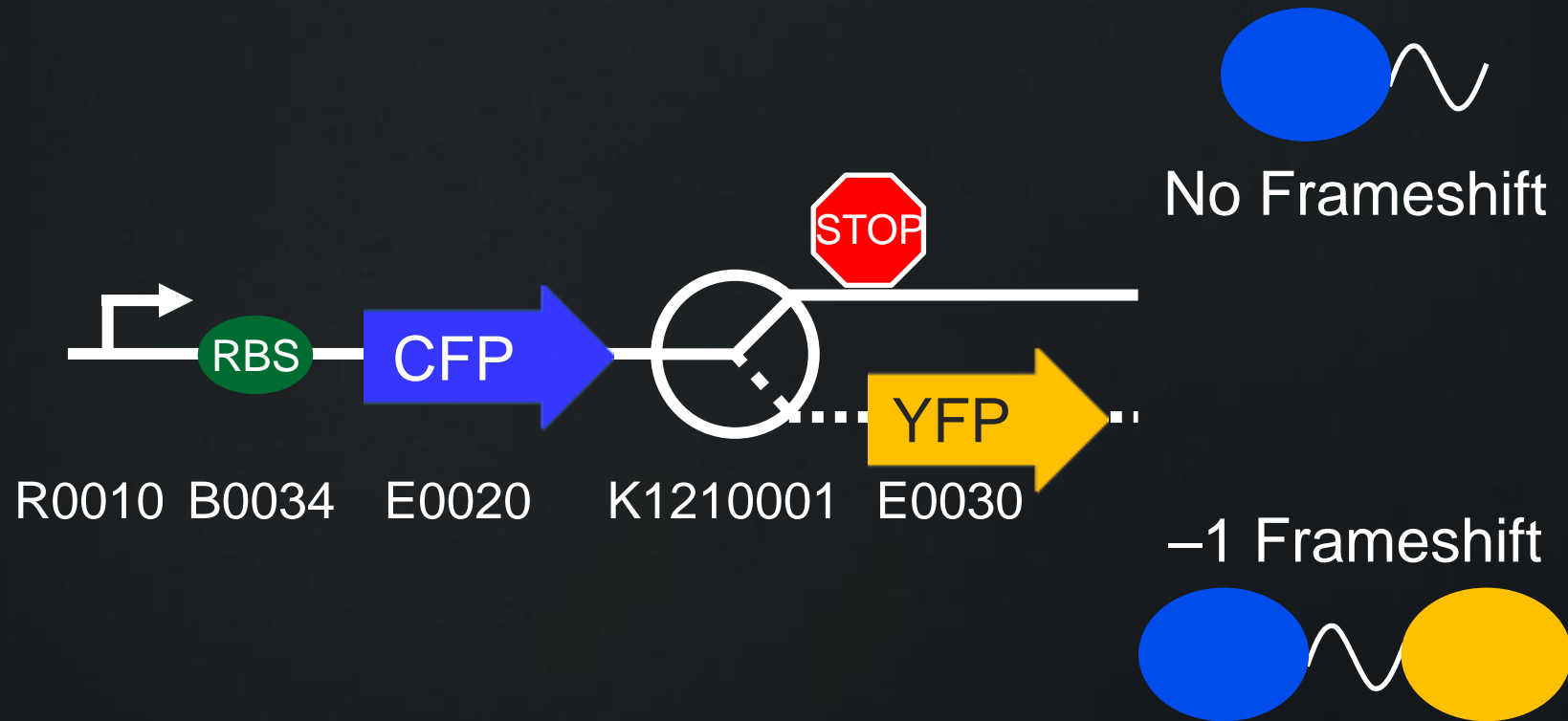
    Use the --man option to see sample data for testing - and program examples
```

Software is available for download from our wiki!



