A TOOLBOX FOR SITE-SPECIFIC DNA METHYLATION

**ABSTRACT**

Epigenetic modifications, such as DNA methylation, are heritable regulators of gene expression. In mammals, methylated CpG sequences are transcriptionally silenced and aberrant methylation patterns can cause human disease. Targeted enough to block RNA polymerase, and an enzymatically active fusion protein to use as a positive control. Our assay is built into a modular single-plasmid system that allows methylases to be easily cloned, expressed, and tested via an insensitive digestion, quickly measuring the existence and extent of targeted methylation. Additionally, our plasmid includes validated primer-binding sites for methylation-sensitive sequencing, and our E.coli chassis effectively eliminated noise associated with methylation studies. The software models the expected methylation-sensitive digestion band pattern, then compares this prediction against a gel electrophoresis image. We are using this assay to characterize our novel zinc-finger methylase fusion. We included the zinc-finger binding site for non-immune diseases favoring for younger patients with non-lethal diseases.

**WHAT MAKES A GOOD TOOLBOX?**

- **Easy to Use**
- **Open Source**
- **Robust**
- **Non-invasive**
- **Easy to Use**
- **Standardized**

 Mammanian cells have high background and one which we plan on using as a negative control. We included the zinc finger binding site for non-specific methylation, while E.coli have no background.

**HUMAN PRACTICES**

1. Untargeted epitherapies are already FDA approved for certain terminal cancers.
2. The risk/benefit ratio is significantly less favorable for younger patients with non-lethal diseases.
3. Their epi-therapies should be gene-specific.

LIMS is a way of managing lab data. One core, indispensable feature of any LIMS is sample management. A good lab information system will tell you where you put your tube after you’ve lost something. Penn iGEM created an open-source LIMS using the Google Spreadsheet and Forms platform.

**FUTURE DIRECTIONS**

1. Use MaGellin to efficiently screen new TALE-methylase constructs, including chimeras, and characterize our Cas9-methylase
2. Determine if orthogonal CpG methylation can regulate transcription in E.coli

MaCp2 is a mammalian protein that binds to methylated CpG sites and does not bind to unmethylated CpG sites. We have been able to express and purify two different recombinant variants of MaCp2, one of which we predict to be functional and one which we plan on using as a negative control. We predict that MaCp2 binding to methylated CpG sites will be significantly affected in E.coli. We are currently in the process of testing this experiment in a cell free system.

**ASSAY**

**Clone**

1. Design the MaGellin assay for screening site specific DNA methylases.
2. Verify that the assay detects the presence of methylation and that it can differentiate between different levels of methylation.
3. Designed standardized bisulfite sequencing primers for further characterization of site specific methylations.
4. MaGellin is extremly sensitive to off target effects.
5. MaGellin is easy to use, inexpensive and generates data quickly.

**Methylation-sensitive digest simulation**

1. Developed the MaGellin software package that simulates methylation sensitive restriction digest.
2. Developed the software into a robust application for automated gel analysis.
3. Working with Swinyard to make gel analysis open source for DIYbio.
4. The MaGellin assay confirmed what previous studies had shown that the existing zinc-finger methylation fusion have significant off target effects.
5. Construct a novel TALE-methylase, which also shows significant off target effects at high induction conditions.
6. Plan to engineer an orthogonal pre-transcriptional control layer for regulating gene expression in E.coli.

**SUMMARY**

**Software**

- **Easy to use QUD**
- **Find sets and Quantify**
- **Gel image quantification**
- **Bug free**
- **Tool**

- **MaGellin reports TALE-M.SssI methylation both on and off target**
- **MaGellin assys 32 conditions in 2 days; shows TALE-M.SssI is of target with high induction**
- **MaGellin reports fully off target effects of Zinc Finger - M.SssI**

As We had done before with the zinc finger, We followed the MaGellin cloning protocol to remove the TALE binding site from the plasmid. We then varied the induction conditions (0, 2, 6, and 24 hours) for both plasmids. We used MaGellin to report methylation by quantifying the off target and on target bands (as marked above). MaGellin assayed these 64 conditions in 2 days, with a margin of $11,200 by the probe, we pay our DNA sequencing costs.

**MaGellin Toolbox**

- **Novel Targeted Methylation**
- **Non Specific Methylation**
- **Site Specific Methylation**
- **DNA Methylation**

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**Penn iGEM**

**Engineering the Epigenome**

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