# Biotechnology Lab at William Floyd High School

# Teachers Prep for PV92 PCR Cheek Cell Protocol

Excluding the preparation of the agarose gels and any prelab/postlab activities/discussion, this activity is normally a four period or two day lab activity. It is a multistage lab with reagents that are time sensitive and require a minimum three hour amplification in the thermal cycler. In a research lab, you would add the primers and master mix and then immediately place the samples into the thermal cycler. However, it might be possible to have a class set of DNA sitting on ice or refrigerated, waiting for the next class period to complete the activity, before you place the samples into the thermal cycler. We suggest that you pick one class period for your first try. Our Bio-Rad instructions are for one class of up to 32 students using one PV92 kit. Since you are provided only 4 electrophoresis chambers, we suggest dividing your class into 16 teams of two with 4 teams sharing each chamber allowing only 16 of the 32 students to amplify their DNA.

_____ 1. Gather together the following documents and make copies as needed:

- Bio-Rad "Biotechnology Explorer, Chromosome 16, PV92 PCR Informatics Kit, Instructor's Manual
- PV92 PCR Student Instructions and Checklists: These document provides the basic lab instructions for students AND ALSO how to use the micropipettes, how to load the gels, how to place the gel tray into the electrophoresis chamber, how to stain and destain the gels, and much more.
- Student Workstation supplies-pipettes, tips, waste basket, marker, practice pipetting kit, whiteboard.

_____ 2. Visit the Biotechnology website accessible through the science department website (or directly at www.msrandallscience.weebly.com) to see the power points and additional teaching resources for PV92 PCR.

- You may wish to use some of the power point slides with your students.

_____ 3. Prepare the TAE buffers,

- 1x TAE. For pouring the gels. To make 500 milliters of 1x TAE buffer, add 10 mL of 50x TAE to 490mL of distilled water. 1 x TAE buffer may be used to store the gels and refrigerate at 4 $^{o}$C for several weeks.
- 0.25x TAE. For running the gels. To run 4 gels add 6.25mL of the 50x TAE to 1.3L distilled water. To run gels place the gel in an electrophoresis chamber and cover it with the 0.25X TAE: ensure gel is submerged (approximately 225mL). Run gels at 200V for no more than 20 minutes. Monitor gel loading dye progress to get a relative idea of electrophoresis progress.

_____4. Prepare the agarose gels.

- Use the combs that make 8 wells top make 4 gels.
- Dissolve 2g of agarose in 200mL of 1x TAE buffer. This will be enough to pour four gels.
- Boil in microwave until all powder is dissolved mixing frequently. (approx. 3 minutes).
- Allow agarose to cool to 60 $^{o}$C before pouring about 50 mL per gel.
- Place the comb in the top notch position (near the "1" on the ruler). Do not remove until gel has solidified and change to a cloudy appearance.
- Store in 1X TAE buffer in refrigerator.

_____ 5. For 2 minute staining, called quick staining, prepare the 100x Fast Blast stain. Mix 100mL of 500x Fast Blast stain with 400 mL of DI water or distilled water. Close and label the container. Store at room temperature until ready to use. This stain can be reused multiple times.

**Final set up for Lesson #1 Lab:**

1. Aliquot InstaGene™ matrix (cheek cell preparation).* Thoroughly mix the InstaGene matrix by gently shaking or vortexing the bottle several times to resuspend the matrix. Be sure that the matrix is well mixed when you aliquot it. The beads settle out of solution quickly, so gently remix the bottle several times during aliquotting. Pipet 200 µl of InstaGene matrix into each screwcap tube. Distribute one tube to each student team. Each student workstation should get 2 tubes of matrix for 4 students. Refrigerate until ready to use.

2. Set your water bath to 56$^{o}$C. It may take several hours to reach the correct temperature, so allow plenty time.

3. Use your hot plate, thermometer, and a beaker, to create a second water bath at 100$^{o}$C. You will need enough floating space in your water bath for the test tube holders.

4. Prepare and aliquot saline solution (cheek cell protocol). Prepare a 0.9% saline solution. To a 500 ml bottle of drinking water, add 4.5 grams of noniodinated salt. Table or kosher salt is recommended. Invert the bottle until the salt goes into solution. For each student team place 10 ml saline into a separate cup. Each student workstation should have 2 cups of saline.

