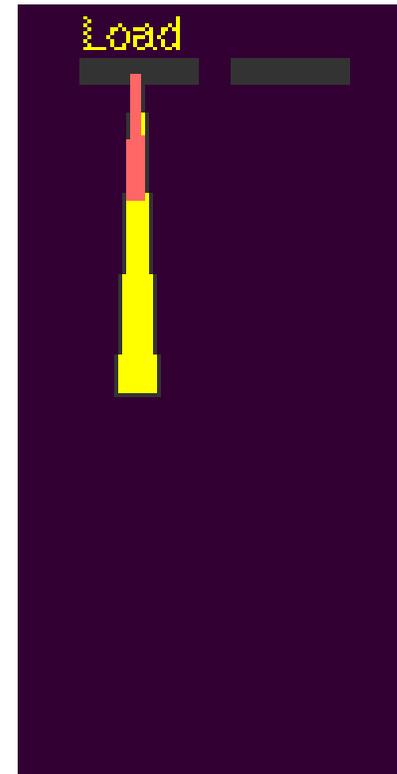


Week 5. Gel Electrophoresis

DNA AGAROSE GEL ELECTROPHORESIS

Department of Microbiology



Definition

- “*Electro*” means electric energy
- “*Phoresis*” is generated from the Greek word “*phoros*” which means to carry through.
- In the electrophoresis, with the help of a constant electric current, molecules are carried through a special matrix called agarose in a defined direction due to their electrical charge.

What is gel electrophoresis?

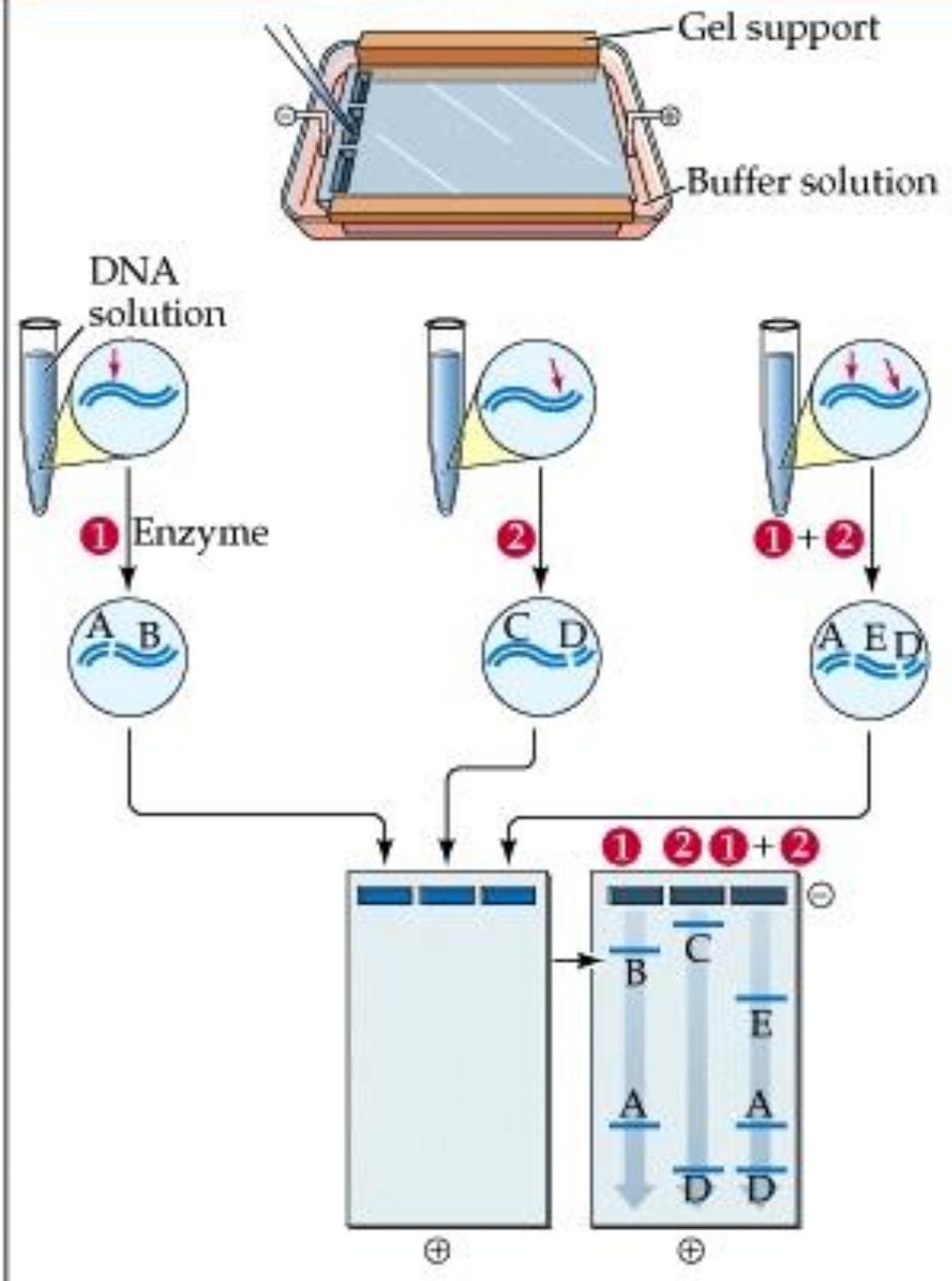
- It is a technique, used for the separation of charged molecules in the laboratory.
- DNA is a negatively charged molecule!
- DNA is moved through an agarose and/or polyacrylamide matrix with the help of a electric current “**running**”

- Gel electrophoresis separates macromolecules (proteins or nucleic acids) from each other, depending on their molecular sizes, electrical charges or other physical characteristics!

Electrophoresis;

- **Separation**
- **Identification**
- **Purification**
- **Restriction analysis**
- **Genotyping (PFGE)**

RESEARCH METHOD



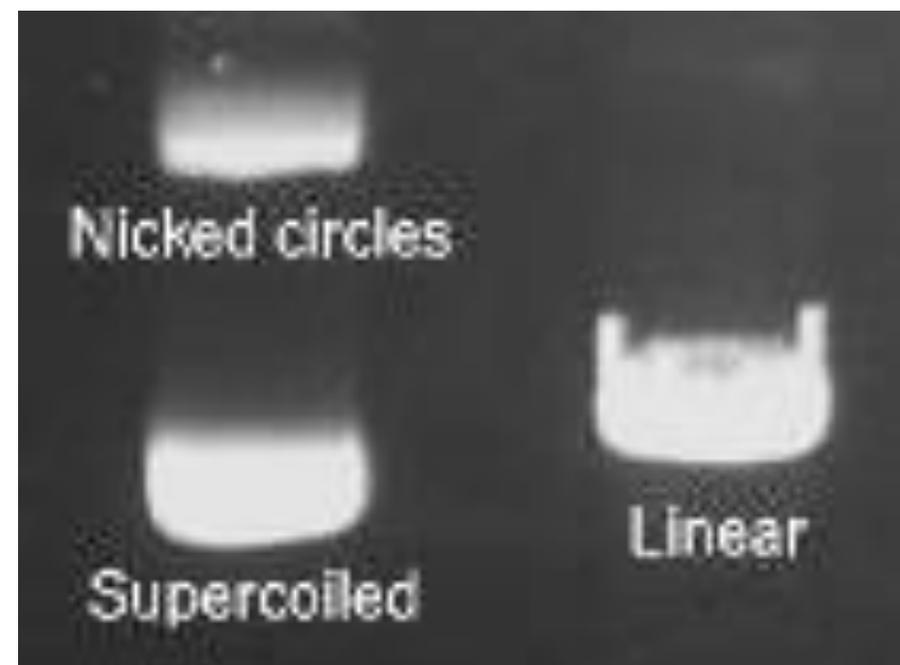
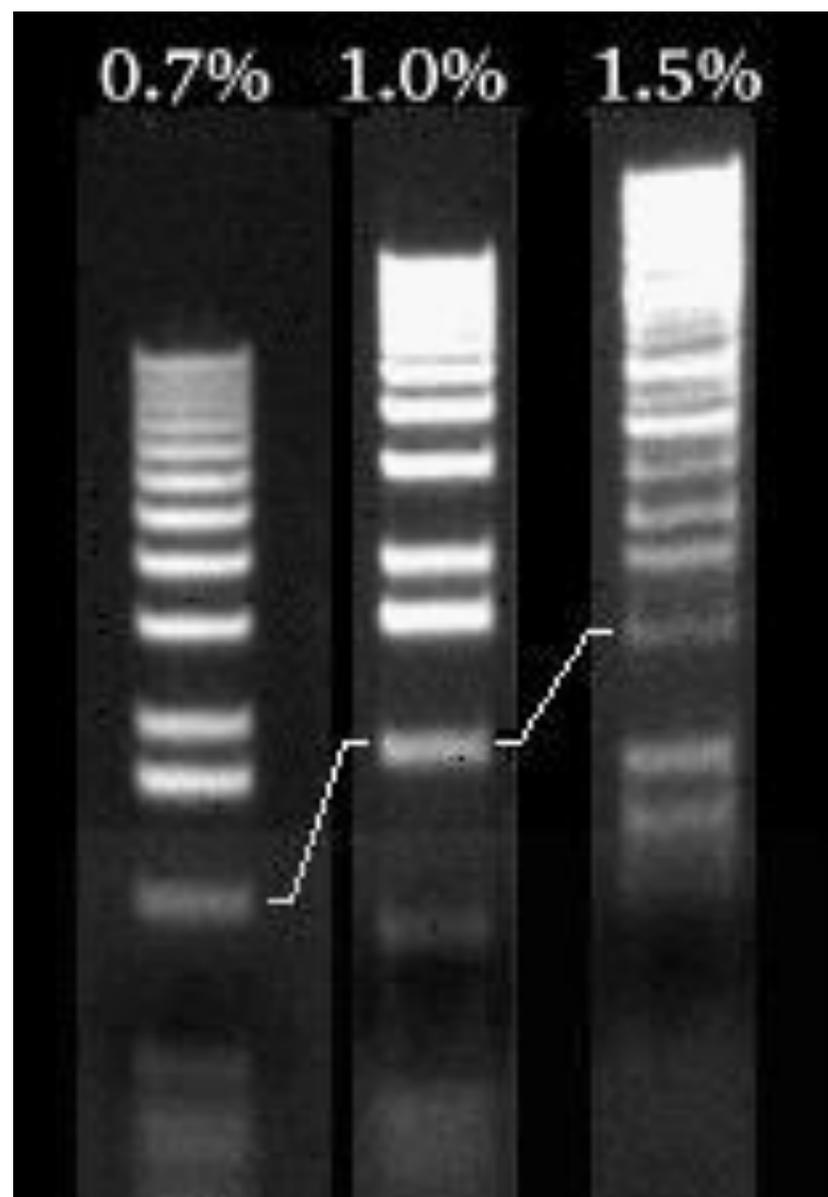
Restriction Analysis

The Electrophoresis Technique;

- **Simple and easy,**
- **Rapid**
- **Separation of DNA those couldnot be separated by techniues such as Density gradient centrifugation**
- **EtBr 1-10 ng, SYBR Gold 20 pg detection sensitivity**

DNA run distances in agarose gels

- **DNA's molecular size**
- **Agarose concentration**
- **DNA's structure**
- **Presence of EtBr in gel and electrophoresis buffer**
- **Applied voltage**
- **Agarose type**
- **Electrophoresis buffer**



Amount of Agarose in Gel (%)	Efficient Range of Separation of linear DNA Molecules (kb)*
0.3	60-5
0,6	20-1
0,7	10-0,8
0,9	7-0,5
1,2	6-0,4
1,5	4-0,2
2,0	3-0,1

Separation (Separation of Molecules)

- Gel is solidified, DNA is loaded into the lanes on the gel “**loading**”
- Electric current is applied to the gel
- Separation is performed regarding 2 factors:
 - Molecule type and size
 - Molecule charge

Agarose

- Agarose is a carbohydrate molecule chain extracted from sea lichen
- Expensive chemical 500 mg 200 \$



