Regulation of the Splicing Factor SRSF10 by Alternative Splicing of an Ultraconserved Exon

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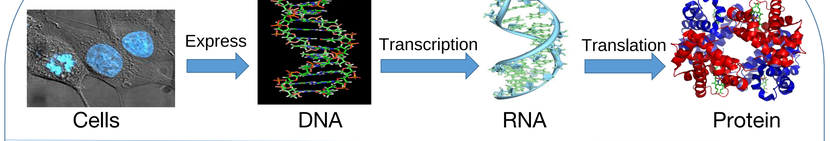
2016 Transfer-to-Excellence Research Experiences for Undergraduates Program (TTE REU Program)

AGTCACCATC CATGTCTCTTCTCACCACCCTCT GAATCTGCATTAGCCAGTCAACTAGCCCTTTCAGCGTCATGTGCC
endless forms most beautiful and most wonderful have been and are being evolved from so simple a beginning

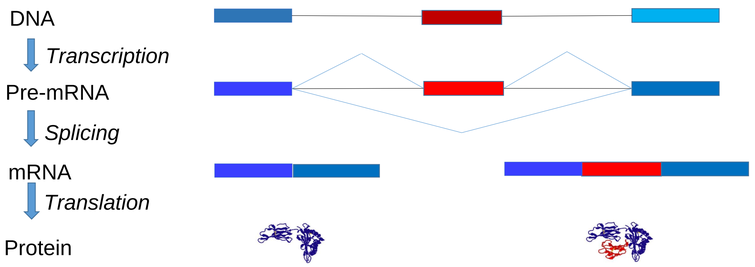
Abstract

During RNA processing, protein factors are often used as a way for cells to manipulate gene expression by regulating mRNA isoform abundance via alternative splicing. The SRSF10 gene sequence, a splicing factor linked to proper cell development in the brains of frogs, has an alternative exon that has been highly conserved over the evolution of-animals. This region is thought to be of crucial importance to the cell because of its apparent mutation sensitivity. A plasmid construct containing a part of the SRSF10 gene was cloned and transfected into human embryonic kidney (HEK) cells to measure mRNA isoform abundance after gene expression for eight hours. A library of mutagenized plasmids was created to then study the effects of single-point mutations on alternative splicing of the SRSF10 reporter to better understand this regulatory mechanism.

Central Dogma

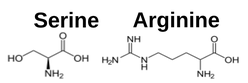


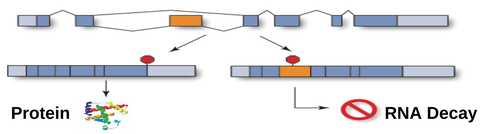
Alternative Splicing

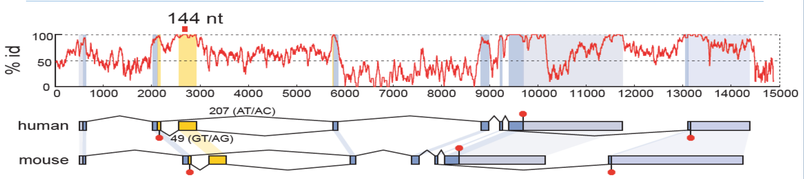


SRSF10: A Serine-Arginine (SR) Splicing Factor

- Splicing factors (**SF**) regulate alternative splicing
- SF production is also regulated by alternative splicing
- **SRSF** family shares commonalities: Serine/Arginine rich, highly-conserved 'poison exons'

