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CANCER RESEARCH CENTER
SCIENCE
EDUCATION
PARTNERSHIP

LAB 1:
DNA PRECUT BY RESTRICTION ENZYMES

Why would anyone want to study DNA? Scientists have learned that the incredible amount of information stored in DNA can answer many questions and solve problems which affect people daily. For example, the forensic analysis of DNA can help convict (or exonerate) suspected criminals, solve cases of poaching of endangered species, and determine which species of salmon is migrating up the Columbia River. The ability to identify specific bacterial strains using a DNA profile allows meat to be tested for the presence of harmful strains of *Escherichia coli* (*E. coli)*, preventing fatal food poisoning. People can be tested for the presence of harmful genes, and someday those genes may be repaired through gene therapy. Biotechnology uses genes to make products humans need for fighting disease. These are all cases in which the analysis of DNA is required.

You will build your skills in working with DNA by learning several techniques that are fundamental to DNA analysis. You will study the DNA of one organism, a virus named lambda (λ). Lambda is a virus that attacks and kills *E. coli* bacteria after using the *E. coli* as a lambda factory. *E. coli* is a kind of bacterium that normally lives in your intestines. The DNA of lambda is a single chromosome about 50,000 base pairs (or 50 kb, kilobase pairs) long. Both lambda and *E. coli* are favorite model organisms, studied by scientists all over the world.

Pieces of DNA the size of the λ chromosome are too big to analyze on the kind of agarose gel that we (and many other research scientists) are using. Lambda has about 50 genes; therefore to study individual genes, it's really useful to cut the DNA up into more manageable size pieces. Proteins called restriction enzymes are used to cut DNA at specific DNA sequences. You can think of these enzymes as molecular scissors. The pieces are sorted out by size using agarose gel electrophoresis. Gel electrophoresis is an example of using the properties of molecules (DNA, in this case) to isolate and study them. In this lab, you will see how this process works using λ DNA samples that have already been cut with restriction enzymes. Keep in mind that DNA from any organism can be studied using these same concepts and technologies. The analysis of DNA by gel electrophoresis is a necessary step in many experiments using DNA.

DNA that has been cut with restriction enzymes is called, "precut DNA" or "DNA fragments." These precut DNA pieces are often used as "DNA markers" and they are labeled according to the enzyme that was used to cut the DNA, i.e. λDNA/EcoRI.

For more information, read the section on the Background Information page (found at the end of the lab section).

Concept Question: How does the size of the DNA fragment affect its movement or migration through the agarose gel during electrophoresis?

Objectives:

1. To understand the physical properties of DNA molecules that allow their separation by agarose gel electrophoresis.
2. To understand the importance of this technique to biotechnology and biomedical research and its applications to DNA fingerprint analysis, forensics, various genome projects, gene discovery, human mutation analysis, food and drug production, and diagnosis of animal pathogens.
3. To provide experience in the techniques of micropipetting, sample preparation and electrophoresis as tools and techniques for gathering, analyzing, and interpreting data.
4. To learn about some of the model organisms used in scientific research.

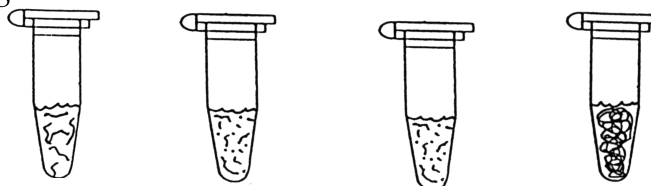
PROCEDURE

1. **Prepare 0.7% agarose gel.** See **How to Make and Stain an Agarose Gel with Fast Blast**™ at the end of this packet.

2. **Prepare samples:**
- Label tubes! (you can use 1, 2, 3, & C (for Control))
- Use the chart as a checklist ✓ so you don't forget to add all reagents.
- Start with the STE buffer, then add DNA. Add Sample Loading Buffer (blue) last.
- WATCH as the tiny volumes go in and out of your micropipet. Think about what you are doing and why.
- Change micropipet tips when you change reagents or if you touch the tip to the liquid in the tubes, to your fingers or anything else except the intended sample.
- WHEN IN DOUBT, CHANGE TIPS!
- Tip: We are using the real research lab names for all the buffers and solutions. You'll need to keep in mind that more than one thing can have "buffer" or "blue" in its name. Double-check names to be sure you're using what you really intend.

Precut DNA Sample Preparation Guide

Note: -- means don't add that reagent to this tube



Add to Tube:	Tube: 1	2	3	C
STE (salt buffer)	10 µl	10 µl	10 µl	11 µl
I (λDNA/EcoRI)	4 µl	--	--	--
II (λDNA/HindIII)	--	4 µl	--	--
III (λDNA/EcoRI+ HindIII)	--	--	4 µl	--
uncut λ DNA	--	--	--	3 µl
Sample Loading Buffer (microcentrifuge tube of blue soln.)	3 µl	3 µl	3 µl	3 µl
Total Volume	17 µl	17 µl	17 µl	17 µl

3. **Mix gently, then centrifuge for 3 sec** — are your tubes balanced?

