A New Set of Synthetic Biology Tools for *Saccharomyces cerevisiae*

Elaine Chang[1], Amanda Hay[2], Zachary Knudsen[1], Brian Landry[1], Brenden McDearmon[1], Alice Meng[1]

1. Department of Biomedical Engineering; Washington University School of Engineering, 2. Department of Biology; Washington University College of Arts and Sciences,
All authors contributed equally to this research

Abstract

- There are few available synthetic biology tools for *S. cerevisiae*
- A new synthetic alternative splicing tool was designed
- The Sex-lethal protein from *Drosophila melanogaster* was used to control 3′ splice site choices
- During this project, new BioBricks were designed and synthesized to ease yeast transformations
- A positive selection marker BioBrick was created
- Two BioBrick vectors were designed to conduct homologous recombination in *S. cerevisiae*

Background

Sex-lethal (SxL)

- Extensively characterized as a splicing regulatory protein in *D. melanogaster*
- Influences splicing by competitively inhibiting the binding of U2 auxiliary factor (U2AF)[1]
- U2AF recruits the U2 snRNP to the 3′ splicing site, a step necessary for spliceosome formation
- SxL binds to a specific form of the poly(Y) U2AF binding site
- SxL preferentially affects the choice of 3′ spliceosome binding, producing altered mRNA and protein[2]

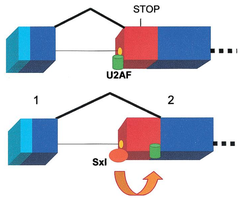


Figure 1: SxL mechanism[3]

Yeast BioBrick Parts

- BioBricks are standardized DNA blocks with defined structure and function that can be manipulated in a consistent manner
- They may be linked to construct new synthetic biological systems
- Currently there are no BioBrick positive selection markers for *S. cerevisiae*
- No existing BioBrick plasmids facilitate chromosomal integration into the *S. cerevisiae* genome

Background

Chromosomal Integration

- *S. cerevisiae* natively has high levels of homologous recombination[4]
- Chromosomal integration creates stable yeast transformants, as DNA inserts are lost at a rate of only 10^{-3} to 10^{-4} per generation[5]
- Transformation efficiencies are nearly equivalent to plasmid transformations[6]

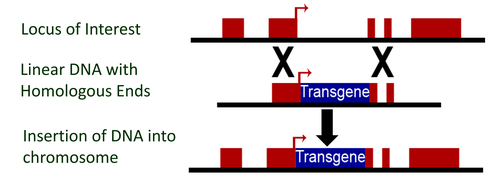


Figure 2: Homologous Recombination

Project Design

Inducible SxL

- SxL was placed under control of the GAL1 promoter

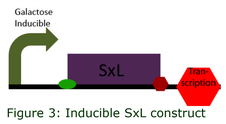


Figure 3: Inducible SxL construct

1. Proof of Concept

- Splicing with SxL results in mRNA containing only YFP
- Splicing without SxL results in mRNA containing only CFP

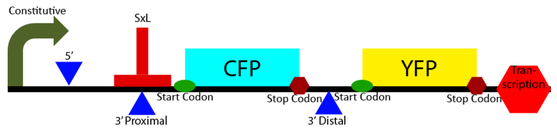


Figure 4: Proof of concept construct

2. Isoform Engineering

- 200bp 5′ fluorescent protein region is alternatively connected to a 3′ region of CFP or YFP.
- Splice site choice influenced by SxL

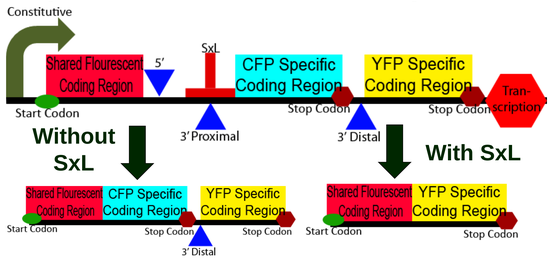


Figure 5: Isoform engineering construct

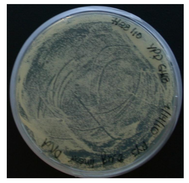
Methods

- DNA constructs were produced by a combination of gene synthesis and BioBrick manipulation
- Chromosomal integration was achieved using homologous recombination
- Positive selection on geneticin and nourseothricin was used
- Double transformants were achieved through yeast mating
- Splicing activity was measured using fluorescence microscopy
- BioBrick parts and plasmids were synthesized by two successive rounds of PCR