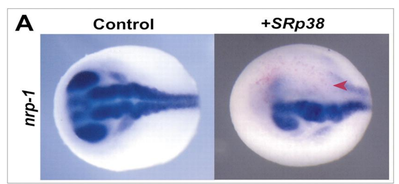




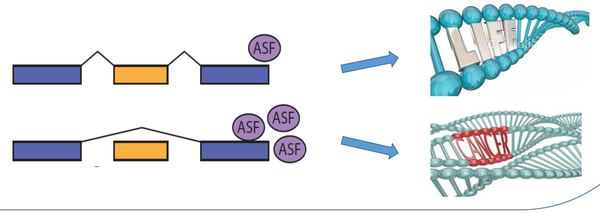


The Importance

- Overexpressing SRSF10 disrupts brain development in frog embryos

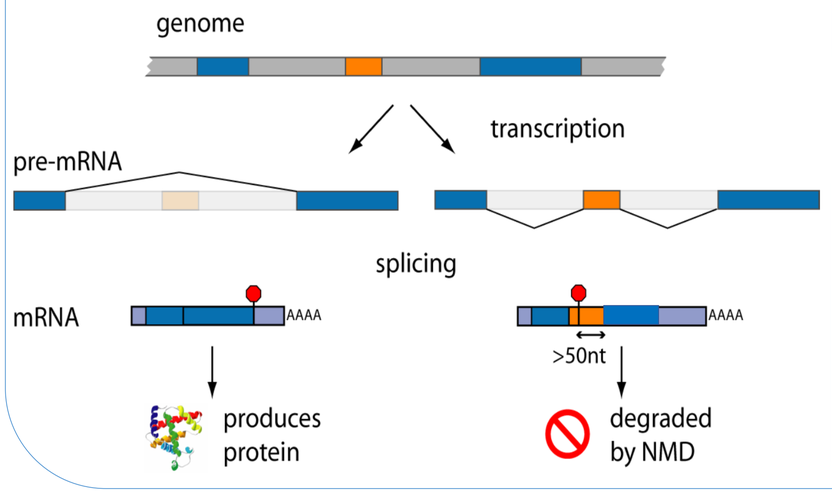


- Improper regulation of splicing can lead to cancer

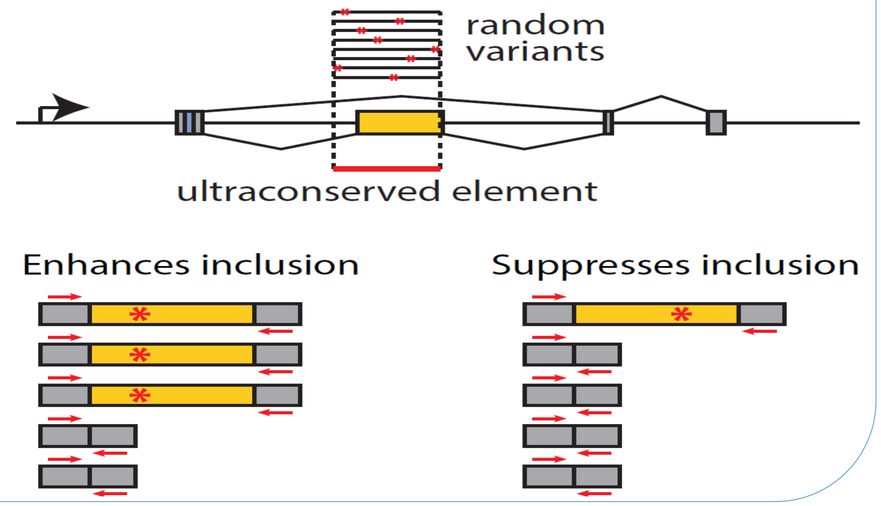


Objective

- Isolate SRSF10 reporter to recreate splicing event
- Measure mRNA isoform ratio

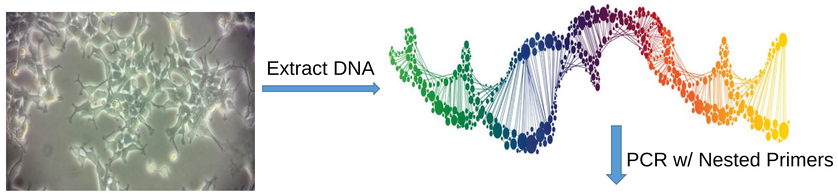


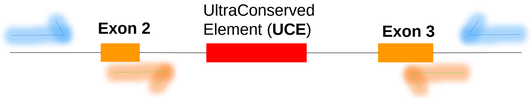
- Create mutant library of conserved 'poison exon' to measure effects on isoform abundance



