Marine Genomics

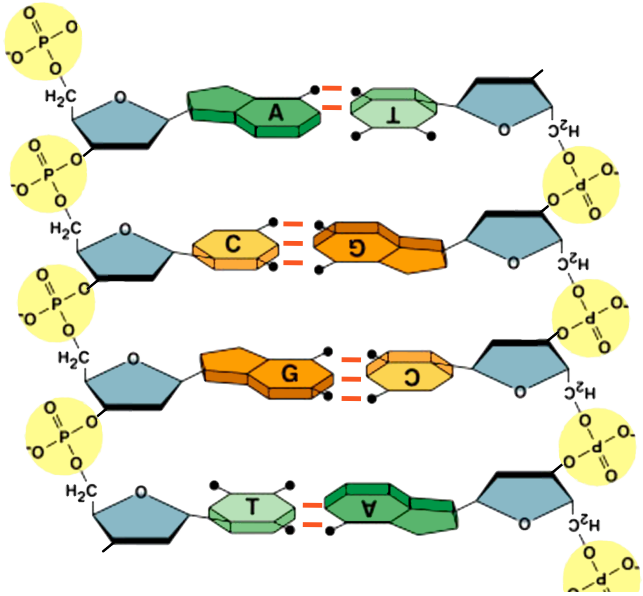
PROTOCOL

Agarose Gel Electrophoresis

compiled by John R. Finnerty

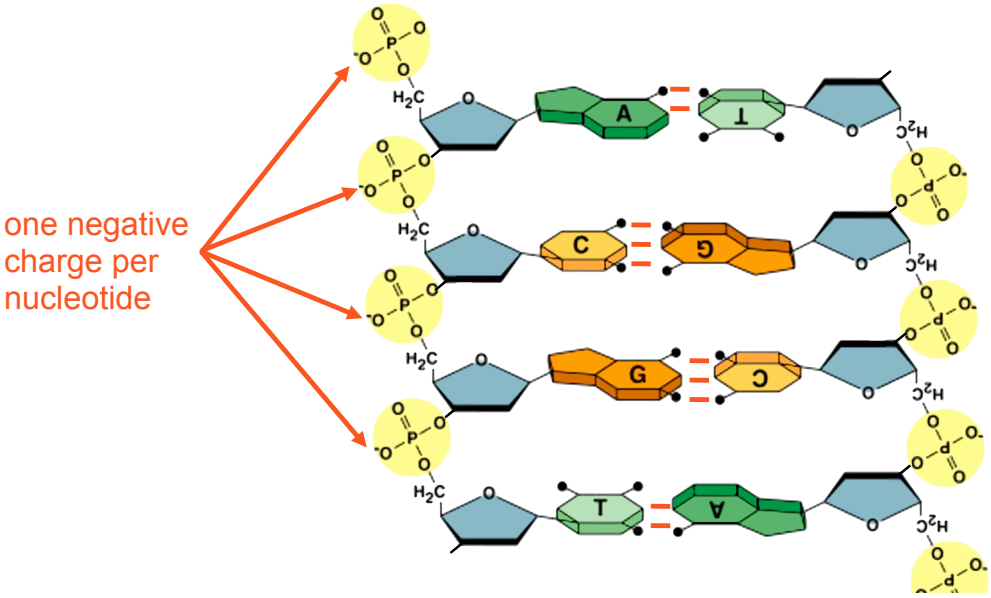
The Principal

In an aqueous solution with moderate pH, DNA and RNA exist as charged molecules because of the phosphate groups along their backbones. When placed in an electric field, they will migrate towards the positively charged pole (the anode).



