**PROTOCOL: ANALYSIS OF PCR PRODUCTS BY GEL ELECTROPHORESIS**

(v. 090308)

**Purpose:** To determine if products were generated by a PCR and if these products are of the appropriate size

**Source/Background Information:**
PCR (DNA) products can be separated by electrophoresis in a matrix comprise of agarose. Smaller molecules move faster and farthest in a gel than do larger molecules. The DNA can be visualized in the gel using a fluorescent dye. In this procedure, you can determine if you have successfully amplified a DNA product using the CHS primers as well as determine the approximate size of the fragment(s) you generated. Subsequent confirmation that the fragment(s) are part of the CHS gene will be determined after the DNA is cloned and sequenced.

**Materials Needed:**

- **Per Student Group**
  - Putative PCR products in reaction mix (from CHS amplification)
    - Tube 1 – positive control reaction
    - Tube 2 – negative control reaction
    - Tube 3 – experimental reaction

- **Other Reagents, Equipment, and Materials**
  - Serological pipettes (various volumes) and ‘pipette gun’ or pipette bulb
  - Micropipettes and Tips (appropriate volumes)
  - Agarose I 500 mg tablets (Amresco; Cat. No. K857)
  - Microwave
  - Hot gloves
  - Disposable vinyl/latex gloves
  - Top loading balance
  - 1X TAE buffer (Amresco)
  - Distilled water
  - 100 bp DNA size ladder (pre-mixed with loading dye)
  - 6X DNA loading dye (Amresco)
  - 50°C water bath
  - Parafilm and scissors
  - Midi-sized gel box (Fotodyne) with 12/14 tooth comb and gel tray
  - Electrophoresis power source with cords
  - UV imaging system (Eagle Eye; Stratagene)
  - Ethidium bromide solution (0.5 µg/ml 1X TAE buffer)
  - 500-ml volumetric flask (with glass cover) containing a magnetic stir bar
Procedure:

IMPORTANT NOTES:

- **Use Extreme Caution!** This procedure
  - includes the preparation of boiling solutions. Use hot gloves to handle these solutions and be sure to wear eye and body protection!
  - uses high voltage during the electrophoresis steps. Be sure that you are aware of the proper set up and use of the equipment in this procedure.
  - involves the use of ethidium bromide, a known mutagen. Use gloves to handle solutions and contaminated with this agent! Dispose of contaminated materials as directed as well as appropriately clean contaminated surfaces!
  - uses ultraviolet (UV) light to visualize stained gels. Wear the appropriate face shield or goggles! Never look at the UV light without eye protection in place! Also, minimize exposure to uncovered skin! UV light will cause severe burns!

- You will receive specific hands-on instructions regarding the use the electrophoresis box, the electrophoresis power supply, and the UV imaging system. DO NOT OPERATE any of these pieces of equipment unless you have been properly trained. If you are unsure on the operation of the equipment at any time after your training, ASK FOR HELP!

- The Eagle Eye (Stratagene) imaging system used in this procedure is no longer commercially available. Other systems do exist and can be used in place of this one.

- Be sure not to contaminate any materials with exogenous sources of DNA/primers. Change pipette tips as often as necessary to avoid cross-contamination. Wipe off the barrels of the mechanical pipettes with dilute bleach and/or 70% ethanol.

1) Place three (3) tablets (500 mg each) of Agarose I in a 500-ml volumetric flask containing a magnetic stir bar. (NOTE: you will be making a 1.5% gel using this procedure. To make a 1% gel, follow the same procedure, but use only 2 agarose tablets. Similarly, for a 2% gel use 4 tablets.)

2) To the flask, add 100 ml of 1X TAE buffer.

3) Place the glass cap on the top of the flask and determine the weight of the flask containing the agarose/buffer solution. Record this value.

4) Stir the solution until the agarose tablets have effectively dissolved.

5) **Use Extreme Caution!** This step, as well as subsequent steps, involves the preparation of boiling solutions. Use hot gloves to handle these solutions and be sure to wear eye and body protection!

