Gel Electrophoresis

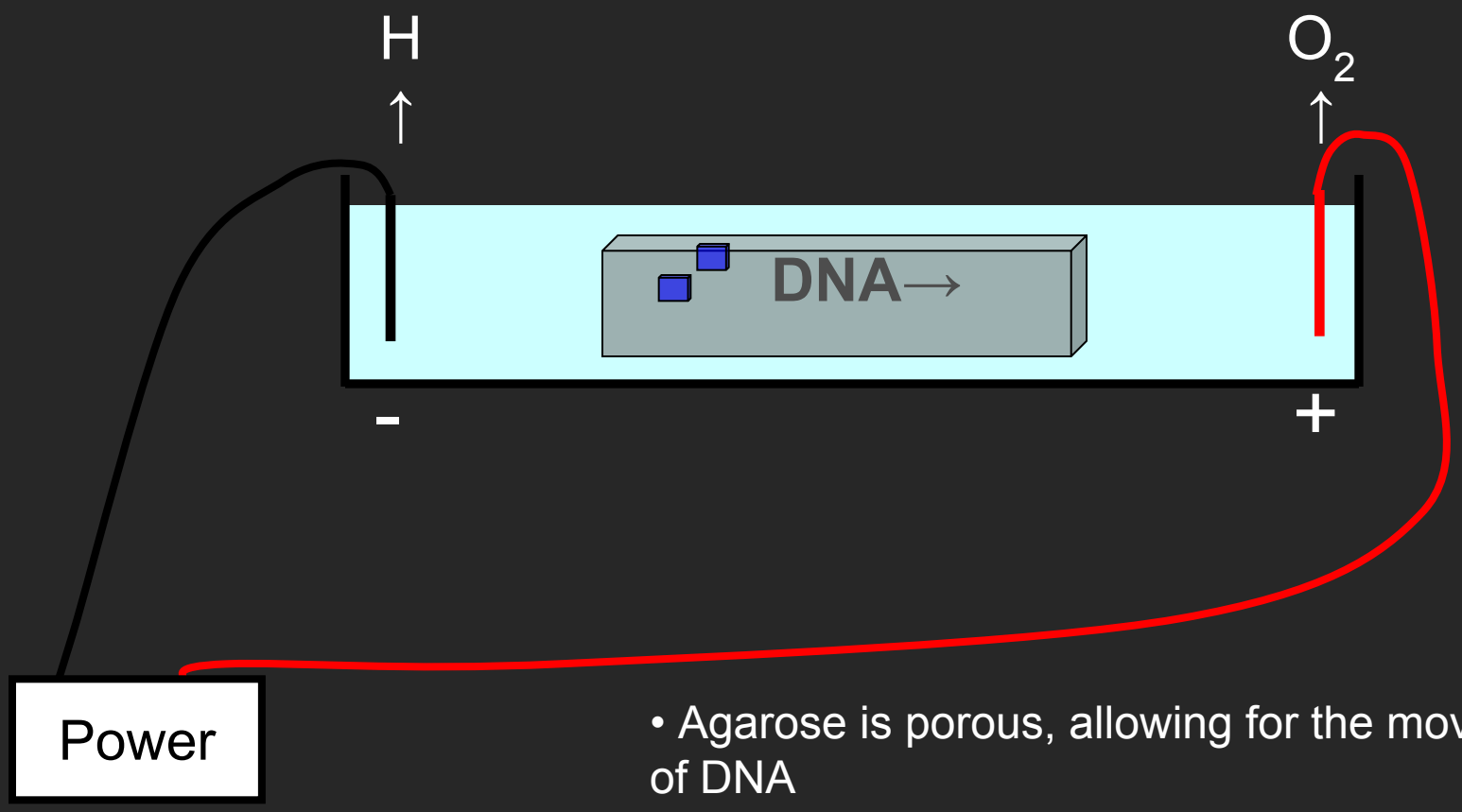
Agarose Gel Electrophoresis

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.

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 charge and size of dyes and DNA strands.

- 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.



- Agarose is porous, allowing for the movement of DNA

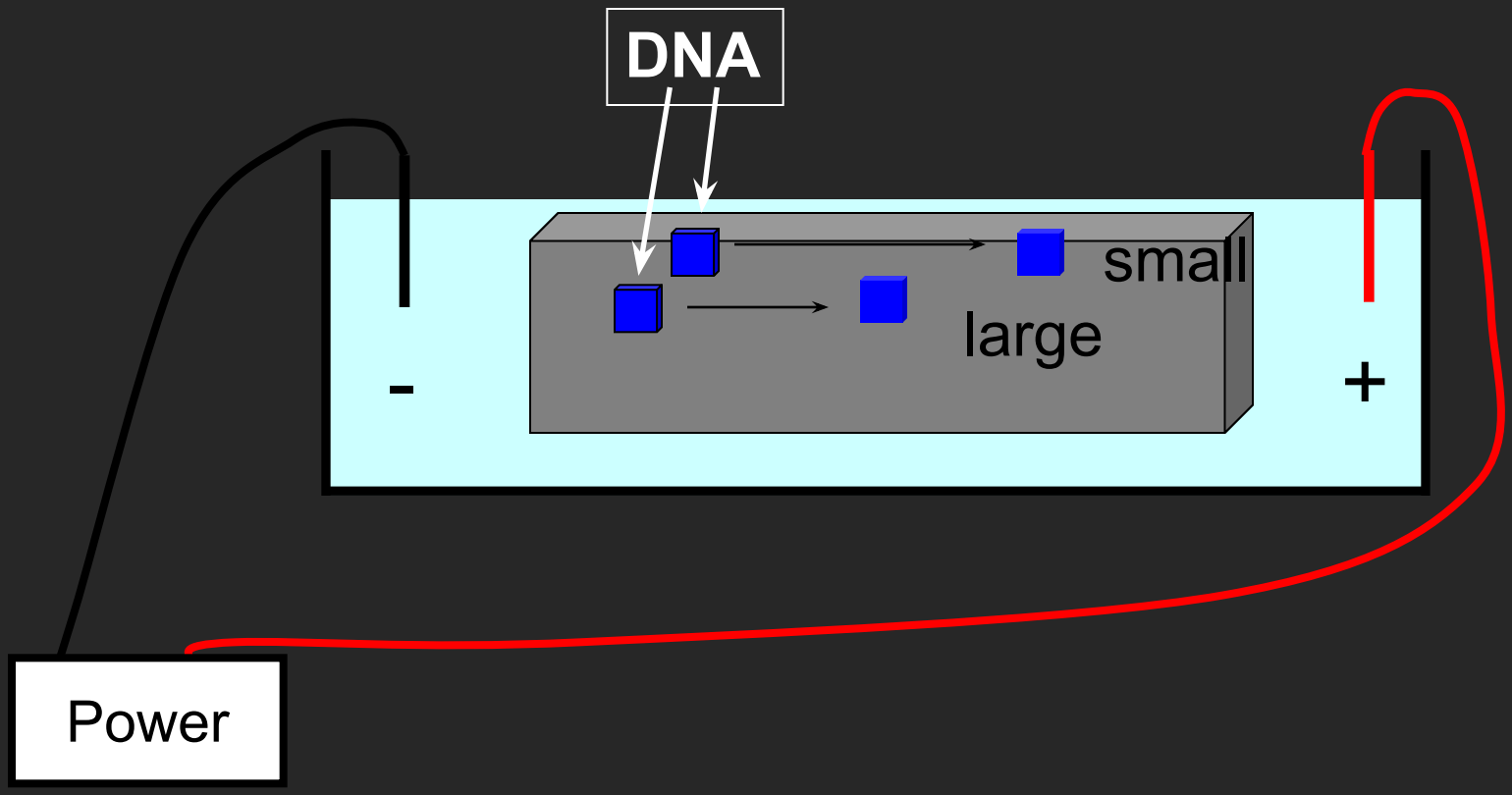
How fast will the DNA migrate?