**Final set up for Lesson #2 Lab:**

1. From the freezer, get one vial of each of the following: master mix, primer mix, +/+ control, -/- control, and +/- control. Allow the reagents to thaw in the refrigerator or in an ice bucket. After thawing, spin briefly in the micro centrifuge before opening vials.

2. Since each gel will be shared by 4 teams, only one set of controls is needed per gel. With a fine point permanent marker, label the top and side of 3 PCR tubes as follows:

    - Label 1 tube "+/+" Label 1 tube "-/-" Label 1 tube "+/-"

- Aliquot the controls as follows:
  Pipet 20 µL of the +/+ control into each of the +/+ tubes,
  Pipet 20 µL of the -/- control into each of the -/- tubes, and
  Pipet 20 µL of the +/- control into each of the +/- tubes.

- Store these control tubes in the refrigerator or on ice until ready to use.
  Refreeze the remainder of any unused controls in their original vials for future use.

3. Turn on the "MyCycler THERMAL CYCLER." It is pre-programed for the PV92 protocol.

4. Label 8 empty snap cap micro test tubes, "MM," and place tubes in a baggie or foam test tube holder. Store empty tubes in refrigerator or on ice.

5. IMMEDIATELY BEFORE LAB OR NO MORE THAN 30 MINUTES BEFORE THE PCR AMPLIFICATION PROCESS, prepare the complete master mix by adding primers. These instructions are for one class of 32 students working in pairs. Procedures Note: Before opening any of the reagent tubes, pulse-spin the contents (~3 seconds) in a centrifuge to bring contents to the bottom of the tubes. Contents often become lodged underneath the caps during shipping. For best results, the following steps should be performed within 15–30 minutes of the PCR reaction. Pipet 550 µl of master mix into a labeled micro test tube. Freeze the remaining master mix for a future lab. For 32 students or 8 student workstations, label 8 micro test tubes "MM" for "Master Mix" and place the tubes on ice. Add 11 µl of the primer mix to the 550 µl of master mix. Vortex 10 seconds to mix. It is imperative that the master mix be evenly mixed after the addition of the primers. The solution should be yellow. The primers are supplied as a concentrated yellow solution in a Tris buffer. Since the primers are much more stable in a concentrated form, add the primers to the master mix just prior to beginning the laboratory exercise — not more than 15–30 minutes before the PCR amplification.

6. Aliquot 45 µl of the complete master mix into the 8 micro test tubes labeled "MM", supplying one tube for each student workstation. Save the remaining complete master mix for the positive control reactions. Place these tubes on ice until they will be used.

7. During the lab, while the students are each transferring 20 µL of the supernatant to their own PCR tubes and adding 20 µL master mix, DESIGNATE 3 STUDENTS TO ADD THE MASTER MIX TO THE +/+, -/-, AND +/- CONTROLS. Each shared gel will have only one set of 3 controls. One tube should be enough for all four gels.

8. The PCR amplification process will take almost 3 hours to complete. However, the machine can be left running overnight. When the process is complete, the machine will refrigerate the samples until you retrieve them the next day from the thermal cycler.

**Final set up for Lesson #3 Lab:**

1. Remove the PCR tubes from the thermal cycler and store in refrigerator until ready for Lesson #3.

2. From the freezer, get the vial of EZ Load molecular mass ruler and allow it to thaw briefly. Label 4 tubes "MMR" and aliquot 11 µL of molecular mass ruler into each of the 4 MMR tubes. Refrigerate until ready for Lesson #3. Refreeze the remainder of the unused MMR in its original vial. The sizes of the DNA standard bands are 1,000 bp, 700 bp, 500 bp, 200 bp, and 100 bp

3. Aliquot PV92 XC loading dye. Label 8 tubes "LD" for loading dye, and aliquot 25 µl into each tube. Distribute to student workstations. (The MMR already has a loading dye in it.)

4. Prepare positive control samples. Add 10 µl of PV92 XC loading dye to each amplified positive control sample (+/+, –/–, +/–). Place the tubes at the teacher's workstation. Either you or a student group will load the positive and negative control samples on each gel, as indicated.

5. The mini centrifuge machines provided have adaptors to be used with the PCR tubes. Instead, you may also use capless micro test tubes as adaptors, if you wish. When ready to begin Lesson #3, students will need to centrifuge their PCR tubes for about 3 seconds.

