Day 2  “Practice Techniques” Carefully follow these procedures

Part A Practice preparation for a digest:

1. Each team is to obtain a set of four microtubes containing different colored water and four empty microtubes (one each of yellow, green, orange, & blue).

2. Using the proper technique, adjust the .5-10 μL micropipettor to 4 μL and add the yellow solution to each microtube as shown in the matrix below. Add the solution by dispensing it onto the side of the microtube in a tiny droplet. Take turns adjusting the micropipettor between solutions, and add the red, blue and green solutions as shown.

<table>
<thead>
<tr>
<th>TUBE</th>
<th>Solution 1 (Yellow)</th>
<th>Solution 2 (Red)</th>
<th>Solution 3 (Blue)</th>
<th>Solution 4 (Green)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>4 μL</td>
<td>5 μL</td>
<td>2 μL</td>
<td>-</td>
</tr>
<tr>
<td>Green</td>
<td>4 μL</td>
<td>5 μL</td>
<td>-</td>
<td>2 μL</td>
</tr>
<tr>
<td>Orange</td>
<td>4 μL</td>
<td>5 μL</td>
<td>2 μL</td>
<td>-</td>
</tr>
<tr>
<td>Blue</td>
<td>4 μL</td>
<td>5 μL</td>
<td></td>
<td>2 μL</td>
</tr>
</tbody>
</table>

3. After you have added all the solutions, show your teacher that you have completed this step correctly. You should have three separate droplets of different sizes on the walls inside your tubes.

4. Mix your solutions by centrifuging for a few seconds. Be sure to properly balance the micro-centrifuge before use.

5. When finished, dry the microtube with a cotton swab or paper towel, leave it for the next group and wait for your turn to practice loading a gel.

Part B Practice loading a gel:

1. Set the gel loading micropipettor to 11 μL, if necessary. Using proper technique, draw up the practice loading dye solution.

2. Again, using the proper techniques demonstrated by your instructor, load the entire contents into a well in a practice agar gel.

3. Gently shake the gel to empty the wells and repeat the loading process as time permits.
Day 3 "Mix and Digest DNA samples"

Carefully follow these procedures:

1. Each lab team is to obtain a set of five different colored tubes in a microtube rack. Assign one team member to each color microtube. If there are fewer than five team members, students can do two microtubes.

2. Each team member must obtain a styrofoam cup 2/3 full of crushed ice and place their tube in it. The prepared DNA and restriction enzymes are unstable at room temperature and therefore must be kept cold at all times. Always keep the tube in the ice except when holding the tube to insert one of the reactants. **Caution: Do not get ice inside your tubes!**

3. Each member should pipette 4 μL of DNA and 5 μL of buffer into their colored tube. The DNA and buffer will be in different locations. Your instructor will direct you to their location. The team member should take the ice cup with their colored tube stuck into the ice to the station identified. Once the DNA and the buffer are in the tube, return to your lab station so the equipment can be rearranged for the next step. **Again, be careful not to get ice into your tube.**

4. The restriction enzyme that each team member is to use is determined by the color of the tube. Each restriction enzyme will have a different location. Your teacher will identify these locations. Take your tube in the ice to the appropriate location and obtain 2 μL of the correct enzyme. Tubes will be color-coded for the restriction enzymes: **Yellow: Bam (B); Green: Eco RI (E); and Orange: Hind III (H); Blue: Control-distilled water (-); and Clear: Unknown (?).** Once the restriction enzyme is in your tube and the tube is back in the ice, return to your lab station and wait until your entire group is finished with this step.

5. Remove tubes from the ice and place them in the microtube rack. One member of the group should take the rack and tubes to the micro-centrifuge and spin as instructed. **Remember to have the micro-centrifuge balanced when spinning.** After spinning, return the tubes to the rack and place it in the water bath set at 37°C. Leave to digest for a minimum of 20 minutes.

6. Obtain one plastic baggie for your group and label it with group number and period in the upper right corner. Your teacher will place your microtubes into the baggies after they have digested for the required time.

7. Return your ice cups and any unmelted ice to the ice chest.
Day 4 "DNA Gel Electrophoresis"

Time may be a problem today. Quickly, but carefully, follow these procedures:

1. Each lab group (team) needs to obtain their tubes and baggie from the teacher.

2. One group at a time, in the order recommended by your teacher, will add 2 μL of loading dye to each tube. Use a clean micropipettor tip for EACH tube. Close the caps and place in the microcentrifuge. When all five tubes are ready, balance the microcentrifuge with an extra tube. Spin the tubes for a count of ten.

3. Use the micropipettor, set to 13 μL, to load the entire contents of the tube into a well of the gel. Record the gel location the team uses by its number on your plastic bag. Place your sample into the well assigned by your teacher. Many great results have been lost because experimenters did not record what went where properly.

4. Leave plastic bag next to the electrophoresis (gel) box. Dispose of the empty microtubes as directed by your teacher.

5. Because class will be over before the run can be completed, your teacher will remove the gels, stain them, and put them into your labeled bag.

Questions:

1. Why did you place each solution on the side of the tube rather than at the bottom?

2. What is the function of the loading dye?

3. Why is it important to remove all of the contents of your tubes before attempting to load a gel?

4. Can you explain what process is causing the production of air bubbles in the tank?

5. Can you explain why you see the color separation in the gel?
DNA RESTRICTION ANALYSIS

1. Draw the DNA fragment lines on your gel onto the drawing below.

2. Why is there separation of the DNA fragments that can be seen on the gel?

3. How can you account for differences in the DNA fragment separation between your gel and the ideal gel?

4. Why are there more DNA fragments in some lanes than in others?

Below is an ideal gel and the gel you produced.

*This analysis is simplified. Banding patterns using other techniques will be different for each enzyme.
Listed below are the base pair lengths of the DNA fragments from the Hind III digested lane:
44,141
37,459
27,479 Label these base pairs on your ideal gel
25,157
23,130

5. When we say a fragment is 25,157 base pairs long, what does that mean?

6. Why did the shorter fragments travel farther down the gel?

7. Examine each team's unknown. Which enzyme does it match? Why?

8. Why does the control lane have no fragments in it?

9. What does a restriction enzyme do?

10. Explain what DNA restriction analysis benefits are to the future of mankind.