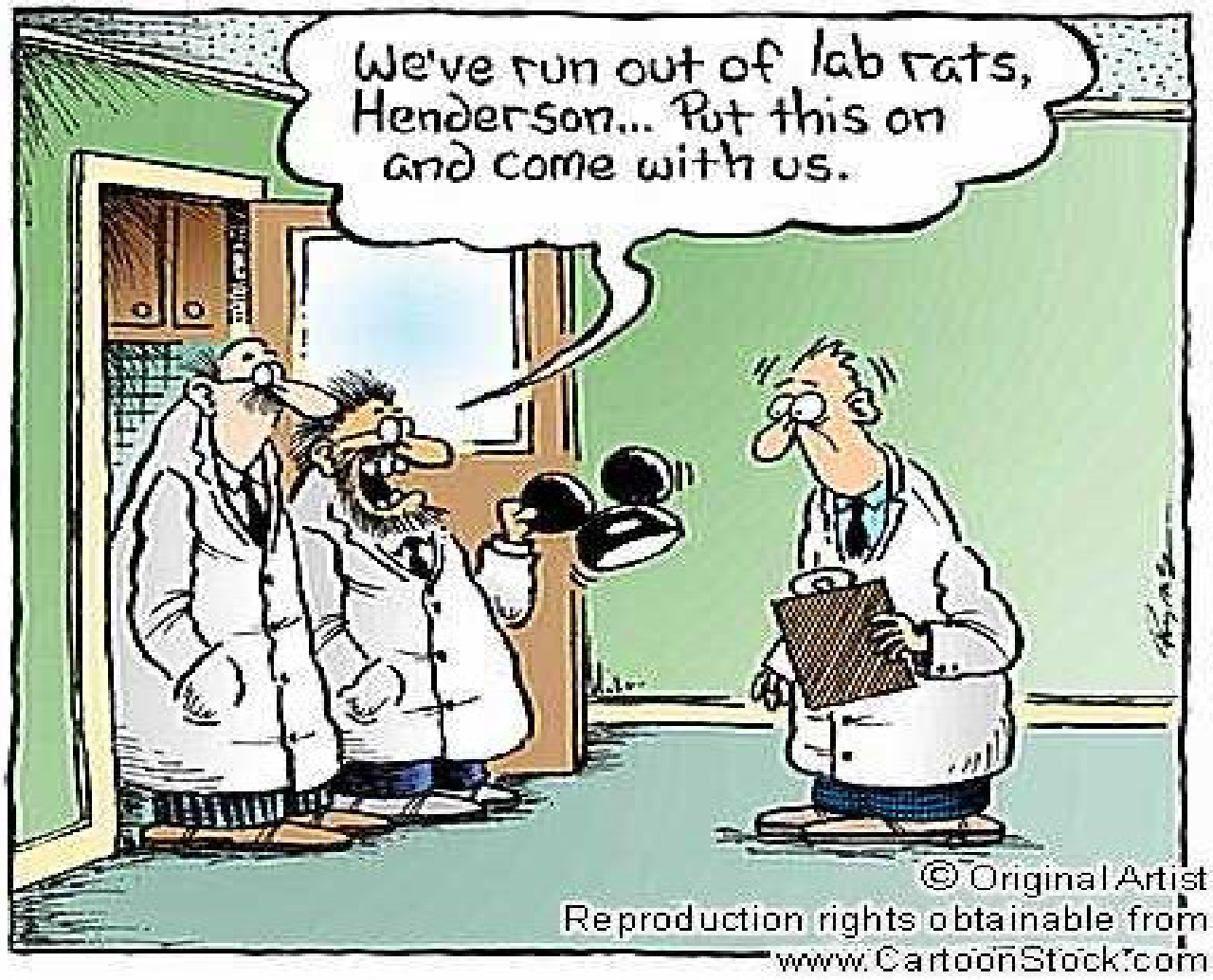
Agarose Gel Electrophoresis Cloning



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PCR reaction

Reagents in a tube

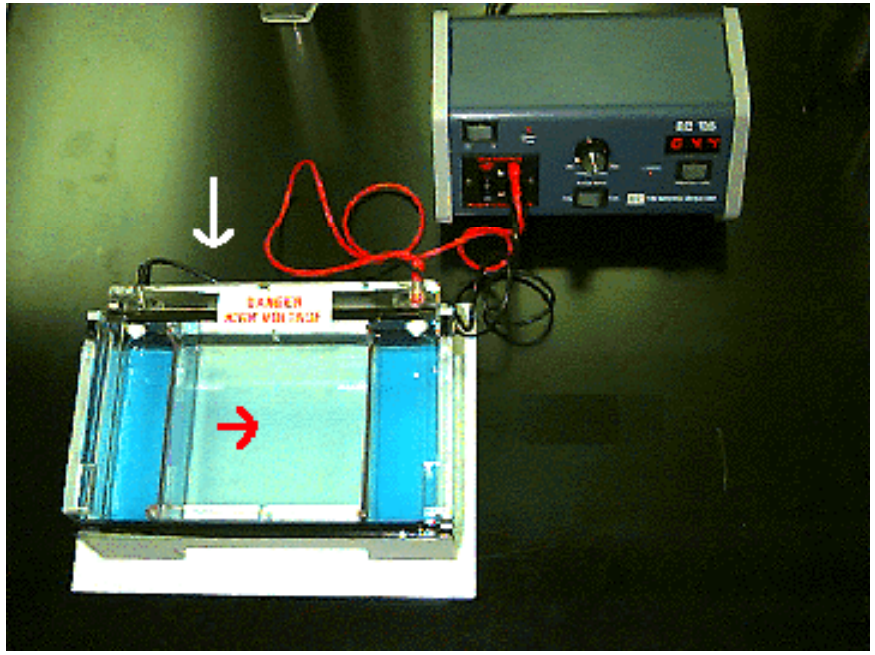
- Sample (DNA)
- Primers (forward and reverse)
- Enzyme (DNA polymerase, *Taq)*
- dNTPs (A, T, G, C)
- Buffer (50 mM KCl; 10 mM Tris-HCl; 1.5 mM MgCl2)
- Water DNAase free water

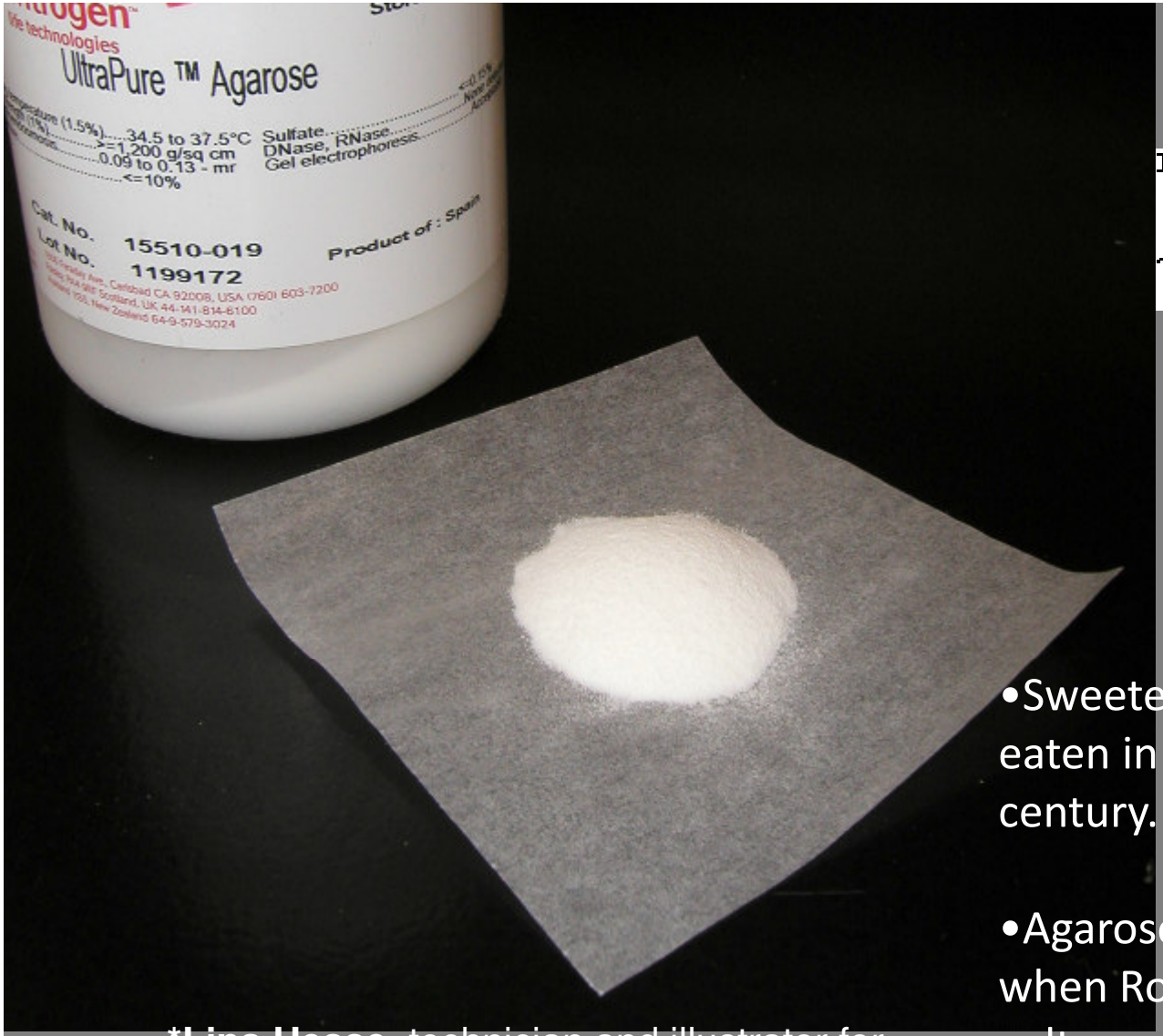
+

- **Gene of interest (Billions of copy)**

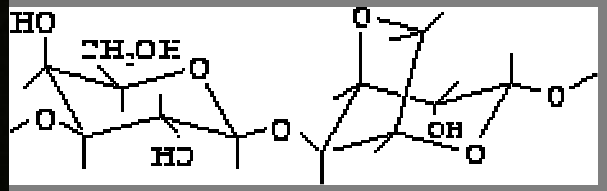
What is Agarose Gel Electrophoresis?

