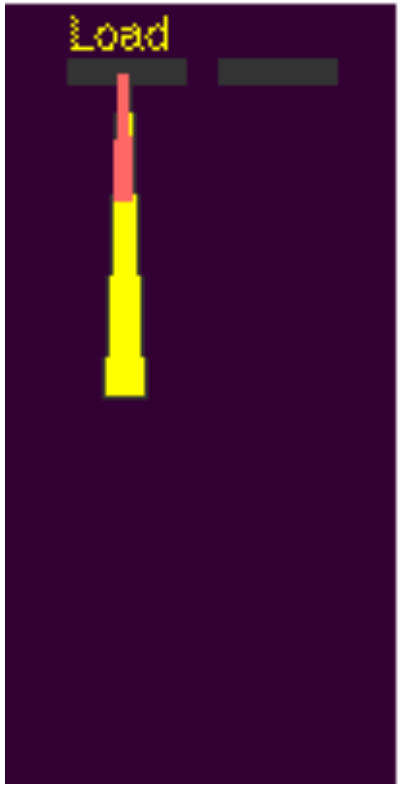
DNA AGAROSE GEL ELECTROPHORESIS

Week 6



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.

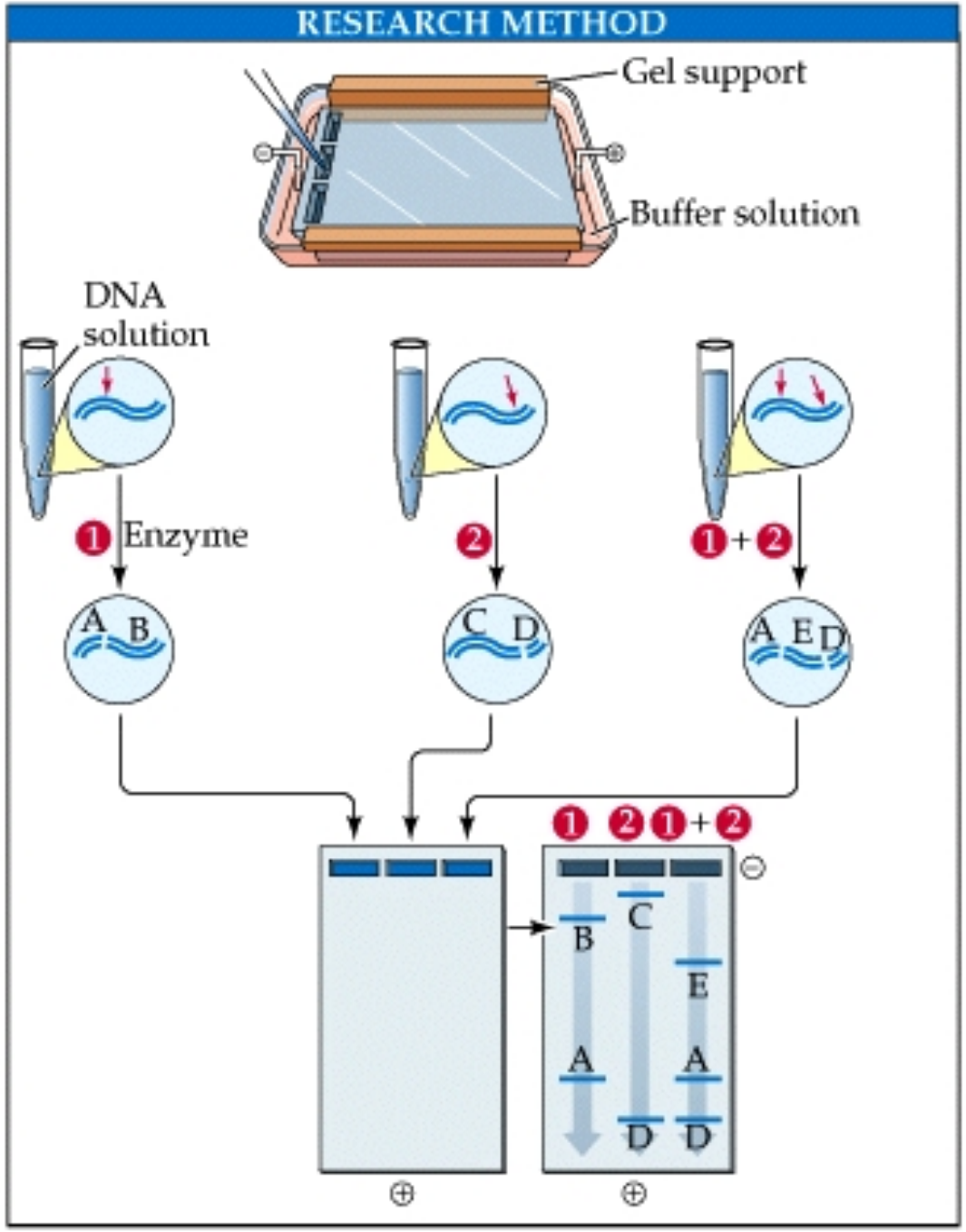
What is gel electrophoresis?

- 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 polyacyrilamide 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**
- **İdentification**
- **Purification**
- **Restriction analysis**
- **Genotyping (PFGE)**



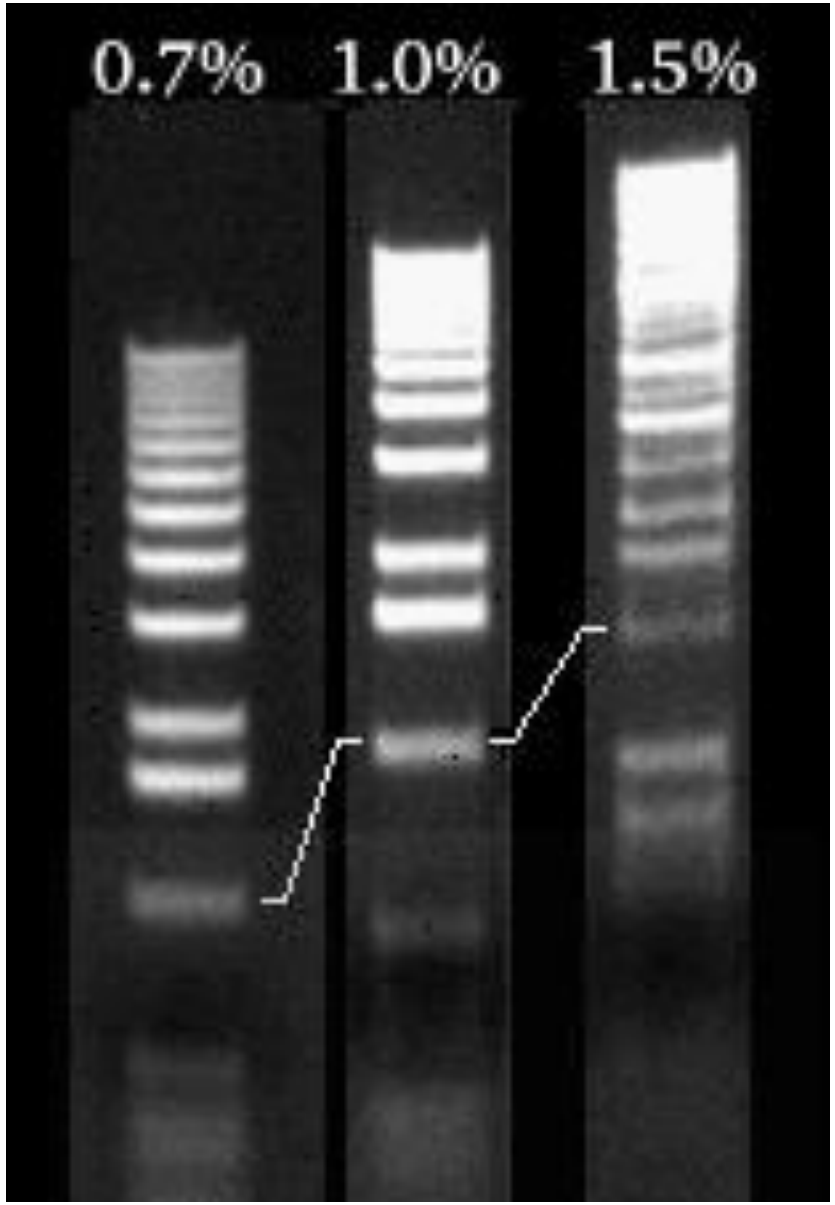
Restriction Analysis

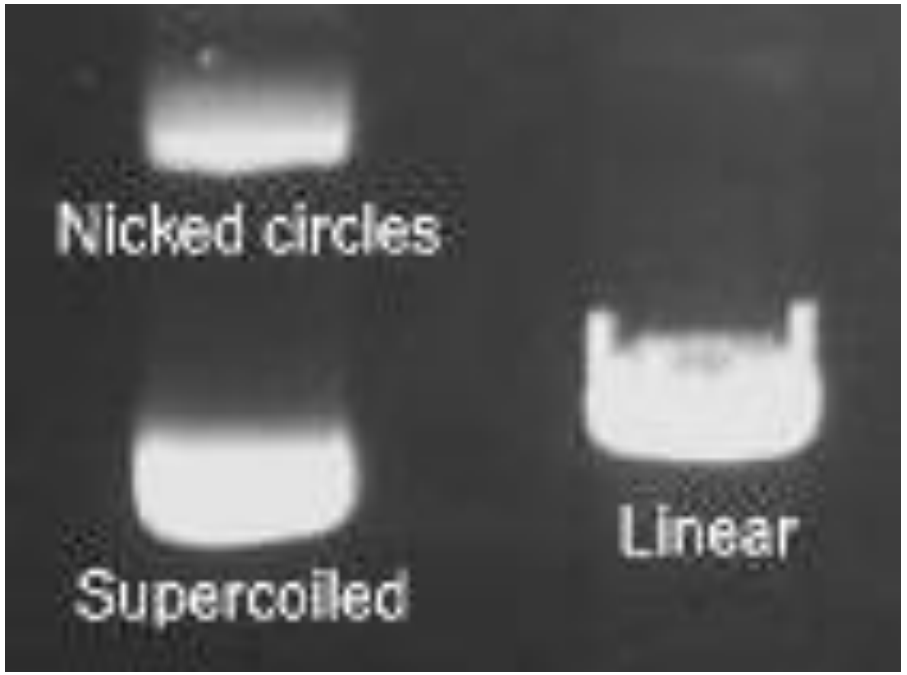
The Electrophoresis Technique;

- **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**


0.7%
1.0%
1.5%


Nicked circles
Supercoiled
Linear

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	4-02
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 jel
- Separation is performed regarding 2 factors:
- Molecule type and size
- Molecule charge