Results

Alternative Splicing Tool

- All three designed constructs were synthesized
- Positive selection of a plasmid was achieved in yeast using geneticin



Figrue 6. Yeast transformed with the Kan′ gene conferring resistance to geneticin

S. cerevisiae BioBricks

- A standard bacterial BioBrick plasmid was designed to conduct chromosomal integration into yeast
- 40bp homologous regions to the Ura3 and His3 loci were used, resulting in Ura- and His- knockouts
- BbsI linearizes the plasmid with non sticky ends to improve efficiency[7]
- Plasmid contains bacterial ampicillin and tetracycline resistance and a high copy replication origin

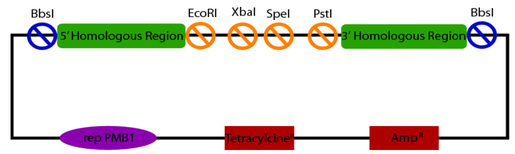


Figure 7. Designed BioBrick vector to facilitate chromosomal integration into *S. cerevisiae*

Results

Fluorescence Techniques

- Established analytical methods to measure splicing activity
- Fluorescence spectroscopy was used in conjunction with ImageJ software[8]

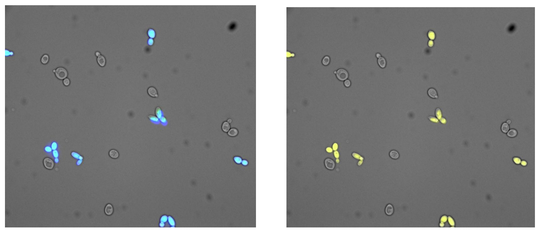


Figure 8. Fluorescent spectroscopy images of control CFP/YFP yeast cells in the CFP (left) and YFP (right) channels

Future Directions

- Implementation of synthesized splicing construct
- Synthesis and characterization of the designed BioBrick plasmids
- Construction of ready-made positive selection BioBrick plasmids
- Application of splicing in solving combinatorial mathematical problems similar to iGEM Davidson 2006
- Alternative isoform applications such as the dynamic modulation of enzyme specificity in biofuel production

Acknowledgments

This research was funded in part by Sigma-Aldrich Co., the Washington University Office of Undergraduate Research, the Washington University Department of Biology, the Washington University School of Engineering, the Summer Undergraduate Research Fellowship, the Howard Hughes Medical Institute, the McKelvey Scholars Program, the Washington University Career Center, and the Tang Lab.

The authors would like to thank the iGEM organization, Dr. Barak Cohen and the Cohen Lab, Dr. Gautam Dantas and the Dantas Lab, Dr. Joseph Jez and the Jez Lab, Dr. Yinjie Tang and the Tang Lab, Lawrence Page, Carol Kohring, Jacob Rubens, and the Horabin Lab for supplying antibodies.

References

1. Merendino et al., 1999. Inhibition of msl-2 splicing by Sex lethal reveals interaction between U2AF35 and the 39 splice site AG, *Nature* v.202 p.838-841
2. Bell et. al. 1988. Sex-lethal, A Drosophila Sex Determination Switch Gene, Exhibits Sex-Specific RNA Splicing and Sequence Similarity to RNA Binding Proteins, *Cell*, v. 55, 1037-1046.
3. Penalva and Sanchez, 2003. RNA Binding Protein Sex-Lethal (Sxl) and Control of Drosophila Sex Determination and Dosage Compensation. *Microbiology and Molecular Biology Reviews*, p. 343–359
4. Rattray et al., 1995. "Multiple Pathways for Homologous Recombination in Saccharomyces cerevisiae." *Genetics Society of America*. 139. 45-56.
5. F. Sherman, 1998. An Introduction to the Genetics and Molecular Biology of the Yeast *Saccharomyces cerevisiae*.
6. Swers et al., 2004. Shuffled antibody libraries created by in vivo homologous recombination and yeast surface display. *Nucleic Acids Research*. 32.3
7. Orr-Weaver et al., 1983. Multiple, Tandem Plasmid Integration in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* , 747-749.
8. ImageJ software. U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2010.