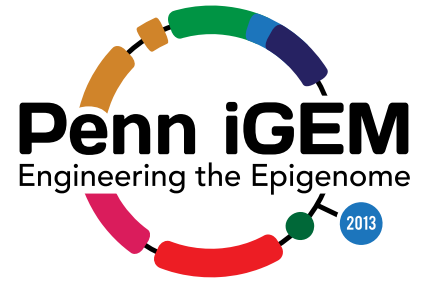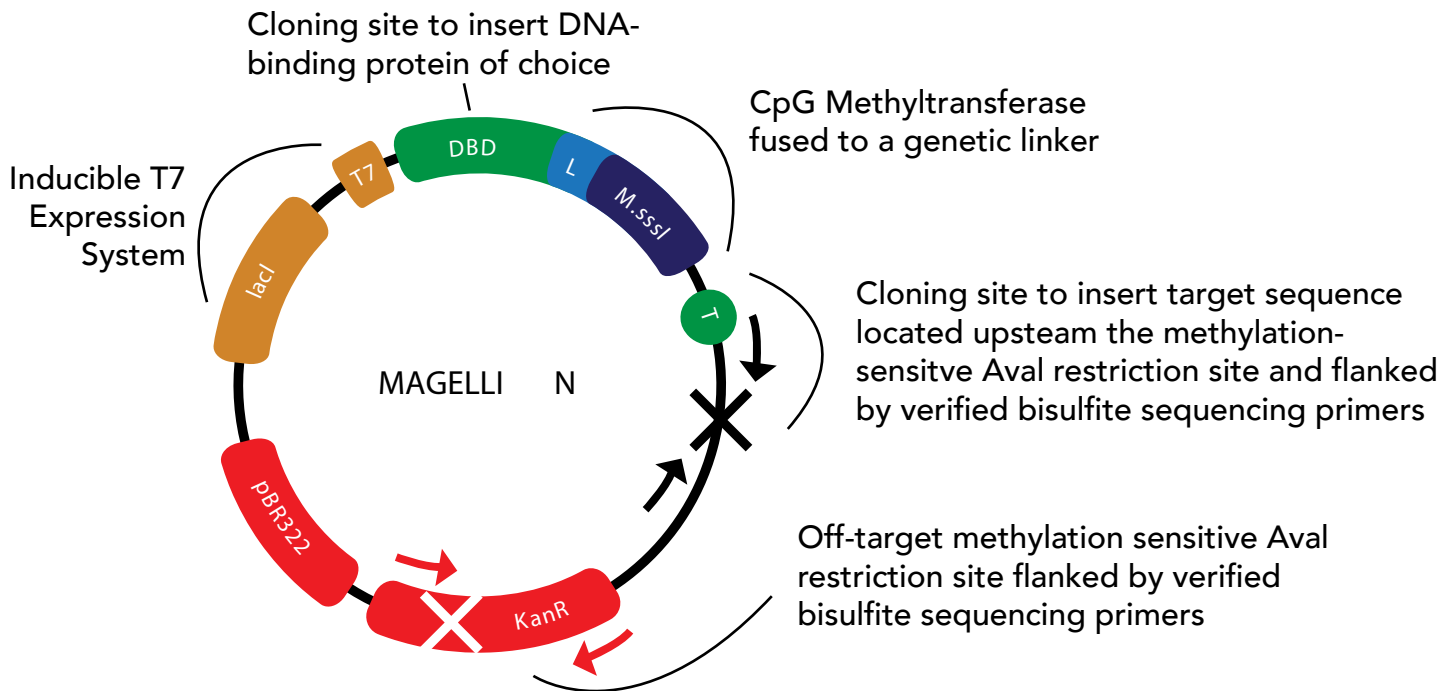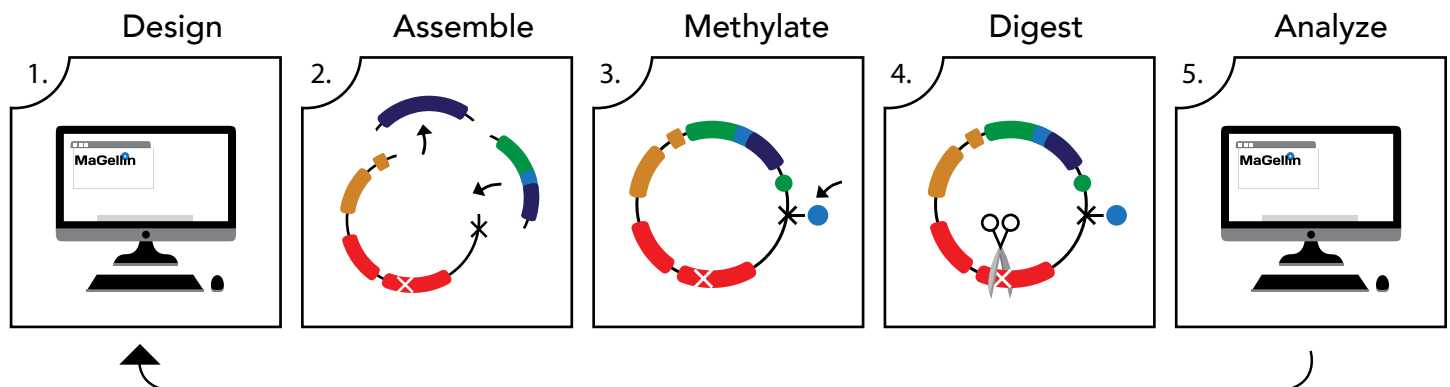# MaGellin

