Electrophoresis of Lambda DNA with the blueGel™ electrophoresis chamber

In this experiment, lambda DNA is cut using the restriction enzymes EcoRI and HindIII. The resulting DNA fragments are then separated using gel electrophoresis. By comparing single and double digestion, interfaces can be identified and the fragment sizes estimated using a DNA ladder.

Biology | Microbiology & genetics | Molecular Genetics

Difficulty level	Group size	Preparation time	Execution time
medium	2	10 minutes	30 minutes

This content can also be found online at:

https://www.curriculab.de/c/68625b671251110002736af2

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Teacher information

Application

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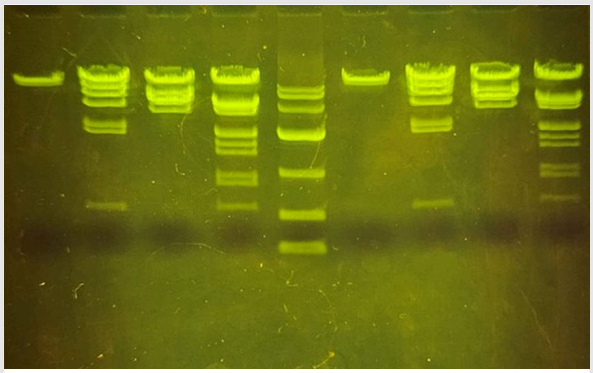

Gel electrophoresis of Lambda DNA with the blueGel system

In order to be able to analyse DNA and RNA fragments, they are separated according to size as standard and made visible by staining. Gel electrophoresis is used for this purpose. Nucleic acids are negatively charged and therefore move towards the positive pole (anode) in the electric field.

The Lambda phage is a bacterial virus that causes *Escherichia coli* attacks. Lambda was discovered in 1950 and is one of the best-researched viruses. Its genome comprises 48,502 base pairs and has been fully sequenced since 1982. As a result, we know which restriction enzymes cut at which points to produce fragments of a defined size.

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Other teacher information (1/4)

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Prior

Pupils should already be familiar with the composition and properties of DNA. They should also know how restriction enzymes work and the behaviour of charged molecules in an electric field. Pupils should also already have a basic understanding of viruses.

Principle

The cut and uncut genetic material of the phage Lambda is separated on an agarose gel according to size. If the SeeGreen™ 3-in-1 agarose tablets are used, the SYBR-Green fluorescent dye contained in the tablets intercalates with the DNA. The dye is excited by the special light of the blueGel™ gel chamber and thus makes the DNA bands visible.

Other teacher information (2/4)

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Learning

The aim of this experiment is to familiarise students with gel electrophoreseis which is a standard method used in a molecular genetics lab, used in a variety of applications, e.g. medicine and forensics.

Tasks

The students prepare an agarose gel of the specified concentration and apply the various DNA samples. The separation can be observed live and documented independently with the help of a smartphone camera.

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Other teacher information (3/4)

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The blueGel electrophoresis system with integrated fluorescent screen and darkroom

Notes on preparation and realisation

- The video on the left shows the production of a gel, the assembly of the system and an example of DNA separation.
- When using GelGreen tablets, please note that the tablet already contains TBE salt. Only 40 ml of deionised water needs to be added per tablet, which is sufficient for preparing 2 gels.
- Use 1x TBE buffer for the running buffer.
- A microwave oven is recommended for boiling the gel, but a hotplate can also be used. Do not exceed 60°C.

Other teacher information (4/4)

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Notes

- As pipetting very small volumes is not easy, it is recommended to practise this procedure with the students in advance.
- The DNA fragments of lambda DNA have "sticky ends" and artefacts can occur during gel electrophoresis: chains that stick together or circular structures that form spontaneously from fragments. These can be easily melted by heating all DNA samples to 70°C for 5 minutes. Immediate cooling with ice (or pre-cooled sample racks in the freezer) prevents these structures from re-forming.
- It is recommended that you also study the operating instructions for the kit used:

Operating instructions of the kit

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Safety instructions

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- The general instructions for safe experimentation in science lessons apply to this experiment.
- Please refer to the safety data sheets of the respective chemicals.
- The SYBR-Green used in the GelGreen tablets is a safe alternative to conventional ethidium bromide. It cannot penetrate the skin, but can penetrate the tissue through open wounds. The use of nitrile gloves is therefore recommended.
- Agarose gels made from the GelGeen tablets can be disposed of in regular household waste.