PROJECT

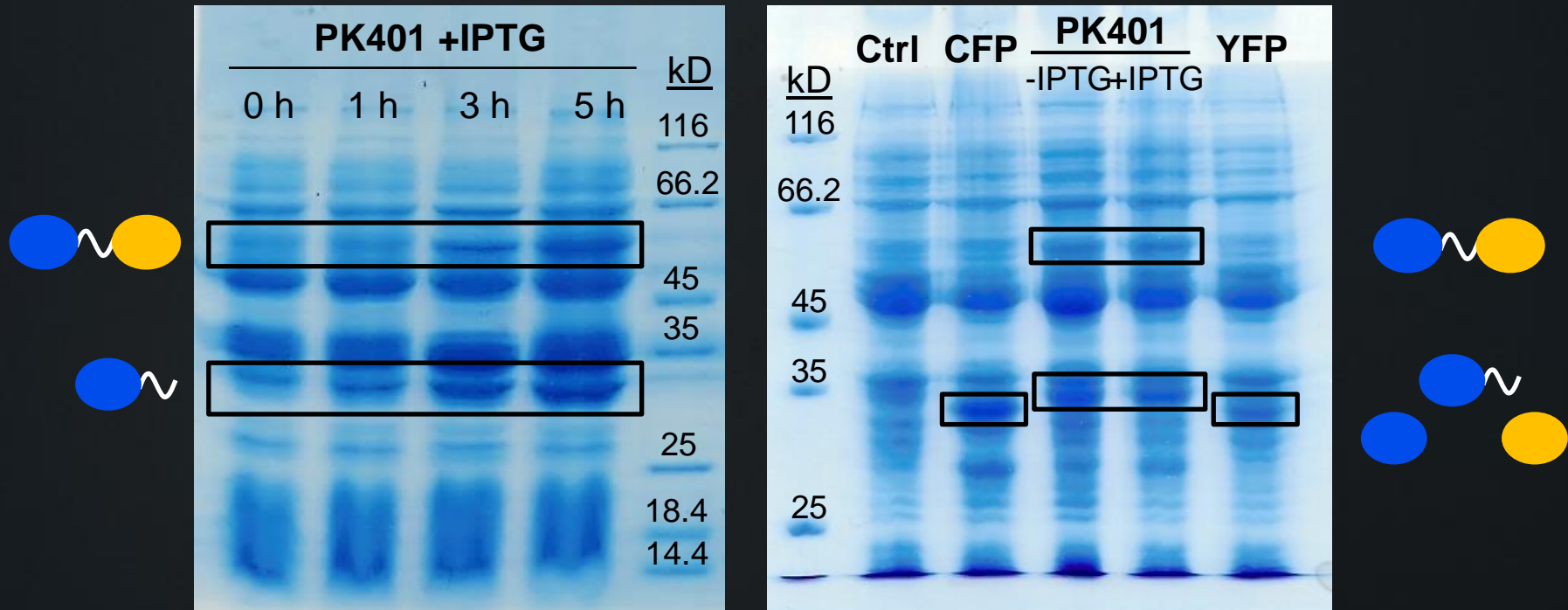
Proof of Principle



BBa_K1210000, PK401 Test Construct

 RESULTS

Reporter Construct Expression

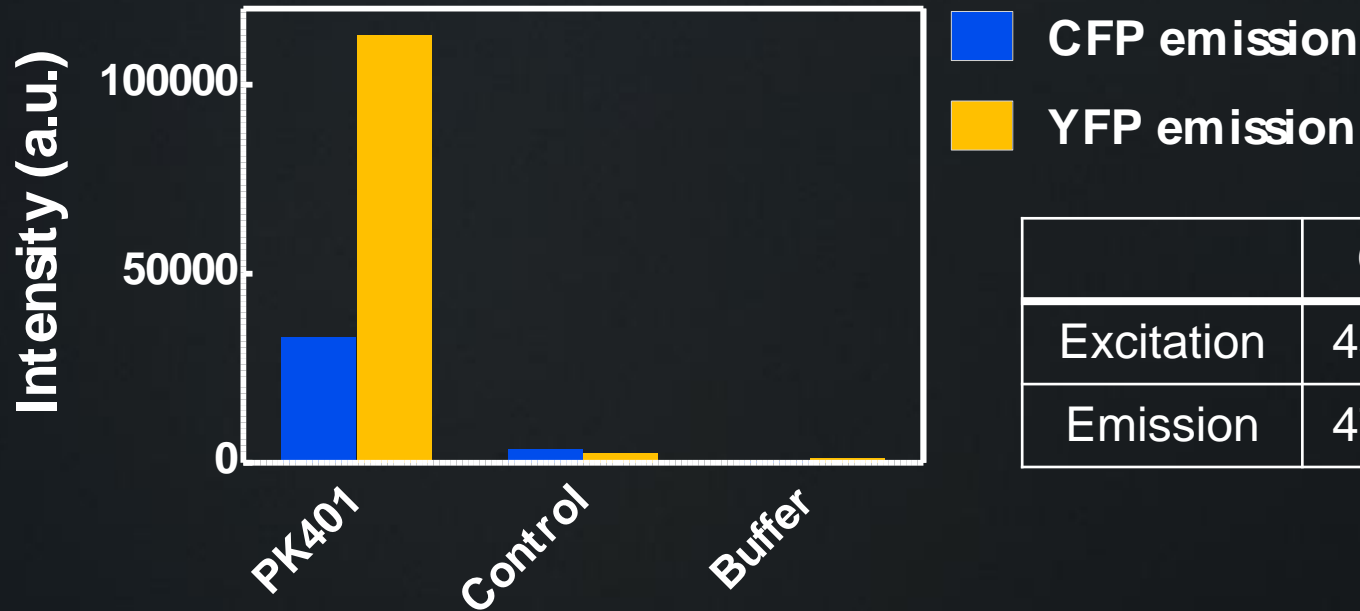


PK401 frameshifting efficiency $21 \pm 5\%$



RESULTS

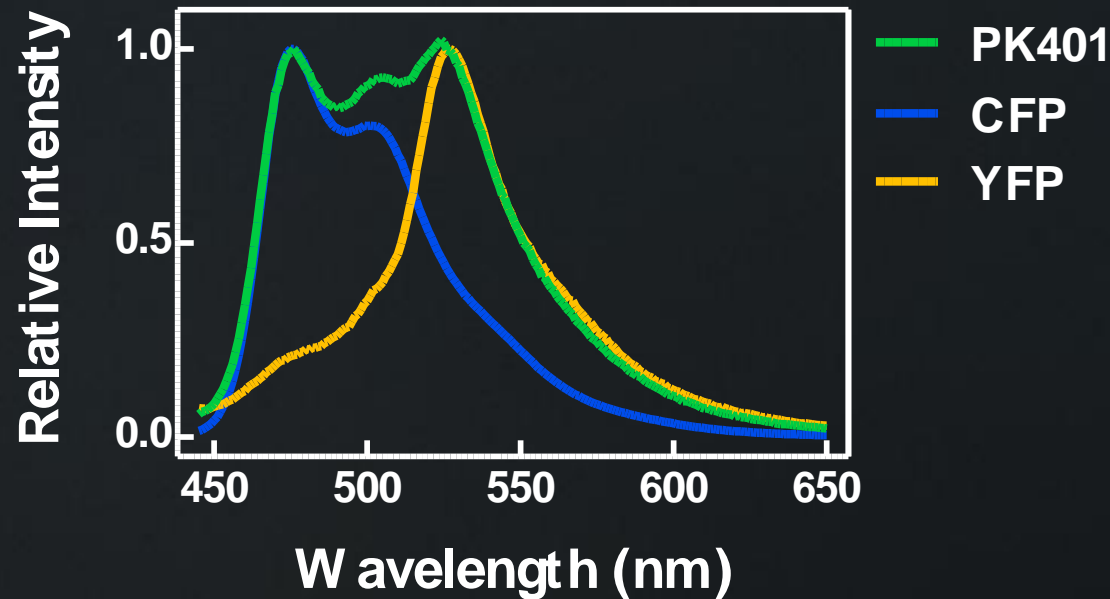
Fluorescence



RESULTS