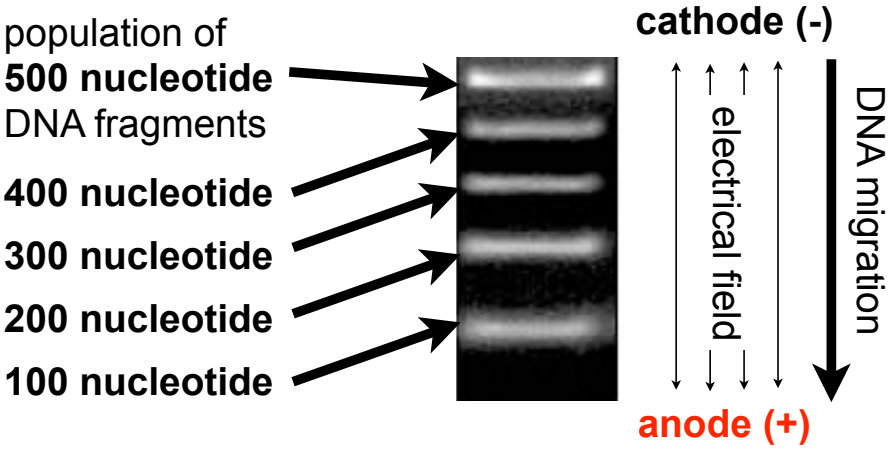
Charge Per Unit Mass

Because there is 1 negative charge per nucleotide, the electrical driving force will be proportionately equivalent for small or large DNA or RNA molecules (e.g., the charge per mass stays the same).



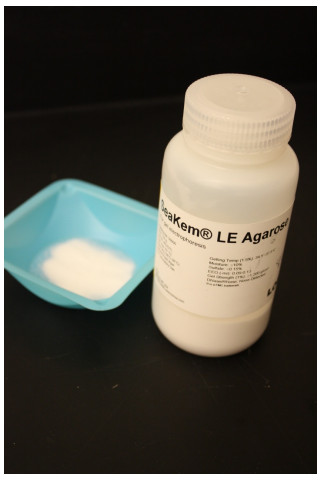
Size Separation on a Porous Matrix

If an electrical field is applied to a salt solution, and submerged in the solution is a porous solid matrix, the pores of the matrix will offer resistance to the movement of the DNA or RNA molecules. Smaller molecules encounter less resistance than larger molecules, and therefore, they migrate faster through the matrix. If sufficient time elapses, DNA or RNA fragments can be easily separated according to their length in nucleotides.

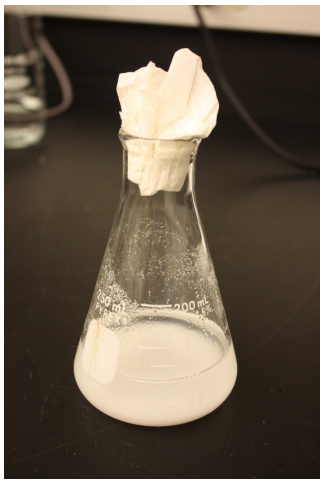


Agarose Gel Electrophoresis

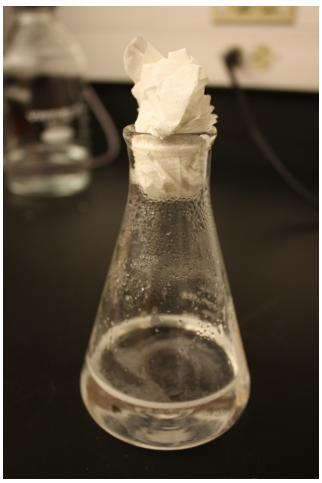
A commonly used matrix for electrophoresis is an “agarose gel.” Agarose is a polysaccharide. It is available as a powder. It is suspended in a solution of running buffer (usually TBE or TAE) and heated until it goes into solution.



1 gram of agarose



1 gram of agarose in 100 ml TBE



after microwaving for 1 minute

Tris-Borate-EDTA (TBE)

This is a solution used as a “running buffer” during electrophoresis. It contains:

Tris: serves to buffer the pH and keep the DNA or RNA soluble

Ethylene-diamine-tetracetic acid (EDTA): chelates divalent cations Mg2+ and Ca2+ which are necessary co-factors for nucleases.

