AGAROSE GEL ELECTROPHORESIS

Modified from Wolbachia FIBR Project, Rochester University

Gel electrophoresis is a widely used technique for the analysis of nucleic acids and proteins. Most every molecular biology research laboratory routinely uses agarose gel electrophoresis for the preparation and analysis of DNA. We will be using agarose gel electrophoresis to determine the presence and size of PCR products and extracted plasmids.

Background:
Electrophoresis is a method of separating substances based on the rate of movement while under the influence of an electric field. Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab. During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is forced through the pores of the gel by the electrical current. Under an electrical field, DNA will move to the positive electrode (red) and away from the negative electrode (black). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose in the gel and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA itself is not visible within an agarose gel. The DNA will be visualized by the use of a dye that binds to DNA.

Purpose: To determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the product.

Materials needed:
- Agarose
- TAE or TBE Buffer
- 6X Sample Loading Buffer
- DNA ladder standard
- Electrophoresis chamber
- Power supply
- Gel casting tray and combs
- DNA stain
- Staining tray
- Gloves
- Pipette and tips

Agarose Gel Electrophoresis Protocol

Preparing the agarose gel (0.7 %)

- Measure 350 mg Agarose powder and add it to a 50ml TAE Buffer in 500 ml flask (the total gel volume well vary depending on the size of the casting tray and the amount of gel solution)
- Melt the agarose in a microwave or hot water bath until the solution becomes clear. (if using a microwave, heat the solution for several short intervals - do not let the solution boil for long periods as it may boil out of the flask).

Add 0.5 ul ethidium bromide (10 mg concentration)

- Let the solution cool to about 50-55°C, swirling the flask occasionally to cool evenly.
- Seal the ends of the casting tray with two layers of tape.
- Place the combs in the gel casting tray.
- Pour the melted agarose solution into the casting tray and let cool until it is solid (it should appear milky white) usually 30 minutes.
- Carefully pull out the combs and remove the tape.
- Place the gel in the electrophoresis chamber.
- Add enough TAE Buffer so that there is about 2-3 mm of buffer over the gel.

Note – gels can be made several days prior to use and sealed in plastic wrap (without combs). If the gel becomes excessively dry, allow it to rehydrate in the buffer within the gel box for a few minutes prior to loading samples.

Loading the gel

- Add 5 μl of 6X Sample Loading Buffer to each 25 μl PCR reaction
- Record the order each sample will be loaded on the gel, controls and ladder.
- Carefully pipette 10 - 20 μl of each sample/Sample Loading Buffer mixture into separate wells in the gel.
- Pipette 5 μl of the DNA ladder standard into at least one well of each row on the gel.

Note – if you are running multiple gels, avoid later confusion by loading the DNA ladder in different lanes on each gel.

Running the gel

- Place the lid on the gel box, connecting the electrodes.
- Connect the electrode wires to the power supply, making sure the positive (red) and negative (black) are correctly connected. (Remember – "Run to Red")
- Turn on the power supply to about 100 volts. Maximum allowed voltage will vary depending on the size of the electrophoresis chamber – **it should not exceed 5 volts/ cm between electrodes!** .
- Check to make sure the current is running through the buffer by looking for bubbles forming on each electrode.
- Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye – this will take a couple of minutes (it will run in the same direction as the DNA).
- Let the power run until the blue dye approaches the end of the gel.
- Turn off the power.
- Disconnect the wires from the power supply.
- Remove the lid of the electrophoresis chamber.
- Using gloves, carefully remove the tray and gel.

Gel Staining (if you added ethidium bromide to your gel after boiling ignore this section)

- Using gloves, remove the gel from the casting tray and place into the staining dish.
- Add warmed (50-55°) staining mix or ethidium bromide stain.
- Allow gel to stain for at least 25-30 minutes (the entire gel will become dark blue with staining mix).
- Pour off the stain (the stain can be saved for future use) or dispose ethidium bromide in appropriate way (check university policy).
- Rinse the gel and staining tray with water to remove residual stain. (if used ethidium bromide you can view your gel under UV light now)
- Fill the tray with warm tap water (50-55°). Change the water several times as it turns blue. Gradually the gel will become lighter, leaving only dark blue DNA bands. Destain completely overnight for best results.

Note – Gels stained with blue stains are stable for long periods. When destaining is complete, remove gel from water and allow the gel to dehydrate. Dark bands can be seen for in a dried gel for weeks or months.

Visualization of the gel

Gels stained with ethidium bromide can be screened and pictured under UV light

Other stain **View the gel against a white light box or bright surface.**

- Record the data while the gel is fresh, very light bands may be difficult to see with time.

Recipes:

EDTA
EDTA (ethylenediamine tetraacetic acid) solution is prepared ahead of time. EDTA will not go completely into solution until the pH is adjusted to about 8.0. For a 500 mL stock solution of 0.5 M EDTA, weigh out 93.05 g EDTA disodium salt (FW = 372.2). Dissolve in 400 mL deionized water and adjust the pH with NaOH. Top up the solution to a final volume of 500 mL.

5XTBE (stock)
Make a concentrated (5x) stock solution of TBE by weighing 54 g Tris base (FW = 121.14) and 27.5 g boric acid (FW = 61.83) and dissolving both in approximately 900 mL deionized water. Add 20 mL of 0.5 M EDTA (pH 8.0) and adjust the solution to a final volume of 1 L. This solution can be stored at room temperature but a precipitate will form in older solutions. Store the buffer in glass bottles and discard if a precipitate has formed.

0.5X TBE (working solution)
For agarose gel electrophoresis, TBE can be used at a concentraion of 0.5x (1:10 dilution of the concentrated stock). Dilute the stock solution by 10x in deionized water. Final solute concentrations are 45 mM Tris-borate and 1 mM EDTA. The buffer is now ready for use in running an agarose gel.

TAE Buffer

4.84 g Tris Base
1.14 ml Glacial Acetic Acid
2 ml 0.5M EDTA (pH 8.0)
- bring the total volume up to 1L with water

Add Tris base to ~900 ml H2O. Add acetic acid and EDTA to solution and mix. Pour mixture into 1 L graduated cylinder and add H2O to a total volume of 1 L.
Note – for convenience a concentrated stock of TAE buffer (either 10X or 50X) is often made ahead of time and diluted with water to 1X concentration prior to use (or 0.5X to safe reagents).

Notes

TBE has a greater buffering capacity and will give sharper resolution than TAE buffer. But TBE is generally more expensive than TAE

6X Sample Loading Buffer

1 ml sterile H2O
1 ml Glycerol
enough bromophenol blue to make the buffer deep blue (~ 0.05 mg)
-for long term storage, keep sample loading buffer frozen.

Ethidium bromide (working solution)
Always buy Ethidium bromide in a solution not powder form

(Ethidium bromide is a powerful mutagen and is toxic. Gloves should be worn at all times)

Ethidium Bromide Disposal

Ethidium bromide is commonly used in molecular biology laboratories. While it is not regulated as hazardous waste, the mutagenic properties of this substance may present a hazard if it is poured down the drain untreated or placed in the trash.

Based on these considerations, we recommend the following disposal procedures for ethidium bromide.

- Gels with ethidium bromide place in biohazard box for incineration or according to the policy of environmental health and safety of the University.

- Aqueous solutions containing >10ug/ml ethidium bromide should be filtered or deactivated using charcoal filtration or chemical deactivation.