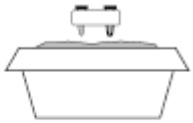
DNA Electrophoresis Lab

Procedure: create a two column table. In the first column, summarize the lab protocol for each day of the lab. You don't need to write every single word of the procedure, summarize each step including enough detail that you or someone else could use your written procedure to replicate the protocol exactly. You may write or sketch the procedure.

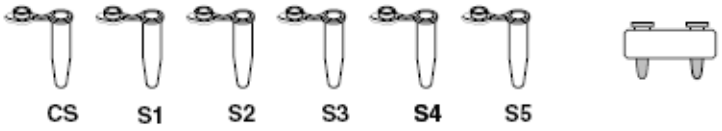
Explanation: In the second column of the table, write an explanation for why *each step* must be completed. Explain what is happening during that step in the procedure. A good way to determine why each step is important is to think about what would happen if you did not do that step.

PROCEDURE FOR RESTRICTION DIGEST (LAB DAY 1)

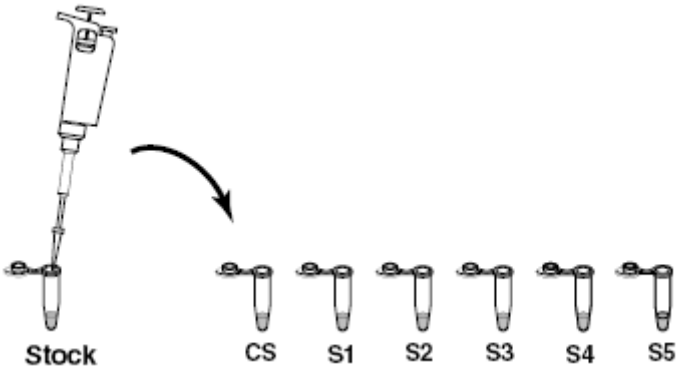
1. Be sure the following materials are at your lab station:
    a. A tube containing the restriction enzyme mix, labeled ENZ, on ice. The enzyme mix contains a mixture of the restriction enzymes EcoRI and PstI.



    b. One of each colored micro test tubes in a foam micro test tube holder. Each tube contains DNA:
        i. green tube CS = crime scene semen
        ii. blue tube S1 = suspect 1 DNA
        iii. orange tube S2 = suspect 2 DNA
        iv. violet tube S3 = suspect 3 DNA
        v. red tube S4 = suspect 4 DNA
        vi. yellow tube S5 = suspect 5 DNA

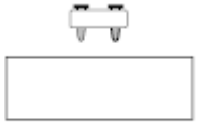


2. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube.



3. Tightly cap the tubes and mix the components by pulsing in the centrifuge to collect all the liquid in the bottom of the tube.

4. Place the tubes in the foam micro tube holder and incubate for 45 minutes at 37°C. Label the foam micro tube holder with your group member's names.



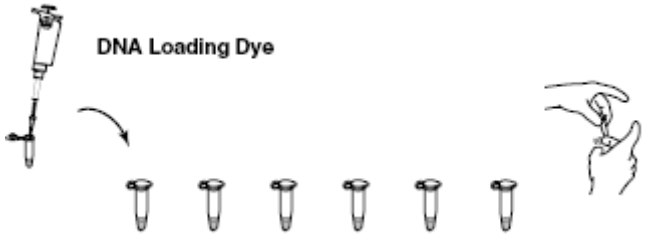
5. While the samples are incubating, cast your agarose gels:
    a. Seal the ends of the gel tray securely with the black dams.
    b. Place the comb into the appropriate slot of the gel tray.
    c. Pour molten Agarose into the gel tray, just to the top of the black dams.
    d. Allow the gel to solidify at room temperature for 10 to 20 minutes—it will appear cloudy, or opaque, when ready to use.
    e. Carefully remove the comb from the solidified gel.
    f. Remove the dams from the edges of the gel tray.
    g. Store gels in a LABELED, sealable plastic bag in the refrigerator.

6. After the incubation period, place the tubes in the refrigerator until the next laboratory period.

PROCEDURE FOR AGAROSE GEL ELECTROPHORESIS (LAB DAY 2)

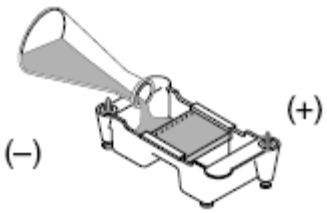
1. Remove your digested DNA samples from the refrigerator. Pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube.

2. 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.



3. Place the agarose gel in the electrophoresis apparatus. Check that the wells of the agarose gels are near the black (-) electrode and the base of the gel is near the red (+) electrode.

4. Fill the electrophoresis chamber with 0.25x TAE buffer to cover the gel, using approximately 275 ml of buffer.

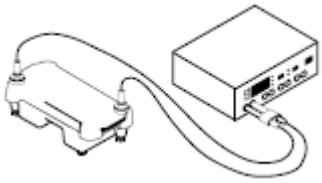


5. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:
    a. Lane 1: M, DNA size marker, **10** µl
    b. Lane 2: CS, green, 20 µl
    c. Lane 3: S1, blue, 20 µl
    d. Lane 4: S2, orange, 20 µl
    e. Lane 5: S3, violet, 20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

6. 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 will match with the red and black jacks on the base.

7. Plug the electrodes into the power supply.

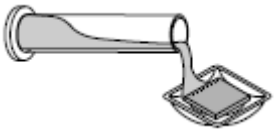
8. Turn on the power and electrophorese your samples at 200 V for 30 minutes or until the loading dye reaches the end of the gel (which ever happens first).



7. When the electrophoresis is complete, turn off the power and remove the top of the gel box.

9. Carefully remove the gel and tray from the gel box. Be careful—the gel is very slippery! Slide the gel into a LABELED staining tray.

8. Add 120 ml of DNA stain to the tray. Cover the tray with plastic wrap. Let the gel stain overnight, with shaking for best results.

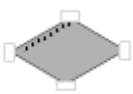


VIEWING RESULTS (LAB DAY 3)

1. Pour off the DNA stain into a bottle.

2. Carefully view your gel using a light source from beneath (i.e. put you gel on the overhead projector)

3. Record your results by copying the locations of the bands of DNA and the wells onto a clear sheet of acetate with a permanent marker.



QUALITATIVE ANALYSIS

1. Attach the plastic sheet tracing of the banding patterns from the DNA gel electrophoresis into your lab book. Be sure it is labeled with a title and a listing of which sample was in which well.

2. Answer the following questions in complete sentences in your lab book:

    a. What can you assume is contained within each band?

    b. What would be a logical explanation as to why there is more than one band of DNA for each of the samples?

    c. Which sample has the smallest DNA fragment? How do you know?

    d. How many restriction sites were there in lane three?

    e. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

    f. What caused the DNA to become fragmented?

    g. What determines where restriction enzymes will "cut" a DNA molecule?

    h. A restriction enzyme "cuts" two DNA molecules at the same location. What can you assume is identical about the molecules at **that location**?

    i. Do any of your suspect samples appear to have *Eco*RI or *Pst*I recognition sites at the same location as the DNA from the crime scene?

    j. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.