

Monday 10/5/09

Colony PCR PrXylR from P. putida mt-2(modified from protocol on 8/4/09)

1. Take a stab of the frozen stock of *P. putida* pWW0 from the IGEM -80C frozen stock (not too much because too much template will lead to unspecific replication) and resuspend it in 50 uL of DI water
2. Mix the primers by making a 50 uM stock. Multiply the nmoles of DNA in each primer by 20, and resuspend in that amount of ultra pure water
 - a. Upstream
 - i. 38.9 nmoles
 - ii. 779 uL of ultra pure water
 - b. Downstream
 - i. 33.7 nmoles
 - ii. 674 uL of ultra pure water
3. Turn on PCR machine to instant incubate at 98 C for the first initial denaturation step
4. Place PCR tubes for run on ice, place tips in -20C freezer
5. PCR reaction KEEP EVERYTHING ON ICE
 - a. Vortex all tubes before starting to make sure everything is well mixed
 - b. For a single reaction mixture
 - i. 37.5 uL Ultra pure water (36.275 uL Ultra pure water was added to this reaction due to primer miscalculation)
 - ii. 10 uL of 5x phusion master mix
 - iii. 1 uL 10 mM dNTP
 - iv. 0.500 uL of primer (0.625 uL of primer A was added for this reaction, used 40 uM to calculate not 50 uM)
 - v. 0.500 uL of primer B (0.625 uL of primer A was added for this reaction, used 40 uM to calculate not 50 uM)
 - vi. 0.5 uL Phusion DNA polymerase
 - c. Combine all ingredients but polymerase for 5 samples
 - i. 225 uL of ultra pure water (217.65 uL of ultra pure water was added to this reaction)
 - ii. 60 uL of 5x phusion master mix
 - iii. 6 uL 10 mM dNTP
 - iv. 3.000 uL of primer A (3.725 uL of primer A was added for this reaction)
 - v. 3.000 uL of primer B (3.725 uL of primer B was added for this reaction)
 - vi. 6 uL of DNA template (from step 1)
 - d. Chill mixture for 15 minutes
 - e. Add 3 uL Phusion DNA polymerase with chilled pipette tip
 - f. Transfer 50 uL of sample to each PCR tube with chilled pipette tip
6. PCR cycle
 - a. 98 C for 30 minutes
 - b. 98 C for 10 seconds
 - c. 50 C to 62 C for 30 seconds

- d. 72 C for 35 seconds (15 seconds per 1 kb for plasmid DNA)
- e. Goto step b four times
- f. 98 C for 10 seconds
- g. 66.7 C for 30 seconds
- h. 72 C for 35 seconds
- i. Goto step f twenty nine times
- j. 72 C for 5 minutes
- k. 4C forever
- l. End