Tuesday 10/13/09

Miniprep of leu landing padb

David started a 1 mL LB +AMP overnight culture and 0.5 mL of this culture was used to inoculate two 4.5 mL culture of LB+100 ug/mL AMP. These culture were started at 7:00PM for a miniprep the following morning.

Wednesday 10/14/09

Miniprep of leu landing pad

A miniprep of the leu landing pad overnight cultures were performed according to the protocol on 7/24/09. The DNA was eluted in 50 uL of ultra pure water.

Nanodrop of leu landing pad

A nanodrop experiment was performed for the leu landing pad according to the protocol on 7/25/09.

Digestion of leu landing pad and PrXylR

The digestion was done according to the protocol on 7/25/09 for a 25 uL reaction volume for the Leu Landing Pad miniprepped today and the gel extraction purified PrXylR samples. The reagents were mixed in the following amounts:

	Sample	
Reagent (uL)	PrXylR 1 and 2	Leu LP 1 and 2
NEB 2 buffer	2.5	2.5
BSA	0.25	0.25
DNA	21.25	21.25
EcoRI	.5	.5
XbaI	XXX	XXX
SpeI	.5	.5

NOTE: PrXylR cannot be digested with PstI because it has a PstI cutsite in one of the regulator protein binding sequences!

No DI water was added because the DNA concentrations are very dilute and both samples were eluted in ultra pure water.

Gel of Digested Revised Pu, GFP generator, PrXylR, Leu LP and uncut Leu LP

A gel was run according to the protocol on 7/27/09 for the PrXylR and Leu LP samples digested today, the uncut Leu LP miniprepped today, and the revised Pu and GFP generator digested on 10/11/09.

Ligation of Pu/GFP generator and PrXylR#2/Leu LP#2

The ligation was performed according to the protocol on 9/8/09 with the inserted to vector ratios determined by volume according to the following amounts:

Component	**1:1** **Volume (20 μL total)**	**1:3** **Volume (20 μL total)**	**1:6** **Volume (20 μL total)**
Ultrapure dH2O	13 μL	9 uL	3 uL (4 uL for PrXylR)
Leu landing pad Or GFP gen	2 uL	2 uL	2 uL
PrXylR or Pu	2 uL	6 uL	12 uL (11 uL for PrXylR because I ran out of sample)
10x Ligase Buffer	2 μL	2 μL	2 uL
T4 DNA ligase	1 μL	1 μL	1 uL

Results

Nanodrop of leu landing pad

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10/14/2009  1:53 PM
Sample ID       ng/uL   A260    260/280 260/230 Constant
Leu LP miniprep #1      7.92    0.158   1.79    1.25    50
Leu LP miniprep #2      43.11   0.862   1.61    0.69    50
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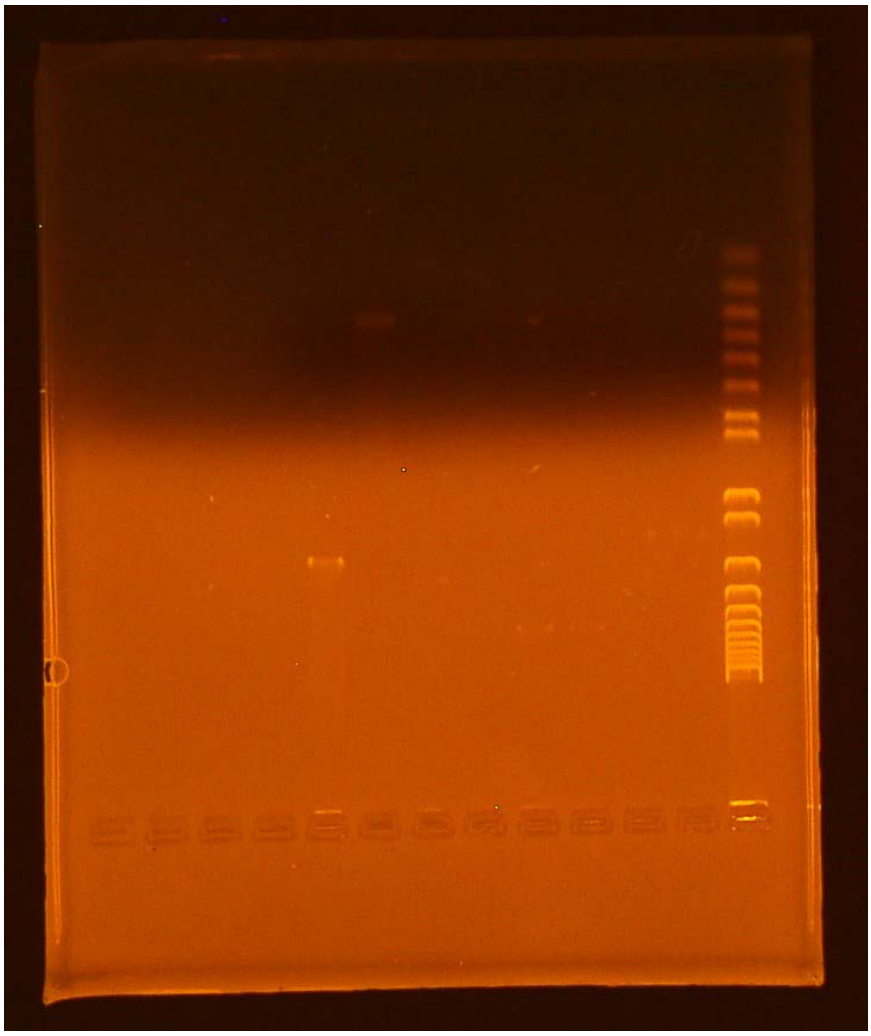
Leu LP miniprep #1 showed a very faint peak and Leu LP miniprep #2 did not show any peak. These samples will be digested and a gel of the digest will be run to check for DNA.

Gel of Digested Revised Pu, GFP generator, PrXylR, Leu LP and uncut Leu LP



Lane 1 (far right): Invitrogen 1 kb plus ladder
Lane 2: PrXylR #1 digested with EcoRI and SpeI (2340 bp)
Lane 3: PrXylR #2 digested with EcoRI and SpeI (2340 bp)
Lane 4: Leu LP #1 digested with EcoRI and SpeI (5800 bp)
Lane 5: Leu LP #2 digested with EcoRI and SpeI (5800 bp)
Lane 6: Uncut Leu LP #1
Lane 7: Uncut Leu LP #2
Lane 8: Revised Pu digest with EcoRI and SpeI (370 bp)
Lane 9: GFP generator digested with EcoRI and XbaI (2100 bp)

All of the samples show very faint signs of DNA. PrXylR #2 and Leu LP #2 will be used for the ligation.