Wednesday 8/12/09

Progress and plan for ligation of Pu RBS GFP

- Pu promoter
  - Currently purified from PCR
  - Digest with EcoRI and SpeI
  - DNA purification
    - Elute in ultra pure water
  - Nanodrop
  - Ligate
- GFP generator
  - Currently minipreped
  - Digest with XbaI and PstI
  - DNA purification
    - Elute in ultra pure water
  - Nanodrop
  - Ligate
- Leu landing pad
  - Currently digested with EcoRI and PstI
  - DNA purification
    - Elute in ultra pure water
  - Nanodrop
  - Ligate

Autoclave

Need sterile flasks for transformation and eppendorf tubes

Pu promoter digestion

Digest according to the protocol on 7/29/09 with a shorter digestion time of 6 hours. Mix the reagents as follows:

Amounts (uL)	Pu promoter 3.3
Ultra pure water	22.5
NEB 2 Buffer	5
BSA	0.5
DNA	20
EcoRI	1
XbaI	XXX
SpeI	1
PstI	XXX

DNA purification of Pu promoter digest 3.3, GFP generator digest 3, and leu landing pad digest

Use the PCR purification kit to purify the digestion products according to the protocol on 8/5/09. Make sure to elute in ultra pure water with no EB buffer.

Nanodrop of Pu promoter digest 3.3, GFP generator digest 3, and leu landing pad digest
Measure the DNA concentration of the purified digests using the protocol on 7/25/09.

Results
Nanodrop of Pu promoter digest 3.3, GFP generator digest 3, and leu landing pad digest

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Sample ID	ng/uL	A260	260/280	260/230	Constant
Pu digested with EcoRI and SpeI	7.09	0.142	1.49	0.76	50
GFP digested with XbaI and PstI	27.07	0.541	1.59	0.61	50
Leu LP digested with EcoRI and PstI	22.62	0.452	1.47	0.58	50

The three samples measured above did not sit in the wash buffer for 5 minutes during the DNA purification. The nanodrop results showed an unpure product with a high absorbance at 230 nm. The remaining digest samples (approximately 20 ul) was purified again adjusting the amount of buffer PB added to 100 uL. The DNA concentration was measured again to show similar results:

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Sample ID	ng/uL	A260	260/280	260/230	Constant
Pu digest EcoRI/SpeI	33.58	0.672	1.56	0.59	50
GFP digest XbaI/PstI	40.57	0.811	1.49	0.61	50
LP digest EcoRI/PstI	33.80	0.676	1.47	0.60	50

A gel can be run to check if the DNA is still there. If there is DNA, the ligation may still work even if the product is impure.