Saturday 8/8/09

Preparation of Electric Competent Cells

Protocol was changed from 7/23/09 to make smaller volume of comp. Cells

Working with Sudarshan on transformation procedure

From Lin Group in Chemical Engineering

1. Inoculate 5 to 10 ml of LB with desired bacteria and cultivate it overnight.
2. Inoculate 50 ml of LB with O/N culture and cultivate until OD600 reaches between 0.4 to 0.9.
3. Pour the cell in a sterile conical tube and chill it on ice for 20 min (or until falcon tube is chilled through).
4. Harvesting - Centrifuge it at 3500 rpm for 15min and discard supernatant.
5. Washing 1 - Resuspend cell pellet with 50ml of cold sterile water, centrifuge it at 3500rpm for 15min, and discard supernatant.
6. Washing 2 - Resuspend cell pellet with 25ml of cold sterile water, centrifuge it at 3500rpm for 15min, and discard supernatant.
7. Washing 3 - Resuspend cell pellet with 2ml of cold sterile water, centrifuge it at 3500rpm for 15min, and discard supernatant.
8. Resuspension - Resuspend cell pellet in 100ul of cold sterile water (if necessary, resuspension in remaining liquid may be adequate).
9. Dilute comp. Cells 1:100 and measure OD_{600}
    - OD_{600} should be larger than 0.200 to have a dense enough solution for transformation
10. Aliquot 40ul.

Retrieving Biobricks from Registry
From the Registry of Biological Parts

1. With a pipette tip, punch a hole through the foil cover into the corresponding well to the Biobrick™-standard part that you want. Make sure you have properly oriented the plate. We recommend that you do not remove the foil cover, as it could lead to cross contamination between the wells.
    a. Biobricks of interest
        i. GFP generator: BBa_E0840-Plate 1 Cell 12O AMP resistance
2. Add 15uL of ultrapure water
3. Remove from well and transfer into an eppendorff tube
4. Place 2 uL of DNA into new chilled eppendorff tube to mix with comp. cells

Transformation

From Lin Group in Chemical Engineering

1. Mix 40ul of comp. cell with 1-2ul DNA.
    - Positive control pSIM5
    - Negative control comp. cells only
    - GFP generator
2. Transfer it to chilled cuvette and set the voltage to 1.8kV. (click both arrow keys at the same time)
3. Electroporate (double-click the charge key)
4. Add 1ml of LB immediately. (SOC gives higher yield.)
5. Cultivate 1hr.
6. Spread 100ul of cell on a plate with beads.
    - Six LB + 100 ug/mL AMP plates are needed
        1. Positive control
        2. Negitive control
        3. Undiluted GFP generator
        4. 1:10 dilution GFP generator
        5. 1:100 dilution GFP generator
        6. Plasmid control
            - Plate 5 uL directly from biobrick resuspension
7. Place in 37C incubator to grow up overnight

Results

Cultivating the comp. cells from the overnight culture was started at 9:40 AM and at 1:15 PM to OD_{600} was 0.6135. After all of the washings, the OD_{600} of a 1:100 diltuion of the comp. cells was 0.491.