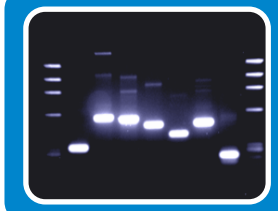


# Agarose gel electrophoresis of DNA

IMBB 2013

**Acknowledgement**

The content of this presentation has been adapted from:

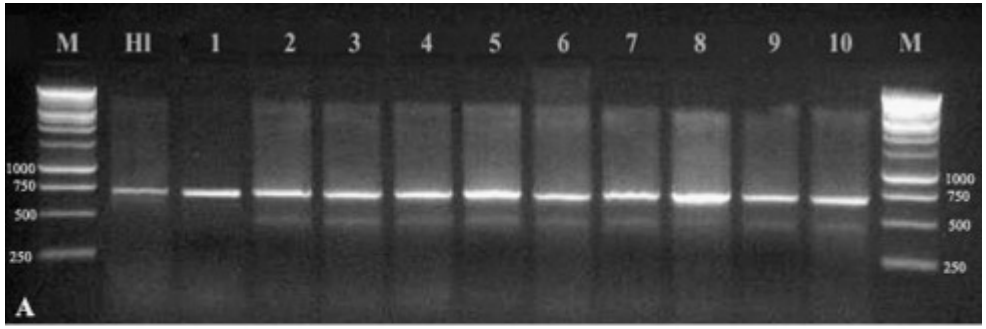
‘Agarose Gel Electrophoresis’ by Michael E. Clark
www.lu-ltspp.ca/.../Gel%20Electorphoresis%20Lecture%202006.ppt

Agarose gel electrophoresis
http://en.wikipedia.org/wiki/Agarose_gel_electrophoresis

# Introduction

Agarose Gel Electrophoresis – uses:

- Estimate the size of DNA molecules
- Analyse PCR products, e.g. in molecular diagnosis or genotyping
- Determine the quality or quantity of DNA
- Purification of DNA



# Introduction

- Nucleic acids are negatively charged molecules in water
- Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field.
- Agarose gel electrophoresis is a simple, cheap and highly effective method for separating, identifying, and purifying DNA fragments.
- We will use agarose gel electrophoresis in the Workshop to determine the size and quality of

    (a) Purified genomic DNA

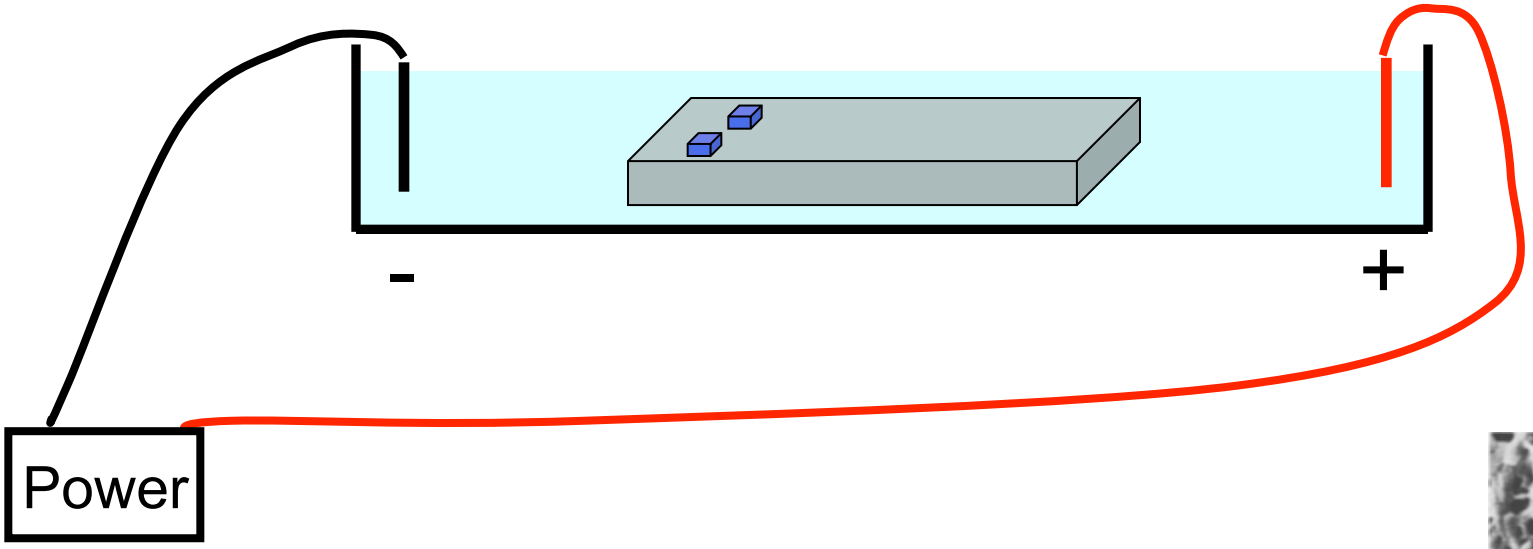
    (b) PCR products

    (c) Restriction enzyme digests of PCR products

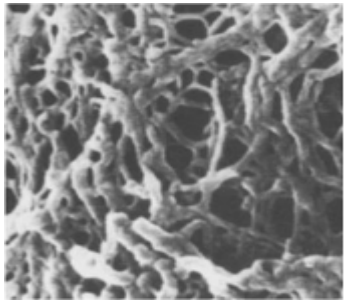
The agarose gel electrophoresis protocol can be divided into three stages:

1. A gel with a DNA dye is prepared with an agarose concentration appropriate for the size of DNA fragments to be separated.
2. The DNA samples are loaded into the sample wells of the gel, and the gel is run at a voltage and time that will achieve optimal separation.
3. The gel is visualized and the image is recorded.

- DNA is negatively charged
- When placed in an electrical field, DNA will migrate toward the positive pole (anode).
- An agarose gel behaves as a molecular sieve, slowing the movement of DNA and separating by size.




Polymerized agarose is porous, allowing for the movement of DNA

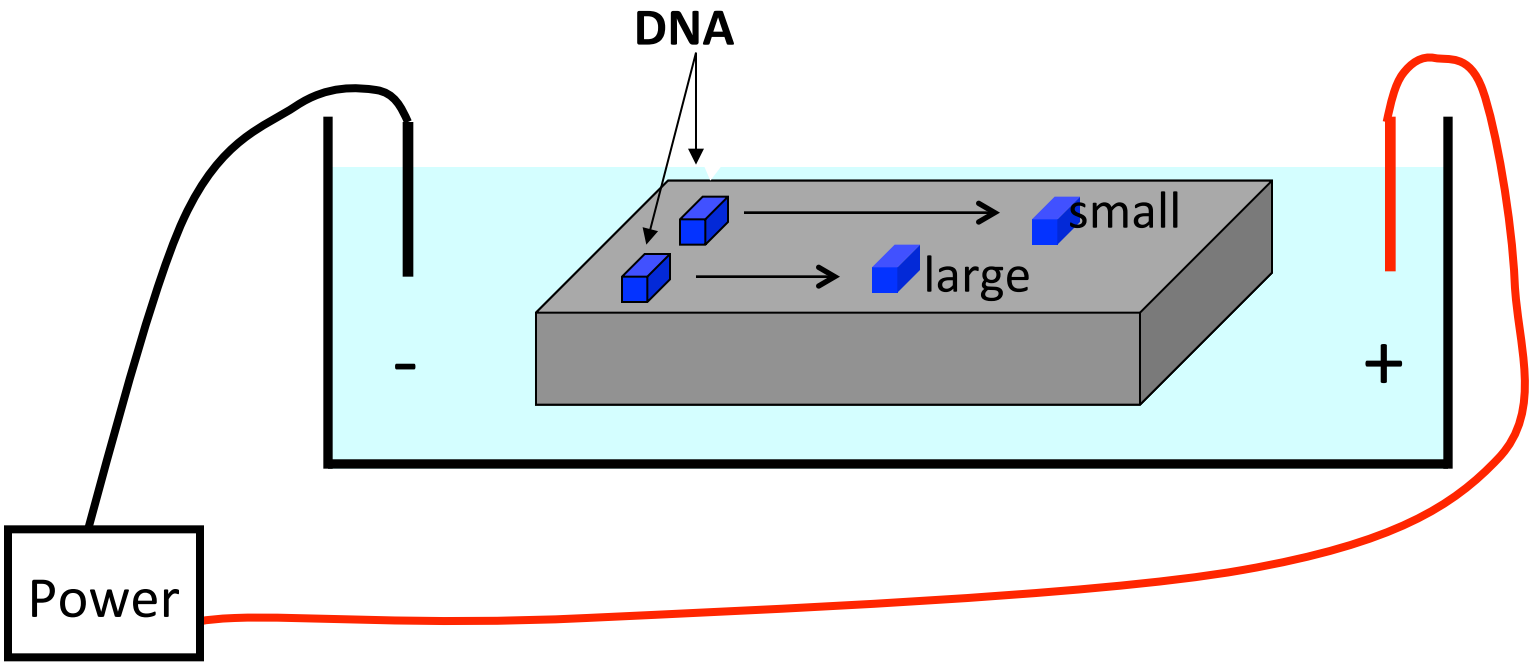


# Background information

## How fast will the DNA migrate?

Speed of migration depends on

- Strength of the electrical field, buffer, density of agarose gel.
- Size of the DNA ! *(Small DNA move faster than large DNA. Gel electrophoresis separates DNA according to size).*

