

Using MaGellin to Screen Methyltransferase-DNA Binding Domain Fusions

By Penn iGEM 2013

- I. Clone sequence of methyltransferase-DNA binding domain fusion into the MaGellin backbone
 - a. Transform MaGellin containing J04450 insert into competent cells
 - b. Grow up and miniprep MaGellin transformation
 - c. Digest of MaGellin backbone and 1ug of assembled methyltransferase-DNA binding domain fusion with appropriate master mix containing restriction enzymes
 - i. 1uL NdeI
 - ii. 1uL NcoI-HF
 - iii. 5uL NEBuffer 2.1
 - iv. 1ug of backbone/insert
 - v. Nuclease-free water to 50uL
 - d. Gel extract MaGellin backbone to remove J04450 insert
 - e. Gel extract or column purify digested methyltransferase-DNA binding domain fusion depending on method of assembly and size of removed ends
 - f. Ligate methyltransferase-DNA binding domain fusion into MaGellin
 - g. Transform fusion into competent cells
 - h. Pick colonies and perform colony PCR to determine success of ligation
 - i. Grow up correct colonies for 16 hours in LB media in the presence of kanamycin
- II. Induce culture with IPTG according to T7 Express Induction Protocol
 - a. Grow overnight culture of methyltransferase fusion cloned into pet26b-sgRNA-target backbone.
 - b. Measure Optical Density on plate reader and dilute to an Optical Density of 0.5
 - c. Add IPTG at 1mM final concentration.
 - d. Grow culture in a shaker incubator at 37 degrees Celsius for 5 hours.
 - e. Miniprep the culture to isolate the plasmid
- III. Miniprep cultures after 5 hours of IPTG induction
- IV. Digest induced cloned methyltransferase-DNA binding domain fusion-MaGellin clone with master mix
 - a. 1uL Aval
 - b. 1uL XbaI
 - c. 5uL NEBuffer 2.1
 - d. 1ug of IPTG-induced plasmid
 - e. Nuclease free water to 50uL
- V. Run digest on gel and image it
- VI. Run image through MaGellin software