Agarose

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

Polyacyrilamide

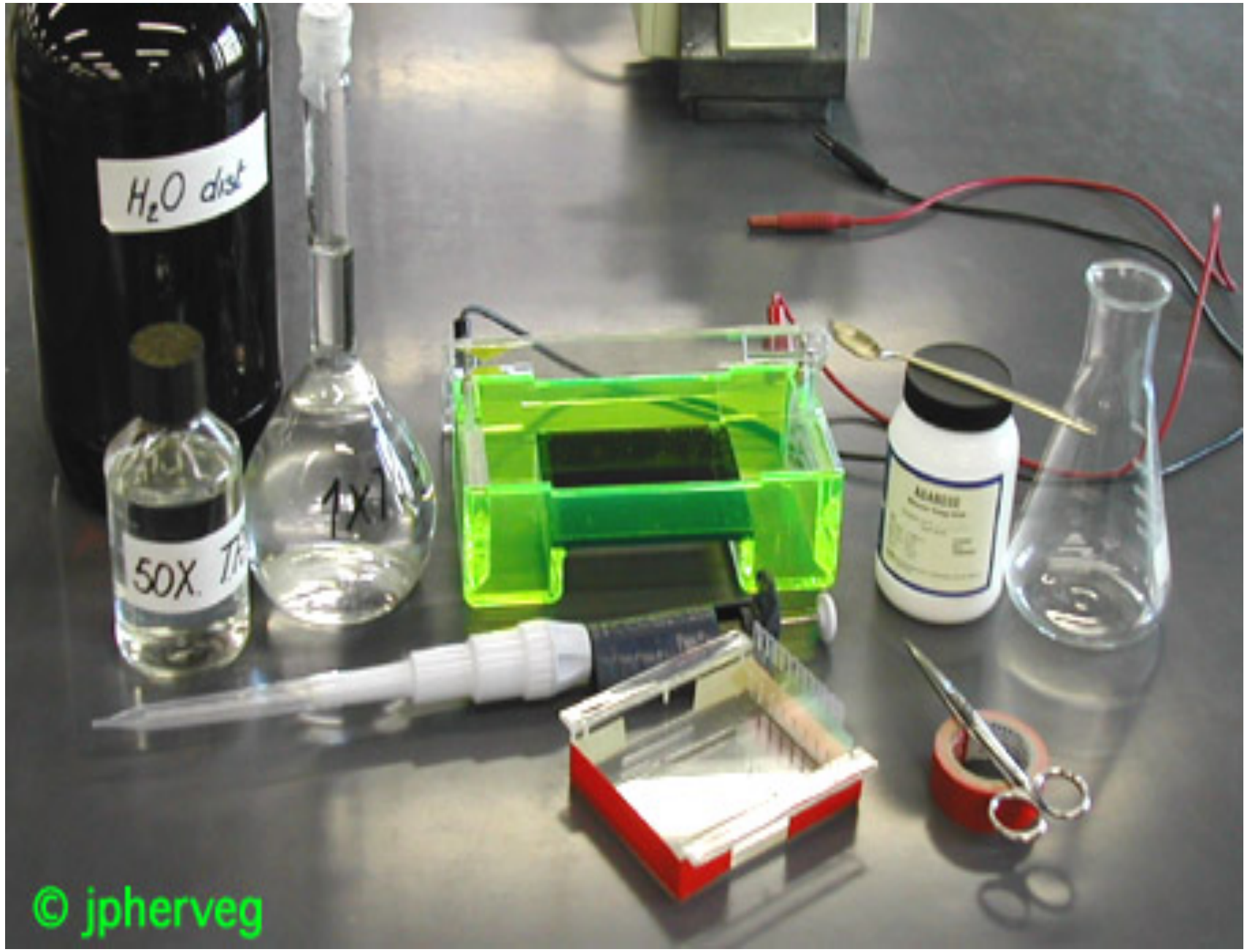
- Material used in skin electrodes and contact lenses
- In both gels pores are formed where the molecules pass through
- Polyacyrilamide is a more synthetic molecule
- With polyacyrilamide number and diameter of pores could be controlled better

Differences

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

Equipments and Expendatures

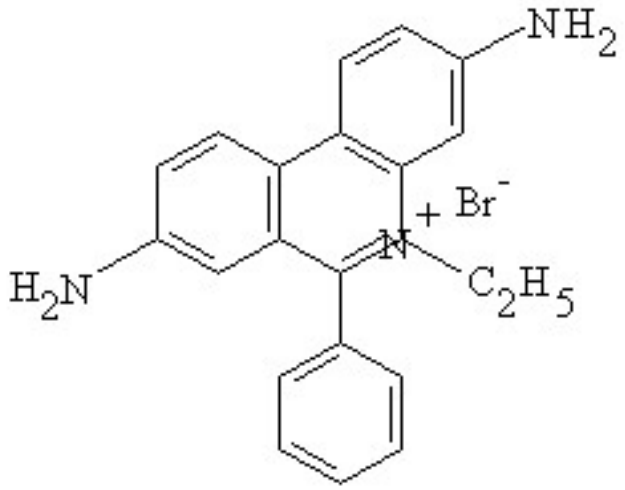
- An electrophoresis tank and power supply
- Gel trays and gel casting equipment (different sizes and UV-transmissible plastic)
- Combs to produce lanes in the gel
- Transilluminator (and UV light source)
- A camera / bioimaging device


H_2O dist
50X
1X
© jpherveg

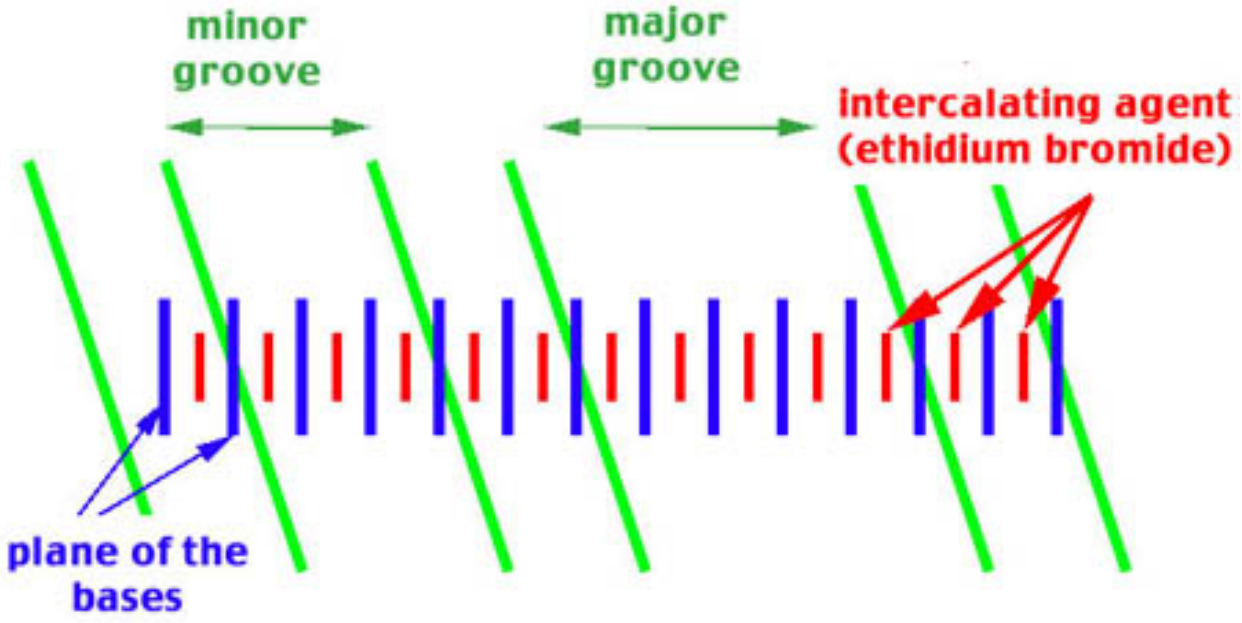
Chemicals

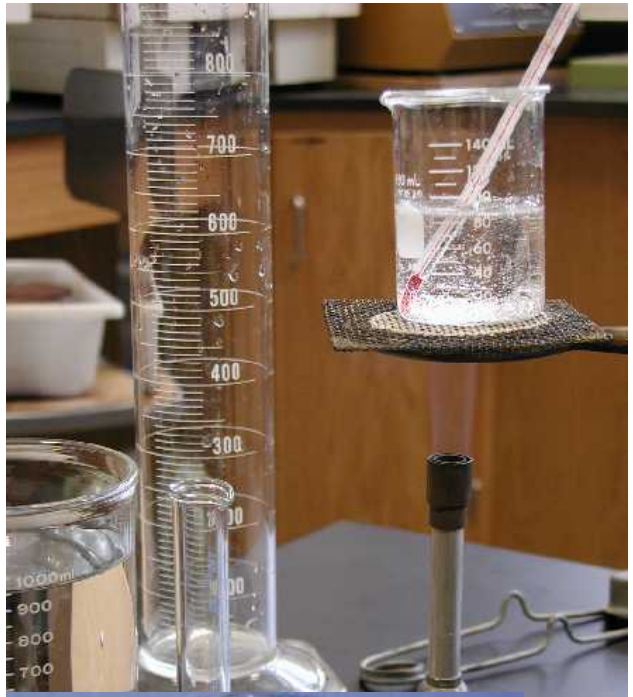
- Electrophoresis buffer
- Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- Loading dye: a concentrating agent (i.e. sucrose) and blue dye to tract DNA run in the gel.
- A fluorescent dye for the staining of nucleic acids i.e. Ethidium bromide.

