Forensic DNA Fingerprinting Kit Quick Guide

Lesson 1 Restriction Digestion

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.

2. Label one of each colored micro test tubes as follows:
   - green tube CS (crime scene)
   - blue tube S1 (suspect 1)
   - orange tube S2 (suspect 2)
   - violet tube S3 (suspect 3)
   - red tube S4 (suspect 4)
   - yellow tube S5 (suspect 5)

   Label the tubes with your name, date, and lab period. Place the tubes in the foam micro test tube holder.

3. Using a fresh tip for each sample, pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored micro test tubes. Make sure the sample is transferred to the bottom of the tubes.

4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipet up and down carefully to mix well.

5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulse-spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.

6. Place the tubes in the foam micro tube holder and incubate for 45 min at 37°C or overnight at room temperature in a large volume of water heated to 37°C.

7. If required, follow the instructors directions to pour a 1% agarose gel.

8. After the incubation period, remove the tubes from the water bath and place in the refrigerator until the next laboratory period. If there is sufficient time to continue, proceed directly to step 2 of Lesson 2.
Lesson 2 Agarose Gel Electrophoresis

1. Remove the digested DNA samples from the refrigerator (if applicable).

2. If a centrifuge is available, pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube or gently tap on the table top.

3. Using a separate tip for each sample, add 5 μl of loading dye "LD" into each tube. Cap the tubes and mix by gently flicking the tube with your finger. Collect the sample at the bottom of the tube by tapping it gently on the table or by pulse-spinning in a centrifuge.

4. Remove the agarose gel from the refrigerator (if applicable) and remove the plastic wrap.

5. Place the agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer* to cover the gel, using approximately 275 ml of buffer for a Bio-Rad Mini-Sub Cell, horizontal electrophoresis chamber.

6. Check that the wells of the agarose gels are near the black (−) electrode and the bottom edge of the gel is near the red (+) electrode.

7. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:
   - Lane 1: M, DNA size marker, 10 μl
   - Lane 2: CS, green tube, 20 μl
   - Lane 3: S1, blue tube, 20 μl
   - Lane 4: S2, orange tube, 20 μl
   - Lane 5: S3, violet tube, 20 μl
   - Lane 6: S4, red tube, 20 μl
   - Lane 7: S5, yellow tube, 20 μl

8. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid of the horizontal electrophoresis chambers will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black.

9. Turn on the power and electrophorese your samples at 100 V for 30 minutes.

* or 0.25x TAE if using the Fast Gel Protocol

Student Manual
Visualization of DNA Fragments

1. When the electrophoresis run is complete, turn off the power and remove the top of the chamber. Carefully remove the gel and tray from the gel box. Be careful — the gel is very slippery. Slide the gel into the staining tray.

2. You have two options for staining your gel:
   
   **Quick staining** (requires 12–15 minutes)
   
   a. Add 120 ml of 100x Fast Blast DNA stain into a staining tray (2 gels per tray).
   b. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use.
   c. Transfer the gels into a large washing container and rinse with warm (40–55°C) tap water for approximately 10 seconds.
   d. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.
   e. Record results.
   f. Trim away any unloaded lanes.
   g. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.

   **Overnight staining**
   
   a. Add 120 ml of 1x Fast Blast DNA stain to the staining tray (2 gels per tray).
   b. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.
   c. Pour off the stain into a waste beaker.
   d. Record results.
   e. Trim away any unloaded lanes.
   f. Air-dry the gel on gel support film and tape the dried gel into your laboratory notebook.