

NEWSLETTER

THURSDAY 16/02/2023

WWW.ITTECH.COM.MY

DNA GEL ELECTROPHORESIS

INTRODUCTION

Gel electrophoresis is a common method to separate and visualize charged biological molecules. During gel electrophoresis, an electric field propels the molecules across the gel. The molecules travel across gel matrix that resembles a web-like mesh.

Scientists commonly perform gel electrophoresis to separate DNA fragments. Since DNA is negatively charged, it migrates toward the positive electrode (Fig. 1). DNA fragments of smaller size migrate faster and further than larger-size DNA. Therefore, this helps scientists to distinguish DNA fragments by their size.

IMPORTANCE OF GEL ELECTROPHORESIS

Each of us is genetically unique, just like our fingerprints! Combined with PCR, gel electrophoresis is used in a technique called DNA fingerprinting to differentiate genetic variations. In forensics, a suspect can be tied to a crime if his DNA pattern matches the one found at the crime scene. In paternity testing, when the DNA patterns of a child match the father's, we could say with certainty they are biologically related. In other cases, the DNA bands can be excised from the gel to be used in other applications— such as DNA cloning. DNA gel electrophoresis has been a powerful tool with numerous applications. We do have to thank Arne Tiselius for this remarkable discovery!



Figure 1

SETTING UP FOR GEL ELECTROPHORESIS

AGAROSE GEL

Agarose is a polysaccharide made of sea kelp. The matrix of agarose gel is made up of pores of different sizes. The higher percent of agarose gel has smaller pore size, allowing smaller DNA to pass and vice versa.

Casting an agarose gel is as simple as making jello! The agarose powder is first dissolved by heating it with buffer. The solution is poured into a casting chamber and a casting comb is placed in the mold to make indentations called "wells" (Fig. 1). After the gel hardens, scientists load DNA samples into the wells using micropipettes (Fig.1).

ELECTROPHORESIS CHAMBER

The chamber is a space that holds the agarose gel and the running buffer. The agarose gel is placed in an orientation that allows the DNA to migrate from the positive to the negative electrode (Fig. 1) once the electric field is applied.









VISUALIZING THE DNA



LOADING DYE

Loading dye is an important component with many functions. It makes the DNA sample denser than the running buffer, allowing the DNA to sink to the bottom of the well. Without the loading dye, the DNA sample floats and dilutes into the running buffer. Because DNA is colourless, loading dye serves as a colour indicator to give a visual confirmation that electrophoresis has taken place. It is important to note that the loading dye does not help with DNA visualization. DNA dye is used for that purpose.

DNA DYE

DNA dye binds in between DNA helices. When DNA is exposed to light of certain wavelengths, it fluoresces. The more DNA is present, the brighter the band. Scientists have different preferences in staining DNA. Some add DNA dye while casting gel. Some stain gels with DNA dye when electrophoresis is done.

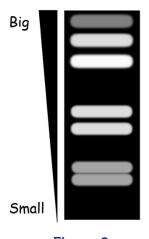


Figure 2

INTERPRETING THE RESULTS OF DNA GEL ELECTROPHORESIS

The DNA molecules migrate along the gel in a straight lane. Million DNA copies of the same size are present on each lane as bands (little rectangles; Fig. 2). The DNA of smaller size travel faster those of larger size. When scientists refer to different band sizes, they mean DNA copies of specific sizes which are presented as multiple bands.

To know the size of each band, DNA ladder is used. DNA ladder is a mixture of DNA molecules of different sizes, giving the impression of ladder on agarose gel (Fig. 2). Since the size of each fragment of the ladder is known, it can be used to estimate the sizes of the unknown DNA fragment by comparing to that of the DNA ladder.

BLUEGEL ELECTROPHORESIS

With the BlueGel electrophoresis system, scientists can visualize the DNA migration while the electrophoresis is still running. This is made possible by the Blue Light transilluminator which is integrated in the unit. Small in size with no need of external power supply, the BlueGel electrophoresis system is the right fit for every molecular lab.