4. **Set up gel.** Obtain 125 ml of 1X TAE. 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, gently remove the comb and dams. Add 1X TAE buffer until the buffer level is about 2 mm 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 test: check pH at each electrode.)

5. **Load 15 µl of your samples** into the wells. On your Record Sheet, indicate which sample is in each well. When all the samples are loaded, close the lid on the gel box. **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 to the + (positive) terminal and the black lead connects to the — (negative) terminal. Usually two

student groups 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.

Start time _______ Total electrophoresis time ________________________

Voltage ________ Current (does it change during the run?) __________

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. If time permits, total electrophoresis time will be about 45 min at 100 volts. If needed, gels can be run only 30 min, but the DNA bands will be less easy to resolve from each other. 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 bromophenol blue has moved about 2/3 of the way through the gel (or half way if time is short).

6. Observations and predictions you can make:
   - What happens to xylene cyanol (the other tracking dye) during the gel run?
   - Do you see bubbles forming at the electrodes? Why or why not? What are they made of?
   - Based on the direction that the DNA molecules are traveling, what can you conclude about the charge on DNA? (DNA is colorless itself. After the run, the staining step with Fast Blast™ will make the DNA bands much more visible. You may have to wait until your gel is finished staining to answer this question.)
   - What's happening to the temperature in the gel box? Why do you think that happens?
   - What is the amount of current [indicated on the power supply by using the switch that selects voltage or mA (milliamps)]?
   - What would happen if you connect your gel box so that the red lead from the power supply is connected to the black end of the gel box?

7. 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 Fast Blast™ as described in **Making and Staining Gels with Fast Blast™.**

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

8. Results: After destaining the gel, record your results on an acetate sheet. After you have traced the wells and the DNA pieces, use a ruler to measure how far each piece traveled (in mm). Make a table to organize your data. Your instructor will show you an example of a table you could use. For each lane, you may see several bands. Be sure to measure each band. After you have measured each band, refer to the restriction map of λ DNA as a guide and see if you can assign sizes to the DNA bands that you see in the gel (acetate sheet). Write your conclusions and further questions below or in your lab journal.

RECORD SHEET: PRECUT DNA (DNA LAB #1)

Loaded, before running

Black (-) end pH:

Red (+) end pH:

After, gel running

Black (-) end pH:

Red (+) end pH:

Put your acetate sheet of results here

STEP 1

prepare gel: 0.7% agarose
add buffer: 125 ml 1X TAE
prepare samples
load 15 μl into wells
optional: record pH at each end

STEP 2

run gel at least 30 min at 100 V
optional: record pH at each end
stain gel in Fast Blast™
destain gel in tap water
record bands above and place your acetate sheet on this page

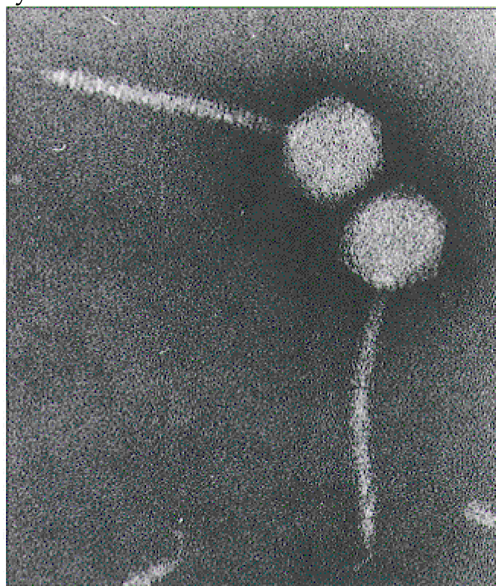
STEP 3

clean up
wash your hands
determine sizes of bands by estimation or by semi-log plot