Polyacrylamide

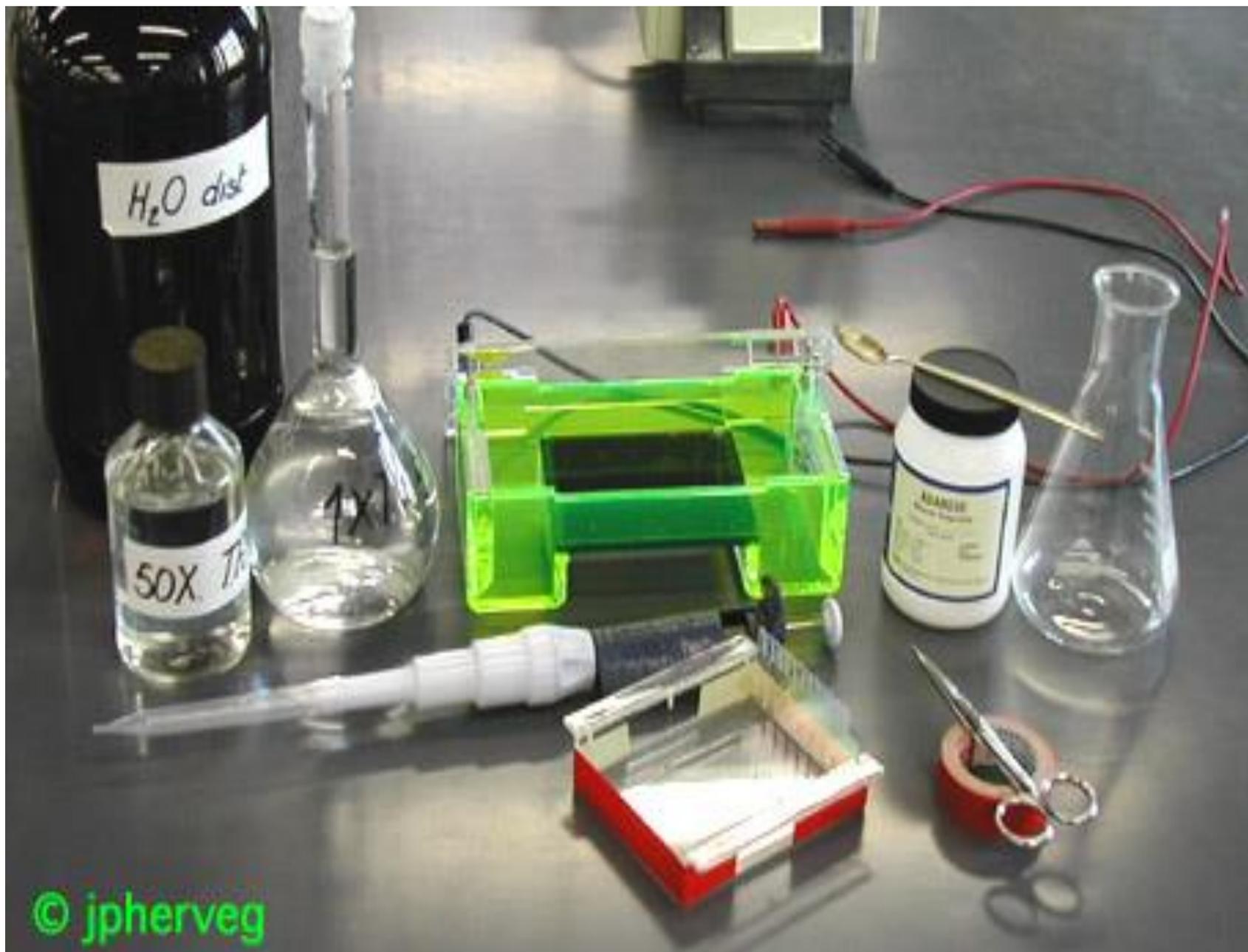
- Material used in skin electrodes and contact lenses
- In both gels pores are formed where the molecules pass through
- Polyacrylamide is a more synthetic molecule
- With polyacrylamide number and diameter of pores could be controlled better

Differences

- Agarose is a natural and pure matrix, on the other hand expensive
- Agarose have more universal pore diameters
- Polyacrylamide is synthetic, however pore diameter and number could be defined

Equipments and Expenditures

- An electrophoresis tank and power supply
- Gel trays and gel casting equipment (different sizes and UV-transmissible plastic)
- Combs to produce lanes in the gel
- Transilluminator (and UV light source)
- A camera / bioimaging device

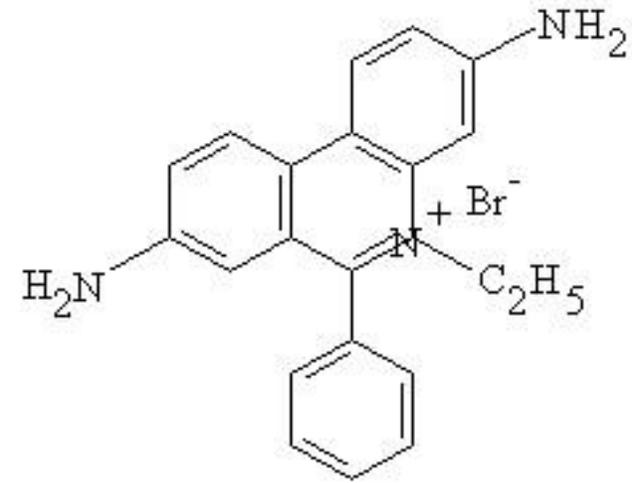


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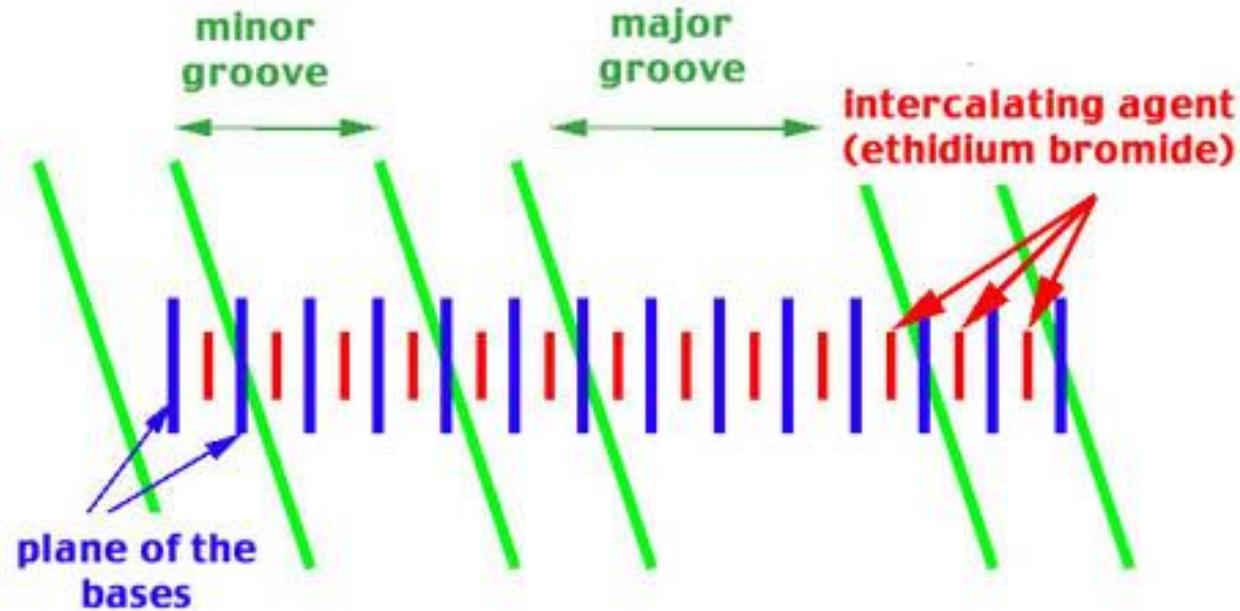
Chemicals

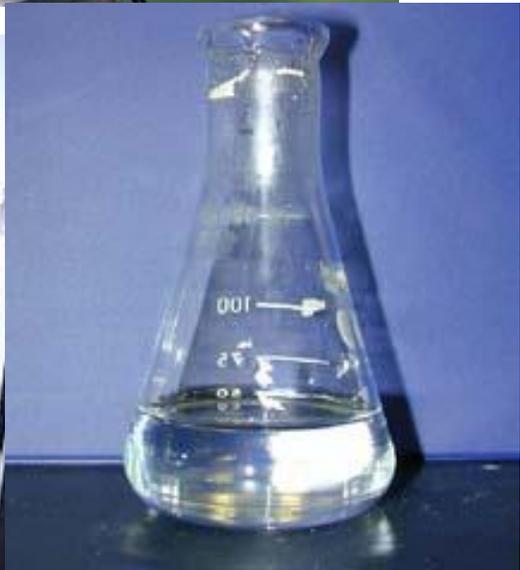
- Electrophoresis buffer
- Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- Loading dye: a concentrating agent (i.e. sucrose) and blue dye to track DNA run in the gel.
- A fluorescent dye for the staining of nucleic acids i.e. Ethidium bromide.

