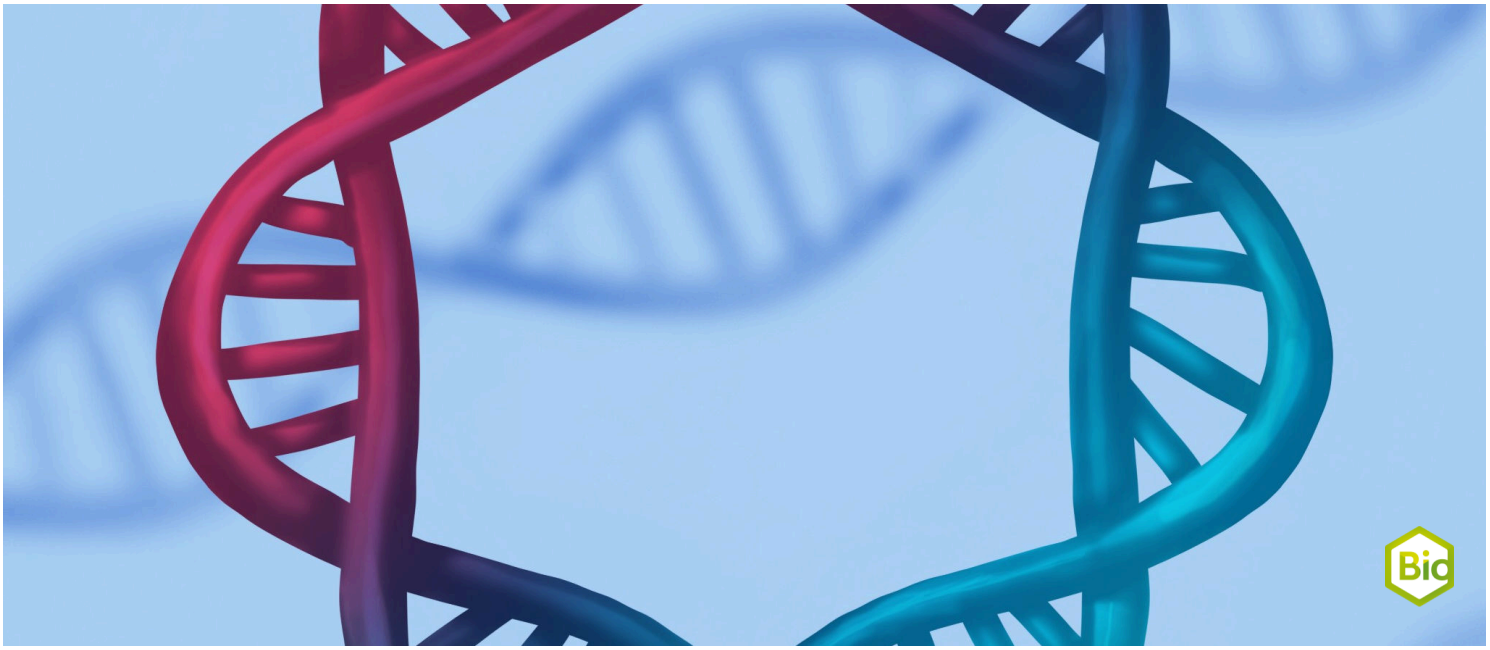
Bacterial plasmid DNA in gel electrophoresis with the blueGel™ electrophoresis chamber



In this experiment, plasmid DNA is cut using the restriction enzymes. The resulting DNA fragments are then separated using 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	20 minutes	40 minutes

This content can also be found online at:

https://www.curriculab.de/c/686bbc7dd7122b000255f9ff

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Teacher information

Application

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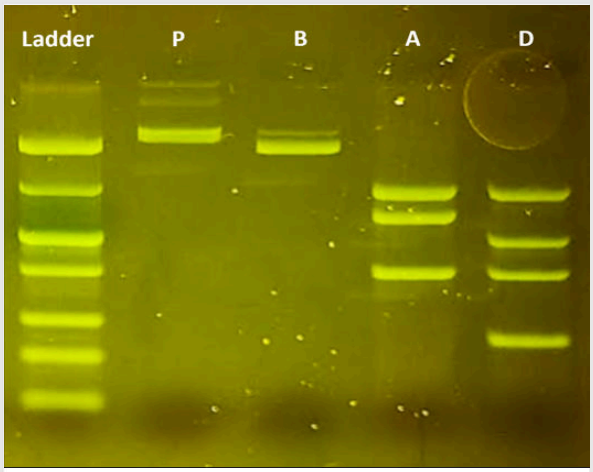

Gel electrophoresis of plasmid DNA with the blueGel™ system

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

The electrophoretic separation of DNA fragments of the pXYZ plasmid is being investigated.

