Penn iGEM
Engineering the Epigenome

D. Cabrera, M. Charawi, D. Fields, B. Kaptur and J. Tycko

iGEM World Championships • November 2nd, 2013
We are intrigued by these two cats.

Copycat

Rainbow
DNA methylation is an epigenetic control mechanism.

- DNA Methylation: chemically modified nucleotides

  \[
  \text{CpG: ATCGAT}
  \]

- Methylated DNA is transcriptionally silenced in mammals

- These modifications are heritable
Engineered DNA Methylation Patterns have two readily apparent applications.

1. **Translational Researchers:** Aberrant DNA methylation is a cause of human disease

2. **Synthetic Biologists:** Pre-transcriptional control layer
DNA methylation is a potential pre-transcriptional control layer for synthetic biology.

DNA Methylation could have advantages as an engineered silencing mechanism:

1. Heritable in mammalian systems
2. Orthogonal in *E Coli*, which have no endogenous CpG methylation
3. Multiplex across different promoters which lack endogenous regulators

*Orthogonal transcriptional silencing*  
*Heritable as cells divide*
We currently lack tools for site-specific DNA methylation.

Methylases catalyze global methylation across the genome.

Crystal structure of M.SssI - CpG methylase from *Spiroplasma* - Biobrick BBa_K1128000

We want a toolbox that is:

- Open source
- Robust
- Standardized
- Inexpensive
- Easy to Use
- Noiseless
What would the toolbox for site-specific DNA methylation include?

- **Tool:** Site Specific Methylase
- **Assay:** MaGellin
- **Automation:** MaGellin Software
Our design for a site-specific methylase is modular.

We hypothesized that a site-specific methylase would have two components:

1. **A DNA binding domain**, which binds to the DNA at a specific site

2. **A methylase**, which attaches a methyl group to the DNA base
We needed an assay to effectively characterize the DNA-binding-domain-methylase fusions we were generating.

- Open source
- Robust
- Standardized
- Inexpensive
- Easy to Use
- Noiseless
Our assay is a standardized, efficient system for assessing site-specific methylation.
Our assay is a standardized, efficient system for assessing site-specific methylation.
Our assay is a standardized, efficient system for assessing site-specific methylation.
Our assay is a standardized, efficient system for assessing site-specific methylation.

**ON TARGET**
Cloning site to insert target sequence located upstream the methylation-sensitive Aval restriction site and flanked by verified bisulfite sequencing primers.
Our assay is a standardized, efficient system for assessing site-specific methylation.

**ON TARGET**
Cloning site to insert target sequence located upstream the methylation-sensitive Aval restriction site and flanked by verified bisulfite sequencing primers

**OFF TARGET**
Off-target methylation sensitive Aval restriction site flanked by verified bisulfite sequencing primers

**Inducible T7 Expression System**

**BinL**

**Cloning site to insert DNA-binding protein of choice**

**CpG Methyltransferase fused to a genetic linker**

**MAGELLIN**
Verified bisulfite sequencing primers elucidate the range of methylation.

<table>
<thead>
<tr>
<th></th>
<th>Unsuccessful Iteration</th>
<th>Successful Primer Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisulfite Converted</td>
<td>X X</td>
<td>X X</td>
</tr>
<tr>
<td>methylated</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
MaGellin detects methylation \textit{in vitro}.

Methylated Plasmid

Unmethylated Plasmid

* Too small to resolve
MaGellin detects different levels of methylation \textit{in vitro}.
MaGellin detects methylation \textit{in vivo}. 
Zinc Finger -M.SssI fusion fully methylates MaGellin plasmid.

<table>
<thead>
<tr>
<th>Contains Binding Site</th>
<th>Induced Expression</th>
<th>Zinc Finger Experimental Conditions</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td>Cut with XbaI, for linearization</td>
<td>X</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>Cut with AvaI, methylation sensitive</td>
<td>X</td>
</tr>
</tbody>
</table>

### Controls

- **X**: Full Methylation
- **X**: No Methylation

![Electrophoresis Image]
We needed a software tool to simulate our experiments and quantify our data.
We took this challenge to the largest college hack-a-thon in the world.

1000+ HACKERS

100+ UNIVERSITIES
The MaGellin Software Package simulates methylation in silico and quantifies gel data.

