Protocols adapted From Lin Group in Chemical Engineering (also see notes on 8/8/09)

Performed by Charlie, Jungho and David

Thursday 10/15/09

1. Inoculate 5 of LB in a 50 mL falcon tube with *E. coli* DH5alpha and cultivate it overnight in the 30 C shaker.

Friday Morning 10/16/09

2. In a **STERILE** flask add 45 mL of LB and the 5 mL overnight culture. Incubate in the 30C shaker for **4 hours** until the OD 600 is between 0.4 and 0.9

Friday Afternoon 10/16/09

3. Pour the cell in a 50 mL falcon tube and chill ice for **20 min**.
    - Ice is located upstairs on the 3rd floor of GGBrown in room 3458 (use lab key labeled ice to open the door around the corner)
4. **EVERYTHING THAT TOUCHES THE CELLS MUST BE CHILLED UNTIL SHOCKING THE CELLS!**
    - Chill sterile DI water on ice
    - Place electroporation cuvettes in the -20C fridge
    - Place 1000 uL and 200uL pipette tips in the -20C fridge
    - Place pipets 25mL, 5 mL, 2 mL in the -20C fridge
5. During the harvesting process (i.e. while you are waiting for the 10 minutes centrifuge cycle) prepare for transformation
    - Place 2 uL of DNA from the 6 ligation reactions into labeled separate 1.5 mL eppendorf tube (take care to use sterile conditions while removing from the container)
        1. Pu/GFP 1:1 insert to vector ligation
        2. Pu/GFP 1:3 ligation
        3. Pu/GFP 1:6 ligation
        4. PrXylR/leuLP 1:1 ligation
        5. PrXylR/leuLP 1:3 ligation
        6. PrXylR/leuLP 1:6 ligation
    - Place 2 uL of a positive control (one that we know the transformation works and has AMP resistance, could use the GFP generator #3 located in the IGEM -20C freezer box (bottom right corner of fridge))
    - Place all tubes with DNA on ice to chill!!
    - Open the electroporation cuvettes and place on ice, label the purple caps with the sample (dont remove the caps, the cuvettes are sterile!)
    - Add 1 mL of LB media to 8-1.5 mL eppendorf tubes
        1. Label the for each ligation, positive control and negative control (comp cells only)

6. Harvesting - Centrifuge it at 3500 rpm for **10min** and discard supernatant in the waste container on the lab bench.
    - When pouring off liquid waste make sure cell pellet if facing up towards the ceiling so it does not get washed out
7. Washing 1 - Resuspend cell pellet with 50ml of cold sterile water, centrifuge it at 3500rpm for **10min**, and discard supernatant.
    - The pellet will be very dense so use a chilled 5 mL pipette to transfer 5 mL of chilled DI water, use the chilled 1000 uL pipette to resuspend the cell pellet and add the rest of the chilled water
8. Washing 2 - Resuspend cell pellet with 25ml of cold sterile water, centrifuge it at 3500rpm for **10min**, and discard supernatant.
    - Resuspend the pellet by stirring on the ice
9. Washing 3 - Resuspend cell pellet with 2ml of cold sterile water, centrifuge it at 3500rpm for **10min**, and discard supernatant.
    - The pellete will be very loose so take precautions not to disturbe it
10. Resuspension - Resuspend cell pellet in 100ul of cold sterile water (if necessary, resuspension in remaining liquid may be adequate).
    - Typically remaining liquid is sufficient

Transformation

1. Turn on the electroporation machine and set to 1800 V
2. Perform 1 electroporation at a time starting with the positive and negative control
    a. Person 1
        i. Transfer 40 uL of compcells to the chilled eppendorf with the DNA using a chilled pipette tip and mix by pipetting
        ii. Place sample into the cuvette by placing the pipet tip into the grove inside the cuvet and slowly pipeting
        iii. Tap on the benchtop to remove any bubbles
        iv. Return the pipet tips to the freezer to keep cold
    b. Person 2
        i. Carry cuvette to electroporation machine
        ii. Remove purple cap and slide inside
        iii. Press pulse twice
        iv. Make sure no popping sounds occur! Note any samples where popping occurs
    c. Person 2/3
        i. Carry cuvette over to the 1.5 mL eppendorf tubes
        ii. Remove the 1 mL of LB from the tube and transfer directly to the cuvette immediately after electroporation to save the cells. Pipette up and down slowly to mix, try not to make foam

iii. Remove 1 mL of culture and place back in the same 15 mL tube
iv. Place the tube in the 37 C incubator to grow out for **1 hr**

3. Repeat this process for each sample
4. For all of the ligation samples after incubation pellet cells at 14000 rpm for 2 minutes (may need to go next door to the gulari lab and use the table top centrifuge, the rotor is stuck in ours)
5. Remove supernatent and resuspend in 100 uL of STERILE DI water
6. Add 100 uL of each to a LB +AMP plate (on the IGEM shelf of the 4C frigde)
7. Use sterile beads and pour about 5 beads onto each plate keeping sterile conditions
8. Stack plates and rotate and shake for about 2 minutes to spread the culture
9. Turn the plates vertical and dump out the beads into the bead waste container (the beads will be baked and reused).
10. Place plate upside down in the 37 C incubator