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

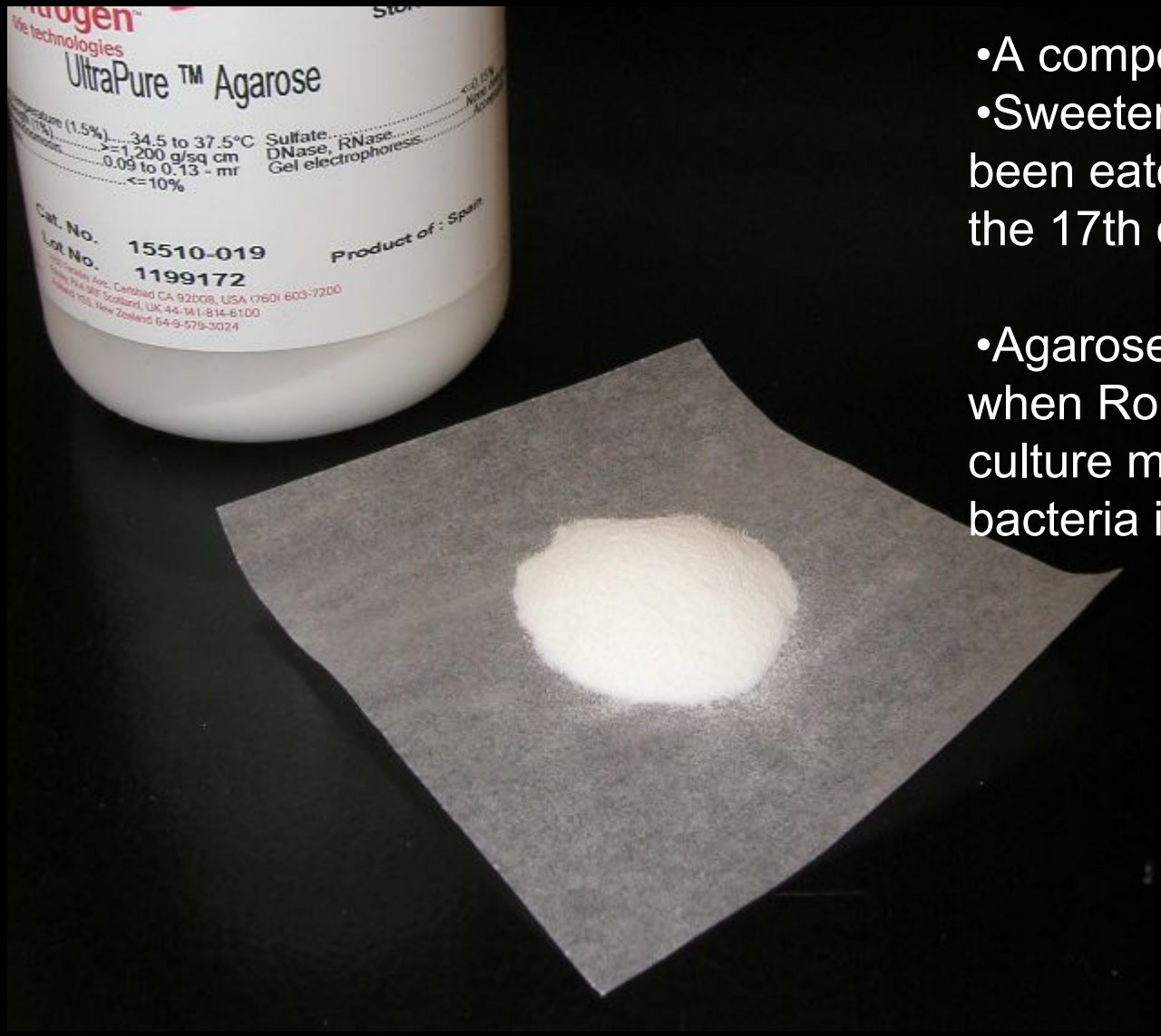
Size of the DNA!

*Small DNA move faster than large DNA

…gel electrophoresis separates DNA according to size



Agarose



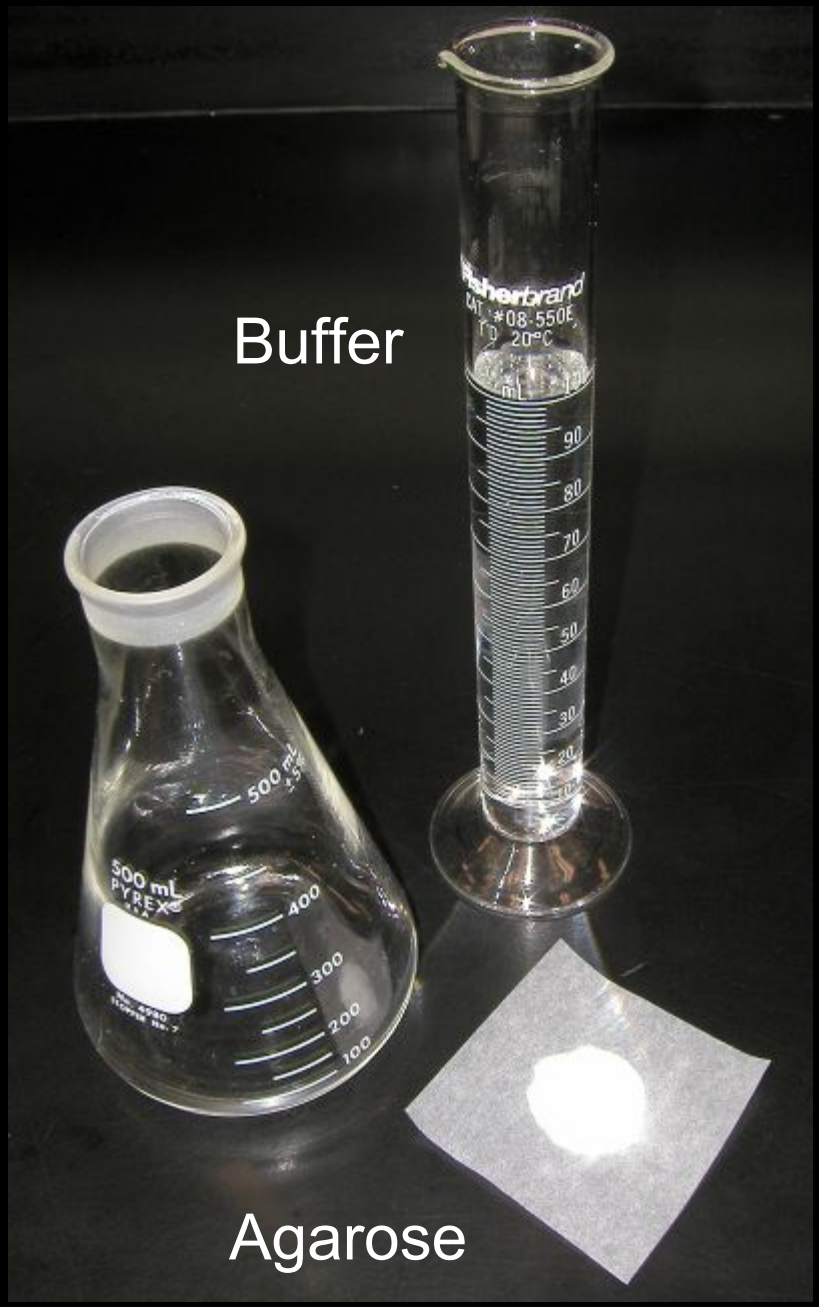
- A compound made of sugars
- Sweetened agarose gels have been eaten in the Far East since the 17th century.

