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

iGEM 2012: Team UTK-Knoxville

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Abstract. Internal Ribosomal Entry Sites (IRES) are an important but poorly understood part of the eukaryotic translational machinery, allowing cap-independent translation initiation. Unfortunately, the Registry of Standard Biological Parts contains few IRESs and even those are poorly annotated. In this work, we submitted five IRESs to the registry and plan to characterize them for relative strength. To do this, we will develop a methodology of determining relative IRES strength, as modeled by the methodology put forth by Kelly *et. al* in the Journal of Biological Engineering (Kelly *et. al*, 2001). Using flow cytometry, we will determine the relative levels of fluorescent protein under the control of each IRES, as expressed in *S. cerevisiae*. This work will not only be useful for other projects within our sponsor lab, but will also serve the greater synthetic biology community by initiating the growth of a library of IRESs and a protocol to allow contribution by all members of the community.

MOTIVATION

BACKGROUND:

1. The Parts Registry has a limited number of yeast parts,
2. The Parts Registry has no IRESs,
3. IRESs included in other parts are poorly documented.

GOALS:

1. Introduce IRESs into the Parts Registry,
2. Create a method of standardizing IRES strength.

Comparison of Traditional vs. IRES-mediated Mechanisms of Eukaryotic Translation Initiation

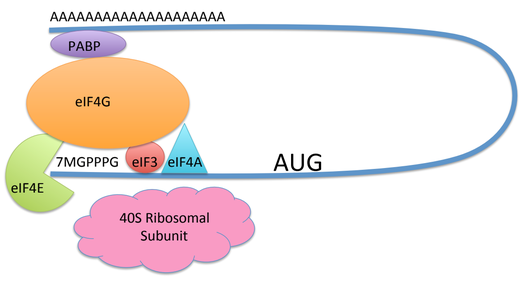


Figure 1: Traditional cap-dependent.

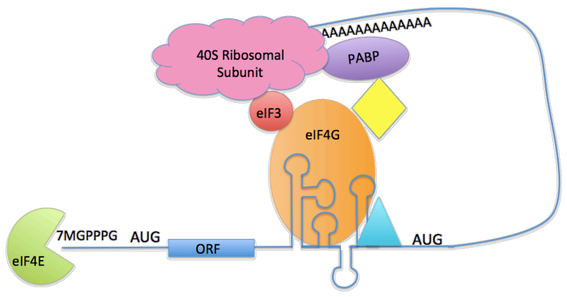
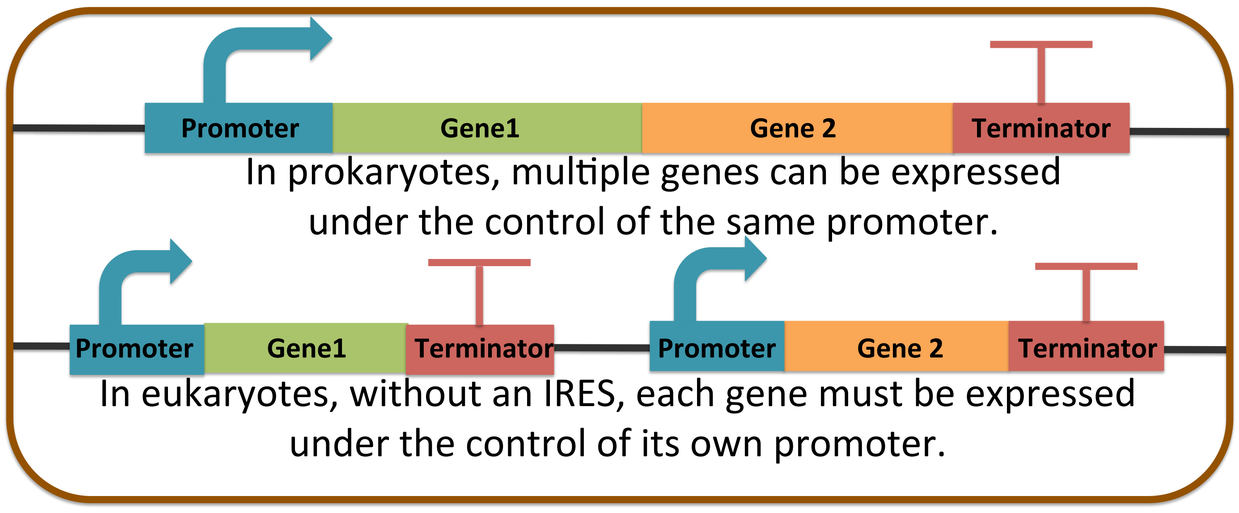


Figure 2: IRES-mediated cap-independent.