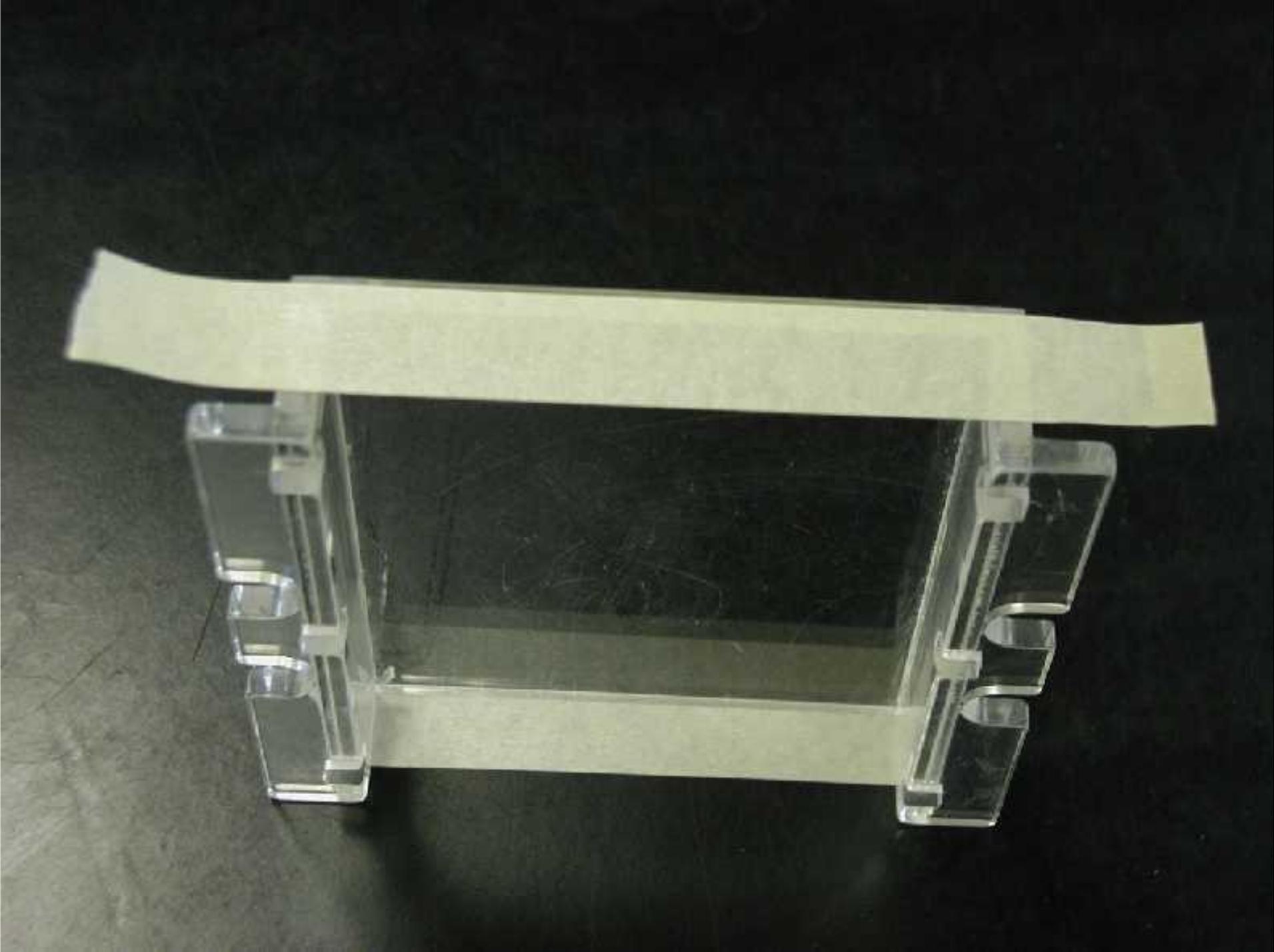
Ethidium Bromide

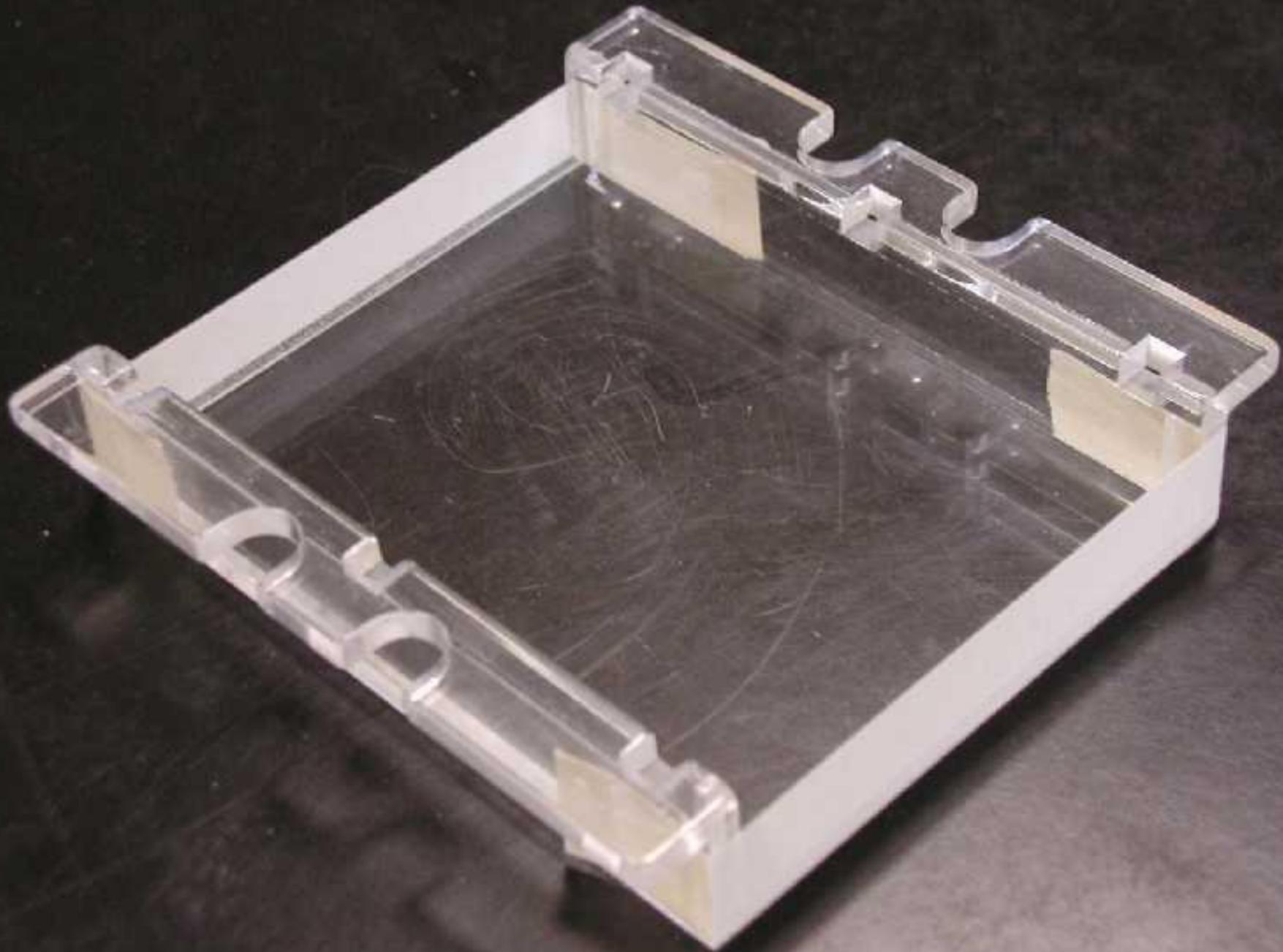


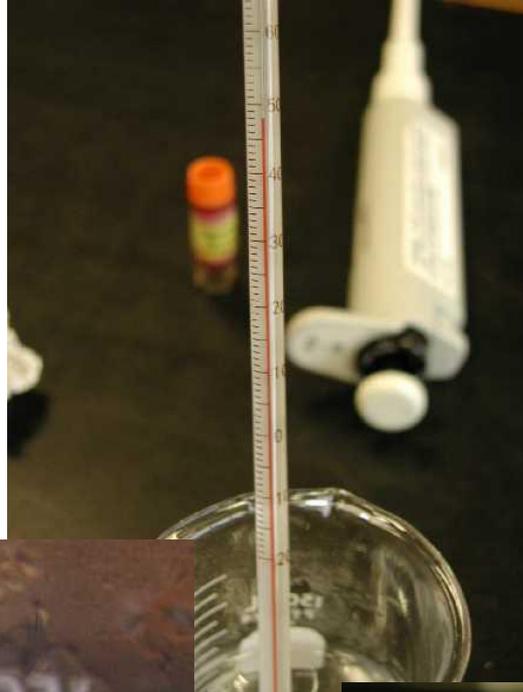
Ethidium Bromide

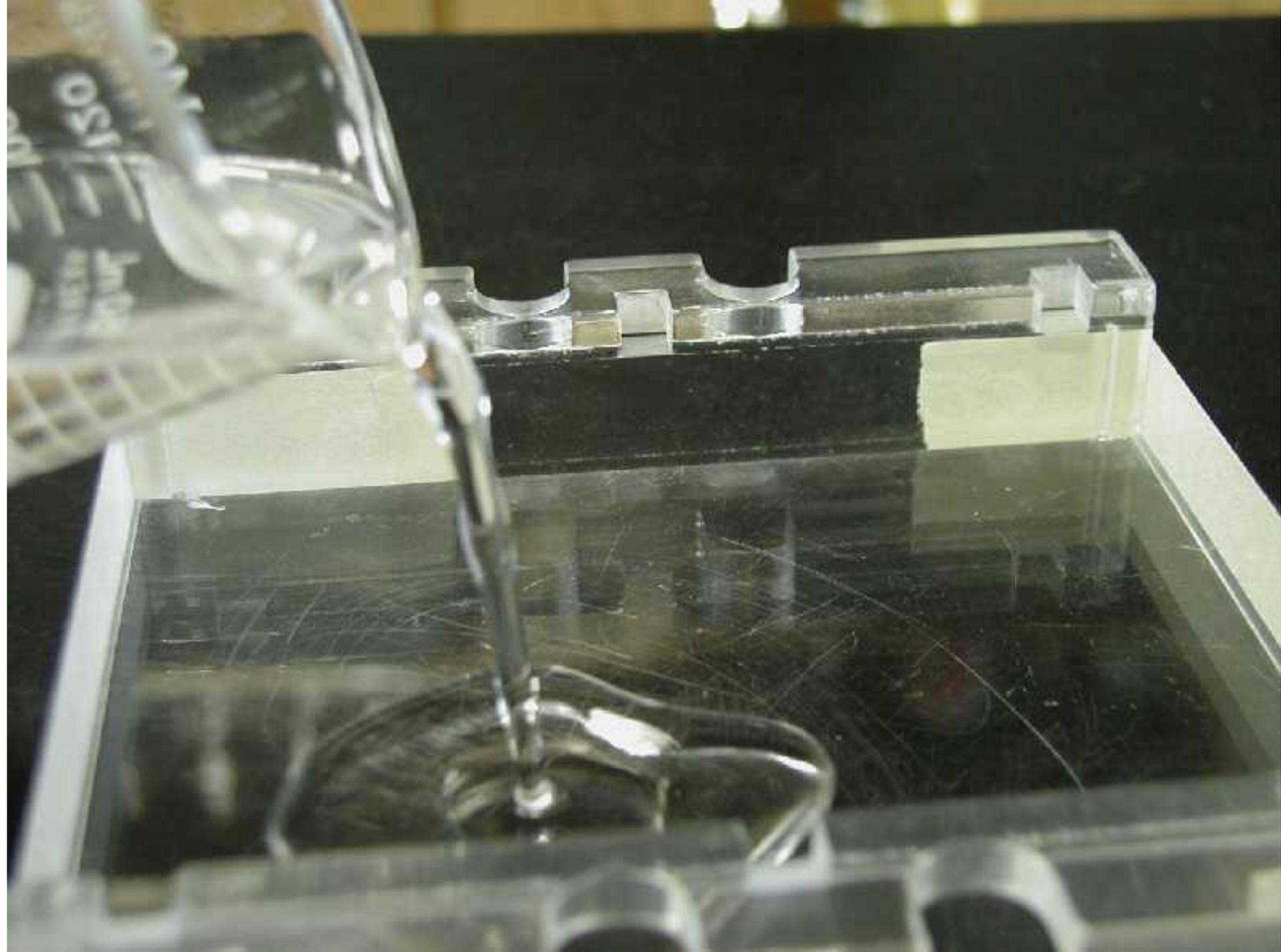


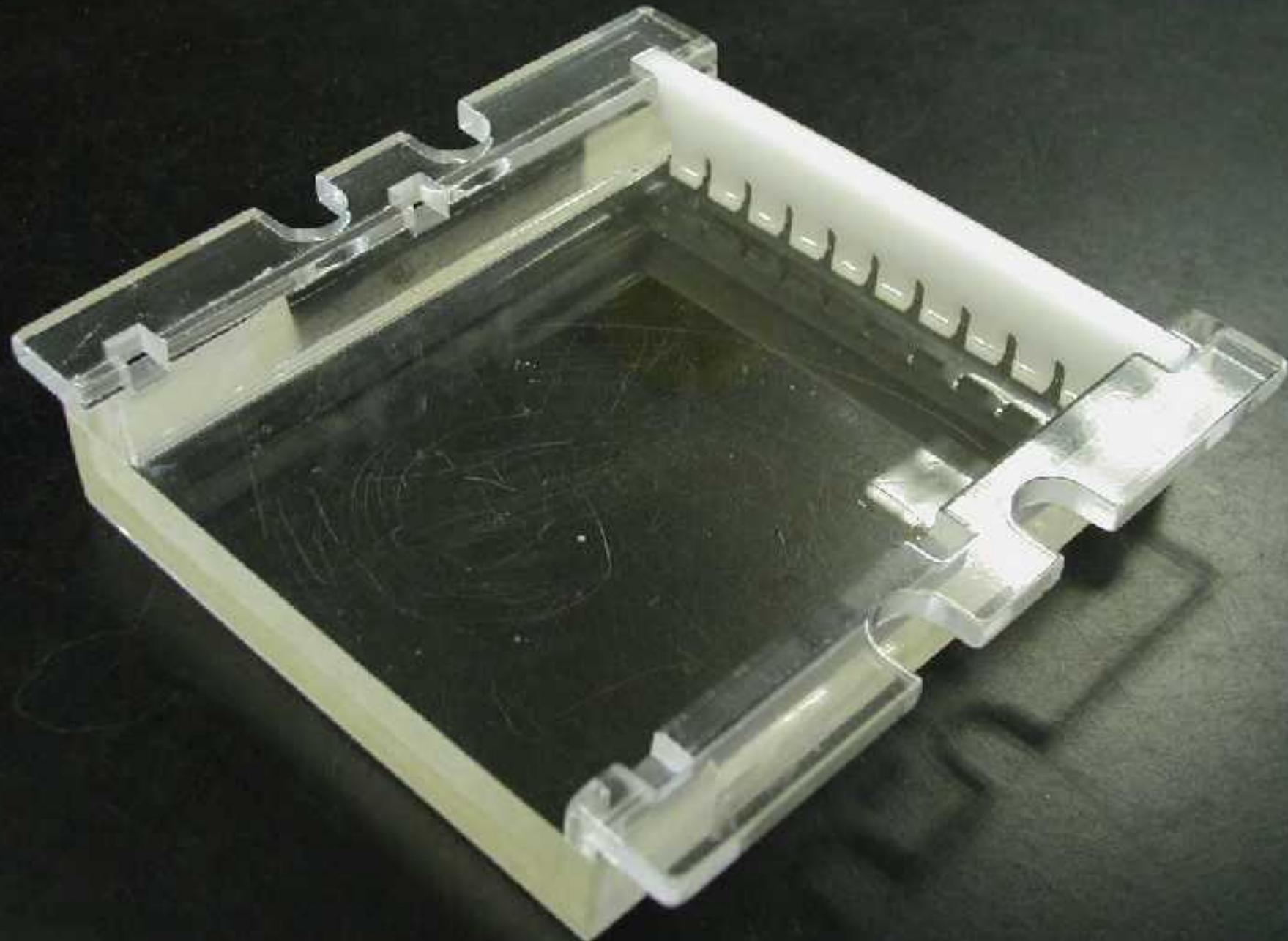


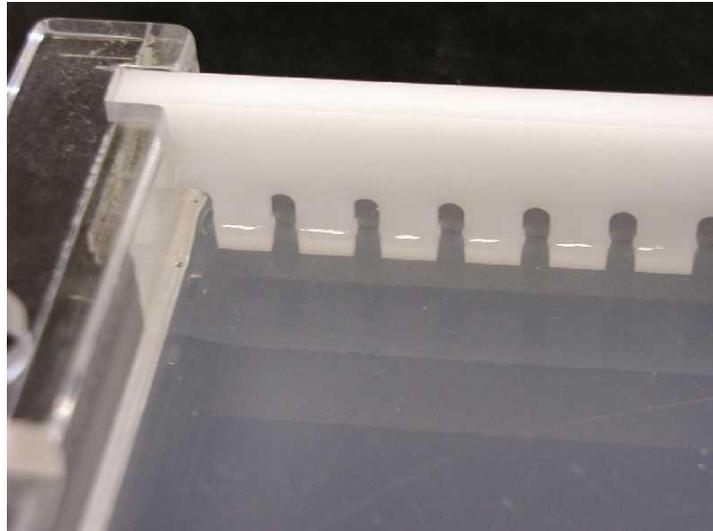
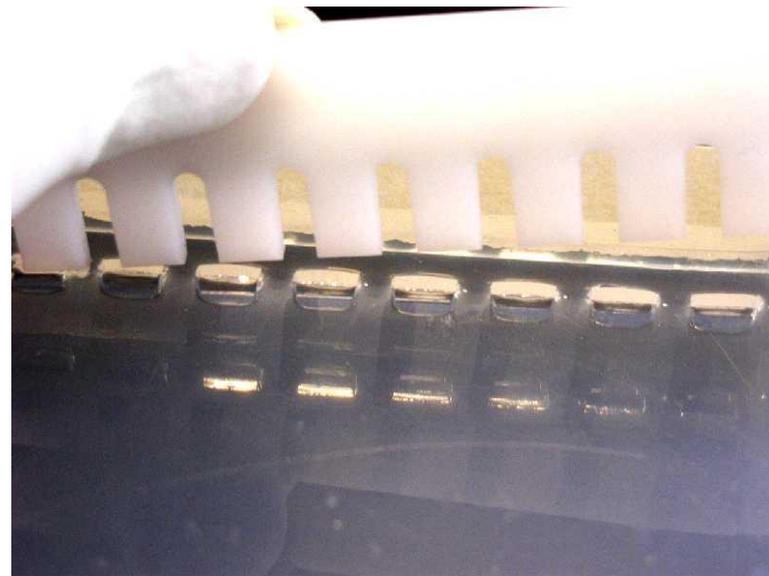
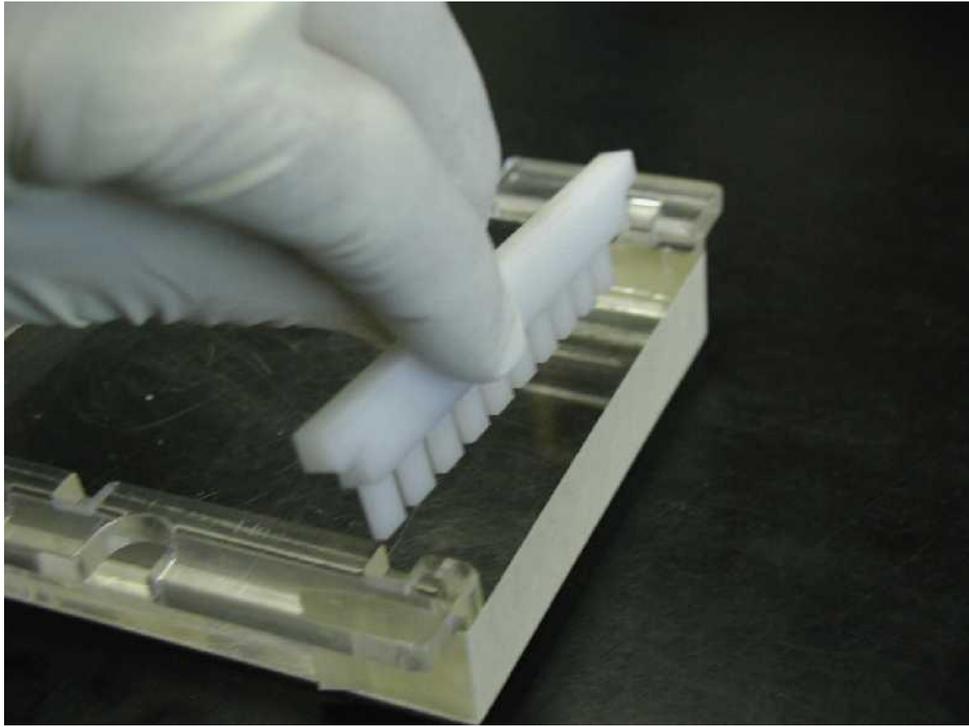