Ethidium Bromid

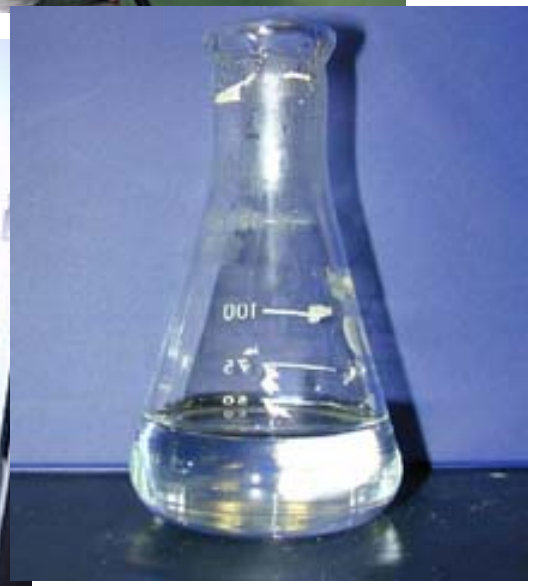


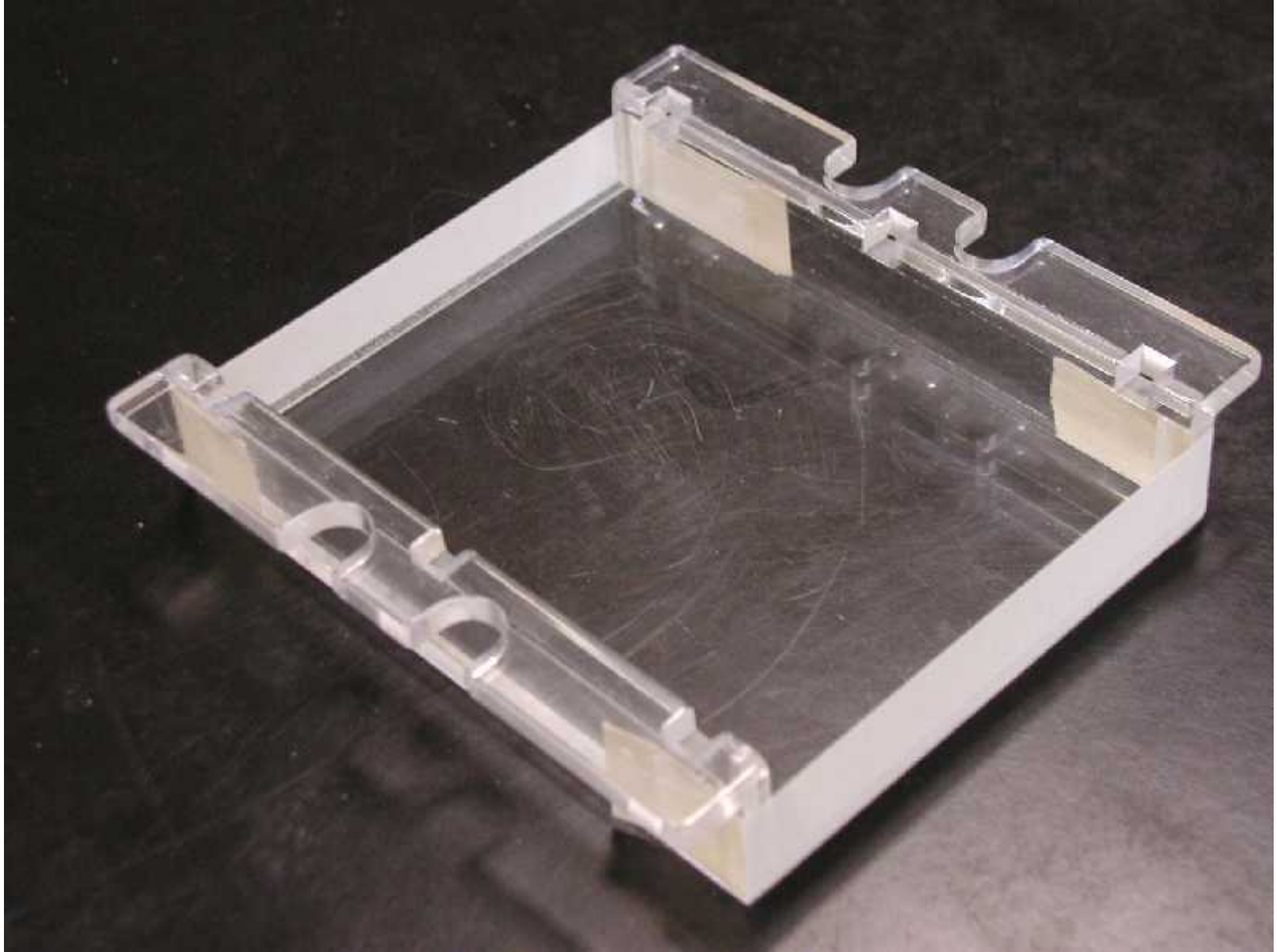
Ethidium Bromide

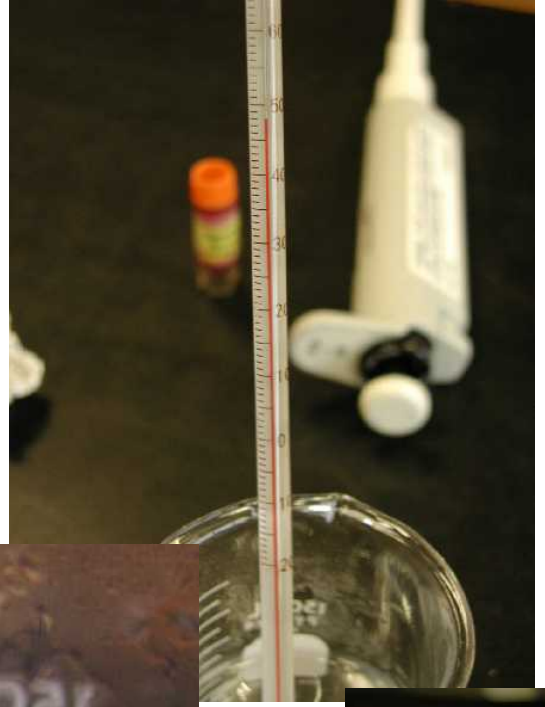


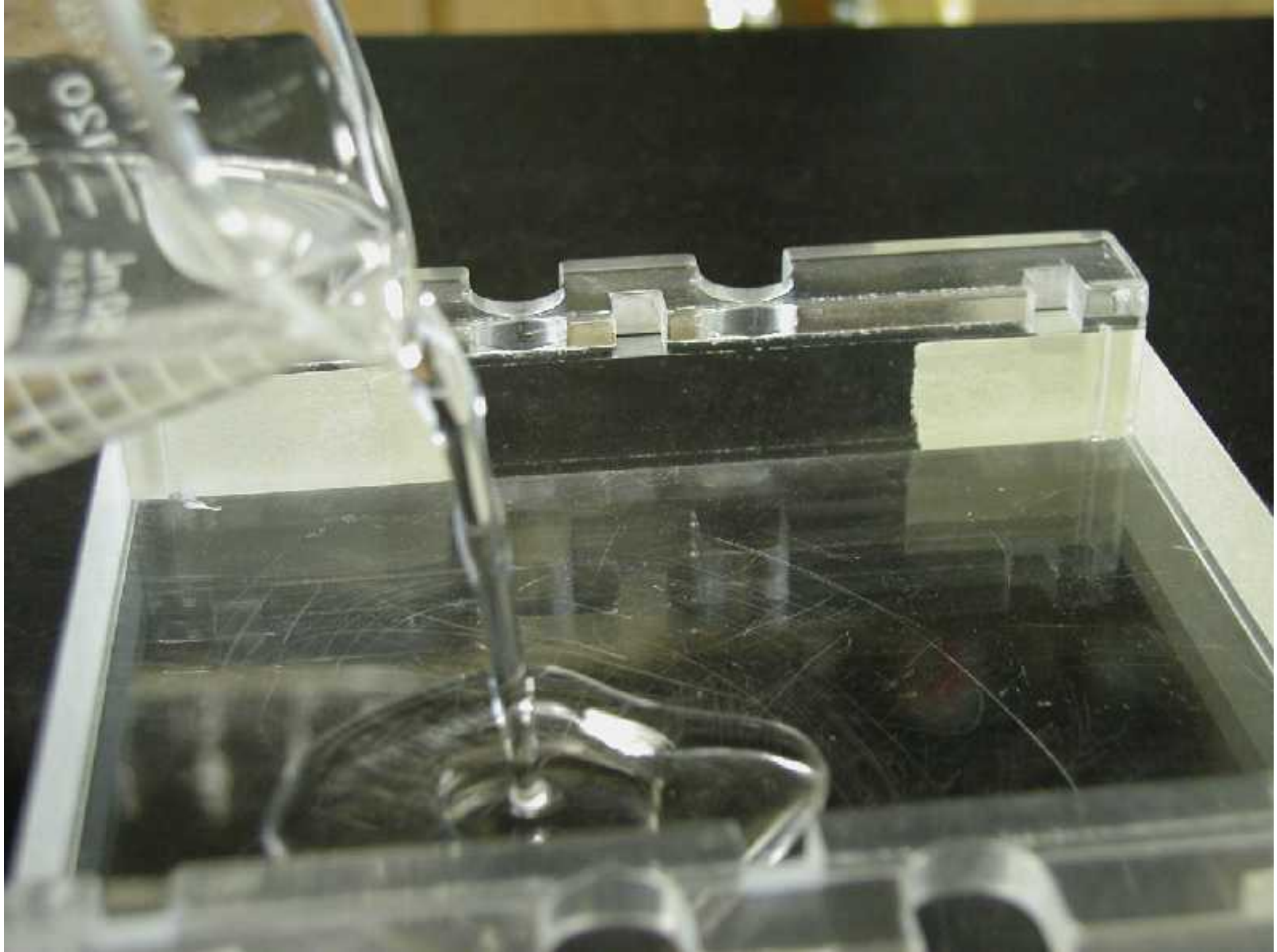


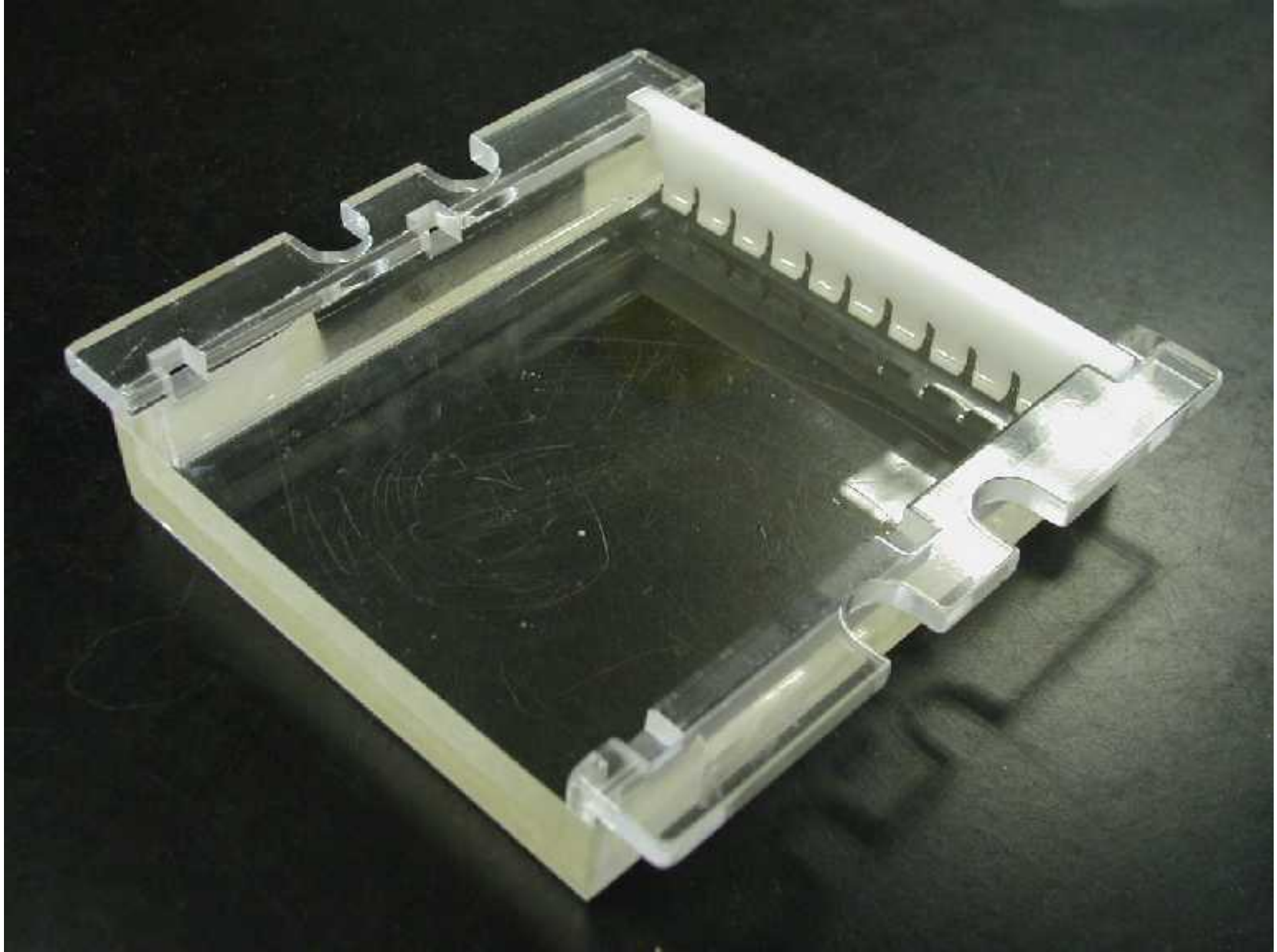

swirl
gently

