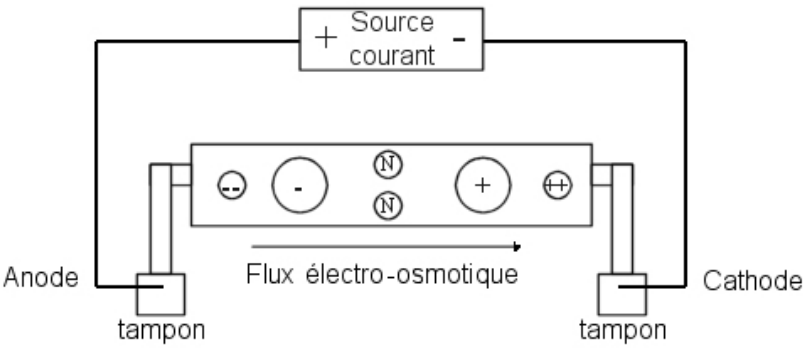
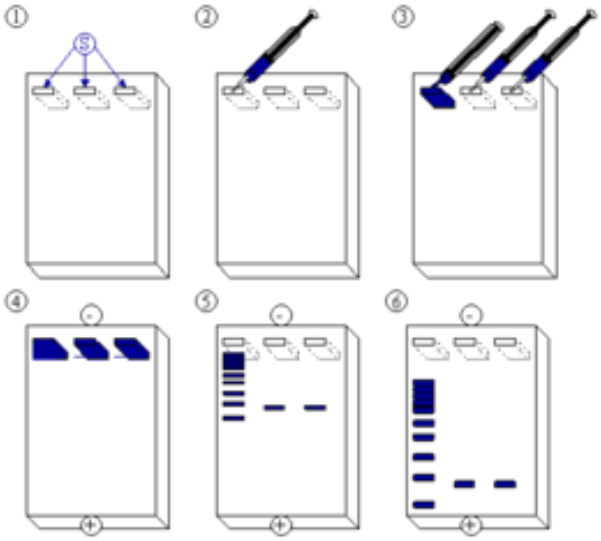
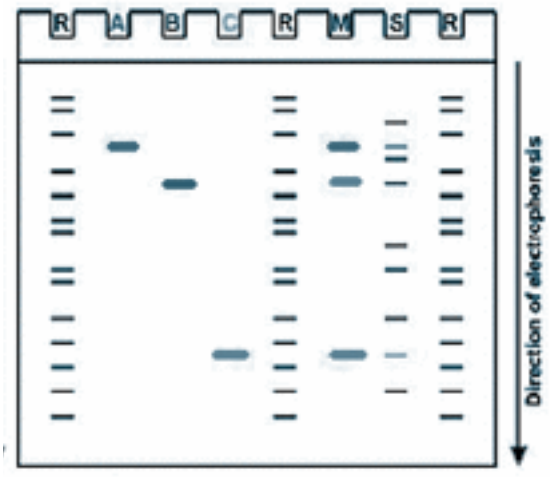
Agarose Gel Electrophoresis for DNA amplification product analysis







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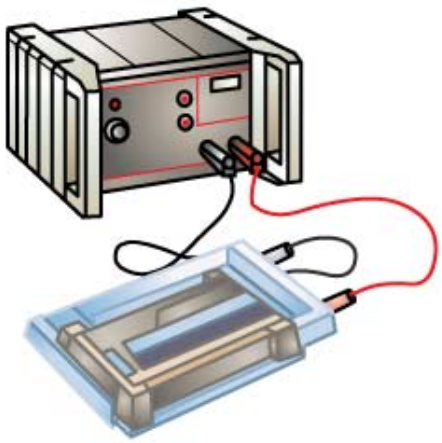
Principle

Gel electrophoresis is a method for separation and analysis of amplified DNA fragments, based on their size and charge. Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through an agarose matrix. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. After the electrophoresis is complete, the molecules in the gel can be stained to make them visible. DNA may be visualized using ethidium bromide which, when intercalated into DNA, fluoresces under ultraviolet light.

