Thursday 8/13/09

Gel of purified digest for Pu-RBS-GFP
A gel of the purified digest of the Pu promoter, GFP generator and leu landing pad was run for both attempts at purification according to the protocol on 7/27/09.

Digestion of Pu promoter, GFP generator, and leu LP round 2
Looking at the digestion program on the newer PCR machine, a weird denaturation time of 5 hours 37 minutes and 39 seconds appeared. I am unsure of how this changed time changed, but the GFP generator and leu landing pad were digested under these conditions. The Pu promoter was digested in the old PCR machine with a normal denaturation time of 20 minutes.

A larger volume of each sample will be digested according to the protocol on 7/25/09 for a shorter time of 6 hours with the following amounts:

Amounts (uL)	Pu promoter 3.1	GFP gen 3	Leu LP
Ultra pure water	35	77	65
NEB 2 Buffer	10	10	10
BSA	1	1	1
DNA	50	8	20
EcoRI	2	XXX	2
XbaI	XXX	2	XXX
SpeI	2	XXX	XXX
PstI	XXX	2	2

Troubleshoot DNA purification
Reading through the QIAqen manual for the PCR purification, two potential problems arose: the pH of the binding buffer was too high or the pH of the water for elusion was too low. Using pH paper to test both reagents, the ultra pure water had a pH greater than 7 (which is ideal for elusion) but the PBI binding buffer had a pH just less than 7 (when ideally the pH should be around 5 for best binding to the column). Upon the QIAgen manual instruction, 10 uL of 3 M sodium acetate was added to 125 uL of PBI buffer which brought the pH of the solution between 3 and 4. This might have been more of a problem with the restriction digest because the NEB 2 buffer used has a pH of 7.8 at room temperature which may have raised the pH so the DNA would not bind to the column. For the PCR reaction, the HF phusion buffer is comprised primarily of DMSO therefore changing pH is not a big concern.

To avoid this complication, instead of using the old reagents in the lab, the new reagents will be mixed in the IGEM PCR purification kit.

A very small amount of digestion product mixed with PBI buffer can also be checked on pH paper before placing in the column to see if the pH needs to be adjusted

Gel of digested Pu promoter, GFP generator, and leu LP round 2

A gel was run according to the protocol on 7/27/09 of the digested DNA for the ligation of Pu-RBS-GFP

DNA purification of digested Pu promoter, GFP generator, and leu LP round 2
DNA purification of the digested Pu promoter, GFP generator, and leu LP round 2 were done according the the protocol on 8/9/09. Before putting the PB buffer mixed with the digest sample in the column, a small amount of sample will be tested with pH paper. All of the washings will be done in eppendorf tubes to save the DNA if it does not get stuck on the column.

Nanodrop of digested Pu promoter, GFP generator, and leu LP round 2
The nanodrop was performed for the digested Pu promoter, GFP generator, and leu LP round 2 according to the protocol on 7/25/09.

Results

Gel of purified digest for Pu-RBS-GFP

Lane 1(top): Invitrogen 1 kb plus ladder
Lane 2: Pu promoter
Lane 3: GFP Generator
Lane 4: leu landing pad

A faint band for the Pu promoter showed up around 500 bp, the proper band for the cut backbone showed up at approximately 2100 bp for the GFP generator and we will assume that the cut part is present, and the leu backbone showed the proper length at about 5800 bp.

Nanodrop of digested Pu promoter, GFP generator, and leu LP round 2

There was still no DNA after the DNA purification even though it appeared in the gel after the digestion. After consulting the Ginko biobrick assembly manual, they do not require DNA purification before ligation. Also, looking up the salts for the NEB 2 digestion buffer and the ligation buffer, they are similar so the left over salts from the digestion hopefully will not interfere with the ligation. The only problem that may arise without purifying the product is having salt in the solution for the electroporation procedure may cause arching. Since only 1 uL

of DNA will be added for 40 uL of salts it should not make a big difference. For these reasons, the ligation procedure will be attempted without purifying the digest.