6. Prepare Fast Blast DNA stain. Fast Blast DNA stain is provided as a 500x concentrate that must be diluted prior to use. The stain can be used as a quick stain when diluted to 100x to allow the visualization of DNA within 12–15 minutes, or it can be used as an overnight stain when diluted to 1x. When an agarose gel is immersed in Fast Blast DNA stain, the dye molecules attach to the DNA molecules trapped in the agarose gel. When the DNA bands are visible, your students can determine their genotypes for the Alu insert. Fast Blast DNA stain is a convenient, safe, and nontoxic alternative to ethidium bromide for the detection of DNA in agarose gels following electrophoresis. Fast Blast contains a cationic compound that belongs to the thiazin family of dyes. The positively charged dye molecules are attracted to and bind to the negatively charged phosphate groups on DNA. The proprietary dye formula stains DNA deep blue in agarose gels and provides vivid, consistent results.

**WARNING** Although Fast Blast DNA stain is nontoxic and noncarcinogenic, latex or vinyl gloves should be worn while handling the stain or stained gels to keep hands from becoming stained blue. Lab coats or other protective clothing should be worn to avoid staining clothes. Dispose of the staining solutions according to protocols at your facility. Use either 10% bleach solution or 70% alcohol solution to remove Fast Blast from most surfaces. Verify that these solutions do not harm the surface prior to use.

- To prepare 100x stain (for quick staining), dilute 100 ml of 500x Fast Blast with 400 ml of distilled or deionized water in an appropriately sized flask or bottle and mix. Cover the flask and store at room temperature until ready to use.

**Note:**

• We recommend using 120 ml of diluted Fast Blast to stain two 7 x 7 cm or 7 x 10 cm agarose gels in individual staining trays provided in the kit (you may want to notch the gel corners for identification). If alternative staining trays are used, add a sufficient volume of staining solution to completely submerge the gels.

• Following electrophoresis, agarose gels must be removed from their gel trays before being placed in the staining solution. This is easily accomplished by holding the base of the gel tray in one hand and gently pushing out the gel with the thumb of the other hand.

• Because the gel is fragile, special attention must be given when handling it. We highly recommend using a large spatula or other supportive surface to transfer the gel from one container to another during the destaining steps involved with the quick staining protocol.

• Destaining (when performing the quick staining protocol) requires the use of at least one large-volume container, capable of holding at least 500 ml, at each student workstation. Each student team may utilize separate washing containers for each wash step, or simply use a single container that is emptied after each wash and refilled for the next wash.

• It is crucial that you shake gels gently and intermittently during the overnight staining in Fast Blast DNA stain; small DNA fragments tend to diffuse without shaking.

• 100x Fast Blast can be reused at least 7 times.

7. We recommend using Bio-Rad's exclusive **gel support film** (catalog #170-2984EDU) to dry agarose gels. Remove the stained agarose gel from its staining tray and trim away any unloaded lanes with a knife or razor blade. Place the gel directly upon the hydrophilic side of a piece of gel support film. (Water will form beads on the hydrophobic side but will spread out on the hydrophilic side of the film.) Center the gel on the film and remove bubbles that may form between the gel and film. Place the film on a paper towel and let the gel dry, making sure to avoid direct exposure to light. As the gel dries it will bond to the film but will not shrink. If left undisturbed on the support film, the gel will dry completely at room temperature after 2–3 days. The result will be a flat, transparent, and durable record of the experiment. Note: Avoid extended exposure of dried gels to direct light to prevent band fading. However, DNA bands will reappear if the dried gels are stored in the dark for 2–3 weeks after fading.

**Getting Started on Allele Server Note:** The Dolan DNA Learning Center web site is continually updated. Some of the following information may change. On your Web browser, go to vector.cshl.org Click on Resources Click on BioServers under Allele Server, click on Register. Registration is free and allows you to set up a personal account. There is no need to register every time you return to this site. Using Allele Server Log in to Allele Server using the username and password you registered. Once you have logged in, follow instructions provided in the pop-up window for using Allele Server. You may also open a new window and go to dnalc.org/help/sad/topic_3.html to get more detailed instructions. Follow the detailed instructions on how to populate the workspace, analyze groups, compare groups, and query the database. Remember that as a registered user, you may store any groups that you loaded in your personal Allele Server database and analyze them at your convenience.