Heat the agarose/buffer solution to boiling in a microwave. Check the solution periodically to ascertain if all the agarose particles have melted. Continue boiling the agarose/buffer solution until all the particles have disappeared. [NOTE: In practice, initially set the timer on the microwave for 2 min. If there are still agarose particles in the solution, continue to boil for short periods of time in the microwave until the particles completely dissolve.]
6) Weigh the flask containing the molten agarose and determine the difference in weight from that measured in step 3.

7) Using a serological pipette, add a volume (in ml) of distilled water to the molten agarose/buffer solution equal to that in the difference in weight determined in step 6. For example, if the flask weighed 350.0 g in step 3 and 341.5 g in step 6, add 8.5 ml of distilled water to the flask containing the molten agarose/buffer solution.

8) Cool the flask to 50°C by placing it for 15 minutes in the water bath equilibrated to this temperature. While the solution is cooling, go to step 9. [NOTE: The agarose solution can remain at 50°C longer than 15 minutes, but significant evaporation will occur if left at this temperature for more than 1.5 hours.]

   Alternative Step: The flask can be place on a magnetic stir plate and cooled by gentle, slow mixing the solution at room temperature. Do not stir so hard as to produce bubbles! The solution will have reached the proper temperature when you can handle the flask by hand and the heat is only mildly irritating. Typically, this takes about 20-30 minutes depending upon the room temperature. However, if you leave the solution too long at room temperature, it will solidify. While the solution is cooling, go to step 9.

9) Prepare the midi-sized gel box being sure to use a 12- or 14-tooth comb. Be sure to place the tray and comb in the proper position. (NOTE: You will receive the proper instruction for using this gel box.)

10) If the agarose was cooled in a water bath, remove the flask, wipe off the outside with a paper towel, and place it on a magnetic stirrer. Operate the stirrer at a low speed for 1-2 minutes to adequately mix the molten agarose/buffer solution.

   NOTE: If you cooled the agarose on a stir plate (Alternative Step 8), you can proceed to the next step.

11) Gently pour the molten agarose into the gel box being sure not to drop the stir bar into the box.

12) Replace the lid of the gel box and allow the agarose to solidify for 20-30 minutes at room temperature.

   NOTE: Immediately rinse the boiling flask with distilled/deionized water to remove traces of agarose before it solidifies in the flask.

13) Once the agarose has solidified in the gel box, open the lid, turn the tray with the wells away from red electrode, then pour 750-800 ml of 1X TAE (running) buffer into the box. Be sure to cover the gel surface with 3-5 mm of buffer.

14) Gently remove the well comb without tearing the gel. (NOTE: Do not remove the comb before adding the running buffer.)

IMPORTANT NOTE: For steps 15 through 18, be sure to use separate tips each time you pipette a sample so as to avoid contamination.

15) On a square of parafilm, place three 2 µl drops of 6X DNA loading dye.

16) To separate drops of dye, add 10 µl of your PCR reaction products from tubes 1-3. Carefully mix the samples by gently pipetting the solution up and down several times.
17) To the well of one lane, add 5 µl of the 100 bp DNA size ladder.
18) To separate wells in the gel near the size standard (100 bp ladder), carefully transfer all the liquid from your sample/dye mixture using a 20 µl pipette tip and a micropipette.
19) Attach the electrode leads from the power supply in the correct orientation (Red goes with red, black goes with black; DNA is negatively charged, the red [positive] electrode lead should be placed at the end farthest from the wells.)
20) Turn on the power supply and set the voltage at 120 volts. (NOTE: You will receive the proper instruction for operating this power supply.)
21) Once the red dye line runs about three-fourths the length of the gel (about 2 hours), turn off the power and remover the electrode leads.
22) Carefully transfer the gel to the ethidium bromide solution. Allow the gel to stain for 15-20 minutes.
23) Remove the gel and visualize the stained DNA on the Eagle Eye imaging system. Save and print the image of your results (NOTE: You will receive the proper instruction for operating this power supply.)