Boric acid: provides a source of ions that allow the buffer to conduct electricity

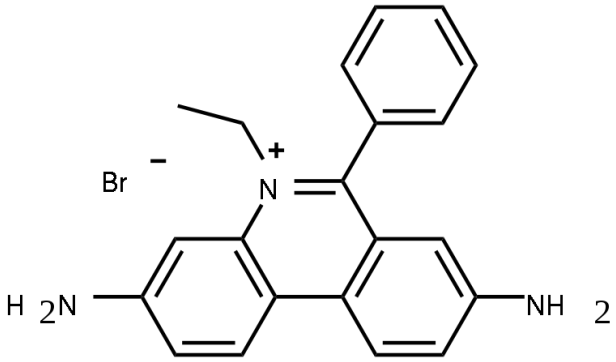
Recipe for 10X concentrated stock of TBE

- 108 g of Tris base (CAS# 37186)
- 55 g of boric acid (CAS# 11280)
- 40 ml of 0.5 M EDTA (CAS# 60004) (pH 8.0)
- bring up to 1 liter with addition of RO or distilled water

- This concentrated stock is diluted with 9 parts of water to make a working strength (1X) stock. It can also be used at 0.5X strength. The same strength TBE should be used to cast the gel and as running buffer during electrophoresis.

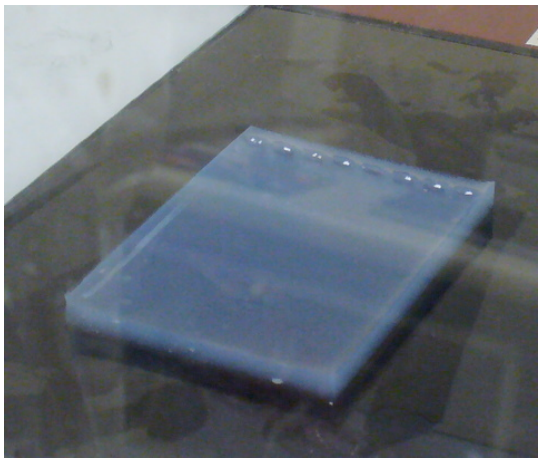
Ethidium Bromide

Ethidium bromide is a planar molecule which will intercalate between the bases on a DNA molecule. Importantly, when exposed to UV light, EtBr will fluoresce bright orange. This dye is therefore useful for visualizing DNA on an agarose gel. This compound has been shown to be mutagenic in the Ames test, so always wear gloves, lab coat, and goggles when handling it.

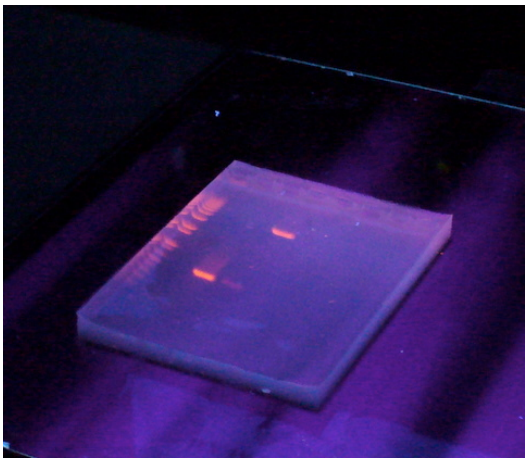


Visualizing the DNA

In order to visualize the DNA, the ethidium-bromide stained agarose gel is exposed to ultraviolet light. Under UV illumination, the ethidium bromide, which is concentrated in the DNA, gives off an orange glow.



