Wednesday 8/5/09

Pu Promoter Colony PCR optimization

Some of Jeremy's suggestions to improve yield for PCR from protocol on 8/4/09

- Increase initial denaturation time from 6 minutes to 10 minutes
- Pick annealing temperature that gave the best yield-53.7 C for first 5 cycles(even though they all looked pretty much the same)
- Use slightly more template (too much will give unspecific amplification)
- If this doesn't work, do 4 reactions today, than the products can be purified and concentrated

Repeat colony PCR of Pu promoter using Jeremy's suggestions

Gel of Pu Promoter Colony PCR optimization

Ran a gel of 5 replicates for Pu promoter colony PCR optimization according to the protocol on 7/27/09

Purification of Pu Promoter from colony PCR on 8/4/09

Protocol is adapted from Jeremy's Protocol for isobutanol evolution gene sequencing of rob

1. Add 5 volumes Buffer PB to 1 volume of PCR sample and mix. DO not need to remove mineral oil

    a. Withdraw 25 uL PCR sample and transfer to eppendorf tube. Add 125 uL buffer PB and mix

2. Place a QI quick spin column (purple) in a provided 2 mL collection tube. Apply prepared mixture to the QIA quick column. Centrifuge column at 13000 rpm for 60 sec.

3. Discard flow through, and place column back into same tube

4. Add 750 uL buffer PE to QIA quick column. Let stand for 5 minutes after adding PE buffer. Centrifuge at 13000 rpm for 1 minute and discard flow through.

5. Centrifuge column for an additional 1 min at 13000 rpm

6. Place QIA quick column into a clean 1.5 mL eppendorf tube

7. To elute DNA, add 50 uL 1:10 diluted Buffer EB to the center of the QIA quick membrane, let column stand for one minute, then centrifuge at 13000 rpm for one minute

    a. Prepare 1:10 diluted buffer EB by adding 100 uL buffere EB to 900 uL ultrapure water in an eppendorf tube. Briefly vortex to mix

Nanodrop of Purified Pu promoter

Used protocol on 7/25/09

Results

Gel of Pu Promoter Colony PCR optimization

No DNA appeared on the gel. Decided to purify product from yesterday's PCR.

Nanodrop of Purified Pu Promoter

Default
8/5/2009 6:35 PM

Sample ID	ng/uL	A260	260/280	260/230	Constant
Pu 1	11.23	0.225	2.03	1.62	50
Pu 2	10.79	0.216	1.99	1.91	50
Pu 3	10.97	0.219	2.14	1.73	50
Pu 4	16.96	0.339	2.00	2.18	50
Pu 5	15.29	0.306	1.83	1.83	50

When the PCR was run earlier today, we picked a lower annealing temperature, when really the higher annealing temperatures yielded more DNA.

Jeremy suggested concentrating DNA to 100 ng/uL for a smaller reaction volume with the ligation step.