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

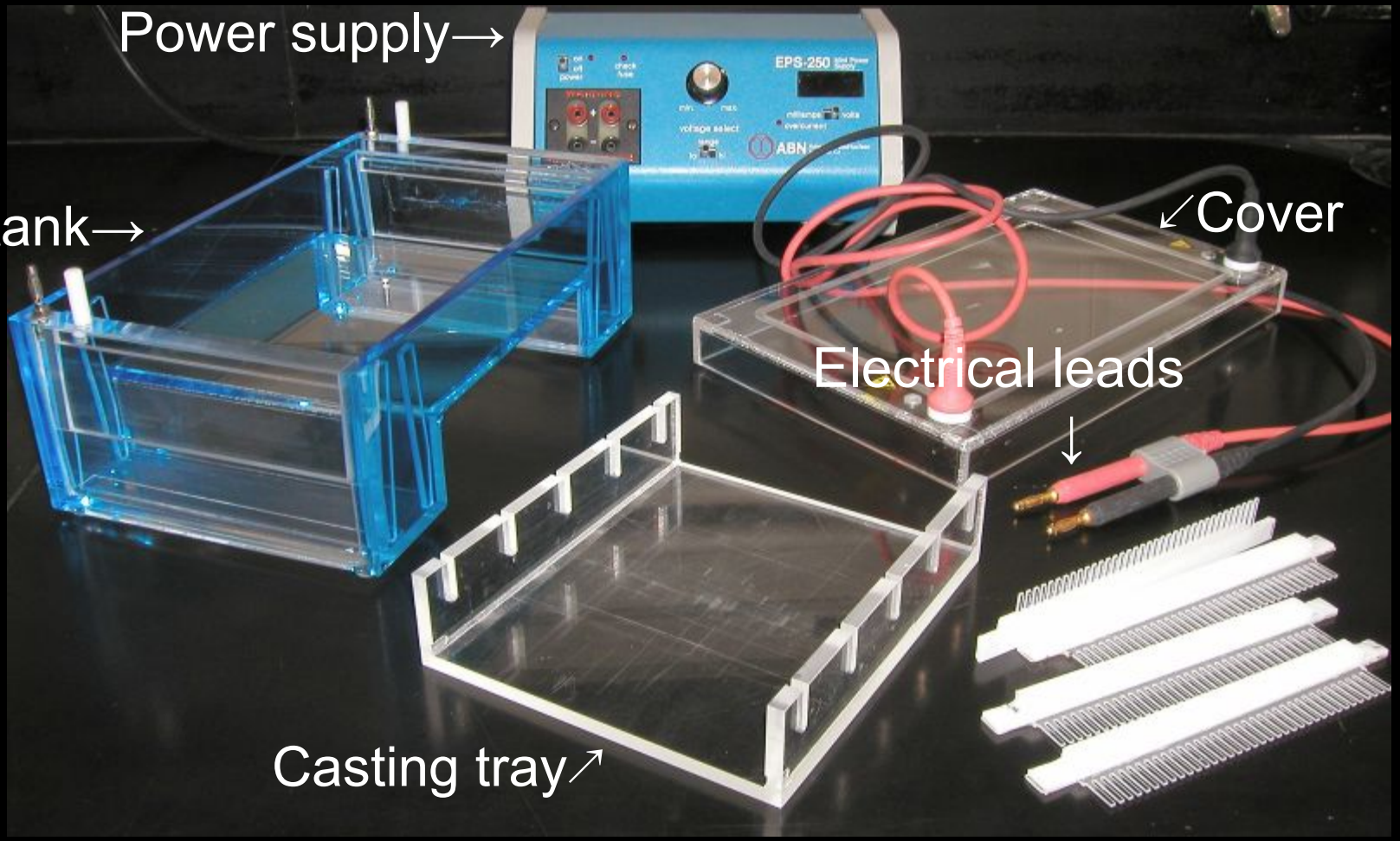
Agarose is a linear polymer extracted from seaweed.

Making an Agarose Gel

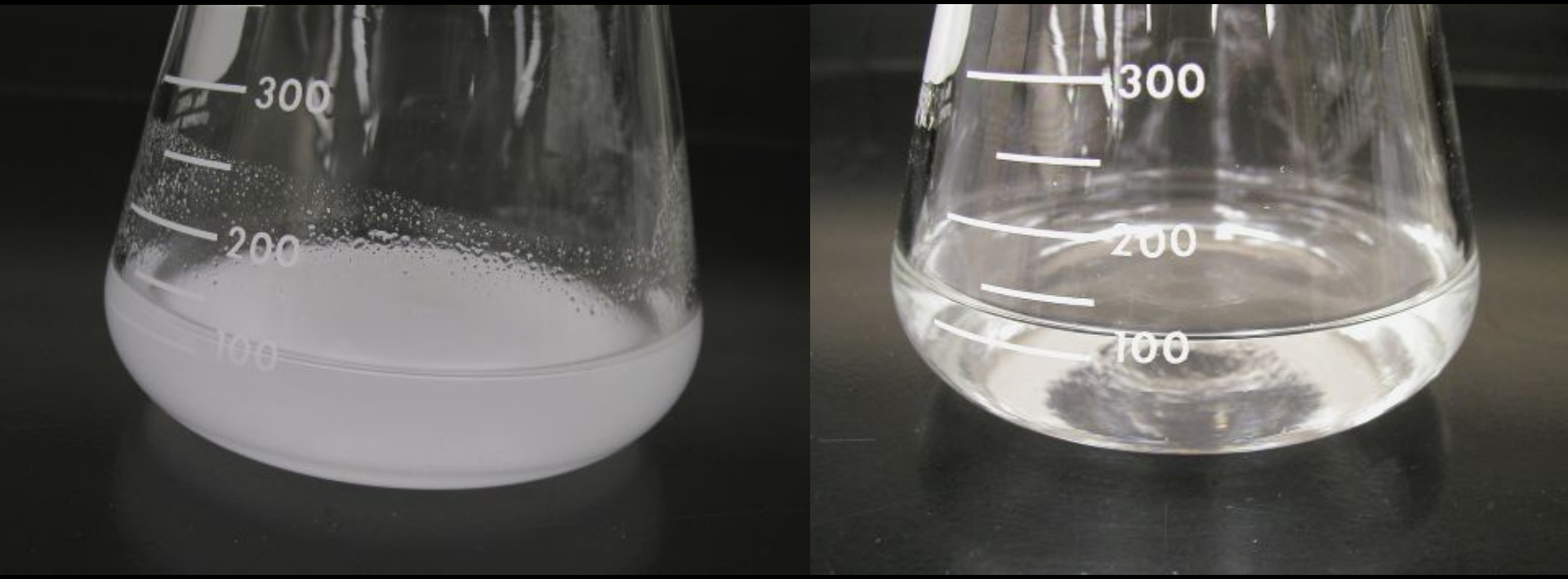
An agarose gel is prepared by combining agarose powder and a buffer solution.



