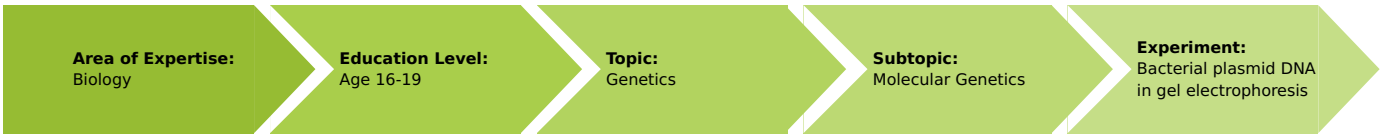
Bacterial plasmid DNA in gel electrophoresis

(Item No.: P8110100)

Curricular Relevance



Difficulty	Preparation Time	Execution Time	Recommended Group Size
Difficult	1 Hour	5 Hours	2 Students

Additional Requirements:

- refrigerator
- precision balance (820 g/ 0.01 g)

Experiment Variations:

Keywords:

biotechnology, chromosome, plasmid, genome, bacterial cell, enzyme, restriction enzyme, agarose, bacteria, electrophoresis, loading buffer, restriction digestion, recombinant DNA, gel, genetics, molecular genetics, molecular biology

Teacher information

Introduction

Important: Please store the consumables with the DNA and buffers immediately upon receiving them in a refrigerator at about 4°C.

These instructions describe the separation of plasmid DNA in agarose gel electrophoresis. During the experiment, uncut (circular) plasmid DNA can be compared to plasmid DNA that has already been cut by way of enzymes (linearised DNA). Naturally, the enclosed uncut DNA can also be used for own restrictions. The required restriction enzymes are not included in the kit. They are available from specialist shops.
The basis of the DNA kit is the plasmid pBR322 which is one of the first plasmids dating back to the 1970s. In the name, p stands for plasmid while B and R stand for Bolivar and Rodriguez, the names of the researchers who constructed it. 322 is a consecutive number of similar, though not identical, plasmids. Other well-known plasmids of this series are pBR325 or pBR328, for example. Details concerning the plasmids can be found in specialist literature or Internet databases.

Practical tips for the lab course:
DNA experiments with this kit system can be easily integrated into the lessons/lab courses since the experiment can be interrupted in between. You can interrupt it at various times, store the samples in a refrigerator and continue when it suits you. Interruptions are possible, for example, after you have completed the steps that are described in sections IV and V. However, the staining step should be performed directly after the actual gel electrophoresis (section VII) since, otherwise, the DNA bands will diffuse in the gel, causing them to become blurry.

Characteristics of plasmids and applications

A plasmid is a circular (covalently closed), always double-stranded DNA molecule which consists of 1,000 to 1,000,000 base pairs and which is located extrachromosomally in the bacterial cell. Therefore is defined as not belonging to the actual genome of the cell. The plasmid contains DNA sequences by which it can utilize the transcription complexes of the host cell. Plasmids occur in bacteria and archaea, in eukaryotes they are rare (in yeast). A cell can house several plasmids. Each plasmid can include several genes and replicated autonomously. The effects of the plasmid on the host cell can be either positive or negative.

Advantages are, e.g.:

- resistance against antibiotics
- toxic effect on competing bacteria or fungi
- exploiting food sources which are otherwise not available to the host cell

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The host cells have evolutionary advantages compared to other bacteria and, thus, can propagate better. Bacteria and plasmids live in symbiosis.
Medical relevance: salmonella which contain the plasmid *Salmonella virulence plasmid*, lead to more frequent and faster deaths to infected animals than salmonella without the plasmid.

Using biotechnological techniques, DNA molecules in plasmids introduced into host cells produce compounds which are useful to humans. In the meantime more than 140 differenct pharmaceuticals can be produced using this technique, e.g. insulin (against diabetes), hormones (to treat disturbances of growth) or enzymes (e.g. against mucoviscidosis).