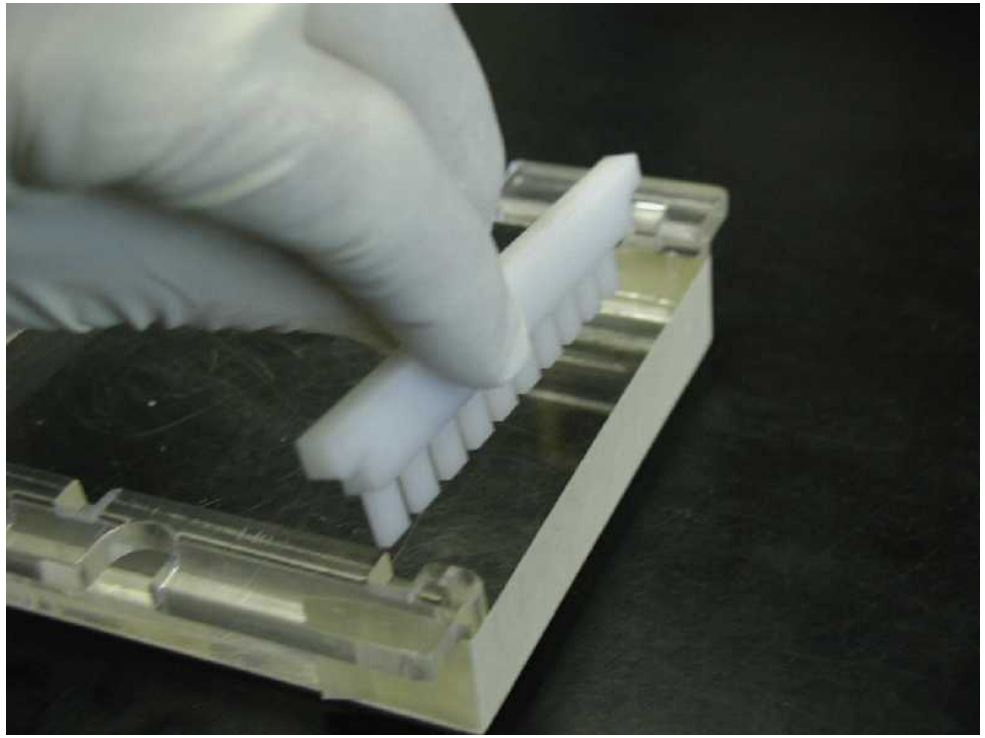


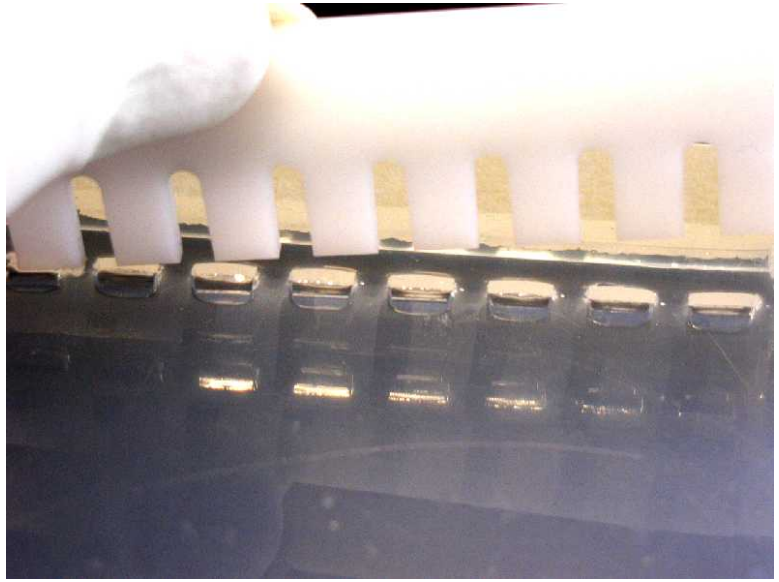


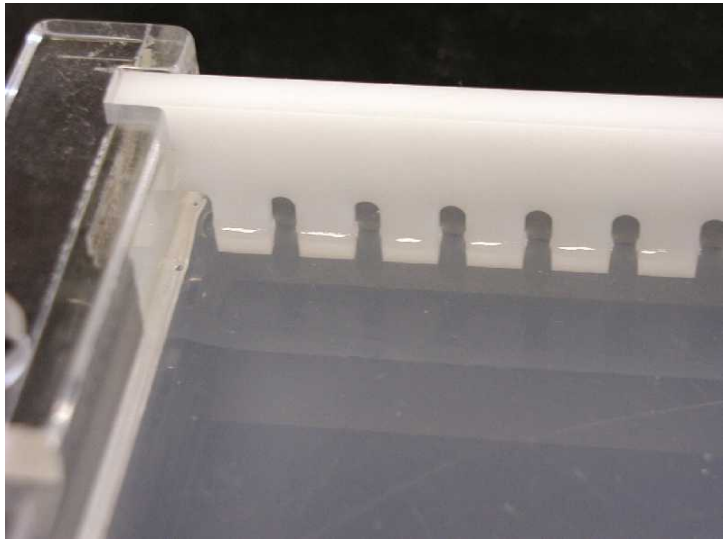


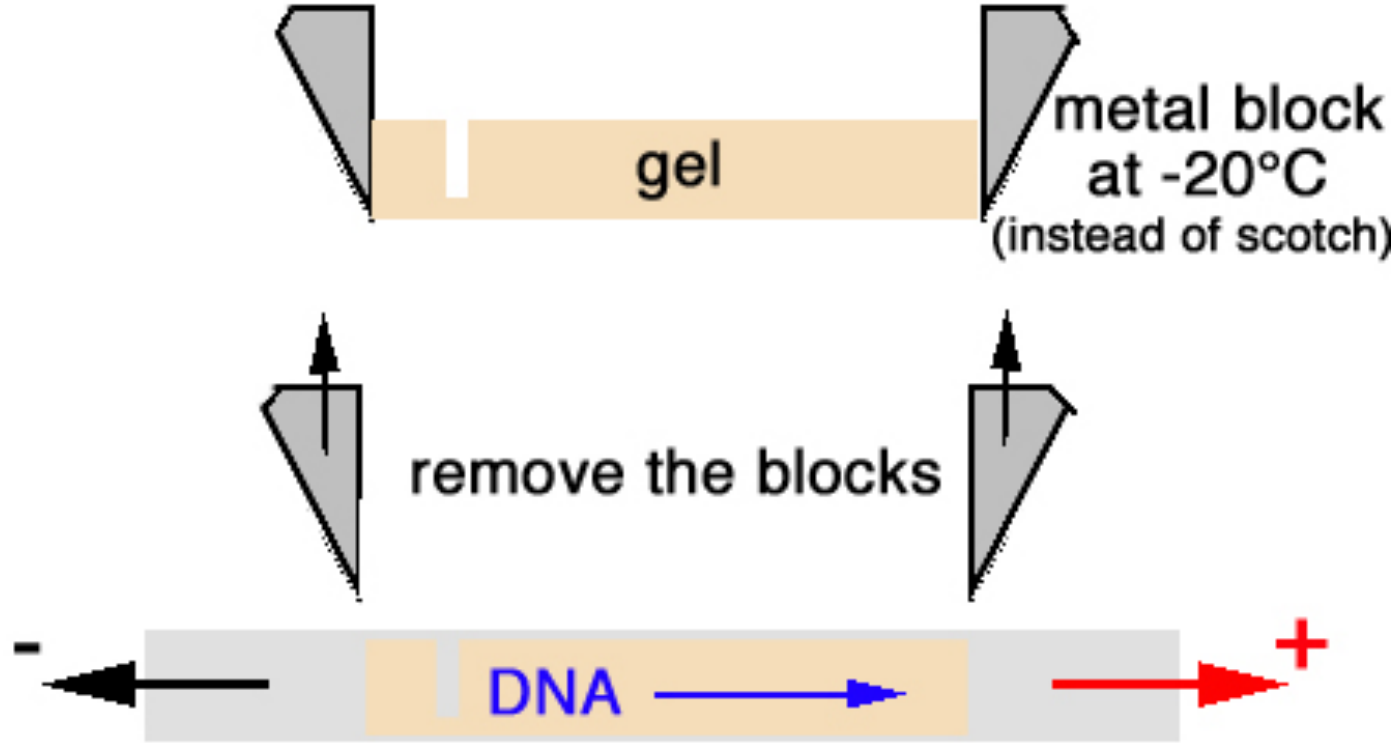


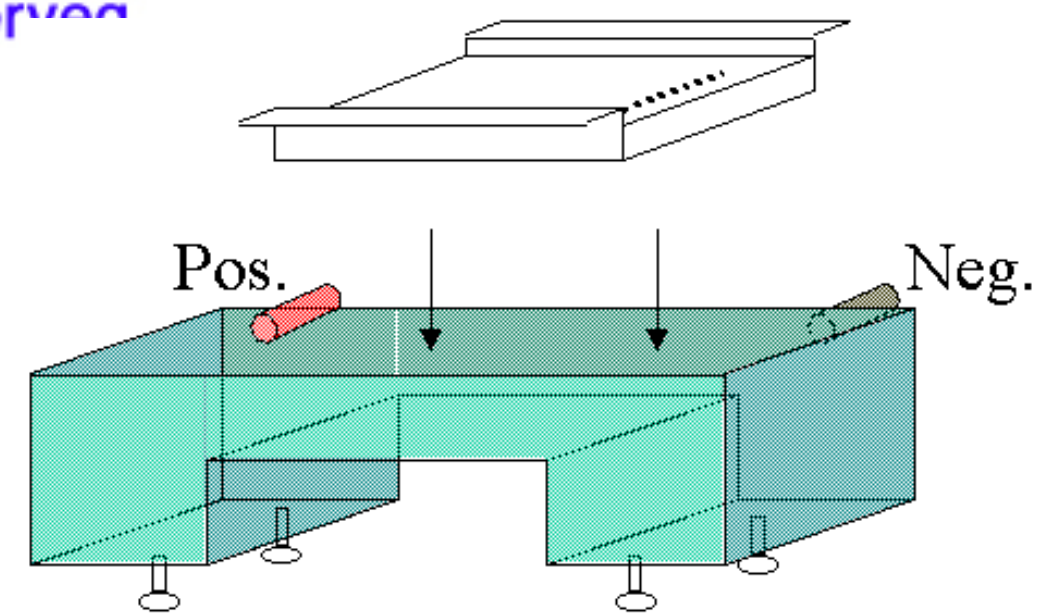


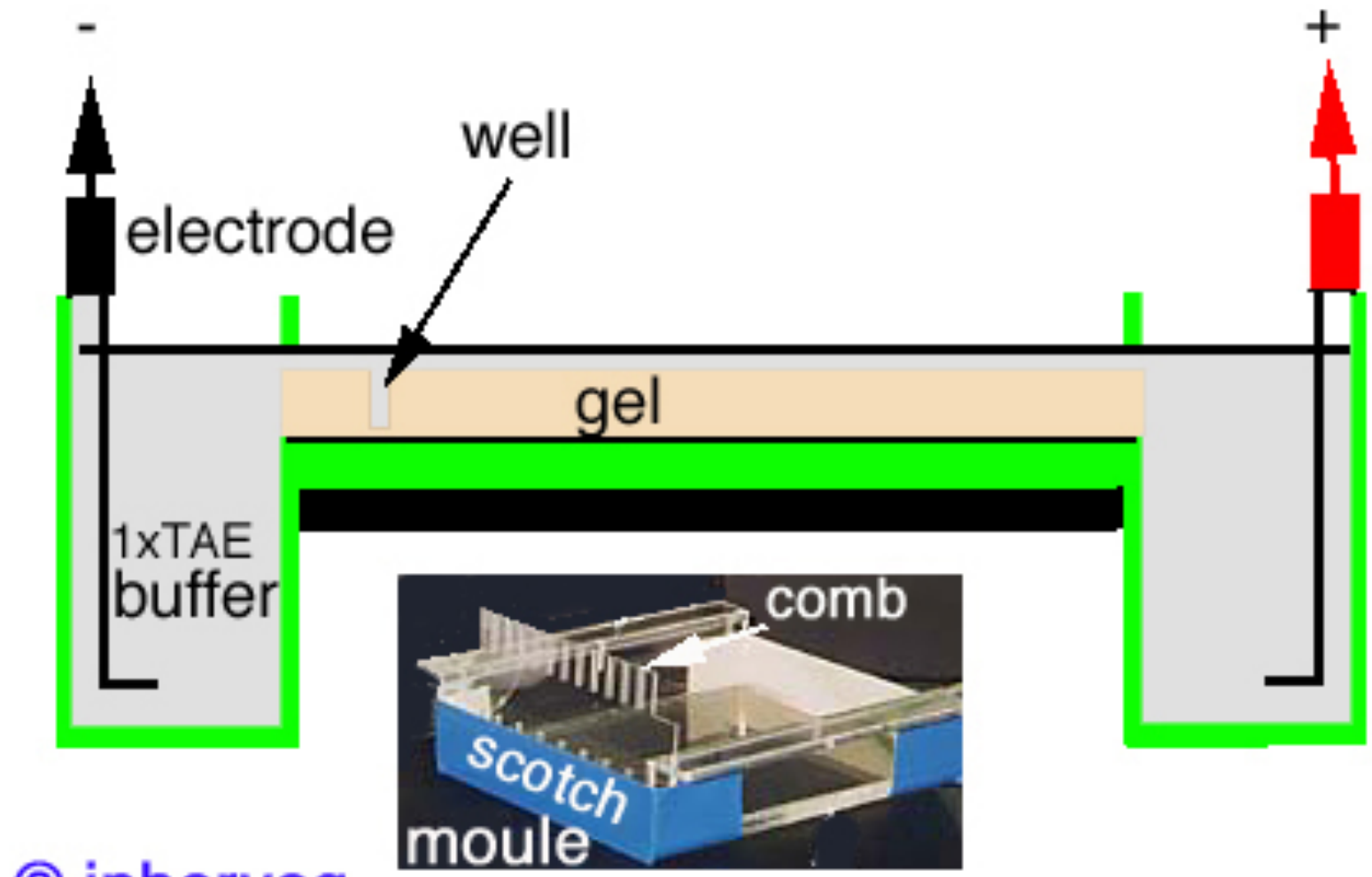