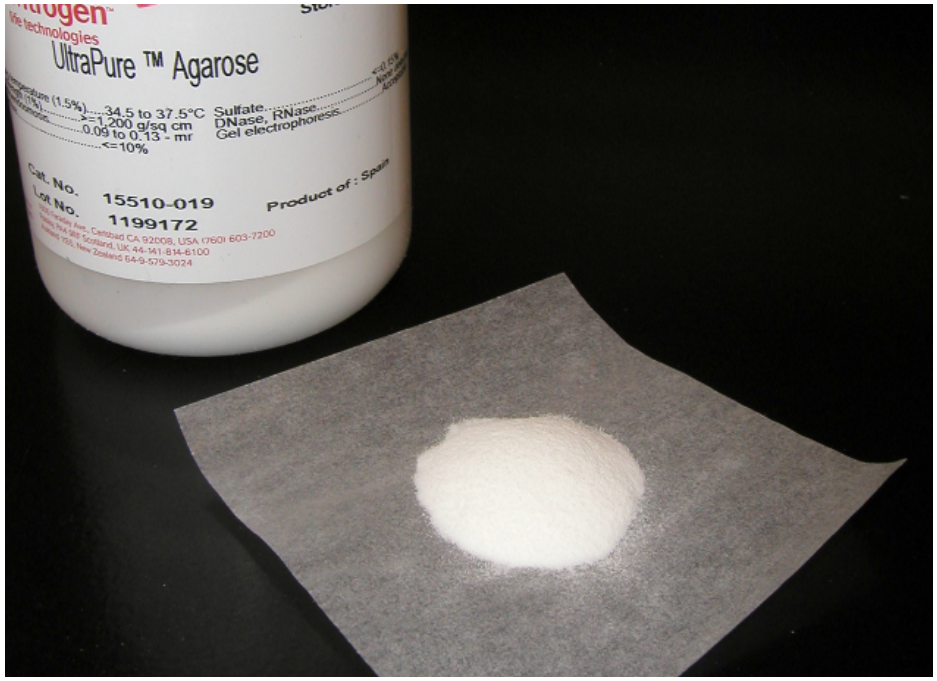



Within an agarose gel, rate of migration of a linear DNA molecule is inversely proportional to the log10 of the molecular weight.

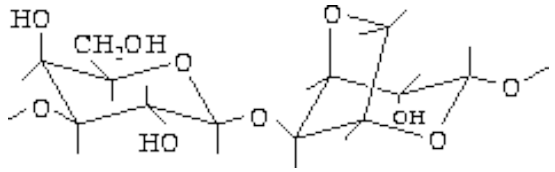
# Movies

Making an Agarose Gel - University of Leicester.flv
Running an Agarose Gel - University of Leicester.flv

