7.13 Experimental Microbial Genetics

Fall 2008

Boot camp day 7: PCR verification of insert size.

You can check the size of your cloned inserts by using PCR. Primers have been designed to anneal just outside the multiple cloning site of pMQ64, so that they will amplify the insert in all of the clones in your colony plasmid prep.

For PCR you need template DNA, primers, nucleotides, DNA polymerase, and the appropriate buffer. To make your life easier, the Platinum PCR Super Mix contains everything except the template DNA and primers. The Super Mix also contains an antibody to Taq polymerase which keeps the polymerase inactive until heated at 94°C for 2 minutes. This means you can set up your reactions on ice or at room temperature. (Be careful-- this is not true for all Taq polymerases or PCR mixes so it's good to get in the habit of setting these reactions up on ice!!)

1. Set up four reactions, one for your 2-4kb prep, one for your 4-8kb prep, one control with pMQ64, and one no-DNA control. (Why are these controls important?) Each reaction should contain
   a. 45 uL Platinum® PCR SuperMix High Fidelity
   b. Primer solution (200 nM final concentration of each is recommended)
   c. Template DNA solution (1–200 ng genomic DNA) (**not in your no-DNA control!**)
   d. Water (to a final volume of 50uL)

2. Cap tubes, mix contents of tubes, pulse centrifuge and load into thermal cycler.

3. PCR program:
   - 94°C for 30 s to 2 min to completely denature the template and activate the enzyme.
   - Perform 35 cycles of PCR amplification as follows:
     Denature 94°C for 15-30 s
     Anneal 55°C for 15-30 s
     Extend 68°C for 1 min per kb
   - Set a final extension step of 68°C for 10 min
   - 4°C forever

4. While your PCR is running, pour a small 1% gel with enough lanes to hold all of your reactions, plus ladder. (Think—what do you expect your gel to look like? Will you see a smear? Bands? How many? What size(s)?)

5. When your PCR is finished, run it out on the gel and image it.
   If you can't run your gel on the same day as your PCR, you can store the PCR reactions overnight at 4°C or -20°C. Make sure they are clearly labeled! Wrap your gel in Saran wrap and save in the cold room for tomorrow.