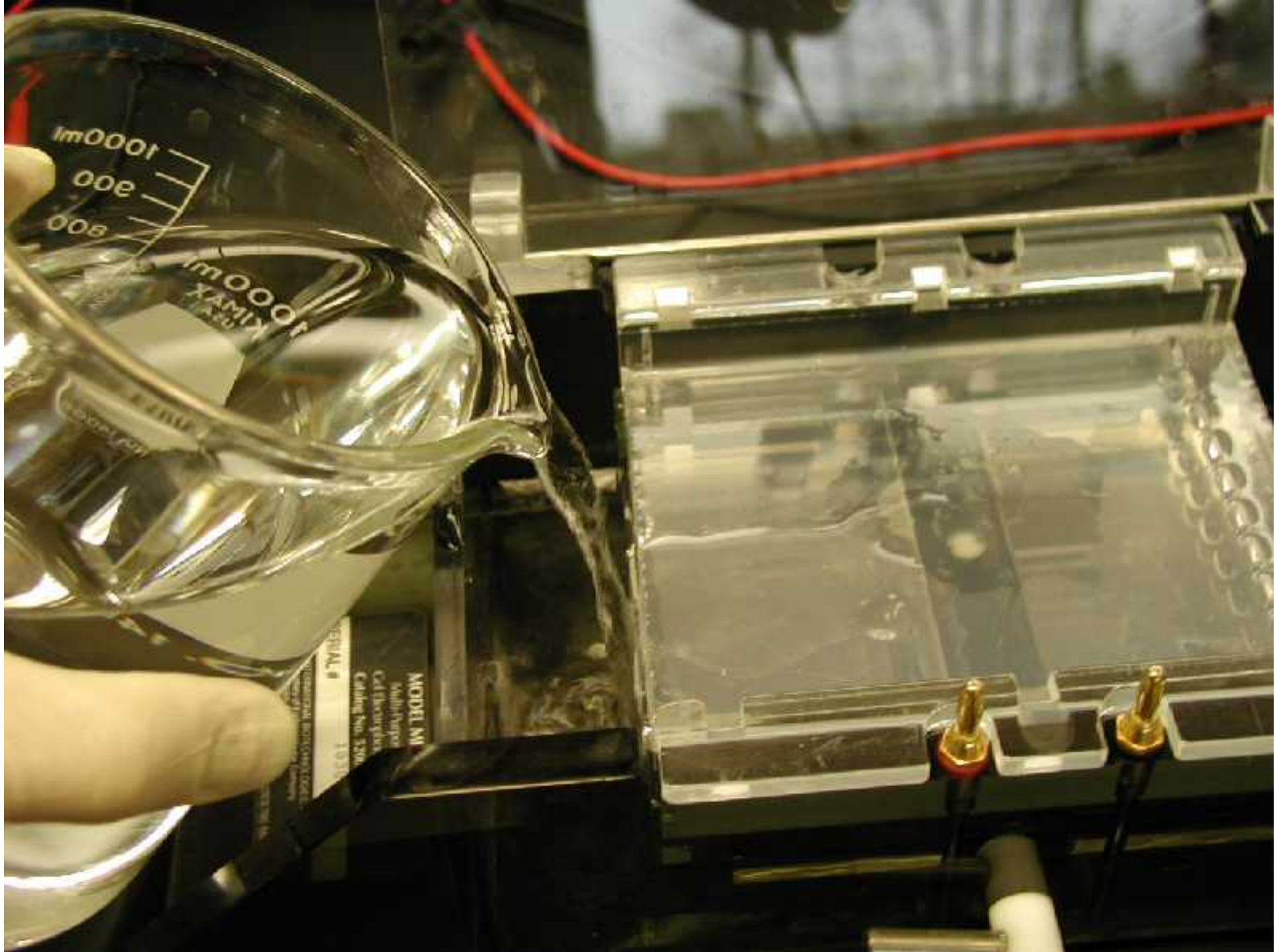


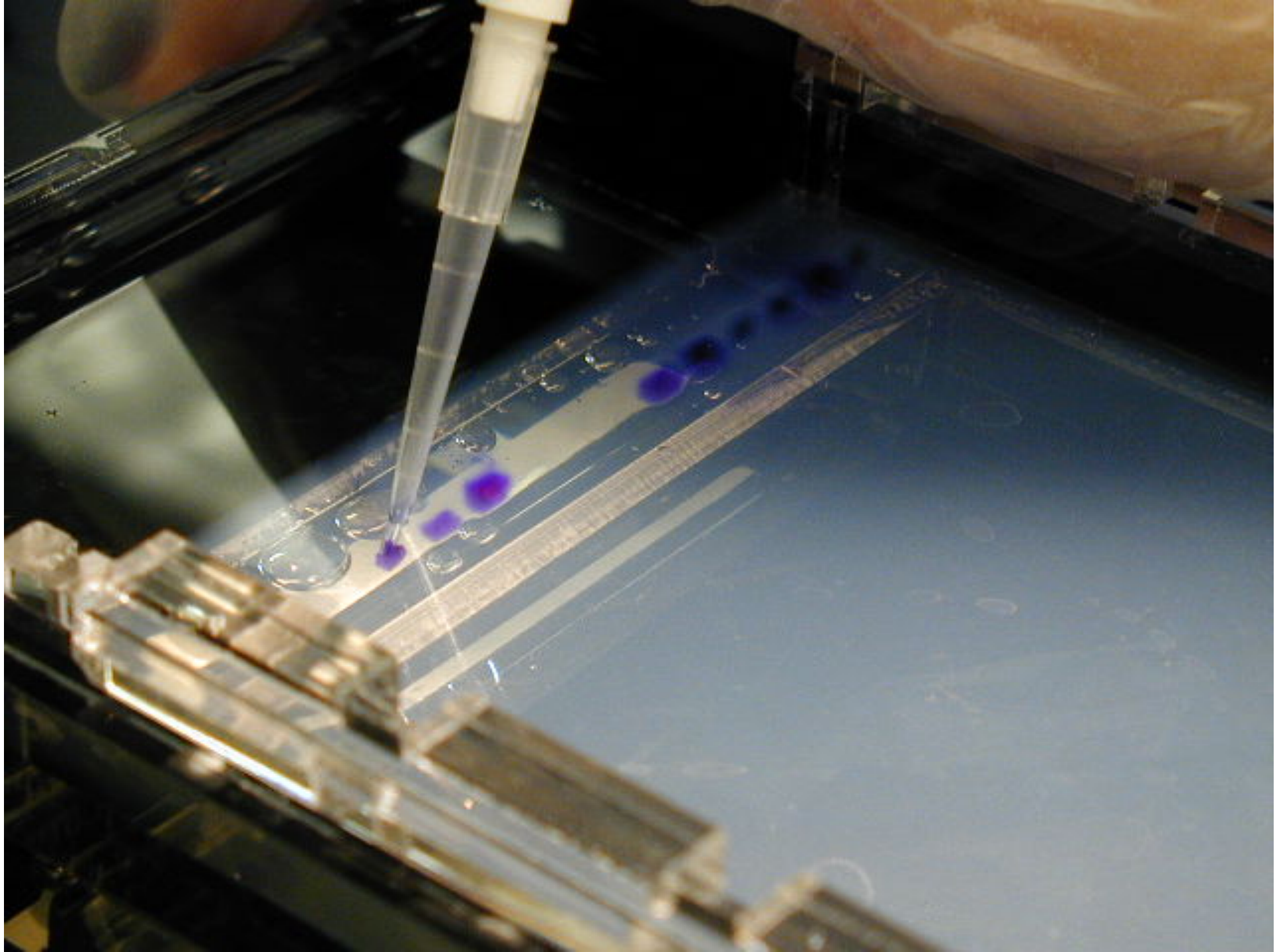


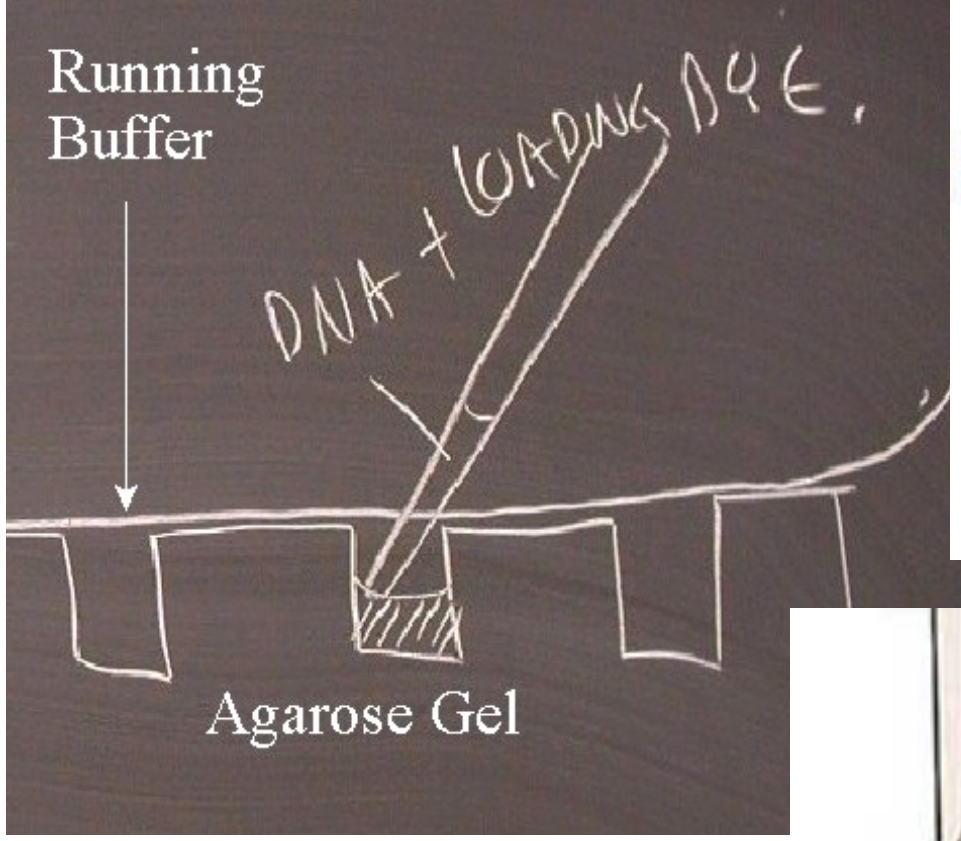

gel
metal block
at -20°C
(instead of scotch)
remove the blocks
-
DNA
+

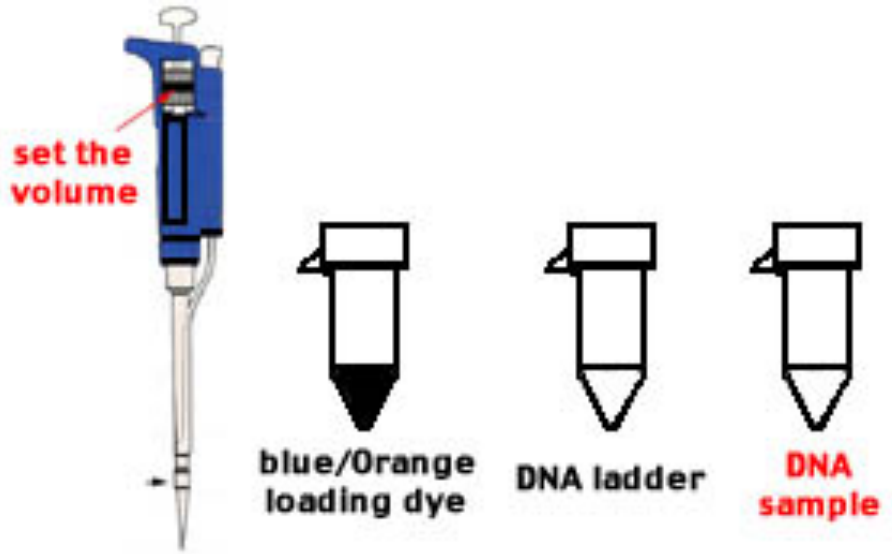

Pos.
Neg.