Agarose is a linear polymer extracted from seaweed



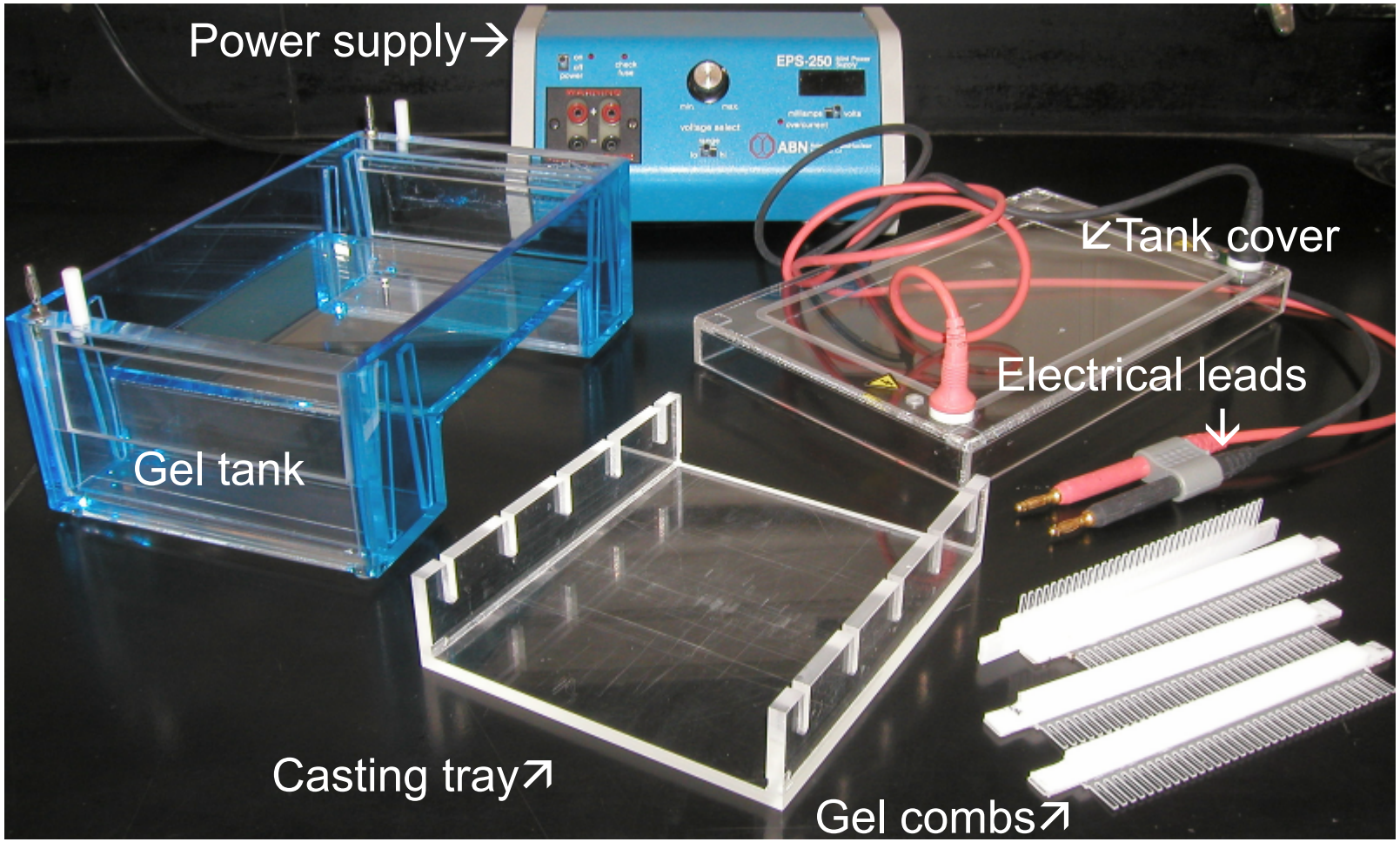

Agarose




D-galactose

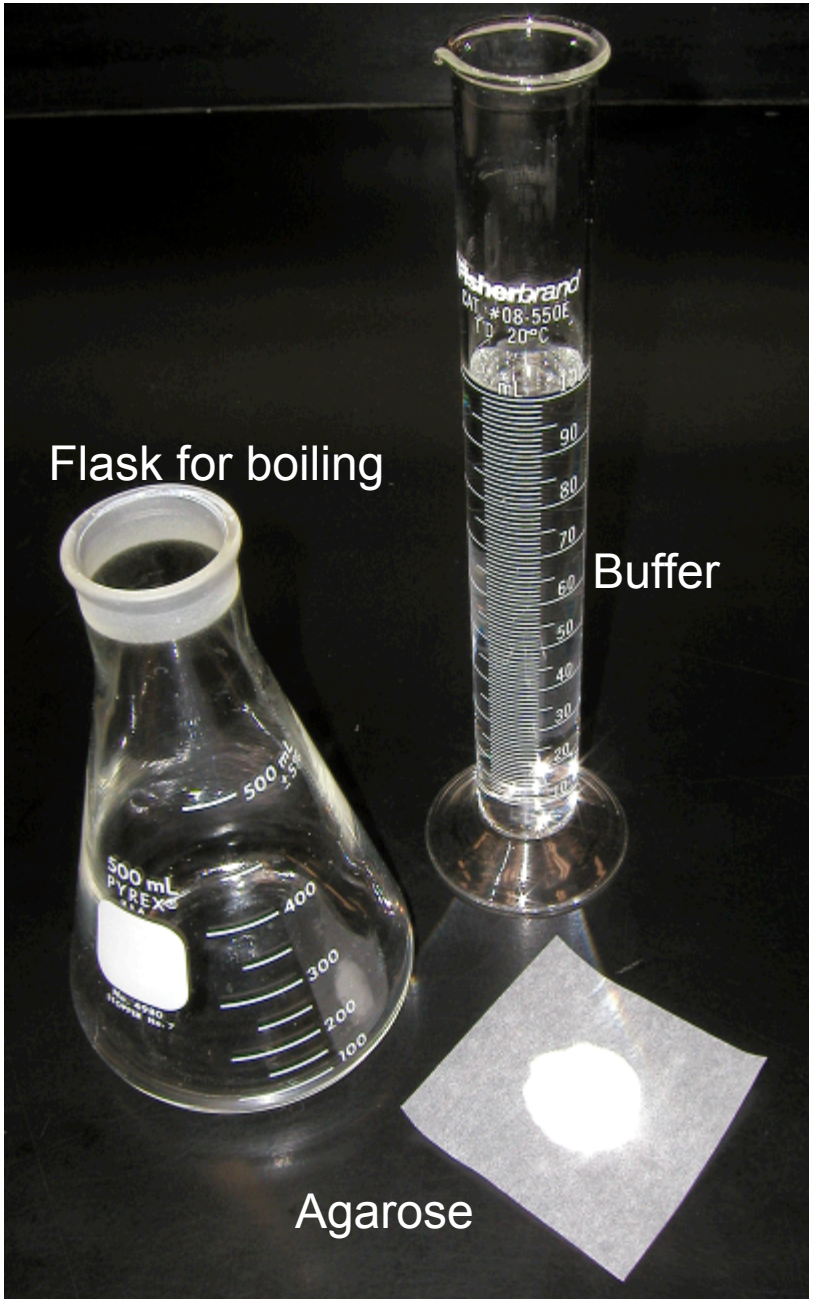
3,6-anhydro
L-galactose

# Electrophoresis equipment

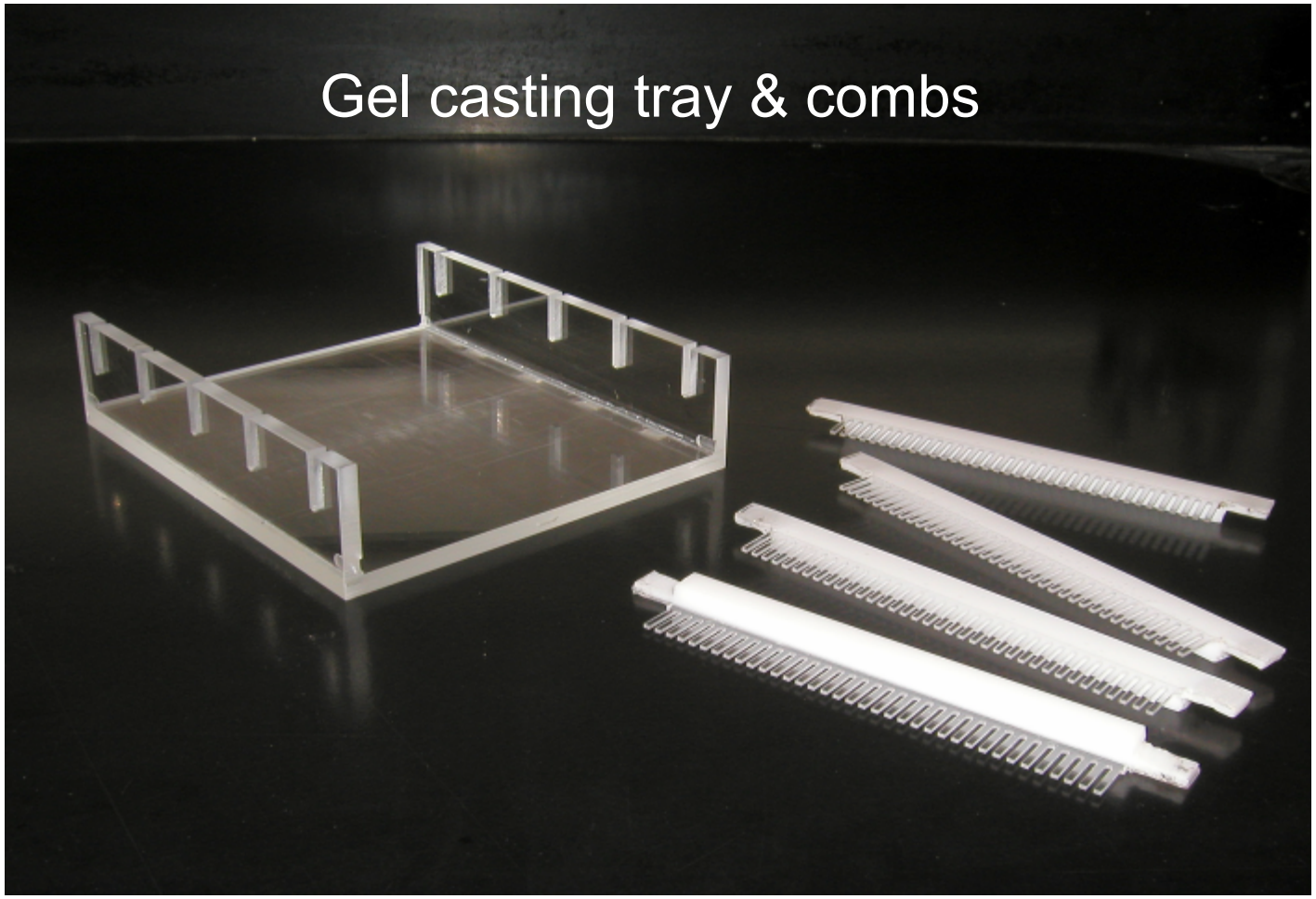


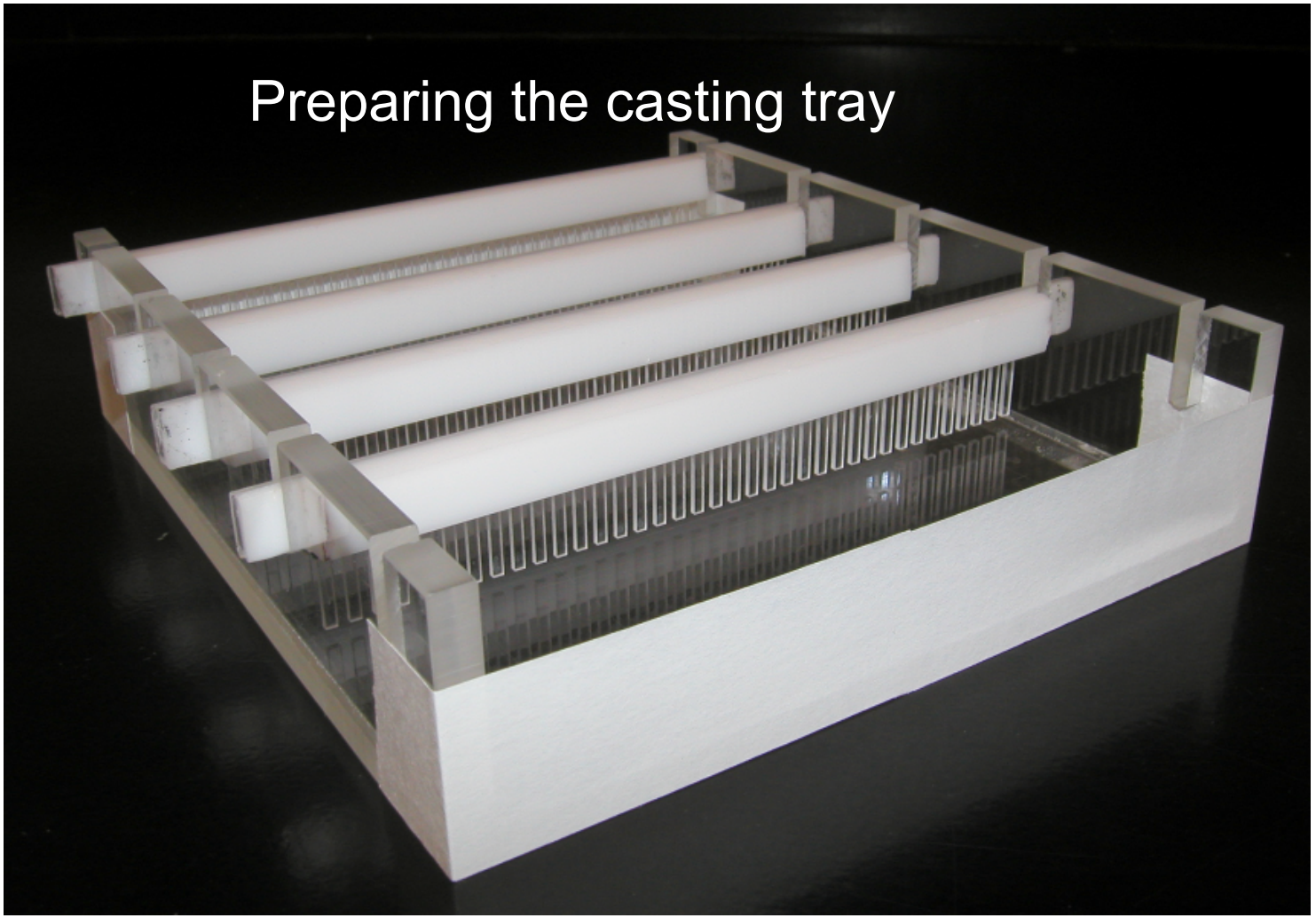
# Making an agarose Gel

An agarose gel is prepared by combining agarose powder and a buffer solution.



