QUESTIONING THE DOGMA IN THE CENTRAL DOGMA

PENN STATE iGEM TEAM
Introduction

- Scenarios that the Central Dogma does not answer
- Answers are needed for rational design

Transcription Initiation
Translation Initiation
Translation Elongation
Bidirectional Promoters

Transcription Initiation
The Background

- Normal promoter sequences contain two hexamers
  - Bind to $\sigma$ factor
  - Control direction of transcription
- Bba_J23102
  - 5’ GTTGACAGCTAGCTCAGTCTAGGTACTGTC TAGCT 3’

![Diagram showing the positions of Hexamer II and Hexamer I with mRNA transcription direction]
What if...

• the promoter is palindromic?
  – which direction is transcribed?

• palindromic promoter:
  – TAAC**TTGACA**ATTATAAAAAAAAAATATAAT**TGTC**AATATTCA
The Design

1. \[ K_{RFP|GFP} = \frac{RFP \text{ fluorescence of Promoter Ligated Reverse}}{GFP \text{ fluorescence of Promoter Ligated Forward}} \]

2.
The Design

Palindromic Sequences

• Bba_K933004
  – TAAC TTGACA ATTATA AAAAAAAAAAATATAATTGTCAA ATATTCA

• Bba_K933005
  – TAACA AACTGT ATTATA AAAAAAAAAAATATAATTGTCAA ATATTCA

• Bba_K933006
  – ATAAC TTGACA ATTATA AAAAAAAAAAAGCCGGTGTCAA ATATTCA
Normalization of Data

- RFP and GFP are expressed unequally
- Calculated constant $K$ to compare fluorescence values

$$\text{Forward Direction} = \frac{((\text{GFP}) \times (K_{\text{RFP}|\text{GFP}}) - \text{RFP})}{((\text{GFP}) \times (K_{\text{RFP}|\text{GFP}}) + \text{RFP})}$$

$$\text{Reverse Direction} = \frac{-((\text{GFP}) \times (K_{\text{RFP}|\text{GFP}}) - \text{RFP})}{((\text{GFP}) \times (K_{\text{RFP}|\text{GFP}}) + \text{RFP})}$$

$$K_{\text{RFP}|\text{GFP}} = \frac{\text{RFP Fluorescence of Promoter Ligated Reverse}}{\text{GFP fluorescence of Promoter Ligated Forward}}$$
The Results

Control BBa_K933003 (BBa_J23102)

Directional Value: 0.969

BBa_K933004

Directional Value: 0.701
The Results

- **BBa_K933005**
  - Directional Value: 0.885

- **BBa_K933006**
  - Directional Value: 0.969
The Results

BBa_J23114

Directional Value: 0.820

TECANM1000: Spectrophotometry: Bulk fluorescence per OD600

Bba_J23114

Relative fluorescence

0 1000 2000 3000 4000 5000 6000 7000

RFP GFP*K(RFP/GFP)
The Conclusion

- Promoter sequences can be bidirectional
- Placement of hexamer sequences effects
  - directionality
  - expression rate

- Watch out for... **promoters with palindromes**!
Multiple Start Codons

Translation Initiation
What if...

• Two start codons exist on a single mRNA strand

• Which is chosen for initiation?
The Design

- Designed with low-initiating RBS sequence
- Removal of all interim stop codons within both frames of reference
The Results

- Construct for start codons within 7 nucleotides
- Calculated approximate relative fluorescence using $K$ constant from promoter data

Fluorescence of sfGFP and RFP by initiated by start codons

$$K_{RFP|GFP} = \frac{\text{RFP Fluorescence of Promoter Ligated Reverse}}{\text{GFP fluorescence of Promoter Ligated Forward}}$$
The Conclusion

- Ribosome overwhelmingly bound to sfGFP-initiating start codon

- Testing with varying RBS translation initiation rates and lengths between codons

- Watch out for...**multiple start codons**!
Codon Optimization

Translation Elongation
The Background

• Codon: 3 nucleotides that code for an amino acid

• Which codon sequence is the best for each amino acid?

<table>
<thead>
<tr>
<th>Threonine</th>
<th>ACT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACC</td>
</tr>
<tr>
<td></td>
<td>ACA</td>
</tr>
<tr>
<td></td>
<td>ACG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alanine</th>
<th>GCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GCC</td>
</tr>
<tr>
<td></td>
<td>GCA</td>
</tr>
<tr>
<td></td>
<td>GCG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proline</th>
<th>CCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCC</td>
</tr>
<tr>
<td></td>
<td>CCA</td>
</tr>
<tr>
<td></td>
<td>CCG</td>
</tr>
</tbody>
</table>
What if...

• Rare codons are translated?
  – Ribosomes read rare sequence

• Charged tRNA not as available with matching codon
  – Translation will be inhibited
  – mRNA degradation may result
• GFP: reference constant

• RFP will vary: amount expressed indicates codon optimization
The Results

Compared with previous data*

- Using 9 repeats-similar results
- GFP differences

Dr. Mike Speer: currently at Cargill
The Conclusion

• Based on previous results and our 6 repeat results for Threonine

  – ACG is optimal codon for DH10B E. coli during exponential growth

• Watch Out For...rare codons!

Educational Outreach

- Presented at local high school
- Molecular Biology Animation
- Prezi Presentations
- Americas East Jamboree high school presentation
• Watch out for **Palindromic Promoters**
  – They transcribe in both directions!

• Watch out for **Multiple Start Codons**
  – They may translate the wrong protein!

• Watch out for **Rare Codons in Coding Sequences**
  – They will slow down translation!
Attributions

• Dr. Tom Richard lab at Penn State
  – Mike Speer

• Dr. Howard Salis lab at Penn State
  – RBS calculator software
Our Team

The Brainy Bunch