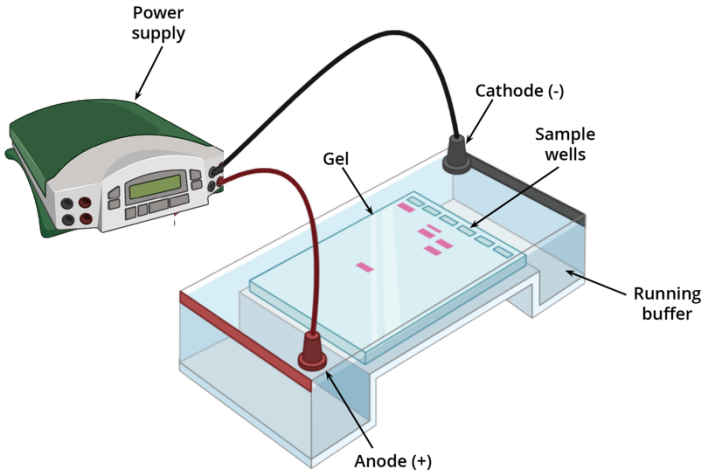
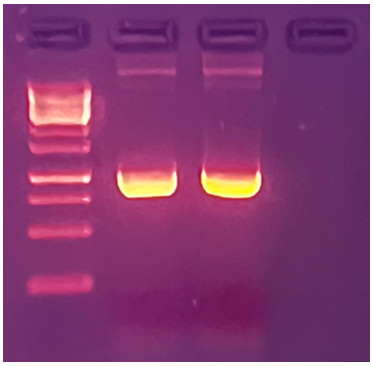
Agarose gel electrophoresis protocol

Background:

Gel electrophoresis allows for the isolation and purification of DNA fragments based on size. Because proteins are much smaller than DNA molecules, and because other cellular components are much larger, standard agarose gel electrophoresis also provides a tool to separate DNA from other junk that may be present in a reaction tube following PCR or restriction digestion. The isolated DNA can then be cut out from the gel and purified using a gel extraction kit. Gel electrophoresis works by passing an electrical current form one end of a buffer chamber to the other across an agarose gel containing the DNA sample. This is accomplished by connecting the cathode (the negatively charged electrode) of a power supply to one end of the chamber and the anode (the positively charged electrode) to the other. To complete the circuit, a current must be formed between these two electrodes by passing through a liquid buffer, usually Tris/Acetic Acid/EDTA (TAE, which provides a greater resolution for fragments > 10 kb) or Tris/Boric Acid/EDTA (TBE, which provides a greater resolution for fragments < 1 kb). Because the phosphate backbone of DNA gives the molecule an overall negative charge, DNA will migrate towards the positive end of an electrified field. During this migration, the density of the agarose will help to separate DNA fragments by size, as small DNA bands have less friction and move through the gel more quickly than larger fragments. This migration is often monitored by the addition of visible loading dyes, as DNA is not visible during electrophoresis. Visualization of the DNA itself occurs via staining post electrophoresis, or through the addition of chemicals like ethidium bromide that bind to the DNA fragments as they migrate through the gel and can be visualized on a gel illuminator with UV light.





When performing gel purification, it is recommended to run the samples more slowly (~10 V/cm distance between the electrodes, for approximately 60 min) for clear, crisp bands. Using a wider gel comb will also allow you to load more of your sample for higher yield. Furthermore, skipping lanes when possible, or using a comb with larger gaps between each lane, will make excision of gel fragments easier if extraction is intended following electrophoresis.

Materials:

- DNA of interest
- Agarose
- **TAE** or **TBE**
- **Ethidium bromide**
- Loading dye
- Gel electrophoresis rig, associated gel cast, and associated combs

Protocol:

*Note: Different size trays will require different running parameters and volumes of agarose. Check with the manufacture accordingly.

- Prepare the volume of agarose gel needed for your gel rig mold by dissolving agarose in TAE or TBE at the proper concentrations for your DNA length using the table below:
  - Note: TAE is better for >10 kb, while TBE is better for <1 kb.

Percent Agarose Gel (w/v)	DNA Size Resolution(kb = 1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

Table 1: Correct Agarose Gel Concentration for Resolving DNA Fragments

  - Note: DNA size resolution is greatest in the middle of these ranges.
- Heat the agarose solution in a 250 mL Erlenmeyer flask using the microwave.
- Let the solution cool to room temperature until no vaporization is visible.
  - Note: This is important to prevent aerosolization of added EtBr, a carcinogen.
- Add 1.5 µL of **Ethidium Bromide** for each 50 mL of agarose solution prepared, swirl to mix, and pour the solution into the gel rig mold.
- Use the well comb to scrape out any bubbles that appear on the surface.
  - Note: If bubbles are left in the liquid, they will form craters when the gel cools.
- Add the well comb to create loading wells and let the gel solidify for ~ 30 min.
  - Note: Check the progress of solidification by looking at the gel from the sides. If it looks cloudy, it is ready, while if it looks clear, it still needs more time.
- Once solidified, gently remove the gel from the mold and then remove the comb.
- Fully submerge the gel in the electrophoresis apparatus tank filled with either **TAE** or **TBE**.
  - Note: Make sure that the loading wells are on the negative side of the apparatus.
- Add the appropriate amount of loading dye to your DNA samples, usually provided with PCR kits, and load your samples into the wells along with a size standard.
- Attach the lid and electrodes, making sure the cathode is on the side with the wells.
- Based on the size of your gel rig, set the mAmps or voltage to the appropriate settings and run for about 1 hour.
  - Note: The proper voltage is typically ~10 V/cm distance between the electrodes.

Media:
Ethidium Bromide

- Add the following ingredients together:
  - 100 mg $C_{21}H_{20}BrN_3$ (ethidium bromide)
  - 10 mL Milli-Q water
    - Store at 4°C

10X TAE (1 L)

- Add the following ingredients together:
  - 48.5 g $C_4H_{11}NO_3$ (tris base)
  - 20 mL 0.5 M EDTA, pH 8.0 (ethylenediaminetetraacetic acid)
  - 11.4 ml CH_3COOH (glacial acetic acid)
  - 1 L Milli-Q water
    - Fill up to 200 mL
    - Store at room temp

10X TBE (1 L)

- Add the following ingredients together:
  - 108 g $C_4H_{11}NO_3$ (tris base)
  - 55 g H_3BO_3 (boric acid)
  - 40 mL 0.5 M EDTA, pH 8.0 (ethylenediaminetetraacetic acid)
  - 1 L Distilled Water
    - Store at room temp