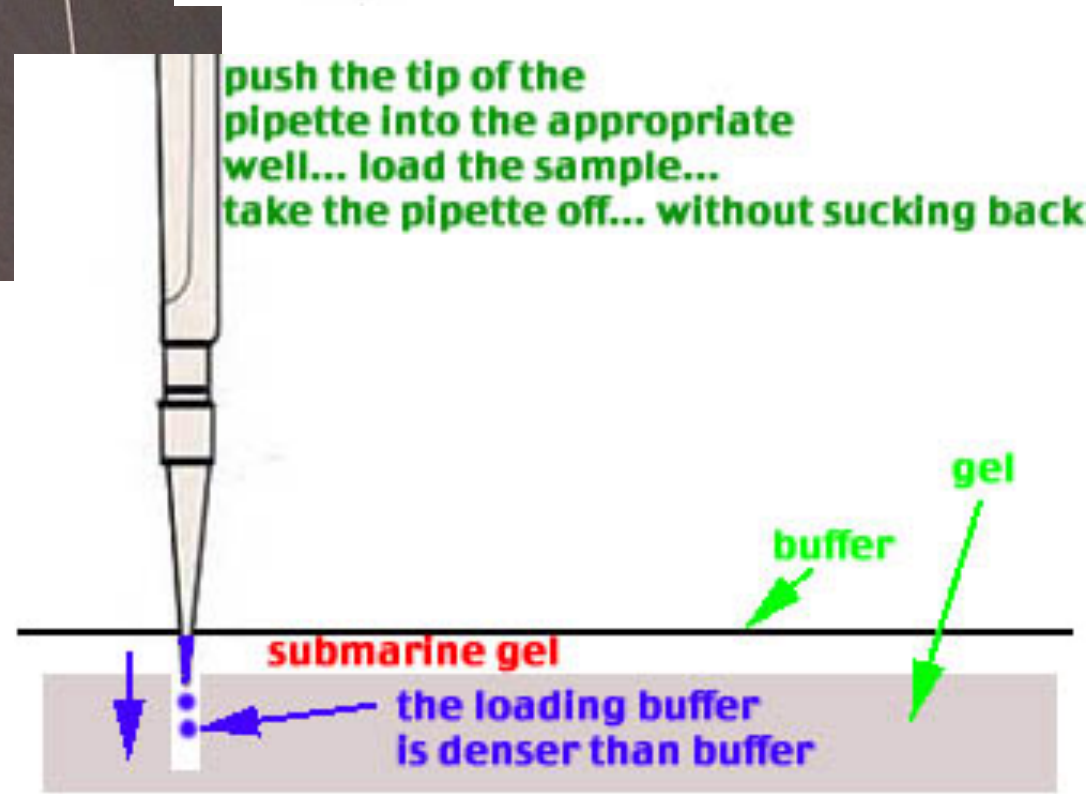


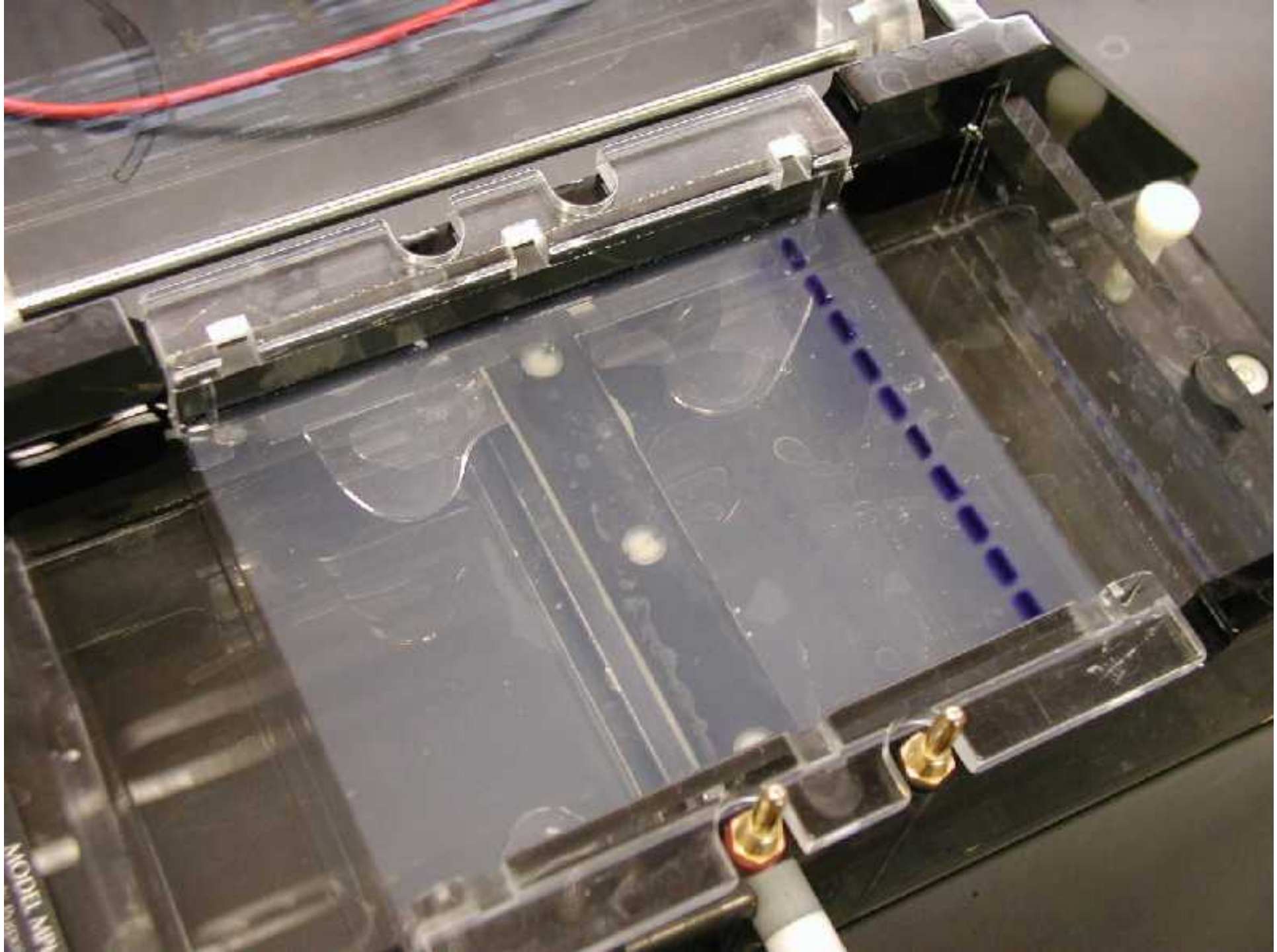

1000lm
900
800
1000lm

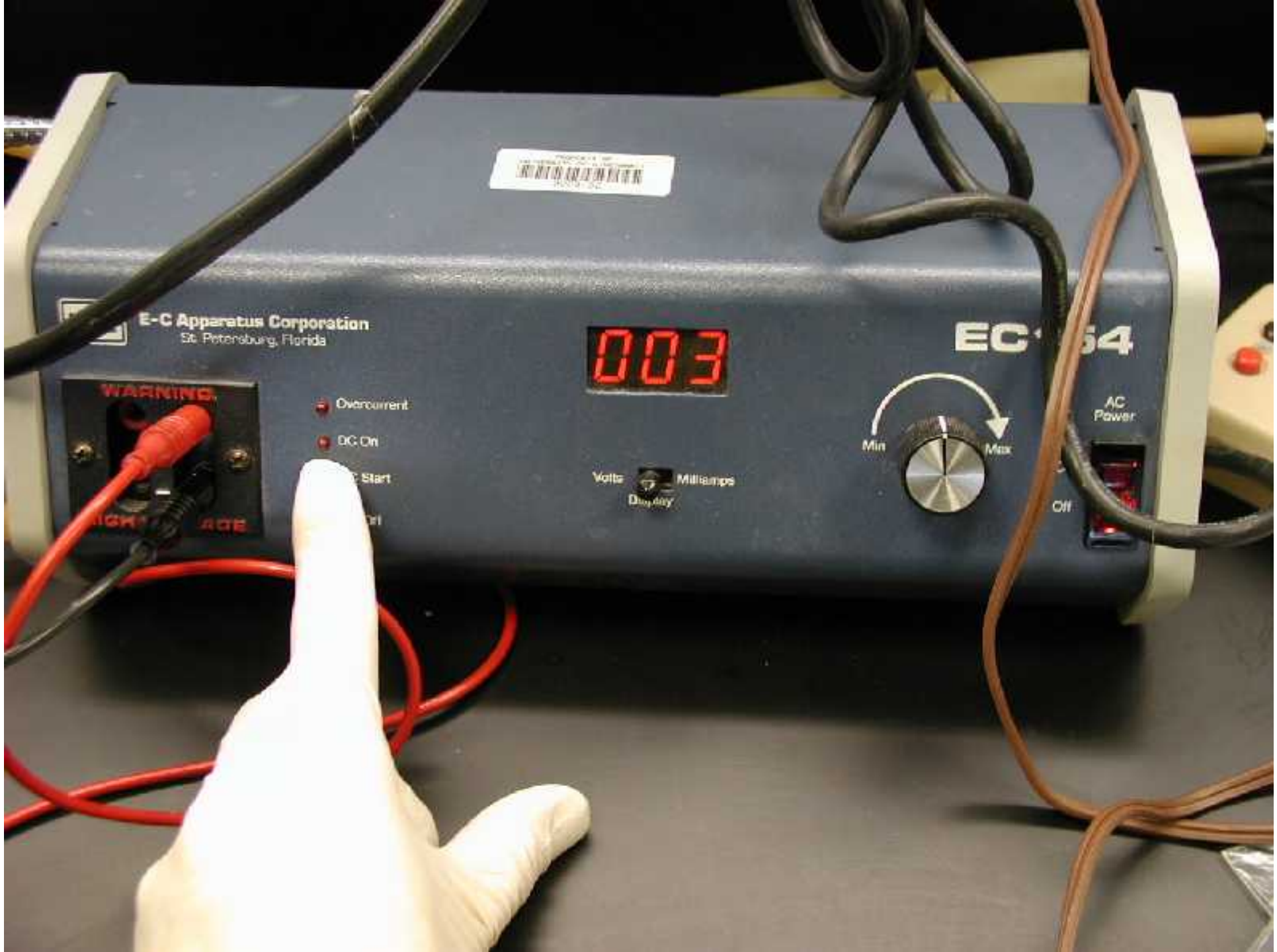


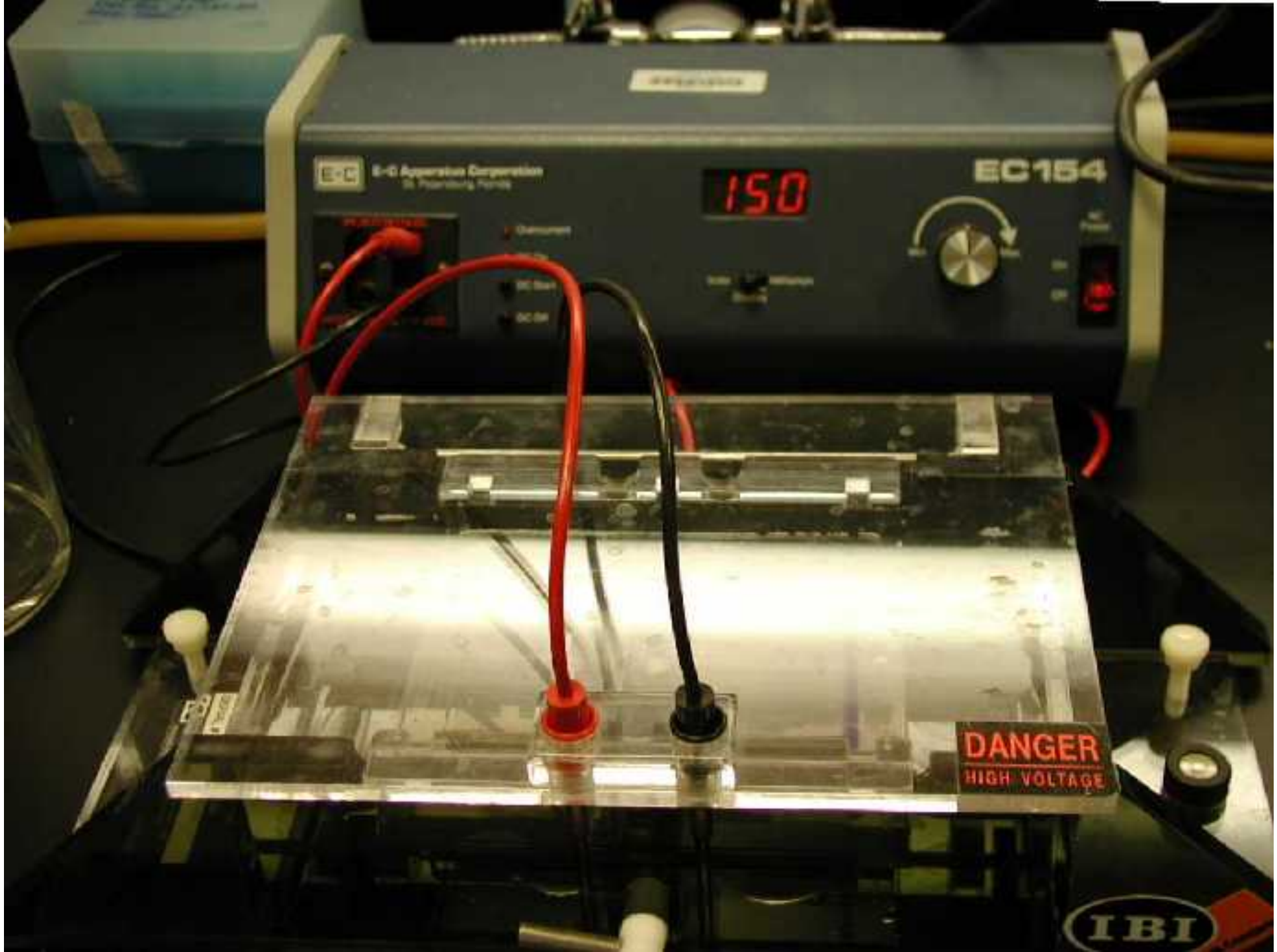

Running
Buffer
DNA + LOADING DYE.
Agarose Gel

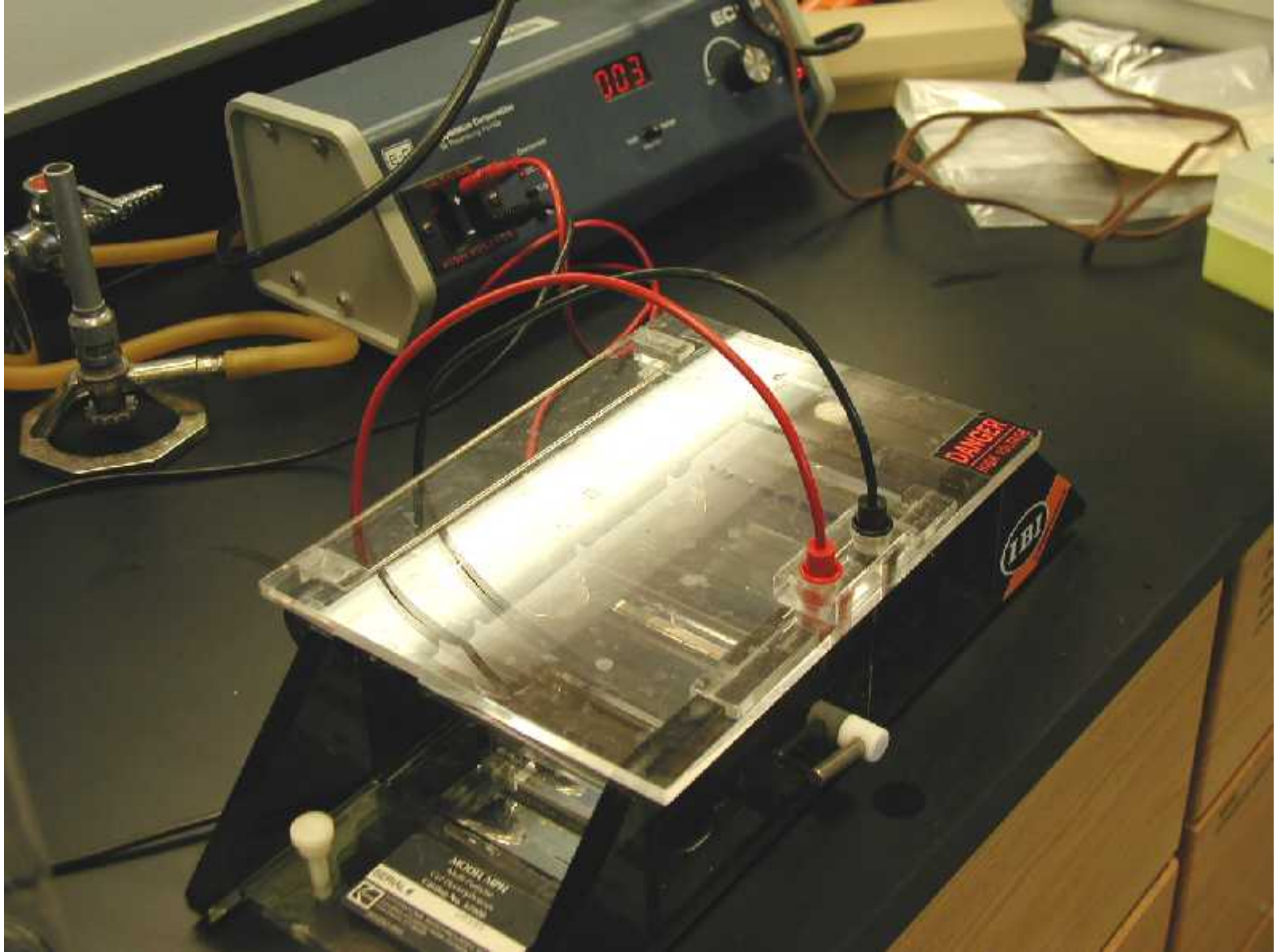

set the
volume
blue/Orange
loading dye
DNA ladder
DNA
sample