Electrophoresis Equipment



Melting the Agarose

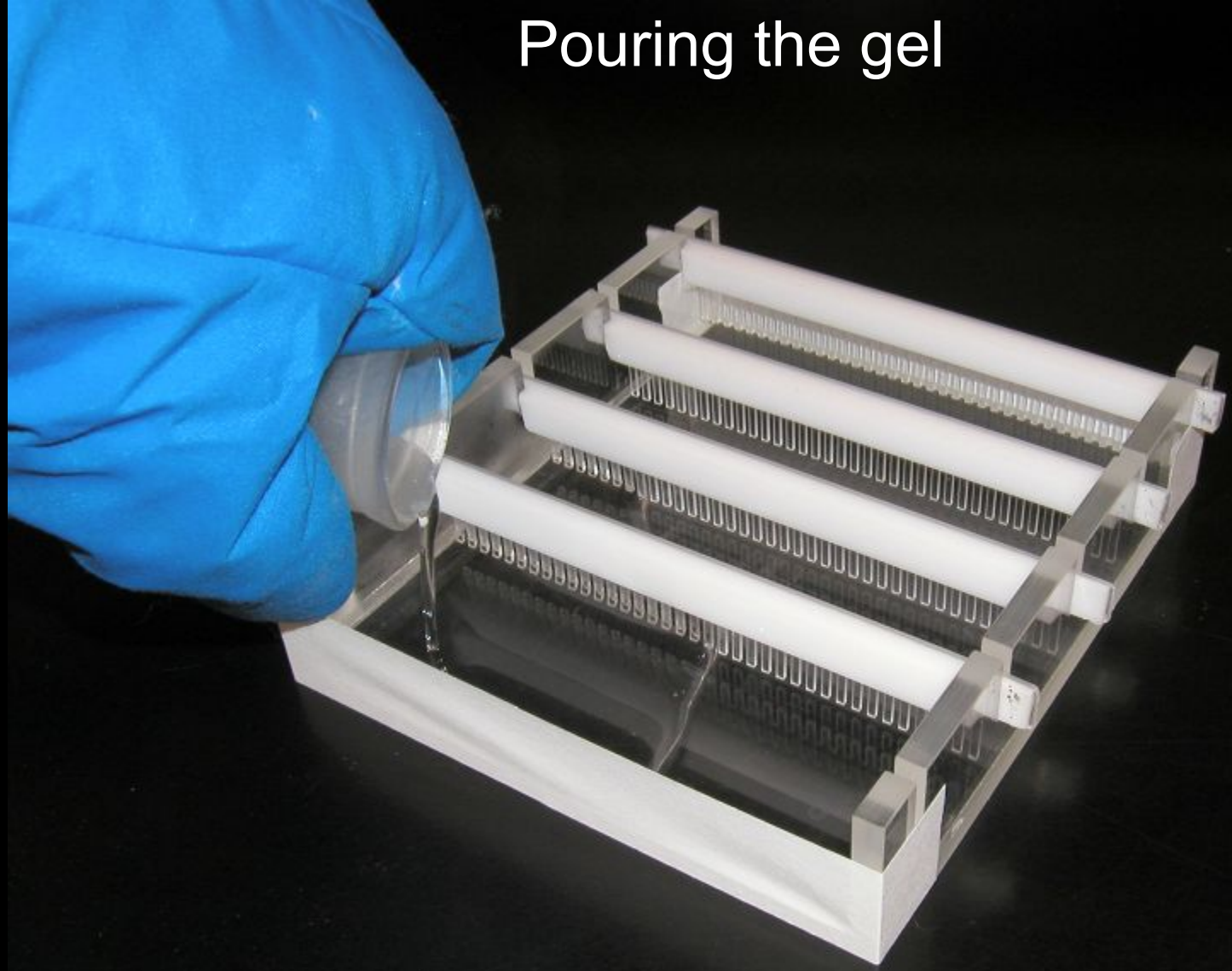


Agarose is insoluble at room temperature (left).
The agarose solution is boiled until clear (right).

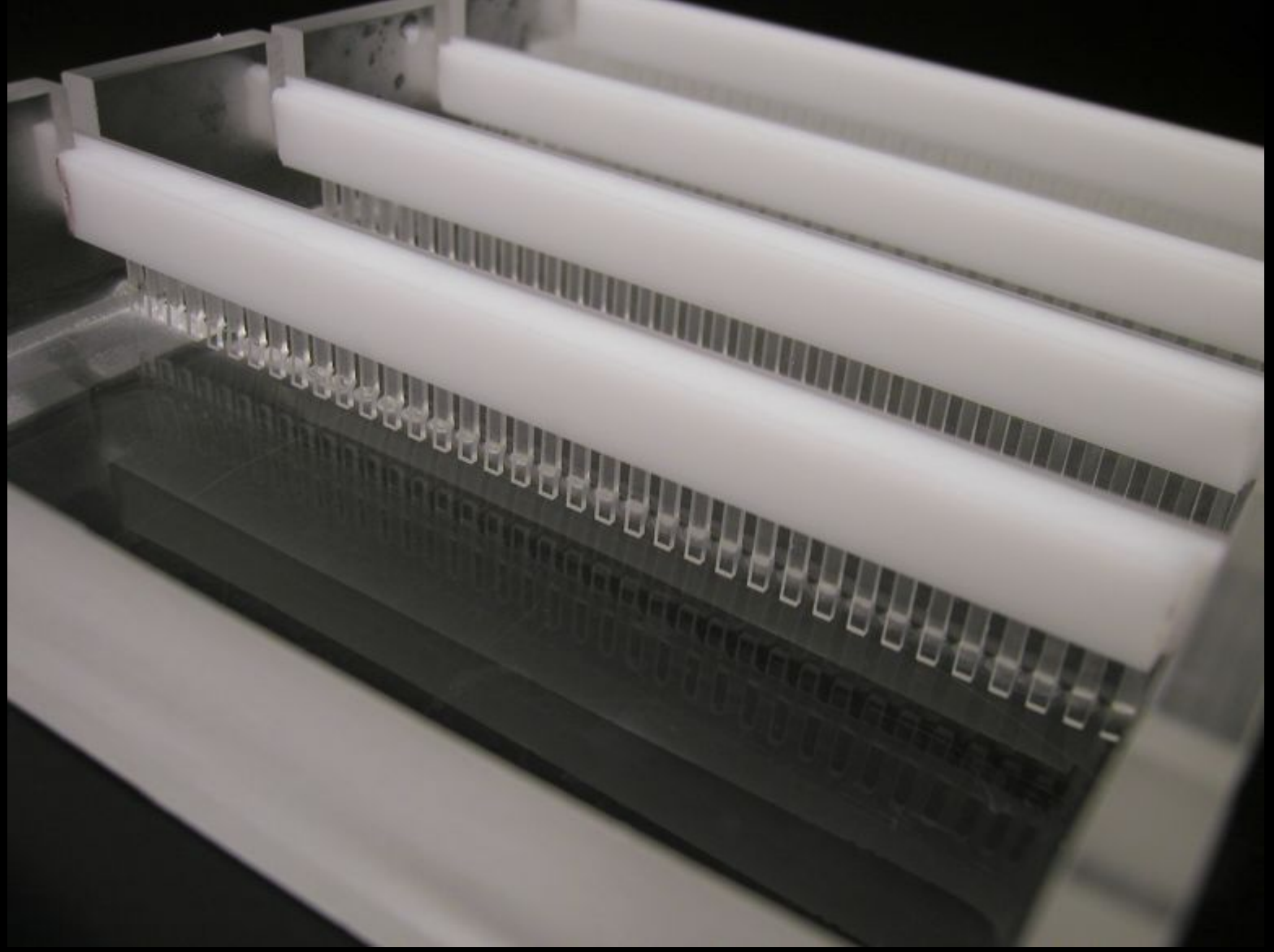
Gently swirl the solution periodically when heating to allow all the grains of agarose to dissolve.

