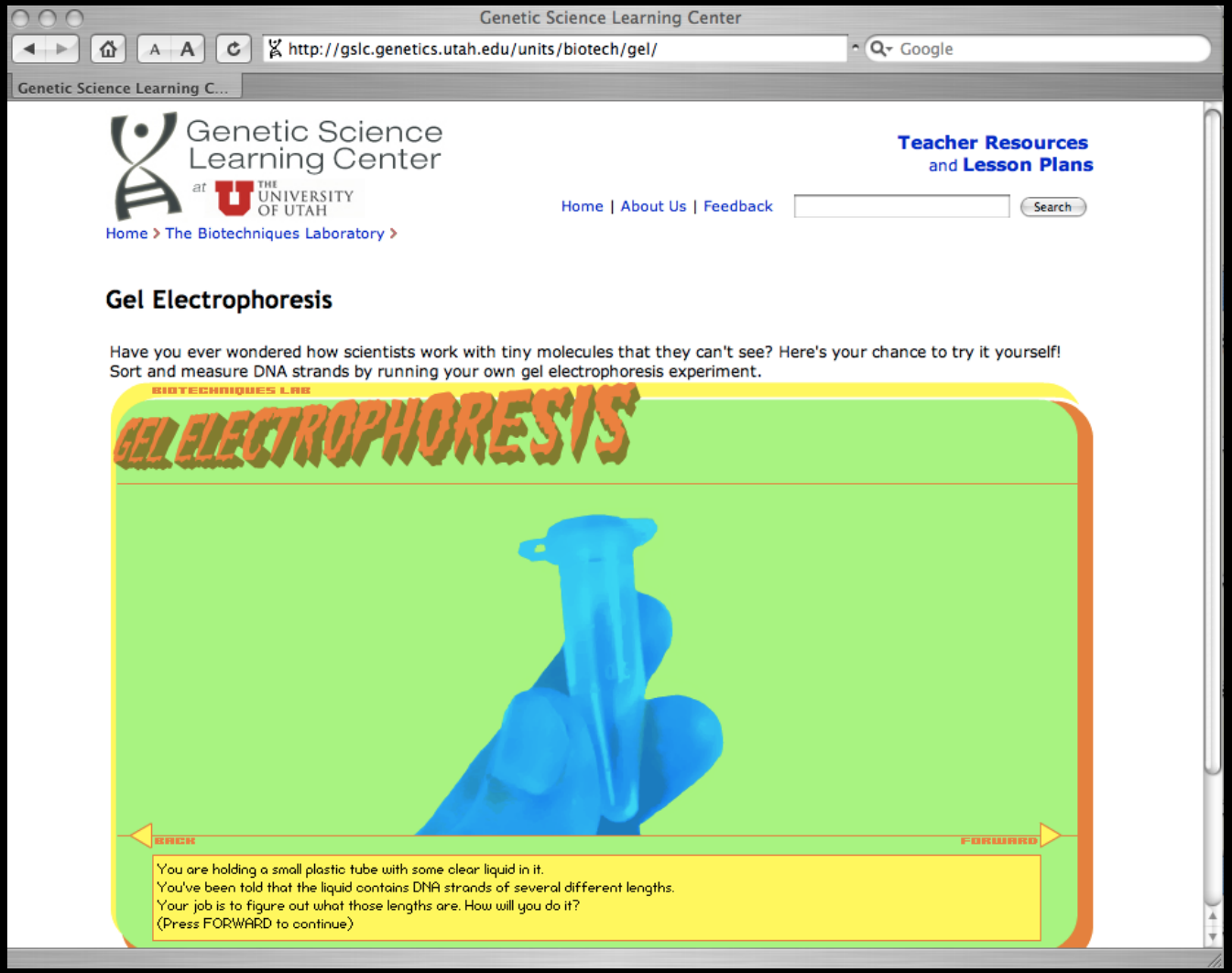
VirginiaTech
Invent the Future

Agarose Gel Electrophoresis

- Gel electrophoresis is a widely used technique for the analysis of **nucleic acids** and **proteins**. Agarose gel electrophoresis is routinely used for the preparation and analysis of DNA. (SDS-PAGE used for protein analyses)
- 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.
- We will be using agarose gel electrophoresis to determine the presence and size of PCR products.

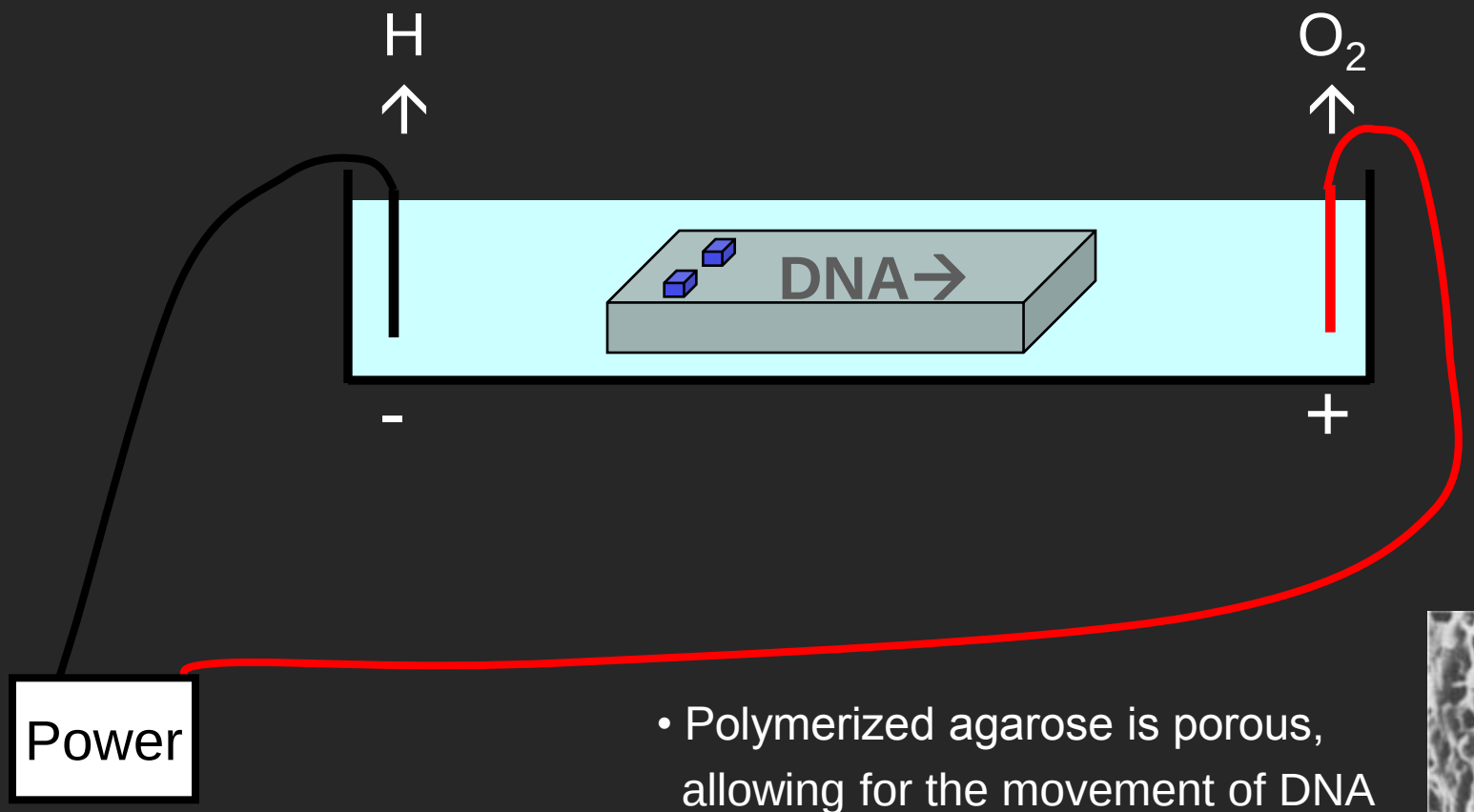
Additional Information on Gel Electrophoresis:

Virtual Gel Electrophoresis



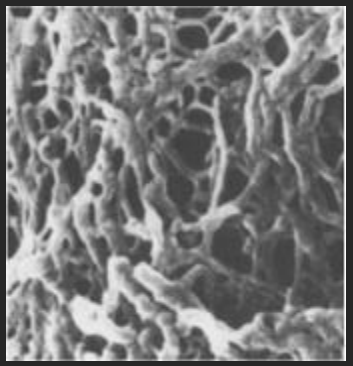
http://gslc.genetics.utah.edu/units/biotech/gel/

- DNA is negatively charged.
- When placed in an electrical field, DNA will migrate toward the positive pole (anode).
- An agarose gel is used to slow the movement of DNA and separate by size.



- Polymerized agarose is porous, allowing for the movement of DNA

Scanning Electron Micrograph of Agarose Gel (1 × 1 µm) →



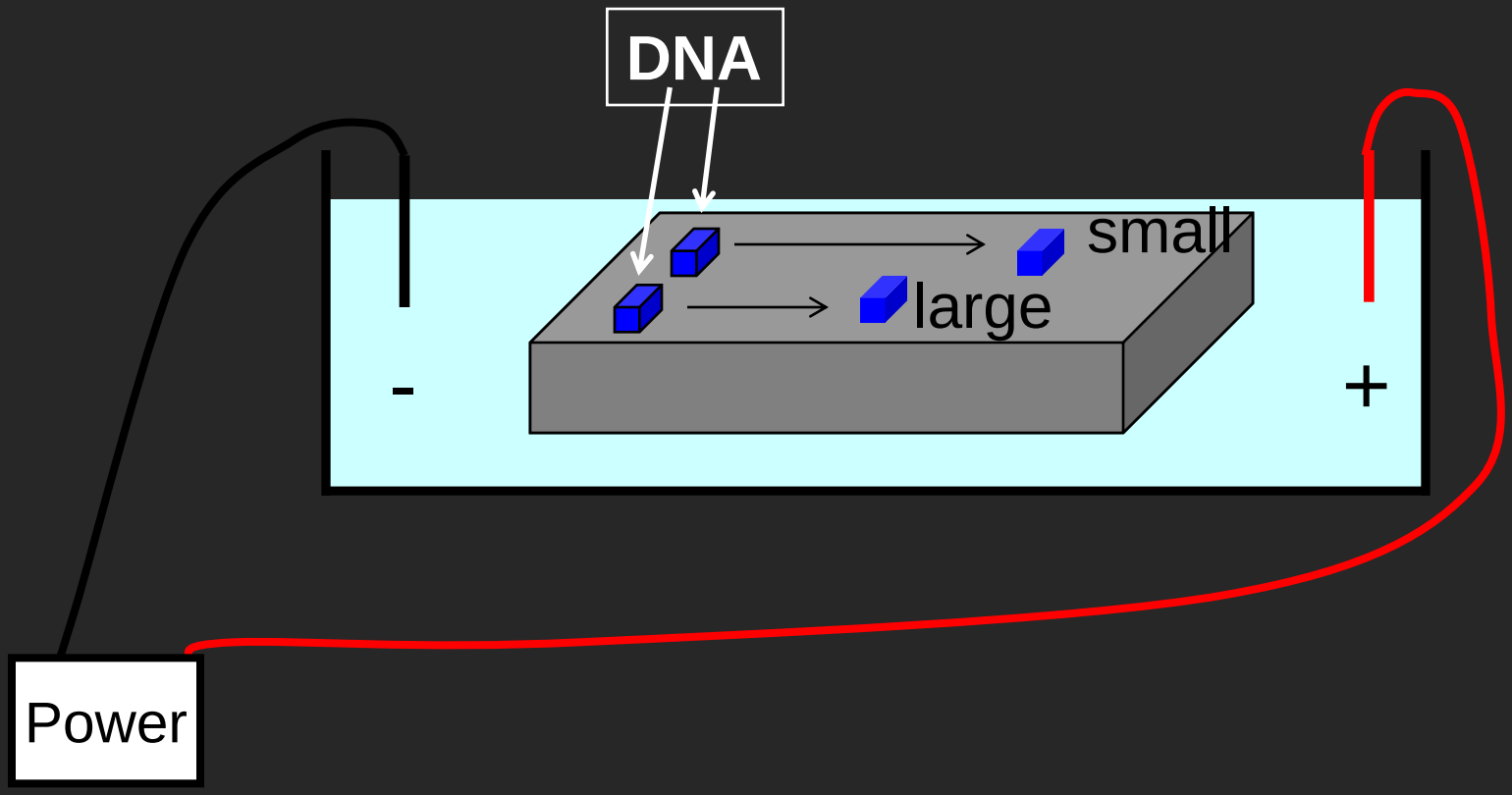
How fast will the DNA migrate?

