Expansion of Basic BioBricks Into Useful Part Families

By The iGEM Team at UC Davis

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Electrical engineer wants to build a simple harmonic oscillator.
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Diagram the circuit.
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Introduction

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We want to make this process easier!
Motivation

Parts Registry is meant to allow synthetic biologists to find the parts they need.

Large selection of both basic and composite parts.

Part families: Groups of parts which stem from one basic part.

Share a common functionality but have different parameters.

Example: Constitutive Promoter Library
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Example: Constitutive Promoter Library
The creation of new part families improves the utility of the Registry.

We set out to develop a process by which basic BioBricks could be expanded into part families. This process would:

- Generate mutant family-members that fill a parameter space with a resolution appropriate for a given circuit.
- Thoroughly characterize these mutants.
- Be accessible enough to encourage other teams to create their own part families.

Lucks et al. 2008
Making Part Families

1. Target Part → Registry Parts

2. Mutagenesis
   - Random
   - Targeted
     - Manual
     - Automatic

3. Screening
   - Cell Death/Survival
   - Plate Reader
   - FACS

4. Characterization
   - Plate Reader
   - Flow Cytometry

Motivation: Approach: Implementation: Assessment
Making Part Families

1. Target Part → Registry Parts → Basic Parts

2. Mutagenesis → Random → Mutagenic PCR


4. Characterization → Plate Reader

Alper et al. 2005
Prototype: GFP

- Useful for prototyping mutagenesis process
  - Small DNA changes can alter phenotype
  - Can help estimate mutation rate
- GFP is a model reporter to prototype
  - Average length
Mutagenic PCR (Error-prone PCR)

- Well known
- Universal
  - Works with any BioBrick using VF2 and VR primers
- Tested 18 variations of mutation protocol to optimize for visual screening

Cirino et al. 2003
**Visual**

- Quickly confirms mutagenesis
- Direct
  - GFP production linked to activity
  - Can make a good estimate at “strong” and “weak” mutants
- Simple

**Plate Reader**

- Fast
- Low error
- Quantitative
Characterization strategy is part and parameter dependent

- High throughput
- Automated
  - Put plate in at night, get data in the morning
  - 2 mutants per day
Integral to all circuits

Easy to screen

Repressible promoters are “tunable”
Implementation

1. Target Part
   - LacI Promoter

2. Mutagenesis
   - Mutagenic PCR

3. Screening
   - Visual Plate Reader

4. Characterization
   - Plate Reader

Ellis et al. 2009
Mayo et al. 2006
Mutate: 3 rounds of error-prone PCR.
Screen: Three-step process

1. Visually screen mutant promoters that have been ligated in front of GFP.

- Fluorescence and promoter activity are directly related.
- We can use a plate reader to measure fluorescence.
2. **Primary Screen** with a plate reader: 84 colonies were selected for screening in liquid culture, such that we had one well for each colony.

- Direct inoculation from transformation plate
- Bottom row contains +/- controls
This graph shows 84 promoter mutants compared to wild type. Green bars represent mutants with activity levels that differ from wild-type by at least 1.5 standard deviations.
3. We then did a **screen in triplicate** in which we selected 28 mutants to see more detailed information about their basal activity levels.

- Mutants are run in triplicate to assess variance.
- If error is large, the screen is repeated to ensure reliable selection.
This graph displays sorted data from a 28-mutant screen. Mutants are nearly linearly spaced, indicating good primary selection.
pBAD regulates repressor transcription.
- When AraC is present, pBAD is repressed.
- Increasing arabinose concentration induces pBAD, driving repressor expression.
- Controlling repressor concentration enables characterization of promoter behavior.
LacI Promoter mutants are characterized one at a time.

