Molecular Detection of *Acinetobacter* bacteria

Experiment 3: Analysis of PCR Products

Objective

To separate the DNA fragments based on their Molecular weight.

Theory

Agarose gel electrophoresis is the easiest and most popular way of separating and analyzing DNA. Here DNA molecules are separated on the basis of charge by applying an electric field to the electrophoretic apparatus. Shorter molecules migrate more easily and move faster than longer molecules through the pores of the gel and this process is called sieving. The gel might be used to look at the DNA in order to quantify it or to isolate a particular band. The DNA can be visualized in the gel by the addition of *ethidium bromide*.

Agarose is a polysaccharide obtained from the red algae Porphyra umbilicalis. Its systematic name is **(1 4)-3, 6-anhydro-a-L-galactopyranosyl-(1 3)-β-D-galactopyranan**. Agarose makes an inert matrix. Most agarose gels are made between 0.7% and 2% of agarose. A 0.7% gel will show good separation for large DNA fragments (5-10kb) and a 2% gel will show good resolution for small fragments with size range of 0.2-1kb. Low percentage gels are very weak (Note:- it may break when you lift them) but high percentage gels are usually brittle and do not set evenly.

Factors Affecting the Movement of DNA:

Voltage Applied

The migration rate of the linear DNA fragments through agarose gel is proportional to the voltage applied to the system. As voltage increases, the speed of DNA also increases. But voltage should be limited because it heats and finally causes the gel to melt.

Buffers

The most commonly used buffers are Tris-acetate-EDTA (TAE) and Tris-borate-EDTA(TBE). The migration rate of DNA fragments in both of these buffers is somewhat different due to the differences in ionic strength. These buffers provide the ions for supporting conductivity.

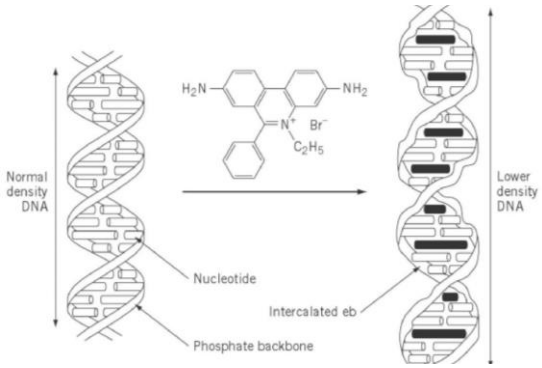
Ethylene-diamine-tetra-acetic acid (EDTA)

Conformation of DNA

DNA with different conformations that has not been cut with a restriction enzyme will migrate with different speeds. The mobility depends on the concentration & size of the DNA, type of agarose used to make the gel, applied voltage, buffer, and the density of super helical twists.

Ethidium Bromide (EtBr)

It is an intercalating agent which intercalates between nucleic acid bases and allows the convenient detection of DNA fragments in gel. When exposed to UV light, it will fluoresce with an orange colour. After the running of DNA through an EtBr-treated gel, any band containing more than ~20 ng DNA becomes distinctly visible under UV light. EtBr is a known "mutagen", however, safer alternatives are available. It can be incorporated with agarose gels or DNA samples before loading, for visualization of the fragments. Binding of Ethidium bromide to DNA alters its mass and rigidity, and thereby its mobility.



Materials Required:

Buffers and Solutions:

- ✓ Agarose solutions.
- ✓ Ethidium bromide.
- ✓ Electrophoresis buffer.

Nucleic Acids and Oligonucleotides:

- ✓ DNA samples.
- ✓ DNA Ladders.

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:

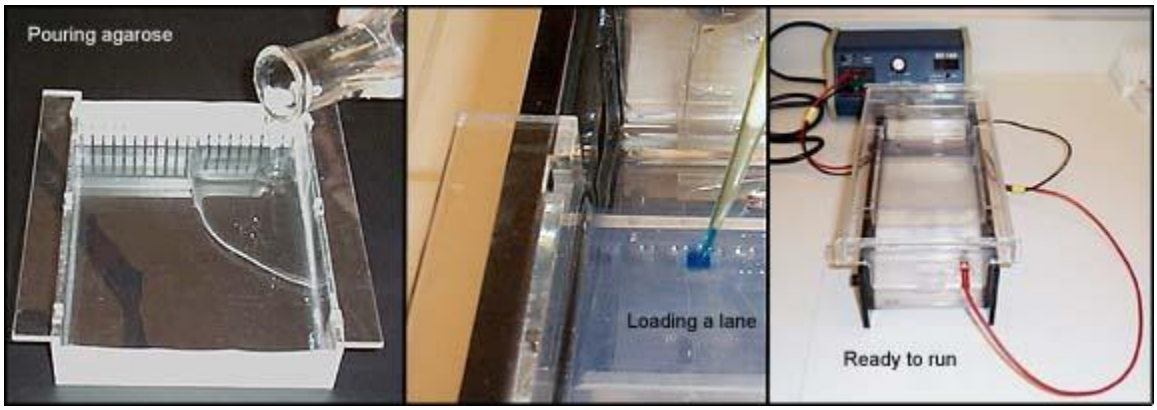
- ➢ An electrophoresis chamber and power supply.
- ➢ Gel casting trays, which are available in a variety of sizes and composed of UV-transparent plastic.
- ➢ Sample combs, around which molten agarose is poured to form sample wells in the gel.
- ➢ Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- ➢ Gel loading buffer: In order to perform gel electrophoresis, DNA samples are mixed with "loading buffer" in 1:6 ratio (e.g. DNA solution 5 ul should be mixed with 1 ul of loading dye). Usually a Loading buffer contains three important components:
  (1) **glycerol**, which allows your sample to "fall" to the bottom of the well when you are loading the gel
  (2) Two dyes called **bromophenol blue & xylene cynol**
  These two dyes components allow viewing the progression of your sample as it migrates down the gel.
- ➢ Ethidium bromide, a fluorescent dye used for staining nucleic acids.

- Gel Doc Imager**,** an integrated system of ultraviolet lightbox enclosed in a chamber linked with CCD camera and a computer to visualize the image on the screen.

Procedure

Preparing and Running Standard Agarose DNA Gels

1. Weigh 0.35 g of Agarose and mix with 50 ml of 1X TBE buffer 0.7%) in a conical flask.
2. Cover the flask with clean wrap and heated in a microwave oven until completely melted.
3. Allow to cool the solution to 60^0C by placing the flask on magnetic stirrer with mixing.
4. Fit the gel tray into the gel casting tray. Make sure that the seal is tight.
5. Insert the well-comb at one end of the tray.
6. After cooling the solution to about 60 ^{o}C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature
7. After the gel has solidified, the comb is removed, using care not to rip the bottom of the wells.
8. The gel, still in its plastic tray, is inserted horizontally into the electrophoresis chamber which is filled with 1X TBE buffer as running buffer in such that the gel is immersed in it.



9. Samples containing DNA mixed with loading buffer are then pipetted into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied. You can confirm that current is flowing by observing bubbles coming off the electrodes. **DNA will migrate towards the positive electrode, which is usually colored red.**
10. Close the lid of the gel tank and attach the electrical leads so that the DNA will migrate toward the positive anode.
11. Run the gel for 10 minutes at 80 volts and next 30 minutes at100 volts.
12. The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes. **Bromophenol blue** and **xylene cyanol** dyes migrate through agarose gels at roughly the same rate as double-stranded DNA fragments of 300 and 4000 bp, respectively.
13. When adequate migration has occurred, **DNA fragments are visualized by staining with ethidium bromide.** This fluorescent dye intercalates between bases of DNA and RNA.
14. After completion of the run, the gel can be stained by immersing the gel in box containing 1XTBE buffer with 10ul ethidium bromide dissolved in it. Keep the gel 20 min in it.
15. After this incubation take the gel and rinse with distilled water. Place the on clean wrap paper.
16. Place the gel on Gel Doc Imager
17. Turn of UV light and observe Genomic and plasmid DNA band of different concentration.
18. Compare approximate sizes with DNA ladder. Save photo as a TIFF file.

Results

a) DNA bands are of what approximate sizes compared to DNA Ladder?
b) How does the DNA band look? Is the DNA a tight band or does it appears to be streaky, displaying signs of degrading and/or shearing?
c) Is the target bacteria present in your sample?