Thursday 8/6/09

DNA concentration of Pu promoter

Protocol from Openwetware

Italicized comments are Jeremy's suggestions

1) Add 0.1 volumes of 3M Sodium Acetate solution to 1 volume of DNA sample.
   i) Pool all 5 PCR products from 8/4/09 together to make a 250 uL sample
   ii) 25 uL 3 M NaAc
2) Add 1ul Glycogen to the DNA sample.

I don't do this because we don't have any glycogen. It doesn't really do much. Glycogen co-precipitates with the DNA, and makes the pellet easier to see after centrifugation

3) Add 2 volumes of 95% EtOH to the DNA Sample.
   i) 275 uL DNA solution
   ii) 550 uL 95% ethanol
4) Store the solution overnight at -20°C or for 30 minutes at -80°C.

   -20C at 1.5 to 2 hours works for me. The DNA actually starts precipitating at 4C. Overnight incubation at -20C is not necessary. -80C will freeze the solution solid, might not be so good for the DNA

5) Centrifuge the solution at maximum speed for least 15 minutes.

Do @ 4C. Be sure to note the orientation of the eppendorf tube in the centrifuge! (See below)

6) Decant and discard the supernatant.

I recommend pipetting the supernatant off. Do not disturb the pellet. The pellet might be very tiny and hard to see or might not even be visible. If this is the case, then you need to guess the position of the pellet in the tube and keep the pipette tip on the opposite side of the tube during pipetting.

7) (Optional) Add 1 ml of 70% EtOH to the pellet and let sit for 5 minutes.

You should shake the tube a little bit to resuspend the DNA.

8) (Optional) Centrifuge the sample at maximum speed for 5 minutes.
9) (Optional) Decant and Discard the supernatant.
10) Air-dry the pellet for 10-15 minutes at room temperature until all liquid is gone.
    If you are in a hurry you can put a 1000 uL pipette tip to an air line and blow-dry the pellet. The air flow rate needs to be extremely low or you can blow out all the liquid and lose your DNA.
11) Resuspend in desired volume of water or buffer
    i) 25 uL ultra pure water

Nanodrop concentrated Pu promoter DNA

Ran nanodrop according to protocol on 7/25/09

Results

DNA concentration of Pu promoter
When performing the DNA precipitation, the DNA remained in the 70% ethanol for about 15 minutes instead of 5 minutes.

Nanodrop concentrated Pu promoter DNA

Default
8/6/2009 9:20 PM

Sample ID	ng/uL	A260	260/280	260/230	Constant
Pu concentrated	53.13	1.063	1.53	0.81	50

There was not a peak at 280 nm. There was a strong absorbance around 230 nm and the reading was a line sloping downward.