# Gel casting tray & combs





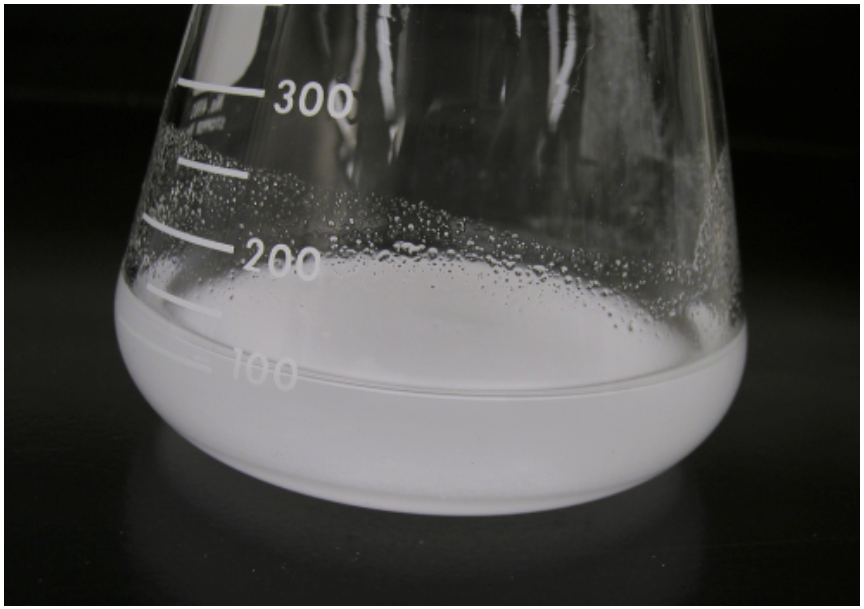

- Seal the edges of the casting tray and put in the combs.
- Place the casting tray on a level surface.
- None of the gel combs should touch the surface of the casting tray.

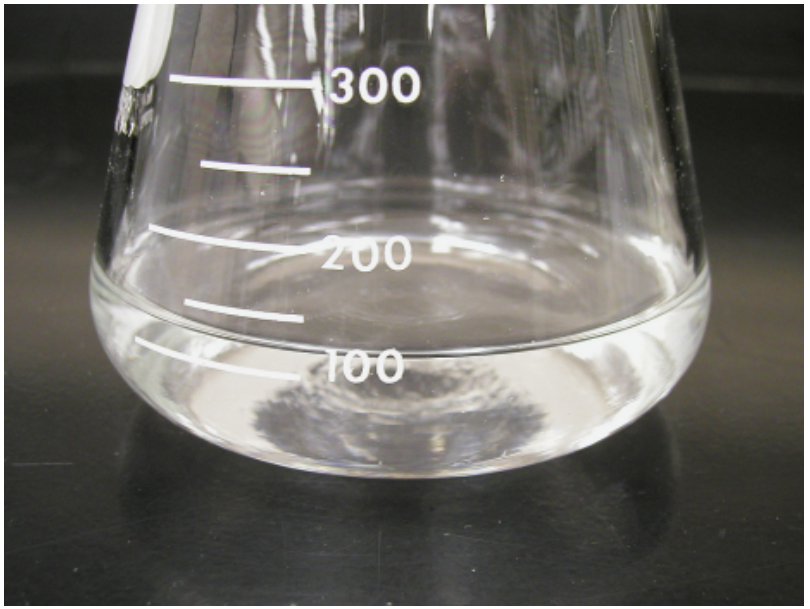
Agarose

Buffer Solution

- Combine the agarose powder and buffer solution (1-2% agarose).
- Use a flask that is several times larger than the volume of buffer.
- Swirl gently to give a uniform suspension (no lumps!).