METHODS

Figure 4. Origin of parts used in this project:

PART	ORIGIN
Backbone (BBa_J63010)	2012 Kit, Plate 1, Well 1C
ADH (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

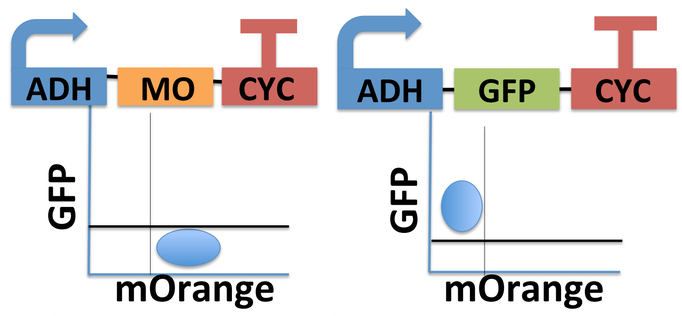


Figure 5. Confirmation of promoter and fluorescent protein functionality.

Figure 6. Proof that IRESs lack promoter activity.

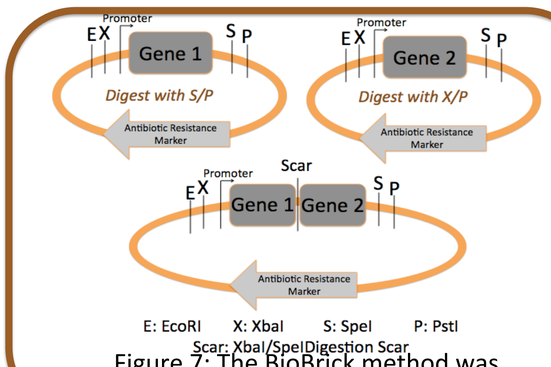


Figure 7: The BioBrick method was used for all cloning.

RESULTS

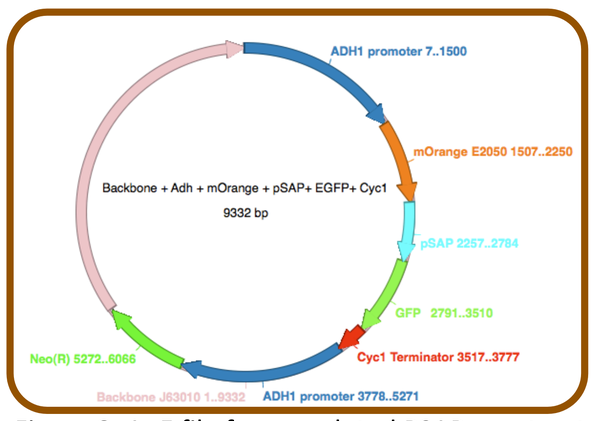


Figure 8: ApE file for completed PSAP construct.

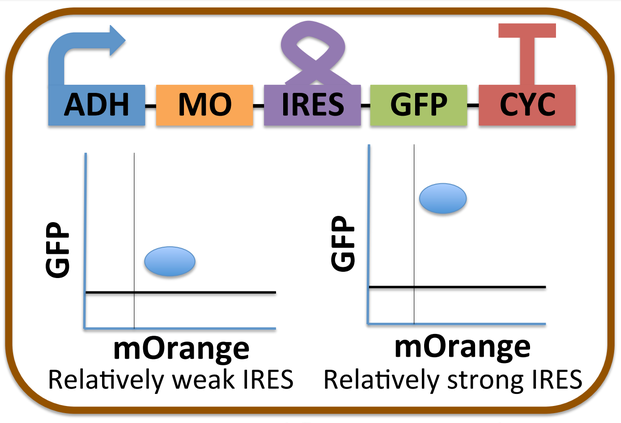


Figure 9. Expected flow cytometry data.

NAME	DESCRIPTION	LENGTH (BP)
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

Figure 10. List of IRES parts submitted to the Parts Registry.

CONCLUSIONS

Problems we encountered:

- Antibiotic resistance
- ADH1 promoter
- Lack of experience with yeast and G418
- Small team
- Limited resources

What we learned:

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

Sponsors

- UTK College of Engineering
- UT-ORNL Joint Institute for Biological Studies
- iGEM
- Duquesne University

NEW ENGLAND BioLabs

IDT INTEGRATED DNA TECHNOLOGIES

References

- Kelly, J. R., Rubin, A. J., Davis, J. H., Ajo-Franklin, C. M., Cumbers, J., Czar, M. J., de Mora, K., et al. (2009). Measuring the activity of BioBrick promoters using an in vivo reference standard. *Journal of biological engineering*, *3*, 4. doi:10.1186/1754-1611-3-4
- Reineke, L. C., Cao, Y., Baus, D., Hossain, N. M., & Merrick, W. C. (2011). Insights into the role of yeast eIF2A in IRES-mediated translation. PloS one, 6(9), e24492. doi:10.1371/journal.pone.0024492
- Zhou, W., Edelman, G. M., & Mauro, V. P. (2001). Transcript leader regions of two Saccharomyces cerevisiae mRNAs contain internal ribosome entry sites that function in living cells. Proceedings of the National Academy of Sciences of the United States of America, 98(4), 1531–6. doi:10.1073/pnas.98.4.1531