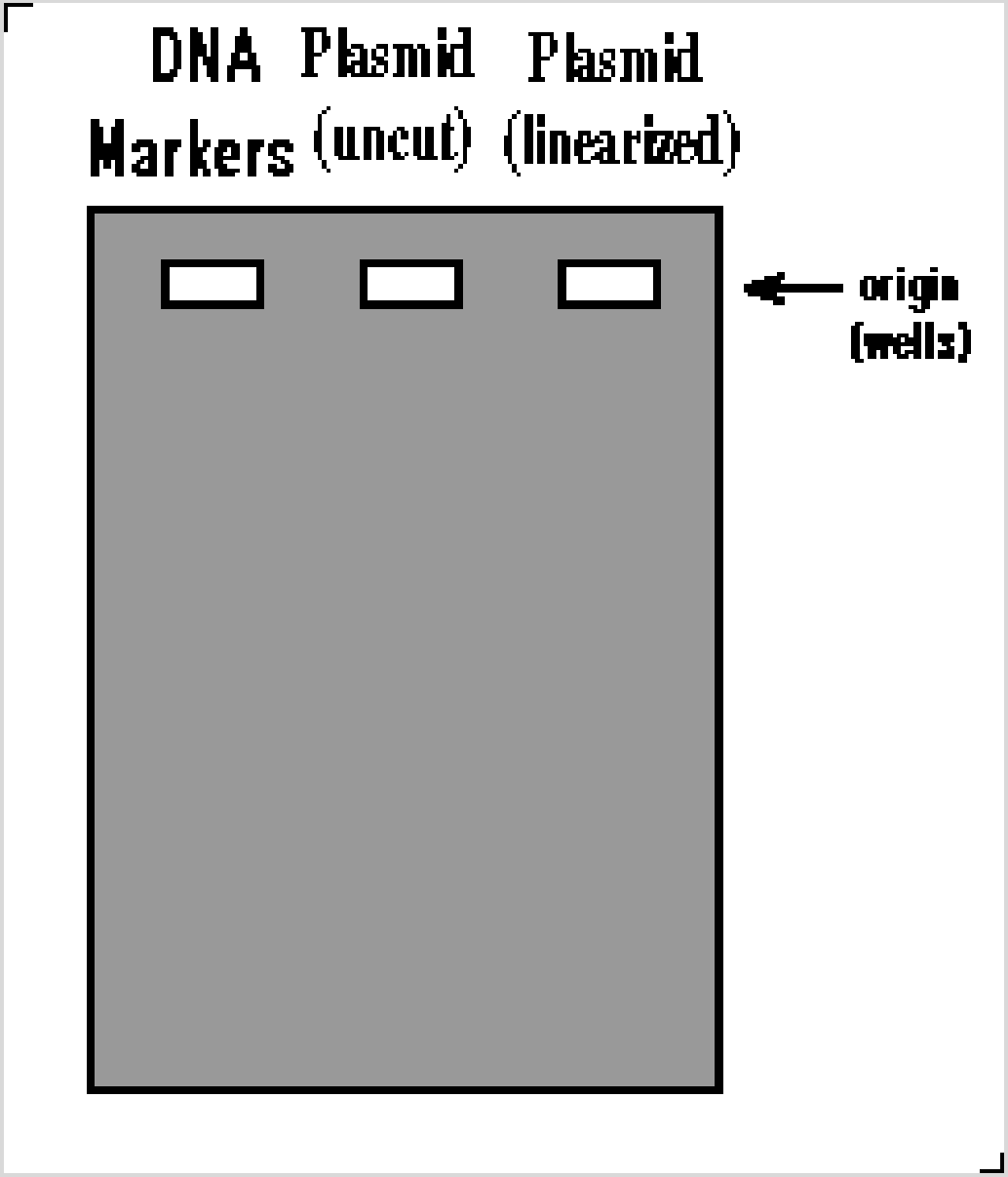
strength of the electrical field, buffer, density of agarose gel…

Size of the DNA!

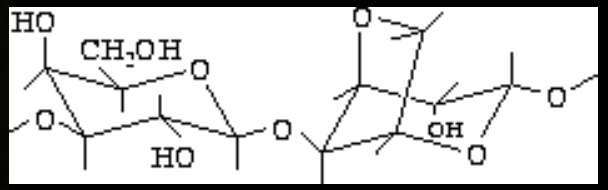
*Small DNA move faster than large DNA

…gel electrophoresis separates DNA according to size



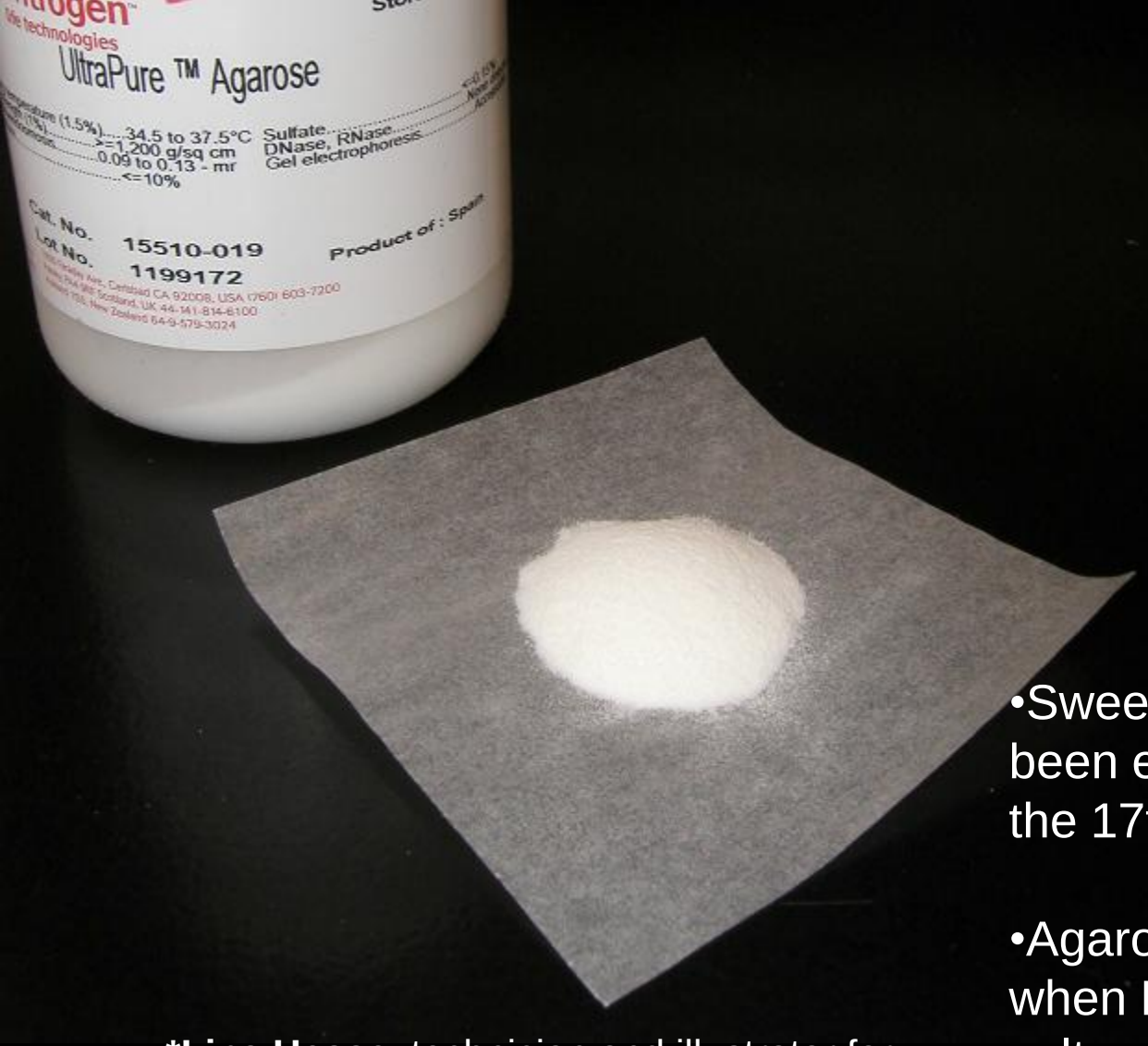

DNA Markers
Plasmid (uncut)
Plasmid (linearized)
origin (wells)