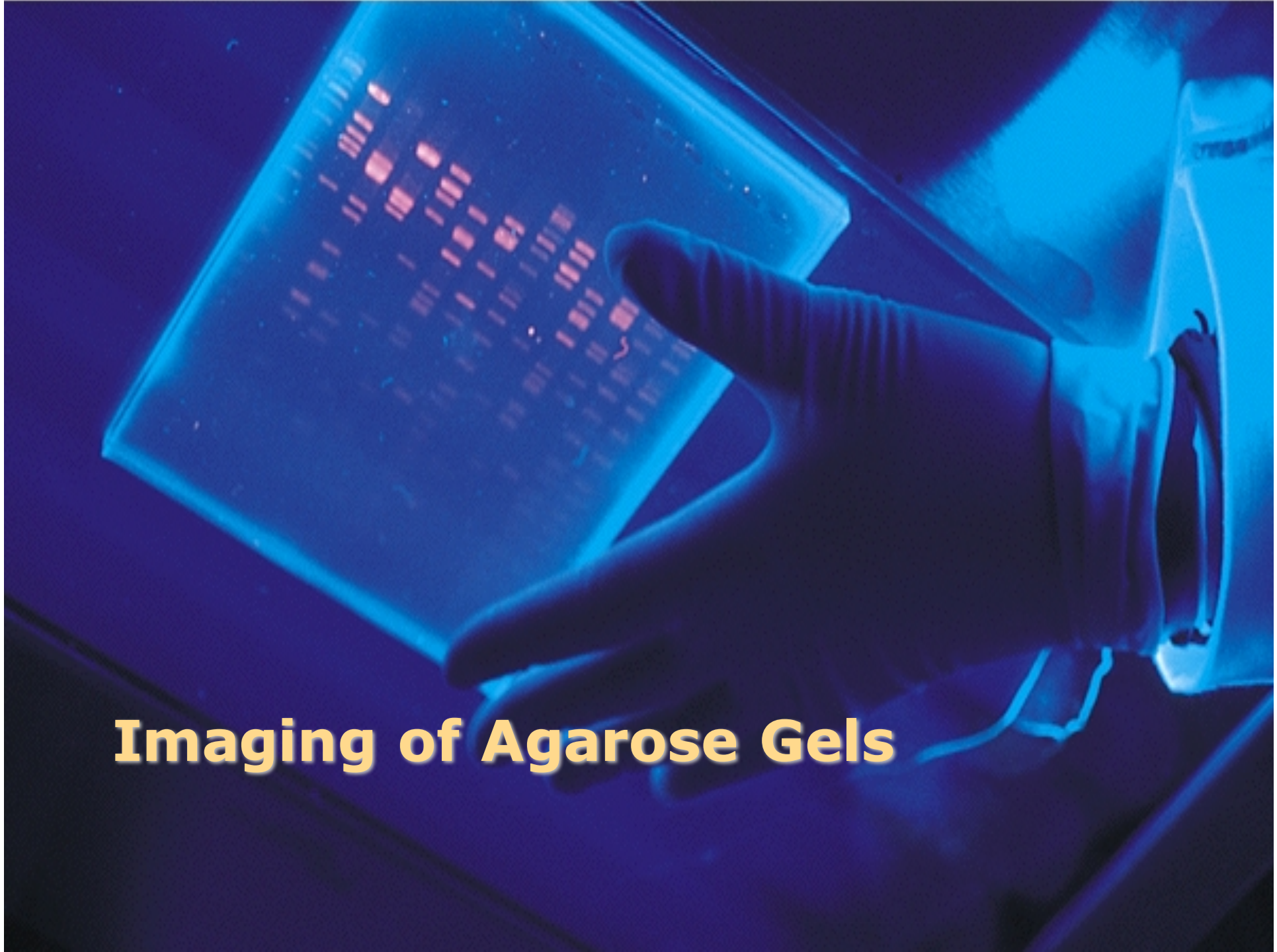

push the tip of the
pipette into the appropriate
well... load the sample...
take the pipette off... without sucking back
gel
buffer
submarine gel
the loading buffer
is denser than buffer




E-C Apparatus Corporation
St. Petersburg, Florida
003
WARNING
Overcurrent
DC On
Volts
Milliamps
Min
Max
AC
Power
Off