Fluorescence



Fluorescence emission from both CFP and YFP from overexpressed PK401 construct



RESULTS

Frameshifting Results

- Demonstrated programmed ribosomal frameshift of BBa_K1210000
- PK401 frameshifting efficiency of $21 \pm 5\%$



RESULTS

Dual-Coded Test Construct

BBa_K1210003



No Frameshifting

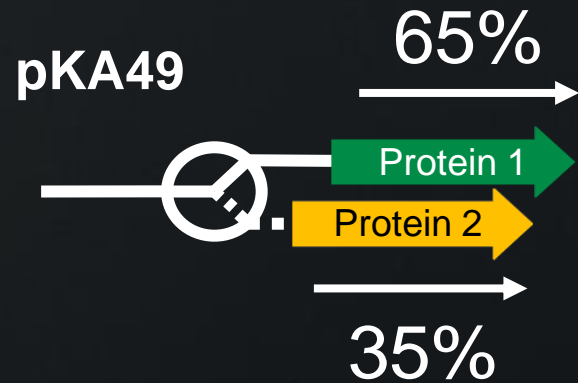
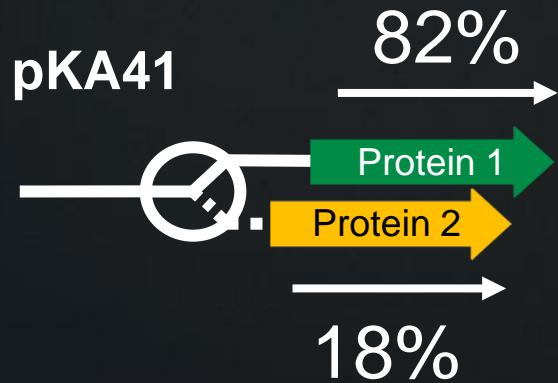
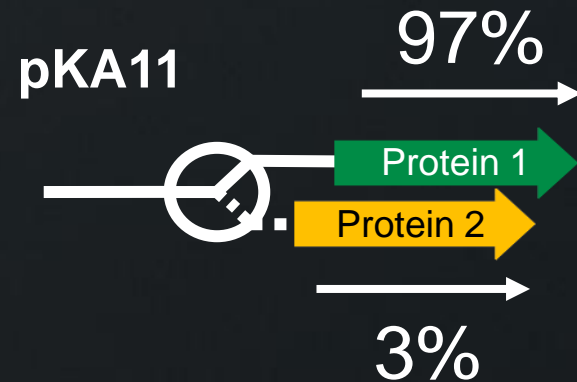
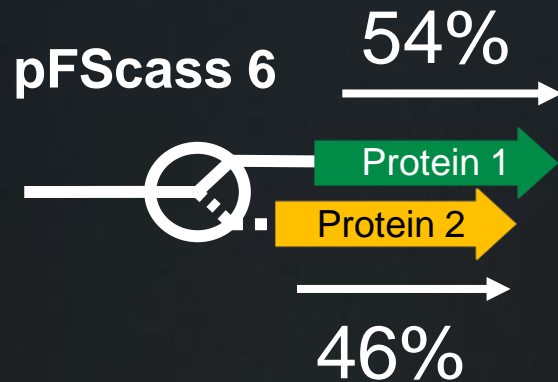


-1 Frameshifting



RESULTS

Dual Coding Proteins in One Nucleotide Sequence

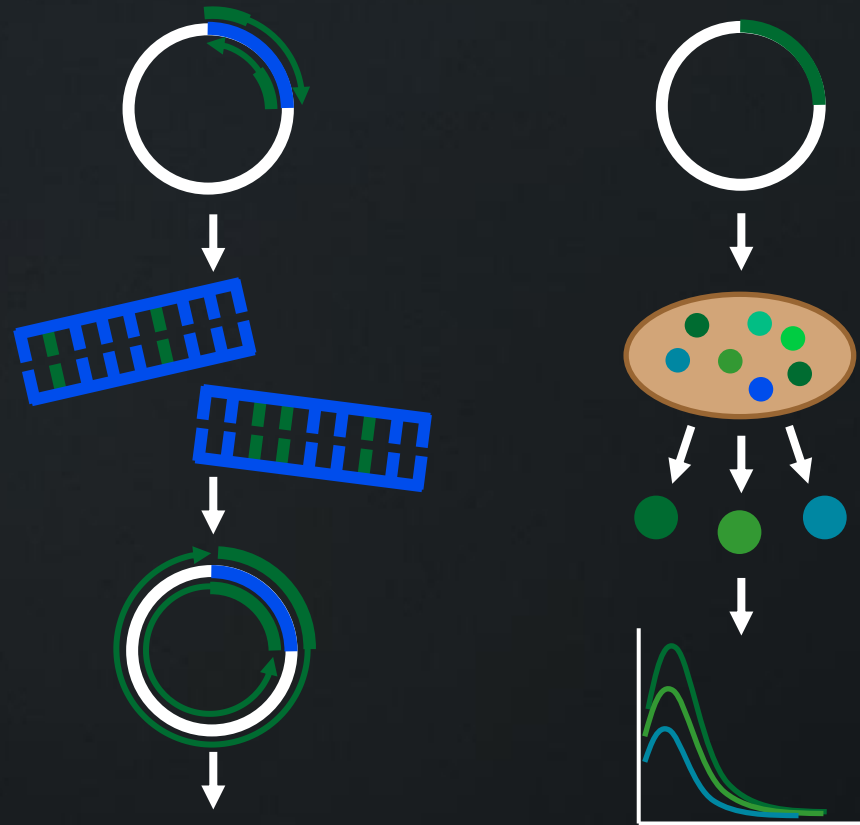


Napthine *et al.* *J Mol Biol* (1999).



