AGAROSE GEL ELECTROPHORESIS.
ADVANCED PRACTICE

Ref. ELECTADVANCED4 (4 practices)

1.EXPERIMENT OBJECTIVE

The objective of this experiment is to develop a basic understanding of electrophoretic theory, and to gain “hands-on” familiarity with the procedures involved in horizontal gel electrophoresis to separate different molecules.

In this case there will be in use DNA fragments, molecular weight markers where it will be possible to observe how the fragments of minor size migrate more rapidly through the agarose gel while genomic DNA, due to his high molecular weight, will migrate slowly.

We will also introduce the technologies of visualization of DNA fragments in the agarose gel. For it, there will be in use a method developed by BioTed that is not toxic unlike the traditional methods (ethidium bromide).

2. INTRODUCTION

Agarose gel electrophoresis is widely used to separate molecules based upon charge, size, and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA, and proteins.

Agarose gel electrophoresis is a procedure used in several areas of Biotechnology, is an analytical process used in biomedical and forensic research laboratories. Of the existing types of electrophoresis, the agarose gel electrophoresis is the most common and used method.

The device of horizontal electrophoresis is essentially a box with electrodes in every end, these are platinum electrodes since it has a very good electrical conductivity. Due to the fact that the platinum electrodes are expensive and fragile, it is necessary to take care when electrophoresis equipments are used.

The way of separation is agarose gel, agarose is a polysaccharide derivative of agar. Original of the alga, the agarose is highly purified to eliminate all the impurities. It is extracted from the same alga that is in use for obtaining the agar used in microbiology. It is a not toxic substance but it must not be ingested.

The gel is made by dissolving agarose powder in a boiling buffer solution. The solution is then cooled to approximately 55°C and poured into a gel tray where it solidifies. The tray is submerged in a buffer-filled electrophoresis apparatus which contains electrodes.

Samples are prepared for electrophoresis by mixing them with components that will give the mixture density, such as glycerol or sucrose. This makes the samples denser than the electrophoresis buffer. These samples can be loaded with a micropipette or transfer pipette into wells that were created in the gel by a template during casting. The dense samples sink through the buffer and remain in the wells.

A direct current power supply is connected to the electrophoresis apparatus and current is applied. Charged molecules in the sample enter the gel through the walls of the wells. Molecules having a

net negative charge migrate towards the positive electrode (anode) while net positivelycharged molecules migrate towards the negative electrode (cathode).

The agarose gel consists of microscopic pores that act as a molecular sieve which separates molecules based upon charge, size and shape. These characteristics, together with buffer conditions, gel concentrations and voltage, affect the mobility of molecules in gels. Smaller molecules move through the pores faster than larger ones.

Molecules can have the same molecular weight and charge but different shapes, as in the case of plasmid DNAs. Molecules having a more compact shape (a sphere is more compact than a rod) can move more easily through the pores. This means that the smaller the linear fragment is, the faster it migrates through the gel. Given two molecules of the same molecular weight and shape, the one with the greater amount of charge will migrate faster. In addition, different molecules can interact with agarose to varying degrees. Molecules that bind more strongly to agarose will migrate more slowly.

The mobility of molecules during electrophoresis is also influenced by gel concentration, and the volume of the agarose gel solution depends upon the size of the casting tray. Higher-percentage gels, as well as thicker gels, are sturdier and easier to handle. However, the mobility of molecules and staining (where applicable) will take longer because of the tighter matrix of the gel.

In order for the molecules of DNA to be visible in the agarose gel, a stain is needed since the DNA does not have color. The most common method to visualize DNA uses ethidium bromide due to its high sensitivity. Ethidium bromide is mutagenic and must be handled with a lot of care. Also, an ultraviolet light source (UV transilluminator) is needed for the visualization. Nowadays, different companies have developed different types of dyes with high sensitivity and a mutagenic power much lower than that of ethidium bromide.

In our case we will use a NONTOXIC method that will allow us to visualize the DNA fragments of blue color, they will be able to be observed during the electrophoresis but a later stain will be necessary to be able to observe all the bands.

3. COMPONENTS

Concentrated Electrophoresis Buffer 10 X (2 bottles 500ml)	2 x 50 ml
Agarose	1.75 gr
Fixed Volume Micropipette 20 µl	1
Micropipette Tips	1
Samples	4
Graduated Cylinders of 50 ml	1
DanaBlue 0.1 %	400 µl
DanaBlue 0.02 %	125 ml

Add 450 ml of distilled water to every bottle of Concentrated Electrophoresis Buffer 10 X to elaborate 2 x 500 ml of Electrophoresis Buffer 1 X , that is the Buffer for working.