Agarose



D-galactose

3,6-anhydro L-galactose



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

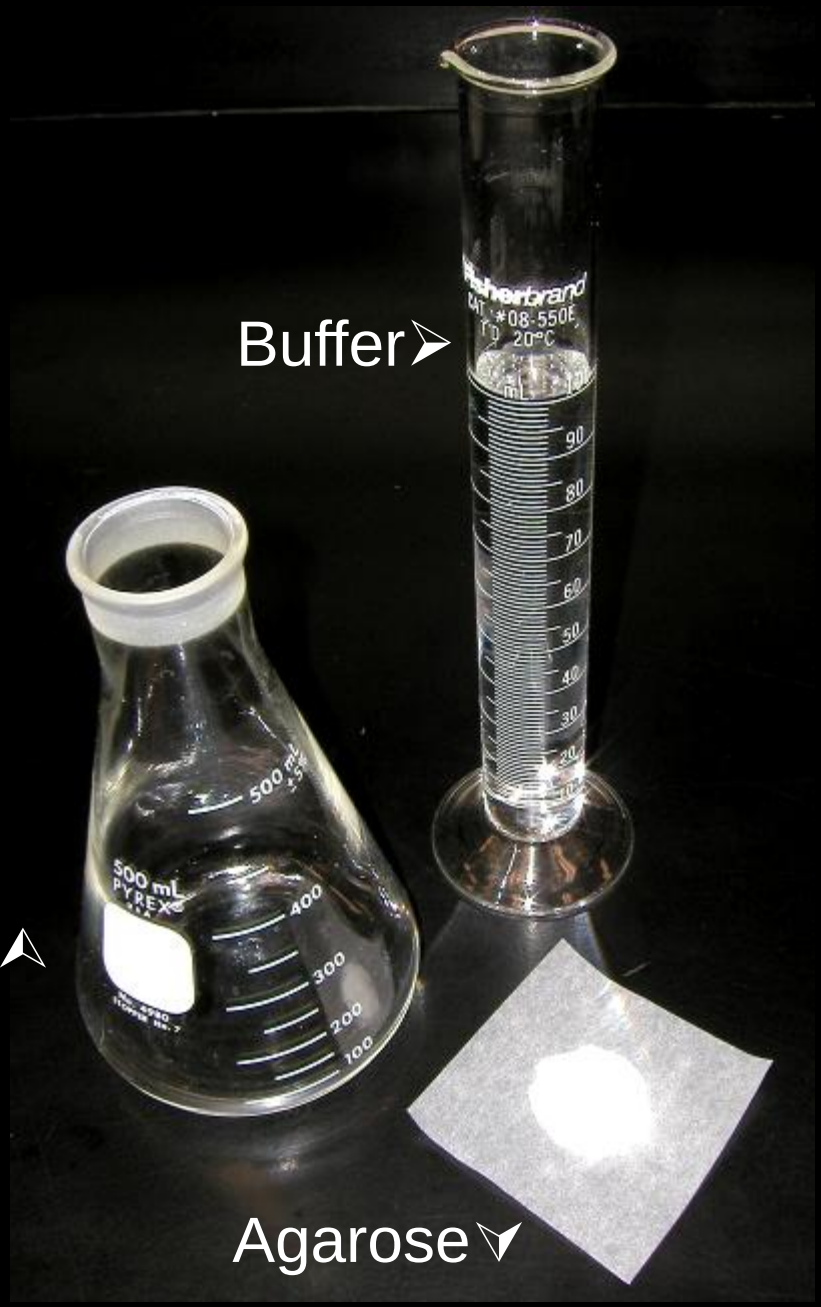
- Sweetened agarose gels have been eaten in the Far East since the 17th century.

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

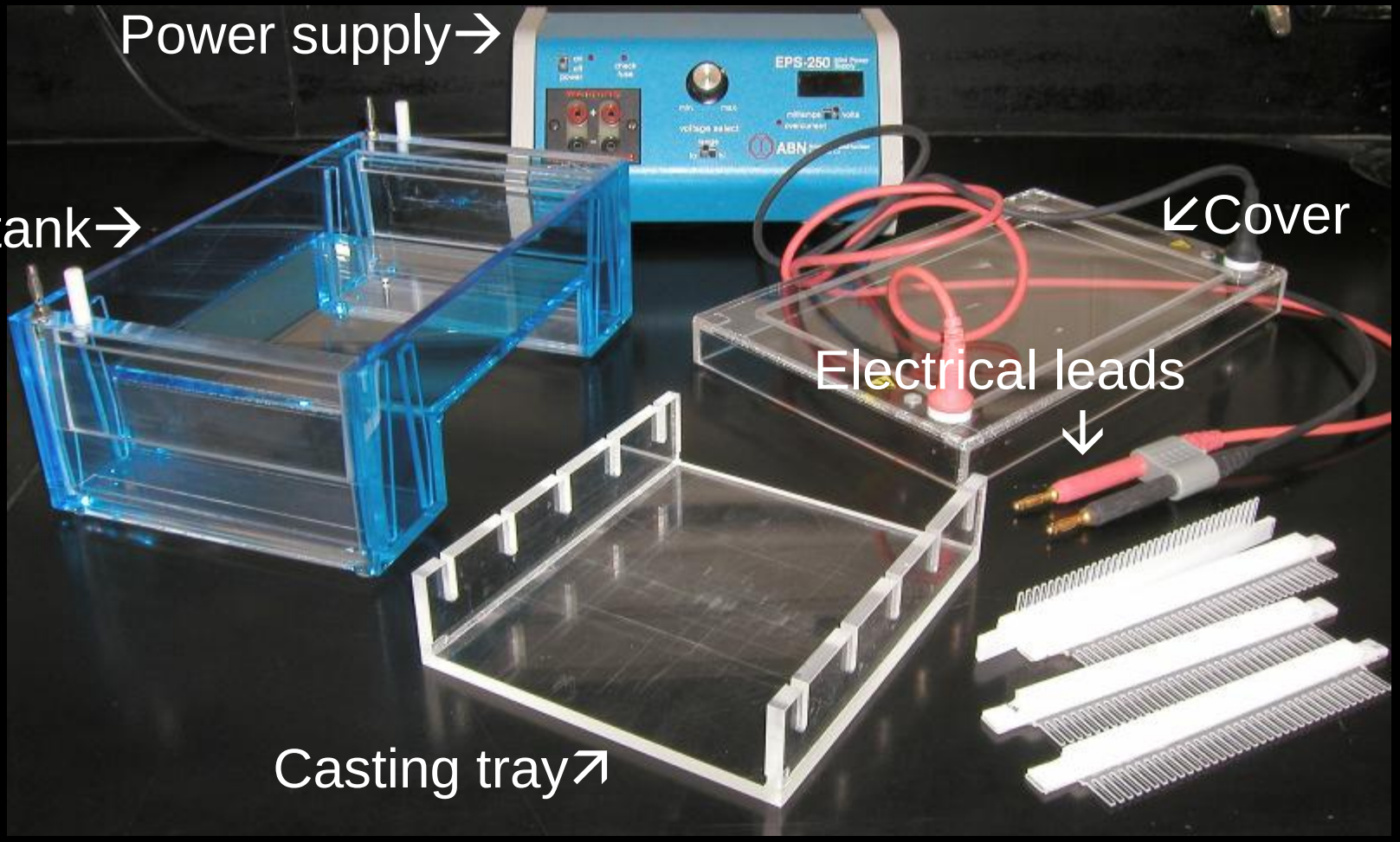
Agarose is a linear polymer extracted from seaweed.

Making an Agarose Gel

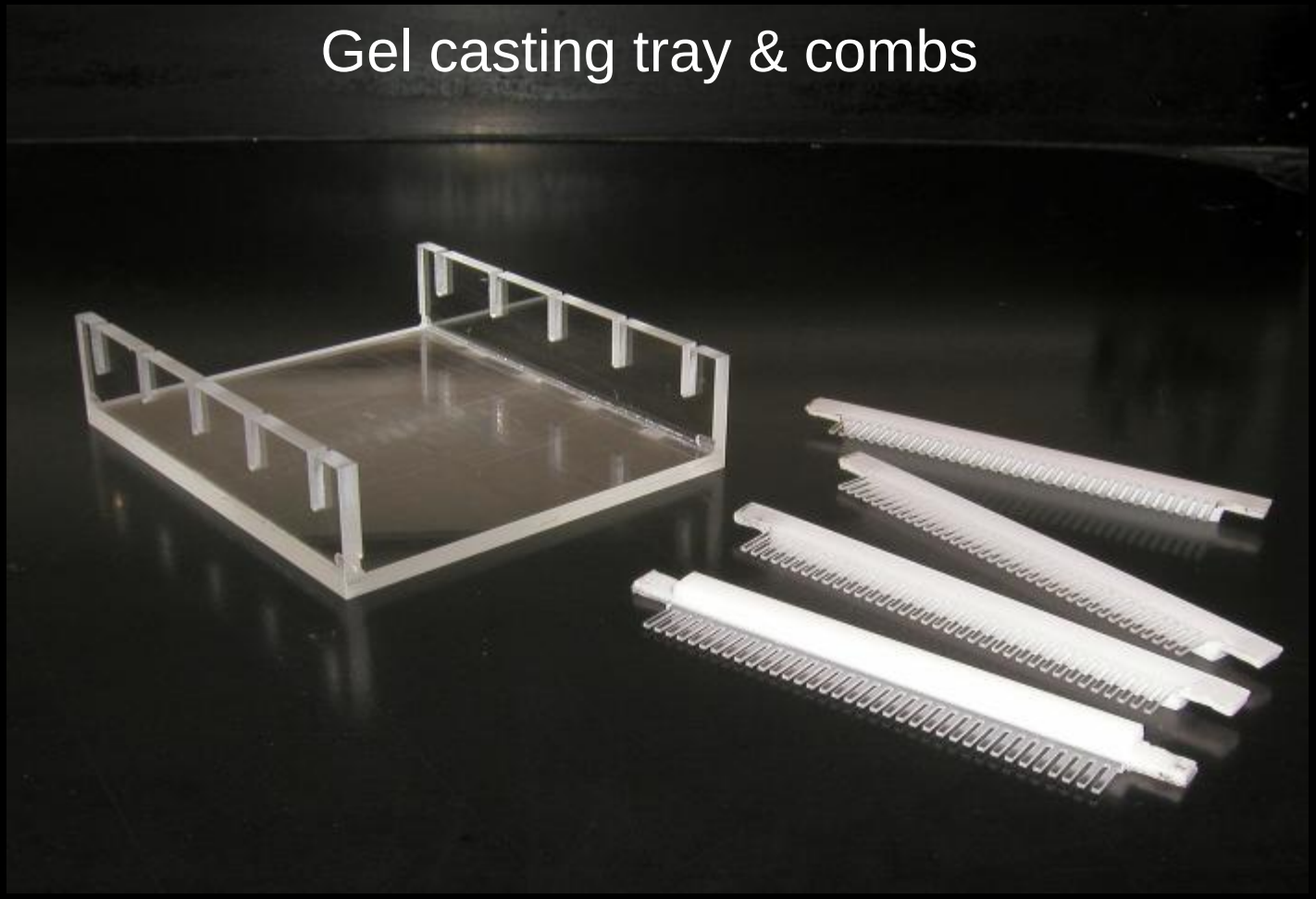
An agarose gel is prepared by combining agarose powder and a buffer solution.