PROJECT

Generating a Pseudoknot Library



Adapted from UC Davis 2011 iGEM Team



RESULTS

Human Practices



HUMAN PRACTICES

Biosafety vs. Biosecurity

- **Biosafety** is keeping “bad bugs” away from people
- **Biosecurity** is keeping “bad people” away from bugs



HUMAN PRACTICES

Current Screening

- 2 step approach to screening

ACGTCCTAGCTAAGCTCGT



 ACGTCCTAGCTAAGCTCGT



DNA Database Comparison



HUMAN PRACTICES

Sequence Screening

- 2 step approach to screening

ACGTCCTAGCTACGCTCGAC



 ACGTCCTAGCTAAGCTCGAC

Frame 2 T R V S P I Stop S G Stop D

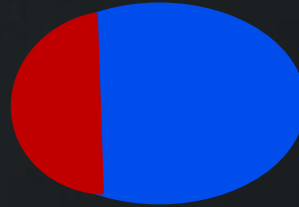
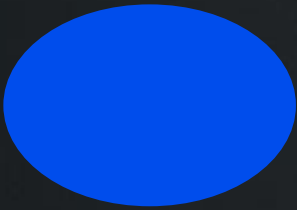
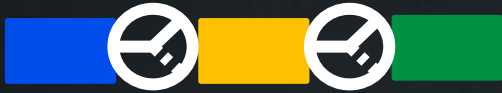


Protein Database Comparison



HUMAN PRACTICES

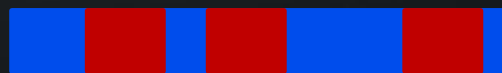
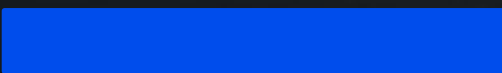
Frameshifts



Native

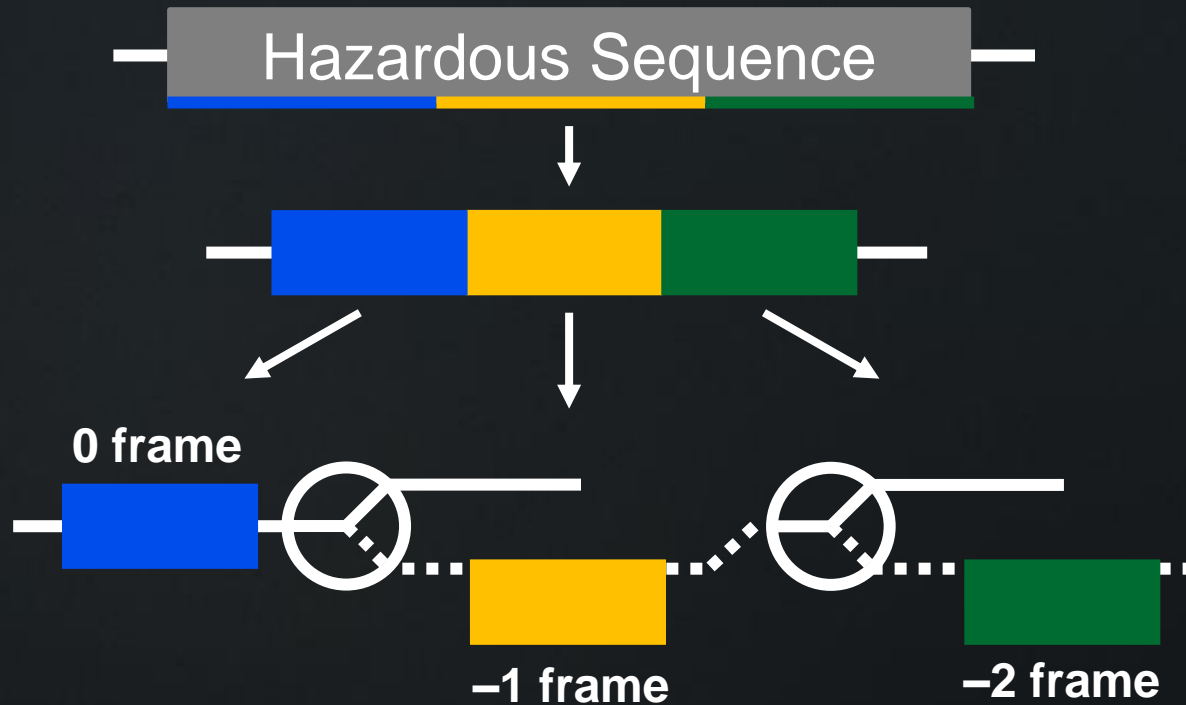
Non-conserved substitutions

Novel



HUMAN PRACTICES

Hidden in Another Frame



HUMAN PRACTICES

Test Sequence Design

CTGTTCTGCCAAAGGAGGTCCCCGCCGGTGTTCATCGCCGCCTTCGGCGCCCCATTCTTCATCTATCTCCTGCTGACCG
TCAAGAAGCTGTAGCGCTACTACTAGAACAAAGATGACCTCCAGCGCTCCTAACTGAACGATAGCGTCAATTAACAGCTCCG
TGGTTTCTTCATCCACATTGTCGTCGTCATCGTGAACAAGCGCCGC TTTAAAGCAGAAAGCGCGCGCACGGAGCGTGGCGT
GCGCGCGCAAGCAAGTGGATGTGATCCTGATGTTGAAAAGCCACGCACCTCTCCTAGCTGCACATCAACACCTTGATACCA
ACAACACCGATTCTACTCGATTATCTCGATCAACATCCTGCGCTTCTTCAAGTATCGCTAACTGTTCCCTGATTTAGATTTAAT
GCTTGATCATGAGCTTCATCCAGAAGCACAAGTTCTAATAAAAGTACCACATCATCTTCAACTGCAAGCTGTGGATCATTCTTCT
TGATTATCTTTAAAGCAGAAAGCGCGCGCACGGAGCGTGGCGTGC GCGCGCAAGCAAGTGGATGTGATCCTGATGTTGAAA
AGCCACGCAC TTGTGCTACATCAAGATCAAGTGCATCTACAAGGAGATCAAGCAGTAGAACATCATCACCATGACCAAGCTG
GAGATCCTGTAATCGCTCAAGCACATCTGCTTCGTGTTGCGCAAGAAGAGCTCGCTGAAGTCCATTTAACTGTAGAAGAATCT
