Gel Electrophoresis Laboratory
School of Biological Sciences

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

Extract.

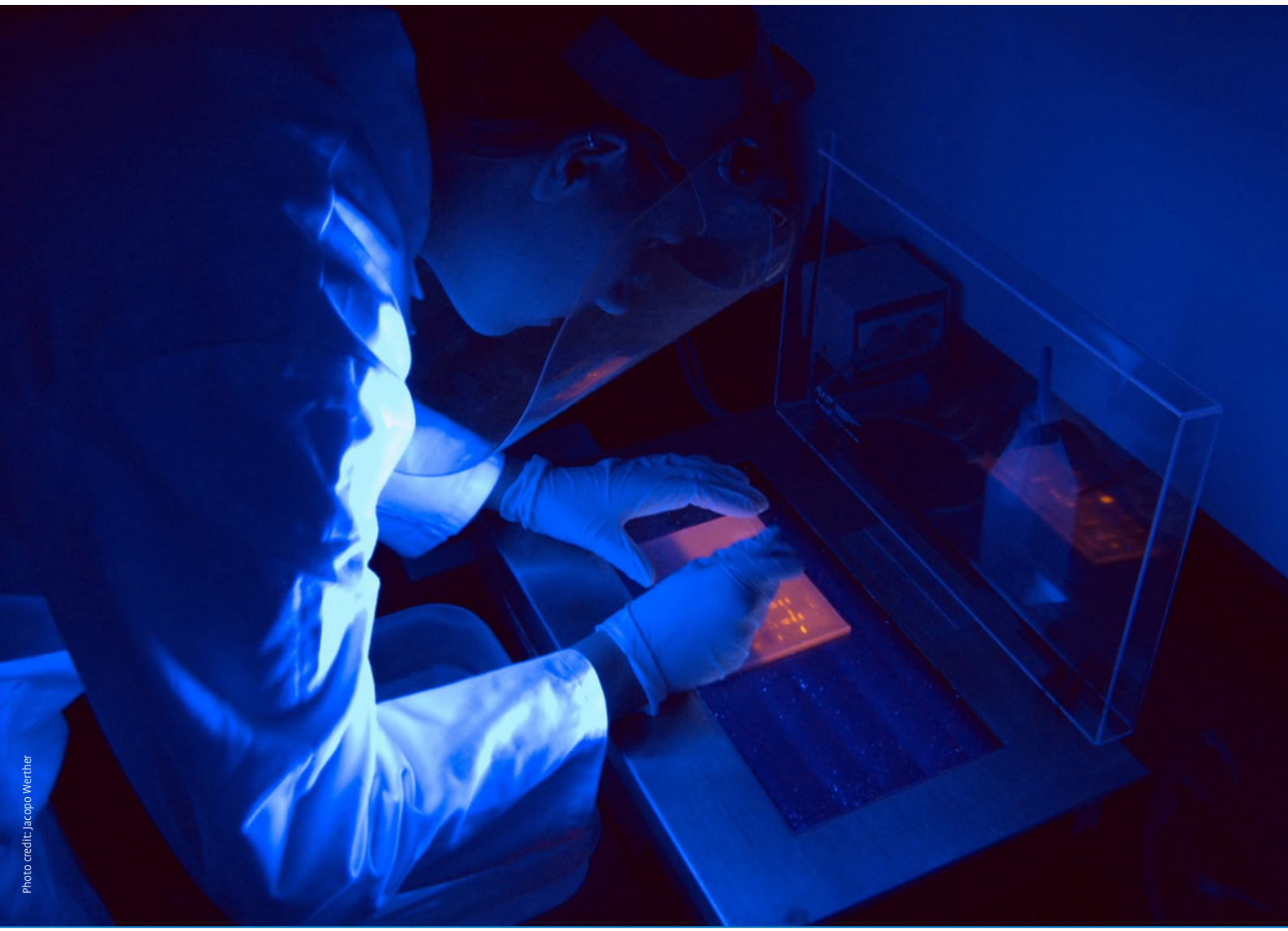


Photo credit: Jacopo Werther

UC SCIENCE

2016

visualising DNA

The Experiment

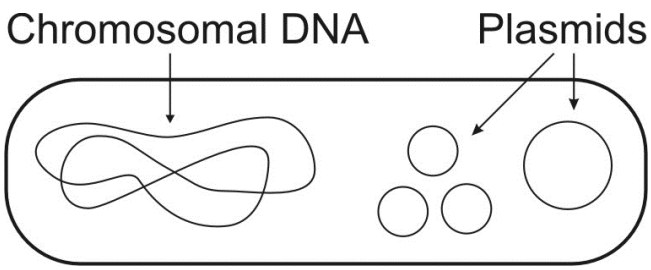
You will undertake a practical activity to reveal how enzymes can be used to cut DNA plasmids in a controlled way, enabling further genetic investigations. You will also view how an electric current can be used on charged molecules to seperate different size pieces.

Gel Electrophoresis

Introduction

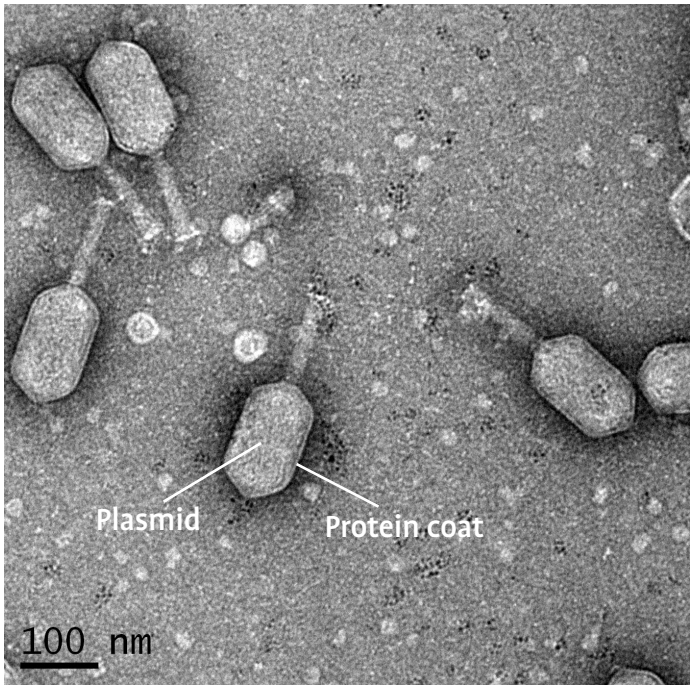
Plasmids – in bacteria

Plasmids are circular pieces of DNA which some bacteria possess, and that are extra to the chromosome. Plasmids are much smaller than the chromosomal DNA (typically a few thousand base pairs), and depending on the individual plasmid, may be present at anywhere from one copy per cell to hundreds of copies per cell. Plasmids are also special because they can be transferred between cells, and so the genes present on plasmids can spread through bacterial populations. A classic example of this spread is antibiotic resistance.



Plasmids – in viruses

Viruses are simple. They consist of a protein coat and a DNA (or RNA) plasmid, which may be either single or double stranded.



Ying-Rong Lin, Chan-Shing Lin (CC BY 2.5)

Restriction enzymes

Restriction enzymes are special DNA cleaving (cutting) enzymes, and have very specific targets. Biologists have identified hundreds of different restriction enzymes, most of which have different target sequences. The two enzymes that are used for this experiment EcoRI and HindIII.