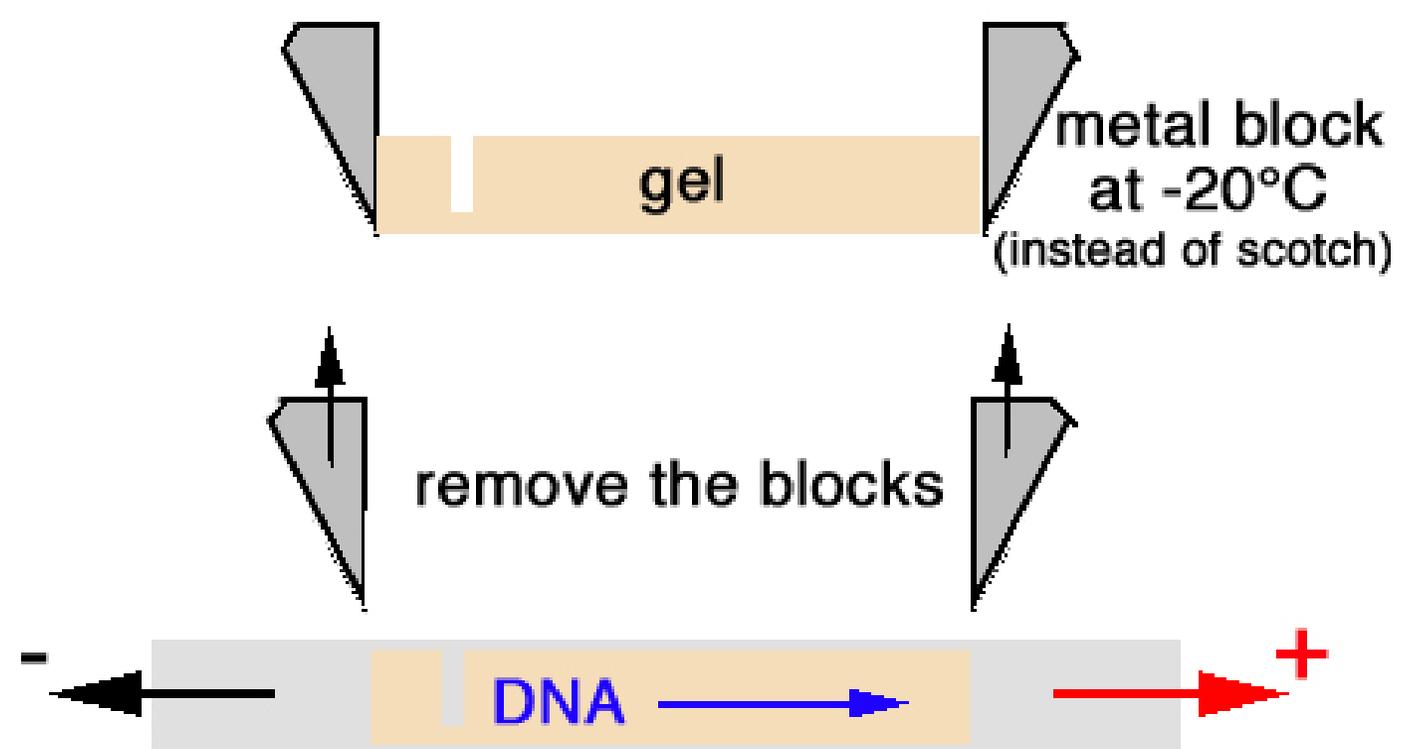




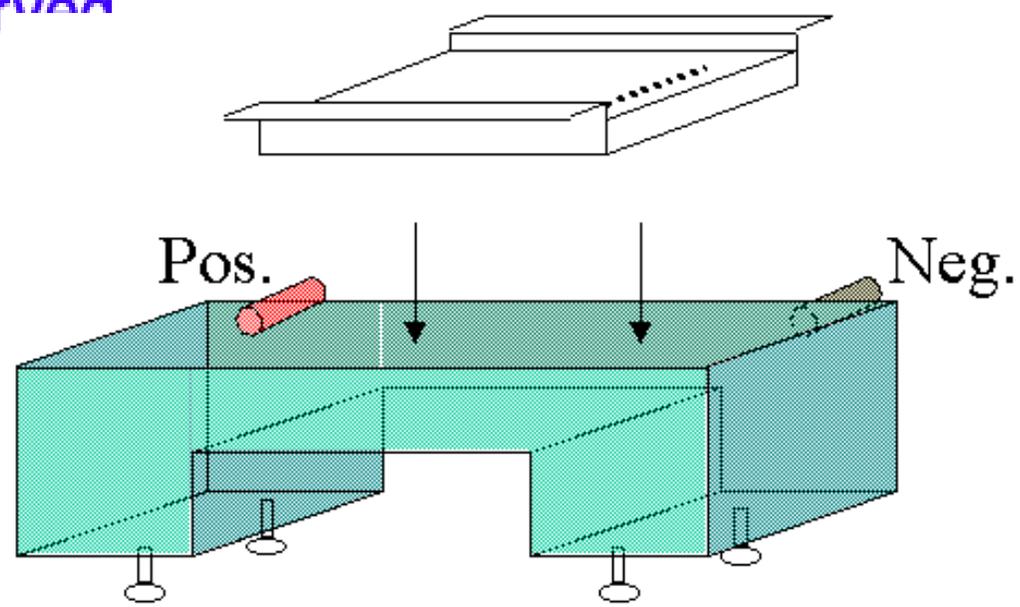


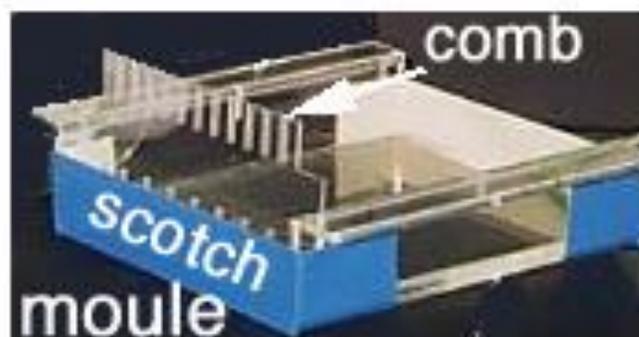
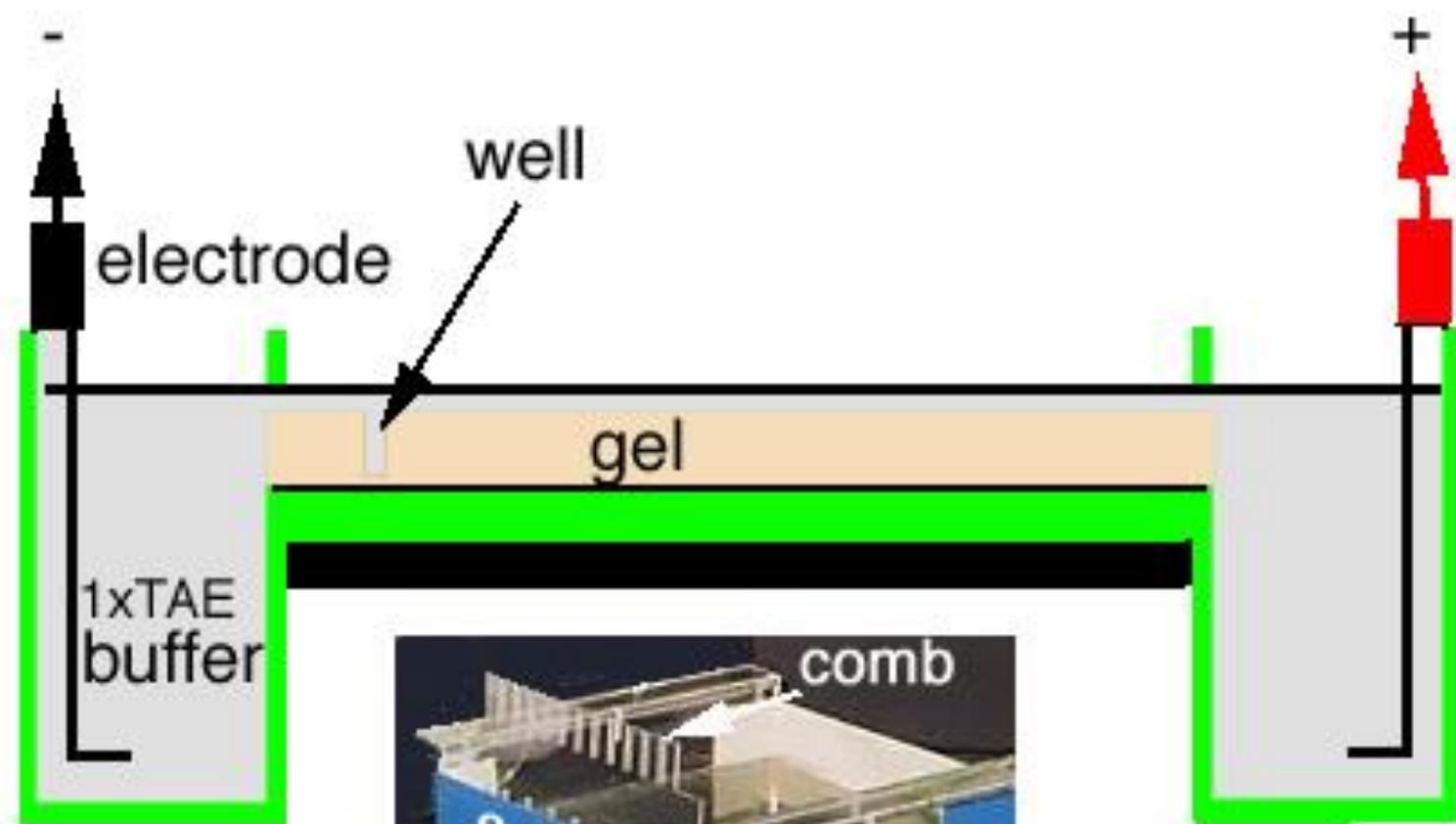