1% agarose gel prior to UV illumination

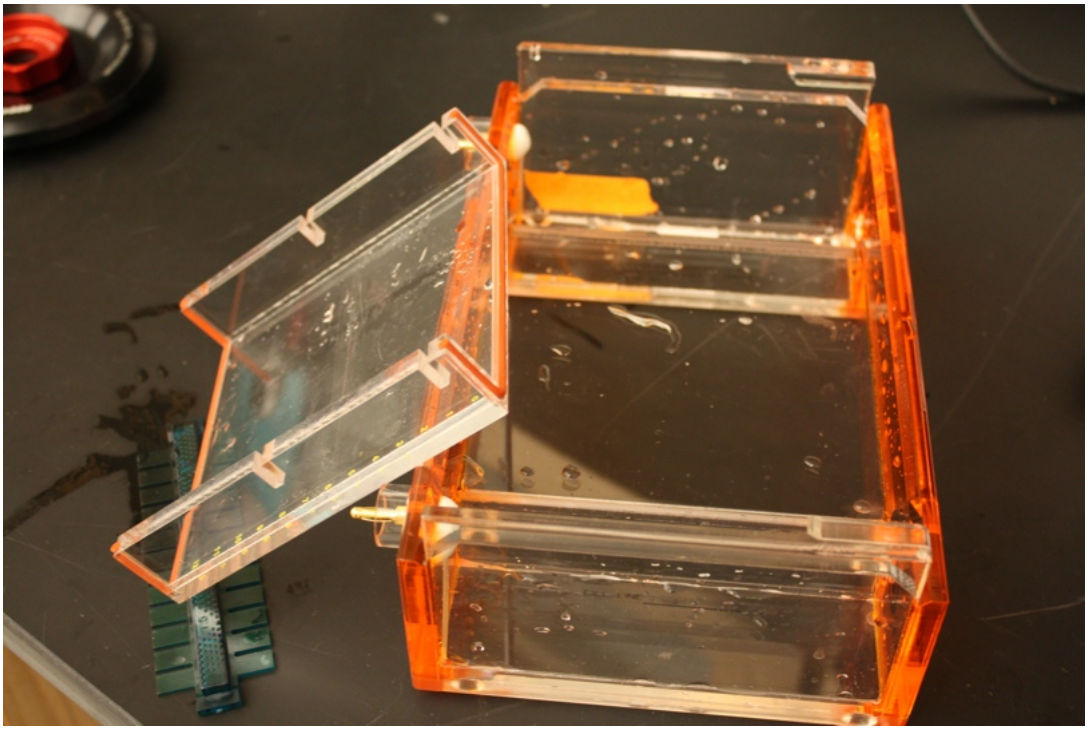


under UV illumination

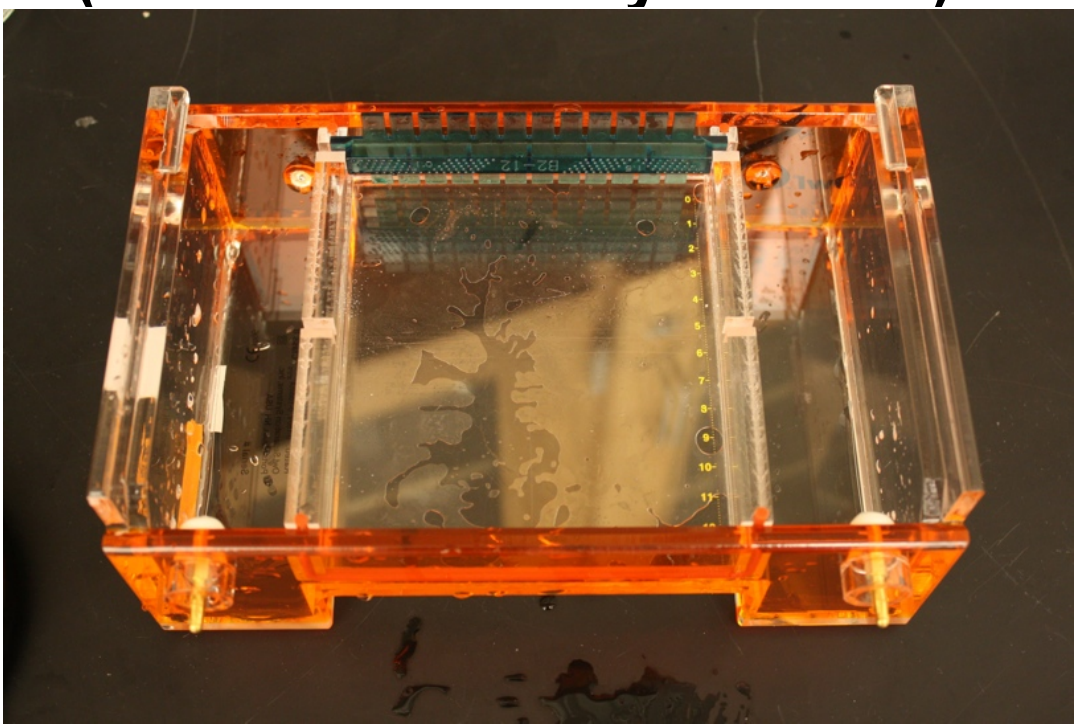
Cast the gel
100 ml of 1% agarose / 0.5X TBE