GTACTACTAACTGATCTACTTCATCAGCAAGAAGACCCACTTCCATCTGCGTGC TTTAAAGCAGAAAGCGCGCGCACGGAG
CGTGGCGTGC GCGCGCAAGCAAGTGGATGTGATCCTGATGTTGAAAAGCCACGCACCTGTTCCAGATCTACGTGATTAACAA
CCGCATCTAATACTCCATCTAAAAGTATCTGTAAAACATCCACATCTTGGTGAAGTTCGAAGTGAAGCTGATGAGCGTCATCC
TGACCTTCCAGTACTTGAAGAACAACGAGCGCAAGCTGCAGAACGTGCTGCACTGCTTGATCAAGTCCCTGAACAACCTGAC
CCTCCAGATCAAGGTGAAT TTTAAAGCAGAAAGCGCGCGCACGGAGCGTGGCGTGC GCGCGCAAGCAAGTGGATGTGATCC
TGATGTTGAAAAGCCACGCACACCAGCTAATAACCAGAAGATCGTGAAGAACTTCTAAACCTAATAATGCATCCAGTGCATCTC
CCGCATTTTGAGCAAGAACATTTAACTAAGTGTGTAAAACCTCCAGTTCTAACTGTTGTCCCTGCTGAAGATCAAGACCT
CCTTCTCCTGAAGATCTAACTGAAGCAGAGCACCATCAACACCCTGAAGCTGCTGGAGCTGTCC TTTAAAGCAGAAAGCGC
GCGCACGGAGCGTGGCGTGC GCGCGCAAGCAAGTGGATGTGATCCTGATGTTGAAAAGCCACGCACATCATCTAAAAGTCC
AAGGCTATCTAATAAAAGAACGCCAGCTGAAGATGAAGGAGAAGTTCTAATTCATCTGGATGACCCGTAGCAAGACTATGCT
GAATAACTATTAAGTAAATCAATTACTAACAGATTAAGATCATCTACATCCTCTTCCCTGAACAACATCTCCATCACTCAG
GTGTGCTGCATCCTGAAGTGCACCTGCTTTAAAGCAGAAAGCGCGCGCACGGAGCGTGGCGTGC GCGCGCAAGCAAGTGG
ATGTGATCCTGATGTTGAAAAGCCACGCACGTTTTCTACTTCTGATCAAGCCACAGCATATCATGAACAATTACCTGTTCTCC
ATCAGCTTGAAGACTATCTCCGAGATCAAGCCCCAGCACATCCAGTCCCCACCCTC



Pseudoknot



0 Frame



-1 Frame



-2 Frame



HUMAN PRACTICES

Test Sequences

Sequence	Total Length (bp)	Codon Changes (%)	Length between PK (bp)
CFP	966	25	180
Staph	1869	0	210
Ricin	2392	25	198
Staph	2450	25	102
CFP	966	16	210
CFP	966	0	180
Staph	2450	0	102
Ricin	2392	0	198
Ebola Matrix Protein	1031	0	0

Sequence	Total Length (bp)	Codon Changes (%)	Length between PK (bp)
CFP	966	16	180
Staph	2450	0	102
CFP	966	0	210
Staph	1869	20	210
Ricin	3139	25	99
Staph	1869	25	210
CFP	966	25	210
Ricin	3139	0	99



HUMAN PRACTICES

Screening Results