EcoRI

```
--G A A T T C--  ⟶  --G          A A T T C--
--C T T A A G--  ⟶  --C T T A A          G--
```

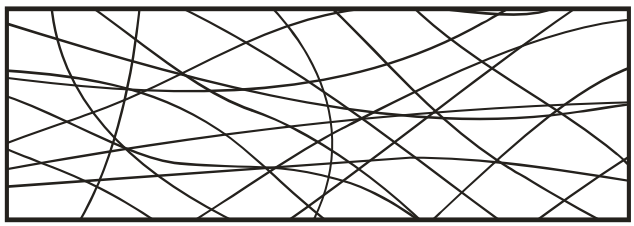
HindIII

```
--A A G C T T--  ⟶  --A          A G C T T--
--T T C G A A--  ⟶  --T T C G A          A--
```

Some restriction enzymes cut both strands of the DNA at the same point these are known as blunt-cutters that leave blunt ends, while others (such as EcoRI and HindIII) cut DNA unevenly, leaving what are called sticky ends, which can be used to stick to a complementary sequence during genetic manipulations.

Agarose electrophoresis of DNA

Agarose (one of the main components of agar) is a polysaccharide, isolated from seaweed. It forms a network of strands in a gel, which acts as a filter to separate molecules by size.

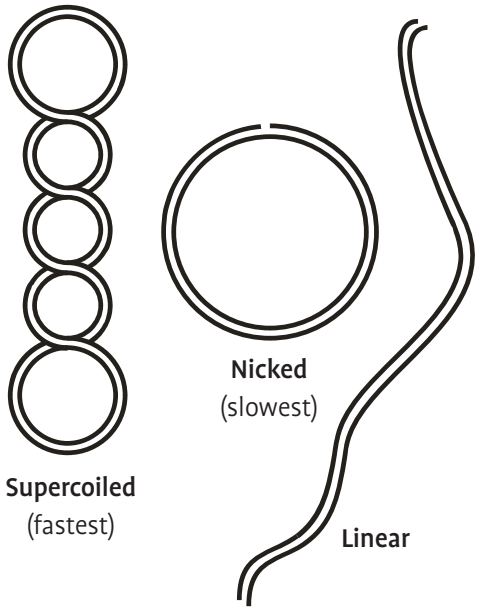


The pores are quite large, so agarose is only useful for separating large molecules, such as DNA (a plasmid of 5,000 base pairs has a molecular weight of about 3.3 million daltons [daltons are also known as g/mol]; glucose is 180 Da, and proteins are usually in the order of 10,000 – 200,000 daltons, so DNA is very large).

When we run a gel, the smallest, most compact molecules run fastest as they can flow through the pores more easily, while larger molecules are held back.

DNA is driven through the gel by an electric current. DNA is heavily negatively charged due to the phosphate groups in its backbone, so when we apply an electric current to a gel, the DNA migrates toward the positive electrode.

Intact plasmid DNA can run on a gel in a few different forms. The plasmid in its natural form is supercoiled, and so very compact. During purification, the plasmid can be nicked, which allows it to relax, and finally, sometimes during purification the DNA can be broken entirely to give linear plasmid.



These different types of DNA migrate at different rates – supercoiled runs the fastest, linear runs in the middle, and nicked plasmid runs the most slowly.

Once DNA has been linearised, it runs in a predictable fashion proportional to its size. Multiple fragments can be identified on a gel as long as they are of sufficiently different sizes (approx 50–100 base pairs).

Pipette practise

Have a go filling the pipette with water and emptying the liquid onto a square of parafilm. Compare the sizes of the droplets.

Practise using the pipette until you are able to consistently produce droplets of the same size.

Cutting the DNA

(already done for you)

In the lab, the λ bacteriophage plasmid (pBR322) was 'cut' with the restriction enzymes Hind III and EcoR I. This is called a restriction digest.

The restriction digest takes about one hour at 37 °C or it can be left at room temperature overnight.

The cut DNA will then be run on a 1 % E-gel pre-cast agarose gel and then viewed directly.

