Biotechnology Lab at William Floyd High School

Teachers Prep for Forensic DNA Fingerprinting Protocol

Excluding the preparation of the agarose gels and any prelab/postlab activities/discussion, this activity is normally a two periods or two day lab activity. It is a multistage lab with reagents that are time sensitive and requires 45 minute incubation at 37 ^{o}C. Our Bio-Rad instructions are for one class of up to 32 students using one DNA fingerprinting kit. Since you are provided only 4 electrophoresis chambers, we suggest dividing your class into 7 teams of four with 2 teams sharing each chamber using a double welled agarose gel.

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

- Bio-Rad "Forensic DNA Fingerprinting Kit, Instructor's Manual
- Forensic DNA Fingerprinting 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 Forensic DNA Fingerprinting.

- 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 30 minutes. Monitor gel loading dye progress to get a relative idea of electrophoresis progress.

_____4. Prepare the agarose gels.

- The recommended agarose concentration for gels in this classroom application is 1% agarose. This concentration of agarose provides good resolution and minimizes run time required for electrophoretic separation of DNA fragments. The recommended thickness for the gel is 0.75–1.0 cm for easy sample loading and gel handling. **Be sure to use electrophoresis buffer, not water, to prepare agarose gels.**
- Use two sets of combs that make 8 wells each to make 4 gels double welled gels.
- Dissolve 2 g 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) and the second notch. 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 distilled water. Close and label the container. Store at room temperature until ready to use. This stain can be reused multiple times.

_____6. For overnight staining, prepare 1X Fast Blast stain. Mix 0.5mL of 500X Fast Blast Stain with 250mL of distilled water per gel. Store at room temperature until ready to use. This stain can be reused multiple times.

Final set up for Lesson #1 Lab:

Rehydrate DNA samples

1. To **rehydrate DNA** samples, carefully remove the stopper and add 200 µl of sterile water to each lyophilized DNA vial. Replace the stopper and vigorously shake the vial. It is critical to dissolve all the powder, some of which may be stuck to the stopper. Allow DNA/buffer samples to rehydrate at room temperature for 15 minutes or until dissolved. Gentle heating at 37°C for 10 minutes may be necessary. You may choose to aliquot and transfer 10 µl the rehydrated DNA/buffer samples to color-coded, labeled 1.5 ml microtubes to make it easier for your students.

- Green tube CS (crime scene)
- Blue tube S1 (suspect 1)
- Orange tube S2 (suspect 2)
- Violet tube S3 (suspect 3)
- Pink tube S4 (suspect 4)
- Yellow tube S5 (suspect 5)

2. Rehydrate lyophilized EcoRI/PstI enzyme mix. To rehydrate EcoRI/PstI enzyme mix, add 750 µl sterile water and swirl to resuspend the enzymes. Allow enzymes to rehydrate on ice for 5 minutes. It is critical that the enzyme mix is kept **on ice**, but not frozen, once it has been rehydrated. **The rehydrated enzymes should be used within 12 hours**.

3. Aliquot enzyme mix. Transfer 80 µl of the rehydrated enzyme mix into each of eight, clear 1.5 ml microtubes labeled **ENZ**.

4. Prepare HindIII lambda digest (DNA standard) and aliquot (optional). Add 20 µl of DNA sample loading dye to the stock tube containing the HindIII lambda digest DNA standard. Heat the standard to 65°C for 5 minutes, then chill on ice — this results in better separation of the standard bands. Label clear microcentrifuge tubes "**S**". Aliquot 15 µl of the DNA standards containing loading dye to 8 clear microcentrifuge tubes labeled "**S**".

5. Aliquot DNA sample loading dye. Label eight clean microcentrifuge tubes "**LD**" for loading dye and aliquot 50 µl of sample loading dye into each tube. Distribute one tube to each team.

6. Prepare the electrophoresis chamber. When the agarose gel has solidified, sample loading and electrophoresis can begin.

a. When placing the gel tray into the electrophoresis chamber, make sure that the sample wells are at the black cathode end. DNA samples will migrate toward the red anode end during electrophoresis. Make sure the tray is fully seated in the chamber.
b. Prepare the required volume of 1x TAE buffer, if you have not prepared it already.
c. Submerge the gel under about 2 mm of 1x TAE buffer.
d. Prepare samples for gel loading. See laboratory protocol in the student section.

Note: Power requirements vary depending on gel thickness, length, and concentration, and on type of electrophoresis buffer used. For this exercise we recommend using a constant voltage of 100 V for 40 min. For a faster electrophoresis protocol use 0.25x TAE buffer which allows the gel to be run in 30 min at 200 V.

7. 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 compare the crime scene and suspect DNA. 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.

- For overnight staining, prepare 1X Fast Blast stain. Mix 0.5mL of 500X Fast Blast Stain with 250mL of distilled water per gel. Store at room temperature until ready to use. This stain can be reused multiple times.

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. There are 4 tupperware containers I the lab cabinet.

• 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. Place each gel in a separate staining container and place containers on the rocker set at 40 on the dial overnight.

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

8. 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.