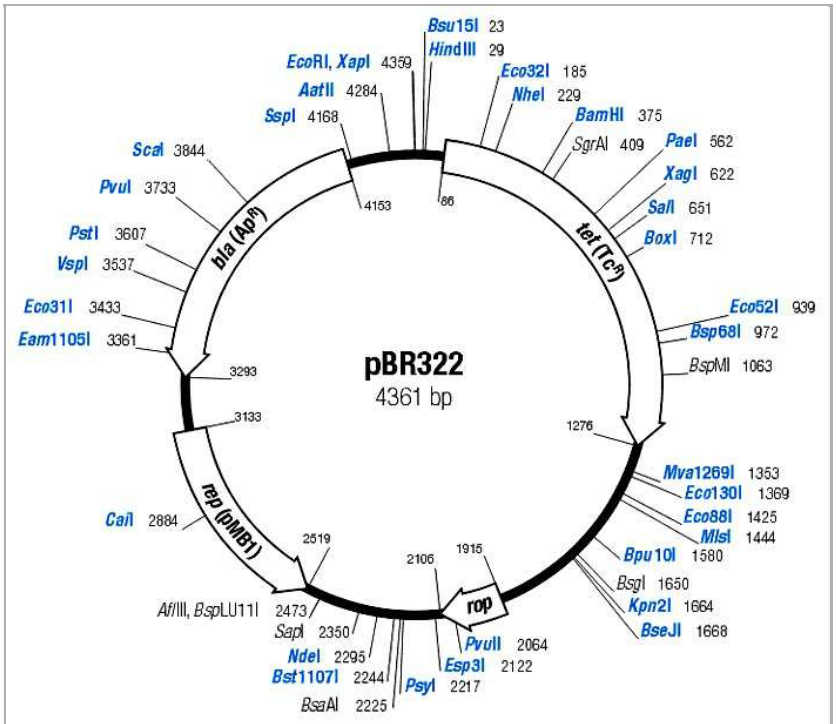

Fig. 1: Schematic representation of the pBR322 plasmid. The origin of replication (rep) and two resistance genes (tet and bla) are shown. In addition, the restriction sites for all rare cutters are also indicated, including EcoR1, however, not of the frequent cutters HinfI and HaeIII.

In order to acquire a plasmid with specific properties the desired gene (DNA sequence) must be cut out of another organism and inserted into a plasmid. This is achieved using restriction enzymes, which are proteins specialized in cutting DNA. Restriction enzymes of different can be found in different living sources. For a specific process the appropriate enzymes, called restriction enzymes, are selected so that the DNA can be cut at the exact desired location. Some enzymes cut both DNA strands at one position creating "blunt ends", other restriction enzymes create "sticky ends" because they cut at slightly different positions for each of both DNA strands.

In this experiments 4 DNA samples of plasmid pBR322 are tested. pBR322 is one of the first artificially constructed plasmids.

One of the samples provided is uncut plasmid both in ccc form (covalently closed circle) and in oc form (open circle). In the other three samples the plasmids were digested (cut) with three different restriction enzymes

- EcoR1 (found in Escherichia coli, creating sticky ends at 5'-G AATTC-3'),
- HinfIII (found in Haemophilus influenzae, creating sticky ends at 5'-G ANTC-3'), and
- HaeIII (found in Haemophilus eqyptius, creating blunt ends at 5'-GG CC-3')

This results in different numbers of fragments of different lengths. These fragments can be visualized using agarose gel electrophoresis for separation and staining to make the fragments visible.

What you can learn about

- Gel electrophoresis
- Bacterial plasmid
- Recombinant DNA
- Restriction enzymes
- Banding pattern
- DNA fragment

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Teacher's/Lecturer's Sheet

Task

In the experiment the DNA of the bacterial plasmid pBR322 in undigested and digested form is investigated using agarose gel electrophoresis.

What students need to know in advance

Students should know and understand chemical safety signs and safety handling procedures. Terms like cell, DNA, plasmid, chromosome, enzymes and biotechnology should be known to them.

Principle

In the experiment 4 different DNA samples from the same organism are investigated: one sample is untreated and the other three treated with different restriction enzymes originating from different bacteria. Therefore all samples contain DNA fragments of different sizes and different numbers. In gel electrophoresis these fragments are sorted because in an agarose gel they migrate at different speeds depending on their size and form. This creates specific banding patterns of the same plasmid digested by different restriction enzymes.

Tips for storage, preparation, setup and experiment procedure

The experiment must not be performed within one session/lab course. It can be stopped at certain experiment process points and the treated samples can be stored in the refrigerator at 4°C for some days.

Storage: Due to the fact that the DNA samples have been freeze-dried, they are extremely stable and can be stored at room temperature for a few days. The same applies to the other components of the kit. However, we recommend storing them in the refrigerator. Only the agarose and TAE buffer should be stored at room temperature.

