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.

**OVERVIEW**

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**PLASMID DESIGN AND FEATURES**

MaGellin consists of a plasmid with the following features:

- **Inducible T7 Expression System**: Allows control over the expression of DNA-binding domains.
- **Cloning site to insert DNA-binding protein of choice**: Enables customization of the DNA-binding domain.
- **CpG Methyltransferase fused to a genetic linker**: Ensures the activity of the methyltransferase domain.
- **Cloning site to insert target sequence located upstream the methylation-sensitive Aval restriction site and flanked by verified bisulfite sequencing primers**: Enables the study of DNA methylation at specific sites.
- **Off-target methylation sensitive Aval restriction site flanked by verified bisulfite sequencing primers**: Provides a control for off-target effects.

**WORKFLOW**

1. **Design**: Design the DNA-binding domain and target sequence.
2. **Assemble**: Assemble the plasmid with the designed DNA-binding domain and target sequence.
3. **Methylate**: Methylate the plasmid using the CpG methyltransferase.
4. **Analyze**: Analyze the methylation levels using agarose gel electrophoresis and included software.
5. **Digest**: Digest the plasmid with methylation-sensitive restriction enzymes to verify methylation sites.
1. Digest BBa_K1128001 (MaGellin backbone) and BBa_K1128002 (linker-M. ssi) 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:
   - Forward: CAGGAGGAATTC[ATG] (add start codon only if not included in gene).
   - Reverse: CTCTAGAAGCGGC (make sure to remove the stop codon).

3. Use EcoRI and XbaI to ligate the DNA-binding protein into the MaGellin backbone, fusing it in frame to the linker-M. ssi construct.

4. Clone in target sequence using BamHI and Xhol.

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

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.

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).

**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 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.