- Electrophoresis: Movement of charged particles through a solution (gel-agarose) under the influence of an electric field
- DNA fragments have a negative (-) charge and will move toward the positive electrode





Agarose



D-galactose

3,6-anhydro L-galactose

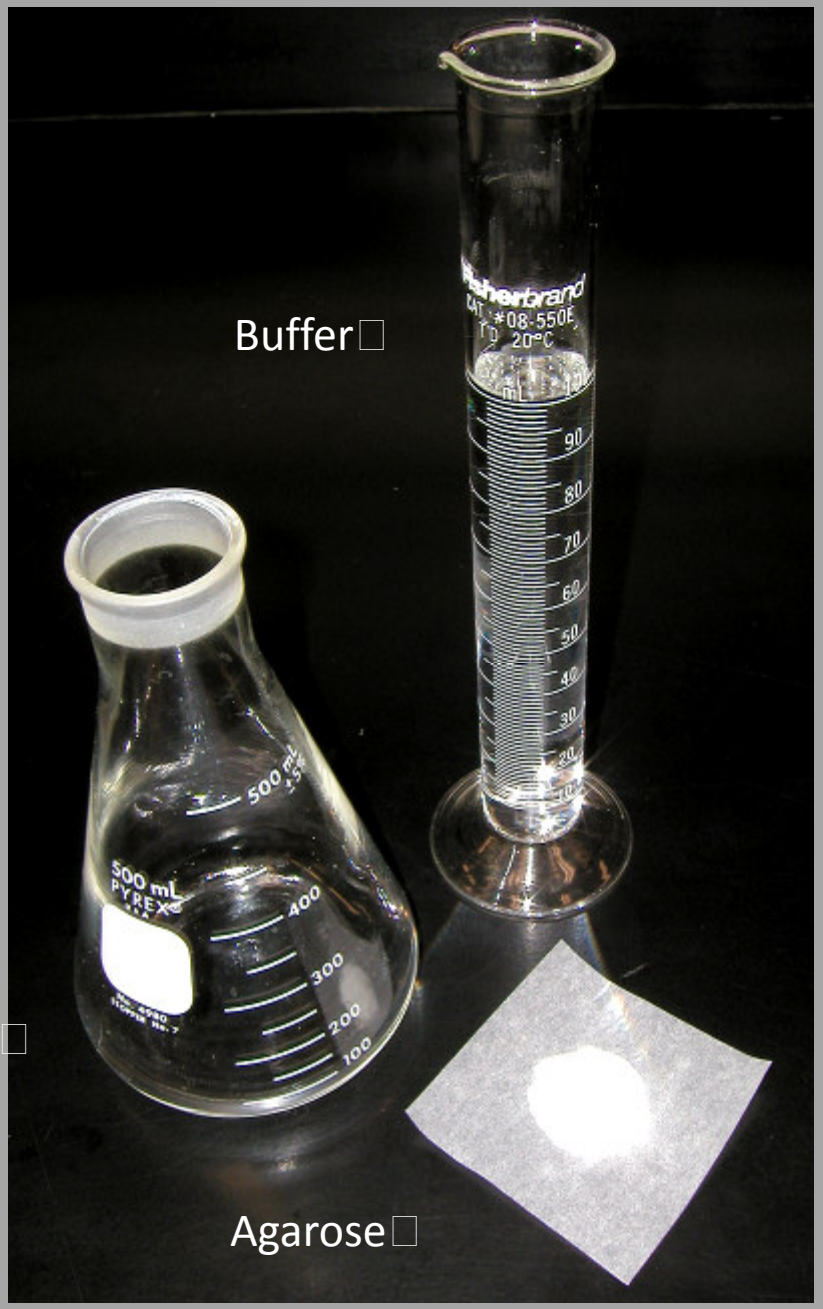
- Sweetened agarose gels have been eaten in the Far East since the 17th century.

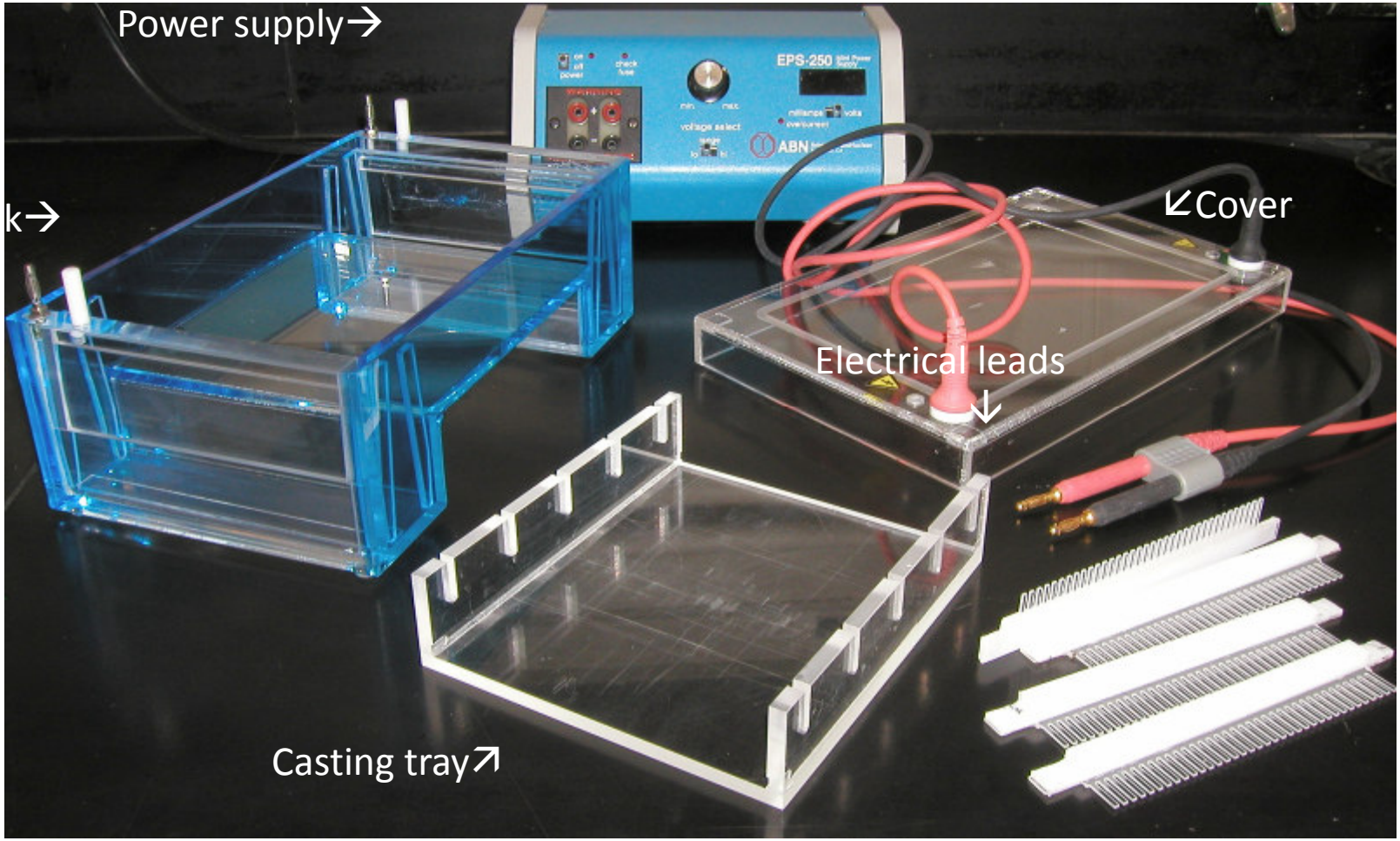
- Agarose was first used in biology when Robert Koch* used it as a culture medium for Tuberculosis bacteria in 1882

*__Lina Hesse__, technician and illustrator for a colleague of Koch was the first to suggest agar for use in culturing bacteria

