RFLP ANALYSIS OF DNA LABORATORY

BIG PICTURE

You will be working with an essential research method widely used in genetics, conservation biology, and forensics. The lab is divided into three sections.

- Part 1: Your lab team will perform an enzyme digest of DNA from confiscated ivory to identify the origin of that ivory.
- Part 2: Your lab team will perform gel electrophoresis of pre-digested samples. You will compare the data to a database of genomic DNA. If you discover new data, you will contribute the data to the database.
- Part 3: Your lab team will analyze gel data from the actual research lab to compare to your samples from part A.

KEY CONCEPTS

When you have completed this lab you should be able to:

- Understand the physical properties of DNA molecules that allow separation by agarose gel electrophoresis.
- Understand how the use of biotechnology and the application of the DNA fingerprint technique (RFLP) can be used to conserve a species.
- Understand the techniques of micropipetting, sample preparation and electrophoresis as tools for gathering, analyzing, and interpreting data.
- Understand how scientists might use a database in scientific research and in conservation of a species.

STUFF TO KNOW BEFORE YOU GO

- **Important:** The restriction enzymes are in very viscous (thick) solutions because they contain 50% glycerol. To pipet these tiny volumes like a "pro," first depress the plunger, then place just the end of the pipet tip into the enzyme solution and release the plunger slowly. When adding the enzyme to the sample tube, place the tip into the droplet containing water, buffer, and DNA. Slowly push out the enzyme; then pipet in and out slowly a few times to rinse all of the enzyme out of the tip.
- **Note:** One unit (U) of activity is usually defined as the amount of enzyme required to digest 1 μg of lambda DNA to completion in 1 hour in the preferred enzyme buffer at the optimal temperature for that enzyme (usually 37°C). Whew!

**** It's important to use the right buffer to provide the best conditions for each enzyme. The enzymes have personalities and work best under different salt conditions. The manufacturer we use calls its buffers REact®. You will be using 10X REact 3® in this lab investigation.**

PART 1: PREPARING THE GEL AND DNA SAMPLES

Your team may want to divide up these responsibilities or you may choose to work on each task as a group. If you choose to work as a group, MOVE QUICKLY FROM ONE TASK TO THE NEXT. Time is short. This must be set up today.

1. **Prepare a 0.7% agarose gel.**

- See "How to Make a Carolina Blu gel." Your teacher will help you with this step.
- When the gel is cool:
  - carefully remove the comb from the gel.
  - store the gel in buffer overnight.
  - See you teacher for instructions on where to store your gel!

2. Prepare the Enzyme digest of the ivory DNA: Tube #6 (see chart 1)

- Keep the restriction enzyme stocks on ice all the time
- Add the reagents in the order listed, adding the enzyme last.
- Check off ✓ each ingredient on the chart as it is added.
- Mix tube contents gently, centrifuge 3 seconds.
- Incubate at 37° for at least 30 minutes or longer.

3. Prepare the pre-digested samples: Tubes 1-5 (see chart 1)

- Label tubes with your team's # and what is in each tube.
- Add reagents in the order given.
- Check off ✓ each ingredient on the chart as it is added.
- Mix all tube contents gently, centrifuge 3 seconds.
- Place on ice.

4. Place all tubes (1-6) in the freezer until the next lab period. See your instructor for where you will place these tubes

Chart 1

TUBE (color or label)	**1 Ladder**	**2 Uncut genomic DNA**	**3 Serengeti**	**4 S.Luangwa**	**5 Etosha**	**6 Ivory Sample**
Sterile distilled H_20	10.5µl	10.5µl	10.5µl	10.5µl	10.5µl	8.5µl
10X REact 3 restriction enzyme buffer	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl
Serengeti sample	—	—	**3µl**	—	—	—
S. Luangwa sample	—	—	—	**3µl**	—	—
Etosha sample	—	—	—	—	**3µl**	—
Uncut genomic DNA	—	**3µl**	—	—	—	—
Ladder (Marker)	**3µl**	—	—	—	—	—
Ivory DNA sample	—	—	—	—	—	**4µl**
Restriction enzyme BamHI	—	—	—	—	—	1µl

