DNA replication/ amplification
Use of Polymerase Chain Reaction—PCR to Identify DNA Polymorphisms

Molecular Biology
Lab 5
Part 2---analysis of PCR amplification fragments

Gels will have been prepared for electrophoresis of your PCR products. I prepared them as you did last time. 1% agarose in !X TAE buffer and added Ethidium bromide directly to the molten agarose.

We will include approximately half the class samples on each gel. Each gel will have a set of controls from individuals known to be +/+, +/-, or -/- for the Alu repeat at locus PV92.

PREPARATION OF SAMPLES

1. Find your PCR samples in the rack.
2. Pipette 25 μl of your sample into a fresh microfuge tube.
3. Add 5 μl of loading dye to your sample.
5. Organize a plan for loading your gel and load your samples accordingly. (Include in your plan, 3 lanes for the control samples and load them)
6. Load standards for each gel.
    1)100 bp ladder (100, 200, 300, 400 etc.)
    *Prepare it by adding 3 μl of the ladder to 17 μl of H_20 and add an additional 5 μl of loading dye.

RUNNING OF THE GEL

Assemble the gel and run ~80 volts for 40 minutes. (Keep an eye on the migration of the dyes).

PHOTODOCUMENTATION AND ANALYSIS

Carefully disassemble the gel rig and transfer the gel (in its casting tray) to a plastic tub. Photograph the gel (Super's lab). If time, we'll print a copy of the gel for everyone.

PV92 Genotyping/Discussion

As we discussed last time, PCR is a sensitive and extremely efficient way to amplify any region of DNA. Make sure you can sketch the basic mechanism of PCR starting with a double stranded region of DNA. What occurs in each of the 3 steps that are repeated 30-40 times. Describe how the number of copies of DNA are amplified logarithmically. Practice designing primers for a given sequence and estimate their melting temperatures. (Chapter 23 of your text).

Some Points to review.

Template preparation:

1. What was the purpose of the matrix solution we placed our cheek or hair cells in?

2. What was the purpose of the 56 degree C incubation?

3. What was the purpose of the 100 degree C (boiling) incubation?

4. What was left in the supernatant after the final spin? Prior to setting up the PCR.

PCR:

1. What are the ingredients needed to perform PCR?

2. Where did you obtain these ingredients?

3. Which ingredients are specific for amplification of PV92? Which are needed for PCR of any DNA region of interest?

4. What occurred during:
    a. The 94 degree/2 minute step?
    b. The 94 degree/1 minute step?
    c. The 60 degree/1 minute step?
    d. The 72 degree/2 minute step?
    e. The 72 degree 10 minute step?

5. Which steps were repeated 40X

5. What was the purpose of the 4 degree hold?

Determine the outcome of the PCR genotyping lab:

The primers used in amplifying PV92 are known to lie 641 bp apart on chromosome 16 in a unique sequence of this region. Predict the amplification products from PV92 (with and without the Alu repeat). Predict how the controls will appear on a gel.

Post electrophoresis

1. Did the control templates work to amplify the PV92 locus? (Did there appear to be general problems in obtaining PCR amplified fragments?)

2. Do the controls reveal an expected pattern of PCR fragments?

3. Did <u>your</u> template work to amplify the PV92 locus? What is your genotype?