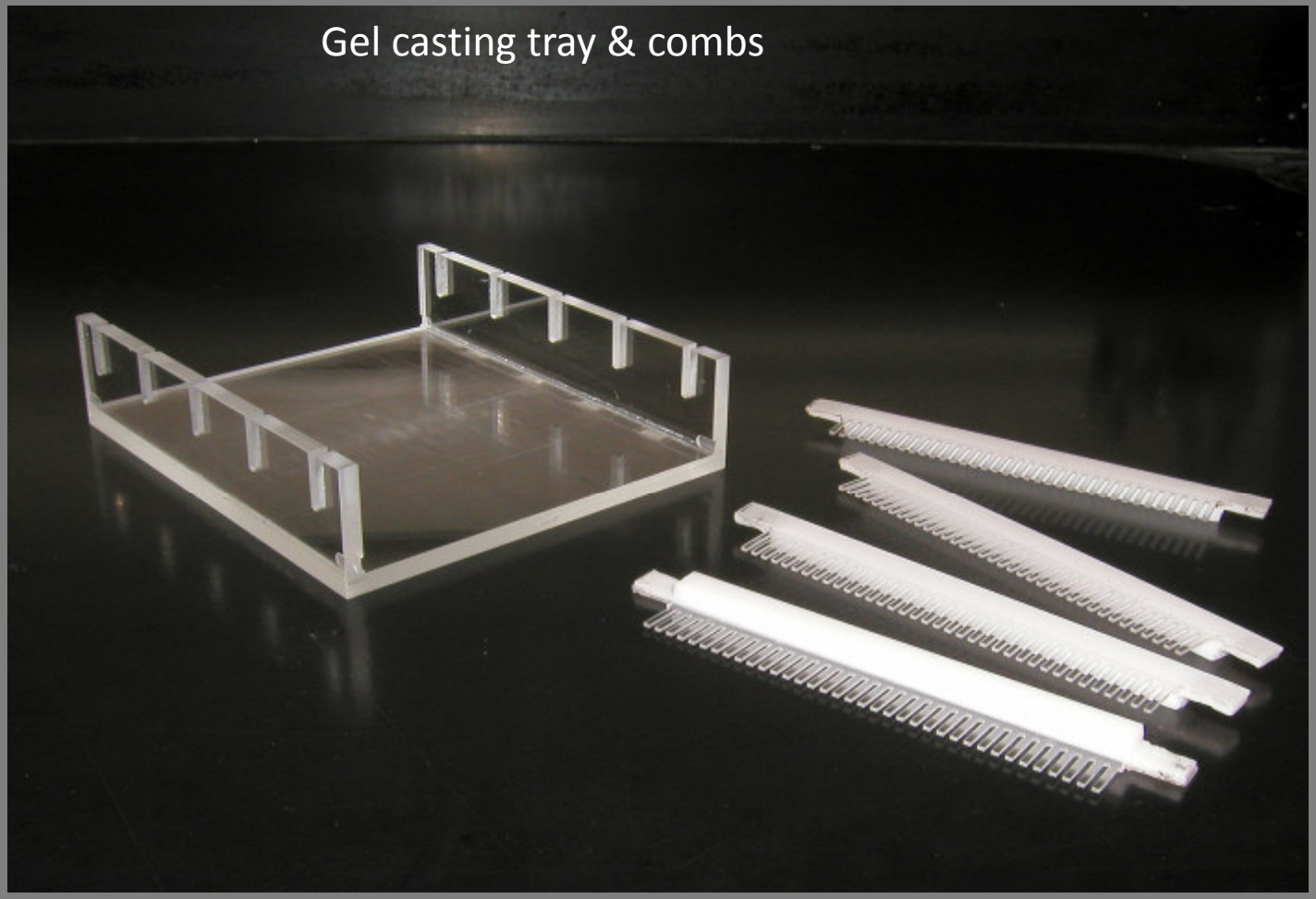
Agarose is a linear polymer extracted from seaweed.

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

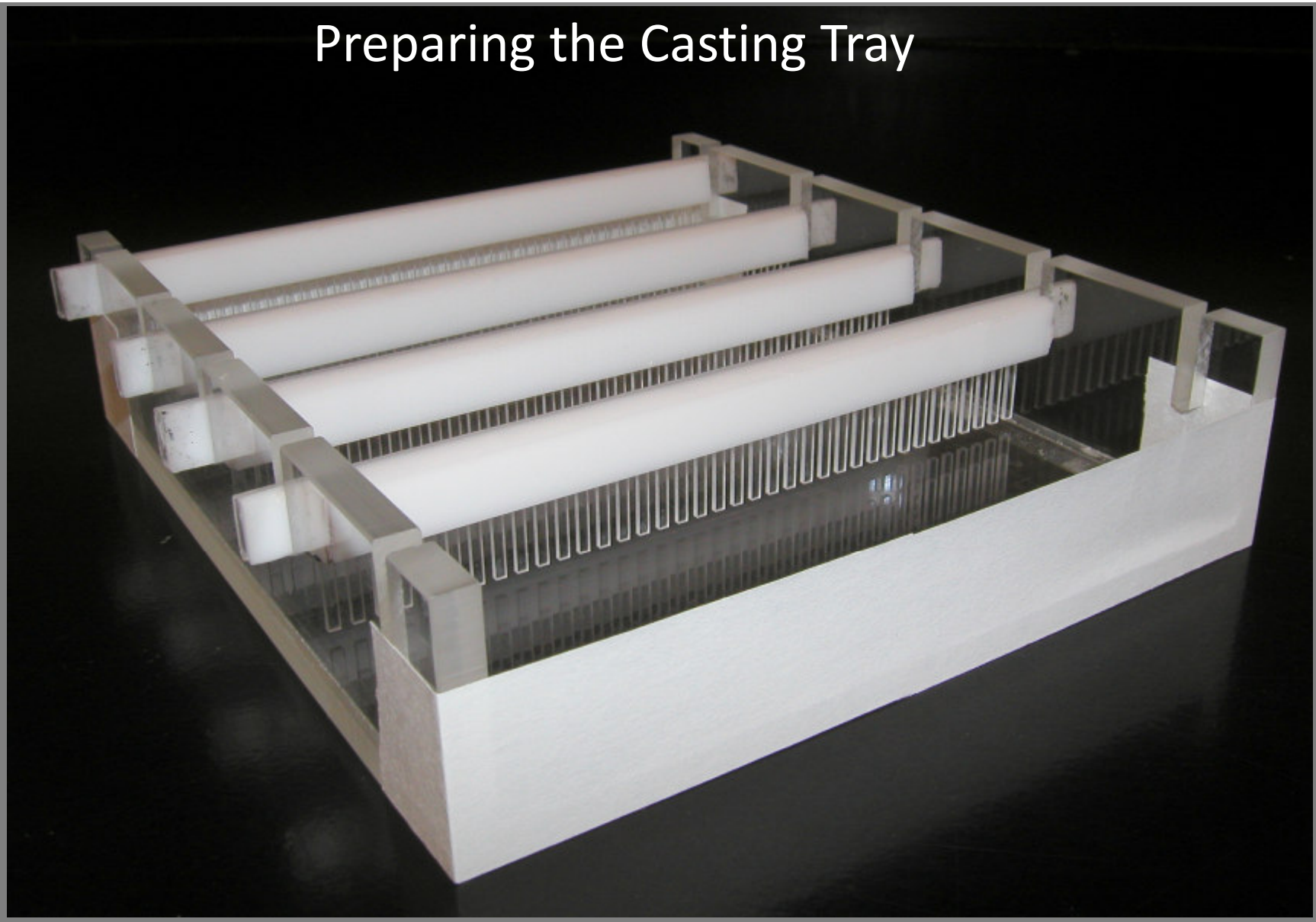



Power supply→
k→
↙Cover
Electrical leads
↓
Casting tray↗
EPS-250
ABN

Gel casting tray & combs



Preparing the Casting Tray



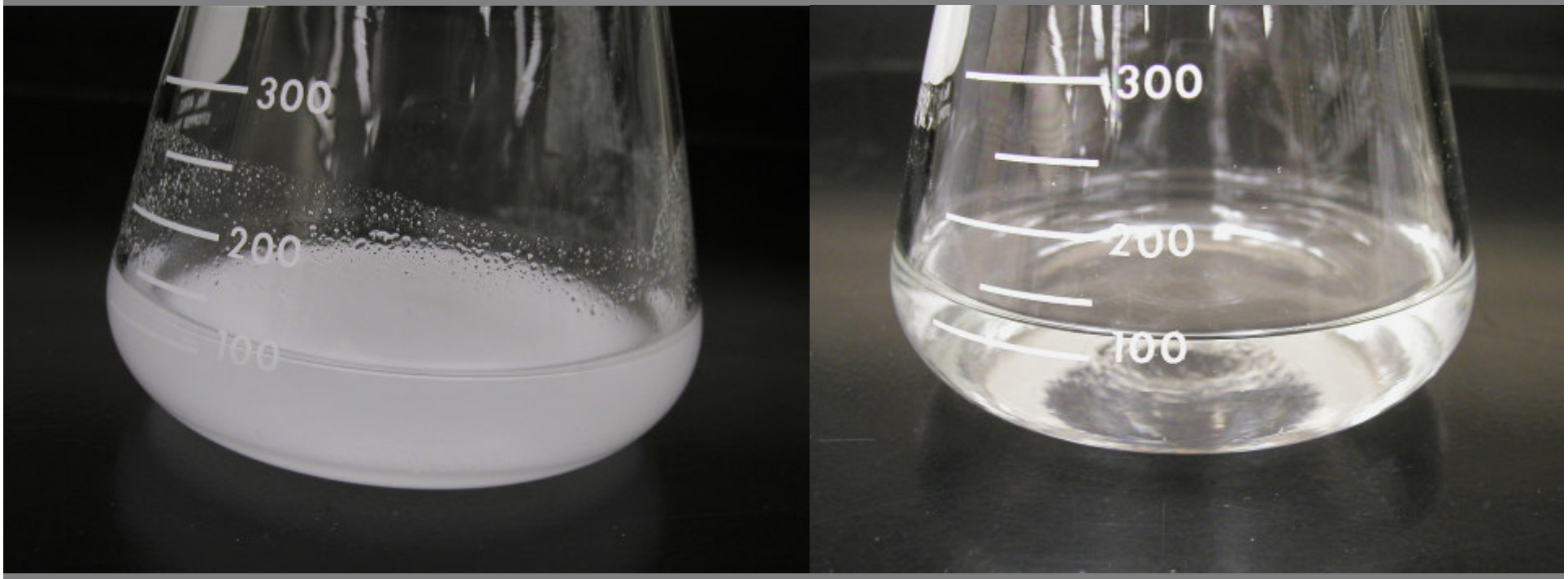
Seal the edges of the casting tray and put in the combs. Place the casting tray on a level surface. None of the gel combs should be touching the surface of the casting tray.