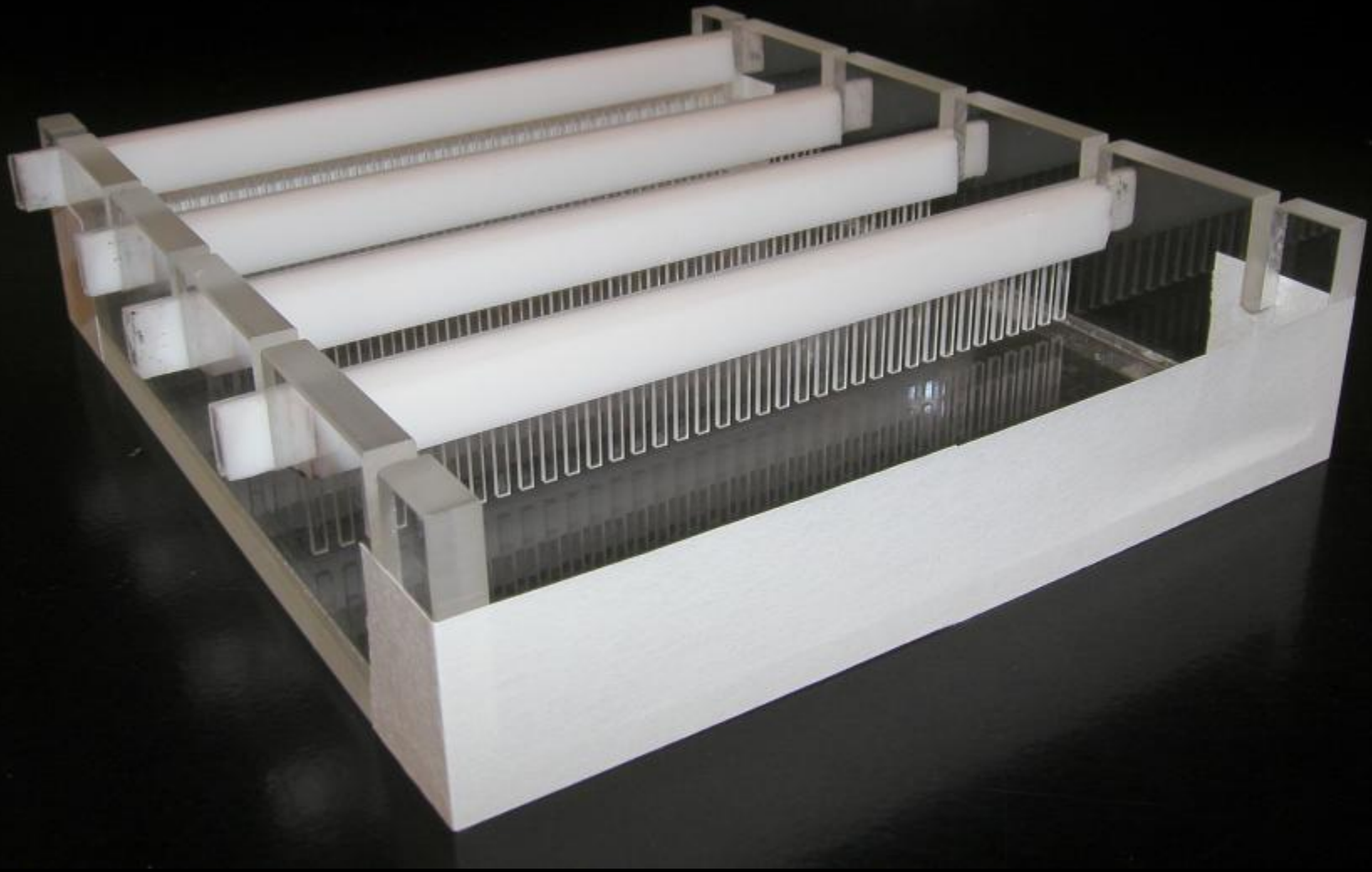
Electrophoresis Equipment



Gel casting tray & combs



Preparing the Casting Tray



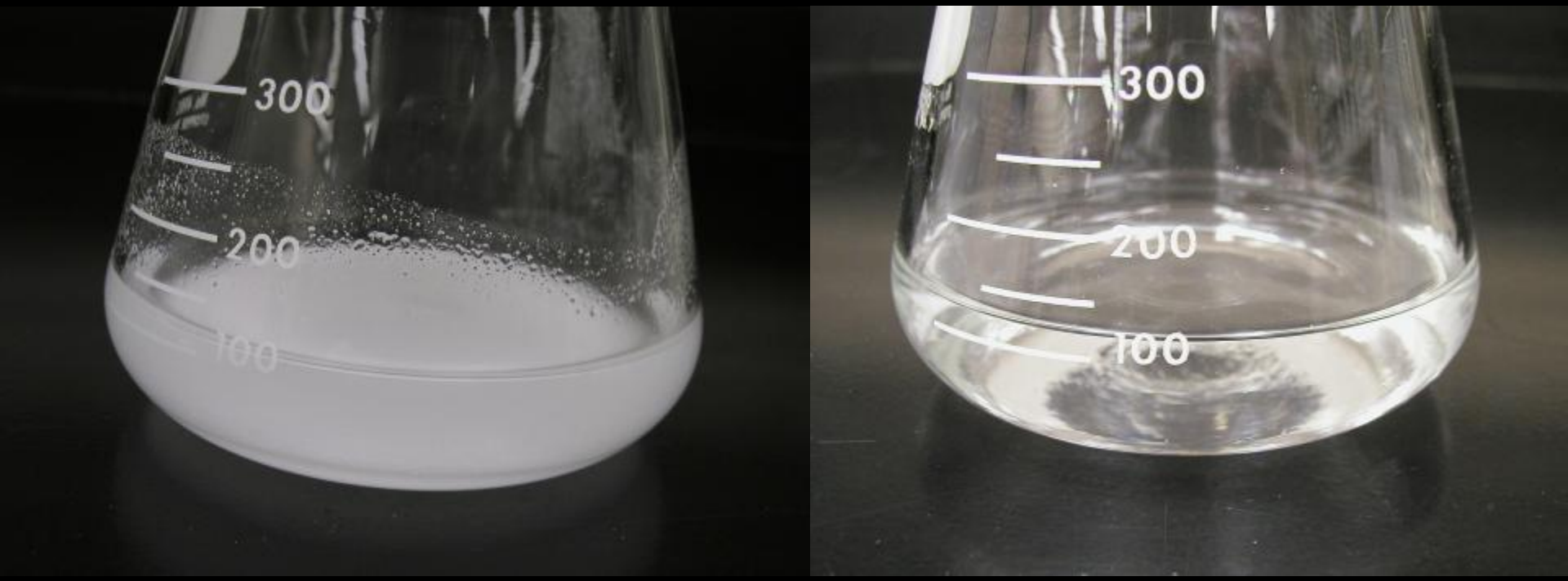
Seal the edges of the casting tray and put in the combs. Place the casting tray on a level surface. None of the gel combs should be touching the surface of the casting tray.

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

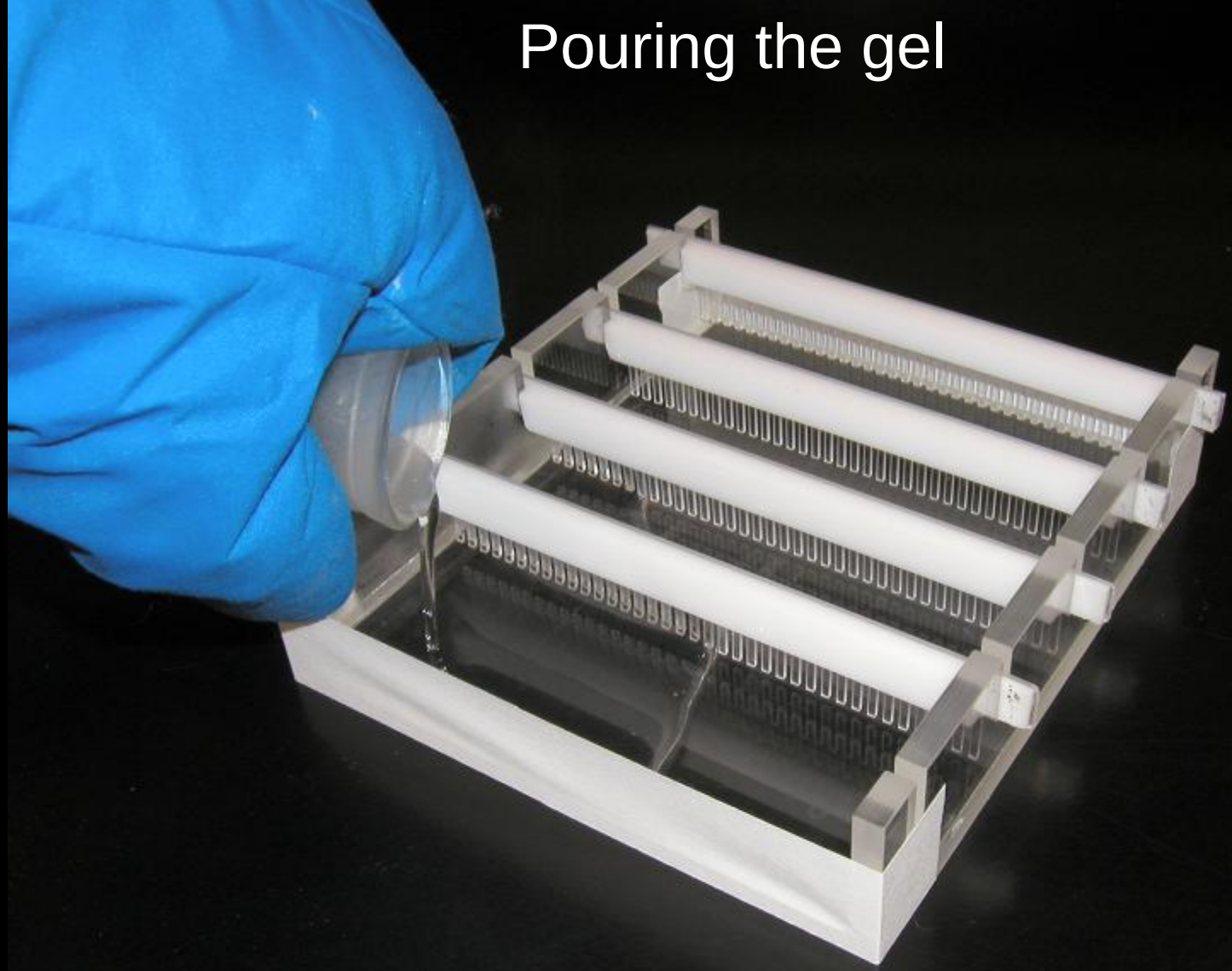


Agarose is insoluble at room temperature (left).
The agarose solution is boiled until clear (right).