- One mutant per plate
- Repressor expression increases with arabinose concentration
- IPTG concentration induces the repressor
Calculation of Relative Promoter Units

\[ RPU = \frac{Fluorescence(\phi)}{Fluorescence(Reference)} \]

\[ OD = \text{Abs}_{600} \propto \text{Cell Number} \]

Use wild-type basal fluorescence as in-vivo reference

Kelly et al. 2009
Results

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Relative Fluorescence vs. Arabinose (wt%)
Results
Results
\[
\frac{dG}{dt} = b_{LacI} + \frac{\alpha_{LacI}}{1 + K_{LacI} (\theta_2 L)^{n_{LacI}}} - K_{deg}^{GFP} G \\
\frac{dL}{dt} = b_{BAD} + \frac{\alpha_{BAD}}{1 + K_{BAD} (\theta_1 A)^{n_{BAD}}} - K_{deg}^L L \\
\theta_2 = \frac{K_{I}^{n_{I}}}{I^{n_{I}} + K_{I}^{n_{I}}} \\
\frac{dA}{dt} = P - K_{deg}^{AraC} A \\
\theta_1 = \frac{K_{L}^{n_{L}}}{L_{A}^{n_{L}} + K_{L}^{n_{L}}}
\]

- \(G = GFP\)
- \(I = IPTG\)
- \(L = LacI\)
- \(b = \text{basal transcription}\)
- \(\alpha = \text{promoter strength}\)
- \(K = \text{binding constant}\)
- \(\theta = \text{bound fraction}\)
• Fit to model
Visualization

Wanted an attractive and free (preferably open-source) solution for plotting data on the web

- 2D: Flot.js
- 3D: ???
KO3D

- Implemented our own 3D plotting library
  - Utilizes HTML 5 Canvas
  - Supports WebGL
- Fully Interactive
- Cross-platform (including smartphones)
- IE support pending
- Fast
Now available on GitHub!

https://github.com/keegano/KO3D
Characterize!

Motivation Approach Implementation Assessment

Registry of Standard Biological Parts

Part: BBa_R0010

promoter (lacI regulated)
This part is an inverting regulator sensitive to LacI and CAP.
It contains two protein binding sites. The first binds the CAP protein, which is generally present in E.coli and is associated with cell health and availability of glucose. The second binds LacI protein.

- In the absence of LacI protein and CAP protein, this part promotes transcription.
- In the presence of LacI protein and CAP protein, this part inhibits transcription.
- LacI can be inhibited by IPTG.
- LacI is coded by BBa_C0010

Usage and Biology
This is a direct copy of bases 0365739 through 0365540 of the E. coli K-12 MG1655 genome, Genbank NC_000913 in reverse complement form. It is the natural promoter for the LacZYA operon. It includes the tail end of the LacI gene coding region, but no promoter region for that partial gene.

Sequence and Features

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</table>

Assembly Compatibility: 10 12 21 23 25
Proposal

Part: BBa_R0010

**promoter (lacI regulated)**
This part is an inverting regulator sensitive to LacI and CAP.

It contains two protein binding sites. The first binds the CAP protein, which is generally present at low glucose. The second binds LacI protein.

- In the absence of LacI protein and CAP protein, this part promotes transcription.
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- LacI is coded by BBa_C0010

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**Assembly Compatibility:**

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**Parameters**

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<td>positive_receptors</td>
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**Variants**

- BBa_17154
- BBa_1714076
- BBa_K075387
- BBa_K252002

**Twins**

- BBa_17154 Deleted
- BBa_1714076 Deleted
- BBa_K075387 Planning
- BBa_K252002 Available
Considerations

- Reporters
  - GFP vs RFP? LacZ?

- Growth Conditions
  - LB vs. minimal media
  - Glucose

- Bacterial Strain
  - DH5a vs. BW22826
Future Goals

- Encourage other iGEM teams to start their own part families
- Formalize mutant libraries into part families.
  - RFC Draft in Progress
- Further characterize our LacI promoter mutants
- Generate part families for the TetR and Lambda cl repressible promoters.
We have:

- Optimized and implemented a pipeline for expanding basic parts into part families
- Proposed solutions to promote part families on the Registry
- Implemented rich web applications for the visualization of scientific data


We would like to thank our advisors and sponsors!

Faculty Advisors:
Dr. Marc Facciotti
Dr. Ilias Tagkopoulos

Graduate Mentors:
Russell Neches
Andrew Yao
Linh Huynh
Mike Starr
Thank you for attending our presentation!