IRESistible: Novel Parts for Use in *S. cerevisiae*

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Get it? ... IRESistible LOL
Outline

- Who we are
- Motivation
- What is an IRES
  - How does IRES mediated translation differ from cap dependent translation
- Applications
- Completed work
- Proposed work
- Conclusions
Who we are
The University of Tennessee, Knoxville

Go Big Orange!!
Who we are

- We are UT's inaugural iGEM team
- Morgan, Katie, and Akshitha were the core members

Genesis Minter
Brandon Wilbanks
Who we are

Michael Wierzbicki works with *E. coli*

Adam Thompson works with *S. cerevisiae*

Dr. Cong Trinh was our primary advisor

Dr. Dan Close works with IRESs
Motivation

In prokaryotes, multiple genes can be expressed under the control of the same promoter.

In eukaryotes, each gene must be expressed under the control of its own promoter.
What is an IRES?

Internal Ribosomal Entry Site

Hey! That looks like me!

What is an IRES?

- Viral Infection
- Nutritional Starvation
- Cell Stress
- Mitosis
- Hypoxia
Motivation

- **Why**
  - The Parts Registry has no IRESs
  - IRESs included in other parts are poorly documented

- **Goals**
  - Introduce IRESs to the Synthetic Biology community because IRESs:
    - Allow for protein expression under one promoter
    - Drastically reduce the size of the construct
    - Reduce likelihood of recombination
  - Create a method of standardizing IRES strength
Traditional mechanism:
Cap dependent translation initiation

- eIF4E
- eIF4G
- eIF3
- eIF4A
- 40S Ribosomal Subunit
- 7MGPPPG
- PABP
- AUG

`AAAAAAAAAAAAAAAAAAAAAAAAA`

5' eIF4E

3' PABP
IRES mechanism:
Cap independent translation initiation
Application

- How can synthetic biologists use IRESs
  
  - reporter genes
    
    - example: pIRES commercial vector
    
    - example: AIDS kittens

Those cats are almost as IRESistible as we are!
Schematic representation of S. cerevisiae BLYES. Estrogenic compounds cross the cell membrane and bind to the estrogen receptor.

# Completed work

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Length</th>
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<tbody>
<tr>
<td>BBa_K813000</td>
<td>YAP1 - Yeast Genomic IRES</td>
<td>164</td>
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<tr>
<td>BBa_K813001</td>
<td>URE2 - Yeast Genomic IRES</td>
<td>167</td>
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<td>BBa_K813002</td>
<td>HAP4 - Yeast Genomic IRES</td>
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<td>pSAP - Yeast Genomic IRES</td>
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<tr>
<td>BBa_K813004</td>
<td>p150 - Yeast Genomic IRES</td>
<td>348</td>
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## Completed work

<table>
<thead>
<tr>
<th>PART</th>
<th>ORIGIN</th>
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<tbody>
<tr>
<td>Backbone (BBa_J63010)</td>
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<td>ADH1 (BBa_J63005)</td>
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<td>cyc1</td>
<td>Trinh Lab</td>
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<tr>
<td>All IRESs</td>
<td>S. cerevisiae genomic DNA</td>
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</table>
Completed work

Completed IRES Characterization Construct
9400 bp
Comparison

Construct with IRES
9,400 bp

Construct without IRES
10,559 bp
Proposed work
Proposed work

- ADH1
- mOrange
- IRES
- GFP
- Cyc

- Relatively weak IRES
- Relatively strong IRES
Proposed work

Hey I don’t work that way!
Conclusions

Problems

- Antibiotic resistance
- ADH1 promoter
- Limited experience with *S. cerevisiae*
- Small team
- Limited resources
Conclusions

What we learned

- IRESs
- Yeast techniques
- BioBrick
- Wiki
- Flow cytometry
- Research project management
Acknowledgments

- The University of Tennessee, Knoxville College of Engineering
- UT-ORNL Joint Institute for Biological Studies
- The University of Tennessee, Knoxville Office of Research
- IDT
- NEB
- The Parts Registry
- iGEM
- Duquesne University
- Dr. Cong Trinh

Thanks!