7.13 Experimental Microbial Genetics

Fall 2008

Bootcamp Day 3 –Purification of Genomic DNA from *Pseudomonas aeruginosa* PA14 using the QIAGEN Genomic-Tip Kit and preparation of agarose gels

A. Purification of Genomic DNA from *Pseudomonas aeruginosa* PA14 using the QIAGEN Genomic-Tip Kit

Sample preparation and lysis protocol:
This protocol is suitable for use with QIAGEN Genomic-tip 100/G, and up to $2.2x10^{10}$ cells. Overloading the tips by using more cells than recommended will decrease the performance of the kit.

Before you start:

- Add 7µL RNase A to 3.5mL Buffer B1.
- Thaw aliquots of lysozyme (100mg/mL) and Proteinase K stock solution.
- Put buffer QF at 50°C

1. Transfer 2.5 mls of cells to a 15mL conical tube and pellet by centrifugation at 3000-5000g for 5-10 minutes. Completely remove all supernatant.

2. Resuspend the pellet in 3.5mL Buffer B1 **with RNase A added**. Mix by vortexing at top speed until suspension is **COMPLETELY** homogeneous.

3. Add 80 µL lysozyme and 100µL Proteinase K, and mix. Incubate at 37°C for 45 min.

4. Add 1.2mL Buffer B2, and mix very thoroughly by vortexing for a few seconds. Incubate at 50°C for 30 minutes. Lysate should be clear after this step—if not, extend the incubation time.

5. During incubation, equilibrate a QIAGEN Genomic-tip 100/G with 4mL Buffer QBT. (Suspend the tip over a collection tube using the provided blue adapter.) The buffer will flow through the tube by gravity. Allow the tip to drain completely.

6. After incubation, vortex sample from step 4 for 10 seconds and promptly apply it to the equilibrated Genomic-tip. Allow it to drain by gravity flow. This step and the upcoming drip steps take a LONG time. During these drip steps prepare your agarose gels for tomorrow! See procedure below.

7. After the sample has dripped through, wash the Genomic-tip with 7.5mL Buffer QC. Repeat after the first wash drips through.

8. Place the tip over a clean 15 ml Falcon tube. Elute the genomic DNA with 5mL Buffer QF. Using Buffer QF prewarmed to 50°C will increase yields. Allow the tip to drain by gravity flow.

9. Precipitate the DNA by adding 3.5mL isopropanol to the eluted DNA in the collection tube. Mix by inverting several times. You should be able to see the DNA precipitate!!!! Yes it's that fluffy looking white stuff! Oooooh!!!

10. Add 300uL TE buffer to a clean Eppendorf tube. Cauterize the end of a clean and sterile glass capillary tube in your Bunsen burner flame and pick up the precipitated DNA using a capillary tube - the DNA should stick to the end of it. Transfer the DNA to the TE buffer. If the DNA won't come off, break off the end of the capillary tube in the Eppendorf tube. Normally you would allow the DNA to resuspend in the TE overnight at room temperature and would quantify the DNA the next day using a spectrophotometer (see protocol posted on the class Stellar site), but this time, please your tube in the rack on the table by the clinical centrifuge. We will quantify your DNA for you by Mon.

B. PREPARATION OF AGAROSE GELS

1. Make 175 mls of a 1% agarose solution in 1X TAE buffer in an Erlenmeyer flask. **Do not make your agarose in water!**

Agarose in on the chemical shelves in chem. room
1x TAE buffer is in carboys above the sinks
Erlenmeyer flasks are on the glassware shelves in chem. room.

2. Bring slurry to a boil in a microwave (heat about 1.5 minute on high power), swirl, and repeat as necessary until no "streaks" or chunks remain.

*** Be careful with molten agar and agarose. It can boil over suddenly and cause nasty burns. Watch it in the microwave so it doesn't boil over. If it does, clean it up!!**

3. While agarose is microwaving, prepare one medium and one small gel cast. The medium gel should be made with a comb that has at least 12 teeth (to analyze pilot partial digest) and the small one should use a comb with 8 wider teeth (for gel extraction of insert genomic DNA and linarized, phosphotased pMQ64 vector).

4. Once agarose is completely dissolved, add ethidium bromide to a final concentration of 0.1µg/ml. Swirl to mix.

WEAR GLOVES when using EtBr! It is a mutagen. The EtBr is in the chemical hood in the lab. It is a stock of 10 mg/ml or 10,000x. Use the pipettemen labeled EtBr to add EtBr to your gel. Discard the EtBr contaminated pipette tip into the waste jar in the chemical hood labeled EtBr solid waste.

5. Pour molten agar into a gel box.

Make sure you've put the comb in! Get rid of any bubbles.

6. The agar is completely set when it becomes opaque (< 20 min). Once this is true, wrap the gels individually in saran wrap and store them in the cold room until Mon. Keep the combs in the gels for now.