# Melting the agarose

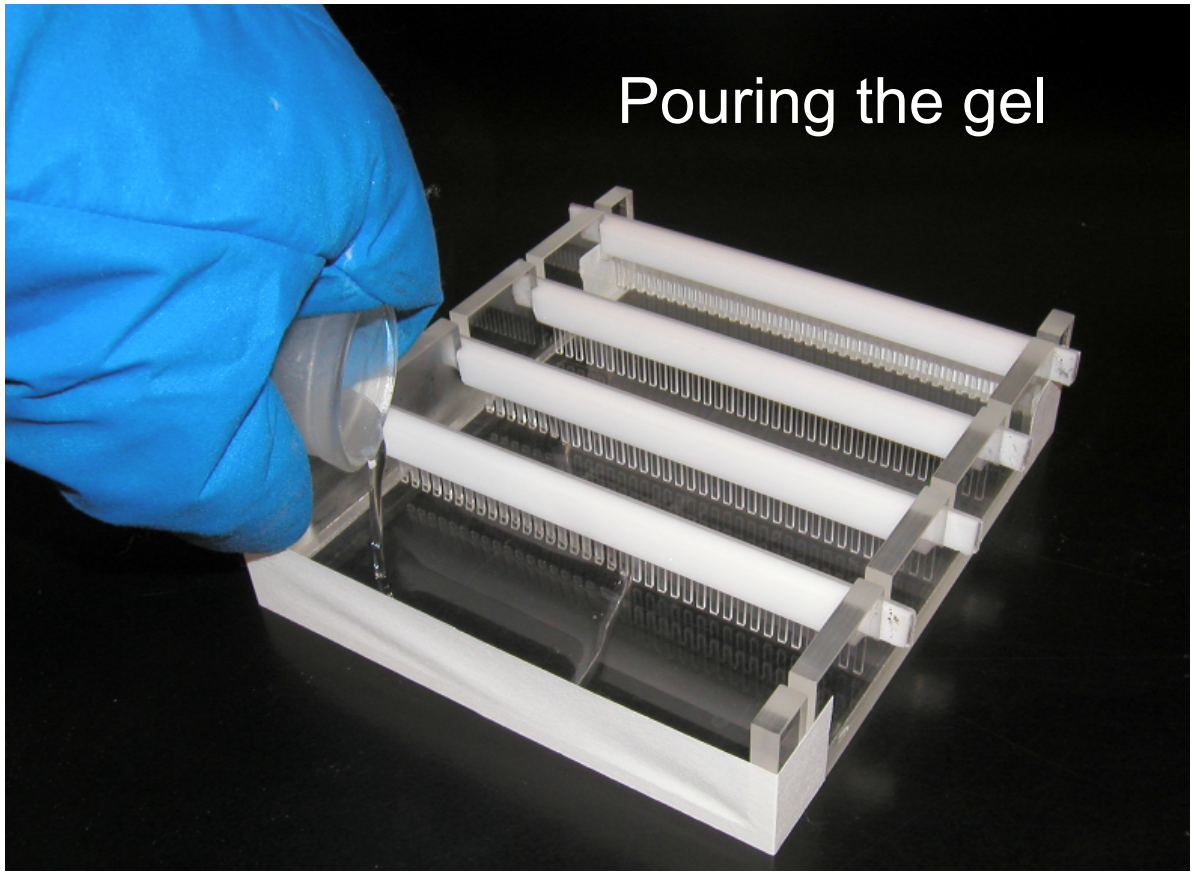


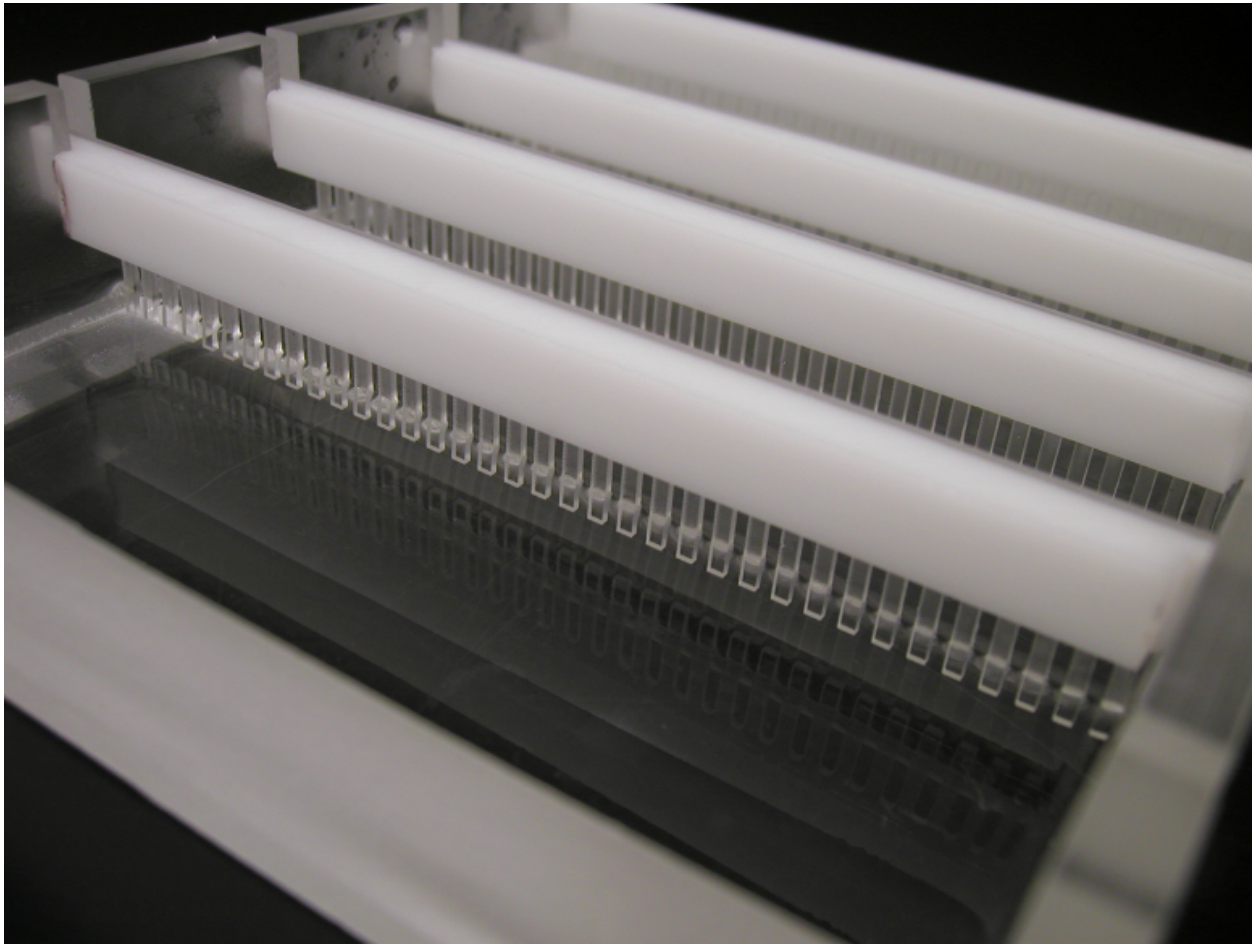

Agarose is insoluble at room temperature (left)

The agarose solution is boiled until clear (right)

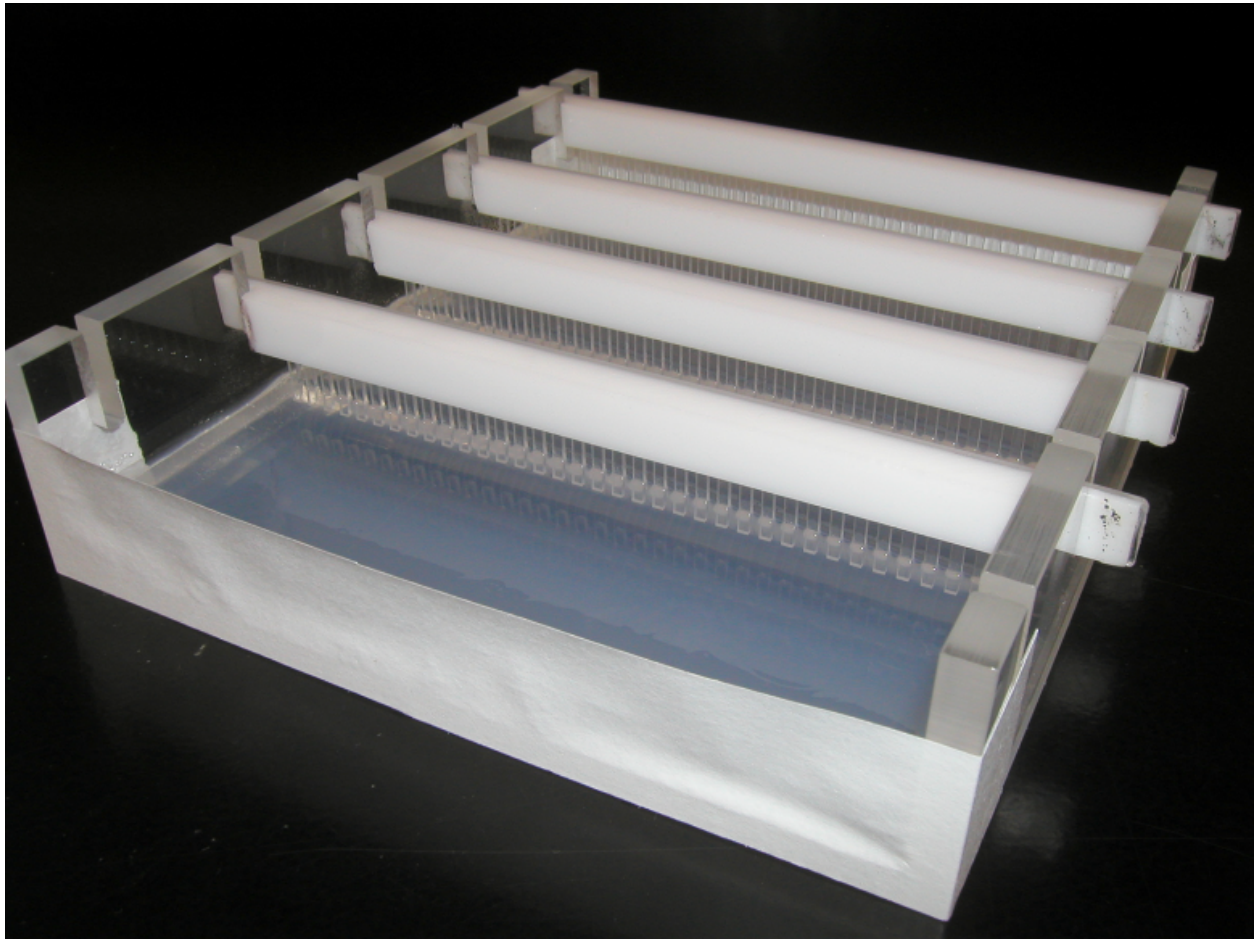
- The easiest way to boil the mixture is with a microwave oven.
- Gently swirl the solution periodically when heating to allow all the grains of agarose to dissolve.
- Be careful when boiling - the agarose solution may become superheated and may boil violently if it has been heated too long in a microwave oven.