Agarose

Buffer Solution

Combine the agarose powder and buffer solution. Use a flask that is several times larger than the volume of buffer.

Melting the Agarose



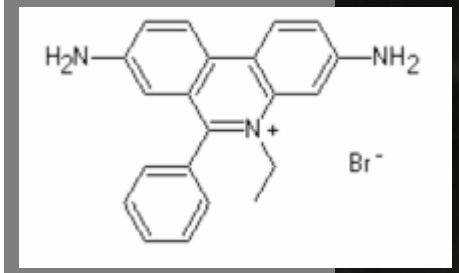
Agarose is insoluble at room temperature (left).
The agarose solution is boiled until clear (right).

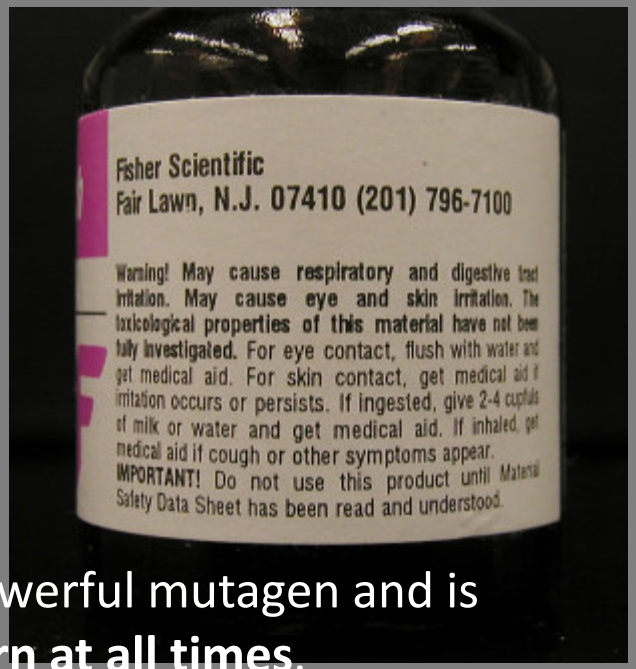
Gently swirl the solution periodically when heating to allow all the grains of agarose to dissolve.

***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.

Staining the Gel

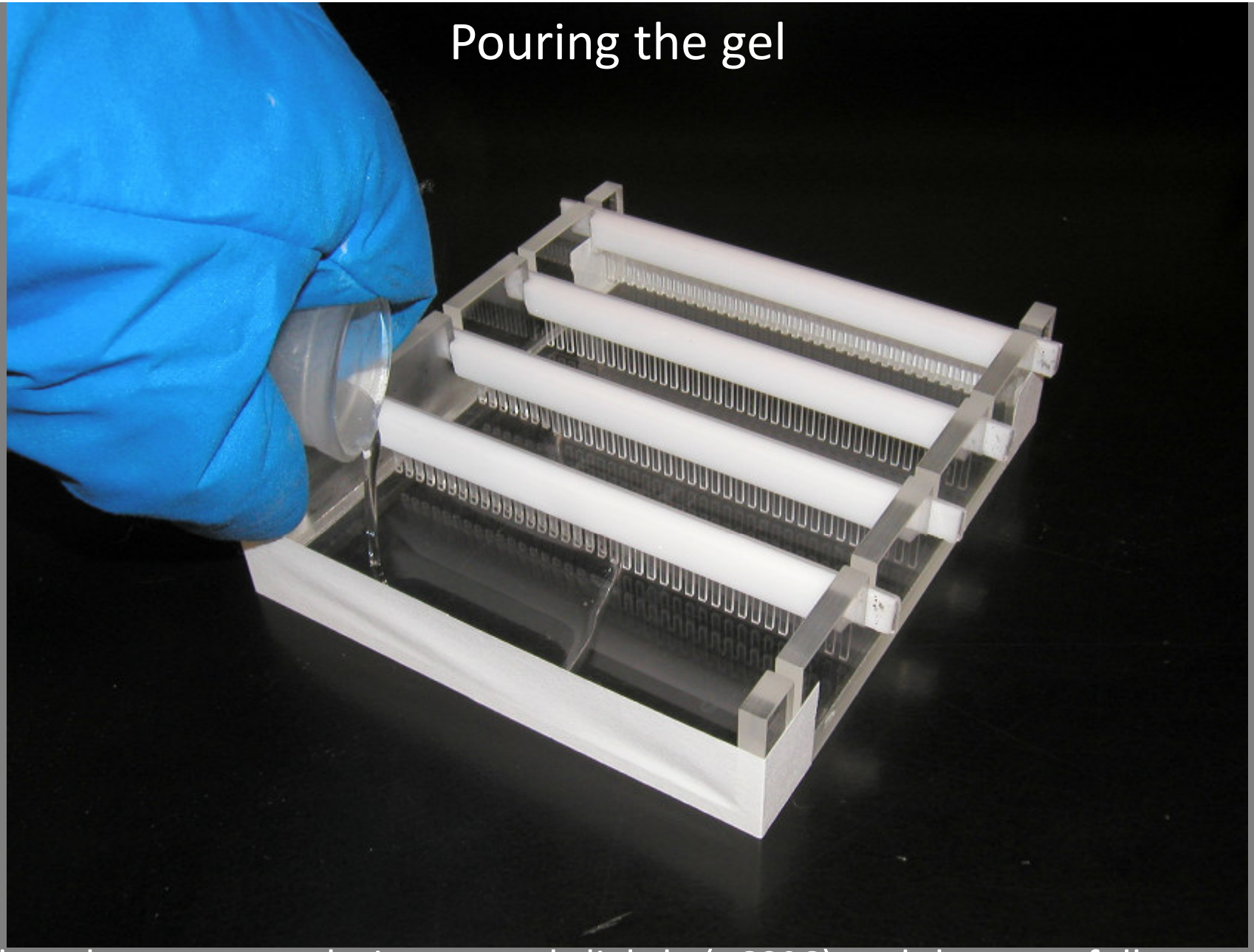
- Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a Gel.
- Ethidium bromide can be added to the **gel** and/or **running buffer** before the gel is run or the gel can be stained after it has run.



