THE UNIVERSITY of TENNESSEE KNOXVILLE

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

UTK-Knoxville iGEM team
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October 13, 2012

BIG ORANGE BIG IDEAS

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

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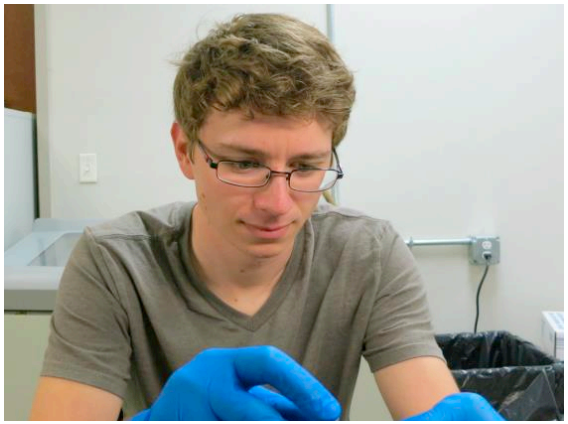
Who we are

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

Genesis Minter

Brandon Wilbanks

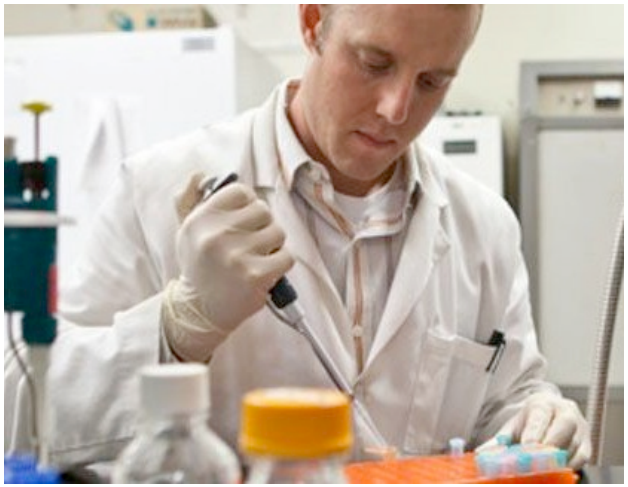
Who we are



Michael Wierzbicki works with *E. coli*

Adam Thompson works with *S. cerevisiae*

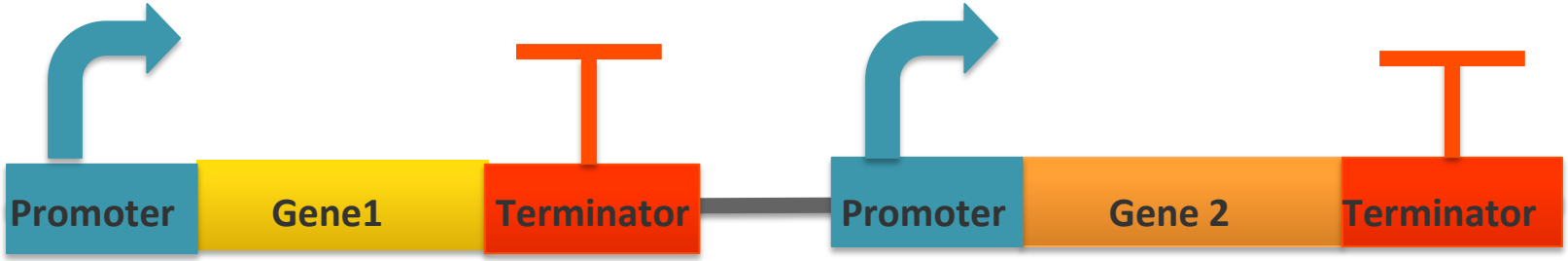
Dr. Cong Trinh was our primary advisor



Dr. Dan Close works with IRESs

Motivation

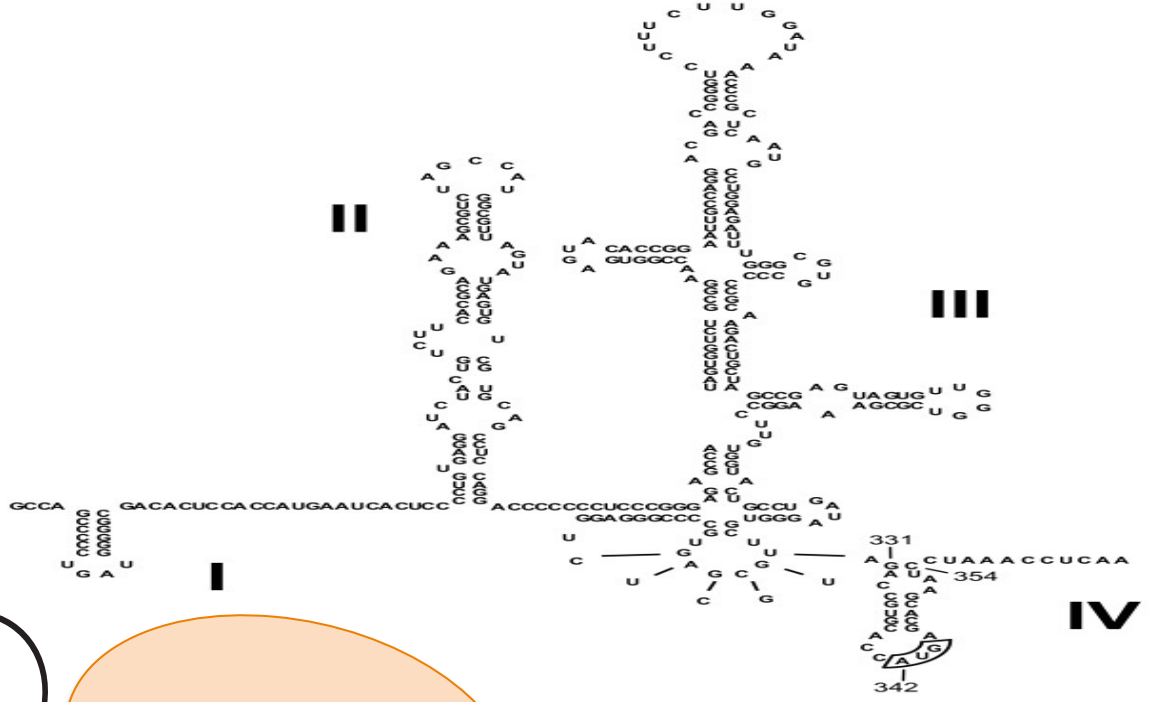
In prokaryotes, multiple genes can be expressed under the control of the same promoter.