Restriction-Based Methylation Analysis

OVERVIEW

MaGellin is a platform that streamlines the process of testing the specificity of DNA binding domains and the activity of methyltransferase-DNA binding domain fusion proteins. This technology combines the use of methylation-sensitive restriction enzymes with a powerful software package to identify and quantify the location and amount of CpG DNA methylation. The one-plasmid design ensures simple customization of the DNA-binding domain and targeting sequence, easy readout by agarose gel electrophoresis and analysis by included software, and pre-designed bisulfite sequencing primers.

PLASMID DESIGN AND FEATURES

Cloning site to insert DNA-binding protein of choice

CpG Methyltransferase fused to a genetic linker

Inducible T7 Expression System

MAGELLIN

DBD
T7
L
M.ssI
lacI
T
pBR322
KanR

Cloning site to insert target sequence located upsteam the methylation-sensitve AvaI restriction site and flanked by verified bisulfite sequencing primers

Off-target methylation sensitive AvaI restriction site flanked by verified bisulfite sequencing primers

WORKFLOW

Design	Assemble	Methylate	Digest	Analyze
1.	2.	3.	4.	5.

WET LAB PROTOCOL

ASSEMBLE

1. Digest BBa_K1128001 (MaGellin backbone) and BBa_K1128002 (linker - M.SssI) with EcoRI and PstI. Ligate K1128002 into the K1128001 backbone.
2. PCR amplify DNA-binding protein of choice. Use the following 5' extensions on the PCR primers:
3. Forward: CAGGAGGAATTC[ATG] (add start codon only if not included in gene).
4. Reverse: CTCTAGAAGCGGC (make sure to remove the stop codon).
5. Use EcoRI and XbaI to ligate the DNA-binding protein into the MaGellin backbone, fusing it in frame to the linker-M.sssI construct.
6. Clone in target sequence using BamHI and XhoI.