1. Material



- Horizontal Gel Electrophoresis chamber
- Gel form and comb
- Electrophoresis power supply
- Microwave oven
- Balance
- Graduated cylinder
- Beaker or Erlenmeyer flask
- 20 µl pipettes
- Disposable sterile pipette tips with filter
- Eppendorf 1.5 mL tubes
- Parafilm
- Plastic wrap or aluminum foil
- Agarose. Ref.: UltraPure™ Agarose 500g 16500500 Invitrogen
- Distilled water
- Tris Base. Ref.: T1503 SIGMA
- EDTA Ref.: T5134 SIGMA
- Glacial acetic acid A6283 SIGMA
- Masking tape, if needed to seal gel form
- Ethidium bromide (EtBr) solution. Ref.: GEPBET02-AF Eurobio (dropper flask, 5 x 5 ml - 0,7 mg/ml).
  Caution: ethidium bromide is mutagen and potentially carcinogenic; wear coat, gloves and safety glasses.
- Loading buffer*. Ref.: 6X DNA Loading Dye RO611 Fermentas
- Molecular Weight marker. Ref.: SmartLadder MW-1700-10 Eurogentec
- DNA loading buffer is used to facilitate the loading of DNA sample in the wells and to track the progression of gel electrophoresis. Usually a DNA loading buffer contains at least one dye (orange G, bromophenol blue, xylene cyanol FF or bromocresol green) and a high-density reagent (glycerol, sucrose or Ficoll 400). DNA loading buffer containing two dyes, mostly bromophenol blue and xylene cyanol FF, is very common for DNA gel electrophoresis. Bromophenol blue migrates very fast in the agarose gel and corresponds to the migration of 300 - 500 bp long DNA fragment in 1% agarose gel. Xylene cyanol FF migrates comparatively slow and corresponds to the migration of 4000 - 5000 bp long DNA fragment in 1% agarose gel. Due to the high density reagent added, the DNA sample settles at the bottom of the well.

2. Reagents

TAE (Tris-Acetate-EDTA)

Solution	Reagent	Quantity	Final concentration
TAE 50X	Tris base 2M	1210 g	2 M
	EDTA 0,5M	500 mL	50 mM
	Glacial acetic acid	285,5 mL	1,45 M
	H_2O to	5 L	
TAE 1X	TAE 50X	20 mL	Tris 40 mM EDTA 1 mM AA 30 mM
		980 mL	

Prepare an adequate volume of 1X TAE buffer (gel and electrophoresis chamber)

3. Making the Gel

- Select the percentage of gel to be run. Percentage of agarose depends on amplification products size.

Agarose (%)	Effective range of resolution of linear DNA fragments (kb)
0.5	30 to 1
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.4
1.5 2.0	3 to 0.2 2 to 0.1

Table 1. Appropriate agarose concentrations for separating DNA fragments of various sizes
(from Current Protocols in Molecular Biology)

- Choose a beaker or Erlenmeyer flask that is 2-4 times the volume of the agarose preparation
- Weigh out the appropriate amount of agarose

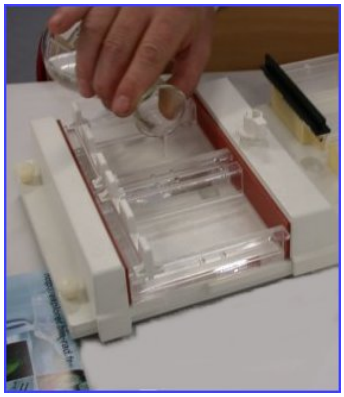
Test	Agarose (%)	Agarose (mg)	TAE 1X (mL)
AB resistance sequencing	1	500	50
GenoType (Hain) Spoligotyping	2	1000	50
MIRU	3	4500	150

- Add the 1X TAE buffer
- Cover the beaker with plastic wrap; pierce a small hole in the plastic for ventilation
- Heat 1 or 2 minutes in microwave to bring the mixture to a boil; watch the solution closely. **Caution: Agar foams up and boils very easily, wear safety glasses**
- Remove the container and gently swirl it to re-suspend any settled agar. **Caution: Protect your hand with a pot holder**
- Reheat the solution until it comes to a boil again
- Remove the container and gently swirl it to re-suspend any settled agar
- If the agar is not dissolved, continue to heat and swirl the solution until dissolved
- Add hot 1X TAE buffer to replace any buffer lost during the heating process; mix thoroughly
- Let it cool until you can comfortably touch the flask (50-60°C) before pouring into the gel plates.
- Ethidium bromide is added to the agarose preparation just before casting the gel to a final concentration of 0.5µg/ml. If using a dropper flask, add 1 drop for 50ml of gel. **Caution: Never boil agarose with EtBr**
- Place tape securely across the ends of the gel form (if needed) and place the comb in the form (choose the comb with teeth number and volume adapted to your samples)
- Pour cooled (50-60°C) agar into the form (avoid air bubbles); the bottom 1/3-1/2 of the comb teeth should be immersed
- Immediately rinse and fill the agar flask with hot water to dissolve any remaining agar
- When the agar has solidified, carefully remove the comb