Dissolving the lyophilized DNA can be performed overnight in a refrigerator so that the dissolved DNA is available next day.

The purpose of the gel loading buffer is to weigh down the DNA molecules so that they can sink more readily in the gel pockets. The dye in the gel loading buffer facilitates pipetting into the gel pockets and helps trace the DNA during the migration in the gel.

Lyophilized DNA is transparent and barely visible in the sample tubes.

Dyeing after gel electrophoresis should be performed immediately after separation in the gel to avoid diffusion of the DNA bands in the gel, becoming fuzzy.

A migration distance of 5 to 8 cm is sufficient to separate the DNA fragments. The dye bromophenol blue in the gel loading buffer serves as a tracer for the separation. Electrophoresis can be terminated when the dye has read two thirds to three fourths of the gel length.

Material

Position No.	Material	Order No.	Quantity
1	Kit: Bacterial plasmid DNA in gel electrophoresis	KLA-530-100	1
2	DNA electrophoresis chamber, horizontal	KLA-530-200	1
3	Electrophoresis power supply 100V/200V	65966-93	1
4	Hotplate Magnetic Stirrer with connection for electroniccontact-thermometer, 3 ltr., 230 V	35760-93	1
5	Microliterpipette 2-20 µl	47141-10	1
6	Microliterpipette 20-200 µl	47141-11	1
7	Staining dish, UV permeable, PETG	35023-20	1
8	Grad.cylinder,high,PP,500ml	46288-01	1
9	Water, distilled 5 l	31246-81	1
10	Pipette tips, 2-200 µl, racked	47148-11	1
11	Protecting glasses, clear glass	39316-00	1
12	Erlenmeyer flask,narrow n.,500 ml	36121-00	1
13	Spoon, nickel-plated, 180 mm	33392-00	1
14	Cotton wool, white 200 g	31944-10	1
15	Rubber gloves, size S (7)	39325-00	1
16	Spatula, steel, l=185mm	46952-00	1
17	Magnetic stirring bar 50 mm, cylindrical	46299-03	1

Safety and disposal

Wear gloves and safety goggles!

The safe handling of laboratory equipment and chemicals requires a certain level of fundamental knowledge and safety measures. As a general rule, you should wear a laboratory coat and safety goggles during the experiment. Gloves should also be provided and worn as required. When preparing the agarose gel, wear insulated gloves in order to avoid burning or scaling your hands. The handling of the equipment and the risks that are involved should be known. Particular attention must be paid to the electricity hazards. Ensure that all of the connectors, mains power cables and work surfaces (and your hands) are dry prior to operating the electrical equipment.
Further health and safety measures: Tie your hair back, do not wear any jewellery and wear clothes with tight-fitting sleeves in order to avoid any unwanted contact with the equipment, chemicals, etc.
Waste must be disposed of in accordance with the instructions and with the local rules and regulations.

Potential hazards of the components of the kit
DNA samples
DNA samples include 10% glycerine as well as the dye bromophenol blue with a concentration of 0.25%.
In accordance with the directive (EC) 1272/2008, the substance or mixture has not been rated as hazardous.
In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.

Electrophoresis buffer, 50x concentration
The following information refers to the concentrated electrophoresis buffer. This means that it does not necessarily apply to the diluted buffer (working solution).
Rating in accordance with the directive (EC) 1272/2008:

Hazard information
H315: Causes skin irritation
H319: Causes serious eye irritation
H335: May cause respiratory irritation

Safety information
P280: Wear protective clothing and eye protection.
P261: Avoid breathing dust/fume/gas/mist/vapours/spray.
P302+P352: If on skin: Wash with plenty of water and soap.
P305+P351+P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

Agarose
In accordance with the directive (EC) 1272/2008, the substance has been rated as non-hazardous.
In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.
Recommendation: Wear gloves and protective goggles. Avoid contact with the skin and eyes. Avoid the formation of dust. Do not breathe the agarose.

DNA staining solution (200x concentration)
In accordance with the directive (EC) 1272/2008, the aqueous solution has not been rated as hazardous.
In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.
Recommendation: Wear gloves and protective goggles. Avoid contact with the skin and eyes.