The influence of an electric field separates DNA pieces of different sizes after the plasmid has been cut with restriction enzymes.

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Other teacher information (1/4)

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Prior knowledge

Students should already be familiar with the composition and properties of DNA. They should also be familiar with the function of restriction enzymes and the behaviour of charged molecules in an electric field.

Principle

The undigested and predigested DNA plasmid is separated on an agarose gel and according to the size of the DNA fragments. 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 how gel electrophoresis works. They will also learn how restriction enzymes can be used to analyse a DNA molecule and how plasmids are structured.

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 how to setup gel electrophoresis and run the gel.
- 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.
- Plasmid DNA is naturally present in a circular, superhelical form. If it is cut with one or more restriction enzymes, linear DNA fragments with so-called "sticky ends" (overhanging ends) are produced, if the enzymes generate such ends. These sticky ends can lead to artefacts 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 holders in the freezer) prevents these structures from re-forming.
- Also study the operating instructions for the kit: Click here

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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 SeeGreen™ tablets is a safe alternative to conventional ethidium bromide. It cannot penetrate the skin, but can enter the tissue through open wounds. The use of nitrile gloves is therefore recommended.
- Agarose gels made from GelGeen™ tablets can be disposed of in regular household waste.

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Student information

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Motivation

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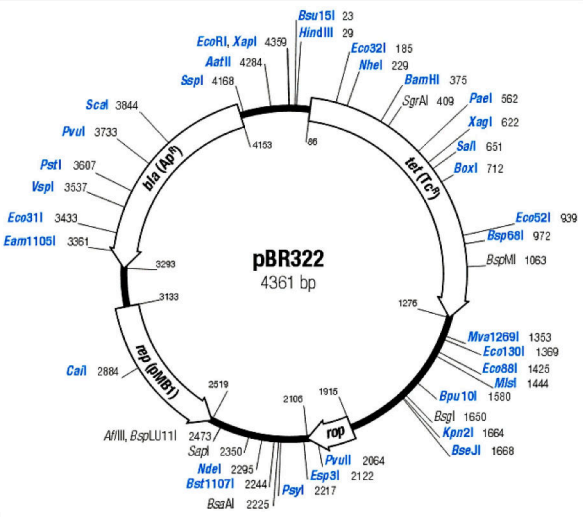

Plasmid map of pBR322

Knowledge about plasmids is important for understanding the evolution and adaptation of microorganisms in their environments, combating antibiotic resistance and enabling modern biotechnological applications. Today's medicine and biotechnology would hardly be conceivable without plasmids. Alongside the lambda phage, the plasmid pBR322 is the most studied and most frequently used DNA molecule.

The most commercially successful application of plasmids to date is the industrial production of insulin. The gene for insulin is incorporated into the plasmid. The plasmid serves as a vector and is introduced into bacterial cells. The bacteria take up the plasmid, multiply and produce large quantities of human insulin with the introduced gene.

Theory (1/6)

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Plasmids - powerful tools in genetic engineering

What are plasmids?

Plasmids are small, ring-shaped DNA molecules in bacteria that are replicated independently of the chromosome. They are often not vital, but can give bacteria advantages.

Plasmids in the laboratory

In the laboratory, we use plasmids to store and replicate important genetic information. Using common genetic engineering techniques, we can insert useful DNA directly into plasmids. These modified plasmids can then be used in bacteria or other cells to multiply.

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Theory (2/6)

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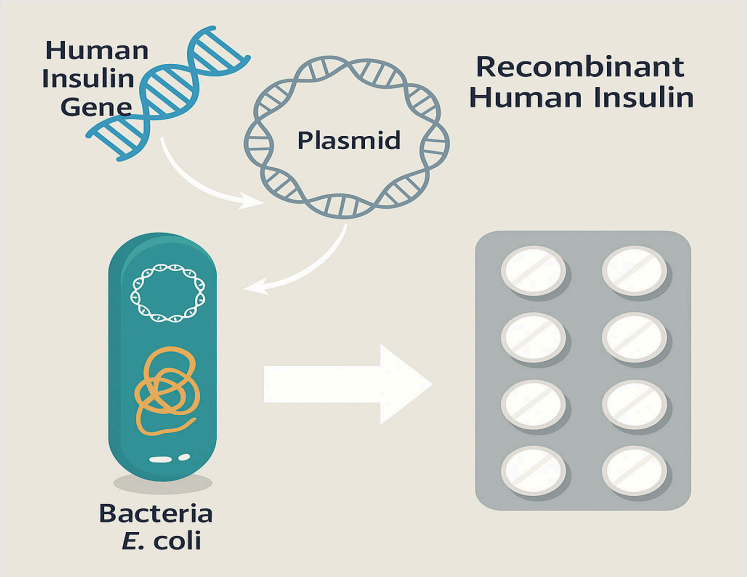

These modified plasmids can then be introduced into bacteria or other cells, where they multiply independently. The cells then utilise the genetic information contained in the plasmids. This enables them to produce certain desired substances. A well-known example is the production of **human insulin** which is used for the treatment of **diabetes**.

