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We are intrigued by these two cats.

Copycat

Rainbow
DNA methylation silences transcription.

- DNA Methylation: chemically modified nucleotides
- Methylated DNA is transcriptionally silenced
- These modifications are heritable
Engineered DNA Methylation Patterns have two readily apparent applications.

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

2. **Synthetic biologists**: heritable transcriptional control
Aberrant DNA methylation is implicated as a cause of many human diseases.

- The “hallmark of cancer” (Szyf, Cancer Lett., 2004)

- Implicated in heart disease, obesity, Down syndrome, Alzheimer's and more (Robertson, Nature Reviews Genetics, 2005)
DNA methylation is a potential pre-transcriptional control layer for synthetic biology.

DNA Methylation is:

1. Independent of any one promoter or transcription factor

2. Orthogonal in *E. coli*

3. Heritable in mammalian systems

*Orthogonal in E.coli*  
*Heritable in mammalian cells*
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
- Inexpensive
- Easy to Use
- Standardized
- Noiseless
What would the toolbox include?

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

The site specific methylase has two components:

1. An anchor, 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.
Our assay is a standardized, efficient system for assessing site-specific methylation.
Verified bisulfite sequencing primers elucidate the range of methylation
MaGellin detects methylation *in vitro*.
MaGellin detects different levels of methylation in vitro
MaGellin detects methylation \textit{in vivo}
Qualitatively analyzing MaGellin data is not as automated as we would like.
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
...and designed an automated software package that predicts and quantifies MaGellin assay results.

**Inputs**
- Gel Image
- DNA Sequence

**Process**
- MaGellin Software
  - Gel Name: TALE-M.Ssp Induction
  - Plasmid Name: TALE-M.Ssp MaGellin
  - Sequence (Paste a Sequence):
    - TGGGAATTGGAGCCGCTGTACGGTTAACCTG
    - TGGGCTTTTGGCTTTGTTTAACCTG
  - Target Sequence: TTAGCCCAGGACGACTT
  - Aval
  - Bgl II

**Output**
- Quantified Results
  - 1
  - 2
  - 3
  - Intensity (au)
  - Construct
Novel TALE-M.SssI fusion reduces non-specific methylation in presence of DNA Binding Site
Wet Lab Accomplishments

Assay

MaGellin

Automation

MaGellin Software

Tool

TALE-Methylase
Future Directions

- Use bisulfite sequencing to determine exact range of methylation induced by TALE-Methylase

- Determine if orthogonal CpG methylation can regulate transcription in *E. coli*
We took many opportunities to introduce our research to a wide-ranging audience.

During the past 4 months, we:

1. Published a bioethics article titled *Epigenetic Therapy: Too Soon to Treat?*
2. Introduced synthetic biology principles to epigenetics researchers
3. Promoted general interest in synthetic biology through community outreach
4. Shared an open source Laboratory Information Management System (LIMS)
We examined the ethical considerations surrounding the development and applications of epigenetic-based human therapies for non-lethal disease.

**Epigenetic Therapy: Too Soon to Treat?**

- Methylation inhibitors are already approved for terminal cancer.
- The risk:benefit ratio is not favorable for younger patients of non-lethal epigenetic disease.
- Treatments should be gene-specific.
- Germline transmission should be unacceptable.
- Dependable genome-wide methylation assays must be developed.
We introduced synthetic biology principles to epigenetic researchers.

- Met with epigenetics researchers to introduce synthetic biology and explain our project.
- Maintained communication throughout the design process.
- Presented finished product to Simmons lab and Bartolomei lab at Penn and discussed future applications.
We promoted general interest in synthetic biology through community outreach.

- Led DNA extraction experiment for children at Science Discovery Day
- Taught incoming engineering students about the principles of synthetic biology
- Reached out to new Penn students at 3 poster presentations
We designed a sample management system that is free, easy to use, and available to download.

- Minipreps, primers, strains can become disorganized and easily confused.
- Consolidation of information about a given sample in a single database fosters collaboration and speeds team communication.
- Commercially available Laboratory Information System (LIMS) software can be expensive and difficult to customize.

Photo Credit: Stanford School of Medicine
Our sample LIMS system encompasses the basic features an iGEM team needs.

- Reagents
- Plasmids
- Primers
- Strains

Straightforward forms simplify the entry of sample information into the database.

A query form enables advanced searches for user-friendly viewing of database information.
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.SsI with Linker – Bba_K1128002
We constructed a toolbox for site-specific methylation and made it accessible to the community.

- Assay for methylation
- Software to analyze methylation
- Novel TALE-MSSI fusion protein construct
- Analysis of epigenetic therapy ethics
- Community Outreach & LIMS Development
We would like to acknowledge and say THANK YOU to:

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