***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.

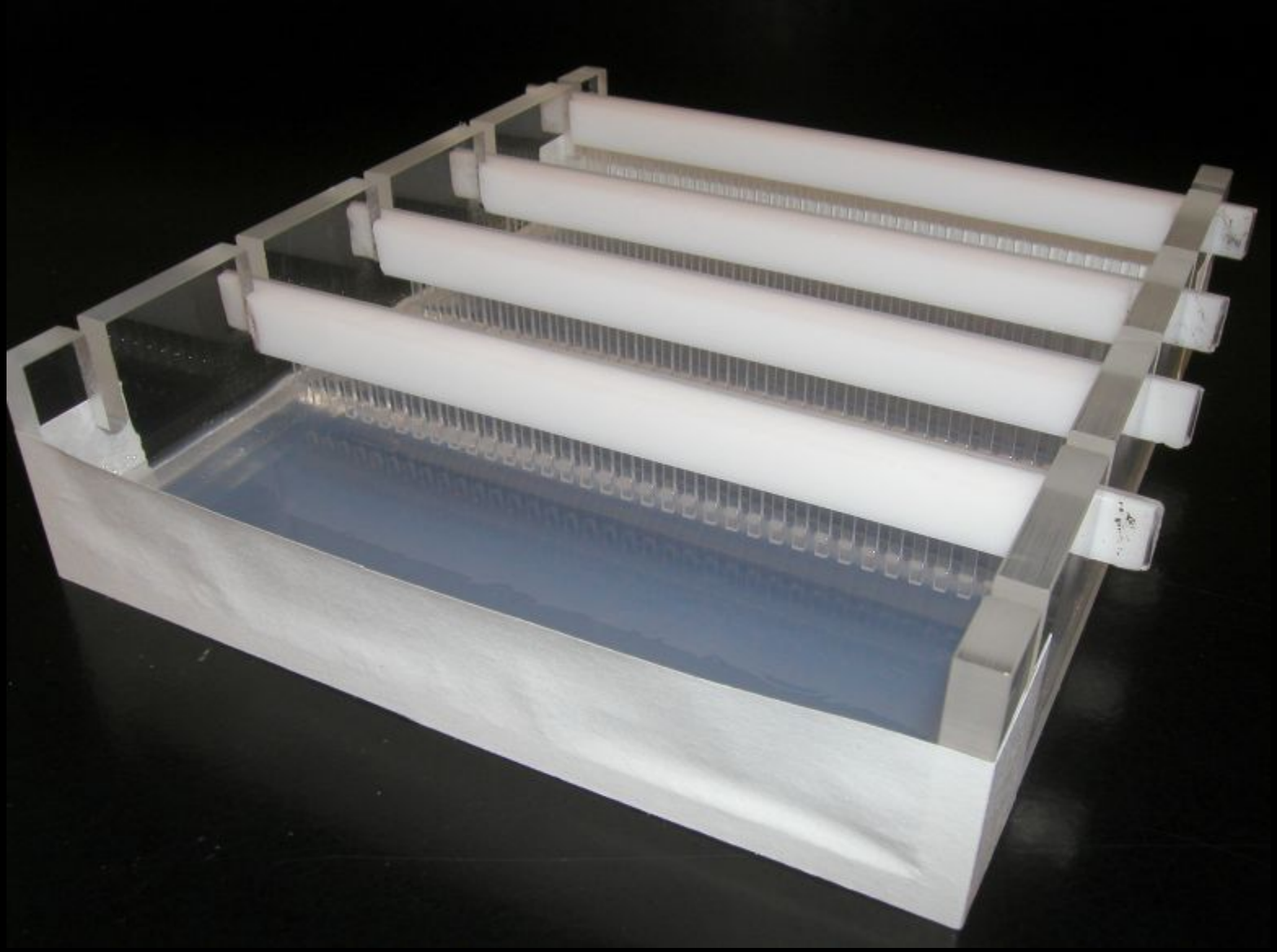
Pouring the gel



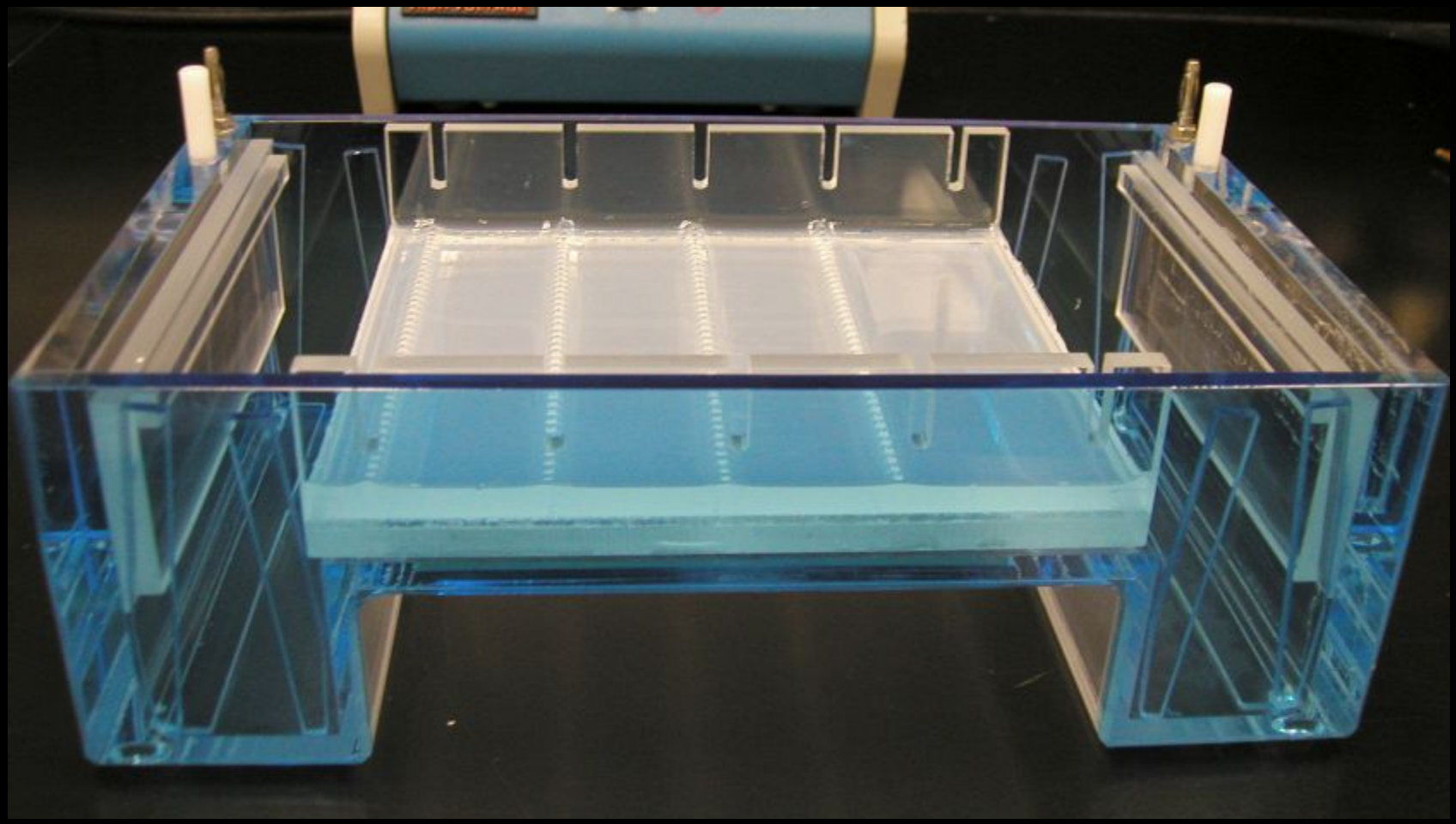
Allow the agarose solution to cool slightly (~60ºC) and then carefully pour the