Bacterial Decoder

University of Illinois iGEM 2009

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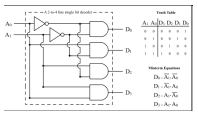
Illinois iGEM

Synthetic Biology hopes to create ideas and constructs using the vast knowledge compiled on biological entities. The University of Illinois iGEM team is a *student-run* team of undergraduates who have worked with bacterial genetic circuitry in efforts to create a novel regulation mechanism in *E. Coli.*

Goal: Cell Decoder

A decoder is a logic construct, that allows for the de-processing of coded information. According to which permutation of the possible combination of binary inputs, the decoder activates a specific and unique location.



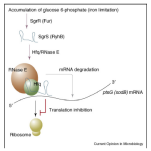
A 2:4 Decoder

In a bacterial cell, it is rare to find a regulation mechanism that functions similar to a decoder.

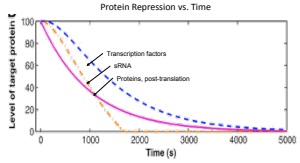
Approach: Regulatory RNA

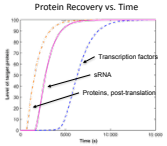
In all three domains of life, regulatory RNA plays a major role in gene regulation. A specific class of regulatory RNA in bacteria was chosen to pursue the composition of the bacterial decoder: negatively regulating, Hfq-binding, *trans*-encoded sRNA.

By simple antisense interaction, these sRNAs bind to their target mRNA transcript, and inhibit translation by recruiting a Degradosome complex and/or Occluding ribosome binding.



sRNA Advantages



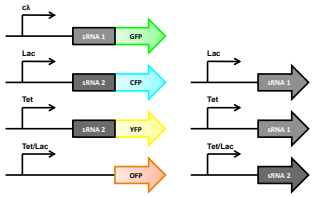


REFERENCES

"Regulation of gene expression by small non-coding RNAs: a quantitative view", Yishai Shimoni, Gilgi Friedlander, Guy Hetzroni, Gali Niv, Shoshy Altuvia, Ofer Biham & Hanah Margalit, 2007

Urban JH, Vogel J. Translational control and target recognition by Escherichiacoli small RNAs in vivo. Nucleic Acids Res. 2007;35(3):1018-37. Epub 2007 Jan 30.PubMed PMID: 17264113.

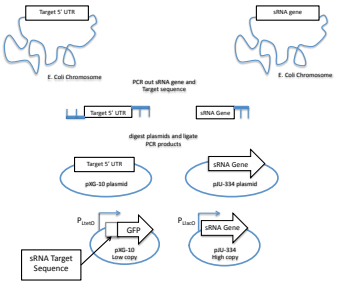
Proposed Decoder



We propose a 2-to-4 binary decoder schematic using two small RNAs, an IPTG-induced promoter, an aTc-induced promoter, a constitutive lambda promoter, a hybrid promoter activated by both IPTG and aTc, and four fluorescent protein outputs.

When neither IPTG nor aTc are present, only GFP is produced. When only IPTG is present, CFP is produced, and sRNA1 is transcribed to prevent translation of GFP. Similarly, when only aTc is present, YFP is produced, and sRNA1 is again transcribed to prevent translation of GFP. When both IPTG and aTc are present, OFP is produced, and both sRNA1 and sRNA2 are transcribed to prevent translation of the other three fluorescent proteins.

Method



In order to test sRNA gene regulation, we synthesized both the sRNA gene and its target sequence by PCR using the E. coli chromosome as a template. We then digested and inserted the sRNA gene sequence into a high-copy plasmid and transformed the plasmid into E. coli Top10 cells. The target sequence of the sRNA was also digested and placed before a GFP reporter gene in a low-copy plasmid. This plasmid was transformed into E. coli Top10F' cells. Finally, we co-transformed both of the plasmids into E. coli Top10F' cells. We cultured these cells for two hours, and then took fluorescence readings to measure the amount of sRNA repression on the GFP reporter gene.

Results

We were able to submit 14 novel BioBricks to the Registry their respective target sequences. When fully induced by IPTG we found regulation levels to be be greater than the un-induced system by a factor of 20 . Below is the MicF and OmpF target sequence pair repressing expression of a GFP molecule.

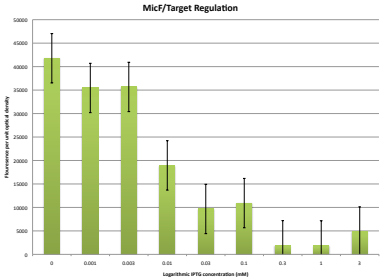


Plate readings were taken over 9 IPTG concentration levels ranging from 0mM to 3mM. 9 samples of each concentration were used to gain an accurate measurement of the fluorescence levels. These levels have been normalized by the optical density at the time of the measurement and plotted on a logarithmic scale. Standard error bars are included.

BioBrick Construction

Construction of the SRNAs

Because sRNA molecules are short and form higher order structures our sRNA BioBricks were designed to keep a blunt end on the 5' site so as not to interfere with folding and binding events. To do so while adhering to the assembly standards each sRNA was ligated to the Lac promoter so future synthetic biologists may use them while adhering to iGEM standards. Typically sRNA molecules are the only genes to be expressed under a promoter so this setup should not limit the BioBricks use or application in the future.

Construction of the sRNA Target Sequences

The target sequences were introduced into the plasmids and submitted using the Freiburg standard. The Freiburg standard creates six base pairs of scar coding instead of eight base pairs. This was important to our project because it kept the GFP in frame with the target sequence (which contains the RBS and start codon). Otherwise, the GFP would be shifted by two base pairs resulting in an insignificant, meaningless string of amino acids instead of GFP. Although the Freiburg standard typically calls for the use of a specific backbone, we were able to use the standard BioBrick backbone with this construct.

Normal	Freiburg Assembly Standard
...ACtactagagCA...	...ACtactagCA...
8bp → Frame shift	6bp → No frame shift

Discussion

Our results indicate that small RNAs can be used successfully to control gene expression. Repression via sRNAs is effective for use in an engineered genetic circuit and it can be advantageous to use them over transcriptional regulators due to the rate of protein expression and recovery. We hope that future iGEM teams will use the BioBricks we have submitted when designing repressor systems or when implementing more complex logic systems in bacteria.

BioBricks

Conclusions

Over the course of our project, we have:

- Constructed 6 sRNA-coding genes and 8 sRNA target sequences and submitted them to the Parts Registry as BioBricks.
- Verified that a sRNA is able to cause repression of a gene of interest by taking fluorescence readings of cells transformed with both an sRNA gene and the corresponding sRNA target sequence-GFP construct.
- Introduced genes and corresponding target sequences for negatively regulating, Hfq-binding, *trans*-encoded sRNA molecules as a new class of BioBricks within the registry.

Acknowledgments

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