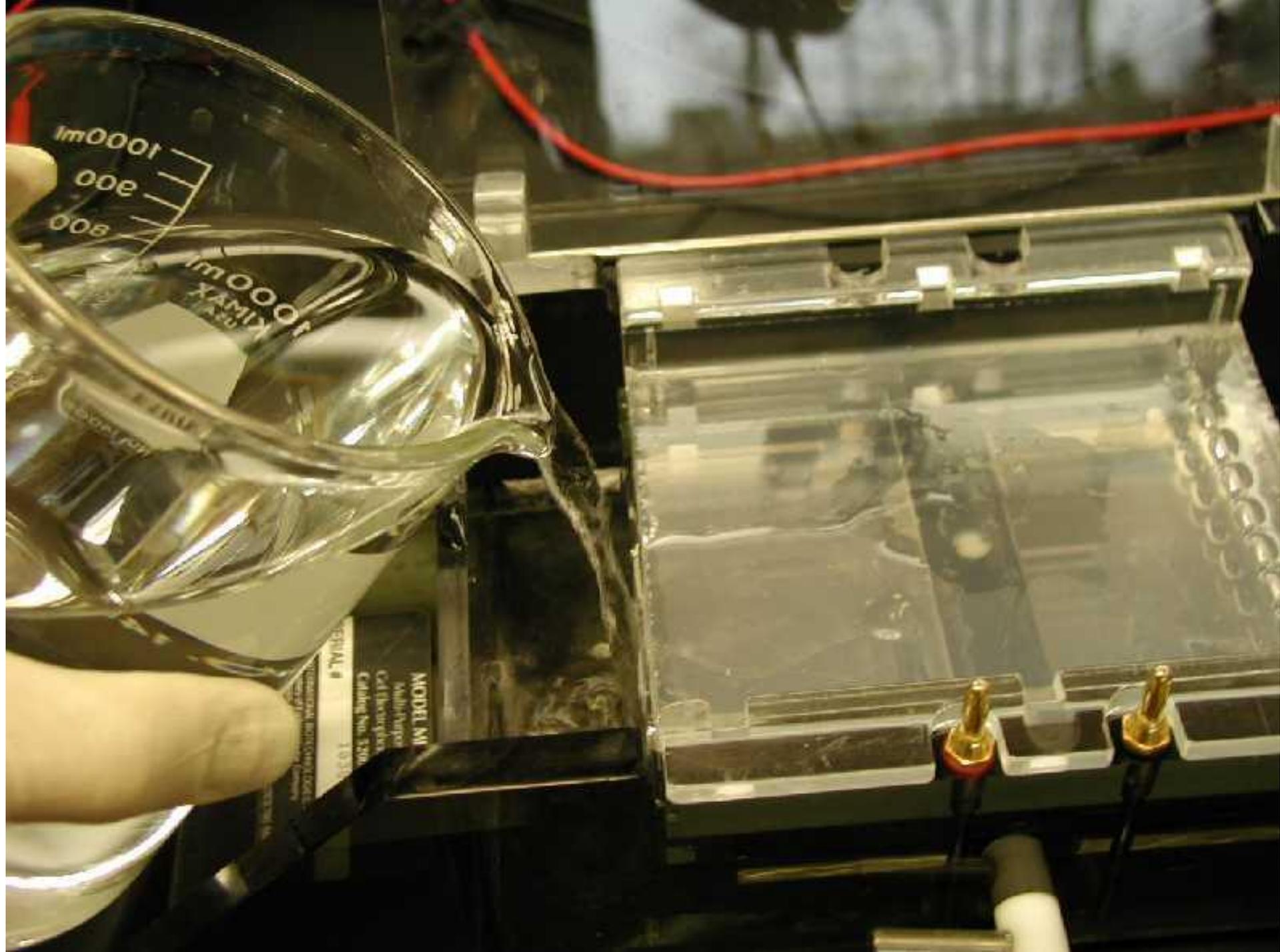


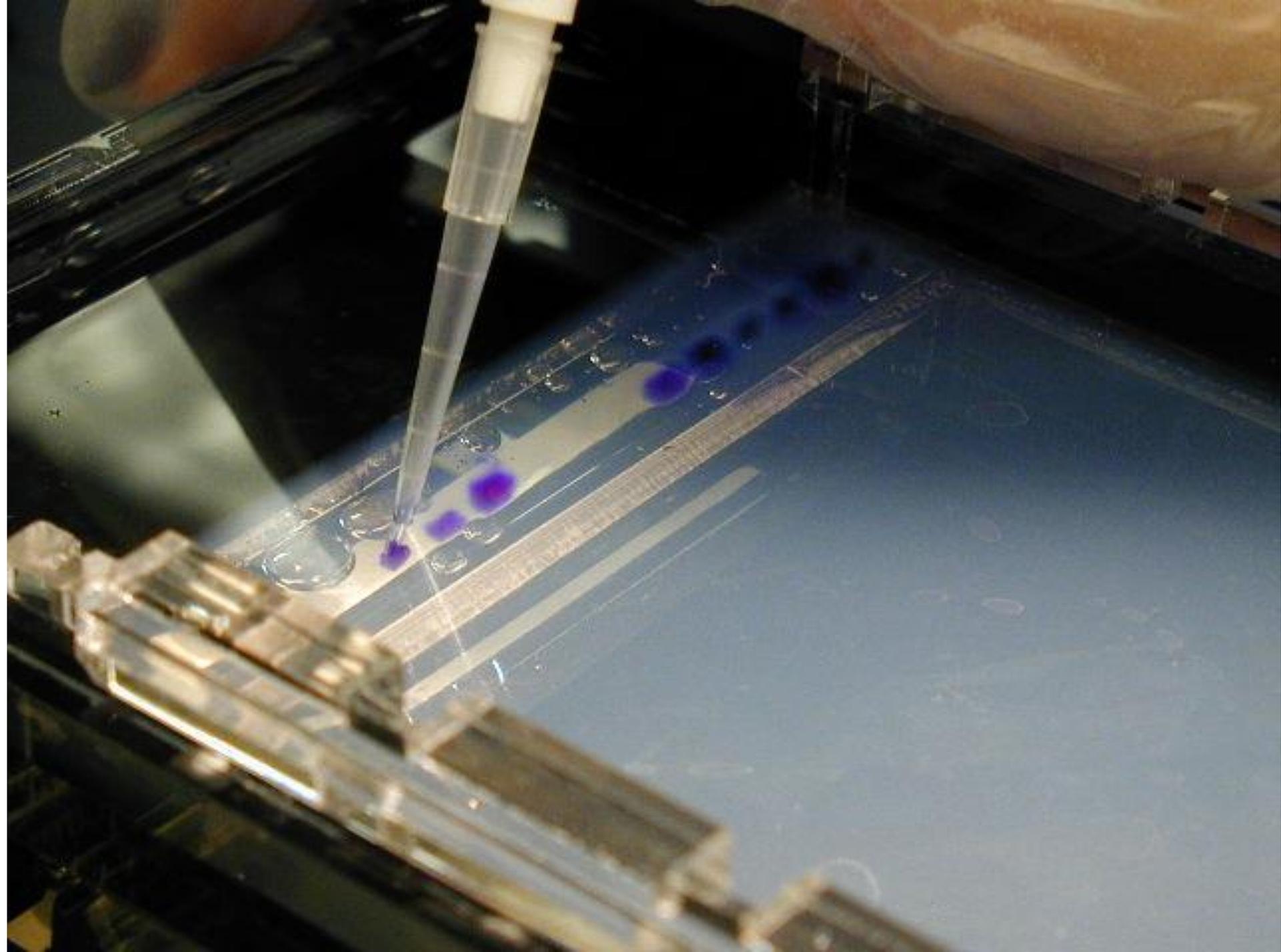
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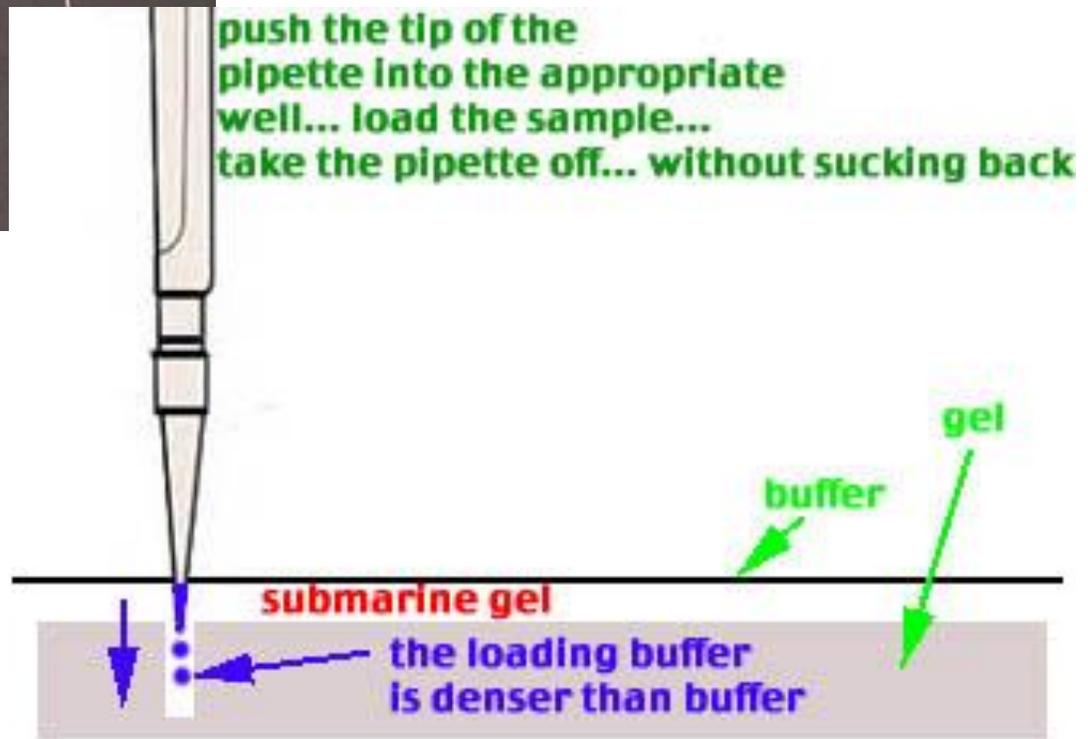
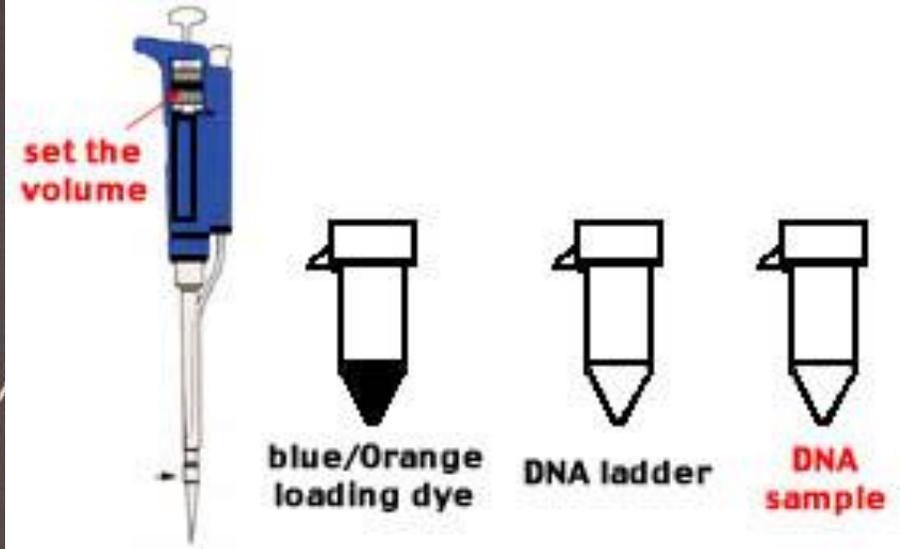
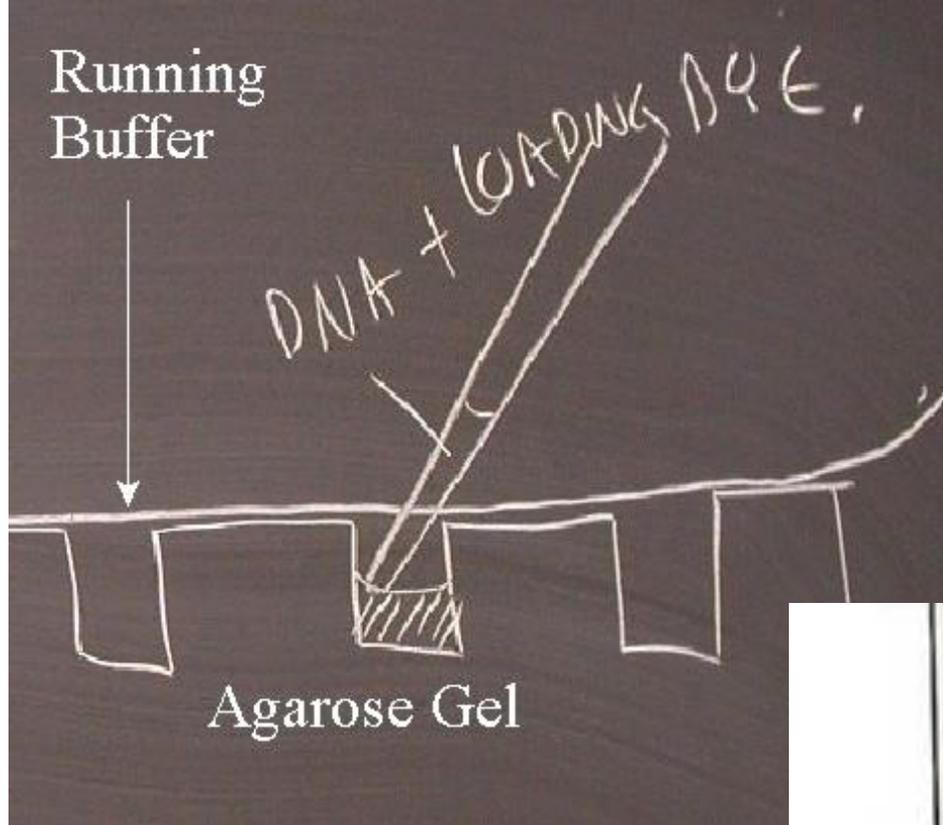