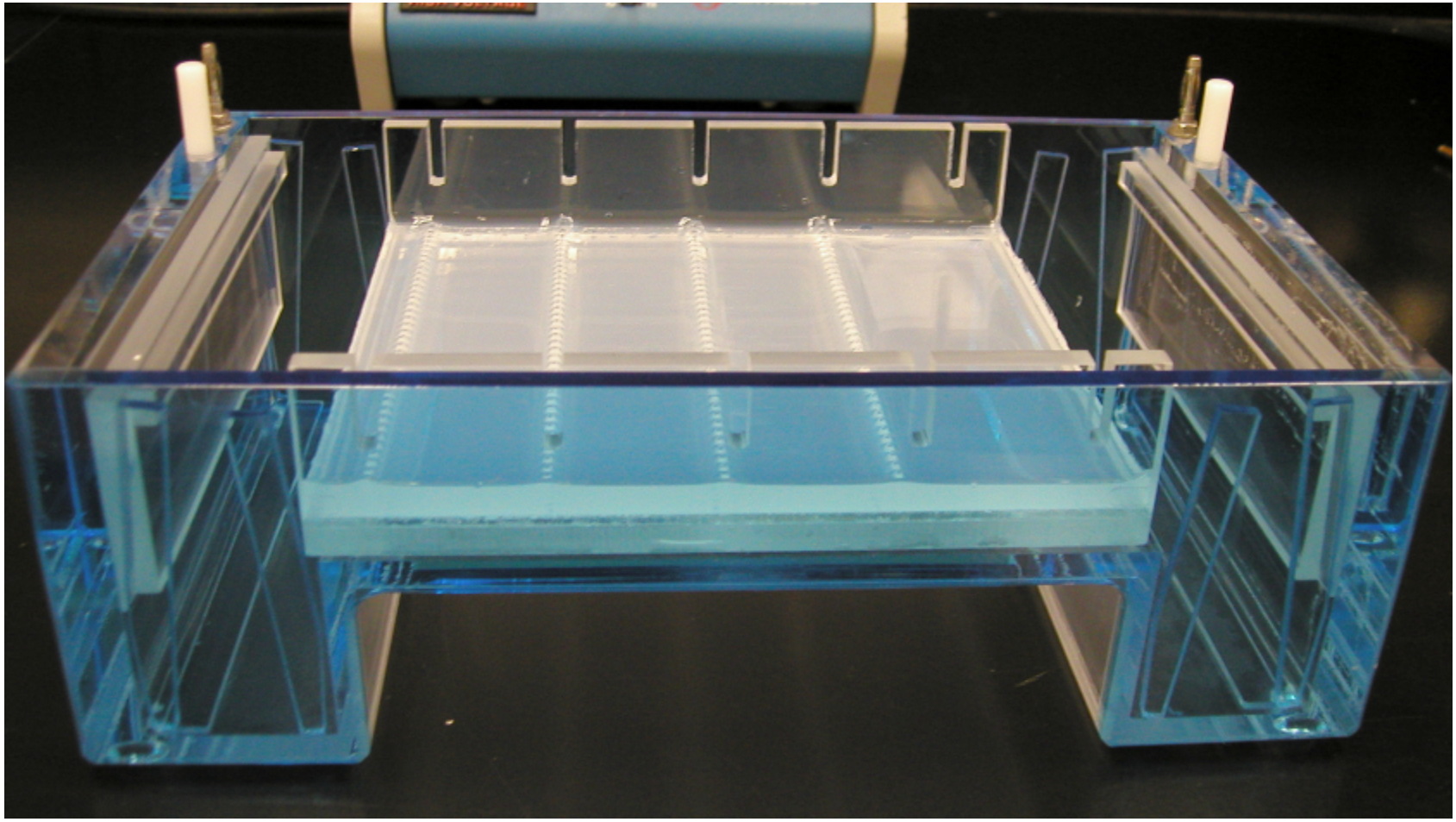

- Allow the agarose solution to cool by placing the flask in a water bath set at 55$^{o}$C.
- Add GelRed DNA dye, and mix by swirling.
- Then carefully pour the melted agarose solution into the casting tray.
- Avoid air bubbles.



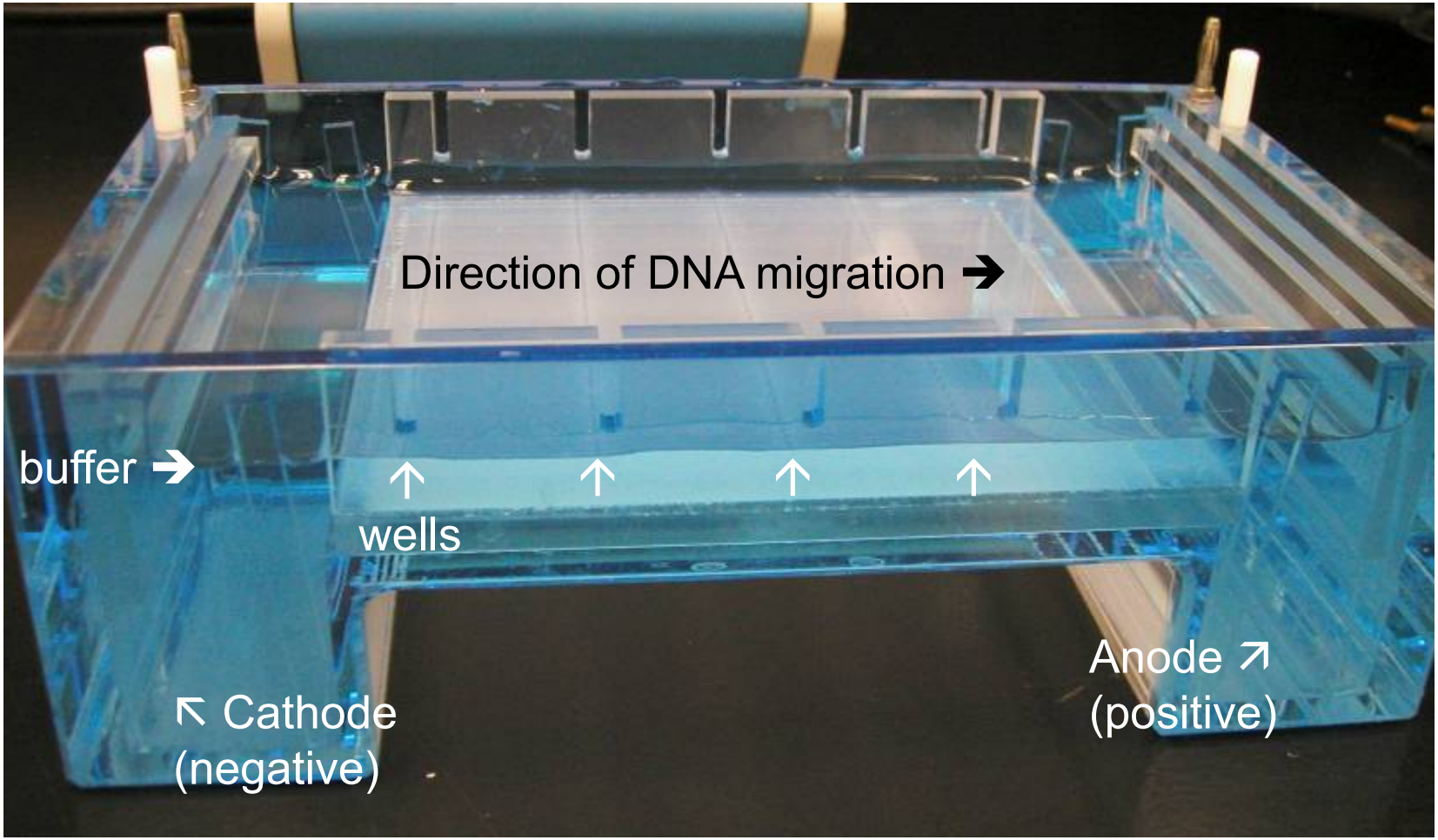
- Each of the gel combs should be submerged in the melted agarose solution.
- Remove air bubbles with a pin or pipette tip.



- When cooled, the agarose polymerizes, forming a flexible gel. It should appear lighter in color when completely cooled (30-45 minutes).
- Carefully remove the combs and tape.
- The gel can be used immediately or stored (wrapped in cling film) in a fridge for a few days before use.



Place the gel in the electrophoresis chamber.

