

**Monday 10/19/09**

*Nanodrop of miniprep Pu/GFP and PrXylR/LeuLP ligation products*

A nanodrop was performed for the 7 samples that were miniprep yesterday according to the protocol on 7/25/09

*Digestion of miniprep Pu/GFP and PrXylR/LeuLP ligation products*

A digest will be performed for all of the samples miniprep yesterday according to the protocol on 7/25/09 for 6 hours. The reagents will be mixed in the following proportions:

| Amounts (uL)     | Pu GFP<br>1:1 #1 | Pu GFP<br>1:1 #2 | Pu GFP<br>1:3 #1 | Pu GFP<br>1:3 #2 | Pu GFP<br>1:6 #1 | Pu GFP<br>1:6 #2 | Pr LeuLP<br>1:3 |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-----------------|
| Ultra pure water | 40.75            | 40.75            | 40.75            | 40.375           | 41               | 40.2             | 38.5            |
| NEB 2 Buffer     | 5                | 5                | 5                | 5                | 5                | 5                | 5               |
| BSA              | 0.5              | 0.5              | 0.5              | 0.5              | 0.5              | 0.5              | 0.5             |
| DNA              | 2.75             | 2.75             | 2.75             | 3.125            | 2.5              | 3.3              | 5               |
| EcoRI            | 1                | 1                | 1                | 1                | 1                | 1                | 1               |

*Gel of digested Pu/GFP and PrXylR/LeuLP*

A gel was run of the above digestion samples with the cut backbone used in the ligation as a reference according to the protocol on 7/27/09.

*Submitting Parts to the Registry*

1. In a single PCR tube dilute DNA
  - a. The concentration cannot be lower than 2ng/uL
  - b. Add 2 uL of DNA to 8 uL of ultra pure water
2. Wrap the PCR tube with label tape and give each tube a number
3. Cap the PCR tube and place inside a 50 mL falcon tube
4. Follow the instructions for submitting DNA on the registry

**Results**

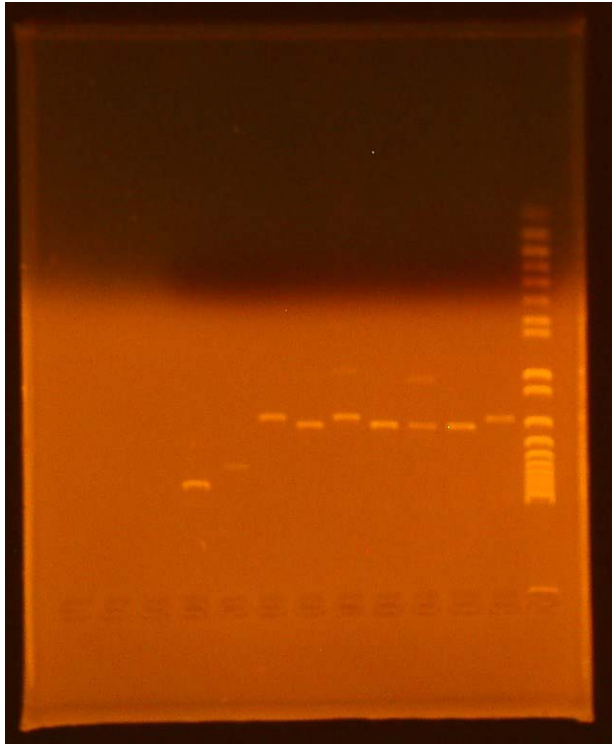
*Nanodrop of miniprep Pu/GFP and PrXylR/LeuLP ligation products*

**10/19/2009 9:42 AM**

| Sample ID              | ng/uL  | A260  | 260/280 | 260/230 | Constant |
|------------------------|--------|-------|---------|---------|----------|
| Pu/GFP 1:1 #1 ligation | 182.22 | 3.644 | 1.90    | 2.30    | 50       |
| Pu/GFP 1:1 #2 ligation | 183.83 | 3.677 | 1.86    | 1.84    | 50       |
| Pu/GFP 1:3 #1 ligation | 181.75 | 3.635 | 1.90    | 2.30    | 50       |

|                           |        |       |      |      |    |
|---------------------------|--------|-------|------|------|----|
| Pu/GFP 1:3 #2 ligation    | 166.33 | 3.327 | 1.92 | 2.32 | 50 |
| Pu/GFP 1:6 #1 ligation    | 202.72 | 4.054 | 1.89 | 2.02 | 50 |
| Pu/GFP 1:6 #2 ligation    | 148.52 | 2.970 | 1.83 | 1.49 | 50 |
| PrXylR/leuLP 1:3 ligation | 108.05 | 2.161 | 1.92 | 2.28 | 50 |

*Gel of digested Pu/GFP and PrXylR/LeuLP*



- Lane 1 (far right): Invitrogen 1 kb plus ladder
- Lane 2: GFP backbone used in the ligation
- Lane 3: Pu 1:1 #1
- Lane 4: Pu 1:1 #2
- Lane 5: Pu 1:3 #1
- Lane 6: Pu 1:3 #2
- Lane 7: Pu 1:6 #1
- Lane 8: Pu 1:6 #2
- Lane 9: LP backbone used in the ligation
- Lane 10: PrXylR 1:3

The expected length of the Pu/GFP ligation is approximately 2450 bp

- Pu: 370 bp
- GFP: 2080 bp

The expected length of the PrXylR/leuLP ligation is approximately 8140bp

- PrXylR: 2340 bp
- leuLP: 5800 bp

*Submitting Parts to the Registry*

Pu/GFP 1:1 #1 ligation and PrXylR 1:3 were submitted to the registry as Bba\_K270002 and Bba\_K270001 respectively.