Wednesday 7/22/09

Media Preparation

- Liquid LB for comp. cell preparation
  - 16 g of LB Broth Lennox (does not contain agar)
  - DI H20 to 800 mL
- AMP LB plates for transformation
  - 2 x 16 g of LB Broth Miller (contains agar)
  - Fill each flask with DI water to 400 mL
- 800 mL DI Water

Start overnight culture for comp. cell preparation

- Innoculate 40 mL of LB with E. coli DH5α frozen stock from IGEM -80C freezer stock in a sterile 250 mL flask
- Place in the 30C shaker to grow out overnight

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Preparation of E. coli DH5α Comp. Cells

Modified from Lin Group in Chemical Engineering
Retrieved from
"https://webapps.itcs.umich.edu/chelinlab/index.php/Preparation_of_Electric_Competent_Cells"
Media

- LB
  - 40 mL for ON innoculation
  - 200 mL for cultivation
- Sterile DI water
  - 265 mL for washing

1. In the morning, start two cultures with 20 mL of the ON E. coli DH5α in 100mL LB in sterile 500 ml flasks and cultivate until OD600 reaches between 0.4 to 0.9.
2. Pour 40 mL of the cell in 50 mL falcon tubes and chill it on ice for 15-30min.
   - At this time make sure all of the supplies needed for the experiment are chilled
3. Harvesting - Centrifuge it at 4200 rpm for 15min and discard supernatant.
4. Washing 1 - Resuspend cell pellet with 40ml of cold sterile water, centrifuge it at 3500rpm for 15min, and discard supernatant.
5. Washing 2 - Resuspend cell pellet with 20ml of cold sterile water, centrifuge it at 3500rpm for 15min, and discard supernatant.
6. Washing 3 - Resuspend cell pellet with 2ml of cold sterile water, centrifuge it at 3500rpm for 15min, and discard supernatant.
7. Resuspension - Resuspend cell pellet in 100ul of cold sterile water if needed.
8. Aliquot 40ul.
9. Dilute 1:100 and measure OD_{600}

- OD_{600} below 0.200 will require reconcentrating

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. Enterobacteria phage T4 Lysis Device: BBa_K112808- plate 3 well 6G
        ii. Lambda phage lysis device: Bba_K112022-plate 3 well 24E
        iii. Bacteriophage lysis cassette:Bba_K124017-plate 3 well 20M
        iv. RBS:Bba_J61101-plate 1 well 5L
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 of Biobricks

1. Mix 40ul of comp. cell with 1-2ul DNA.
    - Samples for each biobrick of interest
    - Positive control with pSIM5
    - Negative control
    - Plasmid control (not enough cuvettes so plate plasmid directly)
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 AMP plate (All biobricks have AMP resistance, 17 plates total)
    - One positive control
    - One negative control
    - One plasmid control for each biobrick
    - No dilution, 1:10 dilution and 1:100 dilution for each biobrick transformation
7. Place in the 37 C incubator to grow out overnight

Results

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The comp. cells were cultivated for 4 hours and grew out to an OD_{600} of 0.544 and 0.645 for the two flasks. After washing, the comp. cells were condensed to two eppendorff tubes. These tubes had an OD_{600} of 0.469 and 0.339 after a 1:100 dilution and were used for the transformation. One mL of left over comp. cells were mixed with 110 uL of pure 0.22 um filter sterilized glycerol and were placed in the IGEM box in the -80C freezer. However, these cells were not

used in the next few days so they are probably no longer viable. The plasmids extracted from the registry were stored in J. Minty box 2 and the T. Reesei box in the -20C freezer.