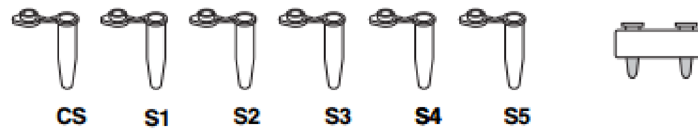
**Lab station protocol sheets**

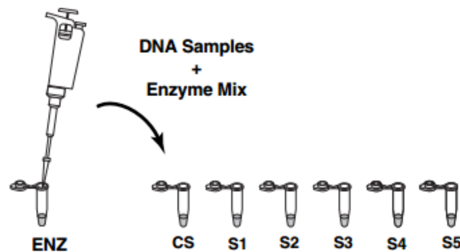
**Forensic DNA Fingerprinting Lab Activity**

**Part I: Restriction Enzyme Digest (Lab Protocol)**

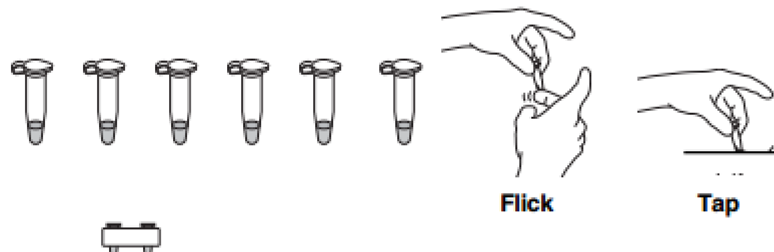
1. Your team of **four students** should have **six 1.5 ml microcentrifuge sample tubes** containing DNA from a crime scene and **80 µl of Restriction enzyme mix**(ENZ) on ice with your name, date, and lab period.



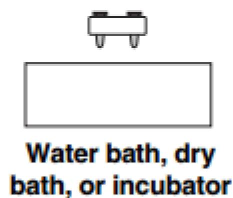

2. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipet up and down carefully to mix well




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




4. Incubate the tubes for 45 min at 37°C or overnight at room temperature in a large volume of water heated to 37°C.



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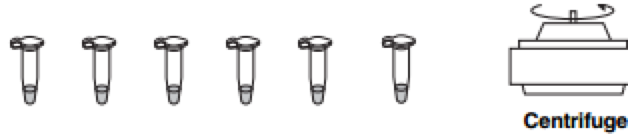
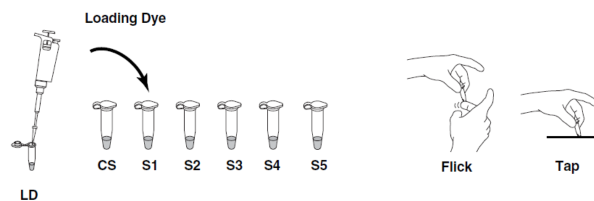
## Part II: Gel Electrophoresis (Lab protocol)

1. Remove your DNA samples from the incubator and place in the micro test tube holder. If a centrifuge is available, place the DNA and pulse-spin the tubes (~3 seconds at 2,000 x g) to bring the condensation that formed on the lids to the bottom of the tubes.

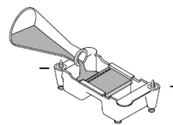



2. Using a new tip for each sample add 5 µl of sample loading dye "LD" to each tube. Tightly cap each tube. Mix the components by gently flicking the tubes with your finger. If a centrifuge is available, pulse-spin the tubes to bring the contents to the bottom of the tube. Otherwise, gently tap the tubes on the table top.




3. Obtain an agarose gel (either the one you poured or one pre-poured by your teacher). Place the casting tray with the solidified gel in it, onto the platform in the gel box. The wells should be at the cathode (–) end of the box, where the black lead is connected.

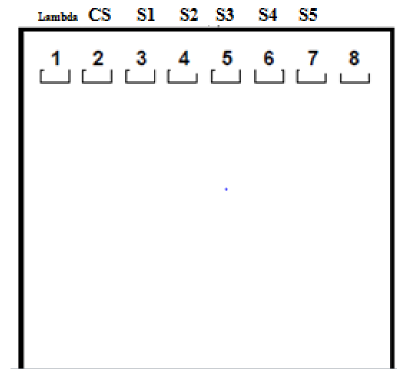
4. Pour ~275 ml of electrophoresis buffer into the electrophoresis chamber, until it just covers the wells.