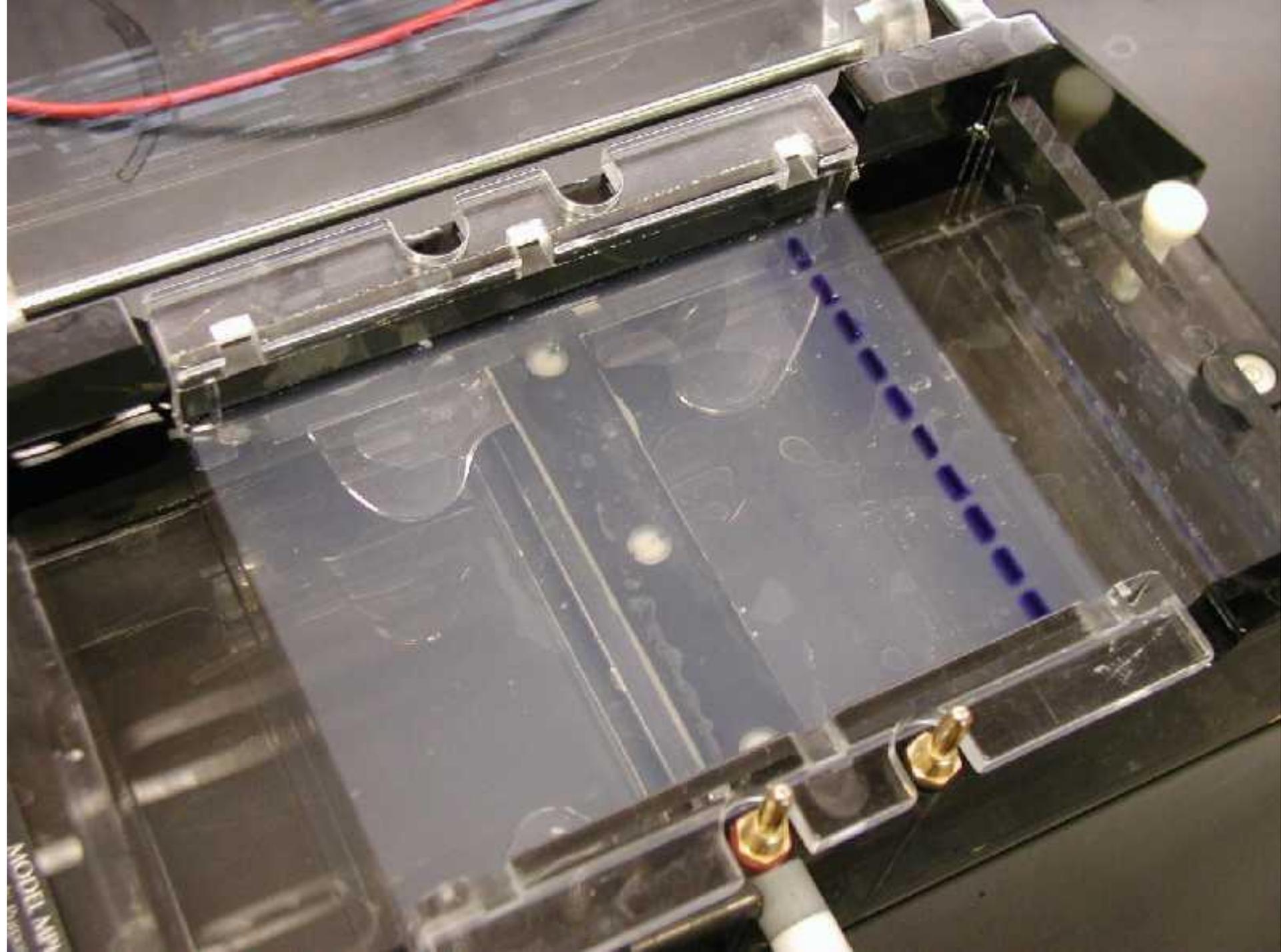


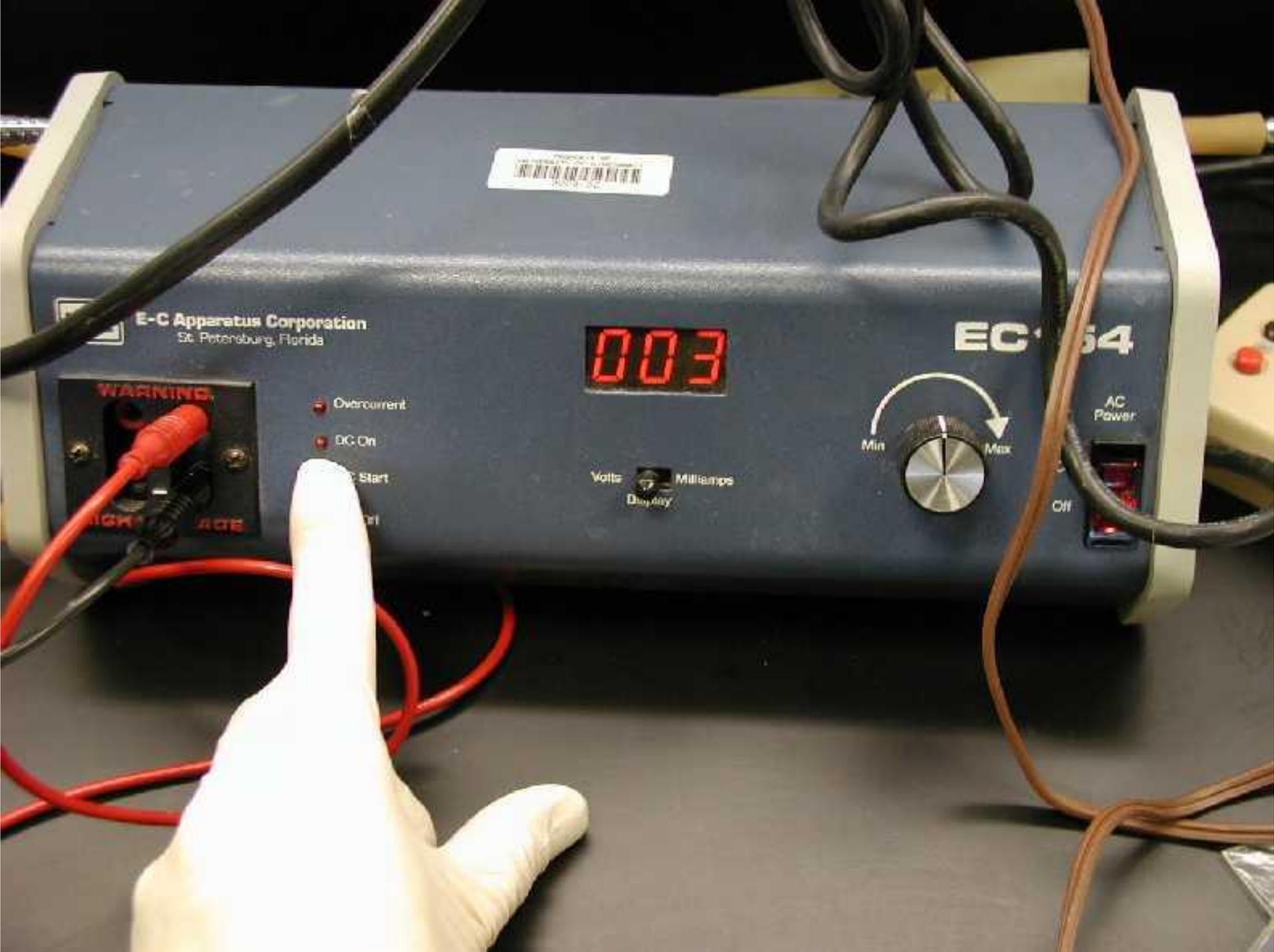
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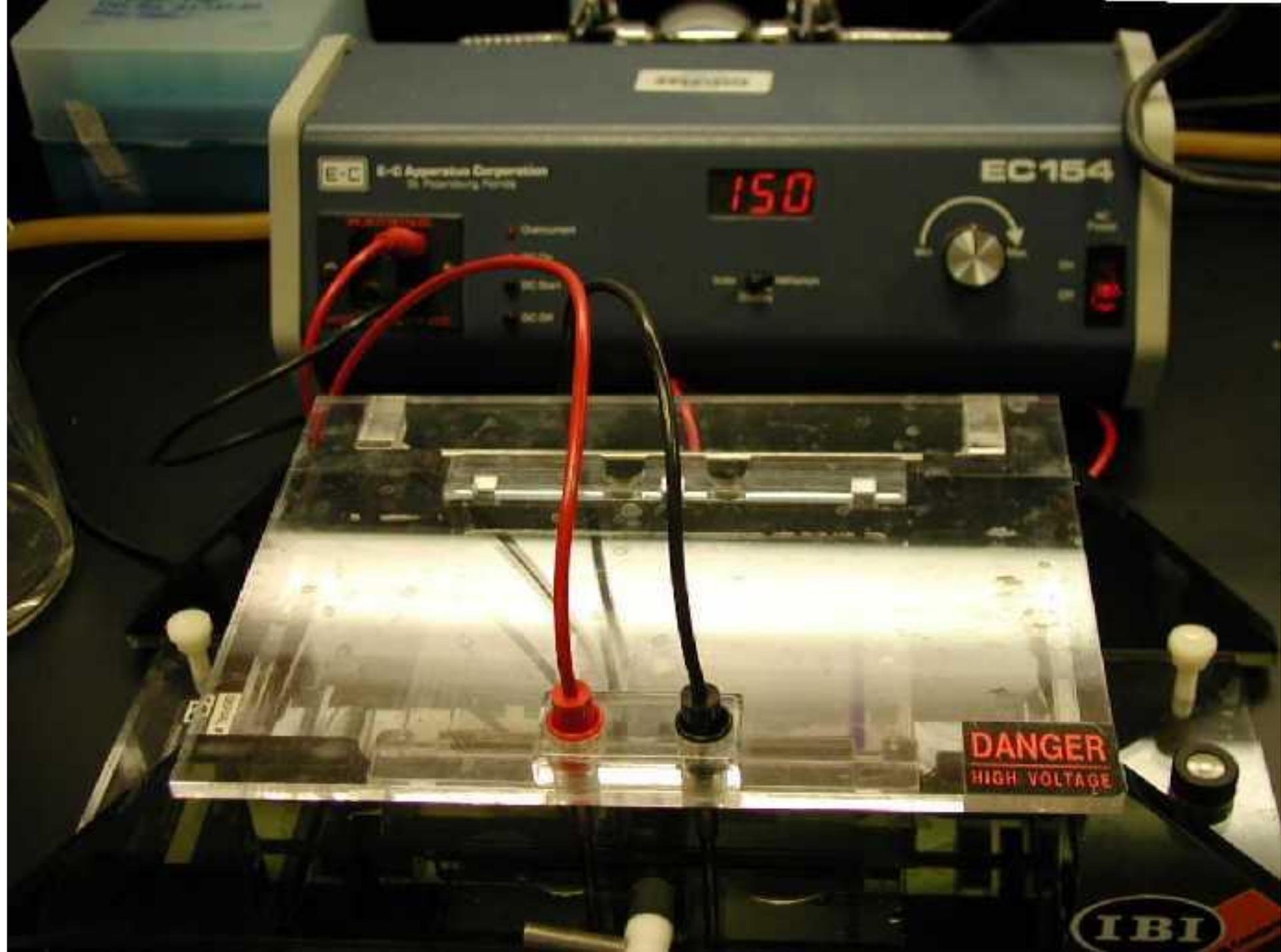