**Inputs**
- Raw Gel Image
- Plasmid DNA Sequence

**Process**
- In silico methylation simulation and identification of target sites
- MaGellin software interface

**Output**
- Quantification of on-target and off-target effects
- Bar chart showing normalized band intensity (a.u.) for different bands.
MaGellin screened 64 conditions in 2 days and reported zinc finger’s off target activity.

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional Assay</td>
<td>2 weeks (+ primer optimization time)</td>
<td>$11,000</td>
</tr>
<tr>
<td>MaGellin</td>
<td>2 days</td>
<td>$60</td>
</tr>
</tbody>
</table>

Representative gel from study
TALE effectively eliminates blanket methylation.
TALE-M.Sssl is Prone to Off Target Activity.

MaGellin greatly accelerates the design, build test cycle.
Future Directions.

- Use our assay to test split reconstitution approach to building site specific TALE methylase

- Determine if orthogonal CpG methylation can regulate transcription in *E. coli*

![MeCP2](attachment:MeCP2.png)
We took many opportunities to introduce our research to a wide-ranging audience.

*During the past 4 months, we:*
We took many opportunities to introduce our research to a wide-ranging audience.

During the past 4 months, we:

1. Published a bioethics article in “the premiere peer-reviewed undergraduate bioethics journal”

- The risk:benefit ratio is not favorable for younger patients of non-lethal epigenetic-associated disease
- Treatments should be gene-specific
- Germline transmission should be unacceptable
- Dependable genome-wide methylation assays must be developed

**The Potential of Epigenetic Therapy and the Need for Elucidation of Risks**

**Josh Tycko, Danielle Fields, Daniel Cabrera, Mahamad Charawi, Bradley Kaptur†**

Epigenetic phenomena are known to be a root cause of many common diseases. To date, the FDA has approved four epigenetic therapies that show promising results for prolonging lives of terminal cancer patients. However, there is a relative lack of knowledge about long-term epigenetic effects, especially those that affect future generations. We propose a heightening of standards for epigenetic therapy: therapies should be targeted to specific genes in specific cells and cannot affect the germline and patients’ epigenomes should be sequenced before and after treatment. Moreover, further research should be performed to answer questions about transgenerational epigenetic effects, to analyze the effects of altered epigenomes in the long term, and to develop superior assays for screening epigenomes. We highlight current research in the field, including the work of the Penn IGEM group.

**Epigenetics Background**

**Introduction.** The code of life is more than a sequence of A, C, G, and T. Muscle cells in the human heart contain the same DNA as skin cells in the foot, yet these two cell types behave in radically different ways. Both contain the DNA for over 20,000 human genes but express only the ones needed for their own form and function. These differences in gene expression are modulated by epigenetic controls. Epigenetics refers to any heritable chemical modification of DNA that alters expression without changing genetic sequence. Neurodevelopmental disorders, immunodeficiency, cancer, and other diseases can result when these mechanisms go awry.

**Methylation.** In humans, enzymes called methyltransferases add methyl groups to short DNA sequences abundant in the genome called CpG sites. Methyl groups block transcription factors (gene activators) from binding to DNA and performing their normal function. Although epigenetic factors do not change the sequence of DNA, they can affect the phenotype, the observable characteristics of the organism. Specific patterns of methylation are necessary for a cell to modulate the level of expression of each of its genes.

**Epigenetic Diseases**

**Cancer.** DNA methylation has been referred to as the “hallmark of cancer” (Szyf 2004). Abnormal methylation patterns throughout the genome cause blockage of tumor suppressor genes have been linked to many types of cancer. For instance, breast cancer generally exhibits inactivation of the gene BRCA1. In sporadic (i.e. non-familial) cases, this suppression is usually caused by hypomethylation rather than mutation of the gene (Rice 2006). In other cases, hypomethylation causes overexpression of the fap endonuclease 1 gene and lead to breast cancer in some patients (Singh 2008).

**Neurological.** Methylation abnormalities have been linked to a wide range of diseases. Fragile X syndrome, one of the leading genetic causes of intellectual disability, is characterized by hypermethylation, which disrupts the production of protein necessary for normal brain development. Patients suffering from this disorder are at risk for autism, ADHD, decreased IQ, infertility in females, and disordered facial features (Jacquemont 2011).

