Tuesday 8/11/09

Digest of GFP generator

A digest of the 3 GFP generator colonies minipreped was performed according to the protocol on 7/25/09 with a shorter digestion time of 6 hours. The reagents were mixed in the following proportions:

	Sample	
Reagent (uL)	GFP single digest	GFP double digest
DI water	39.5	38.5
NEB 2 buffer	5	5
BSA	0.5	0.5
DNA	4	4
EcoRI	1	XXX
SpeI	1	1

Gel of GFP generator

A gel of the GFP generator was run according to the protocol on 7/27/09.

Pu promoter colony PCR round 3

There was only a small amount of Pu promoter left from the PCR of round 2. To make the downstream steps easier, the PCR will be repeated for 12 replicates by doubling the reaction volume for the protocol on 8/9/09. If the samples are still dilute, there will be enough DNA to concentrate a few of the samples and still have some left over if it does not work.

Gel of Pu promoter colony PCR round 3

A gel of the Pu promoter colony PCR round 3 was run according to the protocol on 7/27/09.

DNA purification of colonly PCR round 3

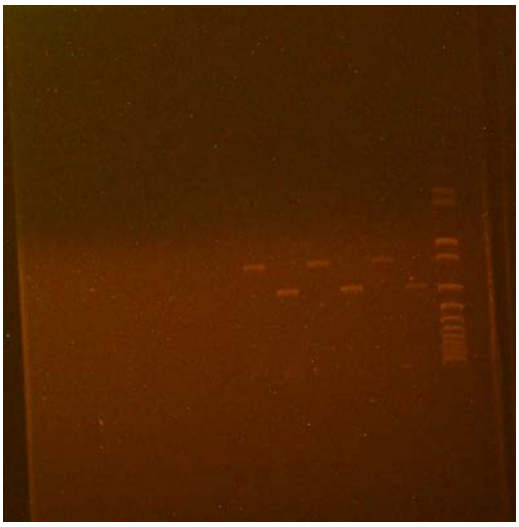
A QIAqen PCR purification kit was used to purify the PCR product according to the protocol on 8/5/09.

Nanodrop of Pu promoter colony PCR round 3

A nanodrop of the Pu promoter round 3 colony PCR was performed according to the protocol on 7/25/09.

Results

Digest of GFP generator



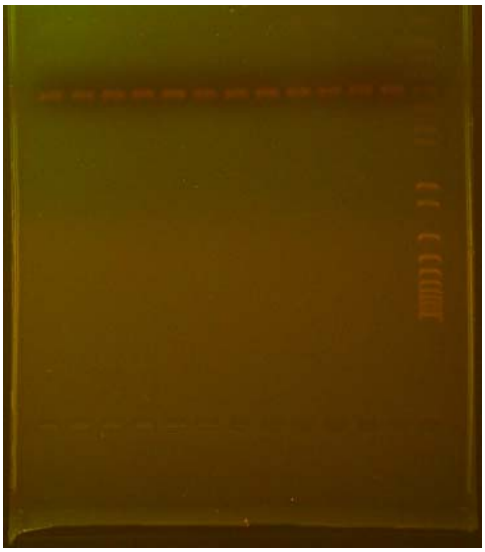
Lane 1: Invitrogen 1 kb plus ladder (far right)
Lane 2: GFP generator colony 1 cut with SpeI
Lane 3: GFP generator colony 1 cut with EcoRI and SpeI
Lane 4: GFP generator colony 2 cut with SpeI
Lane 5: GFP generator colony 2 cut with EcoRI and SpeI
Lane 6: GFP generator colony 3 cut with SpeI
Lane 7: GFP generator colony 3 cut with EcoRI and SpeI

Digestion lengths

- GFP generator
    - Part: 878 bp
    - Plasmid backbone: 2079 bp

The digestion length of the plasmid cut with one restriction enzyme was the expected length of approximately 3000 bp. The double digest only showed the length corresponding to the plasmid backbone indicating the part was cut out. The part may not be visible due to a combination of the short length and DNA concentration.

Pu promoter colony PCR round 3



Lane 1: Invitrogen 1 kb plus ladder (far right)
Lane 2: Pu promoter 3.1
Lane 3: Pu promoter 3.2
Lane 4: Pu promoter 3.3
Lane 5: Pu promoter 3.4
Lane 6: Pu promoter 3.5
Lane 7: Pu promoter 3.6
Lane 8: Pu promoter 3.7
Lane 9: Pu promoter 3.8
Lane 10: Pu promoter 3.9
Lane 11: Pu promoter 3.10
Lane 12: Pu promoter 3.11
Lane 13: Pu promoter 3.12

This PCR reaction yield strong bands on the gel indicating a higher DNA yield.

Nanodrop of Pu promoter round 3 colony PCR

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Sample ID	ng/uL	A260	260/280	260/230	Constant
Pu prom 3.1	23.09	0.462	2.02	1.62	50
Pu prom 3.2	23.46	0.469	1.75	1.61	50
Pu prom 3.3	26.69	0.534	1.75	1.54	50
Pu prom 3.4	22.58	0.452	1.96	1.61	50
Pu prom 3.5	20.34	0.407	1.91	1.56	50
Pu prom 3.6	21.97	0.439	1.82	1.61	50
Pu prom 3.7	20.03	0.401	1.84	1.82	50
Pu prom 3.8	22.50	0.450	1.79	1.56	50
Pu prom 3.9	22.71	0.454	1.86	1.98	50

Pu prom 3.10	21.26	0.425	1.82	1.82	50
Pu prom 3.11	21.67	0.433	1.63	1.67	50
Pu prom 3.12	20.74	0.415	1.84	1.86	50

All samples had a good absorbance peak at 260 nm and can be used for further experiments. Since sample 3 had the highest DNA concentration it will be used for the digestion and ligation.