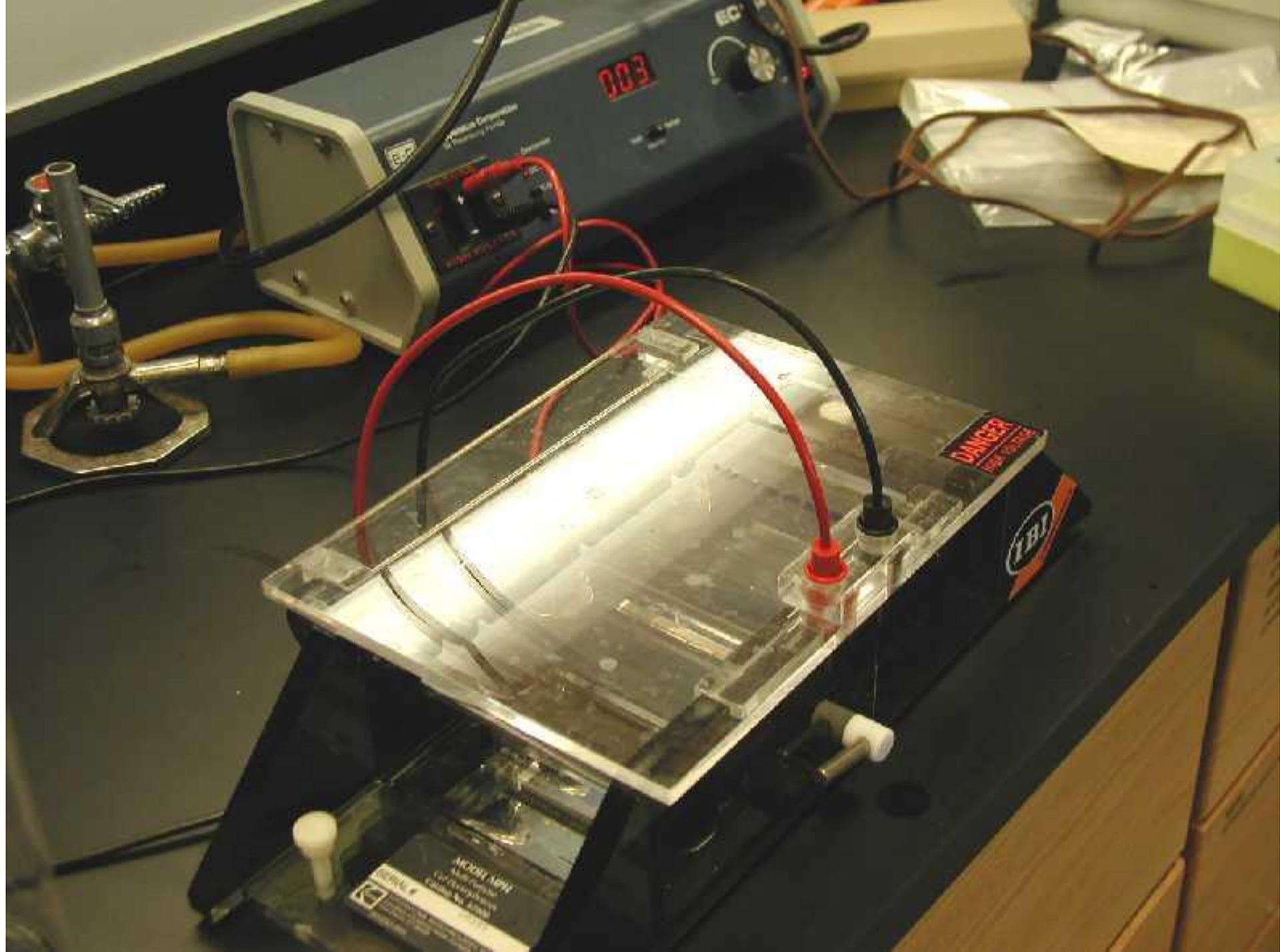














Imaging of Agarose Gels

welder masks equipped
with glass windows

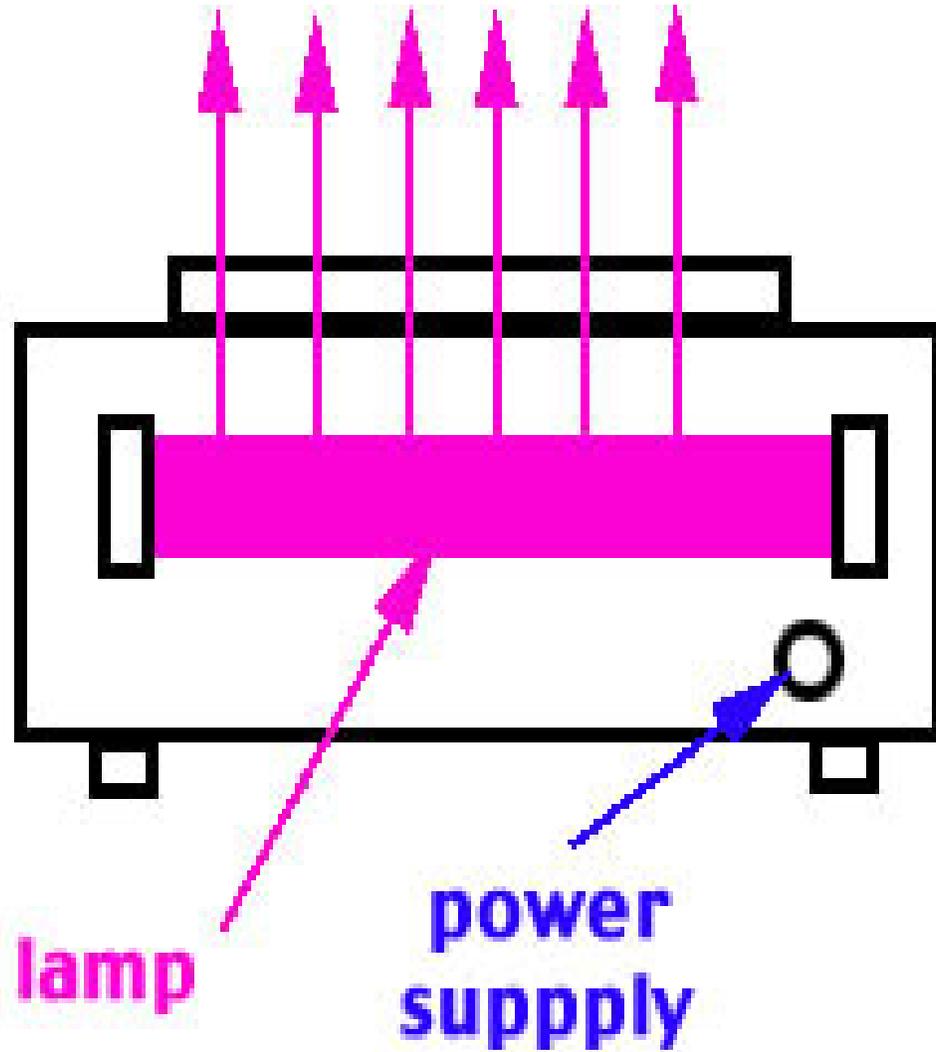
camera



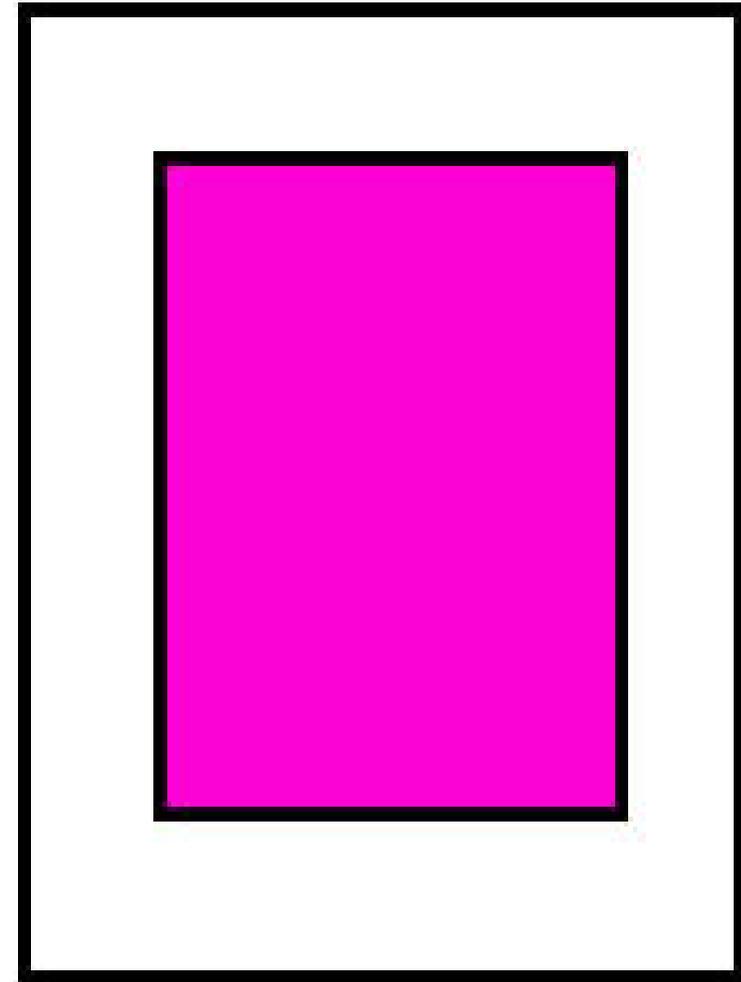
UV transilluminator

on/off switch

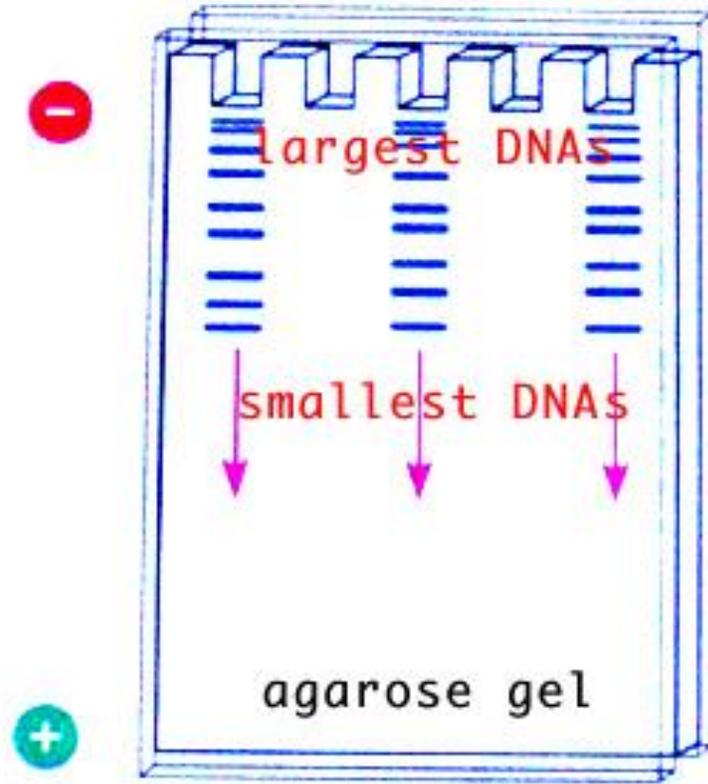
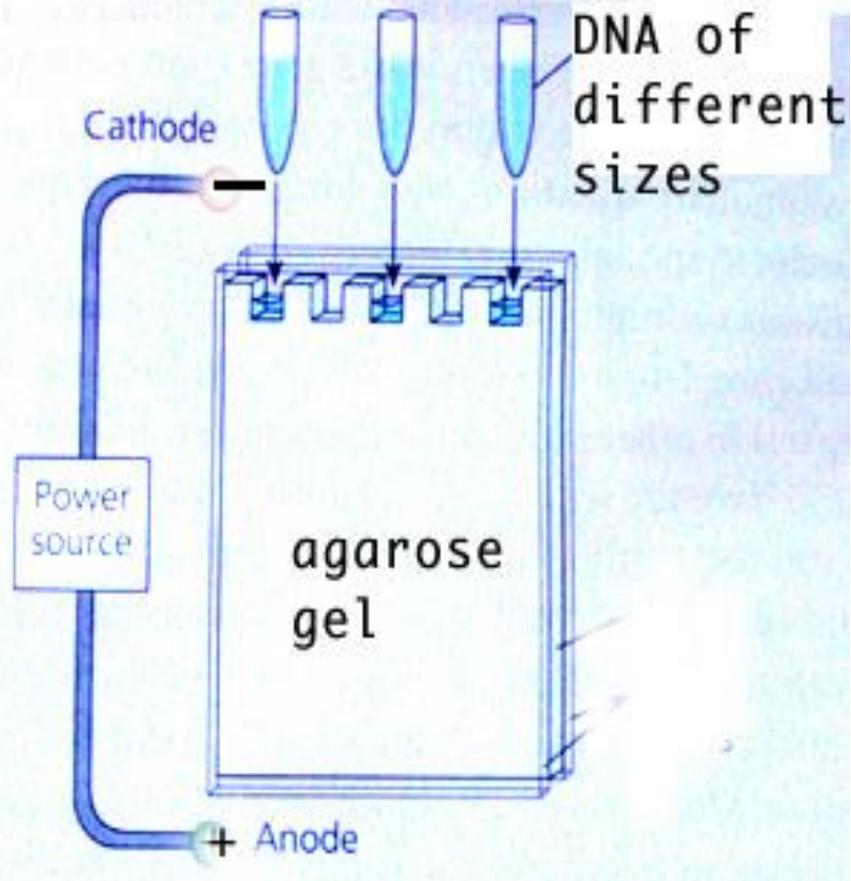
UV transilluminator

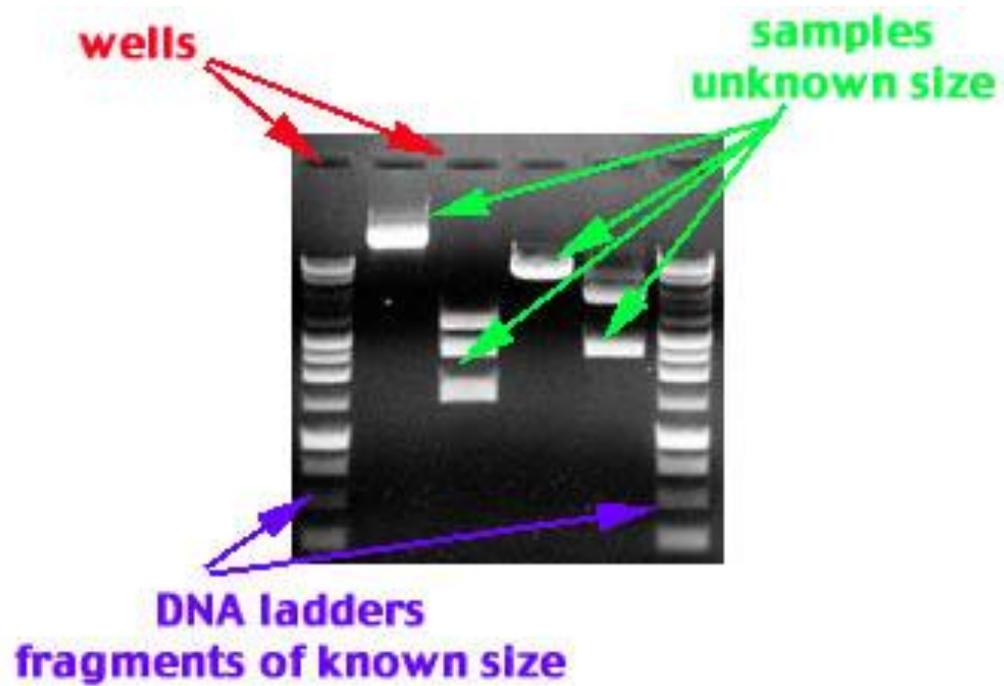
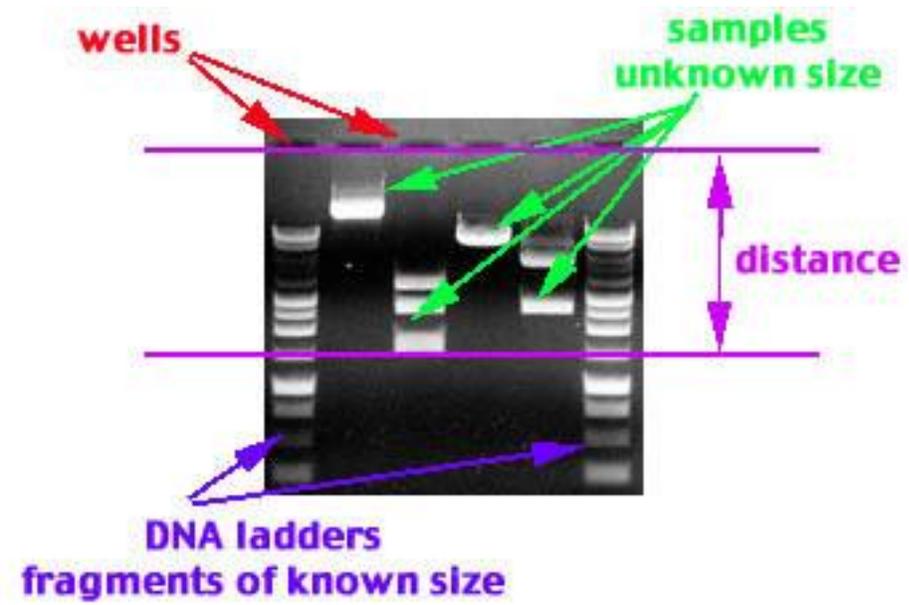