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Student information

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Theory (1/4) / Motivation

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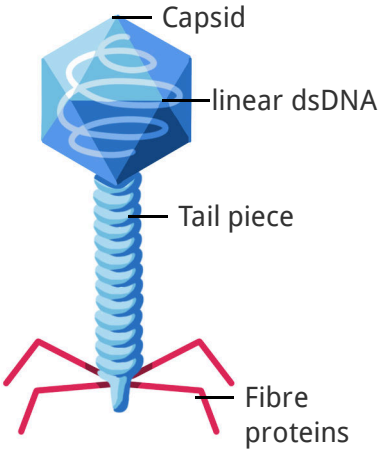

Schematic representation of the phage Lambda

Viruses are exciting and special structures. Although they all contain the genetic material to be able to reproduce, they are unable to do so without a host. They also do not have their own metabolism. For this reason, they are not categorised as living organisms, but are considered "close to life".

To date, around 11,300 virus species have been scientifically described. However, it is estimated that several hundred thousand more virus species exist worldwide. There could be over 320,000 undiscovered species in mammals alone.

The bacteriophage Lambda analysed here is based attacks only the bacterium *E.coli*, i.e. it is harmless to humans. Lambda was discovered in 1950 and has been intensively researched ever since.

Theory (2/4)

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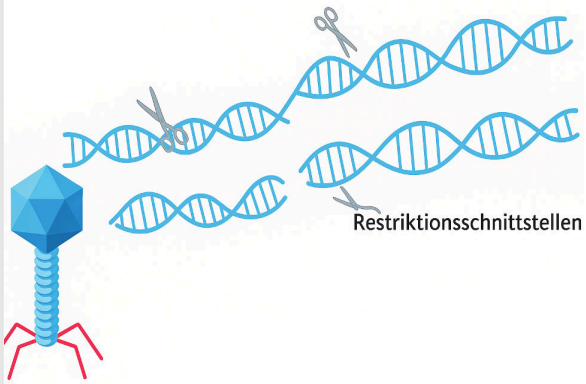

Restriction enzymes are molecular scissors that recognise certain DNA sequences and cut them at these sites. Each enzyme has a specific recognition sequence, e.g. EcoRI (GAATTC) or HindIII (AAGCTT). DNA fragments of different lengths are produced during cutting. Some enzymes produce so-called **sticky ends** - short, protruding single strands that easily combine with matching sequences. These are useful for joining DNA strands together, but can distort the gel pattern during the experiment. For this reason, the DNA is heated in order to dissolve unstable connections and obtain clear results.

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Theory (3/4)

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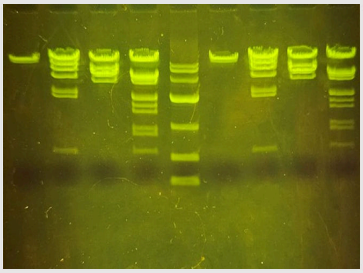
Einschränkung Enzym(e)	Nr. der Fragmente
Keine	1
HindIII	8
EcoRI	6
EcoRI + HindIII	~14



Restriction enzymes like **EcoRI** and **HindIII** cut Lambda DNA with its 48,502 base pairs at specific sequences and generate characteristic fragment patterns:

- HindIII cuts Lambda DNA into 8 fragments that are between a few hundred and over 20,000 base pairs long.
- EcoRI produces 6 fragments of different sizes. Some of them are very similar in length and difficult to distinguish in the gel.
- EcoRI + HindIII together create a more complex fragment pattern as their recognition sites can overlap. This results in even more fragments of different lengths.

These fragments can be separated and compared using agarose gel electrophoresis.