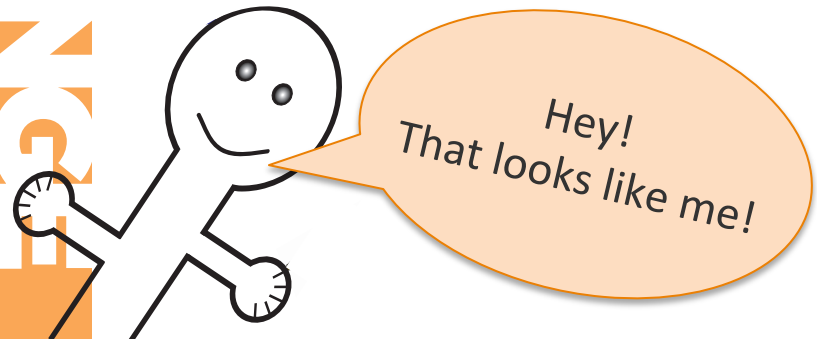


In eukaryotes, each gene must be expressed under the control of its own promoter.

What is an IRES?

Internal Ribosomal Entry Site





Tumban et al. Journal of Negative Results in BioMedicine 2009 8:4 doi: 10.1186/1477-5751-8-4

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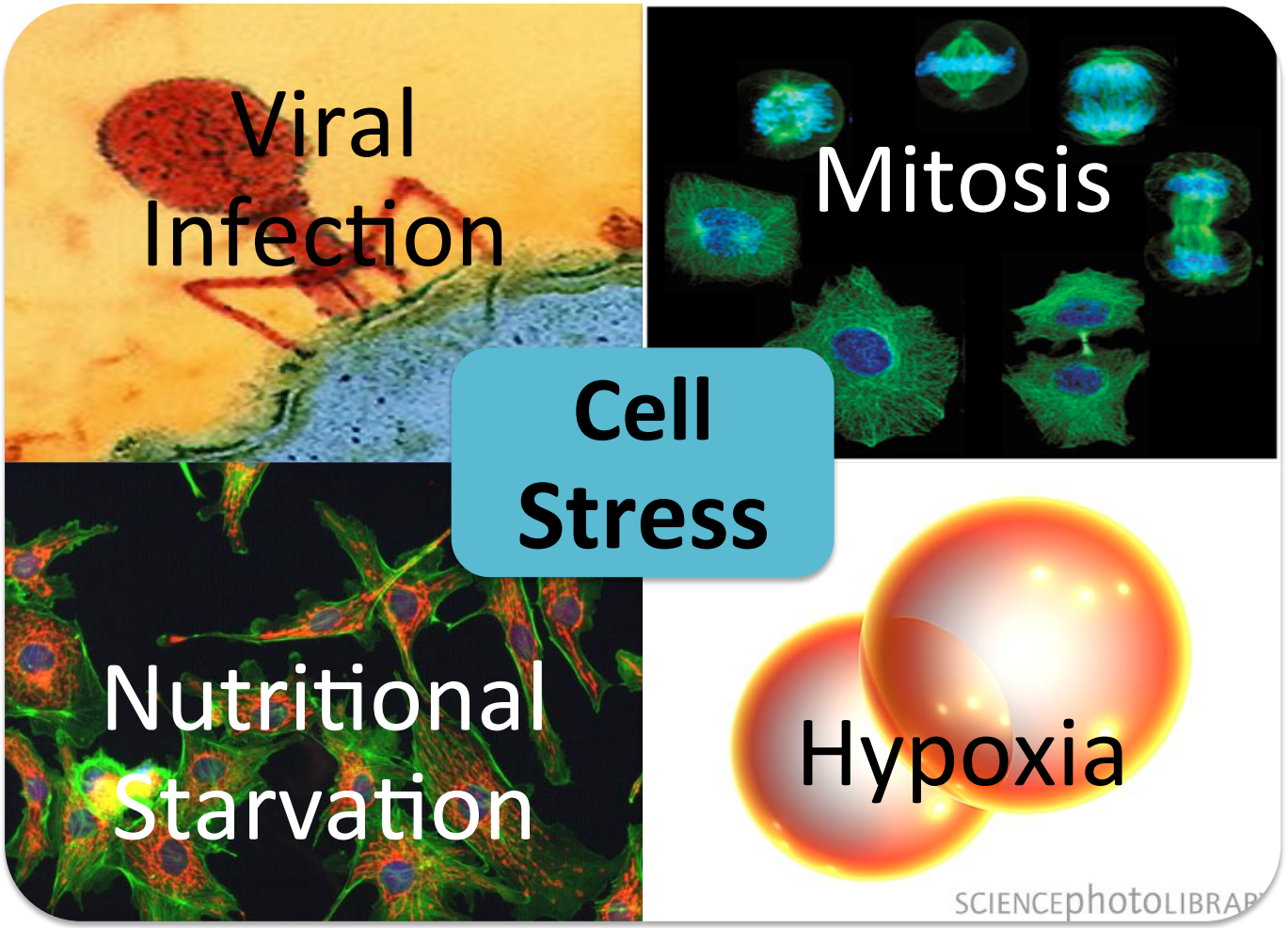
What is an IRES?