METHYLATE

1. Transform the completed MaGellin plasmid into T7 Express (NEB).
2. Induce culture with 1 mM IPTG.
3. Incubate in a shaker at 37C for 5 hours.
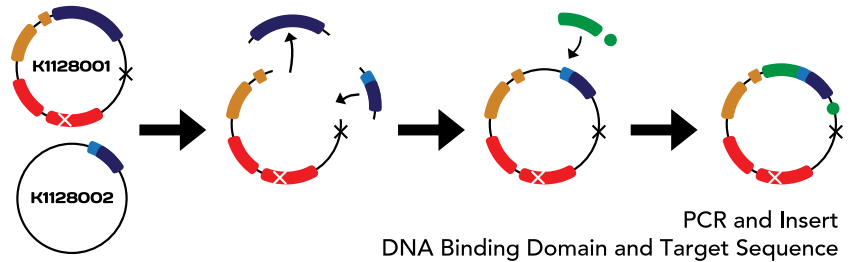4. Miniprep to isolate the plasmid

DIGEST

1. Digest 600 ng of miniprep DNA in a 15 uL reaction with 10 U of both XbaI and AvaI.
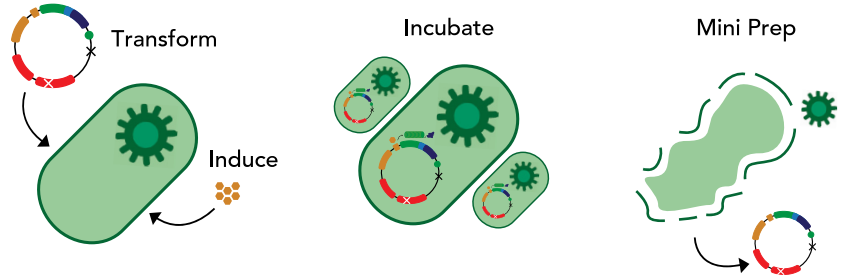2. Incubate reaction for 1 hour at 37C.

ANALYZE

1. Run the entire digestion reaction on a 1% agarose gel.
2. Take a photo of the gel.
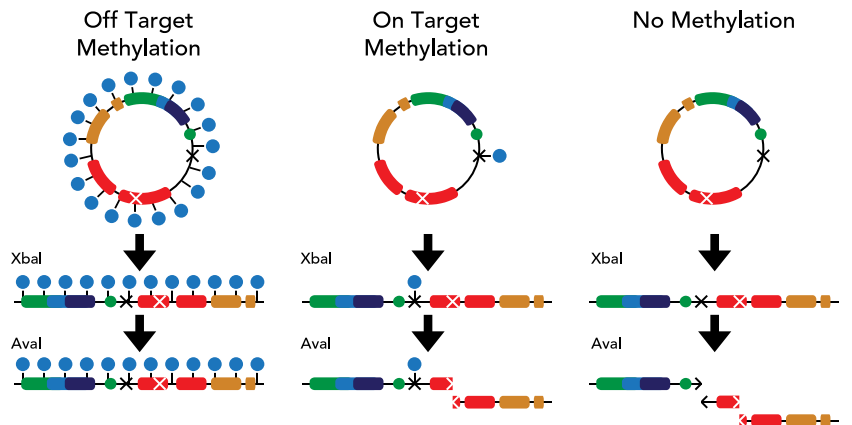3. Upload and analyze the gel photo using the MaGellin Software Package (see protocol below).
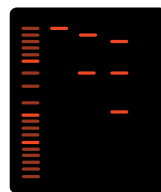
ASSEMBLE

PCR and Insert
DNA Binding Domain and Target Sequence

METHYLATE

Transform   Incubate   Mini Prep

Induce

DIGEST

Off Target Methylation   On Target Methylation   No Methylation

XbaI   XbaI   XbaI

AvaI   AvaI   AvaI

ANALYZE

Run the digests on a gel   Analyze using MaGellin Software

MaGellin

MAGELLIN SOFTWARE PACKAGE PROTOCOL

Upload gel picture from restriction enzyme digest. Fill out all relevant information on the graphical user interface. Enter plasmid and target sequences and select restriction enzymes used. Enter descriptive names for gel and lanes if desired.

1. Press the Analyze button.
2. The MaGellin Software Package will calculate the intensity and position of each band and produce a graph.
3. By comparing with MaGellin's simulated gel, identify whether your enzyme is producing a targeted methylation, untargeted methylation, or unmethylated signature.
4. Use these results to re-design your targeted methyltransferase if necessary.
5. Collaborate with your fellow scientists by sharing your results on SkyDrive.