Lambda DNA Restriction Digestion Analysis

Contents

Background information

Introduction to restriction enzymes

Restriction enzymes, also called restriction endonucleases, are proteins that act like molecular scissors. They recognize specific sequences of DNA—usually 4 to 8 base pairs long—and cut the DNA at or near these sites. These enzymes were originally discovered in bacteria, where by cutting up foreign DNA, they serve as a defense mechanism against invading viruses.

Each restriction enzyme recognizes a unique sequence. For example, EcoRI cuts DNA at the sequence GAATTC, while HindIII cuts at AAGCTT. When DNA is cut with these enzymes, it produces fragments of different lengths depending on where the recognition sites are located.

Scientists use restriction enzymes in molecular biology to map genomes, analyze DNA samples, and prepare DNA for cloning or sequencing. In this lab, you'll analyze Lambda phage DNA that was pre-treated with restriction enzymes and observe the resulting fragments using gel electrophoresis.

Some restriction enzymes can create cohesive (sticky) ends

When restriction enzymes cut DNA, they don't always make a clean, straight cut across both strands. Many enzymes, including EcoRI and HindIII, leave "sticky ends"—short, single-stranded overhangs at the ends of the DNA fragments.

These overhangs are called cohesive ends because they can easily form base-pairs with complementary sequences on other DNA fragments that were cut with the same enzyme. For example:

- EcoRI cuts between G and A in the sequence GAATTC, producing an overhang:

  5'—G AATTC—3'
  3'—CTTAA G—5'

- The resulting AATT overhangs can "stick" to other EcoRI-cut DNA fragments through base pairing.

Sticky ends can be useful in molecular biology because they make it easier to join DNA fragments together. In this lab, however, sticky ends joining together could distort the band patterns we see on the gel. Because the hydrogen bonds that hold sticky ends together are not very strong, thermal energy can make the sticky ends separate. (This is the same principle behind DNA denaturation during PCR.) In today's lab, we will use heat to make sure that sticky ends don't stick to each other.

Lambda DNA and restriction digestion

Lambda DNA comes from bacteriophage Lambda, a virus that infects *E. coli* bacteria. Its genome is about 48,502 base pairs long and is often used in molecular biology because it is easy to handle and produces well-described results in experiments.

When Lambda DNA is treated with restriction enzymes like EcoRI and HindIII, each enzyme cuts in a unique pattern, producing a predictable set of DNA fragments.

- **HindIII cuts Lambda DNA into 8 fragments** with sizes ranging from a few hundred to over 20,000 base pairs.
- **EcoRI produces 6 fragments** of varying sizes, where some of the fragments are very close in size.
- When both enzymes are used together, EcoRI and HindIII generate a more complex pattern with even more fragments, depending on where their recognition sites overlap.

By running restriction-digested DNA samples on an agarose gel, you can visualize the resulting fragments and compare the patterns obtained with different enzymes. This helps illustrate how restriction enzymes work and how they can be used to map DNA or analyze genetic differences.

Expected DNA fragment patterns

Restriction enzyme(s)	No. of fragments	Approximate fragment sizes (bp)
None	1	48,502 bp (full Lambda genome)
HindIII	8	23,130; 9,416; 6,557; 4,361; 2,322; 2,027; 564; 125*
EcoRI	6	21,226; 7,421; 5,804**; 5,643**; 4,878; 3,530
EcoRI + HindIII	~14	Combination of the above

* Smaller fragments, such as the 125 bp fragment, might be hard to detect in gel electrophoresis
** Fragments that are close in molecular weight may appear as a single band on the gel

Technical support

If you have any questions about this kit, contact support@minipcr.com

Experimental workflow

This experiment has multiple steps. Refer to the information below for time requirements.

Steps	Time required
I. Sample preparation	
A. Dispense reagents	10 minutes (can be done days before class)
B. Denature samples	5-10 minutes (must be done right before class)
II. Gel electrophoresis	
C. Prepare gel electrophoresis buffer and agarose gels	20 minutes (can be done before class)
D. Run gel	30-45 minutes

Materials needed

Supplied in kit (KT-1511-01)

- The kit contains enough reagents for 12 gel electrophoresis runs.
- If kept in the freezer, reagents can be stored for 12 months after receipt.
- Reagents for preparing gels, plastic tubes for aliquoting reagents, and pipette tips are sold separately. See next page for details.