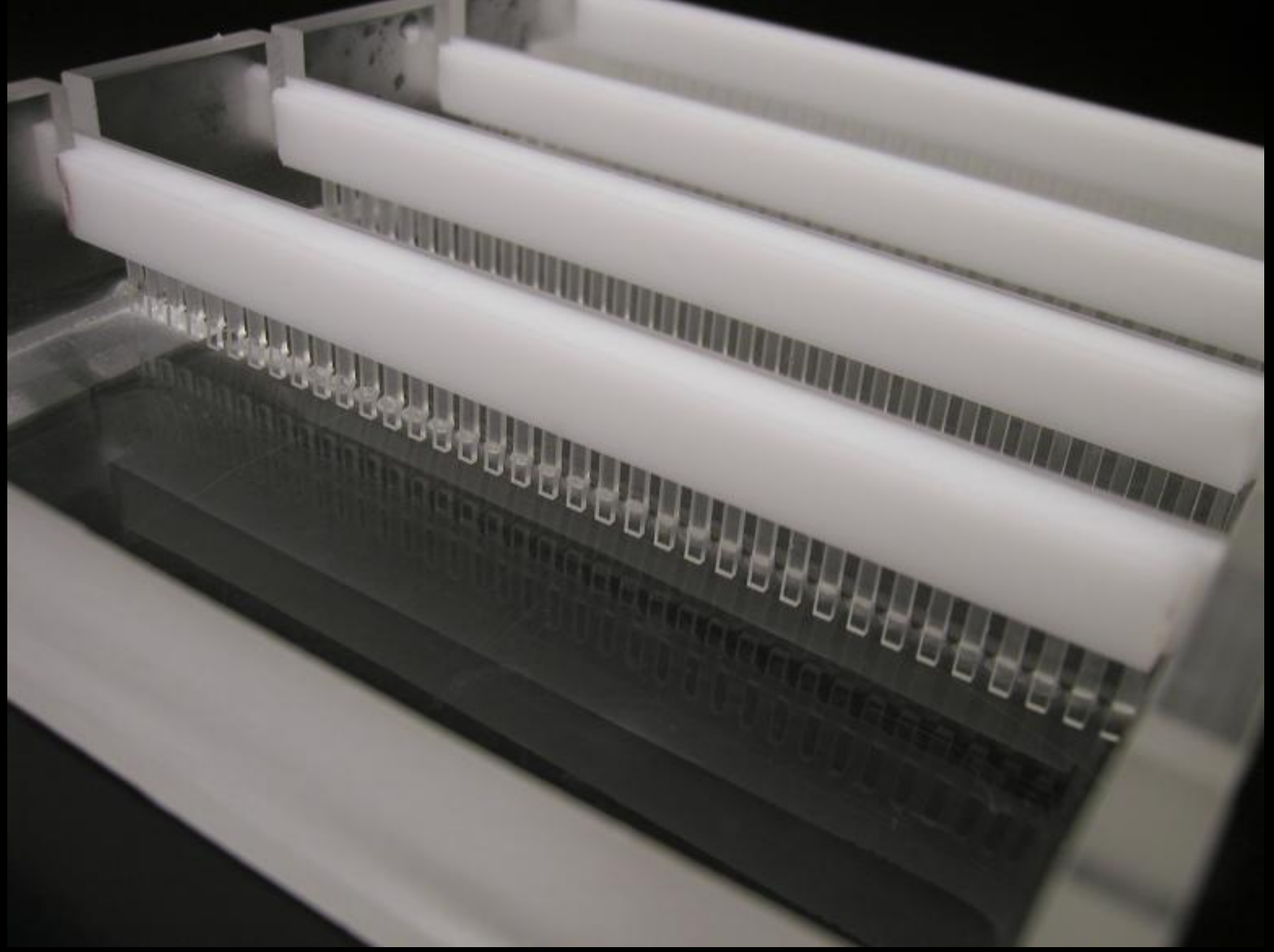
Gently swirl the solution periodically when heating to allow all the grains of agarose to dissolve.

***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.

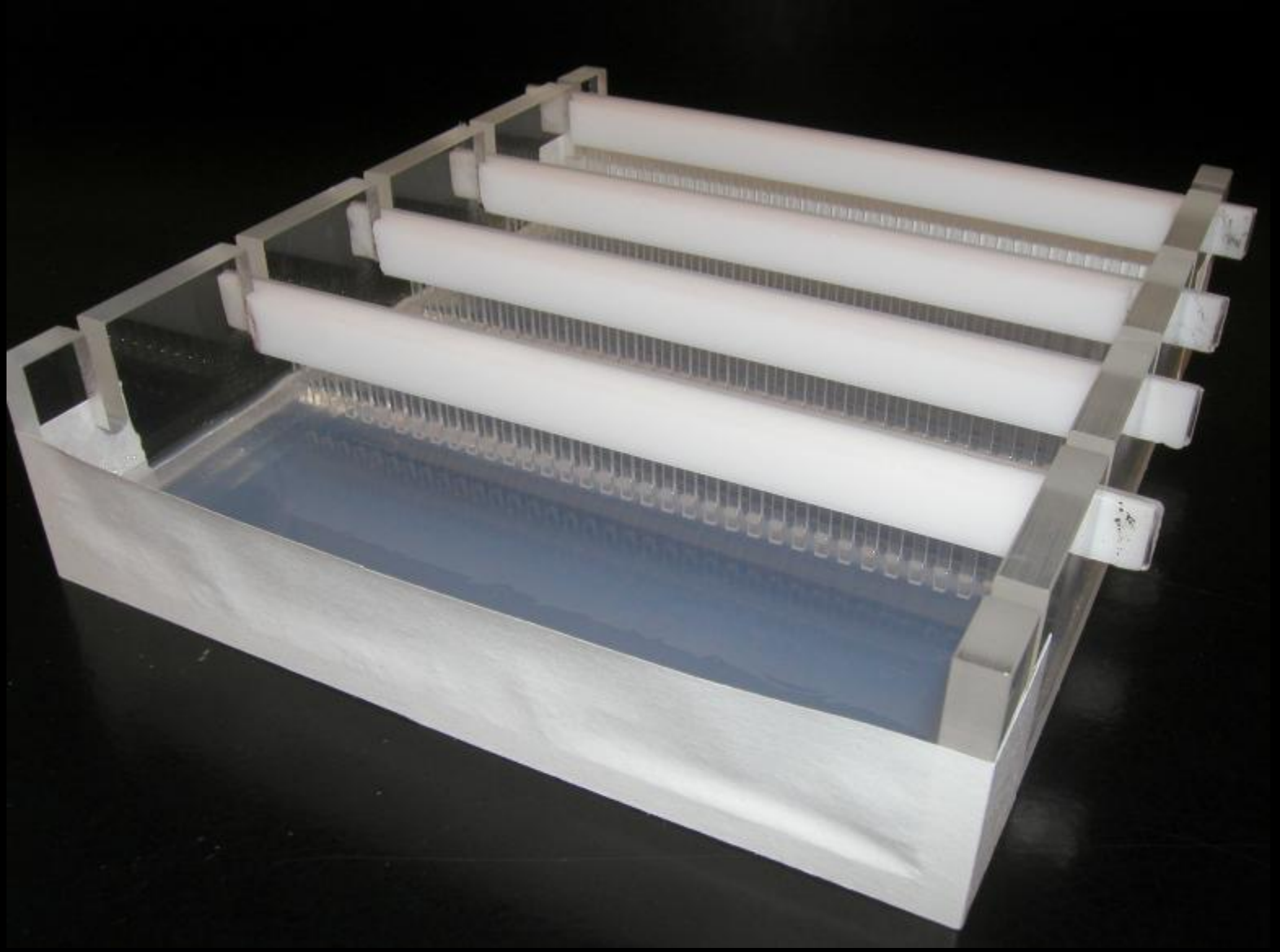
Pouring the gel



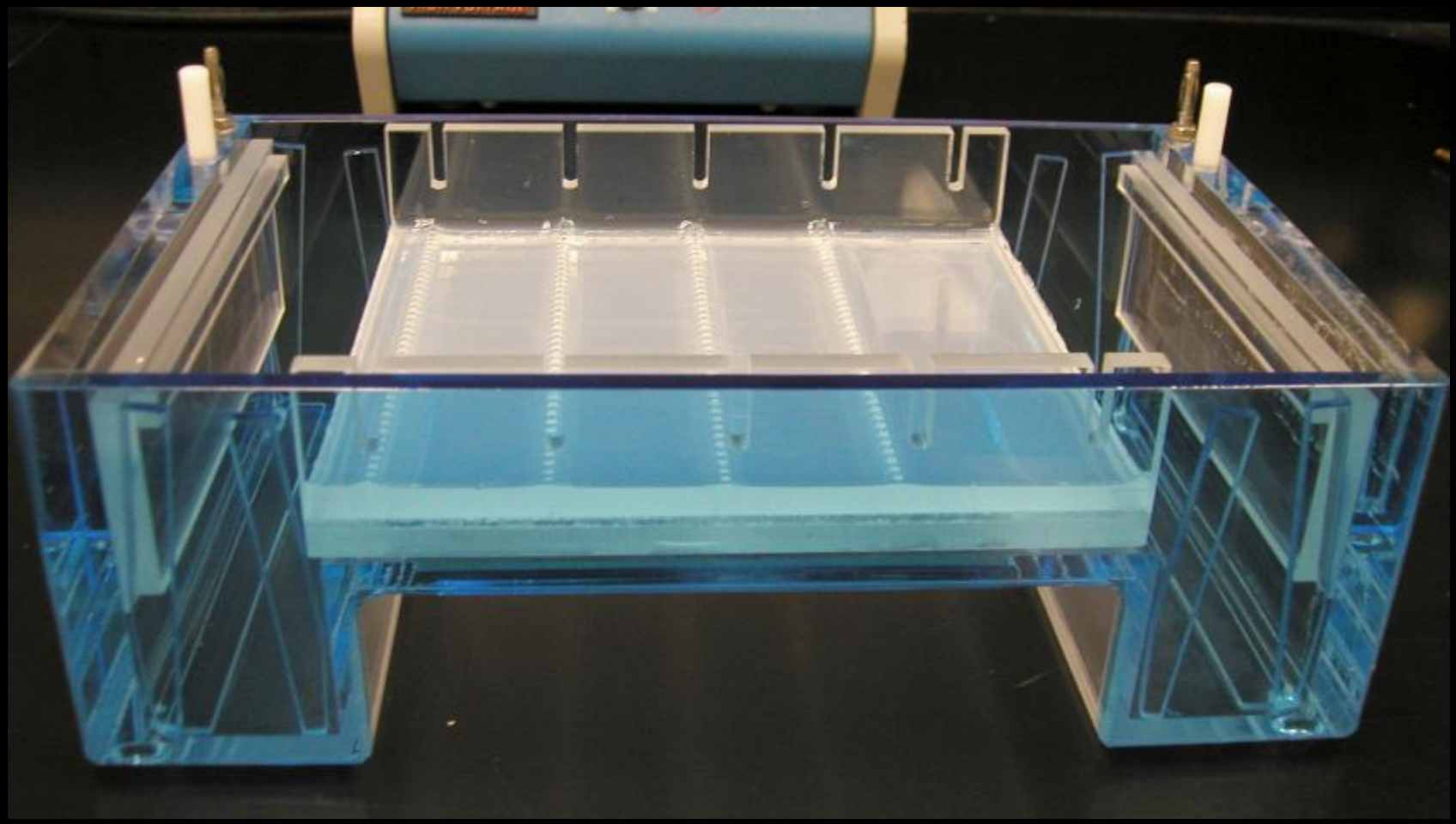
Allow the agarose solution to cool slightly (~60ºC) and then carefully pour the melted agarose solution into the casting tray. Avoid air bubbles.