melted agarose solution into the casting tray. Avoid air bubbles.



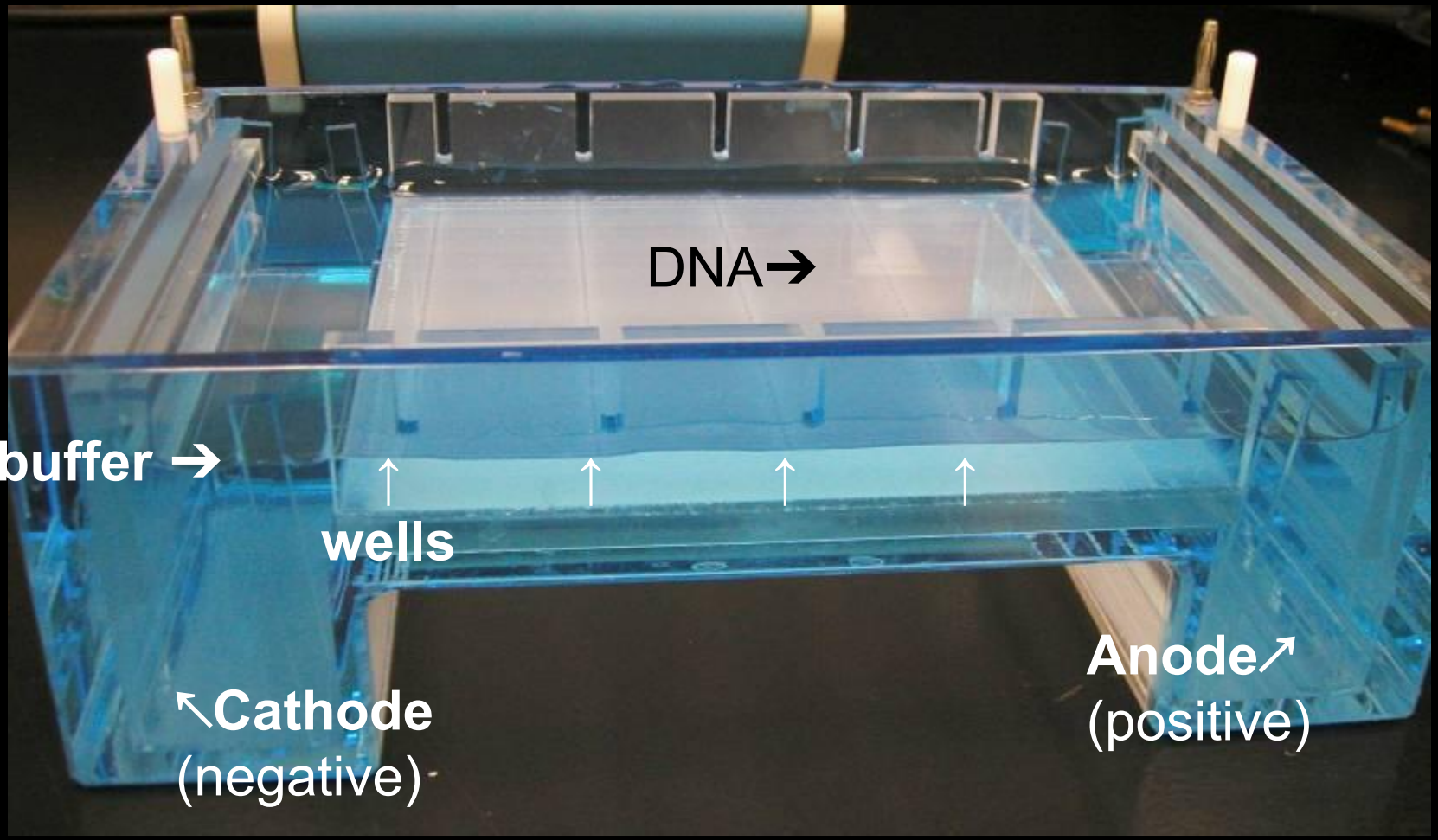
Each of the gel combs should be submerged in the melted agarose solution.



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.