Theory (4/4)

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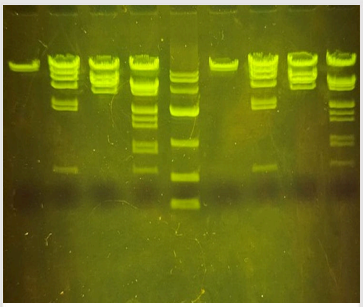

DNA bands in the agarose gel

Gel electrophoresis enables DNA and RNA to be separated and visualised. It utilises the negative charge of the nucleic acids, which migrate to the positive pole (the anode) in the electric field. The agarose gel, which functions like a net, serves as the stationary phase: the higher the agarose concentration in the gel, the narrower the mesh of the net. Small DNA fragments find it easier than large fragments to move through this net to the anode and therefore migrate further in the gel over time.

Nucleic acids are not visible in the gel on their own. They can either be stained with a methylene blue solution at the end of the run or substances can be used that intercalate between the bases of the genetic material. In our experiment, we use a fluorescent dye that intercalates with the lambda DNA fragments. The blue light of the electrophoresis chamber stimulates the dye to glow and thus makes the DNA visible (green-yellow bands, as shown in the photo on the left).

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Tasks

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1. Pour a 0.8% agarose gel: note that the agarose tablet already contains agarose, the fluorescent dye and TBE salt, so only deionised water needs to be added.
2. Load the DNA samples into the gel pockets and start the electrophoresis.
3. Compare the uncut phage DNA with the digested fragments.

Samples from the kit: DNA ladder, undigested lambda DNA, three different lambda DNA digestions

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Equipment

Position	Equipment	Item no.	Quantity
1	blueGel™ gel electrophoresis chamber with power supply and built-in exposure unit	35017-99	1
2	Electrophoresis of lambda DNA	15312-02	1
3	Microlitre pipette 2-20 µl, autoclavable	47141-10	1
4	Tips, plastic (PP) 2-200 µl, 1000 pieces	47148-01	1
5	Safety goggles "classic" - OneSize, unisex	39316-00	1
6	Gloves, rubber, size M, pair	39323-00	1
7	Erlenmeyer flask, boro, wide neck, 100 ml	46151-00	1
8	Graduated cylinder, boro, tall form, 100 ml	36629-00	1
9	Water, distilled, 5 litres	31246-81	1
10	PCR single tubes, 0.2 ml, 100 pieces, in a bag	35928-01	1
11	Stand for 8 x 0.2 ml disposable reaction tubes	37652-01	1
12	SeeGreen™ Agarose Tabs™, 3-in-1 agarose tablets, for gel electrophoresis, for 16 gels	35018-71	1
13	TBE electrophoresis buffer, 1000 ml, 10-fold conc. solution	35018-73	1
14	Graduated pipette, 25 ml, graduation 0.1 ml	36602-00	1
15	Pipette ball, flip model, pipettes up to 100 ml	36592-00	1

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Structure (1/2)

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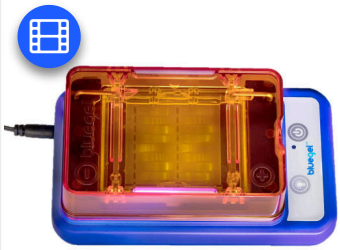

Video: Pouring a gel

- Dilute the 10x concentrated TBE running buffer concentrate to 1x with deionised water (approx. 20 ml buffer is required for one gel).
- Pour a 0.8% agarose gel (see also video 1).

1. Remove one GelGreen Agarose Tab from the pack and place it in the Erlenmeyer flask.
2. Add 40 ml of deionised water to the tablet and boil both (microwave or hotplate). Do not exceed 60°C.
3. Place a gel tray in the casting platform and a comb in the gel tray (the comb is underneath the casting platform). Use the side of the comb with the large teeth.

Structure (2/2)

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Finished gel, ready for loading

4. Pour 20 ml of the liquid gel into the bowl and allow the gel to harden (about 10 minutes).
5. Once the gel has cooled, carefully pull the comb vertically out of the gel.
6. Now place the gel tray in the buffer chamber of the base unit.
7. Add sufficient 1x TBE running buffer to the buffer chamber so that the gel is just overlaid. Not too much, otherwise the separation will take longer.