- Weigh 1.0 g of agarose
- Add agarose to 100 ml of 0.5X TBE in a 250 ml flask
- Block the opening of the flask with a crumpled paper towel.
- Microwave for approximately 1 minute, until the solution becomes clear and begins to boil.
- Add 10 microliters of a 5 mg/ml solution of ethidium bromide.
- Swirl to mix.
- Pour the molten agarose into the gel casting tray, which is supported in the gel box on a flat surface. The gel casting tray should be oriented so that the open ends are closed off by the walls of the gel box.
- Insert a gel comb, making sure that it is fully seated in the slot and the teeth are submerged below the surface of the molten agarose.
- Allow the gel to solidify on the bench top for approximately 1 hour.

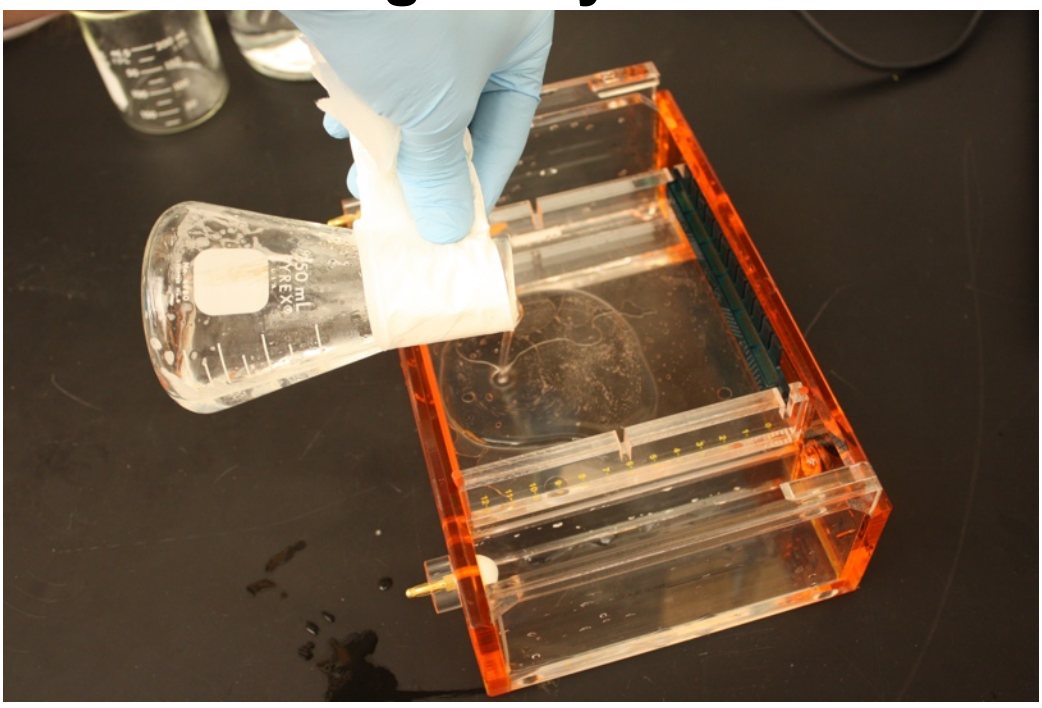
Gel tray leaning against gel box



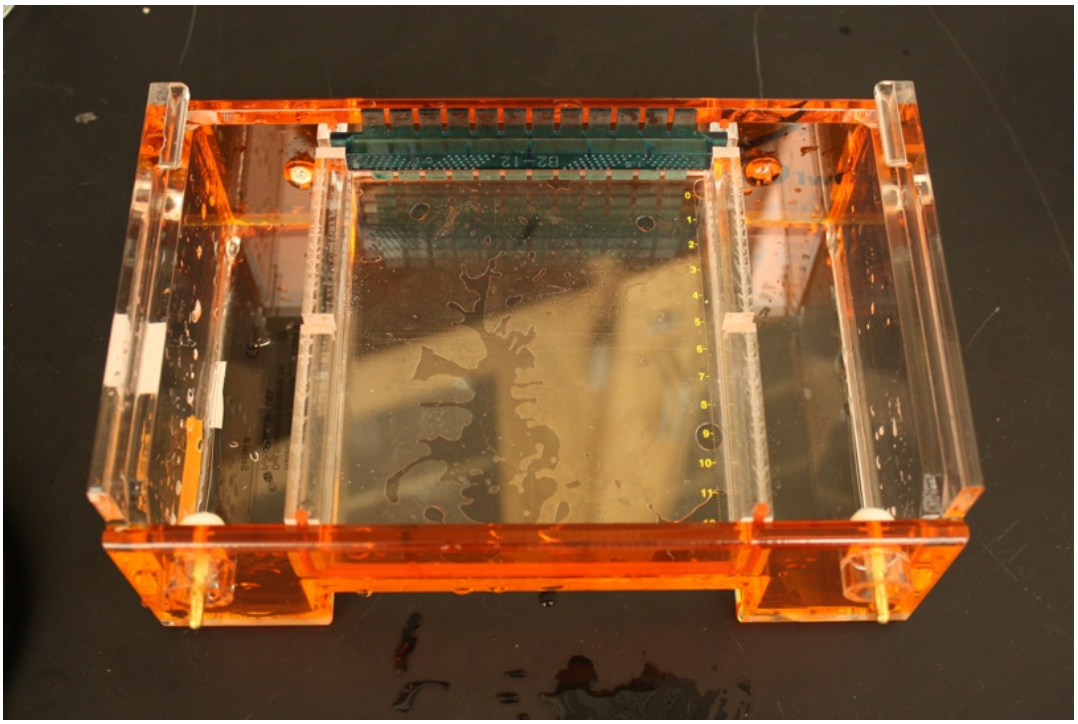
Gel tray seated in gel box (with comb already inserted)



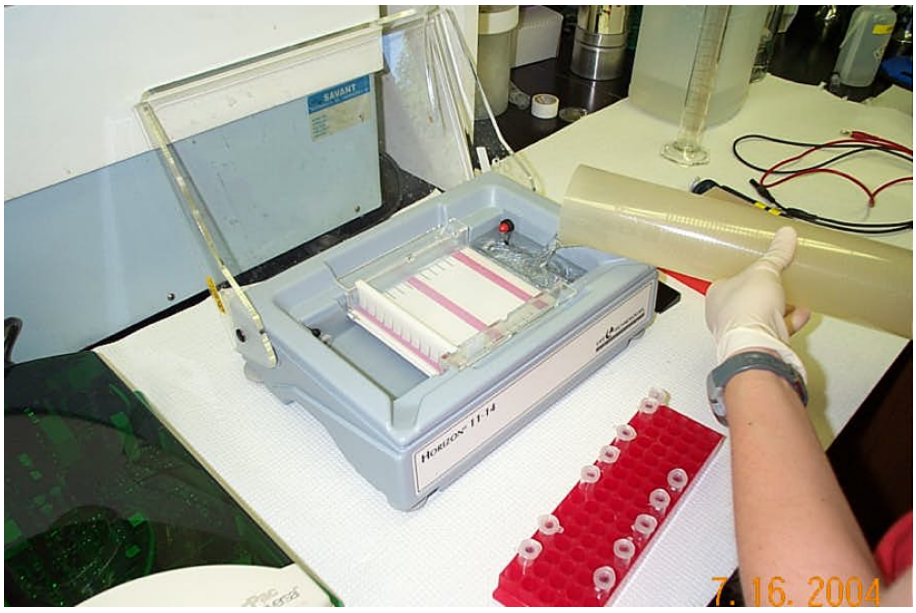
Pouring the molten agarose into the gel tray



