Using MaGellin to Screen Methyltransferase-DNA Binding Domain Fusions

By Penn iGEM 2013

Goal: Screen methyltransferase-DNA binding domain fusions for functionality, specificity, and off-target effects.

Protocol:

1) 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
2) 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
3) Miniprep cultures after 5 hours of IPTG induction
4) Digest induced cloned methyltransferase-DNA binding domain fusion-MaGellin clone with master mix
    a. 1uL AvaI
    b. 1uL XbaI
    c. 5uL NEBuffer 2.1
    d. 1ug of IPTG-induced plasmid

    e. Nuclease free water to 50uL
5) Run digest on gel and image it
6) Run image through MaGellin software