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

After students have digested their "ivory" DNA and performed their gel electrophoresis they will need to analyze both their data and the data from Dr. Comstock's lab. The gels will need to be stained and de-stained at the beginning of, or prior to, this activity. Students can do this during class, or you may choose to do this outside of class.

ANALYSIS OF RFLP DATA FROM ALL SOURCES OF DNA. Presented here are two different options:

Option 1 – Calculation of RFLP lengths using banding patterns and size approximation. This involves estimating band or fragment sizes and is recommended for those whose classroom time is more limited or whose students are not as familiar with graphing.

Option 2 – Calculation of RFLP lengths using semi-log plot. This is recommended for students with strong math skills and tends to be slightly more time consuming.

You will need to choose the appropriate option for your students prior to beginning the analysis. For either option you choose, you will need to do the following:

1. Handout necessary materials for analysis. (Note: both options require a picture of the Comstock gel data and a Data Submission Form DS 571)
2. Introduce the photograph of a gel from Dr. Comstock's lab, pointing out parks from which samples came, presence and reason for two ladders, and base-pair lengths for ladder bands.
3. Have students follow directions for generating base-pair length data from Dr. Comstock's data using either option and record the base pair lengths on data submission form DS 571.
4. Review follow-up questions on Comstock RFLP data with students. ***NOTE: These questions might best be left for homework, depending on time constraints.***
5. Have students obtain information on the country and vegetation regime (the former from a handout, the latter from an overhead transparency) for each park sample and record in the upper portion of the Data Submission Sheet.
6. Review follow-up questions on Comstock RFLP data with students. ***NOTE: These questions might best be left for homework, depending on time constraints.***

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HOW TO MAKE A CAROLINA BLU™ GEL (0.7% agarose for DNA)

Wear goggles and hot gloves when handling hot agarose*

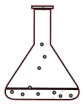
Materials:

- centigram balance
- weigh boats or paper
- bottle or flask, 3X volume of gel solution
- graduated cylinder for agarose solution
- hot gloves and goggles
- microwave oven or hot plate
- gel electrophoresis box & power supply
- 150 ml plastic beaker to hold buffer
- agarose powder
- 1X TAE buffer
- Carolina Blu #1 stain

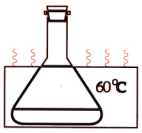
Consult chart on back for amounts of agarose and buffer to use and for useful hints

1. **Add:** ____ grams agarose to ___ milliliters buffer in large Erlenmeyer flask or bottle. (Lid **MUST** be loose before heating!)

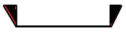
2. **Heat:** Until all particles are dissolved, ~30 sec to 1 min after solution boils. Mix by swirling flask or bottle several times during heating.



3. **To Cool or Store:** Keep flask in a 60°C water bath.

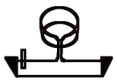


4. **Set up:** Place dams in gel box at each end of gel tray



5. **Add:** Carolina Blu #1: 2 drops to 50 ml agarose — then MIX.

6. **Pour:** 25 ml of agarose into the gel tray. Insert comb at negative (black) end for DNA.



7. **Cool:** Let gel harden 10 min.
   Add: 6 drops Carolina Blu #1 to 125 ml 1X TAE gel buffer.
   Pour: some buffer over top of gel.
   Remove comb & dams gently.

   If you choose to **store the gel before running:**
   Write your name on an acetate sheet and slide it under the gel.
   Store them in a Tupperware container or Ziploc bag with buffer covering all of the gels.
   Gels containing Carolina Blu should be used within 24 hours.

8. **Run:** Add the remaining buffer, load 15 µl samples, and run the gel at 100 volts.

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MAKING & STAINING GELS WITH CAROLINA BLU™

In DNA labs, 0.7% agarose gels are used. This low agarose % will allow the DNA to run faster, thus shortening the electrophoresis time to 30-45 min. Note that these gels will be more fragile than 1% agarose gels used in the Dye Lab.

MAKE THE AGAROSE SOLUTION

1. Wear goggles. Obtain a bottle with a **loosened cap** or an Erlenmeyer flask. The container's volume should be about 3X the volume of the solution to prevent boiling over.
2. Use Table 1 to calculate the amount of agarose and buffer you will need. Add the agarose powder to the buffer and mix.

   Note: Wear hot gloves. Agarose will boil over quite easily! ***Beware of steaming hot agarose.!***
3. To dissolve the agarose, heat the mixture to boiling in microwave or on a hot plate for ~30 seconds to 1 min after the mixture begins to boil. Swirl the bottle occasionally as it heats.
4. Swirl the bottle to see if agarose is dissolved. If any clear floating particles are visible, heat it for another 30 seconds and check again.
5. When the agarose is dissolved, add the appropriate amount of Carolina Blu #1 to the solution and swirl to mix. See Table 1. *Note: Carolina Blu must be added* ***after*** *the agarose has dissolved!*
6. Place the hot container in a 60°C water bath (Rival Hot Pot works well) or oven to hold the melted agarose at the right temperature for pouring gels throughout the day.

Table 1

No. gels @ 25 ml each	agarose	1X TAE	HEAT & DISSOLVE THEN ADD CAROLINA BLU #1	Carolina Blu #1
2	0.35 gm	50 ml		80 μl (2 drops)
4	0.7 gm	100 ml		160 μl (4 drops)
10	1.75 gm	250 ml		400 μl (10 drops)

POUR THE GEL

7. You can measure 25 ml or just fill the tray, with dams in, to the edge with agarose. You do **not** have to cool the agarose to pour the gel in the Horizon 58 gel boxes. They can take the heat. You do, however, need to pour the agarose *slowly* so that it does not leak under the dams.