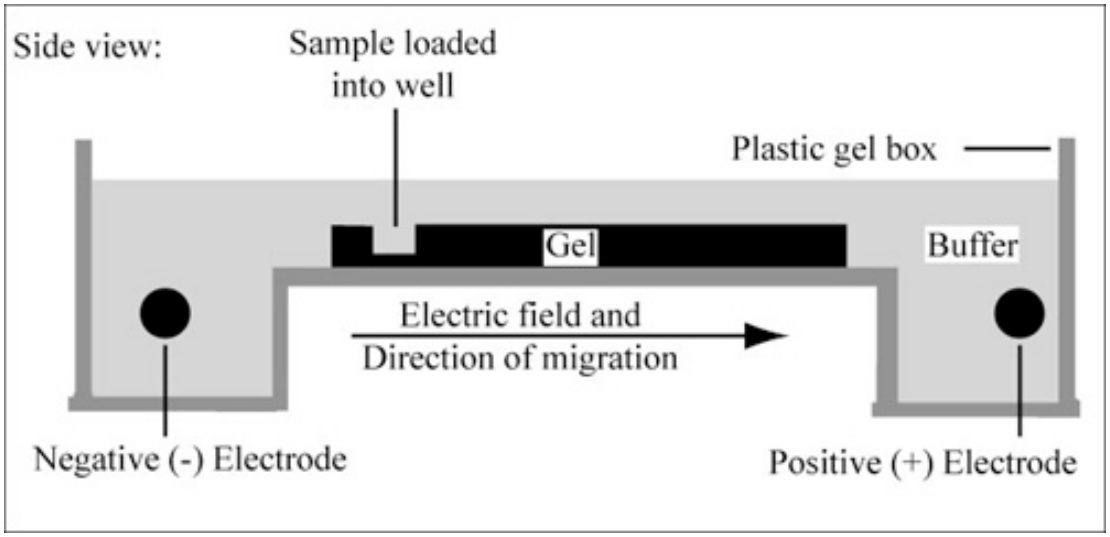
Allow the gel to harden for > 1 hr



Rotate the gel 90 degrees so that the wells are perpendicular to the flow of current.

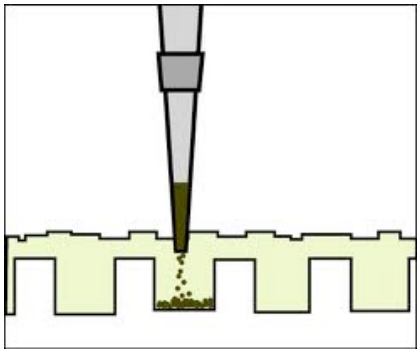


Fill the buffer chambers with 0.5x TBE so that the gel itself is slightly submerged.