PART 2: RUNNING THE GEL

1. **Set up the gel.**

- Obtain 125ml of 1X TAE buffer containing Carolina Blu-1.
- If your gel was made earlier, place the gel into the gel box, sample wells near the negative (black) electrode and top side of the gel facing up. If your gel was poured today, add a little buffer around the comb, and gently remove the comb and dams.
- Add buffer with Carolina Blu until the buffer level is about 2mm above the top of the gel.
- Put the gel box near the power supply before loading samples.
- Once you start loading samples in the next step, you should avoid shaking or moving the gel box. (Optional: check pH at each electrode.)

2. **Remove tubes 1-6 from the freezer**. Place them on the lab bench until they thaw.
3. **Then add 3 µl Sample Loading Buffer** to each. If necessary, centrifuge again.

TUBE (COLOR OR LABEL)	**1** **Ladder**	**2** **Uncut genomic DNA**	**3** **Serengeti**	**4** **S.Luangwa**	**5** **Etosha**	**6** **Ivory Sample**
Sample Loading Buffer(microcentrifuge tube of blue soln.)	3µl	3µl	3µl	3µl	3µl	3µl
Total Volume	18µl	18µl	18µl	18µl	18µl	18µl

4. **Load 15 µl of your samples** into the wells.
   - On your record sheet, or in our journal indicate which sample is in each well.
   - When all the samples are loaded, close the lid on the gel box.
5. **Beginning Gel electrophoresis**

- **Check that the power supply is turned off and the voltage is turned all the way down.**
  - Then attach the electrodes of the gel box to the power supply, making sure that the red lead connects the positive (+) terminals and the black lead connects the negative (—)terminals.
  - Usually two student teams will be sharing one power supply. If one group starts their run first, just turn off the power supply while connecting the second gel box. Adjust the power supply to ~100 volts.

6. **Record the following information in your journal**:

Start time _____ Total electrophoresis time_____

Voltage _____ Current (may change during the run) _____

7. **Running the gel**
   - Electrophorese for at least 5-10 min to make sure the samples have entered the gel from the wells. Your teacher will tell you if this is the stopping point for today or how long to run your gel.
   - The ideal electrophoresis time will be 45 min at 100 volts. Less time will result in poor separation of the DNA bands.

- You can monitor the general progress of the electrophoresis by watching the tracking dyes included in the sample loading buffer, bromophenol blue (at this pH it's purple) and xylene cyanol. It's best to run the gels until the bromophenol blue has moved about 2/3 of the way through the gel (or halfway if time is short).

8. **Termination of electrophoresis**:

- After 45 min or at the time your instructor indicates, **turn off the power supply**.
- **Then** detach the electrical leads (optional: record the pH at each end of the gel box).
- Note the positions of the tracking dyes on your record sheet.
- Carefully transfer the gel to a staining dish bearing your group's names or initials.
- Stain the gel with Carolina Blu-2, as described on the blue "How to make a Carolina Blu gel" sheet.

9. **Clean up** your lab station, dispose of designated materials as directed. Wash your hands.

PART 3 – CALCULATION OF RFLP LENGTHS USING BANDING PATTERNS AND SIZE APPROXIMATION

- Obtain a small acetate sheet for each member of your group.
- Place the gel on a light source.
- Carefully place the acetate over the top of the gel.
- Trace the outline of the wells.
- Number the wells.
- Trace the DNA fragment lengths as accurately as possible.
- Tape your completed acetate sheet into your journal.
- Label the marker fragments with the number of kilobase pairs they represent.
- Locate an unknown fragment you are trying to measure. Use a straight edge or ruler to compare the location of the unknown band to the known marker bands.
- Estimate the size of the unknown band based on the comparative location to the nearest markers.
- Share data with your table partners and calculate the average base pair length of each unknown fragment.
- Record the data in your Data Submission Form (DS571).