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Results

Gel with banding patterns:

Fig. 2: Electrophoretic separation of pBR322 DNA (stained with methylene blue): Lane 1: pBR322, undigested (ccc form); lane 3: pBR322 EcoRI; lane 5: pBR322 Hinf I; lane 7: pBR322 Hae III; lane 9: length marker, applied in addition (not included in the kit as standard). All values in base pairs (bp).

Note: The undigested plasmid pBR322 (ccc form: covalently closed circle) migrates more quickly through the gel than the linearised plasmid which was digested by EcoR1 at one position. The "balled" conformation of this plasmid encounters less resistance in this separation matrix which is why it displays slightly higher mobility in the gel.

Table 1: Fragment lengths for specific restriction digests (in base pairs (bp)):

pBR322-DNA EcoRI	pBR322-DNA HinfI	pBR322-DNA HaeIII
4.361	1.632	587
	517	540
	504	502
	396	458
	344	434
	298	267
	221	234
	220	213
	154	192
	75 (1)	184
		124
		123
		104
		89
		80,64,57
		51,21,18,
		11, 8 (2)

(1): Only visible with optimum loading and sensitive staining. (2): Individual, low-molecular fragments no longer separate in most cases.

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Bacterial plasmid DNA in gel electrophoresis **(Item No.: P8110100)**

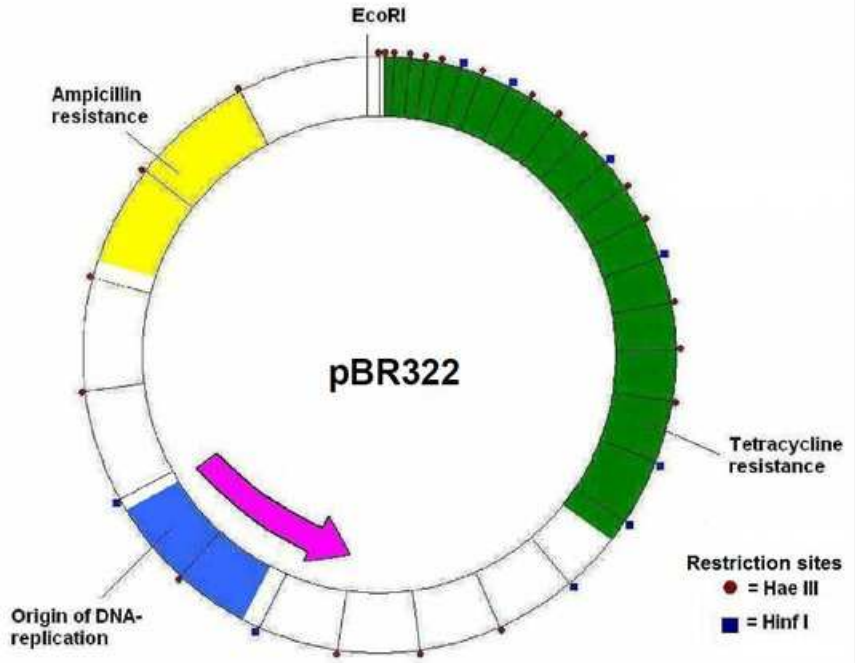
Introduction

Principle and applications

The purpose of this experiment to make students familiar with the most common of techniques used in molecular biology, agarose gel electrophoresis. This method allows you to visualize DNA and particularly relatively small fragments of DNA and to separate the DNA fragments from one another.

This method is used in many practical applications, from determining if a certain fragment of DNA, e.g. a part of a gene is available, to determining family relationships (e.g. in paternity tests), to determining if a crime suspect is the culprit, to cleaning a DNA fragment from other DNA, just to give a few examples.

For gel electrophoresis, a DNA sample very familiar to researchers in molecular biology is used, a bacterial plasmid. A plasmid is a DNA double strand, which is a closed circle, is not part of the bacterial genome, and can be manipulated by inserting DNA fragments from other genomes, like genes that provide resistance against antibiotics, as is the case in our plasmid, called pBR322. In includes two resistance genes. In addition, to allow for manipulating the plasmid's DNA, it includes restriction sites for restriction enzymes which can cut (digest) the plasmid into fragments:



Schematic representation of the pBR322 plasmid. The origin of replication (rep) and two resistance genes (tet and bla) are shown.

These fragments are separated according to their size (length) in gel during electrophoresis.