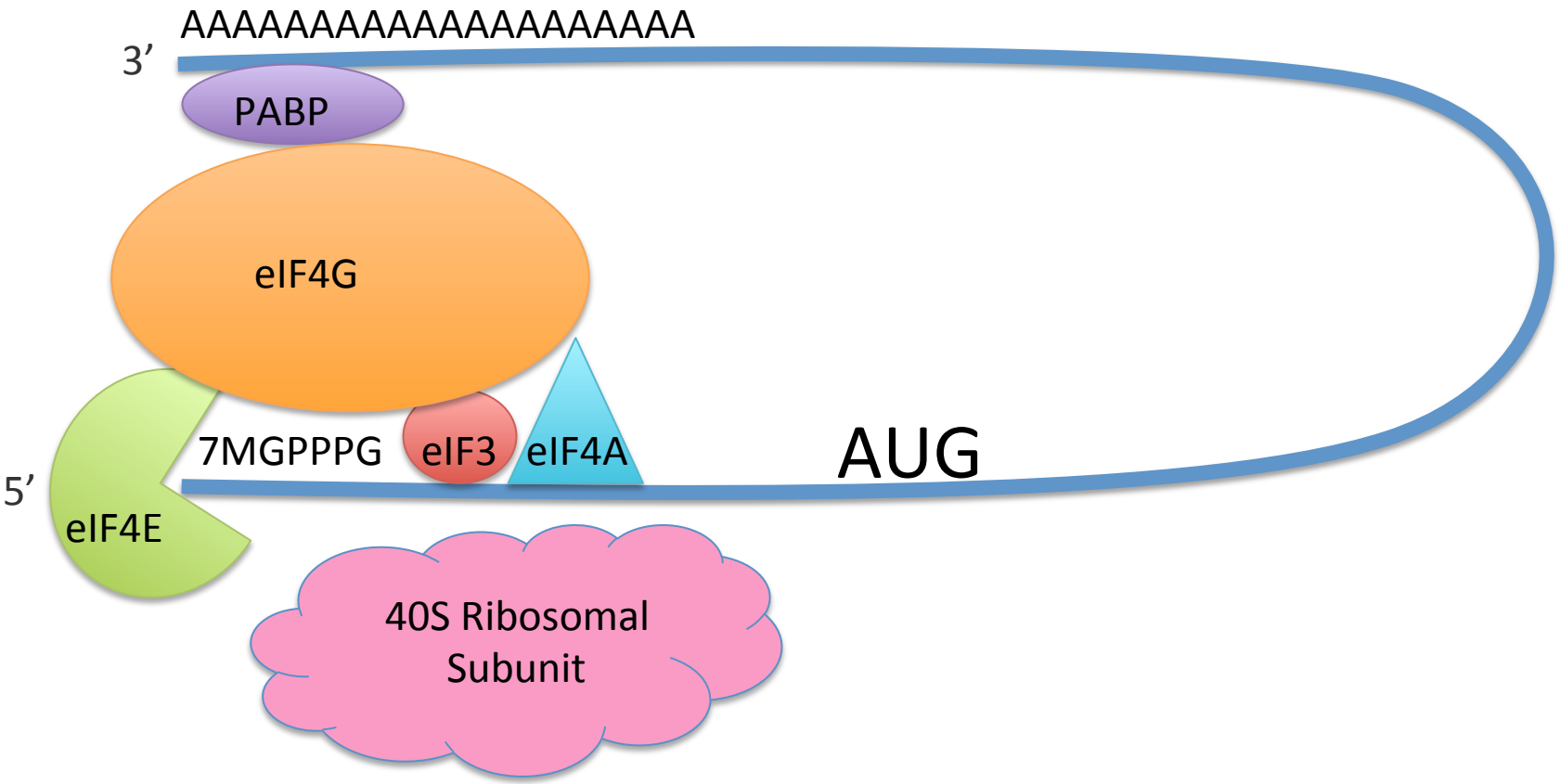


Motivation

- **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