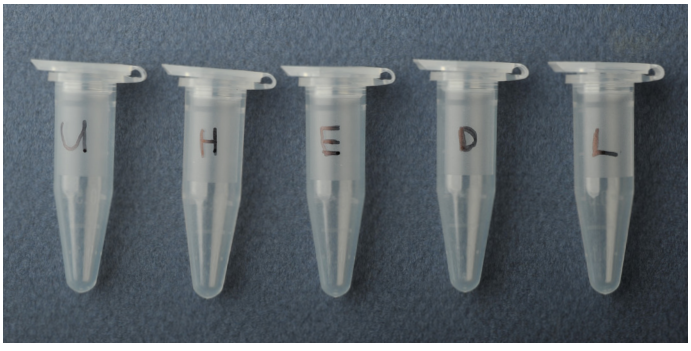
DNA fragments obtained can then be analysed to calculate a restriction map for the plasmid.

The five eppendorf tubes* are labelled as:

- **U** (Uncut)
- **H** (HindIII)
- **E** (EcoR I)
- **D** (Double)
- **L** (Ladder)

A DNA ladder is a sample of known standard lengths of DNA. All the tubes contain 10 µL of liquid.

* You may have to shake or centrifuge the tubes so that the liquid settles to the bottom of the tube.



How to prepare and run the gel

1. Prepare the samples

Make up your samples to 20 µL by adding 10 µL distilled water to each of the tubes using a micropipette.

Using a quick wrist flicking motion get all the liquid to the bottom of the tube so that you can pipette it out.

2. Prepare your E-gel-EX

Take the gel out of the foil packaging.

Carefully remove the comb by lifting it from both sides without bending it.

3. Load the gel

Pipette your samples and ladder into the desired wells; try to avoid introducing bubbles into the wells.

Fill any empty wells with 20 µL of distilled water.

Write down which well you loaded each sample into on page 6.

4. Run the gel

Place the gel on the grey iBase, right side first, sliding it across and pressing on the left side.

Use programme 7 "***Run E-Gel EX***" programme (find this by pressing Mode then the up arrow then press ***Go*** when you are ready).

5. Watch progress

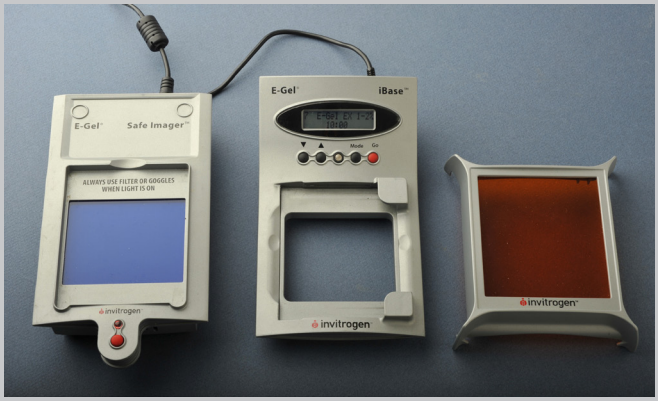
You can watch the progress of the gel using the Safe-Imager and the orange screen: place the iBase on top of the Safe-Imager with the orange screen over the gel, then press the red button on the Safe-Imager to turn on the light – your DNA bands will light up.

6. View results and photograph

When the gels are finished (10 minutes), have a look at each of them using the Safe-Imager, and take photos if desired.

The DNA will diffuse over time, so be sure to examine your gels shortly after they finish running.

Add 10 µL of distilled water to all 5 of the eppendorf tubes using a micropipette. Then transfer the solutions to the gel wells.