Thanks to this biotechnological process, human insulin can now be produced in large quantities and high quality.

Theory (3/6)

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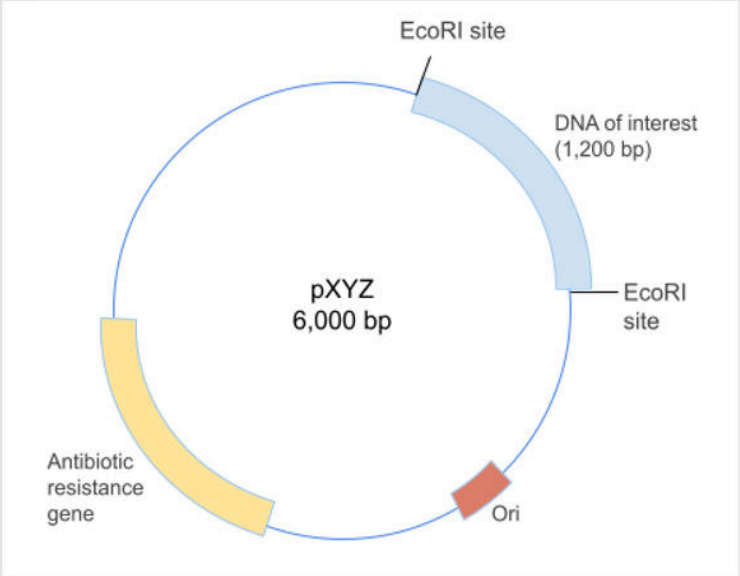

Plasmid maps provide an overview of the structure of a plasmid. They contain

- the total length in base pairs (bp)
- cloning sites for the insertion of DNA
- the origin of replication (Ori)
- marker genes such as antibiotic resistance for selection

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Theory (4/6)

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Restriction enzymes are molecular scissors that cut DNA at very specific points. They recognise a specific base sequence, which is usually 4 to 8 base pairs long. Each of these enzymes has a specific recognition sequence.

Here are some common restriction enzymes and their recognition sites:

- The restriction enzyme EcoRI cuts at the recognition site GAATTC
- BamHI cuts within GGATCC
- PvuII cuts in the centre of CAGCTG

Theory (5/6)

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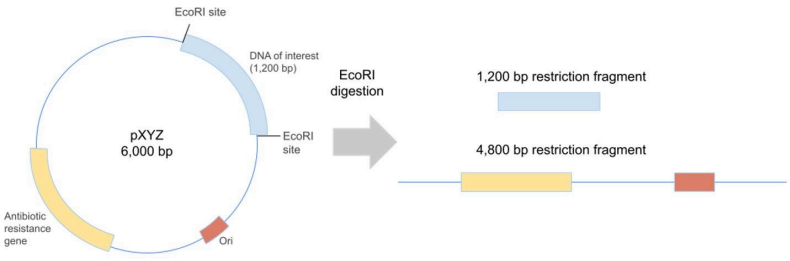
Genetic engineers use restriction enzymes such as EcoRI to cut plasmid DNA and thereby verify its identity. With plasmid **pXYZ** which has two EcoRI recognition sites, two fragments are produced after cutting: a **1,200 bp fragment** (the DNA of interest) and a **4,800 bp fragment** (the rest of the plasmid). These fragments can be visualised by gel electrophoresis and measured to confirm that it is the correct plasmid.



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Theory (6/6)

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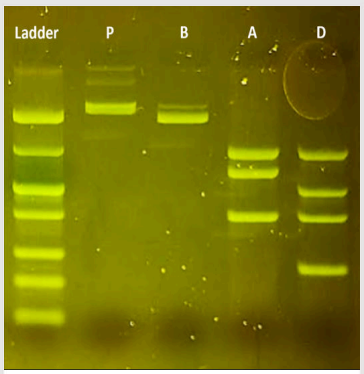

DNA bands in the agarose gel

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 plasmid 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).

Tasks

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1. Pour a 2% 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 electrophoresis.
3. Compare the uncut plasmid with the cut plasmid.

