RFLP ANALYSIS OF DNA
LABORATORY
TEACHER GUIDE

OBJECTIVES: **Elephants, Ivory, Biotechnology, and Global issues**

1. To understand the physical properties of DNA molecules that allows their separation by agarose gel electrophoresis and apply this technique to an engaging scenario.
2. To understand the relevance of this technique to scientific research and global issues.
3. To provide students with further experience with the techniques of electrophoresis, micropipetting, and sample preparation.

SPECIAL INSTRUCTIONS

- In this activity students will perform the restriction digest of "ivory" DNA using BamHI, prepare simulated Genomic DNA samples for electrophoresis analysis, pour a gel, load and run the samples, stain the gel for viewing and analysis.
- DNA+BamHI+React will have to incubate for a minimum of 30 minutes at 37°C then placed in the freezer overnight. However, it is acceptable to allow them to incubate for up to 2 hours and then you will have to put them in the freezer when students are gone.
- Gels should be run for 45-55 minutes at 100-110Volts. If you plan to run at a voltage higher that 100 volts, place 1xTAE buffer in the refrigerator. Place the cold buffer on the gel. This will allow you to run the gels at the higher voltage and hopefully not melt the wells beyond recognition. It is not recommended that you run the gels for less that 45 minutes as you will not have good separation of the bands. This will make it very difficult to identify the source of the "ivory"DNA.
- To save time, you may choose to have the agarose already heated (keep in a 60° water bath until ready to use) and ready for students to pour. Also, just before students begin, you may prepare a tube for each group of premixed 1.5ul REact and 1ul BamHI. Be sure to instruct your students to add the entire 2.5ul volume of REact+enzyme to their "ivory" DNA.
- You may want to use different color microtubes for each of the park DNA samples that you are preparing for loading on the gel.
- Flow-charting of the lab procedure is highly recommended, either as homework or in class with teacher assistance. (*See the flow chart example in the Elephant RFLP lab folder*.)
- Assign lab team members specific tasks. Each member can be assigned a park sample to prepare. The expert micropipettor of the group can do the enzyme digest. In preparation the lab team members can be trained as experts in lab tasks like running the gel box, pouring the gel, setting up the gel box.
- You will find in the *Appendix folder of Alternate Protocols* there are several versions of this lab. The kit version that can be adapted in any time frame that you choose but was written with 55 minute class periods in mind.
- By using these time saving ideas, you will have the opportunity to teach your students about semi-log graphs, and even get them started with some of the graphing and analysis of the Comstock data.
- **NOTE!!** *The DNA used in this lab is λDNA. Using authentic elephant DNA is not an option due to biohazard issues and simply because it is just too precious to scientists in their current research to include in this kit. Still, this lab is meant to be an accurate simulation of the work being done by Kenine and Sam.*

Please put the Freezer Box into a freezer immediately, if you haven't already done so

GENERAL KIT NOTES

1. The Elephant Project DNA labs have been updated as of 2002. Please read through them even if you've used them in the past.
2. Included in the GEL KIT NOTEBOOK are the instructions for DNA Labs 1 & 2, the Electrophoresis Exploration Lab, and the Dye/Indicator Lab. They are on a disk included in the Kit Notebook. They are in both Mac and PC versions. Feel free to adapt these labs for your own use, but please credit SEP when you do.
3. If you choose to have your students measure the pH of the gel box buffer, please do so only in the Electrophoresis Exploration Lab, or once in the Dye/Indicator or DNA Labs. This pH paper is very expensive (over $20 a box) and it's not useful to keep measuring the pH in each lab.
4. The electrophoresis gel box lids crack easily. Please repack the boxes carefully and don't put anything on top of the gel boxes or crush them with the green crate lids. Please pack the gel boxes in two stacks of 4, with nothing on top of them. Gel box lids are $50 each and we've had quite a few broken due to poor repacking.
5. In pouring gels, students can measure 25 ml or slowly pour the agarose directly into the gel casting tray (with dams) up to the edge of the tray. If they pour too quickly, the agarose solution may leak under the casting dams.
6. In running gels, use 100 volts for 45 minutes or until the bromophenol blue (purple at this pH!) is about halfway down the gel. If you run the gels longer or faster (i.e., at higher voltage), they may melt. If the power supply beeps during the run, turn it down one notch.
7. Field Guide to Gels: Don't forget that the DNA Science book has a wonderful gel troubleshooting section on pages 274-275. That portion of the book is included in the Gel Kit Notebook.
8. Included with the Teacher Information in the "Additional Resources" folder is a semi-log plot from Nancy Hutchison's old research notebook. This shows the curving of the line at large or small fragment sizes. You may wish to copy this for your students.

CLASSROOM MANAGEMENT

1. Potential bottlenecks include weighing out agarose, microwaving, getting DNA or enzyme samples, and centrifugation. Try using 2 or 3 stations of the materials that students share. It will help to have multiple balances and to aliquot the DNA and enzymes to multiple tubes.
2. For classroom discussion of results, you can try putting the gels onto the overhead projector. Use the Zap Shot digital camera (SEP has one you could borrow) or a video camera and monitor to share student gels.
3. **Helpful suggestions:**
    - Prepare simulated elephant DNA samples, store in fridge or freezer for up to several days. See the student lab for individual μl volumes.
    - Pour gels, remove (carefully, the wells are fragile), and store up to several weeks in 1X TAE buffer, refrigerated.
    - Prepare DNA digests, allow to digest at 37°C a minimum of 30 minutes up to or as long as overnight, then run or put into freezer.
    - Run gels, stain in Carolina Blu (#2), destain by leaving in a small amount of distilled water in the fridge overnight. This will also make your results easier to see, but there is the risk of losing the smallest DNA bands due to diffusion of the DNA (the tracking dyes will disappear entirely).

- Record results on acetate sheets and then do the semi-log plot another day.
- See the **Teacher Folder Comstock Database Activity Folder** for semi-log plot of this database for comparison.

MATERIALS

The materials provided in the SEP kit are detailed on the Kit Inventory Sheet (in the Teacher's Packet and on the kit crates) and on the Field Guide to the Freezer Box. Use the paper list to check off that you received and returned all the items indicated.

Materials not in the kit that you will need to provide are

microwave oven or hot plate
balance
deionized or distilled water (recommended, but not essential)

Please keep the enzymes on ice or in the freezer box (and in the freezer) at all times. The freezer box protects the samples during transit and stays cold for about 2 hours. Poor storage and handling reduce enzyme activity.

Enzyme Activity: The enzymes are usually at >10 U/μl in activity. One unit (U) of activity is 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!

Field Guide to the Freezer Box
Gel Electrophoresis Kit

	Tube Top	Tube Label	Which means	Simulated Elephant population
DNA (Thaw tube contents and mix thoroughly before using)				
	λ	λ DNA	Uncut lambda DNA	Genomic Elephant DNA
	I	Marker I λ/EcoR I 250 μg/ml	λ DNA cut with EcoR I *(Precut DNA)*	Seregenti
	II	Marker II λ/Hind III 250 μg/ml	λ DNA cut with Hind III *(Precut DNA)*	South Luangwa
	III	Marker III λ/EcoR I +Hind III 250 μg/ml	λ DNA cut with both EcoR I & Hind III *(Precut DNA)*	Etosha
Restriction Enzyme				
	BamH I	BamH I Use REact 3	Restriction Enzyme Activity= 10 U/μl	Use with REact 3 buffer
Restriciton Enzyme Buffers (Use as is, do not dilute)				
	REact 3	10X REact 3 Buffer	10X REact 3 Buffer	Use with BamH I digests