Agarose Gel Electrophoresis

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.

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
- Gloves
- Pipette and tips

Agarose Gel Electrophoresis Protocol

Preparing the agarose gel (1 %)

- Measure 0.5 g Agarose powder and add it to a 50ml TBE Buffer in 250 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 TBE Buffer so that there is about 2-3 mm of buffer over the gel.

Loading the gel

- Add 1 μl of 6X Sample Loading Buffer to each 5 μl from PCR reaction
- Record the order each sample will be loaded on the gel, controls and ladder.
- Carefully pipette 6 μl of each sample/Sample Loading Buffer mixture into separate wells in the gel.

- Pipette 2.5 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 80 volts for 2h. 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.

Visualization of the gel

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

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.