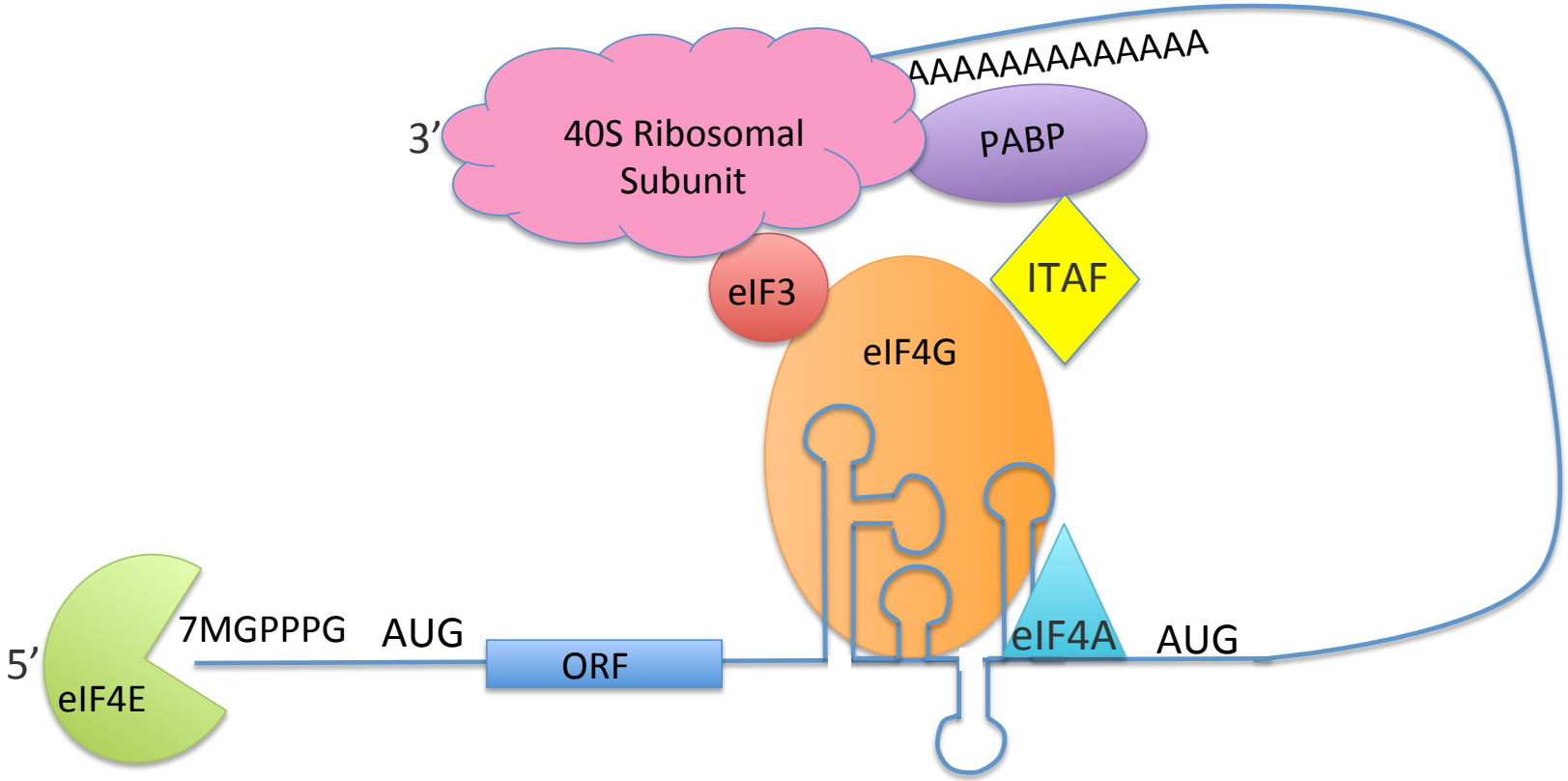
Traditional mechanism:

Cap dependent translation initiation



IRES mechanism:

Cap independent translation initiation



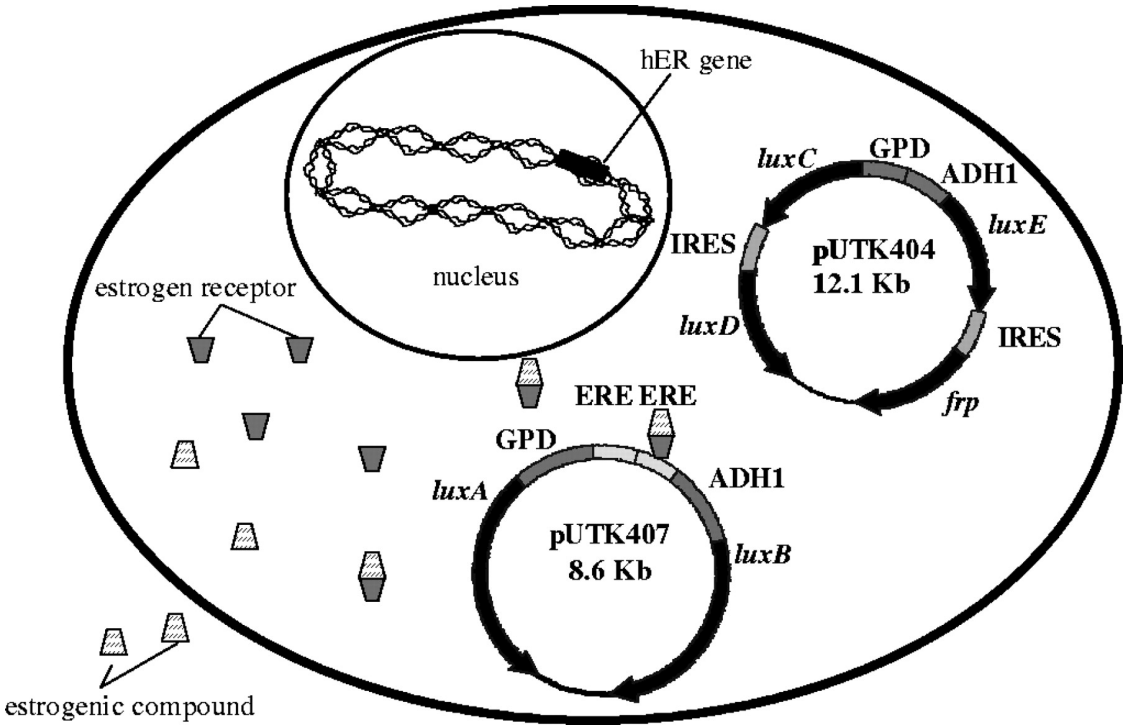
Application

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

Application

Estrogenic Hormone Biosensor

Schematic representation of S. cerevisiae BLYES. Estrogenic compounds cross the cell membrane and bind to the estrogen receptor.



Sanseverino J et al. Appl. Environ. Microbiol. 2005;71:4455-4460

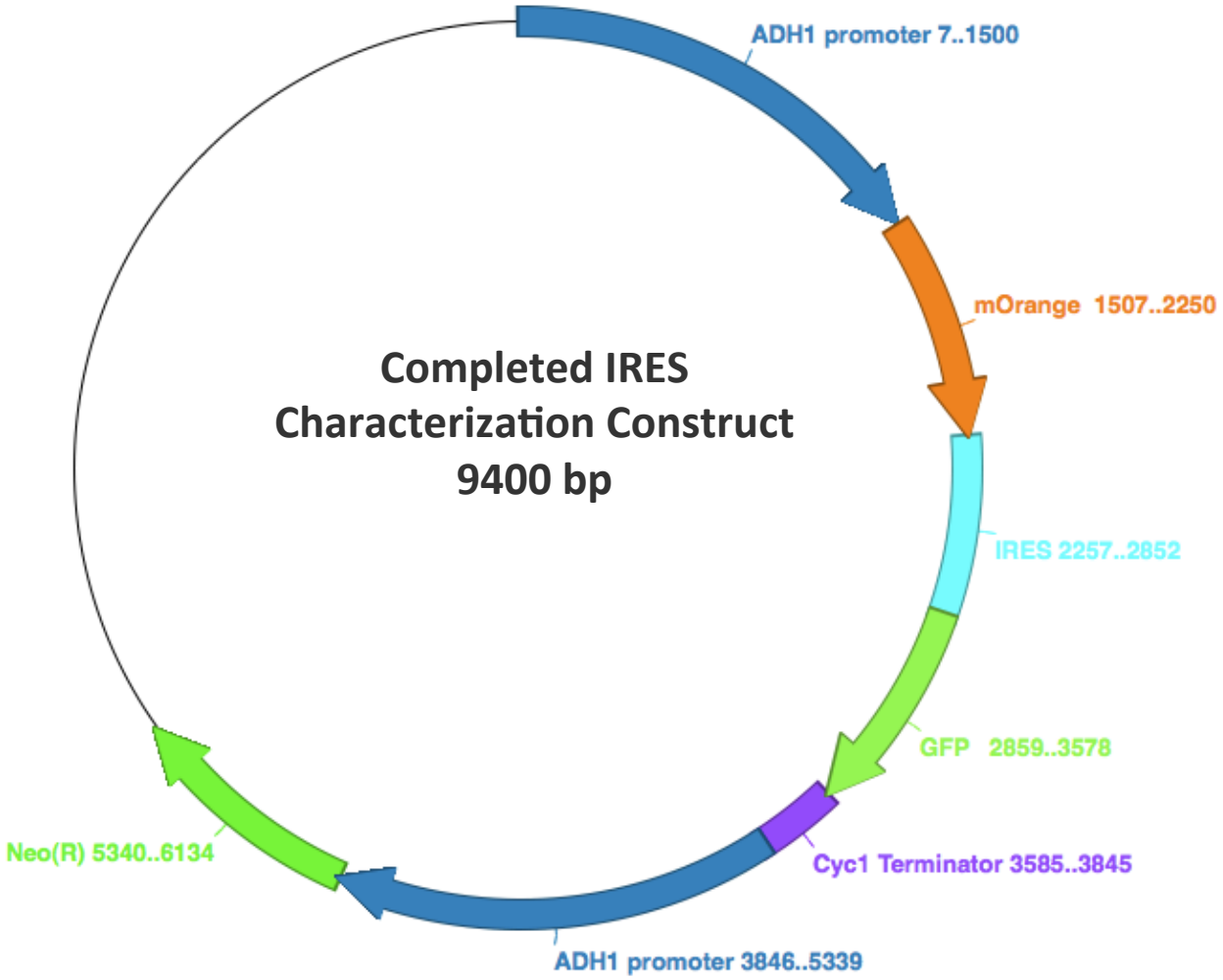
Completed work

Name	Description	Length
BBa_K813000	YAP1 - Yeast Genomic IRES	164
BBa_K813001	URE2 - Yeast Genomic IRES	167
BBa_K813002	HAP4 - Yeast Genomic IRES	270
BBa_K813003	pSAP - Yeast Genomic IRES	528
BBa_K813004	p150 - Yeast Genomic IRES	348