Sequence ID	Threat?	Detected Origin
CFP	FALSE	
Staph	TRUE	Staph
Ricin	TRUE	Ricin
Staph	TRUE	Staph
CFP	FALSE	
CFP	FALSE	
Staph	TRUE	Staph
Ricin	TRUE	Ricin
Ebola Matrix Protein	TRUE	Ebola

All sequences were correctly identified regardless of codon changes and pseudoknot position

Sequence ID	Threat?	Detected Origin
CFP	FALSE	
Staph	TRUE	Staph
CFP	FALSE	
Staph	TRUE	Staph
Ricin	TRUE	Ricin
Staph	TRUE	Staph
CFP	FALSE	
Ricin	TRUE	Ricin



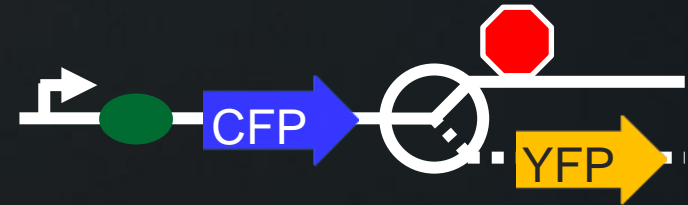
Biosecurity



HUMAN PRACTICES

Summary

- Engineered and submitted frameshifting test construct, BBa_K1210000



- Engineered and currently testing a dual-coded frameshifting construct, BBa_K1210003



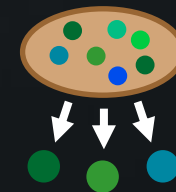
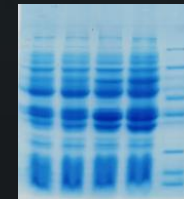
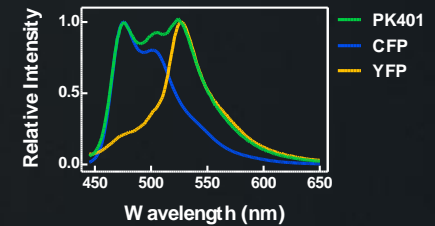
- Submitted PK401 for assembly in other constructs, BBa_K1210001



PROJECT

Summary

- Characterized successful frameshifting by PK401
- Estimated frameshift efficiency supported by published values
- First steps toward a pseudoknot library



RESULTS



Summary

- Collaborated with gene synthesis companies to test biosecurity measures
- Frameshifting element does not pose a biosecurity threat
- Developed software for designing dual-coded protein sequences for use with our pseudoknot part



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FRAMEchanger



Acknowledgements

- Dr. Gaymon Bennett (Fred Hutchinson Cancer Research Center)
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Public Health Agency of Canada, Centre for Biosecurity
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McLean, Fan Mo, Justin Vigar, and Anthony Vuong (University of
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