Reagents and supplies	Provided	Required	Storage
Lambda DNA	120 µl	10 µl per lane	Freezer
HindIII Lambda DNA	120 µl	10 µl per lane	Freezer
EcoRI Lambda DNA	120 µl	10 µl per lane	Freezer
Double Lambda DNA Digest	120 µl	10 µl per lane	Freezer
10K DNA Ladder	120 µl	10 µl per lane	Freezer

Electrophoresis reagents and plastics sold separately

- This activity requires 0.8% agarose gels with a fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®) and 1.5 ml or 0.2 ml microtubes for dispensing and heating DNA samples.
- The Learning Lab Companion Kit (KT-1510-01) provides sufficient reagents to prepare and run 16 gels when using the blueGel™ or Bandit™ electrophoresis systems, as well as plastic tubes.
- Alternatively, bulk electrophoresis reagents and plastics (tubes, pipette tips) are available for purchase from miniPCR bio.
- Gel electrophoresis reagents and plastics can also be purchased from other suppliers.

Required equipment

- This lab requires a 70 °C heat source (*e.g.*, water bath or heat block)
- This lab is compatible with any horizontal gel electrophoresis system in combination with:
  - A fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®)
  - A transilluminator that is compatible with the DNA stain used. Fluorescent DNA stains typically require blue light (~470 nm) or UV (~260 nm) illumination.
- The table below outlines equipment from miniPCR bio that meets these requirements.

available at www.minipcr.com

Item
Heat source for 70 °C incubation A **miniPCR thermal cycler** is a suitable option
Gel electrophoresis and visualization system Option 1: blueGel™ OR GELATO™ electrophoresis systems with integrated blue light transilluminator Option 2: Bandit™ STEM Electrophoresis Kit paired with the Viewit™ Illumination Kit Option 3: Bandit™ STEM Electrophoresis Kit paired with a blueBox blue light transilluminator
Micropipettes and tips 2-20 µl adjustable micropipette

Other materials supplied by user

- Distilled water
- Microwave or hot plate
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker
- Ice

Sample preparation

Protective gloves and eyewear should be worn for the entirety of this experiment.

Dispense reagents (up to 1 week before lab)

- DNA samples can be dispensed up to one week in advance and stored in the refrigerator.
- This kit provides sufficient reagents for 12 gel runs.

Materials needed

From the lab kit (stored in the freezer):

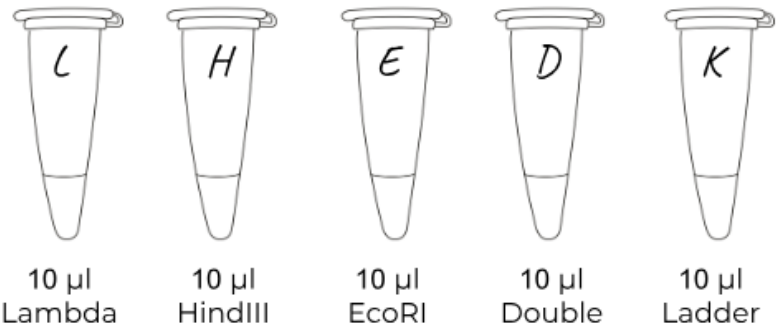
- Lambda DNA
- HindIII Digest
- EcoRI Digest
- Double Digest
- 10K DNA Ladder

Supplied by user:

- Plastic tubes for dispensing reagents (1.5 ml or 0.2 ml tubes)
- 2-20 µl micropipette and tips
- Fine-tipped permanent marker

1. Thaw reagents by placing tubes at room temperature.
2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.
3. When you open each tube, check for liquid stuck inside the cap. If necessary, put the cap back on and repeat step 2.
4. Dispense the following reagents into plastic tubes that fit in your 70 °C heat source. If using a miniPCR thermal cycler for denaturation, use 0.2 ml PCR tubes.
5. Label the upper sidewall of each tube with the corresponding sample ID.

   - Lambda DNA (L) 10 µl
   - HindIII digest (H) 10 µl
   - EcoRI digest (E) 10 µl
   - Double digest (D) 10 µl
   - 10K DNA Ladder (K) 10 µl



6. Repeat steps 4 and 5 for as many lab groups as required by your class. This kit provides enough reagents for up to 12 groups.
7. If you are preparing the DNA samples more than 24 hours before class, store the tubes in the refrigerator until use. Dispensed DNA samples can be stored in the refrigerator for up to one week.