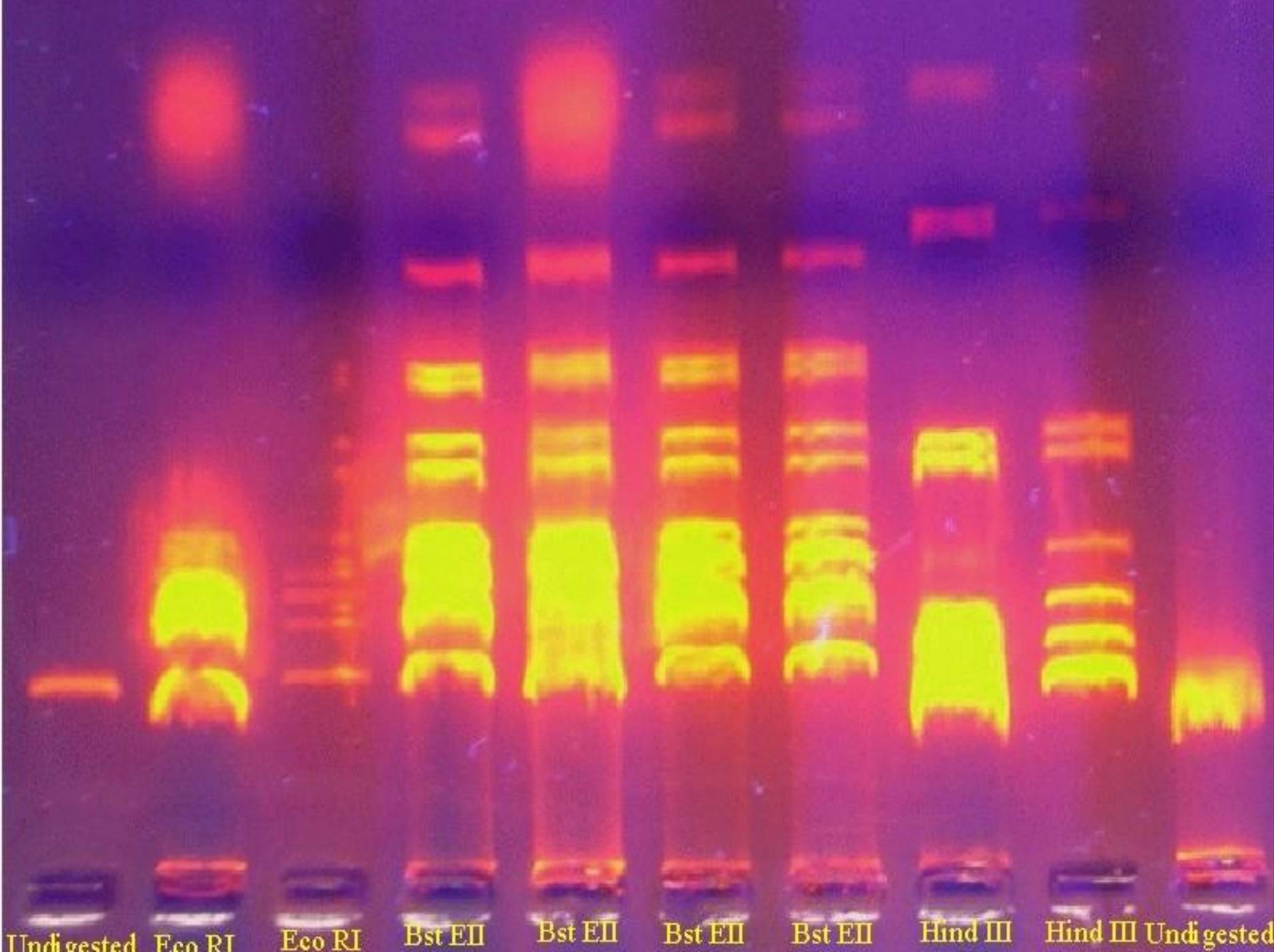
front view



top view







Undigested

Eco RI

Eco RI

Bst EII

Bst EII

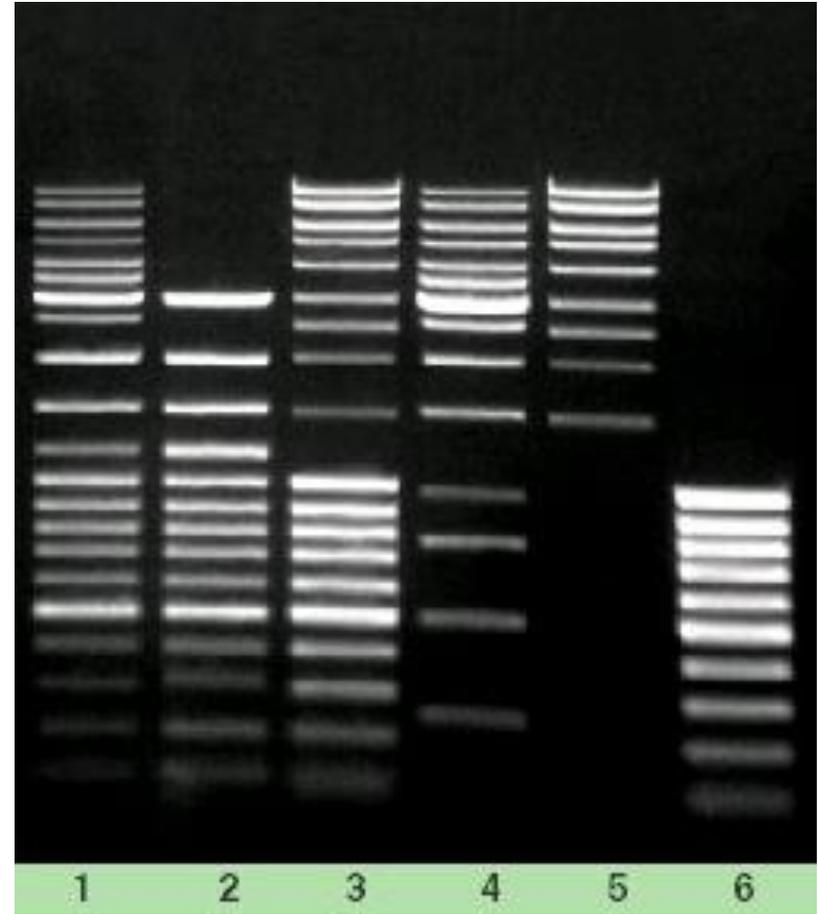
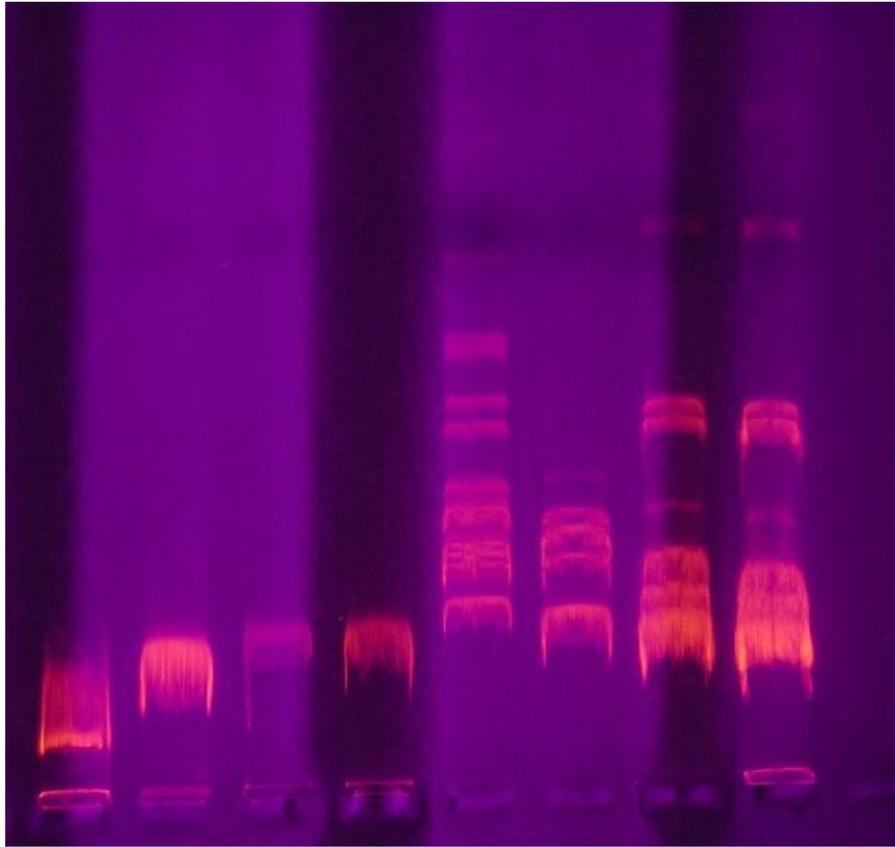
Bst EII

Bst EII

Hind III

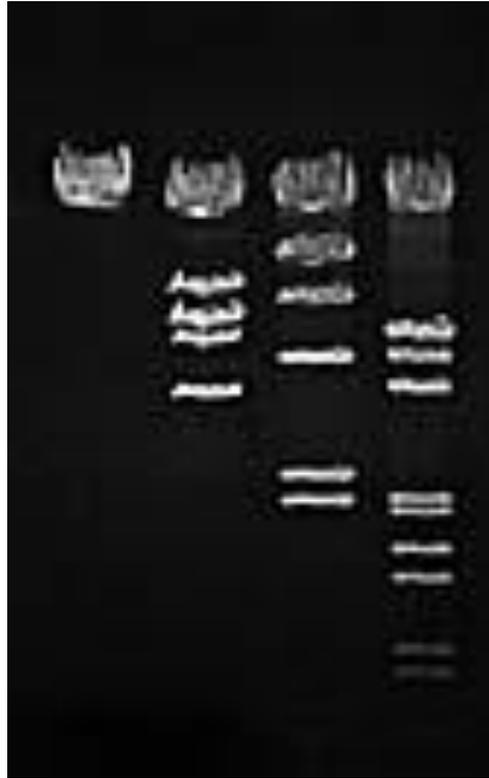
Hind III

Undigested





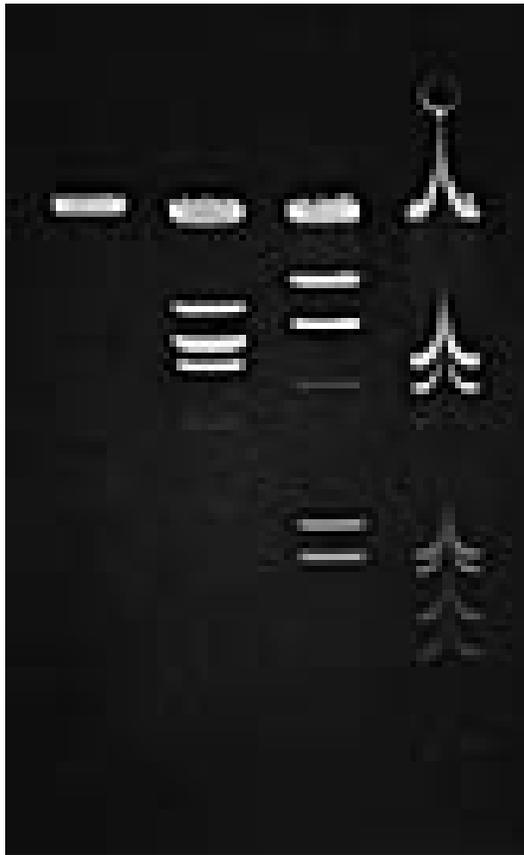
0.8% agarose mini-gel in 1X TBE buffer run at 60 Volts for 2 hours.



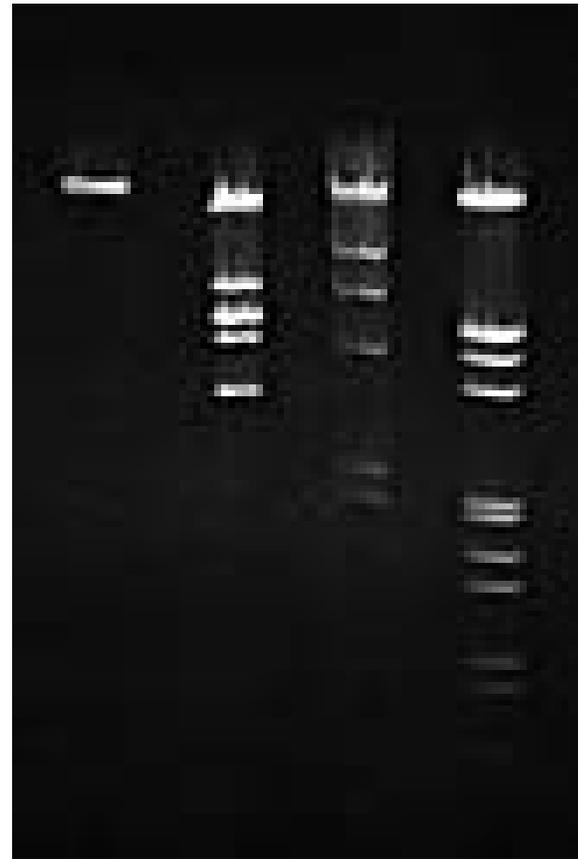
Bands smeared in all lanes. Too much DNA loaded.



During loading, Lane 2 was punctured with the micropipette tip. In more severe cases, DNA is lost through the hole in the well.



Bump in band in Lane 4. Bubble in Lane 4 of agarose gel.



Wavy bands in all lanes. Comb removed before gel was completely set. In this case, comb was removed eight minutes after pouring gel.



Bands smeared in all lanes. Water, instead of 1X TBE buffer, was used to prepare agarose.