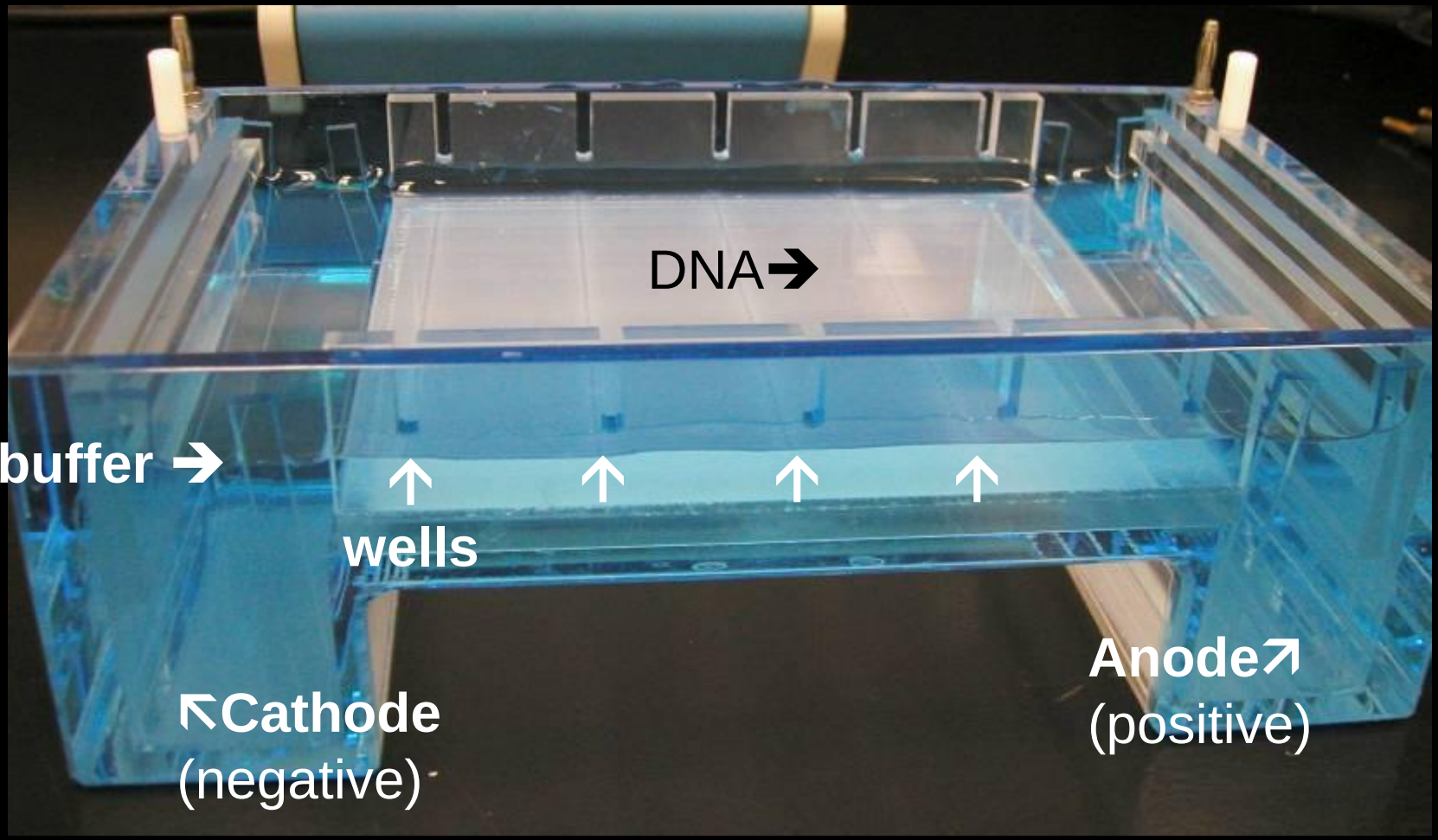
Each of the gel combs should be submerged in the melted agarose solution.



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.



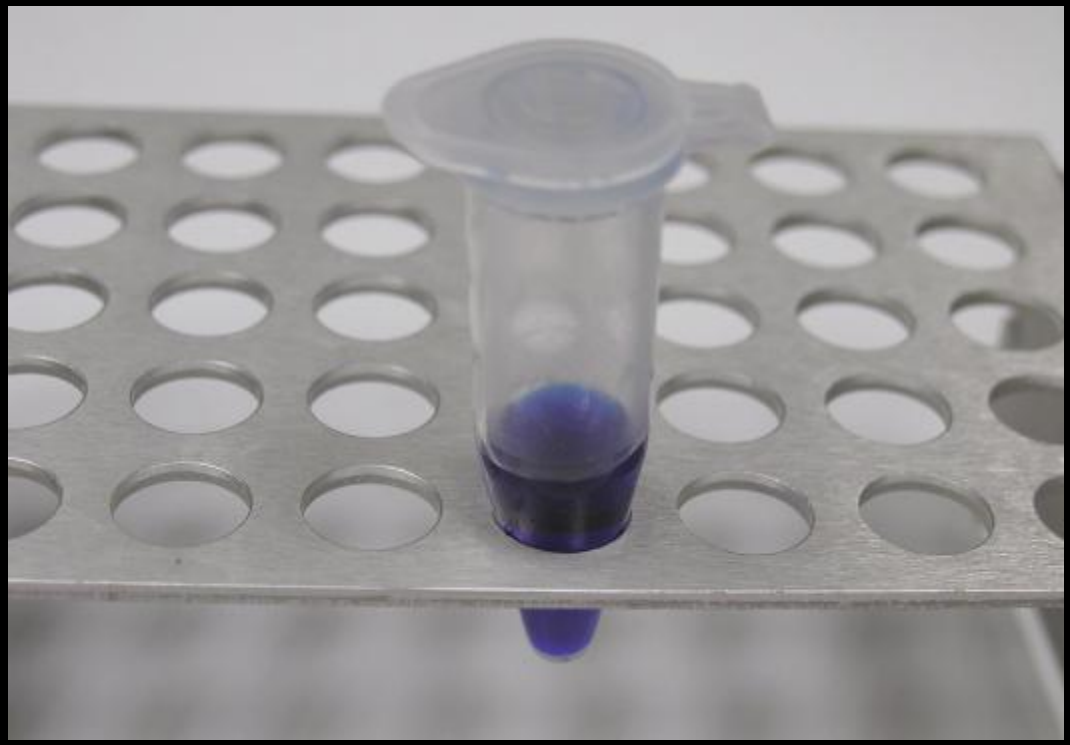
Place the gel in the electrophoresis chamber.



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

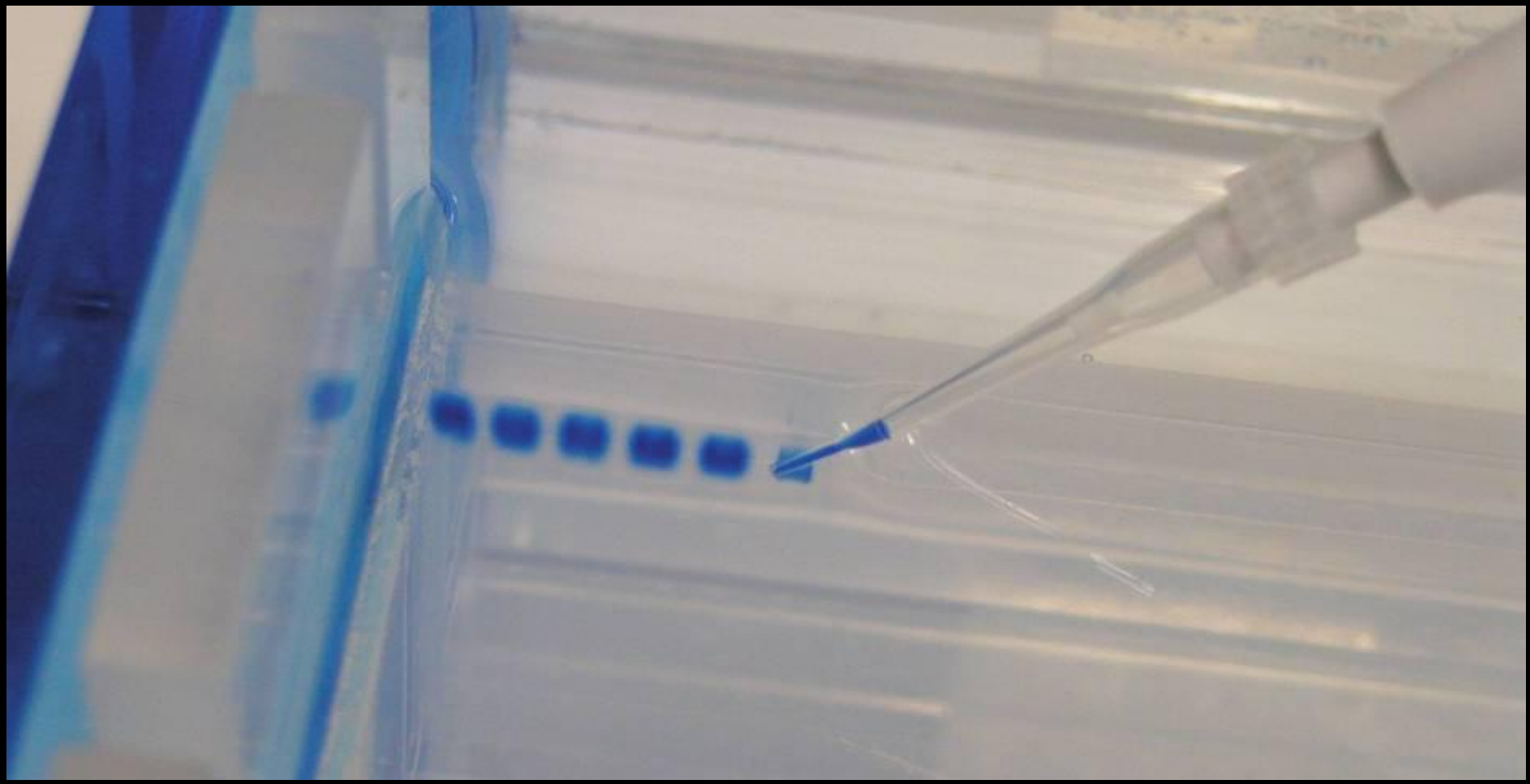
Mix the samples of DNA with the 6X sample loading buffer (w/ tracking dye). This allows the samples to be seen when loading onto the gel, and increases the density of the samples, causing them to sink into the gel wells.



6X Loading Buffer: →

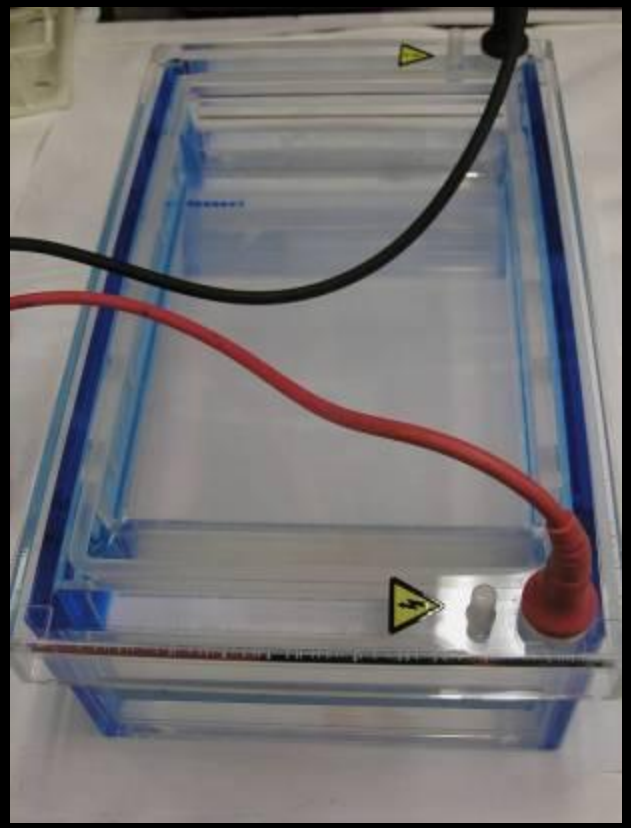
- Bromophenol Blue (for color)
- Glycerol (for weight)