The reason why bacterial plasmids are used in molecular biology is because their hosts, bacteria, can be multiplied easily and quickly, making them perfect work horses.

Material

Student's Sheet

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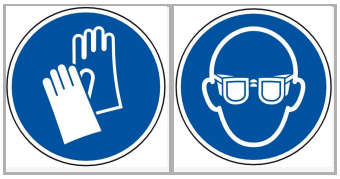
Position No.	Material	Order No.	Quantity
1	DNA-Elektrophoresekammer, horizontal	KLA-530-200	1
2	Electrophoresis power supply 100V/200V	65966-93	1
3	Bakterielle Plasmid-DNA in der Gel-Elektrophorese	KLA-530-100	1
5	Hotplate Magnetic Stirrer with connection for electroniccontact-thermometer, 3 ltr., 230 V	35760-93	1
6	Microliterpipette 2-20 µl	47141-10	1
7	Microliterpipette 20-200 µl	47141-11	1
8	Staining dish, UV permeable, PETG	35023-20	1
9	Grad.cylinder,high,PP,500ml	46288-01	1
10	Water, distilled 5 l	31246-81	1
11	Pipette tips, 2-200 µl, racked	47148-11	1
12	Protecting glasses, clear glass	39316-00	1
13	Erlenmeyer flask,narrow n.,500 ml	36121-00	1
14	Spoon, nickel-plated, 180 mm	33392-00	1
15	Cotton wool, white 200 g	31944-10	1
16	Rubber gloves, size S (7)	39325-00	1
17	Spatula, steel, l=185mm	46952-00	1
18	Magnetic stirring bar 50 mm, cylindrical	46299-03	1

Safety information



Wear gloves and safety goggles!

Please follow the directives of your instructor in respect to electrical instruments (power supply and electrophoresis instrument) as well as how to handle chemicals. Please also follow the regulations concerning disposal of chemicals.

The safe handling of laboratory equipment and chemicals requires a certain level of fundamental knowledge and safety measures. As a general rule, you should wear a laboratory coat and safety goggles during the experiment. Gloves should also be provided and worn as required. When preparing the agarose gel, wear insulated gloves in order to avoid burning or scaling your hands. The handling of the equipment and the risks that are involved should be known. Particular attention must be paid to the electricity hazards. Ensure that all of the connectors, mains power cables and work surfaces (and your hands) are dry prior to operating the electrical equipment.
Further health and safety measures: Tie your hair back, do not wear any jewellery and wear clothes with tight-fitting sleeves in order to avoid any unwanted contact with the equipment, chemicals, etc.
Waste must be disposed of in accordance with the instructions and with the local rules and regulations.

Set-up and procedure

Set-up

Content of the consumables kit:

- pBR322 DNA, uncut (ccc and oc form visible in the gel): 10 µg lyophilised
- pBR322 DNA, EcoRI, cut: 10 µg lyophilised
- pBR322 DNA, HinfI, cut: 10 µg lyophilised
- pBR322 DNA, HaeIII, cut: 10 µg lyophilised
- Gel loading buffer for dissolving the freeze-dried plasmid DNA, contains bromophenol blue as a colour marker: 0.5 ml (ready for use)
- Agarose: 6 g
- TAE buffer (50x concentration): 50 ml
- DNA staining solution (200x concentration): 1.5 ml

1) The gel loading buffer is used to "load" the DNA molecules so that they can sink into the gel pockets. The dye in the gel loading buffer facilitates the transfer of the buffer into the gel pockets by way of a pipette and it is also used to monitor the progress of the electrophoresis.
2) Freeze-dried DNA is transparent and, therefore, hardly visible in the vessels (transparent film).

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Procedure

Dissolving the DNA in the gel loading buffer

Dissolve the DNA in the gel loading buffer in a sterile manner, if possible. The final concentration of the dissolved DNA depends on the sensitivity of the DNA staining.
For staining with methylene blue, as is intended for this kit, the DNA must be dissolved as follows:

pBR322 DNA, uncut	+ addition of 110 µl of the gel loading buffer
pBR322 DNA, EcoRI, cut	+ addition of 130 µl of the gel loading buffer
pBR322 DNA, HinfI, cut	+ addition of 110 µl of the gel loading buffer
pBR322 DNA, HaeIII, cut	+ addition of 110 µl of the gel loading buffer

The dissolving process takes approximately 15 minutes. Shake the vessels vigorously in between on a laboratory shaker or by hand. At the end of the dissolving process, spin them briefly or bounce the vessels briefly on the desktop so that the entire liquid collects at the bottom of the vessel.
Tip: The dissolution of the DNA can also take place overnight in a refrigerator so that the dissolved DNA will be available the next day.