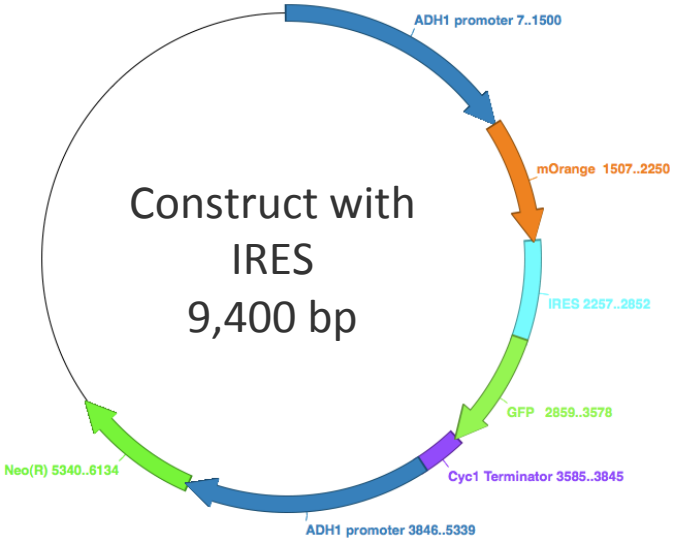
Completed work

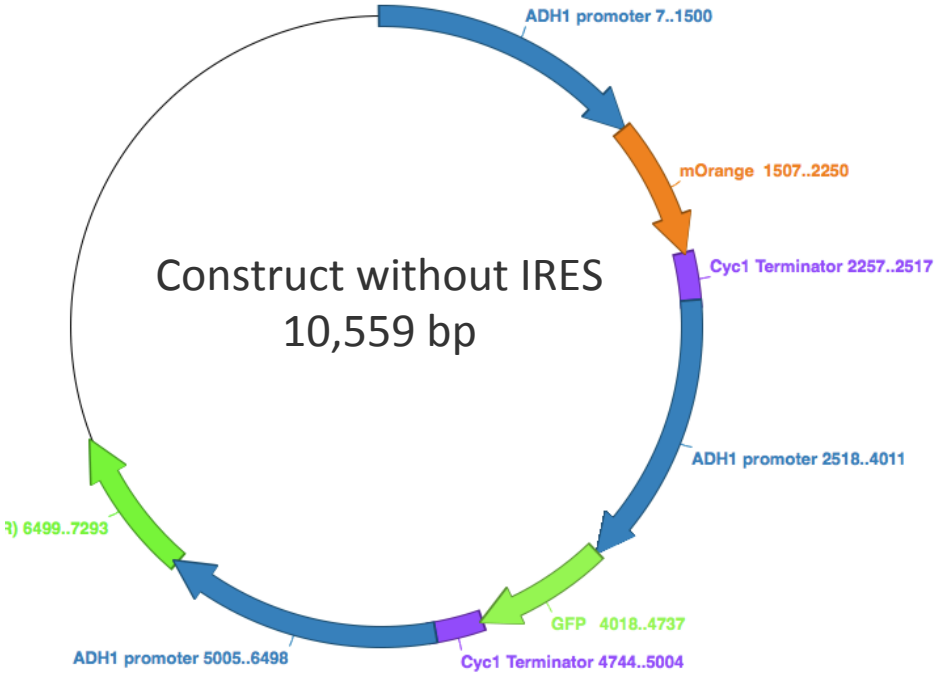
PART	ORIGIN
Backbone (BBa_J63010)	2012 Kit, Plate 1, Well 1C
ADH1 (BBa_J63005)	2012 Kit, Plate 1, Well 1C
mOrange (BBa_E2050)	2012 Kit, Plate 2, Well 13N
GFP (BBa_I13522)	2011 Kit, Plate 2, Well 8A
cyc1	Trinh Lab
All IRESs	S. cerevisiae genomic DNA