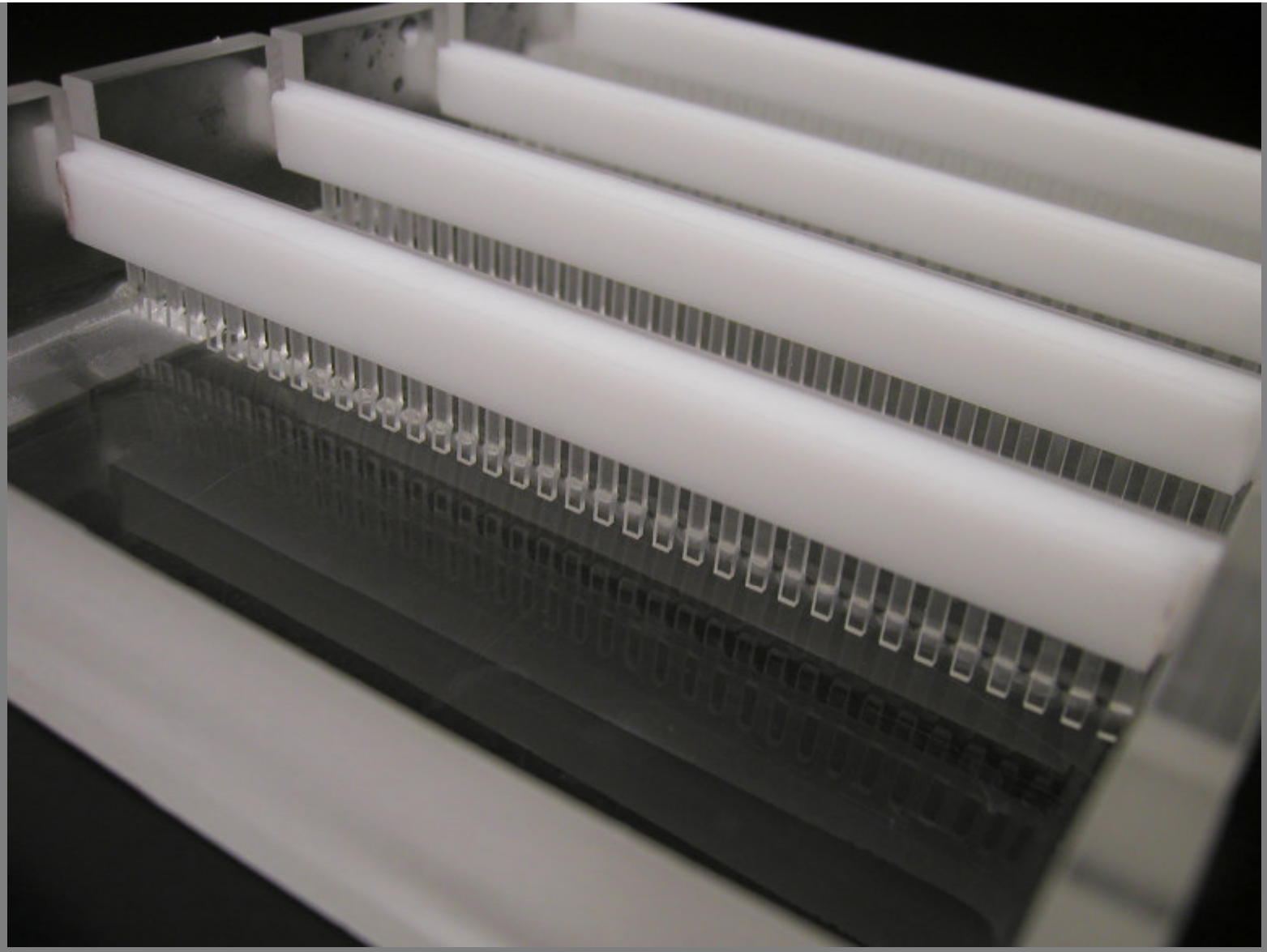


***CAUTION! Ethidium bromide is a powerful mutagen and is moderately toxic. **Gloves should be worn at all times**.

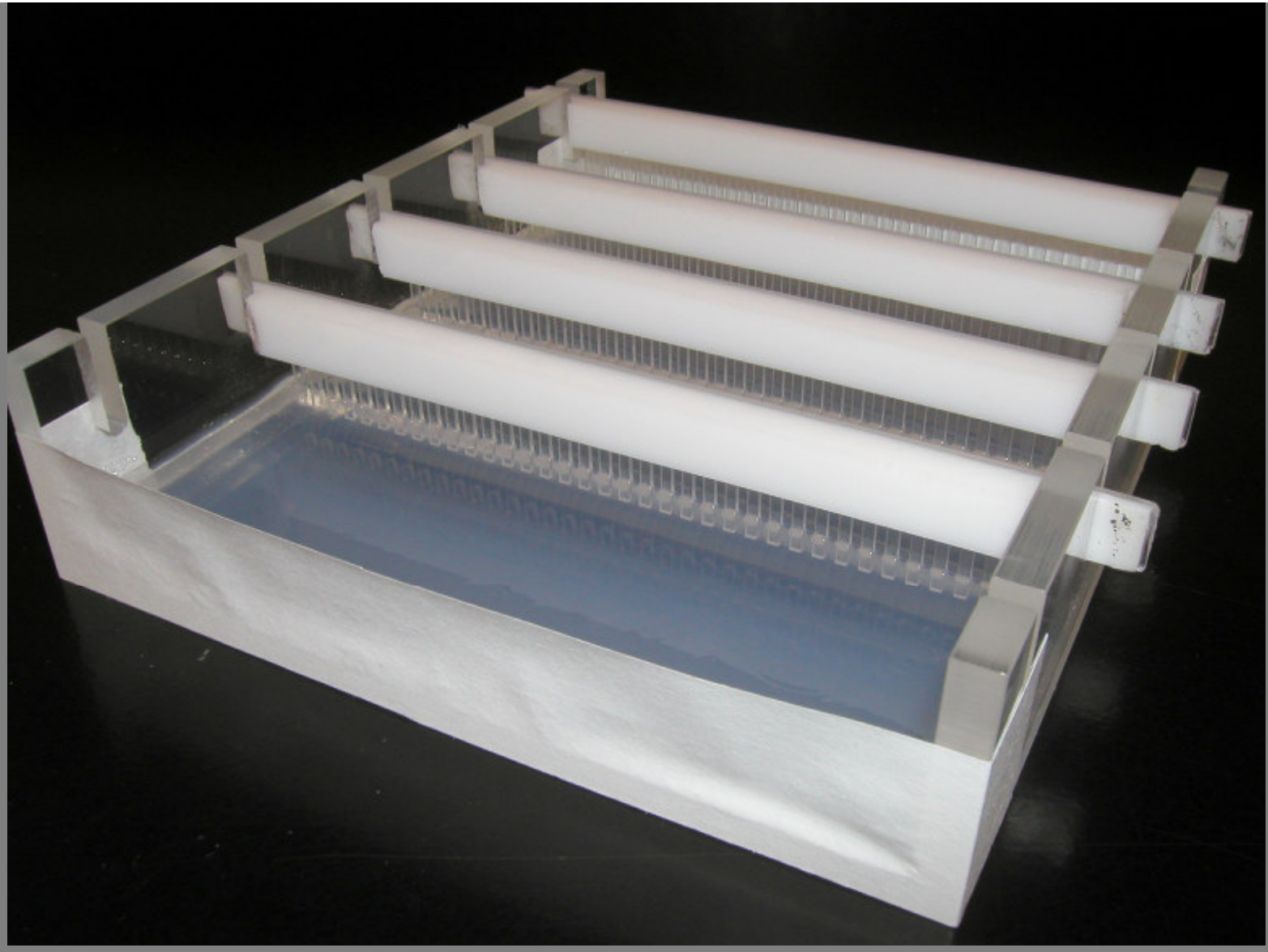
Pouring the gel



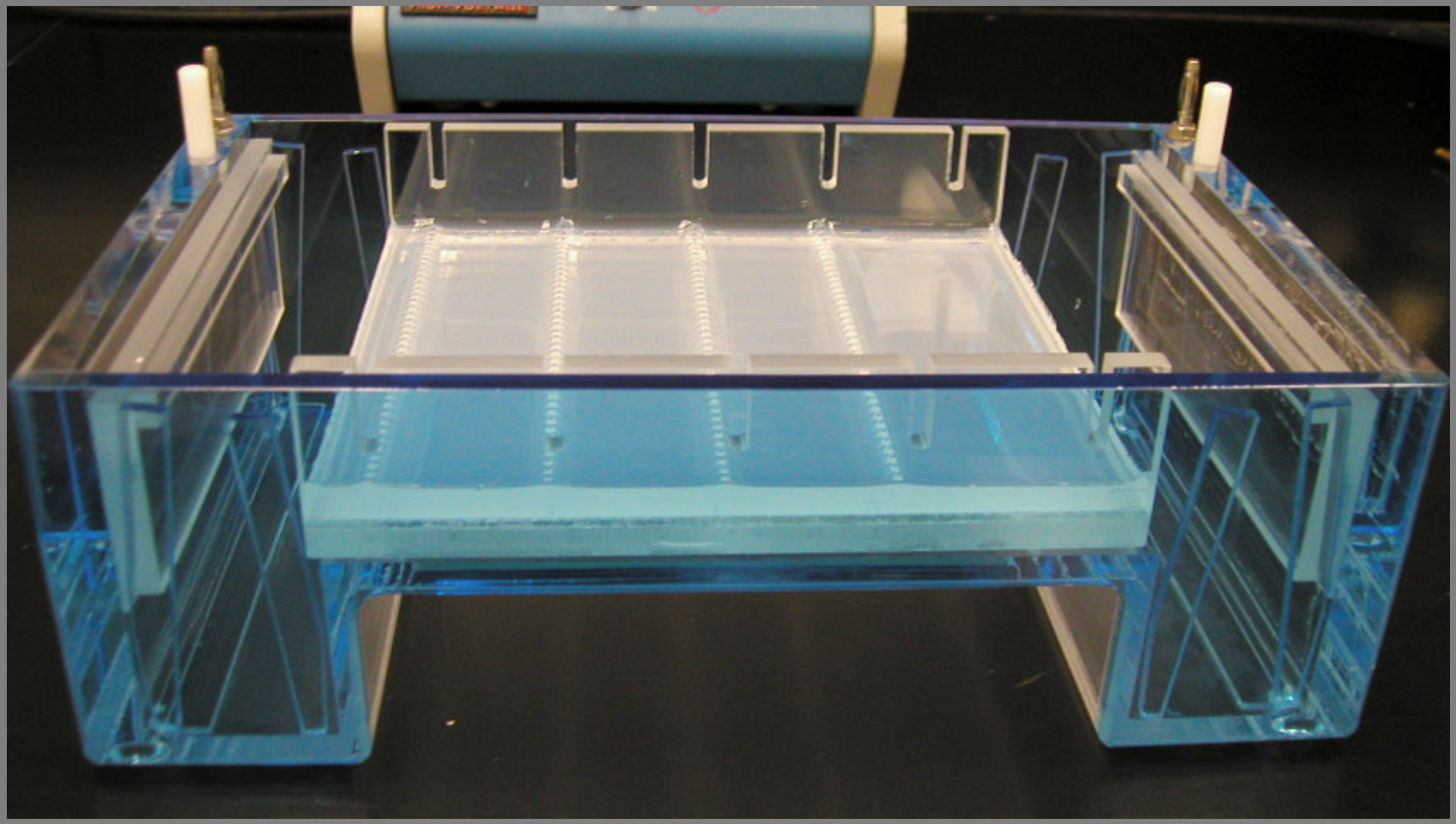
Allow the agarose solution to cool slightly (~60ºC) and then carefully pour the melted agarose solution into the casting tray. Avoid air bubbles.