Loading the Gel



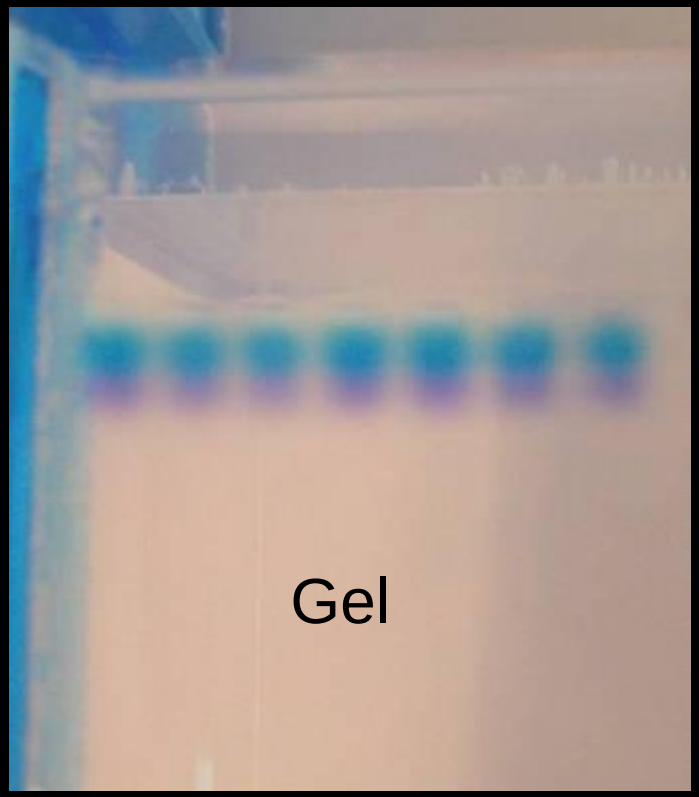
Carefully place the pipette tip over a well and gently expel the sample. The sample should sink into the well. Be careful not to puncture the gel with the pipette tip.

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
(-)



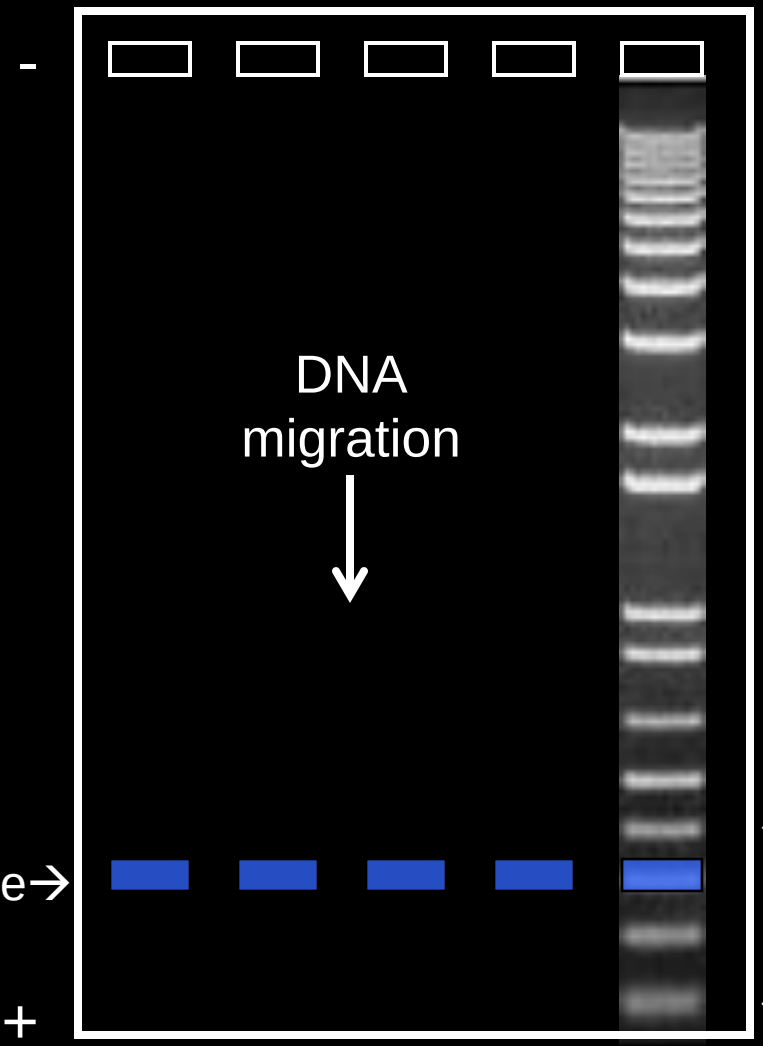
← wells
← Bromophenol Blue

DNA
(-)
↓

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.

DNA Ladder Standard



Note: bromophenol blue migrates at approximately the same rate as a 300 bp DNA molecule

bromophenol blue→

Inclusion of a DNA ladder (DNAs of know sizes) on the gel makes it easy to determine the sizes of unknown DNAs.

As an alternative to purchasing costly DNA ladders, one can be created using meal worm (*Tenebrio molitor*) DNA and a restriction enzyme.

DNA exercise VII: Characterization of satellite DNA from the Meal Worm Beetle

Behavioral Objectives

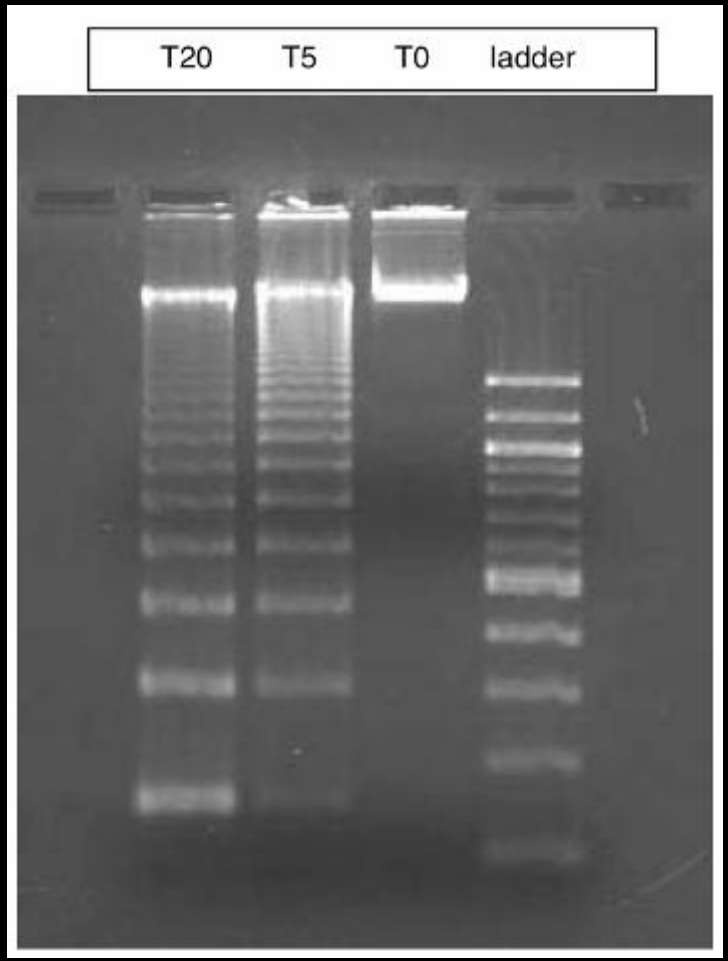
1. To become familiar with the life cycle of the Meal Worm Beetle, *Tenebrio molitor*.
2. To be able to distinguish between the different classes of DNA sequences found within a eukaryote's genome.
3. To gain some familiarity with the methods used for extracting nucleic acids from complex eukaryotes.
4. To appreciate the power of restriction enzymes for studying structural aspects of an organism's genomic DNA.

Introduction

So far in this laboratory course, restriction endonucleases have been used to analyze and characterize relatively small DNA molecules (e.g. the genome of phage lambda). However, restriction endonucleases can also be used to investigate certain structural aspects of much larger DNA molecules, such as those that comprise the genomes of higher eukaryotes. The genomes of higher eukaryotes possess three classes of DNA sequences. Those that are present only once per haploid genome are referred to as unique (nonrepetitive) sequences, and can account for between 10-80% of a eukaryote's genome. Sequences that are repeated from 10 to 10,000 times per haploid genome are called moderately repetitive DNA sequences, and can constitute anywhere from 10-50% of a eukaryotic genome. The final class of DNA sequences are those that are referred to highly repetitive, and are typically repeated in excess of a 100,000 times; these sequences may constitute 10-80% of a eukaryote's genome.

Highly repetitive DNA sequences are located primarily within centromeres and telomeres, and possess repeat units ranging from 5-300 bp that are often arranged one after the other in large tandem arrays. Because these sequences often possess higher levels of AT or GC nucleotides than unique sequence DNA, they tend to band separately from unique sequence DNA during equilibrium density-gradient centrifugation, forming a "satellite" band. Thus, highly repetitive DNA is often referred to as satellite DNA.

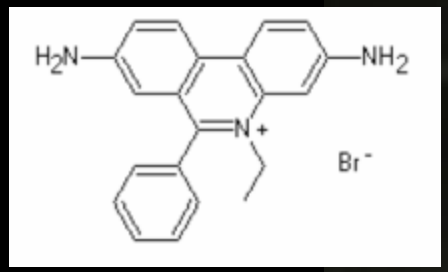
The Yellow Meal Worm Beetle (*Tenebrio molitor*) is an insect that possesses a complex developmental cycle beginning with eggs, laid by the female adult beetle, that hatch within about two weeks to form relatively long-lived larvae. These larvae undergo a number of molting (instar) stages over the course of about 8-9 months producing pupae that transform into adult beetles after about a month. The meal worm beetle often infests grain and grain products such as cereals and although it is not a major food pest, due to its relatively slow life cycle, when encountered its larvae commonly cause alarm due to their relatively large size. Meal Worm larvae are frequently raised for use as fishing bait and for feeding certain types of birds.



http://people.uis.edu/rmosh1/DNAexerciseVIIa02.pdf

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**.