**Psychological.** Epigenetic mechanisms can also impact psychological states. In an animal study, rat pups that received better maternal care in the form of licking, grooming, and arch-back nursing had lower levels of methylation at the glucocorticoid receptor gene. These rats displayed less intense responses to stressful situations than those who received poor maternal care. The researchers were able to eliminate these differences via epigenetic interference (Weaver 2004).

**Epigenetic Therapy**

**Fundamental Advantages.** The aforementioned epigenetic roots of disease are attractive targets for therapy. Aberrant DNA methylation patterns are more easily reversible than genetic mutations. In the case of cancer, epigenetic therapy coaxes tumor cells to return to a healthy state, rewiring their methylation patterns so the cells express genes that halt their cancerous uncontrolled growth. Traditional chemotherapy strategies, on the other hand, aim to kill cancer cells and are fundamentally more toxic to patients since healthy cells are also harmed.

**Recent Successes.** There have been some exciting clinical successes with the first generation of epigenetic
During the past 4 months, we:

1. Published a bioethics article in “the premiere peer-reviewed undergraduate bioethics journal”

2. Shared an open source Laboratory Information Management System (LIMS)

- Minipreps, primers, strains can become disorganized and easily confused
- Commercially available Laboratory Information System (LIMS) software can be expensive and difficult to customize
- Handle Plasmids, Primers, Strains
We took many opportunities to introduce our research to a wide-ranging audience.

**During the past 4 months, we:**

1. Published a bioethics article in “the premiere peer-reviewed undergraduate bioethics journal”
2. Shared an open source Laboratory Information Management System (LIMS)
3. Introduced synthetic biology principles to epigenetics researchers

---

**Article**

**The Potential of Epigenetic Therapy and the Need for Elucidation of Risks**

Josh Tycko, Danielle Fields, Daniel Cabrera, Mahamad Charawi, Bradley Kaptur

---

**Bartolomei Lab**

**Simmons Lab**
We took many opportunities to introduce our research to a wide-ranging audience.

<table>
<thead>
<tr>
<th>Number</th>
<th>Activity</th>
<th>Source/Details</th>
</tr>
</thead>
</table>
| 1      | Published a bioethics article in “the premiere peer-reviewed undergraduate bioethics journal” | The Potential of Epigenetic Therapy and the Need for Elucidation of Risks  
Josh Tycko, Danielle Fields, Daniel Cabrera, Mahamad Charawi, Bradley Kaptur |
| 2      | Shared an open source Laboratory Information Management System (LIMS)      |                                                                                  |
| 3      | Introduced synthetic biology principles to epigenetics researchers         | Bartolomei Lab  
Simmons Lab                                                                 |
| 4      | Promoted general interest in synthetic biology through community outreach  |                                                                                  |
Our most valuable parts are well-characterized BioBricks.

These include:

1. Magellin Plasmid Backbone – BBa_K1128001
2. CpG Methylase M.SssI – BBa_K1128000
3. CpG Methylase M.SssI with Linker – BBa_K1128002
We constructed a toolbox for site-specific methylation and made it accessible to the community.

- Robust
- Inexpensive
- Noiseless
- Open Source
- Easy to Use
- Standardized
We would like to acknowledge and say THANK YOU to:

Our Collaborators

Bartolomei lab, Penn Med
Chow lab, Penn SEAS
Simmons lab, Penn Med
Zhao lab, Penn Med
Penn Genome Fronteirs Institute
Goulian lab, Penn Dept of Biology
Henry Ma & Sevile Mannickarottu, Penn SEAS
Eric Kauderer-Abrams
iGEM HQ

Our Advisors

Spencer Glantz
Michael Magaraci
Avin Veerakumar
Dr. Jordan Miller
Dr. Orkan Telhan
Dr. Brian Chow

Our Sponsors

New England Biolabs, Integrated DNA Technologies, Mack Institute for Innovation Management at Wharton, Penn Center for Undergraduate Research and Fellowships, Addgene, Jerome Fisher Program in Management and Technology, UPenn College Office, Biomatters, Penn Engineering, Penn Epigenetics, Qiagen, Red Bull
Penn iGEM
Engineering the Epigenome
Questions
Published COBRA assay agrees with MaGellin that TALE methylates off target site.
Dcas9 effectively eliminates blanket methylation.