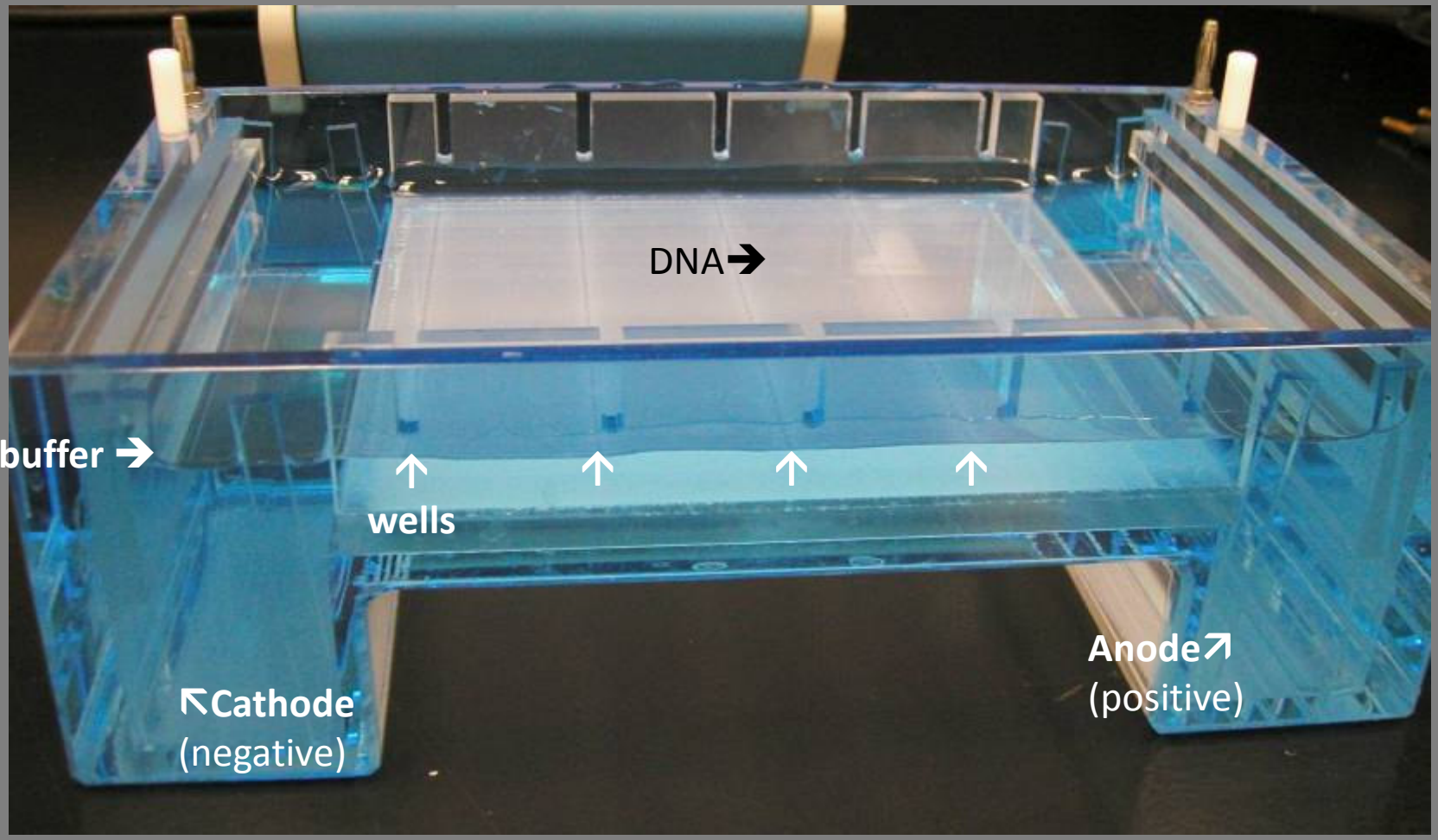
Each of the gel combs should be submerged in the melted agarose solution.



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.



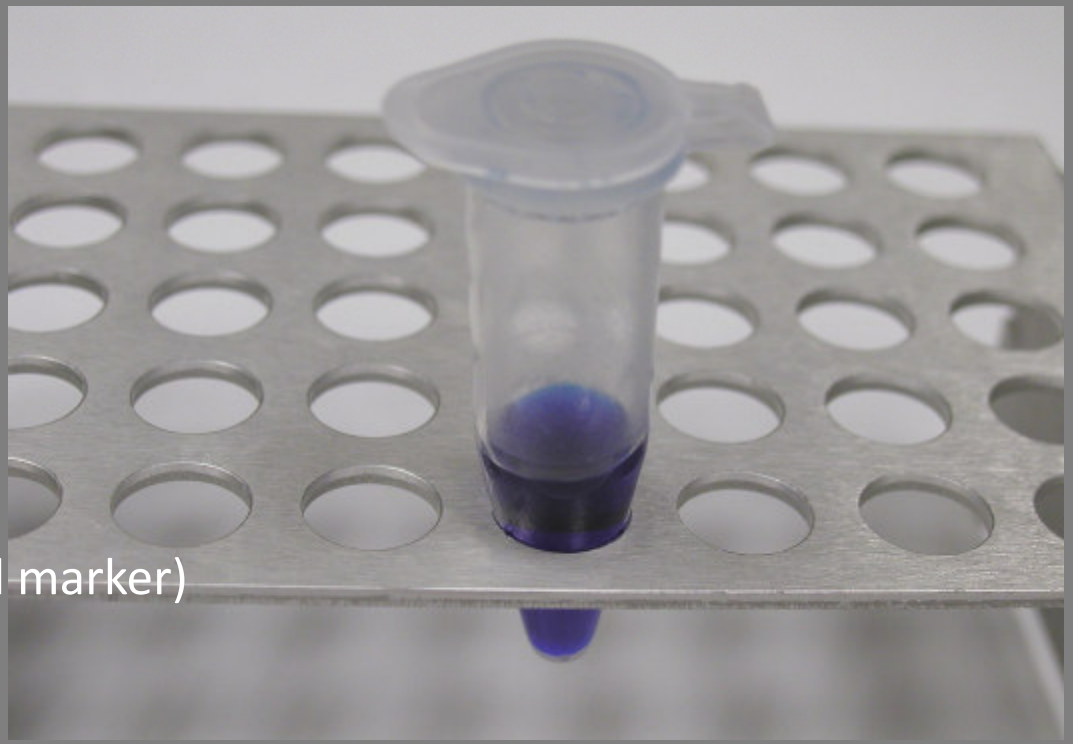
Place the gel in the electrophoresis chamber.



Add enough electrophoresis buffer to cover the gel to a depth of at least 1 mm. Make sure each well is filled with buffer.

Sample Preparation

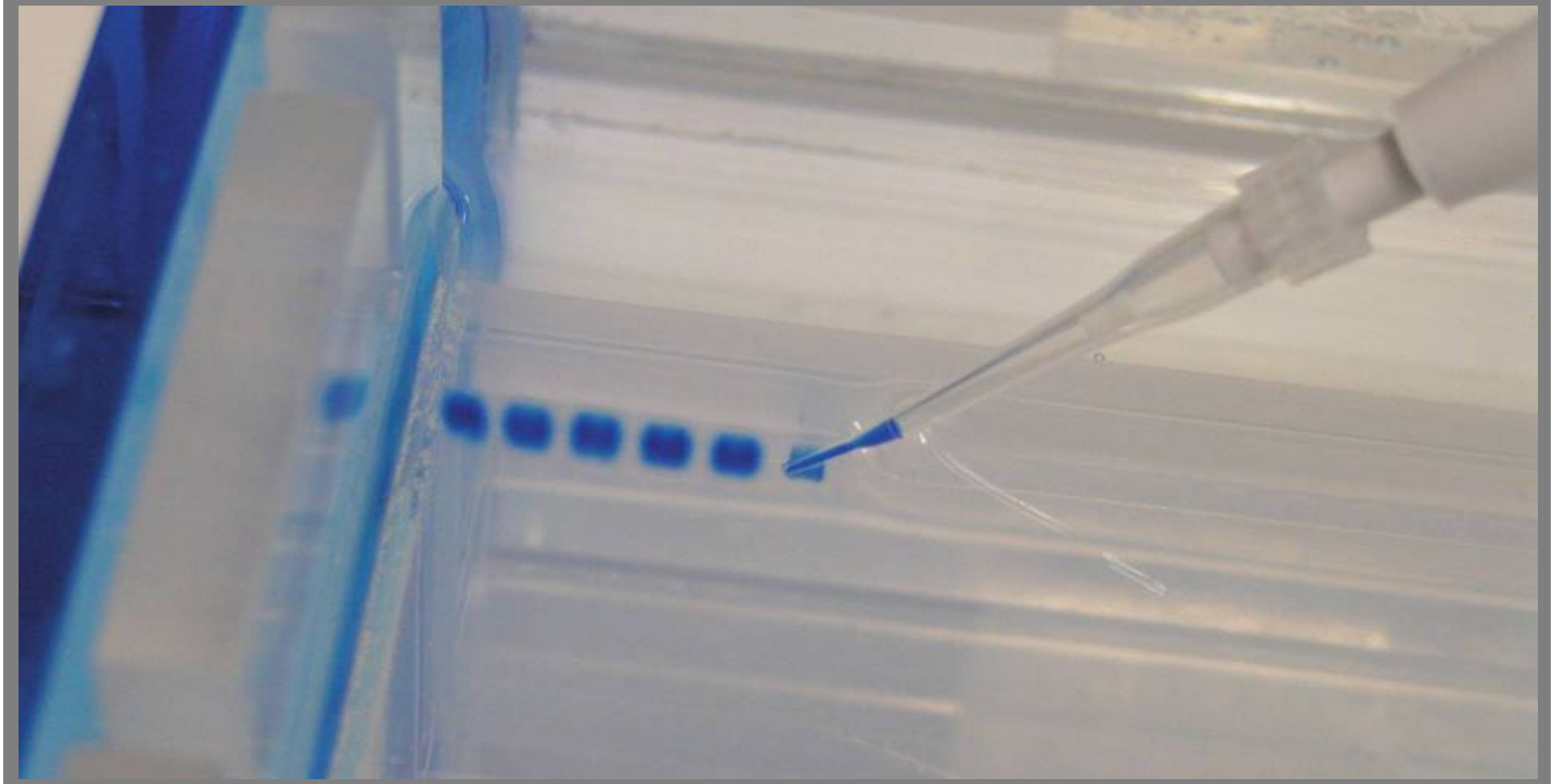
Mix the samples of DNA with the 6X sample loading buffer (w/ tracking dye). This allows the samples to be seen when loading onto the gel, and increases the density of the samples, causing them to sink into the gel wells.