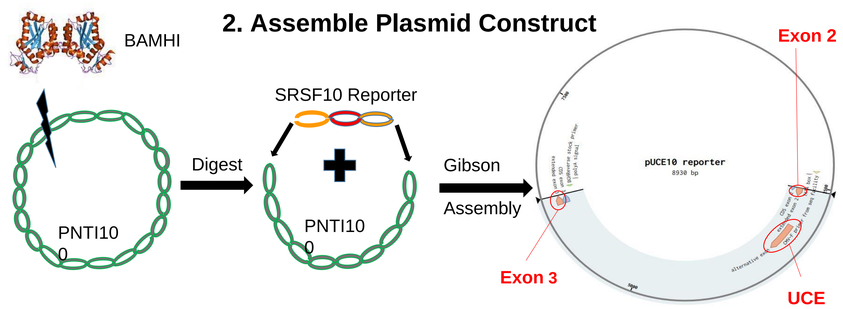
Methods

1. Isolate SRSF10 Reporter

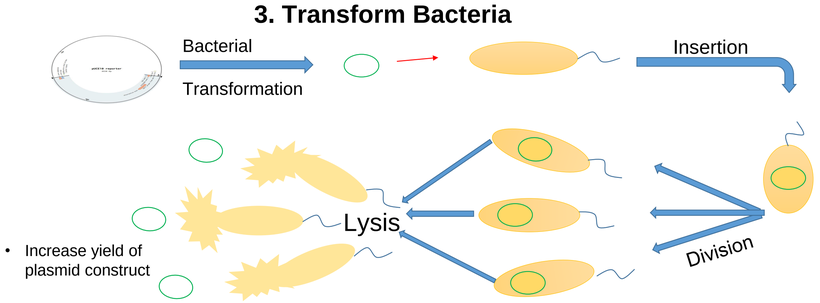




2. Assemble Plasmid Construct

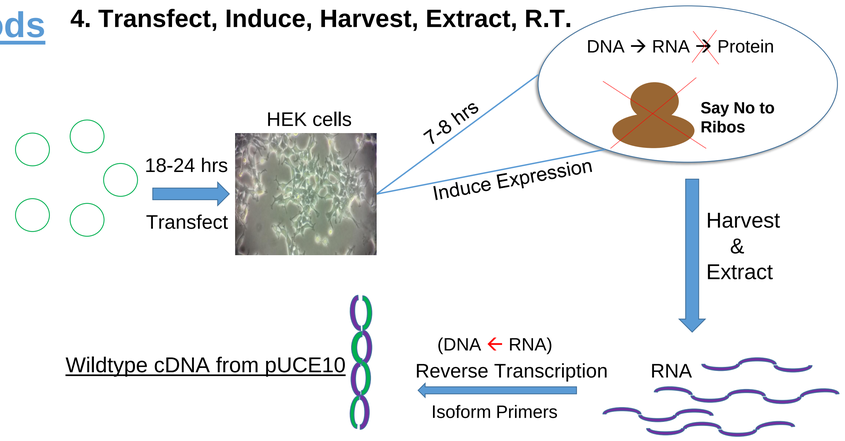


3. Transform Bacteria



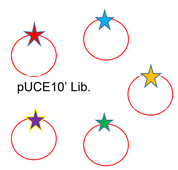
- Increase yield of plasmid construct

4. Transfect, Induce, Harvest, Extract, R.T.



5. Single-Point Mutagenesis of 'poison exon'

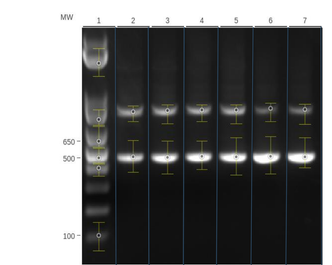
- Repeat steps 3 and 4 to increase variability of mutant library and gather mutant cDNA for analysis.



Data Analysis & Conclusion

- Recapitulated natural splicing event
- Measured isoform ratio of Wildtype (Wt) and Mutant library (M)
- 500 ng of Wt plasmid lowers long isoform abundance as compared to 250 ng of Wt.
- Mutant library lowers long isoform abundance even more

Future Experiments:
- Create larger mutagenized library
- Purify longer isoforms for RNA-Seq



% Exon Included	37	33	29	29	15	17
	Wt250 ng		Wt500 ng		M250 ng	

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Support Information
This work was funded by National Science Foundation Award ECCS-1461157 & ECCS-0939514

References
1. Lareau, L.F., Inada, M., Green, R.E., Wengrod, J.C., Brenner, S.E. (2007). Unproductive splicing of SR genes associated with highly conserved and ultraconserved DNA elements. *Nature. Vol. 446. Pages 926-929.* doi: 10.1038/nature05676
2. Lewis, B.P., Green, R.E., Brenner, S.E. (2002). Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. *PNAS. Vol. 100. Pages 189-192.* doi: 10.1073/pnas.0136770100

Acknowledgements
I'd like to thank all my colleagues at the Ingolia/Lareau lab, all of my fellow TTE REU interns, and of course the E3S REU staff