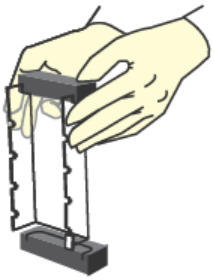
4. PRACTICE

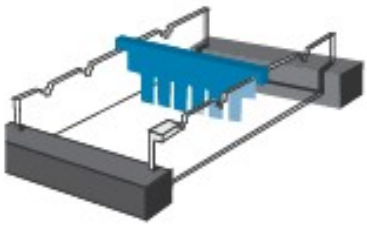
4.1 AGAROSE GEL PREPARATION

A) Preparing the gel bed

1.a) Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams.

2.b) Place a well-former template (comb) in the middle set of notches. Make sure the comb sits firmly and evenly across the bed.





B) Preparing agarose gels

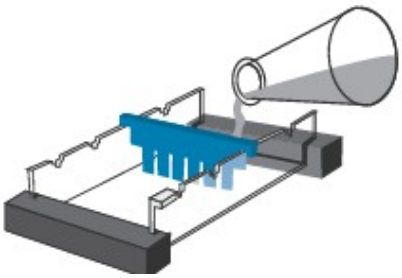
1.b) Use a beaker o erlenmeyer of 100 ml for preparing gel solution:

2.b) **For 7 x 7 cm casting tray:** add **32 ml of Electrophoresis Buffer 1 X + 0.30 gr of agarose**, Swirl the mixture to disperse clumps of agarose powder.
For 7 x 10 cm casting tray: add **42 ml of Electrophoresis Buffer 1 X + 0.40 gr of agarose**, Swirl the mixture to disperse clumps of agarose powder.
NOTE: verify that the 450 ml of distilled water have been added to the Concentrated Electrophoresis Buffer 10 X.

3.b) Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles. The most rapid method is the utilization of a microwave, also a hot plate can be used, in both cases, for dissolving the agarose**, we have to take the solution to boiling.**

4.b) **Cool** the agarose solution **to 55°C** with a careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume (to accelerate the process it is possible to cool placing the beaker under a water faucet and shake it).

5.b) Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.

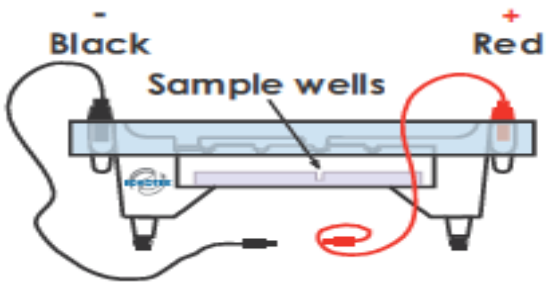


6.b) Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. You can accelerate the process putting the gel at 4ºC. The gel can remain at 4ºC to be used another day.

C) Preparing the gel for electrophoresis

1.c) After the gel is completely solidified, carefully and slowly remove the rubber dams or tapes from the gel bed.

2.c) Place the gel (on its bed) into the electrophoresis chamber, properly oriented, with the wells nearer to the negative pole (black color).



3.c) Fill the electrophoresis apparatus chamber with **300 ml of Electrophoresis Buffer 1 X.** ***The electrophoresis buffer can be used for 2 electrophoresis experiments, once finished the electrophoresis you can store the buffer used, in a packing different to the supplied, to use it in a new electroforesis.***

4.c) Make sure the gel is completely covered with buffer.

5.c) Remove the comb by slowly pulling it straight up. Do this carefully and evenly to prevent tearing the sample wells.

6.c) Proceed to loading the samples and conducting electrophoresis.

4.2 SAMPLE DELIVERY (GEL LOADING) AND CONDUCTING AGAROSE GEL ELECTROPHORESIS

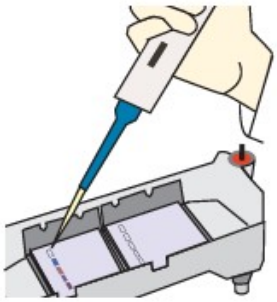
Note: If you are unfamiliar with loading samples in agarose gels, it is recommended that you practice sample delivery techniques before conducting the actual experiment, or carry out the complete experiment before realizing it with the students.

A) Electrophoresis samples. Check the sample volume. Sometimes a small amount of sample will cling to the walls of the tubes. Make sure the entire volume of sample is at the bottom of the tubes before starting to load the gel. Briefly centrifuge the sample tubes, or tap each tube on the tabletop to get the whole sample to the bottom of the tube.