Sample denaturation (right before gel electrophoresis)

1. Place tubes H, E, and D in your 70 °C heat source. Tubes L and K need not be denatured..

Kit Reagent	Suggested tube label	Denaturation required?
Lambda DNA	L	NO
HindIII Lambda DNA	**H**	**YES**
EcoRI Lambda DNA	**E**	**YES**
Double Lambda DNA Digest	**D**	**YES**
10K DNA Ladder	K	NO

2. Heat tubes for 5 min.
3. Cool tubes by placing them finely crushed ice for at least 3 min.
4. Proceed to gel electrophoresis.

Gel electrophoresis

A. Prepare gel electrophoresis buffer and agarose gels

1. Prepare electrophoresis buffer.
   - For the blueGel and Bandit electrophoresis systems, 30 ml of TBE buffer per gel.
   - For other systems, refer to the manufacturer's instructions for the volume needed.

2. Prepare 0.8% agarose gel(s) with fluorescent DNA stain..
   - This lab kit is compatible with any molecular grade agarose and fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®).
   - The volume of gel needed varies based on the gel electrophoresis system you are using.
   - If using gel electrophoresis reagents from miniPCR bio, gels can be prepared up to five days in advance. Store prepared gels at room temperature in an airtight container protected from light. Do NOT soak the gels in buffer or wrap them in paper towels.

B. Run gel

This kit is compatible with any horizontal gel electrophoresis system. Refer to the manufacturer's instructions for the operation of your specific gel electrophoresis system.

Detailed operating instructions for miniPCR electrophoresis systems

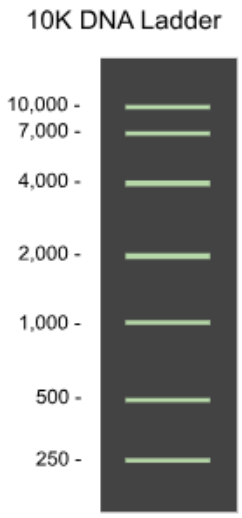
blueGel

https://links.minipcr.com/blueGelRun

Bandit

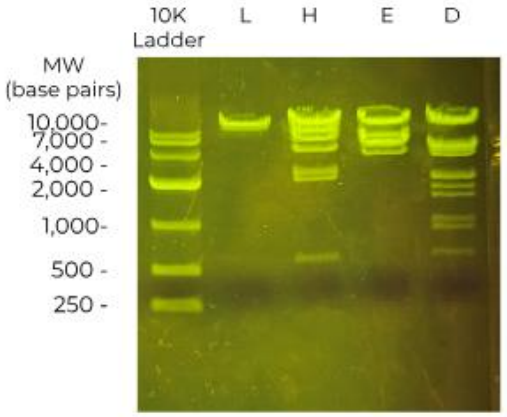
https://links.minipcr.com/BanditViewit

1. Place the prepared gel into the electrophoresis chamber.
2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel™ or Bandit™ electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.
3. Use a micropipette to load 10 µl of 10K DNA Ladder in the first well.
4. Changing pipette tips each time, load 10 µl of each DNA sample in its own well.
   - Lambda DNA (Tube L)
   - HindIII Digest (Tube H)
   - EcoRI Digest (Tube E)
   - Double Digest (Tube D)
5. Run the gel for 30-45 minutes.
   - The blueGel and Bandit electrophoresis systems run at a fixed voltage.
   - If using another gel electrophoresis system, set the voltage in the 70-90 V range. Visualize the DNA samples using a transilluminator.
6. To visualize the DNA samples, turn on the blue light in your electrophoresis system or move the gel to a transilluminator.
7. If necessary, continue running the gel until there is sufficient separation to interpret the results.



C. Expected results

Compare the bands in the 10K DNA Ladder to your Lambda DNA samples to obtain size estimates.



This image represents results obtained after a 30 minute run using a blueGel electrophoresis system.

D. Unexpected gel electrophoresis results and troubleshooting

Faint or missing bands. Insufficient DNA or a gel that is run too long may produce this result.

- Ensure that you are using the correct loading amount per well
- Check the gel during the run to monitor migration progress

Smearing of DNA bands. Overloaded wells or degraded DNA can produce smearing, as can old or incorrect gel electrophoresis buffer.

