Sunday 8/9/09

Colony PCR Pu Promoter
Slightly modified protocol from 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. Turn on PCR machine to instant incubate at 95 C for the first initial denaturation step
3. Place PCR tubes for run on ice
4. 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. 36.275 uL Ultra pure water
        ii. 10 uL of 5x phusion master mix HF
        iii. 1 uL 10 mM dNTP
        iv. 0.625 uL of primer A (0.5 uM concentration from 40 uM stock)
        v. 0.625 uL of primer B (0.5 uM concentration from 40 uM stock)
        vi. 1 uL of DNA template (from step 1)
        vii. 0.5 uL Phusion DNA polymerase
    c. Combine all ingredients but polymerase for 6 samples
        i. 217.65 uL of ultra pure water
        ii. 60 uL of 5x phusion master mix
        iii. 6 uL 10 mM dNTP
        iv. 3.75 uL of primer A
        v. 3.75 uL of primer B
        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

5. PCR cycle
    a. 95 C for 10 minutes
    b. 98 C for 30 seconds
    c. 58 C for 30 seconds
        i. This annealing temperature gave highest DNA concentration in last PCR
    d. 72 C for 15 seconds
    e. Goto step b four times
    f. 98 C for 10 seconds
    g. 69 C for 30 seconds
    h. 72 C for 15 seconds
    i. Goto step f twenty nine times
    j. 72 C for 10 minutes
    k. 4C forever
    l. End

Gel of Pu Promoter colony PCR
Ran a gel according to the protocol on 7/27/09.

DNA purification of Pu promoter round 2
DNA purification of the Pu promoter from colony PCR today was done using the protocol on 8/5/09.

Nanodrop of Pu promoter round 2 and leu landing pad
The DNA concentration was checked using the nanodrop according to the protocol on 7/25/09

Miniprep of GFP generator
The miniprep of the GFP generator was performed according to the protocol on 7/24/09

Nanodrop of GFP generator miniprep
The nanodrop of the GFP generator was performed according to the protocol on 7/25/09

Restriction Digest of Pu promoter, GFP generator and leu landing pad
The restriction digest was performed according to the protocol on 7/25/09

The reagents were mixed in the following proportions:

	Sample		
Reagent (uL)	Pu	GFP	Leu LP
DI water	2.5	38.5	32.5
NEB 2 buffer	5	5	5
BSA	0.5	0.5	0.5
DNA	40	4	10
EcoRI	1	XXX	1
XbaI	XXX	1	XXX
SpeI	1	XXX	XXX
PstI	XXX	1	1

Results

Transformation of the GFP generator

The transformation of the GFP generator was a success and 3 colonies were chosen off of the no dilution plate to make a frozen stock and start a 5 mL culture with 100 ug/mL AMP for a miniprep

Gel of Pu Promoter colony PCR
The UV lamp was broken today so no picture was taken. Using a hand-held UV lamp, all lanes showed DNA just under 500 bp so the PCR was successful.

Nanodrop of Pu promoter round 2 and leu landing pad
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Sample ID	ng/uL	A260	260/280	260/230	Constant
Pu 2.1	36.70	0.734	1.67	0.94	50
Pu 2.2	18.29	0.366	1.86	2.39	50
Pu 2.3	13.14	0.263	1.77	2.05	50
Pu 2.4	40.73	0.815	1.65	0.83	50
Pu 2.5	16.17	0.323	1.70	1.97	50
Pu 2.6	50.11	1.002	1.56	0.75	50
leu landing pad	54.45	1.089	1.82	2.07	50

Pu 2.1, Pu 2.4 and Pu 2.6 did not have a strong peak at 260 nm and will not be used for further experimentation.

Pu 2.2 will be used for the digest because it has the most DNA

Nanodrop of GFP generator miniprep
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Sample ID	ng/uL	A260	260/280	260/230	Constant
GFP gen 1	124.39	2.488	1.87	1.74	50
GFP gen 2	185.51	3.710	1.82	1.36	50
GFP gen 3	153.86	3.077	1.89	1.88	50

GFP gen 2 looked less pure than GFP gen 1 and 3. GFP gen 3 will be used for the digestion because it has the most DNA.