Safe Imager iBase Orange filter screen

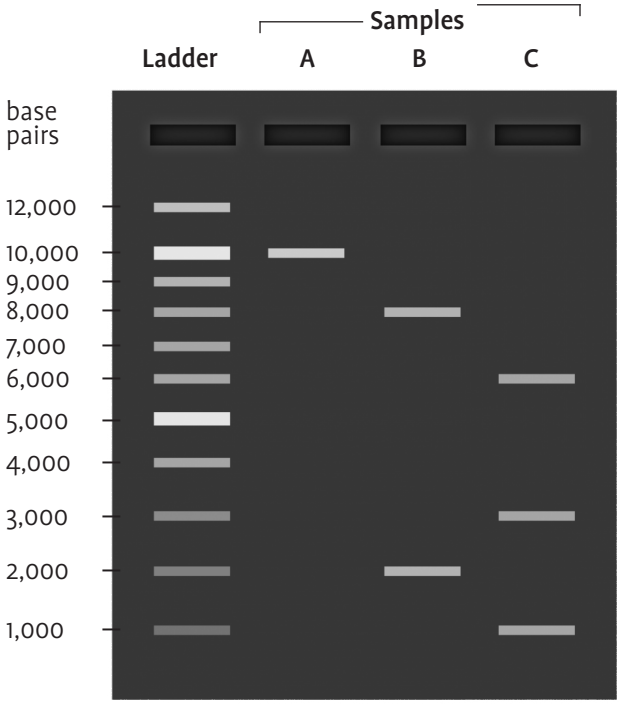
Combined unit ready to run gel.

Location of your samples in the gel

Sample	Gel lane
U (Uncut)	
H (HindIII)	
E (EcoR I)	
D (Double)	
L (Ladder)	

Understanding DNA markers

A DNA marker or ladder is a standard of known lengths of DNA, it allows you to determine the lengths of DNA from unknown samples.



Estimate the fragment lengths in the samples above:

A:

B:

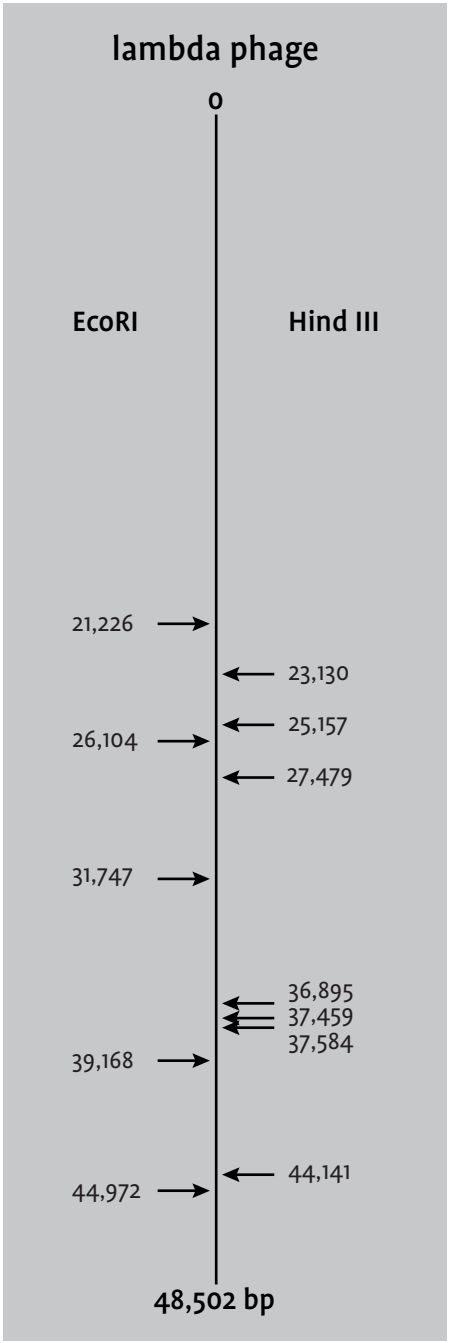
C:

Where should the bands be?

Examine the restriction map below to complete the table (at right) before plotting where the bands of DNA should be on your gel.