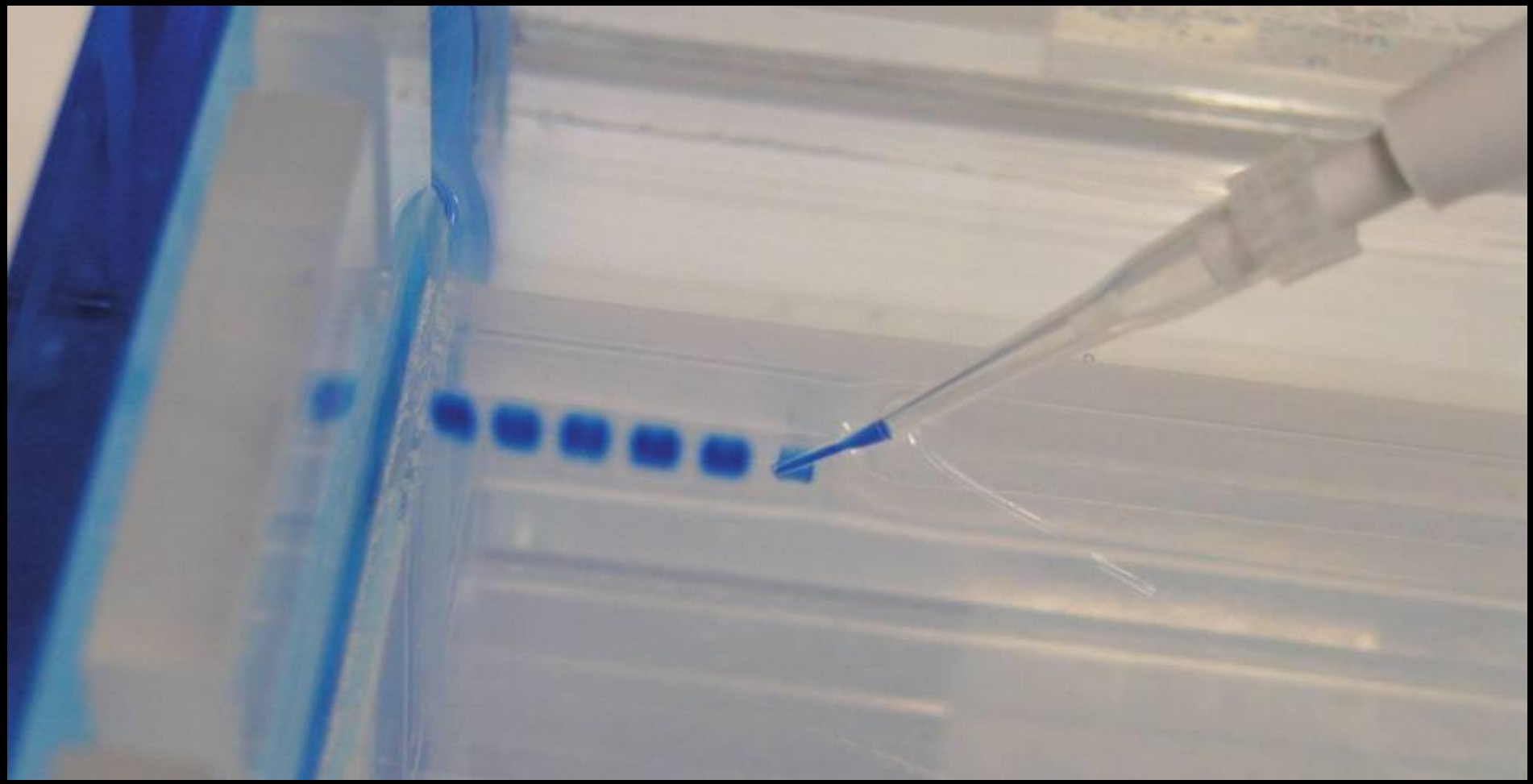


Place the gel in the electrophoresis chamber.



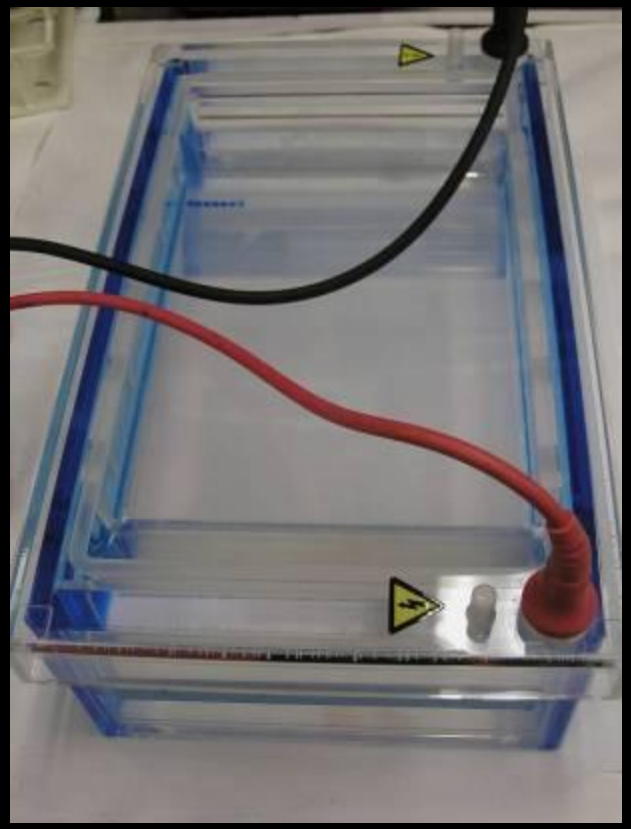
Add enough electrophoresis buffer to cover the gel to a depth of at least 1 mm. Make sure each well is filled with buffer.

Loading the Gel



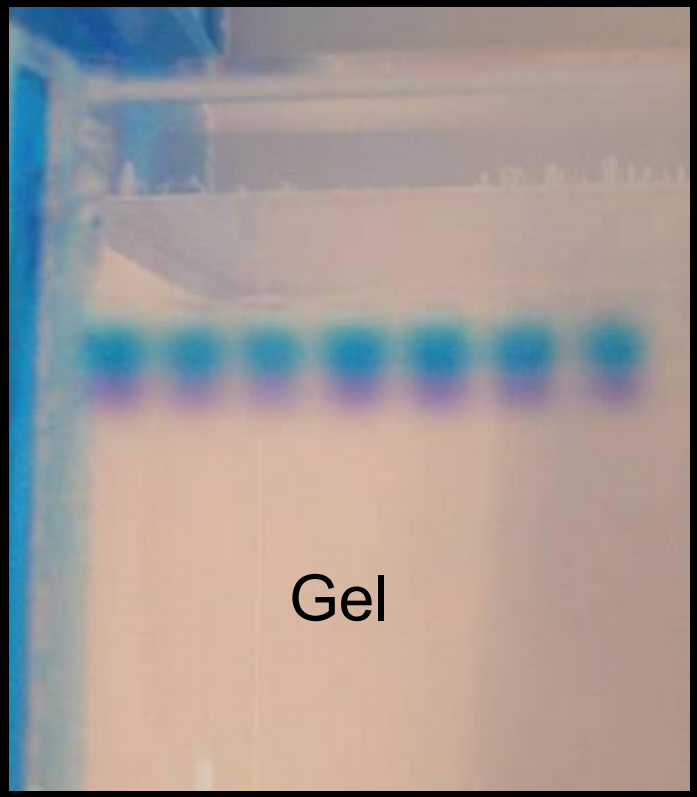
Carefully place the pipette tip over a well and gently expel the sample. The sample should sink into the well. Be careful not to puncture the gel with the pipette tip.