6X Loading Buffer: →

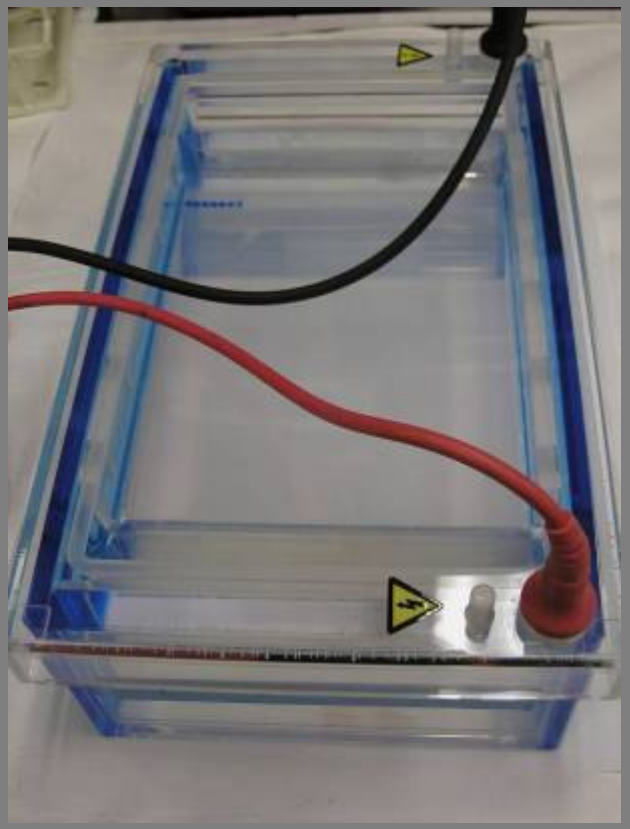
- Bromophenol Blue (for color and marker)
- Glycerol (for weight)

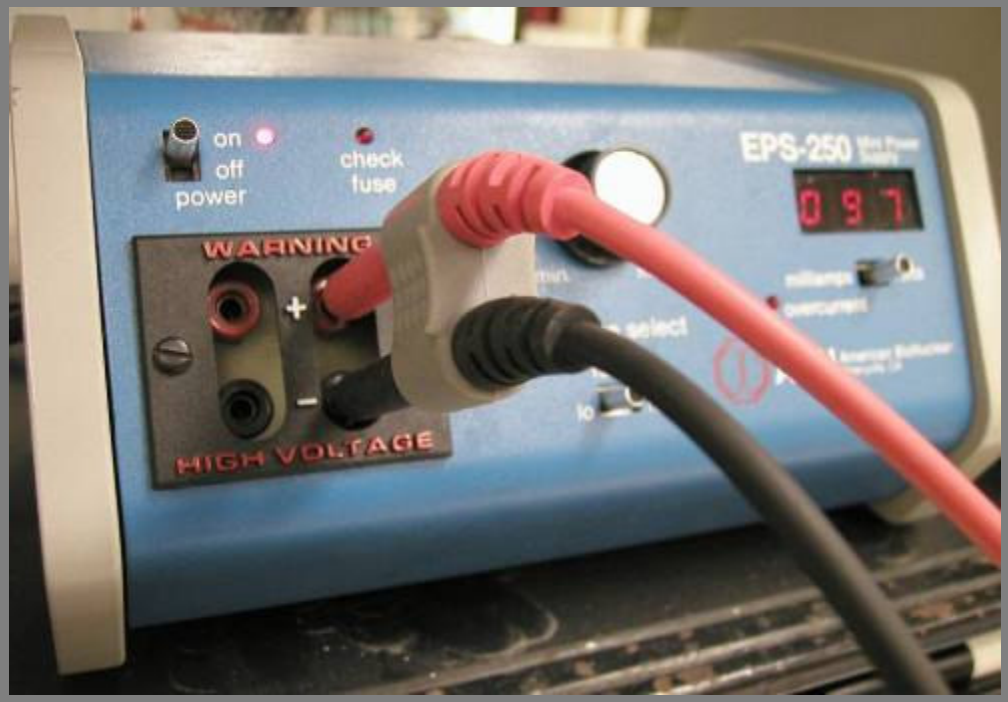
Loading the Gel



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

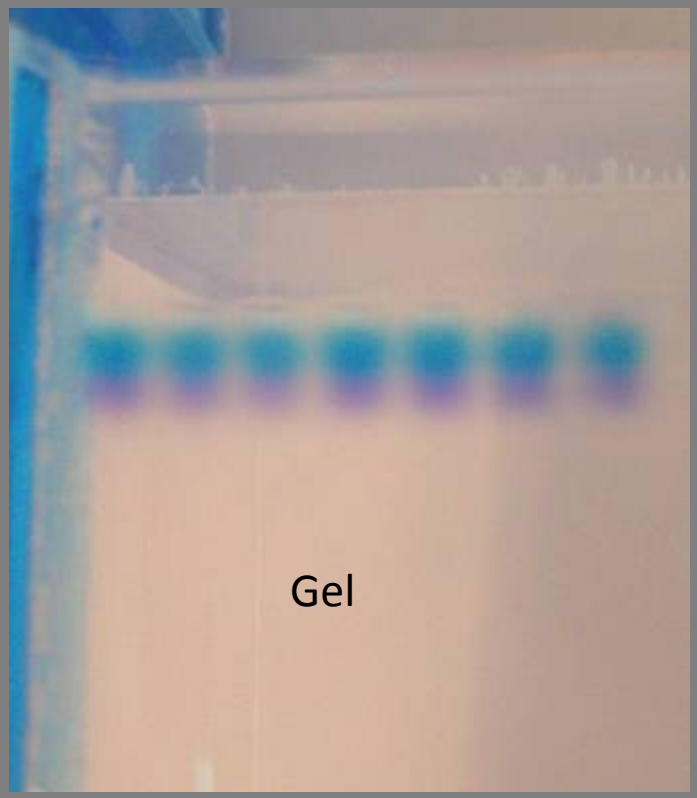




Place the cover on the electrophoresis chamber, connecting the electrical leads. Connect the electrical leads to the power supply. Be sure the leads are attached correctly - DNA migrates toward the anode (red). When the power is turned on, bubbles should form on the electrodes in the electrophoresis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

Cathode
(-)