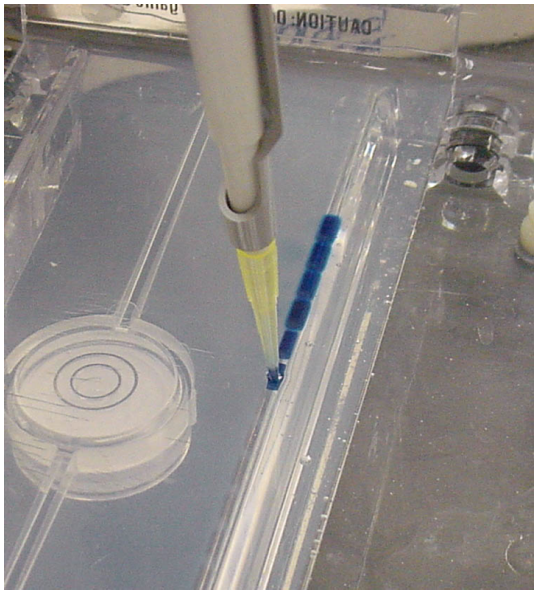


Mix 5 volumes of your sample + 1 volume of 6x loading dye

(e.g., 5 microliters sample + 1 microliter loading dye)



Your pipet tip should be in the well itself, or just above the well. Go slowly and be sure the sample is filling the well.

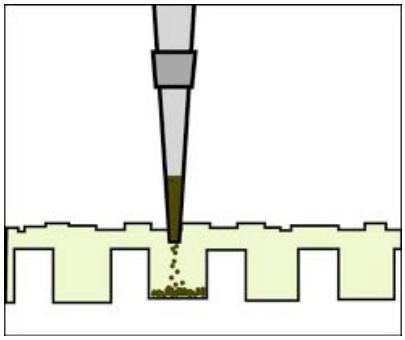


What is loading dye?

- Loading dye accomplishes two objectives.
  1. It makes your sample more dense, so it sinks to the bottom of the well. Reagents used to make the sample more dense include glycerol or 60% sucrose.
  2. It helps you track the sample's progress during electrophoresis. Visible dyes that migrate at consistent rates through the gel during electrophoresis include bromophenol blue, xylene cyanol, cresol red, or yellow food color.

	Migration rate = a doubled-stranded DNA fragment of _ bp	
Dye	**in TBE running buffer**	**in TAE running buffer**
Bromophenol blue	3030 bp	4160 bp
Xylene cyanol	220 bp	370 bp
Cresol red	125 bp	?
Yellow food color	?	?

Carefully pipet the samples into the wells of the agarose gel



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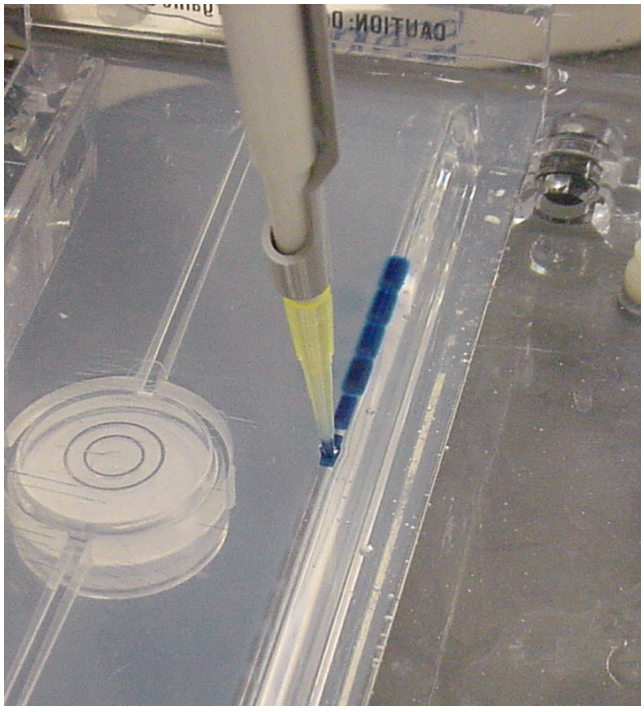

Plug in the electrodes and run the gel at a voltage drop* appropriate to the size of the bands you are trying to detect

***volts per cm from cathode to anode**

length of DNA fragments	voltage drop
< 1 kilobase	5 V / cm
1-12 kilobases	4-10 V / cm
> 12 kilobases	1-2 V / cm

Run the gel until the bromophenol blue and xylene cyanol are separated by 2-3 cm

Visualize and photograph the DNA on a UV-transilluminator