5. Obtain the tube of HindIII lambda digest (DNA standard). The loading dye should already have been added by your instructor.

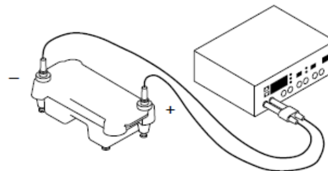
6. Using a clean tip for each sample, load the samples into the 8 wells of the gel in the following order:

| | |
|---|---|
| Lane 1: | HindIII lambda digest (DNA standards), clear tube, 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 |




7. Secure the lid on the gel box. The lid will attach to the base in only one orientation: red to red and black to black. Connect the electrical leads to the power supply.

8. Turn on the power supply. Set it to 200 V and electrophorese the samples for 30 minutes.




9. When electrophoresis is complete, turn off the power and remove the lid from the gel box. Carefully remove the gel tray and the gel from the gel box. Be careful, the gel is very slippery. Nudge the gel off the gel tray with your thumb and carefully slide it into your plastic staining tray.

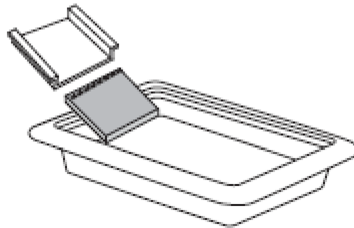
## Directions for Using Fast Blast DNA Stain

**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.**

### Protocol 1: Quick Staining of Agarose Gels in 100x Fast Blast DNA Stain

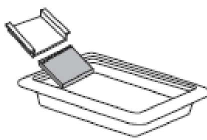
**Overnight procedure may be used instead with 1X Fast Blast-See Instructor**

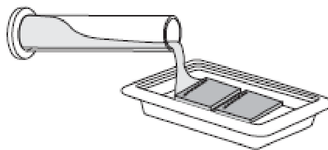
1. **Mark the staining tray with your initials and class period.** You will stain 2 gels per tray.

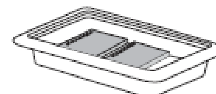


2. **Stain gels (2–3 minutes)**

   - Remove each gel from the gel tray and carefully slide it into the staining tray. Pour approximately 120 ml of 100x stain into the staining tray. If necessary, add more 100x stain to completely submerge the gels. Stain the gels for 2–3 minutes, but not for more than 3 minutes. Using a funnel, pour the 100x stain into storage bottle and save it for future use. **The stain can be reused at least 7 times.**



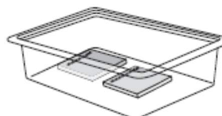




2–3 minutes

3. **Rinse gels**

   - Transfer the gels into a large container containing 500–700 ml of clean, warm (40–55°C) tap water. Gently shake the gels in the water for ~10 seconds to rinse.

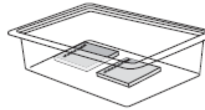


10 seconds

4. **Wash gels**
   - Transfer the gel into a large container with 500–700 ml of clean, warm tap water. Gently rock or shake the gels on a rocking platform for 5 minutes. If no rocking platform is available, move the gels gently in the water once every minute.

### 5. Wash gels

- Perform a second wash as in step 4.




### 6. Record and analyze results

- Examine the stained gels for expected DNA bands. The bands may appear fuzzy immediately after the second wash, but will begin to develop into sharper bands within 5–15 minutes after the second wash. This is due to Fast Blast dye molecules migrating into the gel and binding more tightly to the DNA molecules.

- To obtain maximum contrast, additional washes in warm water may be necessary. Destain to the desired level, but do not wash the gels in water overnight. If you cannot complete the destaining in the allocated time, you may transfer the gel to 1x Fast Blast stain for overnightstaining.

### Dry the agarose gel

To create a permanent record of the experiment place the gel directly upon the hydrophilic size of a piece of gel support film. (Water will form beads on the hydrophobic side of a piece of gel support 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 in a well-ventilated area, 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 for the experiment. Tape the dried gel into your laboratory notebook.

**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.**