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Procedure (1/2)

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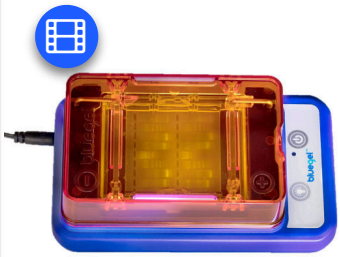

Video: Load and run the gel

Loading and running the gel (see also the video on the left):

1. Set the 2 µl - 20 µl pipette to 10 µl and place a yellow pipette tip on the pipette. **Important:** Use a new pipette tip for each sample.

2. Load the DNA samples onto the gel in the following order:

- Lambda DNA, native (tube L)
- Lambda DNA, HindIII cut (tube H)
- Lambda DNA, EcoRI cut (tube E)
- Lambda DNA, EcoRI / HindIII cut (tube D)
- 10K DNA ladder

Procedure

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Gel chamber with darkroom and smartphone

3. **Gel run**

- Place the cover on the base unit and start the run by pressing the "On/Off" button.
- Unfold the darkroom and carefully place it over the lid (see also the photo on the left).
- Press the light button to activate the blue light and you can now follow the separation of the samples. Using a smartphone or tablet, you can also film or photograph the separation process. After 30-45 minutes at the latest, the separation is complete.

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Report

Task 1

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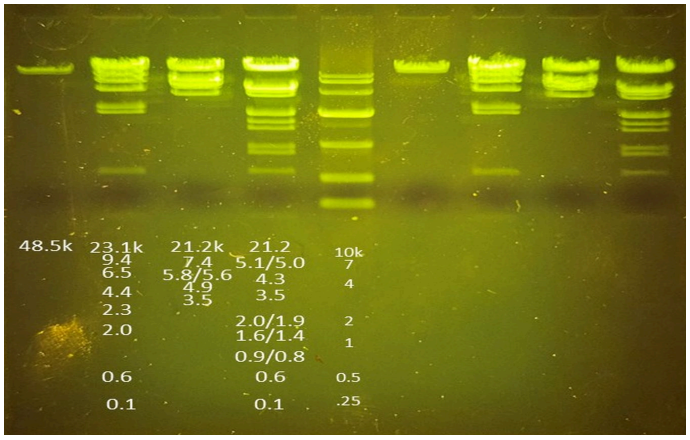
Compare your results with the data in the table and the photo shown.

Lambda DNA native	Lambda DNA EcoRI	Lambda DNA HindIII	Lambda DNA EcoRI / HindIII
48.502	21.226	23.130	21.226
	7.421	9.416	5.148
	5.804	6.557	4.973
	5.643	4.361	4.268
	4.878	2.322	3.530
	3.530	2.027	2.027
		564	1.904

Fragment sizes with optimal staining and separation (in base pairs [bp])



Separation of Lambda DNA

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Task 2

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What happens during gel electrophoresis?

- ○ Lambda DNA fragments become visible without dye.
- ○ Separation of DNA fragments according to their charge.
- ○ Separation of DNA fragments according to their size.
- ○ The higher the agarose concentration, the worse the current is conducted through the chamber.

Check

Task 3

PHYWE excellence in science

How does the combination of two restriction enzymes (e.g. EcoRI and HindIII) help to map the DNA more precisely?

- ○ It creates more cuts, resulting in smaller and more distinguishable fragments.
- ○ It extends the DNA to make it more visible.
- ○ It protects the DNA from further enzymatic reactions.
- ○ It reduces the number of fragments so that the gel image is simpler.

Check

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Task 4

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Why is undigested lambda DNA used as a control?

- [ ] Because undigested DNA is more stable
- [ ] In order to have a comparison sample to the cut DNA
- [ ] To check how many enzymes were used
- [ ] To see whether the restriction enzymes have cut correctly

Check

Slide	Score / Total
Slide 21: Principle of gel electrophoresis	0/1
Slide 22: Restriction enzymes	0/1
Slide 23: Function of undigested lambda DNA as a control	0/2

Total amount 0 / 4

Solutions Repeat

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