Casting the agarose gel

1.4% agarose gel is most suitable for the preparation of the DNA samples (which are actually PCR products). It can be prepared with the supplied TAE electrophoresis buffer (50x concentration). The buffer must be diluted with distilled water at a ratio of 1:50 beforehand.

1. Weigh the required quantity of agarose for a 1.4% gel and fill it into an Erlenmeyer flask.
2. Measure the corresponding volume of the 1x concentrated electrophoresis buffer in a graduated cylinder and add it to the agarose.
3. Heat the agarose in a microwave oven or in boiling water until it has completely liquefied. Remove the Erlenmeyer flask repeatedly in between and shake it in order to ensure the homogeneous fusion of the agarose. **Caution:** Wear kitchen gloves when holding the hot Erlenmeyer flask. In addition, superheating (boiling retardation) may occur so that the agarose may splash out of the flask (protective goggles!).
4. Let the liquid agarose cool down to approximately 80°C. Then, cast the gel.
5. Place a comb in the cast and let the agarose gel solidify (approximately 20 minutes).
6. Overlay the gel with the electrophoresis buffer and carefully remove the comb.
7. The teeth of the comb have created gel pockets in the solid agarose. Later, the samples will be filled into these pockets by way of a pipette.

Tip:
If you want to wait until the next day for performing the actual electrophoresis, leave the comb in the gel, overlay the gel with the electrophoresis buffer and cover everything with cling film so that the gel will not dry up.

Note:
The electrophoresis buffer can be used several times. Store it at room temperature.

Applying the samples for gel electrophoresis

Fill 10 µl of the various plasmid samples as well as of the DNA length marker into the pockets of the agarose gel. Insert the tip of the micropipette into the pocket, but ensure to not pierce the bottom of the pocket. Then, let the sample "flow" carefully into the pocket. The samples that are dissolved in the gel loading buffer are heavier than the electrophoresis buffer so that they sink into the pockets easily.

Attention:
Please note that the above-mentioned pipetting recommendations refer to standard mini- or midi-size gel formats with a pocket width of approximately 3.5 mm and a migration length of approximately 50 mm.
The volumes that are stated in section IV apply to staining with methylene blue. If you want to perform more sensitive DNA staining, the volumes can be reduced accordingly.
We recommend using a test gel prior to the actual experiment in order to see whether your volumes are suitable for your gel format. If not, adapt the volumes accordingly.
The samples that have been prepared for the electrophoresis can be stored in the refrigerator for several days if you want to perform the actual electrophoresis at a later time.

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Agarose gel electrophoresis

In general, a migration distance of 5 to 8 cm is sufficient for separating the corresponding DNA fragments. The dye bromophenol blue in the gel loading buffer "indicates" the progress of the separation. The electrophoresis will be stopped when the colour marker bromophenol blue has reached 2/3 to 3/4 of the gel length.
Connect the electrophoresis chamber to a suitable voltage supply unit. The electrophoretic separation of the DNA samples takes place at a voltage of approximately 3 to 5 Volts/cm (distance between electrodes) when using a TAE electrophoresis buffer.
Please refer to the information and notes that are provided in the operating instructions of your electrophoresis apparatus. Your apparatus may not be suitable for the above-mentioned voltages. In this case, a lower voltage must be used.

Staining the agarose gel

1. Prepare the DNA staining solution by diluting 1 ml of the staining concentrate with 200 ml of distilled water. The staining solution can be used several times. Stored in a closed container at 4°C in a refrigerator.
2. After the electrophoresis, transfer the agarose gel carefully with a kitchen spatula into a gel staining dish and pour the staining solution over the gel.
3. Stain the gel for approximately 10 to 15 minutes. Shake the dish from time to time during this process.
4. Then, fill the staining solution into a labelled bottle for storage and store it in the refrigerator. Next, destain the gel with tap water until the background is sufficiently light so that the stained DNA bands contrast well with the background.
5. The blue DNA bands can be documented by taking a photo of the gel with transmitted light (e.g. by way of a light box for slide presentations).

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