Restriction map of λ bacteriophage linear double-stranded DNA

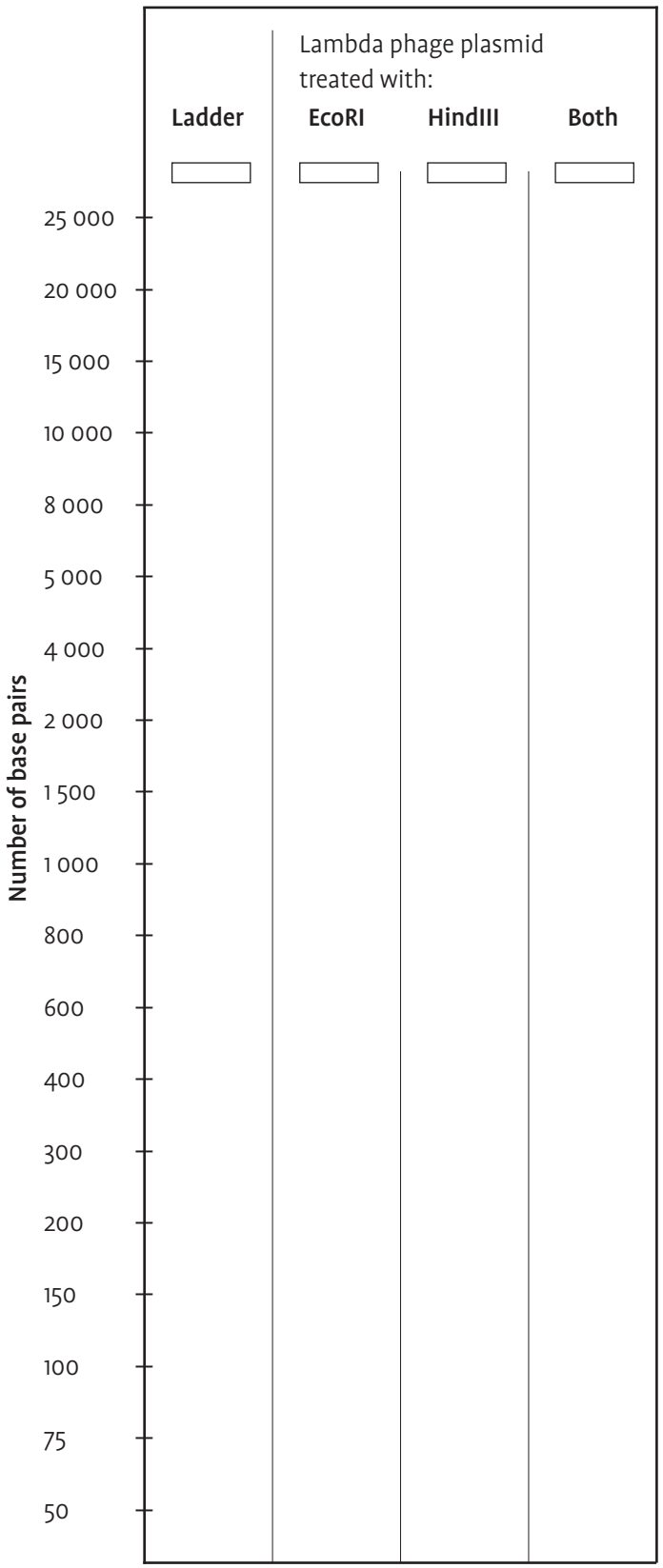
This map shows where the two enzymes, HindIII and EcoRI will cut the plasmid in its linear form.



Complete the table of fragments and sizes

Enzyme(s)	Number of DNA fragments	Sizes of the DNA fragments (base pairs)
Ladder	14	200, 400, 600, 800, 1,000, 1,500, 2,000, 2,500, 3,000, 4,000, 5,000, 6,000, 8,000, 10,000
EcoRI		
HindIII		
HindIII & EcoRI		

Draw in where you expect to find the DNA bands



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