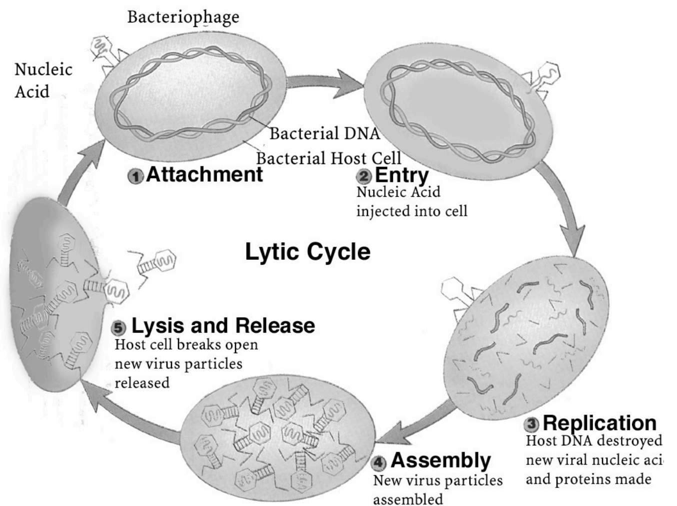
Background Information

Both lambda (λ) and *Escherichia coli* (*E. coli*) are favorite model organisms studied by scientists all over the world. *E. coli* is a kind of bacteria that normally lives in your intestines. Lambda (λ) is the name of a virus that attacks *E. coli*. Viruses that attack bacteria are called bacteriophages or phages, so sometimes we call it phage λ. When λ infects *E. coli*, it first attaches to the bacterial cell and then injects its DNA into the *E. coli*. When lots of copies of the virus are replicated and assembled inside the *E. coli* cell, the cell breaks open (and dies) releasing many new virus particles ready to infect more cells. The name bacteriophage derives from words meaning "to eat bacteria," although we now understand that λ is not eating the bacteria, but causing the cells to break open. On a culture plate of bacteria, colonies of *E. coli* appear white. When phage attack an area of bacteria, dead cells leave a clear spot, which led to the idea of the bacteria being eaten.

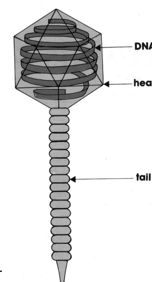
The DNA of lambda is a single chromosome about 50,000 base pairs (or 50 kilobase pairs) long. By the way, the genome of *E. coli* is made up of ~3-5 million base pairs, and the human genome is about 3×10^9 bp long (3 billion base pairs!). Actually, from DNA sequencing, we now know the exact length for the λ strain we're using is 48,502 base pairs. This genome is standard double stranded DNA except for 12 bases at each end that are single stranded. Lambda uses these interesting single stranded regions, called *cos* (for cohesive) ends, to turn its DNA molecule into a circle at the beginning of the infection cycle.



An electron micrograph of two lambda The scale bar stands for 100 nanometers (nm), or 100 billionths of a meter



The life cycle of a virus like lambda
Taken from Biggs Kapicka Lundgren *Biology: the Dynamics of life* 1998 page 507



A schematic drawing of a single lambda
The DNA in the head is wrapped around a protein core. Taken from Ptashne, *A Genetic Switch*, 2nd ed. 1992

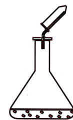
HOW TO MAKE A GEL
0.7% agarose for DNA

Wear goggles and hot gloves when handling hot agarose

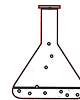
Materials:

- ❑ 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

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

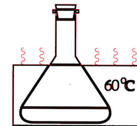


1. **Add:** ____ grams agarose to ____ milliliters buffer in large Erlenmeyer flask or bottle. (Lid **MUST** be loose before heating!)



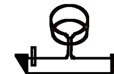
2. **Heat:** Until all particles are dissolved,
~30 sec to 1 min <u>after</u> solution boils.
Mix by swirling flask or bottle several times during heating.

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

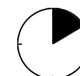


4. **Set up:** Place dams in gel box
at each end of gel tray

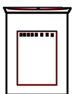
5. **Pour:** 25 ml of agarose into the gel tray.
Insert comb at negative (black) end for DNA.



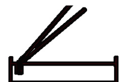
6. **Cool:** Let gel harden 10 min.
Pour: some buffer over top of gel.
Remove comb & dams gently.



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.



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



MAKING & STAINING GELS WITH FAST BLAST™

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

Make the agarose solution

1. Wear goggles. Obtain a bottle 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, loosen the cap on the container or stuff opening with a couple of lab tissues (Kimwipes®), 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.

6. Place the hot container in a 60°C water bath to hold the melted agarose at the right temperature for pouring gels throughout the day.

No. gels @ 25 ml each	agarose	1X TAE
2	0.35 gm	50 ml
4	0.7 gm	100 ml
10	1.75 gm	250 ml

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. If you get bubbles in the gel, use a pipet tip to drag them to the dams so they don't interfere with the samples.

Stain & destain the gel using Fast Blast™ Stain

8. **Stain:** Following the electrophoresis, place the gel into a staining tray. Cover the gel with 30 ml of 100X Fast Blast™ and allow it to sit for **3 minutes**. Pour the stain into the used Fast Blast™ container (stain can be reused 7 times).
9. **Destain:** Transfer the gel to a large container and rinse it with 500 ml of warm (40-55ºC) tap water for 10 seconds. Discard the water. Again, add 500 ml of warm (40-55ºC) tap water and agitate gently for 5 minutes. Discard the water and repeat 5 minute wash in water. Discard the final wash and observe results.
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.