DNA
(-)
↓

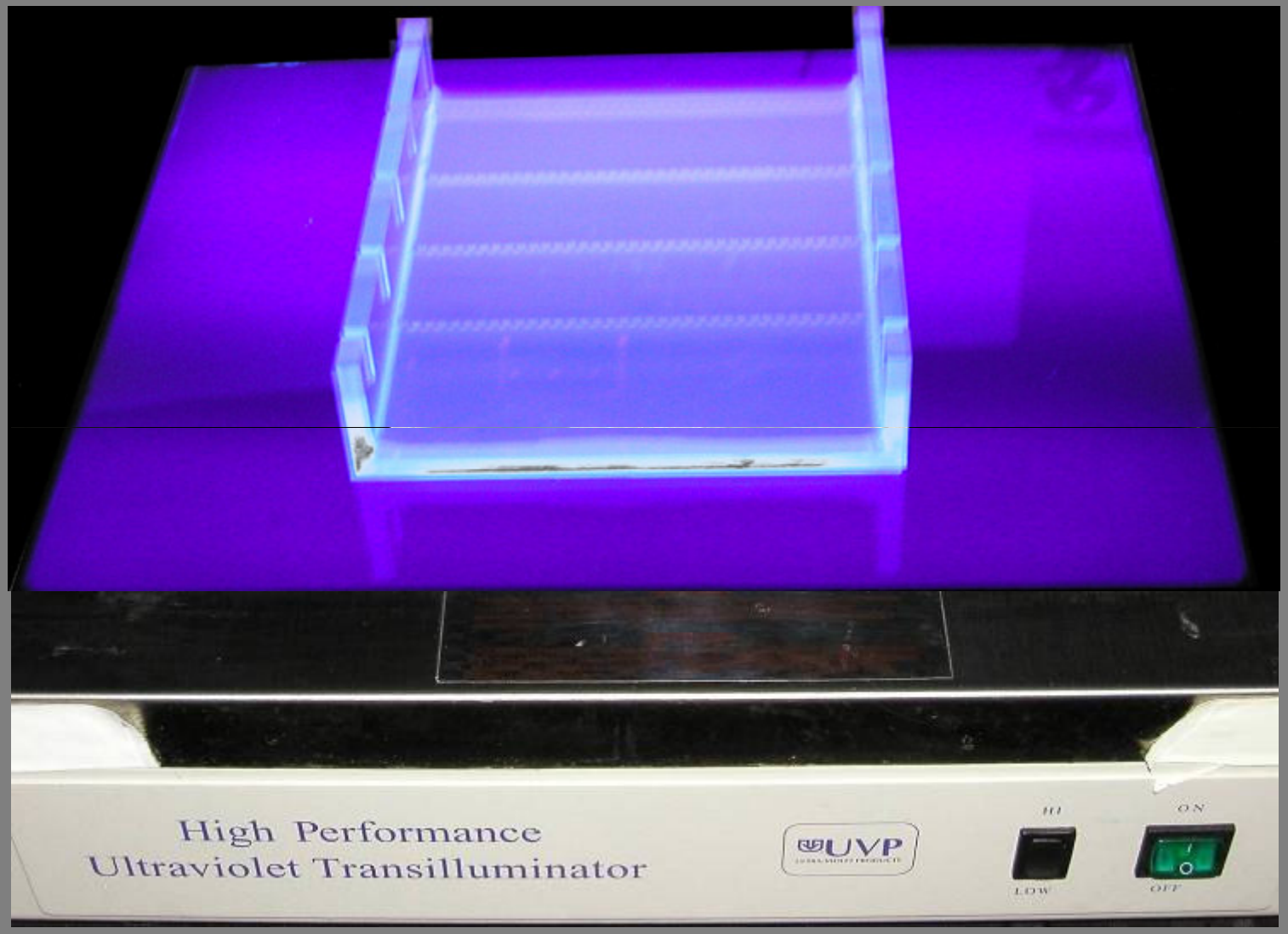


← wells
← Bromophenol Blue

Anode
(+)

After the current is applied, make sure the Gel is running in the correct direction. Bromophenol blue will run in the same direction as the DNA.

Ethidium Bromide requires an ultraviolet light source to visualize



Visualizing the DNA (ethidium bromide)

Samples # 1, 4, 6 & 7 were positive for Wolbachia DNA

Important points to consider for cloning

When little is
much better

- Ethidium bromide

0.5 µl or less
10 mg/ ml

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Reduce UV light exposure

* Take picture

Reduce agarose

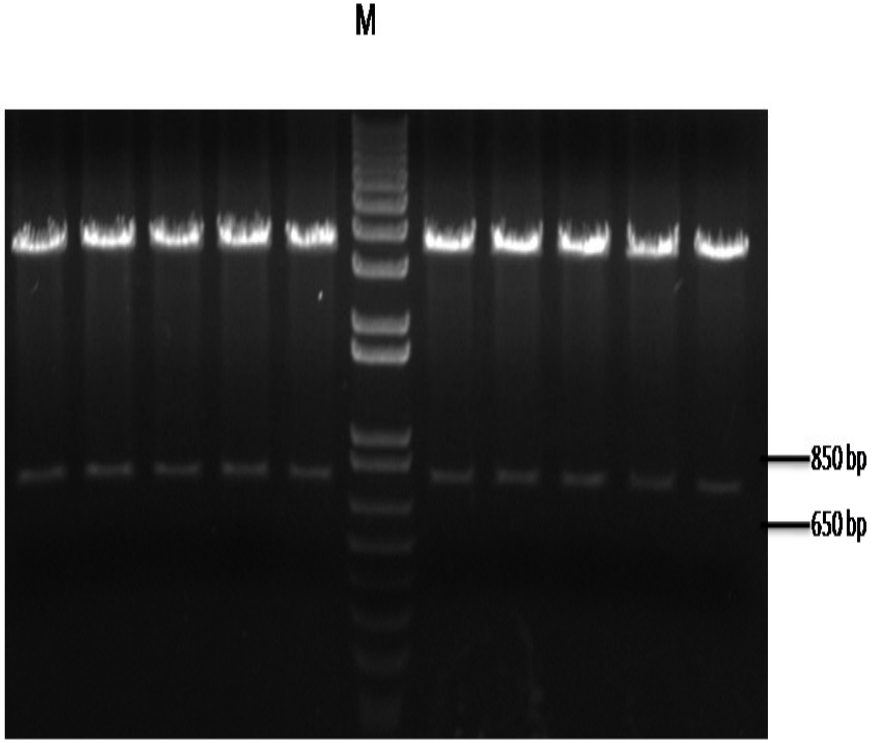
0.7 %

350 mg/ 50 ml

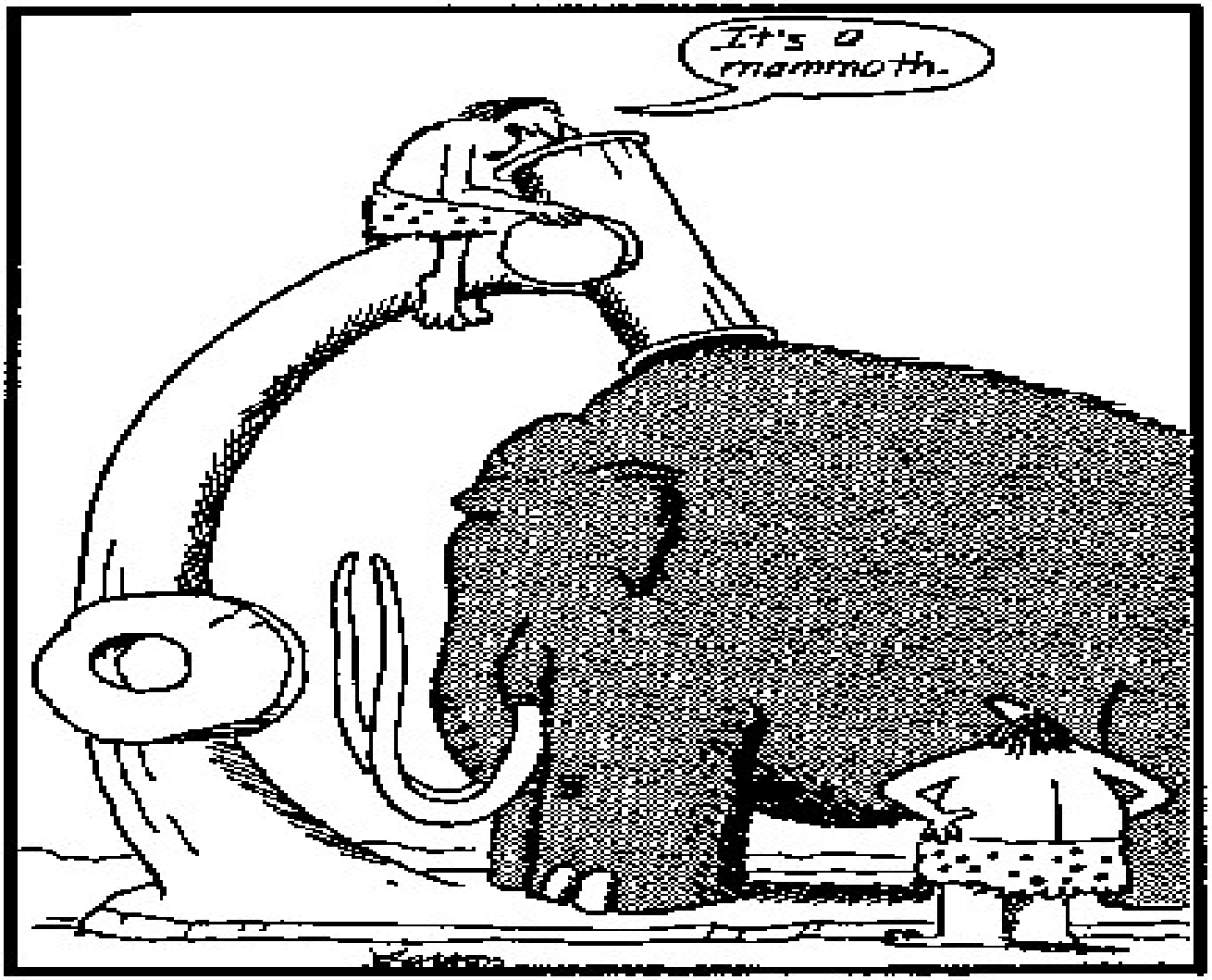
700 mg/ 100 ml

DNA extraction from gel

- Reduce exposure to UV light
- Reduce agarose (don't exceed 400 mg)
- Take one band only
- Not enough DNA (increase PCR volume)
- Use free water for elution



Questions



Early microscope