1.a) We supply 4 different samples presented in 4 tubes, each of a color. Load the samples in the consecutive order:

Well	Sample	Description
1	**Green**	Molecular weight marker
2	**Purple**	Genomic DNA
3	**Red**	Genomic DNA
4	**Blue**	Molecular weight marker

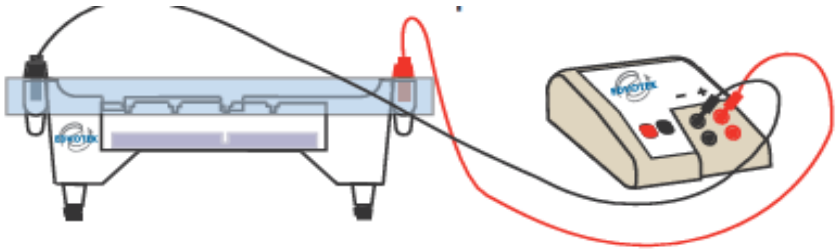
2.b) Sample delivery. **Load 20 µl** of each sample, you must use the Fixed Volume Micropipet 20 µl that is supplied with a micropipet tip.



B) Running the gel

1.b) After the samples are loaded, carefully snap the cover down onto the electrode terminals.

2.b) Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).



3.b) Set the power source at the required voltage and conduct electrophoresis for **75 (30 min) - 150 (20 min) volts.** Check that you see the current flowing properly - you should see bubbles forming on the two platinum electrodes.

4.b) After approximately 10 minutes, you will begin to see separation of the colored dyes.

5.b) After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads, and remove the cover.

4.3 AGAROSE GEL STAINING

a) After the electrophoresis is completed. Wash the gel with water and incubate it with the solution of **DanaBlue 0.02 %** for 15 minutes, for this, use a plastic container that is capable to completely cover the agarose gel. You can reuse this colorant solution some other 10 times more or less.

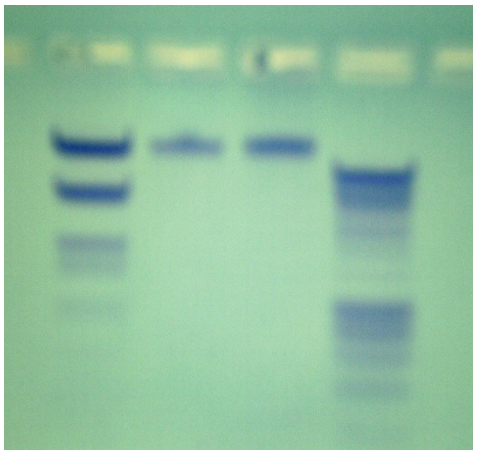
b) Wash the gel with abundant water to eliminate the excess of dye. In this first wash, place the container with the gel under a faucet of current water until the water of the gel goes out almost without blue color.

c) Wash it several times with water, in agitation if possible. It will be observed how every time the bands become more visible.

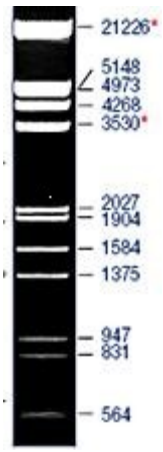
d) Document the gel results. Place the gel in one White Light Box (a piece of white paper can also be used).

5. RESULTS

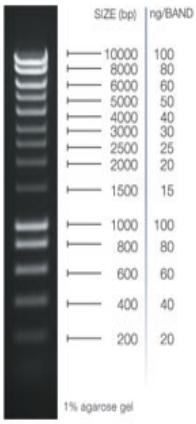
M1 1 2 M2



M1



M2



M1: Molecular weight marker, you can see the size of DNA fragments in the attached figure.

M2: Molecular weight marker, you can see the size of DNA fragments in the attached figure.

1 and 2: Genomic DNA

6. QUESTIONS AND ANSWERS ABOUT THE PRACTICE

You can do a series of questions to the students about the practice:

1. **What conclusion can be drawn from the results of the experiment?** That the genomic DNA has a size of 20-25 Kb (25.000 base pairs) with regard to the molecular weight marker. That the genomic DNA is intact, since it appears as the only intact band. If it was degraded, we would see a "smear".
2. **What is the genomic DNA?** It is the DNA that one finds inside our chromosomes and is where all the information necessary for life is.
3. **On what basis does agarose gel electrophoresis separate molecules?** Agarose gel electrophoresis separates molecules based on size, charge and shape.
4. **Explain migration according to charge.** Molecules having a negative charge migrate towards the positive electrode; positively charged molecules migrate towards the negative electrode.
5. **What would happen if distilled water were substituted for buffer in either the chamber solution or the gel solution?** No ions are contained in distilled water. Ions are required for fluid conductivity and therefore, for the ability of the molecules to migrate through the gel. The buffer serves as driver of the electricity and controls the pH, which is important for the stability of biological molecules.
6. **Towards what electrode will the DNA migrate?** Since the DNA has a negative charge at neutral pH, it will migrate across the gel towards the positive electrode during the electrophoresis.

Do not hesitate to contact us for any doubt or additional consultation, please, contact bioted@arrakis.es