Completed work



Comparison





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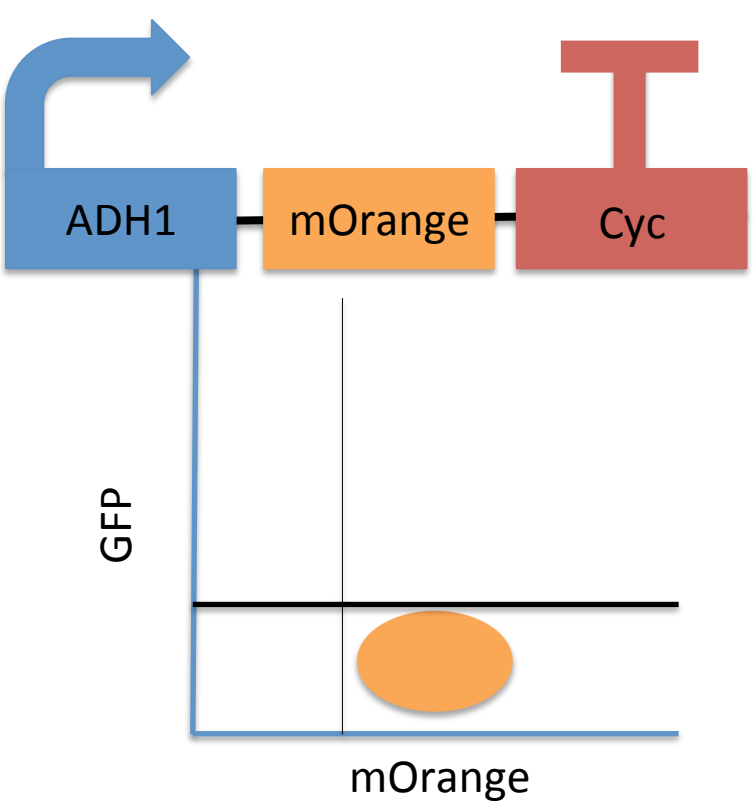
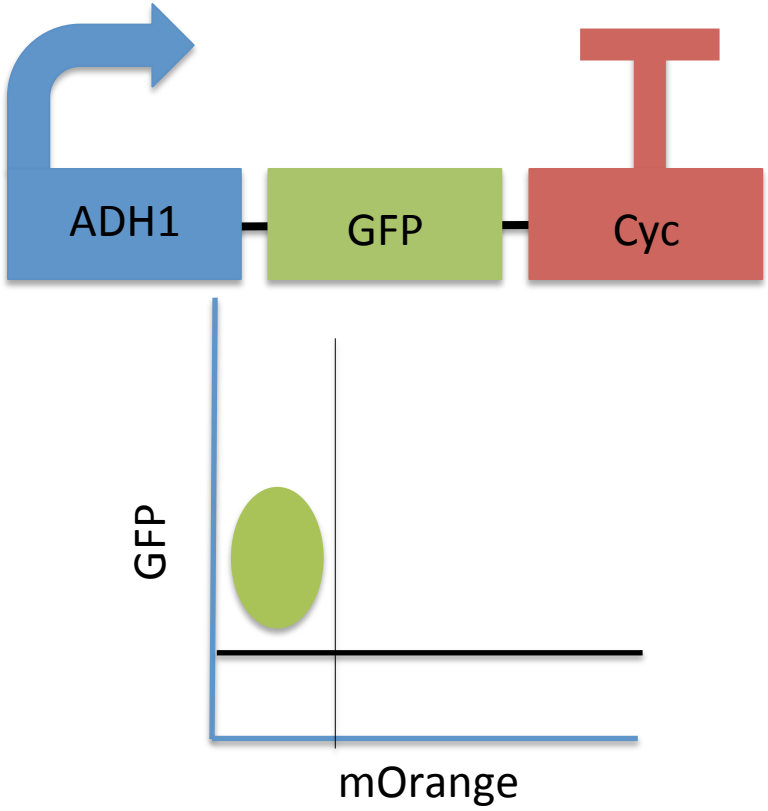
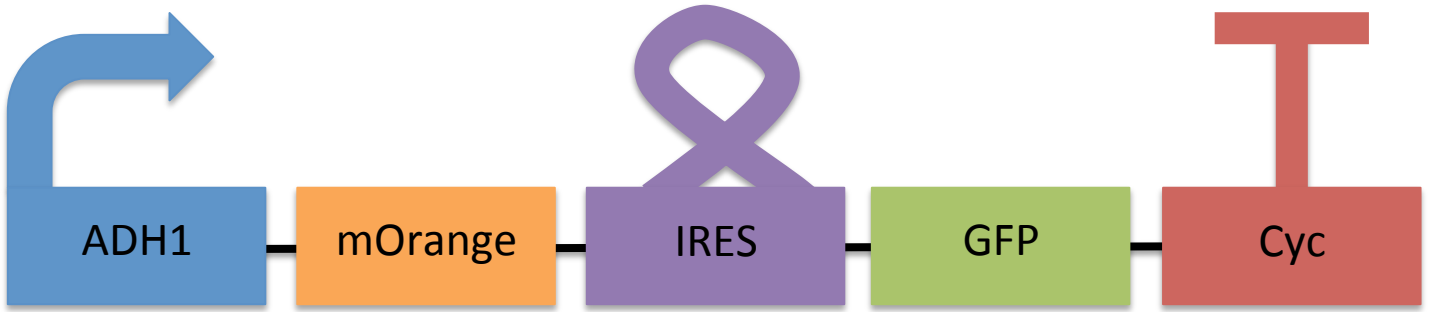
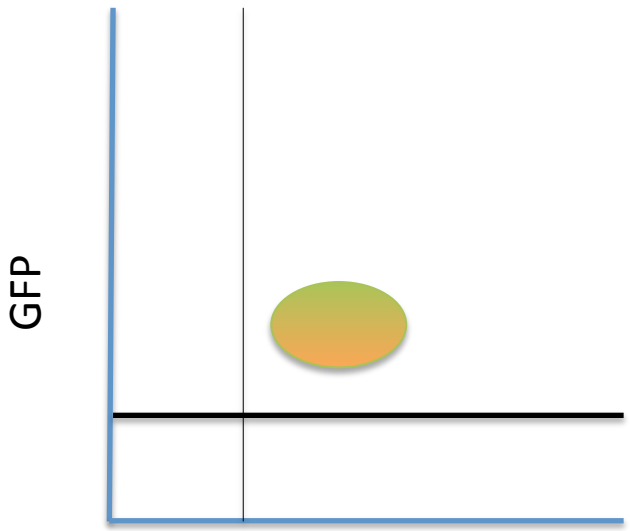
Proposed work