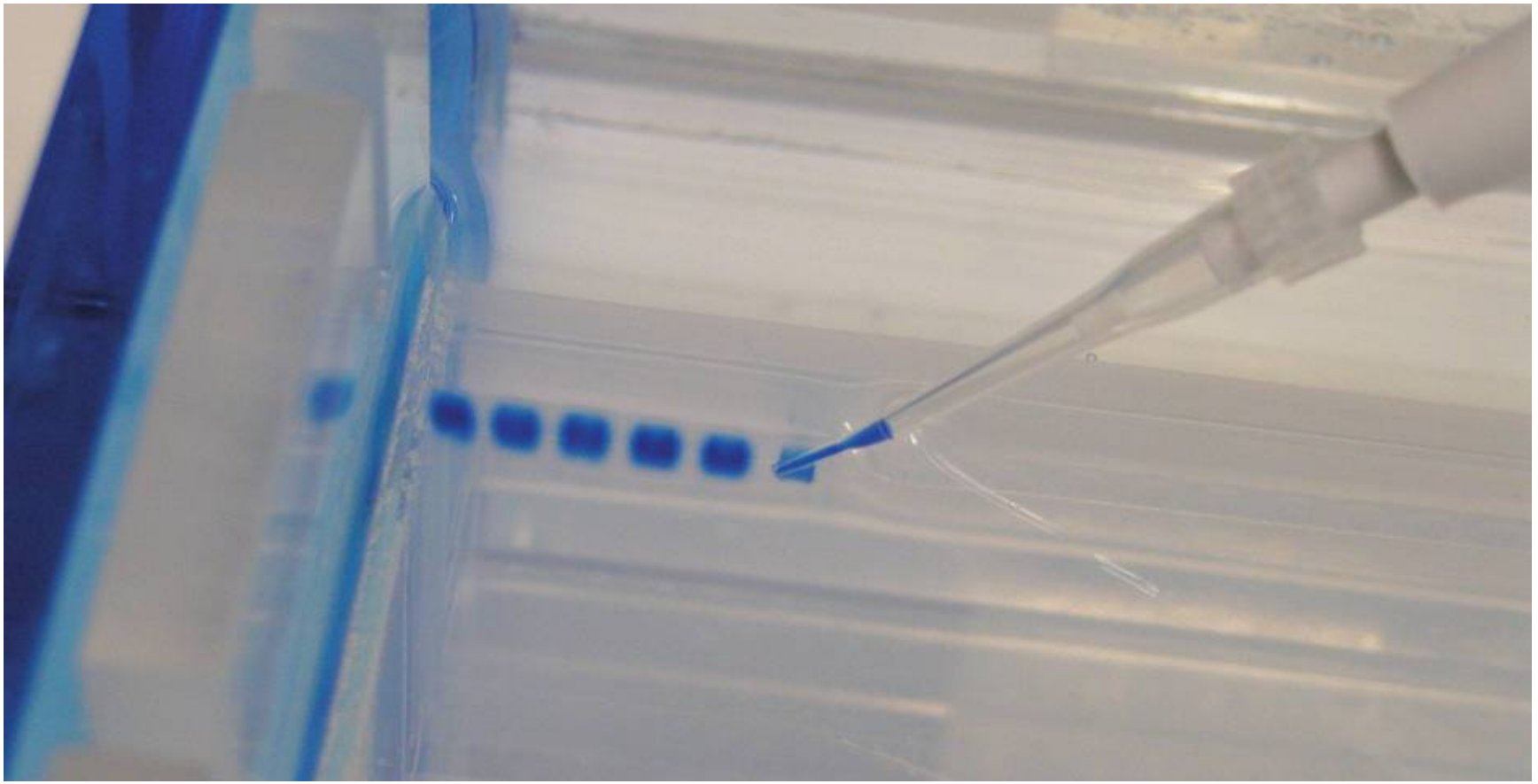



- Add enough electrophoresis buffer to cover the gel to a depth of at least ~2 mm.
- Make sure each well is filled with buffer.

# Sample Preparation

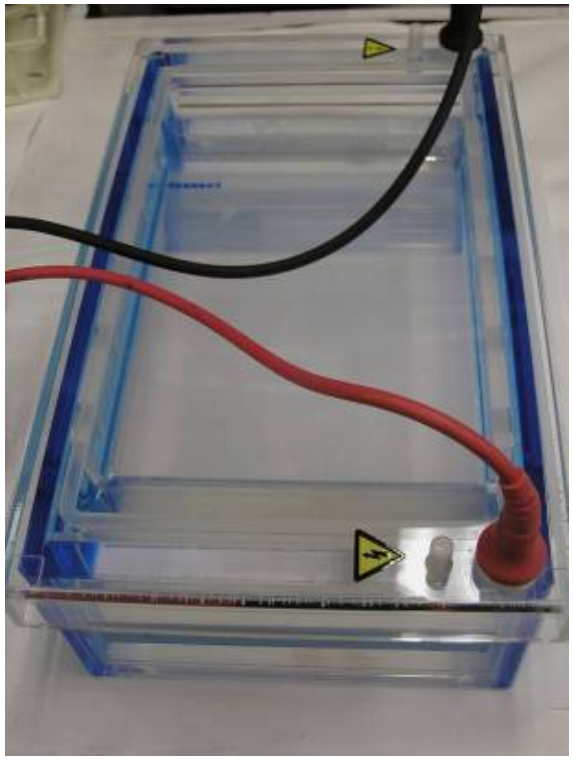
- Mix the samples of DNA with 2X sample loading buffer containing a dye. (Mix 1 part loading buffer with 1 parts DNA sample).
- Approximately 5-10 microlitres (µL) of sample are loaded into a well, depending on the size of the well.
- The dye in the loading buffer allows the samples to be seen when loading onto the gel. Glycerol in the loading buffer increases the density of the samples, causing them to sink into the wells.
- The loading buffer dye, which migrates during electrophoresis, also helps to monitor the progress of the separation.

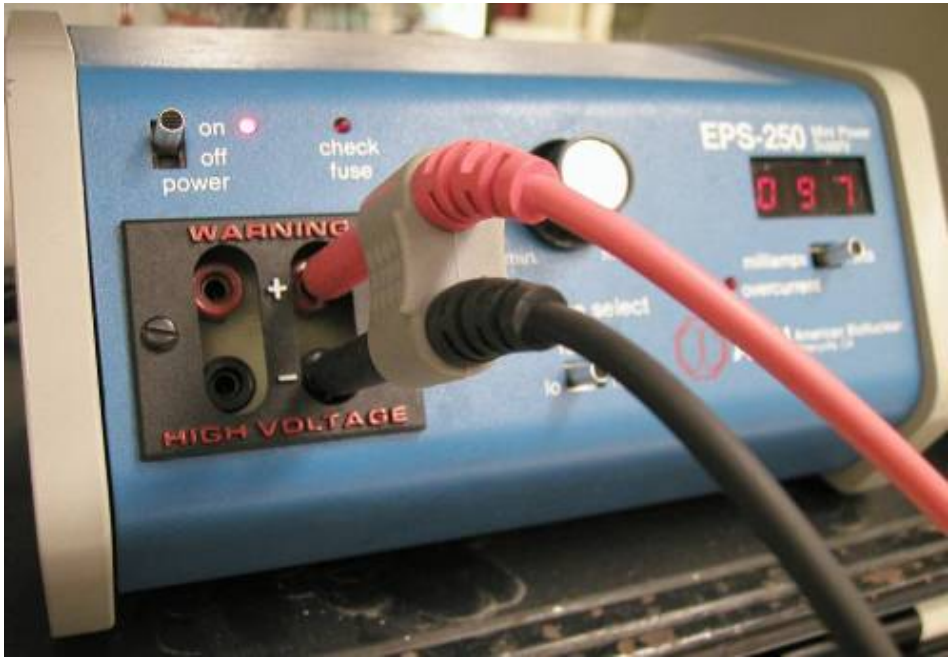
# Loading the Gel



- Carefully place the pipette tip over a well and gently expel the sample.
- The sample should sink into the well.
- Be careful not to puncture the gel with the pipette tip.

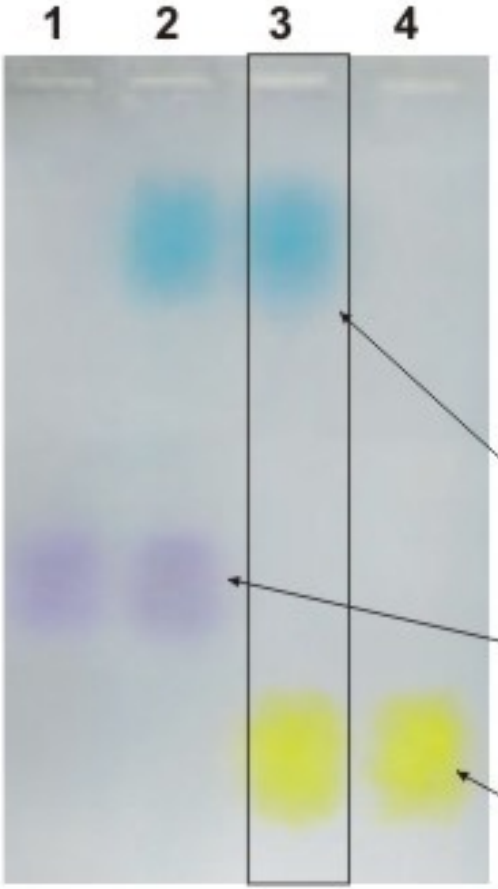
# Running the Gel






- Place the cover on the electrophoresis chamber, connecting the electrical leads.
- Connect the electrical leads to the power supply.
- Be sure the leads are attached correctly - DNA migrates toward the anode (red).
- When the power is turned on, bubbles should form on the electrodes in the electrophoresis chamber.