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
(-)



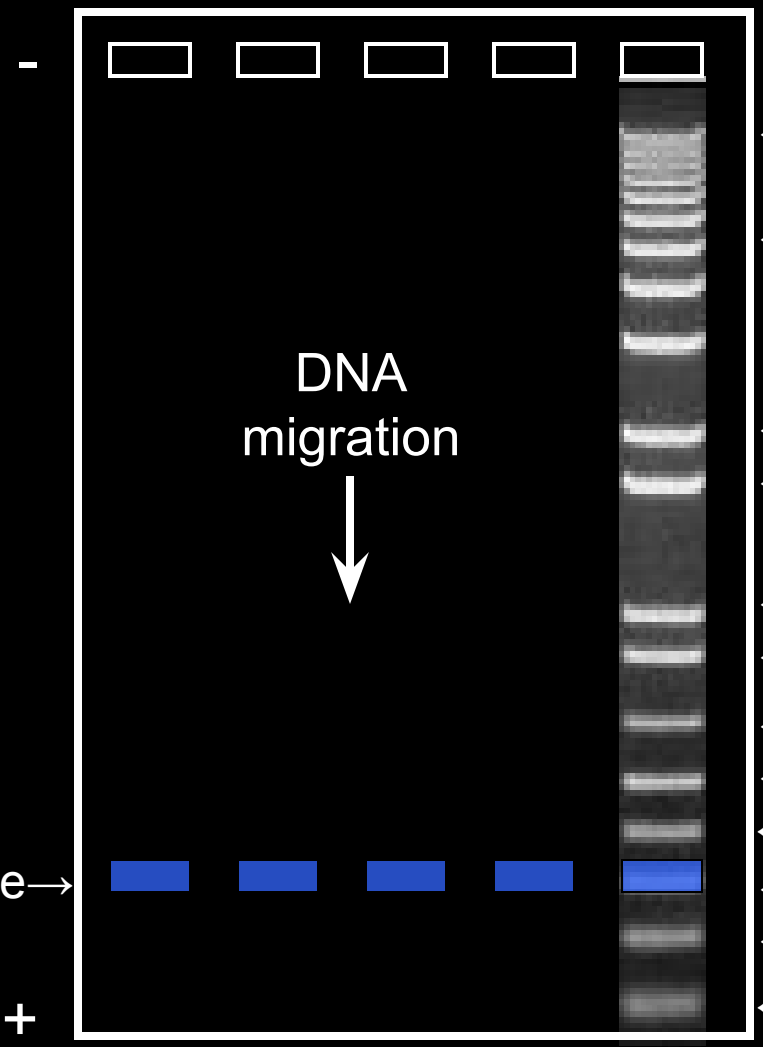
← wells
← Dye

DNA
(-)
↓

Anode
(+)

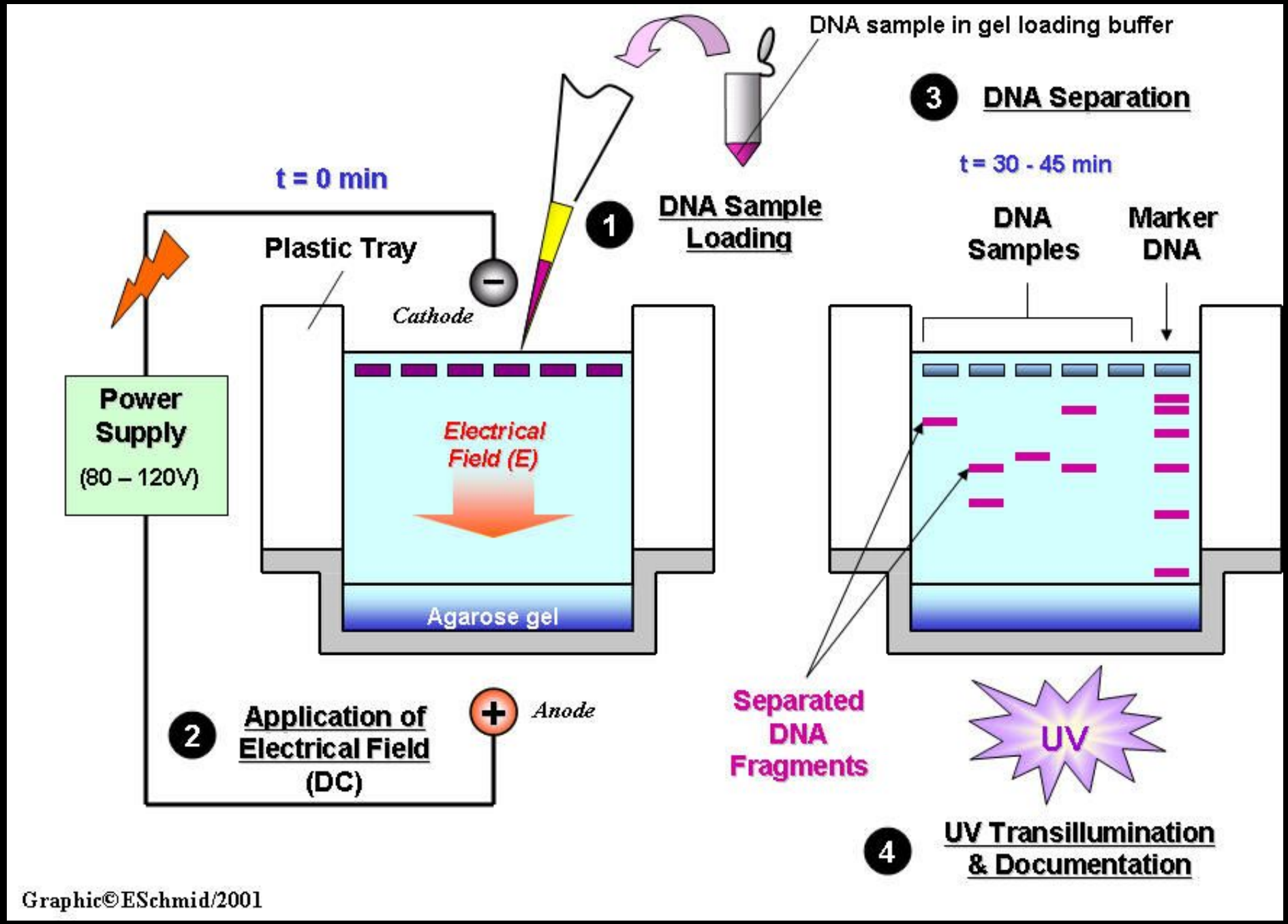
After the current is applied, make sure the Gel is running in the correct direction. Dye will run in the same direction as the DNA.

DNA Ladder Standard



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

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


DNA sample in gel loading buffer
3 DNA Separation
t = 0 min
t = 30 - 45 min
1 DNA Sample Loading
DNA Samples
Marker DNA
Plastic Tray
Cathode
Power Supply
(80 – 120V)
Electrical Field (E)
Agarose gel
2 Application of Electrical Field (DC)
Anode
Separated DNA Fragments
UV
4 UV Transillumination & Documentation
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