- Reduce the amount of DNA per well
- Use fresh DNA that has been denatured for no longer than 5 minutes, then chilled
- Prepare fresh gel electrophoresis buffer

Insufficient band separation. Bands that are too close together or hard to distinguish may reflect an incorrect agarose pore size.

- Ensure that you are using a 0.8% agarose gel
- Run the gel longer

No bands appear at all. This may be the consequence of incorrect gel preparation, in particular if no fluorescent DNA stain is added.

- Ensure that you are using fluorescent DNA stain compatible with the wavelength of your illumination system (*e.g.,* 460 nm excitation for blueGel™ and Viewit™).
- If you are not using All-in-One Agarose Tabs, check that the correct amount of DNA stain was added to your agarose mixture

Technical support

contact support@minipcr.com

Study questions

Pre-lab: Restriction site predictions

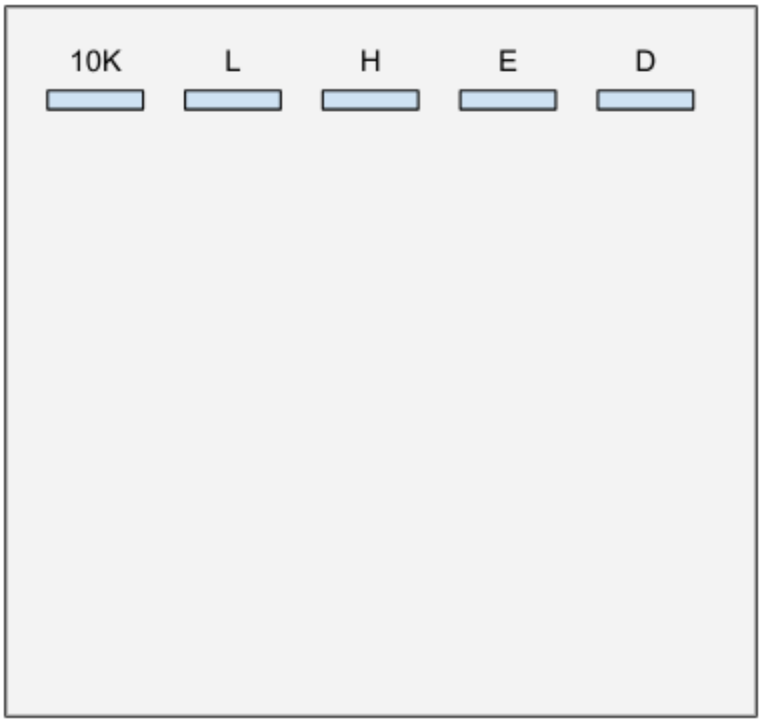
In this activity, you will use bioinformatic analysis to predict restriction sites in Lambda DNA.

1. Download the Lambda DNA sequence from the miniPCR product page, found under the "Downloads" tab.
2. Open the downloaded Lambda DNA sequence.TXT file
3. Copy the entire Lambda DNA sequence. (The header starting with > is optional.)
4. On your web browser, navigate to the NEBcutter page (v3).
5. Paste the Lambda DNA sequence into the large input box on the NEBcutter page.
6. Under "Set preferences" click on "Additional Preferences (enzymes, oligos, etc.)."
7. Under "Oligonucleotide sequences to include," click "Only oligonucleotide sequences defined below."
8. Input **Name: HindIII; Sequence: AAGCTT.** Click "Save Preferences."
9. On the main NEBcutter screen, name the project and click the "Submit" button.
10. If a pop-up window appears, click "Continue."
11. On the next screen, select "Enzyme List" and click **on the grey arrow** next to HindIII. Write down all predicted Cut Positions (these are expressed in bp).
12. Create a new project. Repeat steps 5-11 for **EcoRI** (recognition sequence: **GAATTC**.)
13. Based on your bioinformatic analysis, complete the following table:

Restriction enzyme(s)	No. of DNA fragments	Predicted DNA fragment sizes (bp)
None	1	48,502 (full Lambda genome)
EcoRI		
HindIII		
EcoRI + HindIII double digest		

Post-lab: Data interpretation

1. In the diagram below, sketch the band patterns observed for each lane.
2. Estimate fragment sizes using the 10K DNA Ladder for reference.
3. Below each lane, count the number of visible fragments in each digest.



4. Did the observed restriction fragments match your bioinformatic predictions? Name two experimental variables that may have influenced this outcome, and explain why.

5. Why was it important to include undigested Lambda DNA as a control?