# 6x DNA Loading Dye




**Line 1:** DNA L.Dye Buffer Blue
**Line 2:** DNA L.Dye Buffer Double Blue
**Line 3:** DNA L.Dye Buffer Orange, Blue
**Line 4:** DNA L.Dye Buffer Orange

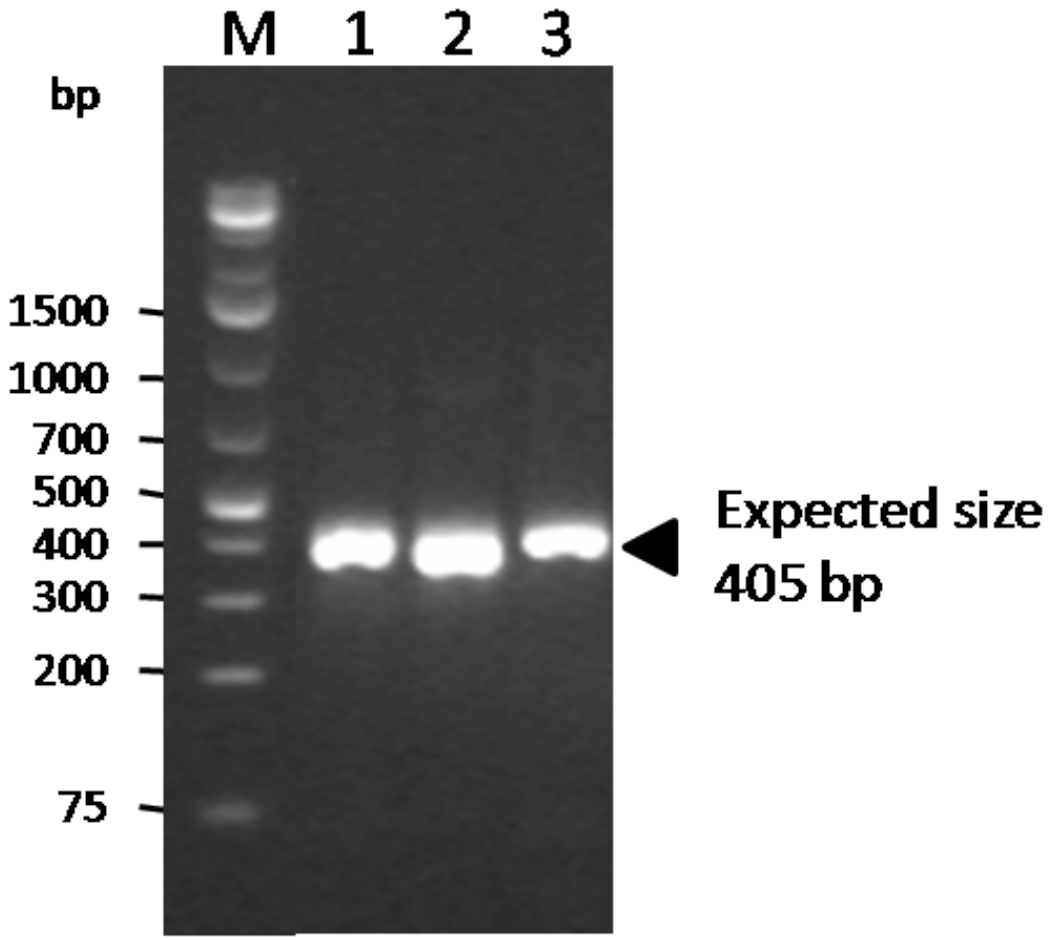
**In 1% agarose gel 1x TBE**

**Xylene Cyanol FF** migrates along with~3500 bp fragments,

**Bromophenol Blue** migrates along with ~300 bp fragments and

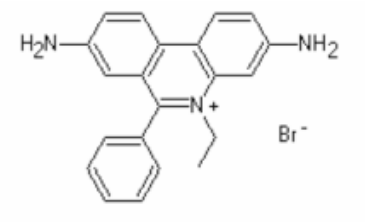
**Orange G** migrates along with ~40 bp

Including a DNA ladder enables the estimation of sample DNA size

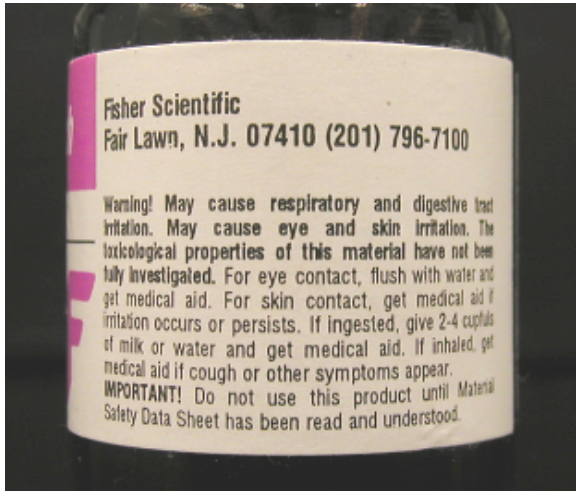


# Staining the DNA

**Ethidium bromide** has been the most commonly used DNA stain for many years.



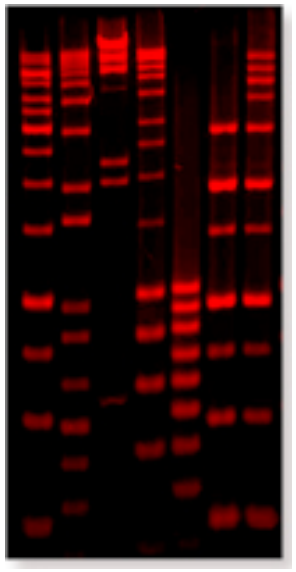





***CAUTION!**

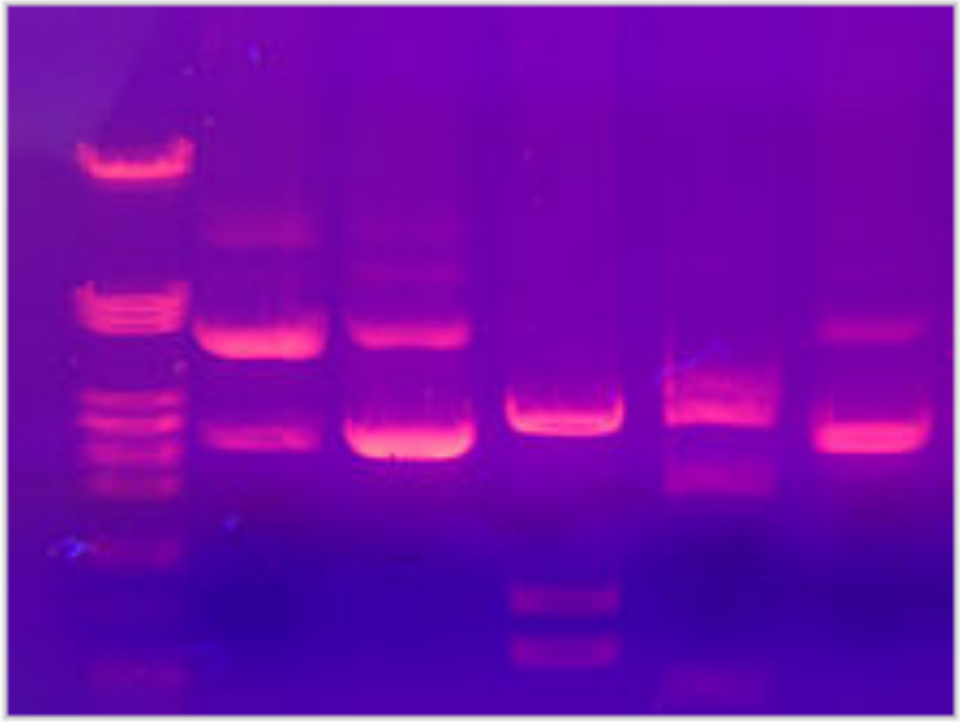
- Ethidium bromide is a **powerful mutagen** and is moderately toxic.
- **Gloves should be worn at all times**.
- Only purchase a solution of ethidium bromide, not powder.
- Dispose of waste correctly.

# GelRed, a safer alternative to Ethidium Bromide

- New generation of safe nucleic acid gel stains
- Cannot penetrate latex gloves and cell membranes
- Non-cytotoxic and non-mutagenic
- Not classified as hazardous waste, thus can be safely disposed of down the drain or as regular trash, providing convenience and reducing cost in waste disposal.

GelRed requires an ultraviolet light or a blue light source to visualize the DNA



A pattern of DNA-bands under UV light

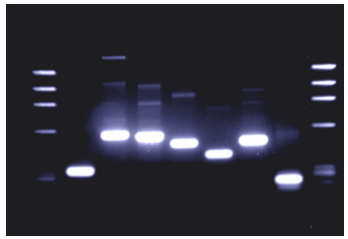
**Further information**

**Books**

Buckingham and Flaws (2007) Molecular Diagnostics
Micklos, Freyer, Crotty (2003) DNA Science: a first course
Sambrook and Russell (2001) Molecular Cloning (3rd edition)

**Websites**

Construct your own electrophoresis chamber
http://learn.genetics.utah.edu/content/labs/gel/gelchamber/
Virtual Gel Electrophoresis
http://gslc.genetics.utah.edu/units/biotech/gel/
Agarose gel electrophoresis - Wikipedia, the free encyclopedia
http://en.wikipedia.org/wiki/Agarose_gel_electrophoresis
Gel Electrophoresis animation
http://www.dnalc.org/ddnalc/resources/electrophoresis.html
Agarose gel electrophoresis animation
http://bcs.whfreeman.com/thelifewire/content/chp16/1601s.swf
Gel Red slide presentation
http://biotium.com/product/product_info/other/GelRed%20&%20GelGreen%20Slides.pdf



Thank you