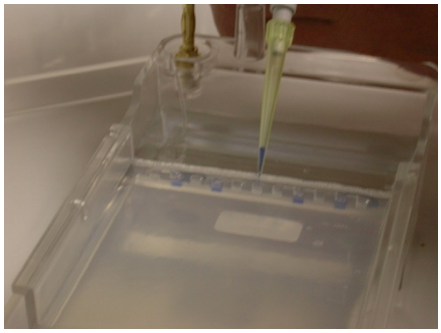
- Remove the tape (if used) from the ends of the gel form
- Place the gel in electrophoresis chamber with the wells closest to the negative

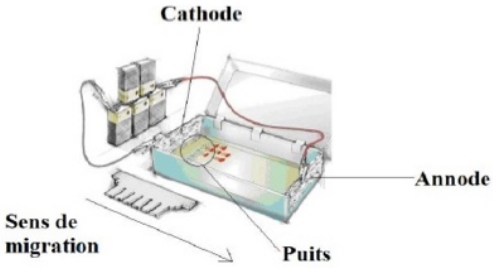
TIP: Gels can be prepared and poured several days before use. After cooling, wrap each gel in plastic wrap. Store gels at room temperature overnight or in the refrigerator for days. Keep away from light (because of EtBr).

4. Loading samples in the wells in the gel



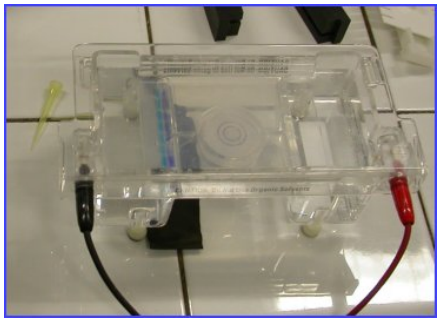
- Make a complete written gel map. If you run a gel with more than one comb, you have to plan at least one lane with molecular weight marker in each comb.
- If using multiple gels, clip the corner of one of them to be able to distinguish one gel from another.
- Place the gel in the chamber. A black or dark surface below the chamber will help you see the wells in the agar
- Fill each half of the chamber, adding solution until the tops of the wells are submerged. Make sure there are no air bubbles within the wells.
- Prepare DNA sample (10% of PCR product) with loading buffer (final concentration 1X) in Eppendorf 1.5 mL tubes .
- Using a clean micro-pipet tip for each sample, pipet the samples and molecular weight markers each into a separate well of the gel.
- Use both hands when loading the samples into the gel; hold the sample loading device in one hand and use your other hand and arm to support and stabilize the hand with the pipet doing the loading
- When loading samples into the gel, be careful to not puncture the bottoms of the wells and to not mix samples between wells.



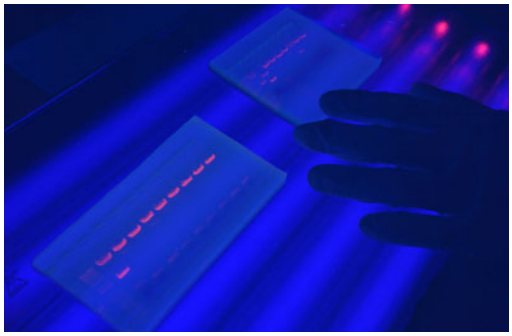


5. Migration

- Place the lid on the chamber and connect the electrode leads to the power supply: the black lead to the negative terminal and red to the positive terminal.
- Turn on the power supply and adjust the voltage to 50-100 Volts (5 to 10 volts/cm of gel).
- Let the gels run from negative to positive. The progress of the separation can be monitored by the migration of the dyes in the loading buffer
- When the dyes have migrated sufficiently, turn off the power supply, disconnect the electrode leads, and remove the chamber lid.

6. Examining  the Gels

- Place the gel on the UV trans-illuminator and look for orange, pink bands of DNA. **!!DO NOT LOOK DIRECTLY AT THE UV LIGHT WITHOUT PROTECTIVE GOGGLES!!**
- Record all of your observations on the gel map.
- Take a picture of the gel to use in the lab.
- Once you are done with the gel, discard it in the trash. If you need or want to save the gel after electrophoresis, it can be wrapped in plastic wrap or put in a plastic bag and placed in the freezer. This stops the diffusion of the color into the gel.
- Rinse the electrophoresis chamber and the gel form with tap water; turn them upside down to dry.



Preparing gels on Monday June 12 2017

Group 1, 2, 3, 4 and 5 :
Prepare each a 1% Agarose small gel (0,5 g in 50 mL TAE) for antibiotic resistance sequencing

Group 6, 7, 8, 9, and 10 :
Prepare each a 3% Nusieve Agarose big gel (4,5 g in 150 mL TAE) for MIRU