Samples from the kit: DNA ladder, undigested plasmid DNA, three different plasmid 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	Bacterial plasmid DNA in gel electrophoresis	15312-01	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	Erlenmeyer flask, boro, wide neck, 100 ml	46151-00	1
7	Graduated cylinder, boro, tall form, 100 ml	36629-00	1
8	Water, distilled, 5 litres	31246-81	1
9	Gloves, rubber, size M, pair	39323-00	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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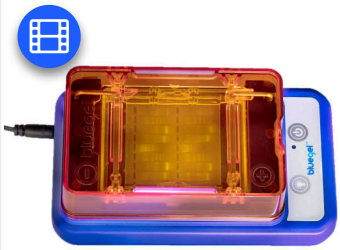

Video: Pouring a gel

- Dilute the 10x concentrated TBE running buffer concentrate to 1x with deionised water (approx. 20 ml buffer is required for one gel).
- Pour a 2% agarose gel (see also video 1).

1. Remove one GelGreen™ Agarose Tab from the pack and add it to 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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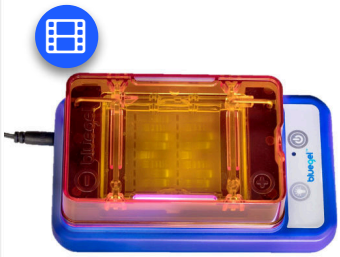

Video: Load and run the gel

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:

- Plasmid DNA (tube P)
- BamHI digestion (tube B)
- ApaLI digestion (tube A)
- Double digestion (tube D)
- 3K DNA ladder (tube L)

Procedure

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Gel chamber with dark hood and smartphone

3. **Gel run**

- Place the cover on the base unit and start the run by pressing the "On/Off" button.
- Unfold the dark hood 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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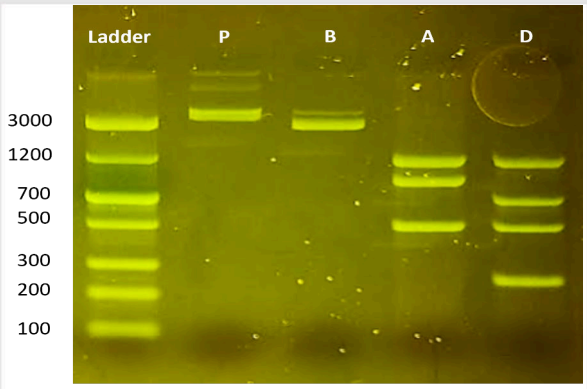
Compare your results with the photo shown and complete the table below with the resulting restriction fragments and their sizes:

Restriction enzyme(s)	# DNA fragments	Restriction fragment sizes (bp)
None	1 (circular)	6,000
EcoRI	2	4,800 and 1,200
BamHI		
XmnI		

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



Separation of the plasmid DNA

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Task 2

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What happens during gel electrophoresis?

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

Check

Task 3

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Which properties of plasmids make them useful DNA carriers in the laboratory? Select all the answers that apply.

- ☐ B. They are easy to manipulate with restriction enzymes
- ☐ D. They are circular
- ☐ C. They contain selectable markers
- ☐ A. They can multiply within bacterial cells

Check

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Task 4

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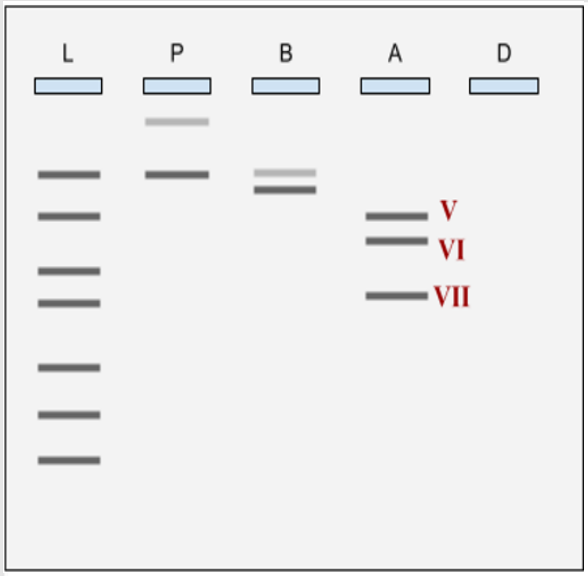
Look at the middle fragment on lane A and compare it with the pattern of the 3K DNA ladder (lane L). What size is this middle fragment likely to be?

- B. 950 bp
- C. 500 bp
- A. 1,250 bp

Check



Slide	Score / Total
Slide 24: Principle of gel electrophoresis	0/1
Slide 25: Properties of plasmids	0/4
Slide 26: Determination of the fragment size	0/1

Total amount 0 / 6

Solutions Repeat

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