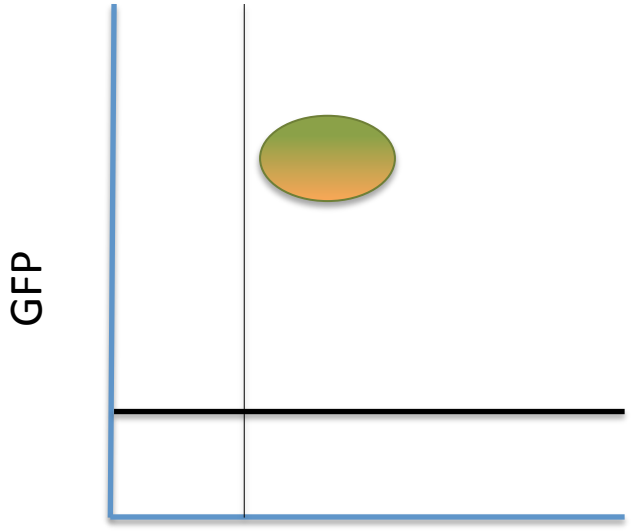


Proposed work



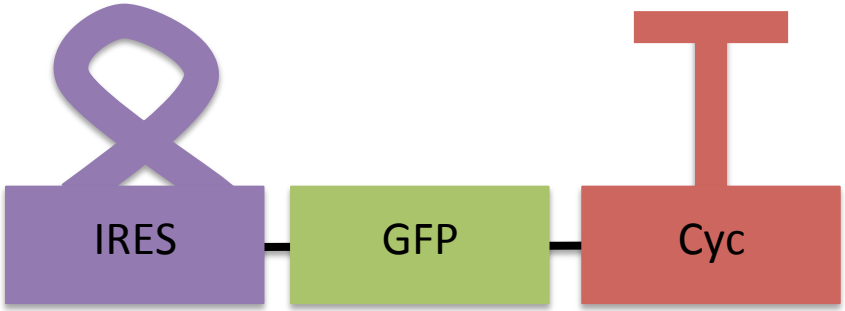


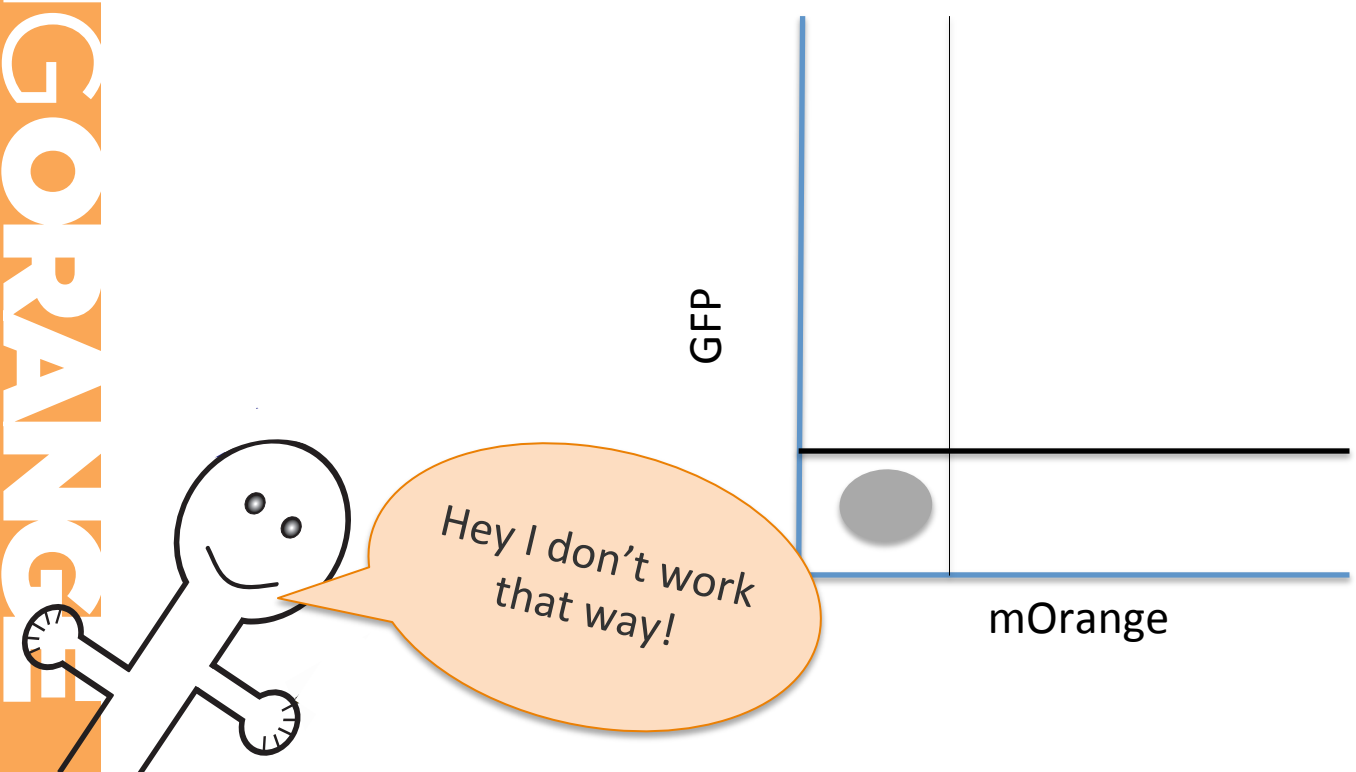
Relatively weak IRES



Relatively strong IRES

Proposed work





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

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