Safer alternatives to Ethidium Bromide

- Methylene Blue
- BioRAD - Bio-Safe DNA Stain
- Ward's - QUIKView DNA Stain
- Carolina BLU Stain

…others

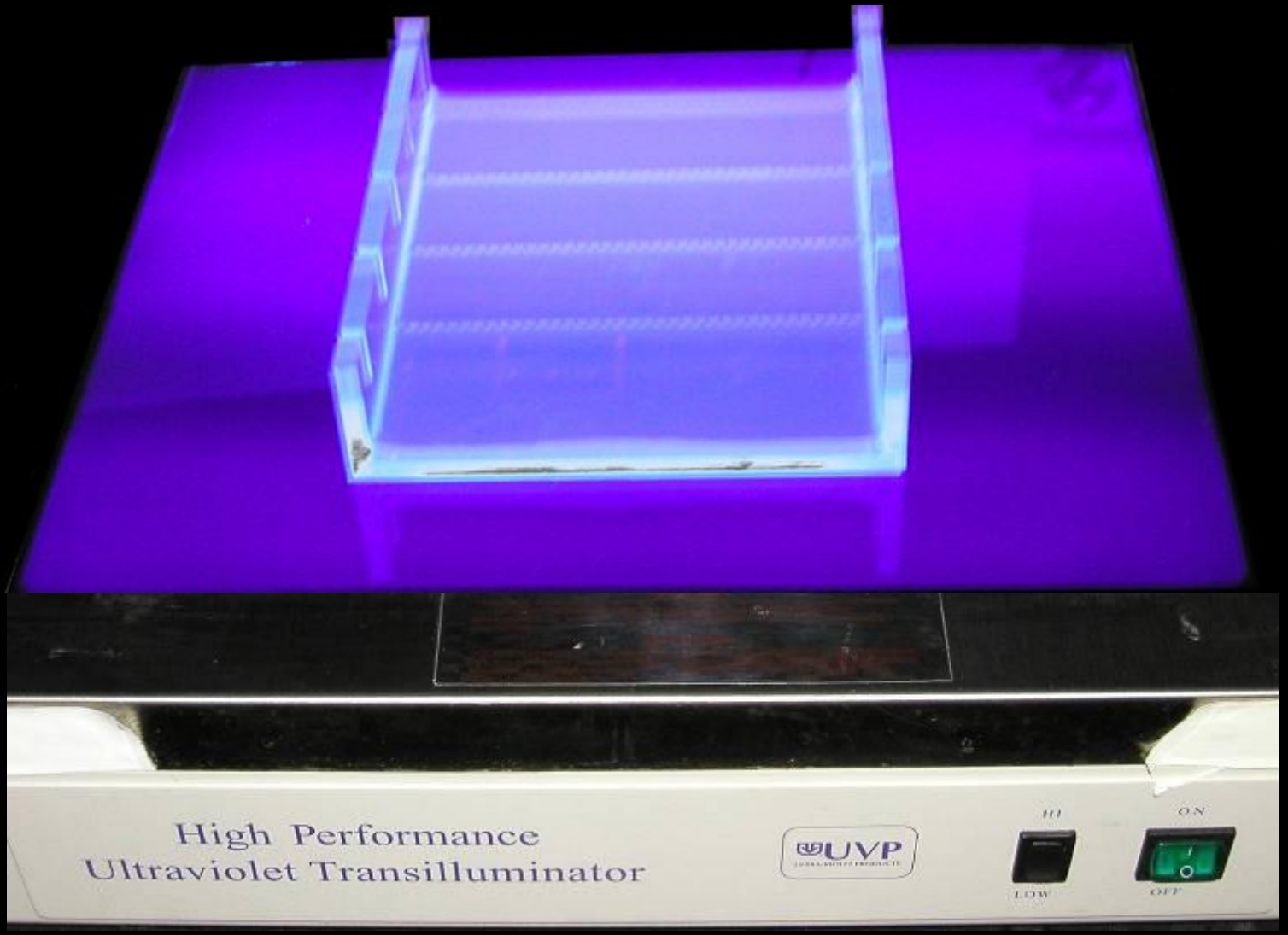
advantages
Inexpensive
Less toxic
No UV light required
No hazardous waste disposal

disadvantages
Less sensitive
More DNA needed on gel
Longer staining/destaining time

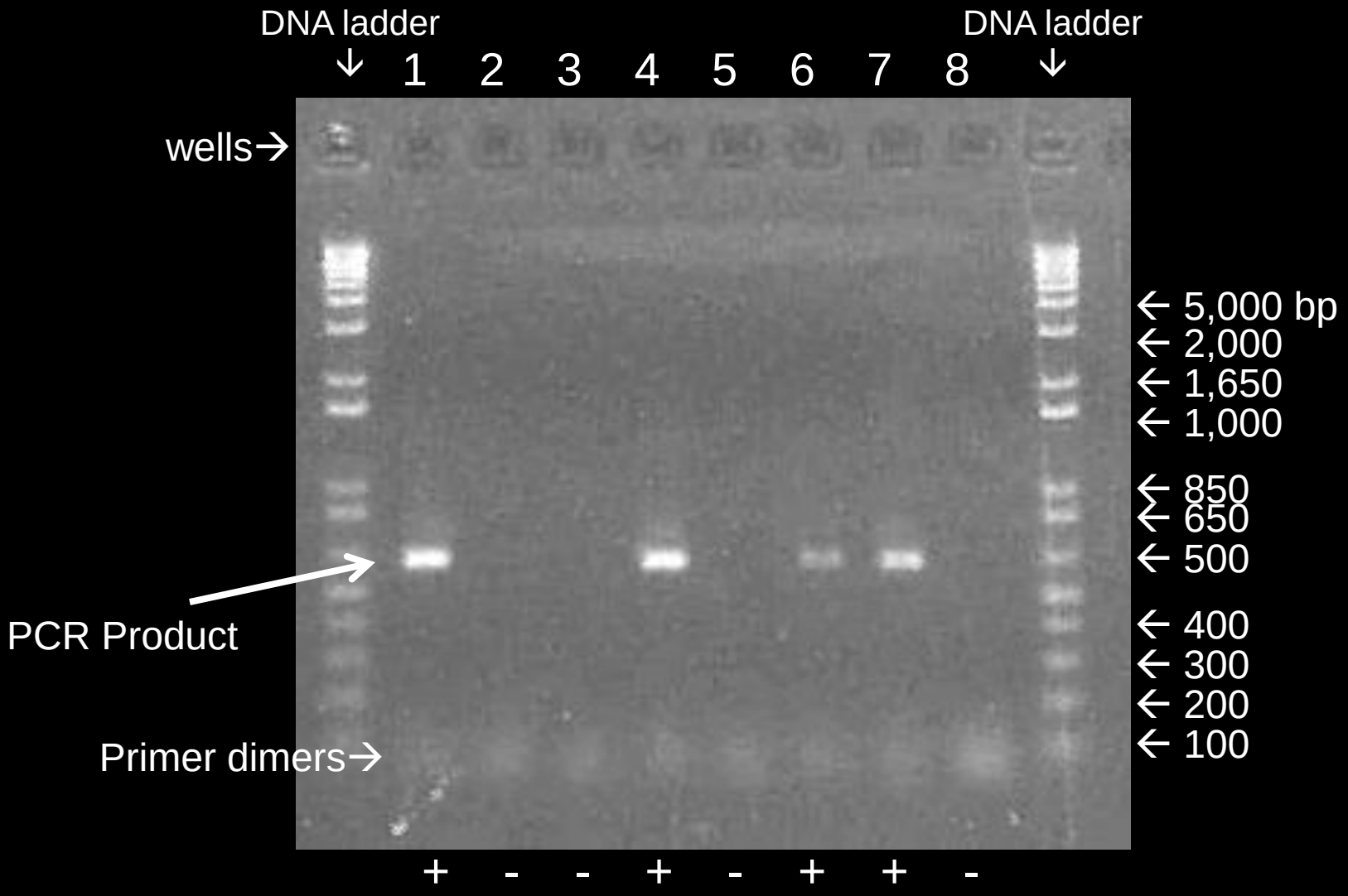
Staining the Gel

- Place the gel in the staining tray containing warm diluted stain.
- Allow the gel to stain for 25-30 minutes.
- To remove excess stain, allow the gel to destain in water.
- Replace water several times for efficient destain.

Ethidium Bromide requires an ultraviolet light source to visualize

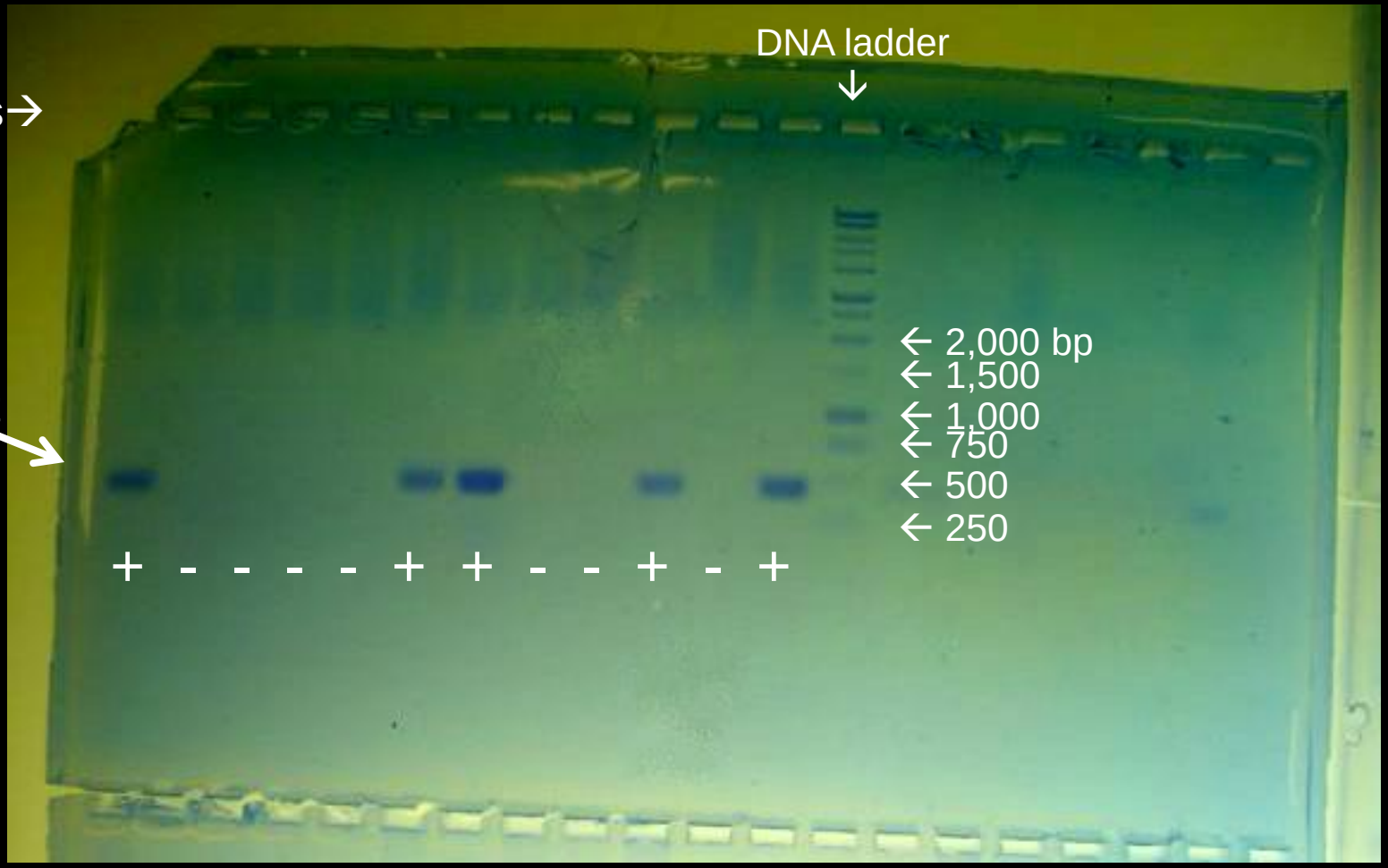


Visualizing the DNA (ethidium bromide)



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

Visualizing the DNA (QuikVIEW stain)



Samples # 1, 6, 7, 10 & 12 were positive for Wolbachia DNA

March 12, 2006