Table 2

Amount of Carolina Blu #1 to add to 1X TAE buffer for running DNA gels

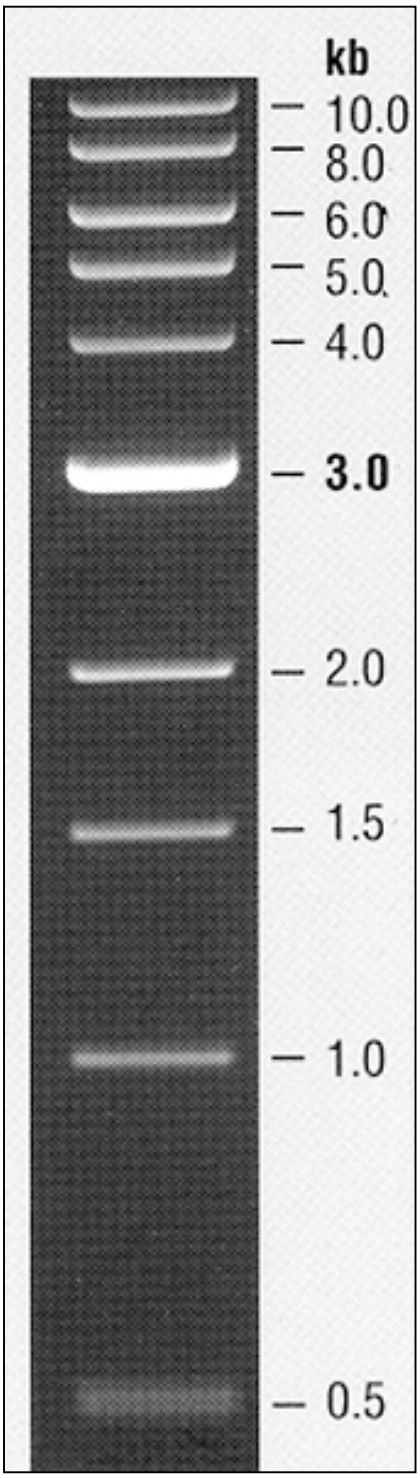
# of gel boxes	1X TAE Buffer volume	Carolina Blu #1 volume
1	125 ml	240 μl (6 drops)
8	1000 ml	1.9 ml (48 drops)

STAIN & DESTAIN THE GEL USING CAROLINA BLU #2 FINAL STAIN

8. **Stain:** Following the electrophoresis, place the gel into a staining tray. Cover the gel with Carolina Blu #2 and allow to sit for 15 minutes. Agitate gently if possible. Pour the stain into a container (it can be reused).
9. **Destain:** Cover the gel with distilled water. (Tap water contains chloride ions that may partially remove the stain from DNA bands.) Occasionally, gently agitate the gel. Change the water 3-4 times over the course of 30-40 minutes. The gel can be left in a little water to destain fully. If the gel destains too much, you can restain it. For best results, monitor the gel during destaining.
10. **Store:** Once destained, the gel can be covered in plastic wrap, placed in a storage bag, or left in the staining tray covered in plastic wrap and stored in the refrigerator. Stored in this manner, bands are visible for 6-8 weeks.

    Note: With these stains and gels, you need about 1.0 μg DNA per lane to see the bands

1 KB LADDER FOR ELEPHANT RFLP ANALYSIS



Additional information can be obtained from: New England Biolabs at:
http://www.neb.com/nebecomm/products/productN3232.asp

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KEY TO SIMULATED ELEPHANT DNA RFLP SIZES AND MARKER SIZES

λ (lambda) is ~48,502 bp, depending on the strain. It has 12 bp single-stranded cohesive (*cos*) or sticky ends with complementary sequence that can bind each other within the same molecule or in another λ molecule. At room temperature, these *cos* ends do like to stick together resulting in changed DNA band sizes. (To prevent this, samples can be heated gently before running on the gel.)

**** means that these are end fragments-they may be faint on gels if the samples are not heated before running.***

Seringeti (λ EcoR I – Marker I)

1. 21,226* left end
2. 7,421
3. 5,804
4. 5,643
5. 4,878
6. 3,530* right end

South Luangwa (λ Hind III – Marker II)

1. 27,500 present when sample not heated before running
2. 23,130* left end
3. 9,416
4. 6,551
5. 4,361* right end
6. 2,322
7. 2,027
8. 564 } faint
9. 125 } "

Etosha (λ EcoR I + Hind III – Marker III)

1. 21,226* left end
2. 5,148*
3. 4,913
4. 4,268
5. 3,530* right end
6. 2,027
7. 1,904
8. 1,584
9. 1,375
10. 947
11. 831
12. 564} faint
13. 125} faint

1 kb Plus ladder (Invitrogen)

1. 12,000
2. 11,000
3. 10,000
4. 9,000
5. 8,000
6. 7,000
7. 6,000
8. 5,000
9. 4,000
10. 3,000
11. 2,000
12. 1,650
13. 1,000
14. 850
15. 650
16. 500
17. 400
18. 300
19. 200
20. 100

Ivory DNA (λ BamHI – student digest)

1. 16, 800
2. 12., 200
3. 7,200
4. 6,500
5. 5,600

COMPARISON OF STUDENT DNA GELS
45 AND 60 MINUTES RUN TIME