Genetics

Lab 10-Part 2

Analysis of PCR products/PV92 genotyping

Agarose gel electrophoresis

 Today we will determine our success in amplifying the PV92 locus in your genome by PCR. If successful, we will know our genotype for the PV92 locus (Recall PCR theory and set up last week.) Each of you should have a PCR sample using either cheek cell or hair follicle cell DNA as template. One or more groups set up a set of control samples from individuals with known PV92 genotypes.

The most common way of analyzing PCR amplification is by directly visualizing the DNA fragments produced. A standard method for analyzing the size and amount of DNA produced in a PCR reaction is **agarose gel electrophoresis**. Agarose is a product of seaweed that can be dissolved and then solidified, forming a gel/matrix for separation of DNA. The gel is submerged in a buffer which allows an electrical potential to be applied. DNA is loaded into wells made in the gel and an electrical current is applied. DNA is highly negatively charged and will migrate through the pores of the gel toward the positive pole. DNA fragments of different sizes will migrate different distances in a given period of time (see below). DNA is typically visualized by staining the whole gel with a dye that is taken up by DNA. One common dye is called Ethidium Bromide. This fluorescent dye binds between the paired bases of the nucleotides and is detectable in UV light. (illustrated by small circles below)



Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the log10 of their molecular weight. In other words, if you plot the distance from the well that DNA fragments have migrated against the log10 of either their molecular weights or number of base pairs, a roughly straight line will appear. (We will do this following the electrophoresis.)

Review animation!

**Procedure:**

**Materials**

* **PCR samples**
* **An electrophoresis chamber and power supply**
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* **Gel casting trays**, which are available in a variety of sizes and composed of UV-transparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
* **Sample combs**, around which molten agarose is poured to form sample wells in the gel.
* **Electrophoresis buffer**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
* **Loading buffer**, which contains something dense (e.g. glycerol) to allow the sample to sink into the sample wells, and one or more tracking dyes, which migrate in the gel and allow visual monitoring of how far the electrophoresis has proceeded.
* **Ethidium bromide**, a fluorescent dye used for staining nucleic acids. *NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling.*
* **Transilluminator** (an ultraviolet lightbox), which is used to visualize ethidium bromide-stained DNA in gels. *NOTE: always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.*
* **Electrophoresis standards/DNA ladders** (DNA fragments of know sizes)

**A. Prepare 1% agarose gel following steps below** ( or..Dr. Super will demonstrate)

 **(USE EYE PROTECTION and OVEN MITTS) \*\*2+ Lab groups will work together on preparation of gels.**

1. Prepare casting tray for gel. Use the masking tape to dam the ends.
2. Place a comb in the casting tray .
3. Weigh agarose for 100 ml gel (1% w/v agarose)
4. Measure 100 ml electrophoresis buffer (1x TAE) in flask (125 ml or larger)
5. Add powdered agarose to electrophoresis buffer, swirl gently and plug with paper towel.
6. Heat in microwave till boiling starts---watch carefully.
7. Remove from microwave and GENTLY swirl
8. Return to microwave and bring just to boil
9. Remove from microwave and GENTLY swirl
10. Check for undissolved crystals---if none stop here. If not dissolved repeat heating.
11. Let cool 5 minutes
12. Put on gloves (latex)
13. Add 1l Ethidium Bromide to the warm agarose. (**Ethidium Bromide is a strong mutagen---take care when using the concentrated solution and wear gloves when handling the gel from this point on.**
14. Pour the gel slowly into the casting tray and allow it to solidify.
15. Place your gel into a plastic box, cover it with electrophoresis buffer. **OR** wrap the gel as instructed.

**B. Prepare DNA samples and molecular weight standard sample (DNA ladders—2 samples/gel)**

1. Find your PCR samples in the rack.

2. Add 5 l of loading dye to your sample.

3. Organize a plan for loading your gel with all samples from your group (Include in your plan, 4 lanes for the control samples)

4. Prepare standards/ladders for each gel.

 1) 15 l or the MMR standard (ready to use0. (1,000 bp, 700 bp, 500 bp, 200 bp, 100 bp)

 2) 20ul of the 100 bp ladder (100, 200, 300, 400 etc.) \*\*Prepare it by adding 3 l of the ladder to 17 l of H20 and add an additional 5 l of loading dye.

**C. Loading and running the gel**

 1. Refer to your plan for samples in your gel. Each lab section will use a separate gel.

 2. Set a micro pipettor to 20 l.

 3. Carefully load 20 l of each student sample into wells of the gel. Dr. Super will demonstrate. Load DNA ladders/standards according to your plan.

 4. Place the lid on the gel, noting the orientation of the electrodes. Run the gel at 80 Volts for ~40 minutes.

5. View on UV transilluminator, noting presence or absence of DNA in your lanes, and differences in migration of DNA.

6. Document and discuss your results. Using the DNA ladder, estimate the sizes of the DNA fragments you amplified.

7. If time allows, plot the migration of the DNA ladder fragments and compare migration of your PCR-amplified fragments.

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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.

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 your template work to amplify the PV92 locus? What is your genotype?