Welcome to the Duke iGEM 2010

Duke Engineering: Where the Magic Happens
Developing a Modular Regulatory Toolkit Using Protein Sequestration

Duke iGEM 2010

• High Schooler: Stefano Fenu

• Undergrads: Kevin Chien, Nicholas Tang, Peter Zhu

• Advisors: Nicolas Buchler, Jingdong Tian
Background

Problems with modern bistable switches:

- Promoter "leakiness" can impact efficiency
- Basal noise can produce signal errors
Goals

- Removal of basal signal expression
- Noise removal
- Fast-acting response to stimulus
- Tunability through library generation
Design

- **Protein sequestration**
  - Can generate drastic response to stimulus
  - Basal signal production can be “buffered out”
  - Ultrasensitivity can be produced
Background

- **Leucine Zippers (bZIPS)**
  - Eukaryotic DNA specific—will only affect recombinant DNA in bacteria
  - DNA binding only occurs when bZIPs are dimerized
Background

- Dominant negatives of bZIPs
- Competitive inhibition
- Higher binding affinity
Design

c-Fos and c-Jun leucine zippers

Bind to dominant negative

pC-Fos/CJun Promoter

Dimerize and bind to cI promoter modified with AP-I binding site
Design
Design

- Heptad repeats of leucine determine specificity
  - Alteration of inter-heptad base pairs or heptad interchanges can generate uniquely specific novel leucine zippers
Novel Promoter

- cl promoter:
  taacaccgt gcgtttgactatttt tacctctgg cggtgataatgttgc

- AP-1 Promoter, also called c-Fos/c-Jun promoter
  ctgactcat gcgtttgactatttt tgacgtca cggtgataatgttgc

- AP-1 Binding Sites:
  ctgactcat
tgacgtca

- cl repressor binding sites:
  tacctctgg
tacctctgg
BioBricks

- **K429000**
  - Promoter testing device

- **K429001**
  - Promoter testing device
Next Step

- Generating libraries of leucine zippers and their dominant negatives
- Incorporation of bZIP switches into existing gene networks
Future Applications

- **Signal amplifier**
  - Generated by ultrasensitive feed-forward loop

- **Higher order logic functions**
  - Uniquely specific bZIP switches can be concatenated indefinitely
High Throughput Expression Screening

- Obtain time lapse expression information from LacZ and GFP expressing colonies in solid culture.
- Efficiently and automatically extract, evaluate, and correlate growth and expression information.
- Goals:
  - Match the high throughput of DNA synthesis machines.
  - Address bottlenecks such as limited sample size of fluorometry and specificity of flow cytometry.
Codon Optimization

- Each amino acid has redundant codons. It is acknowledged that optimal codons help achieve increased protein expression.
- A laboratory DNA synthesizer was used for degenerate oligonucleotide synthesis of gene fragments. By substituting a random base pair every third base pair in the sequence, a combinatorial library of codon variants is generated.

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<td>CCA Pro</td>
<td>CAU His</td>
<td>CGU Arg</td>
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Experimental Setup (GFP)

- A blue light transilluminator and 535nm discriminating filter was optimized for GFP.
- A computer controlled DSLR Camera was for high resolution imaging.
Experimental Setup (LacZ)

- Petri dishes are incubated on the scanning window of a flatbed scanner.
- A macro on the host computer was designed to rescan at every 15 minutes for 12 hours.
pET Expression System

- Synchronized gene expression
- IPTG induces the production of both GFP and polymerase, ensuring full control of transcription.

A. Uninduced - no expression

B. Induced - expression of gene of interest
An automatic thresholding method was used to identify bacterial colonies.
Verification
Parameters

- Certain parameters (Area, Rate, Latency, Half Time, Final Intensity) are estimated in order to rank colonies.
- Colonies can then be picked for further analysis or sequencing.
Latency
Outlook

• Characterize leucine zipper libraries.
• Develop fusion proteins to screen for proteins other than GFP and LacZ.
• Develop expression vectors by tuning transcription factors.
• High throughput sequencing to develop an understanding of the underlying science of codon bias.
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