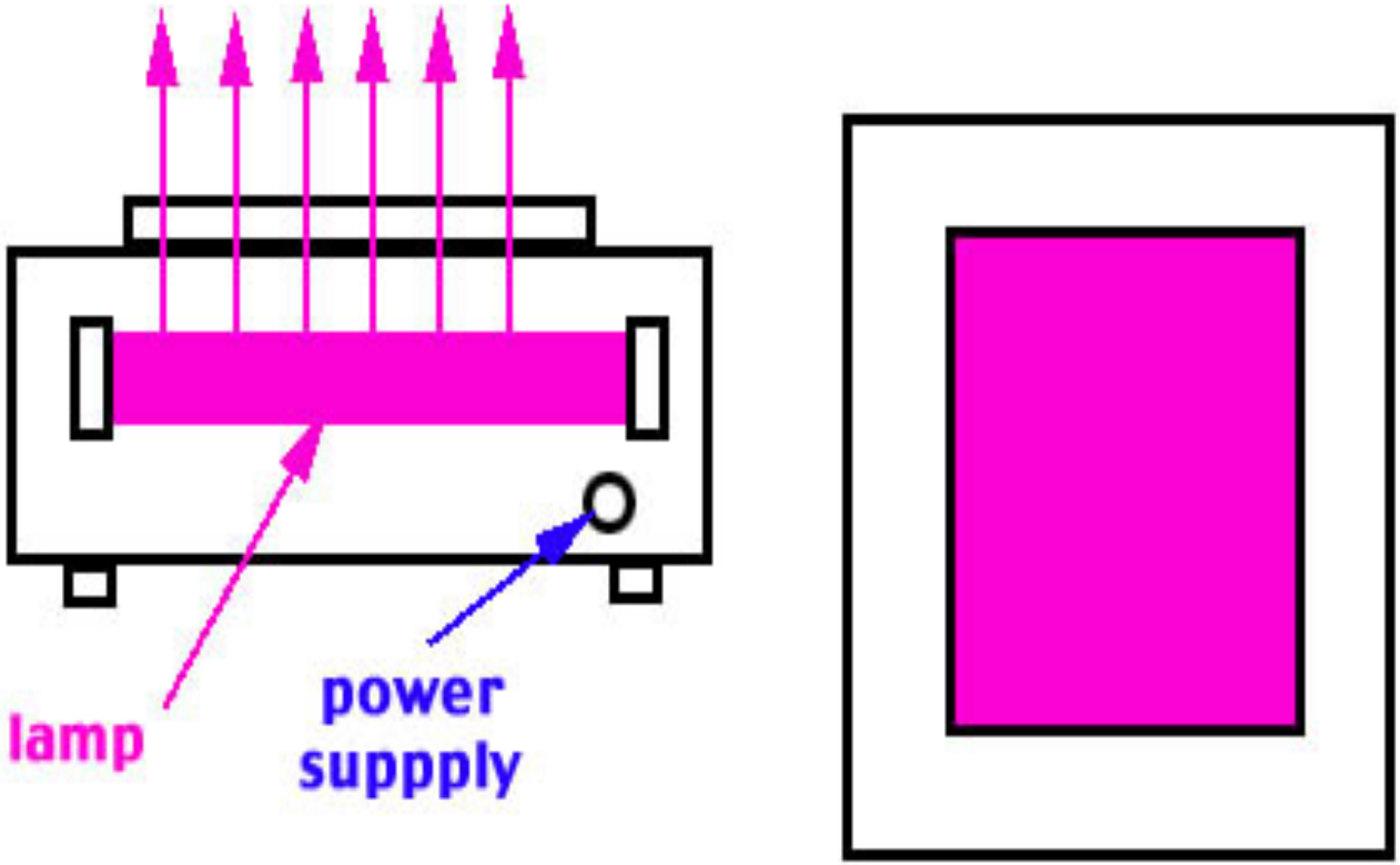

E-C Apparatus Corporation
St. Petersburg, Florida
150
EC154
DANGER
HIGH VOLTAGE
IBI

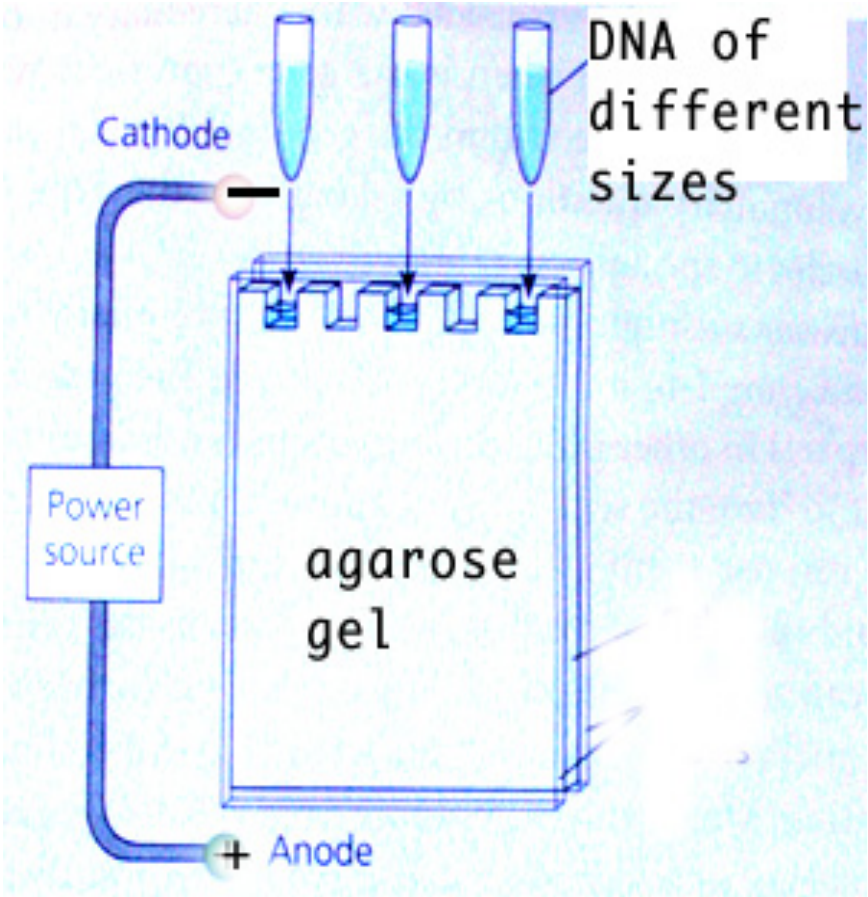

DANGER
IBI

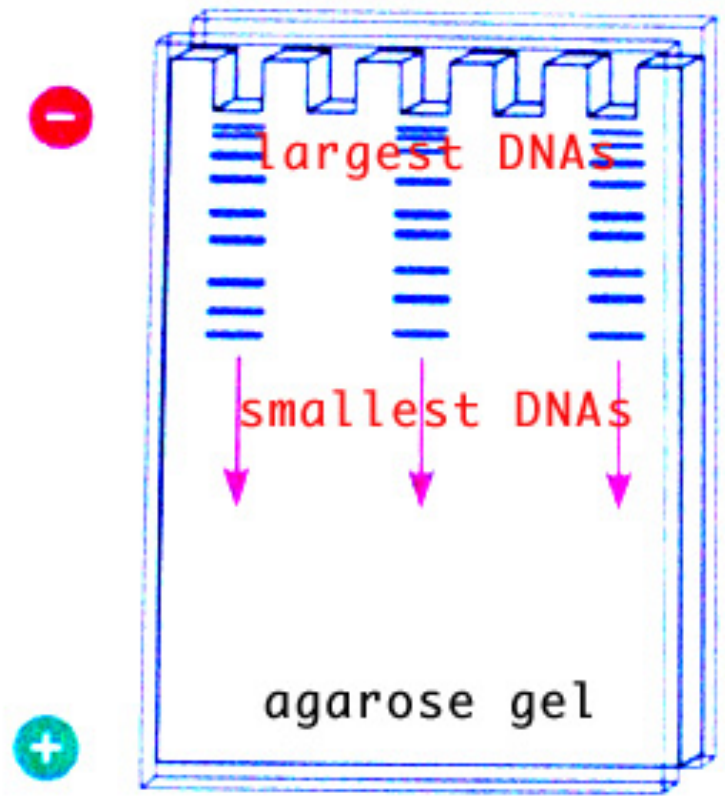

Imaging of Agarose Gels

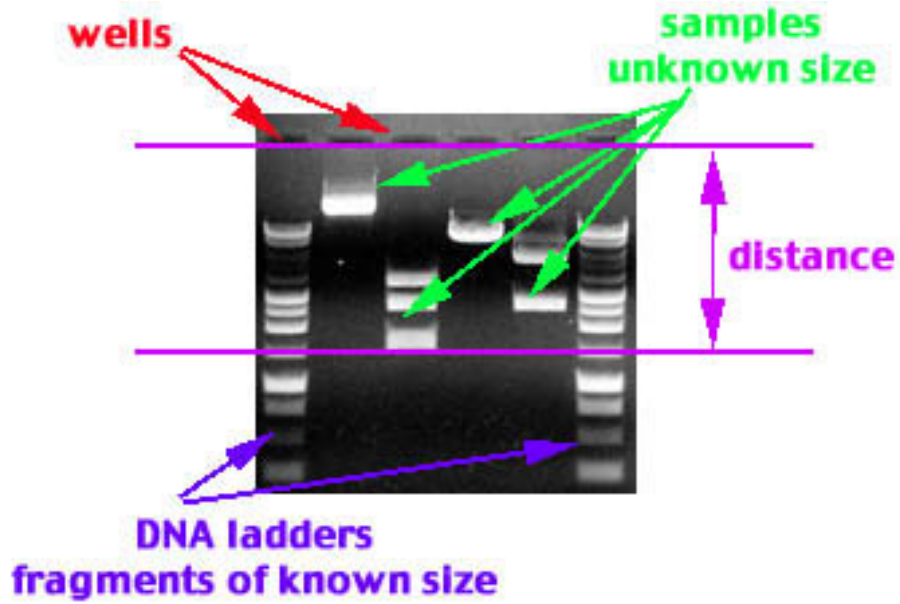

welder masks equipped
with glass windows
camera
POLAROID
UV transilluminator
on/off switch

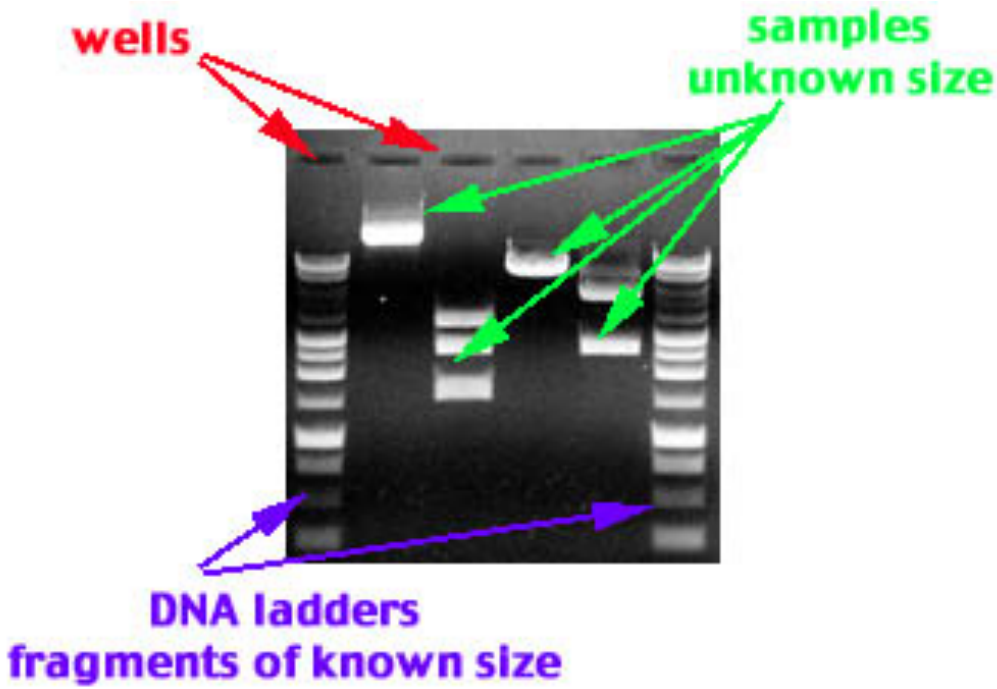
UV transilluminator

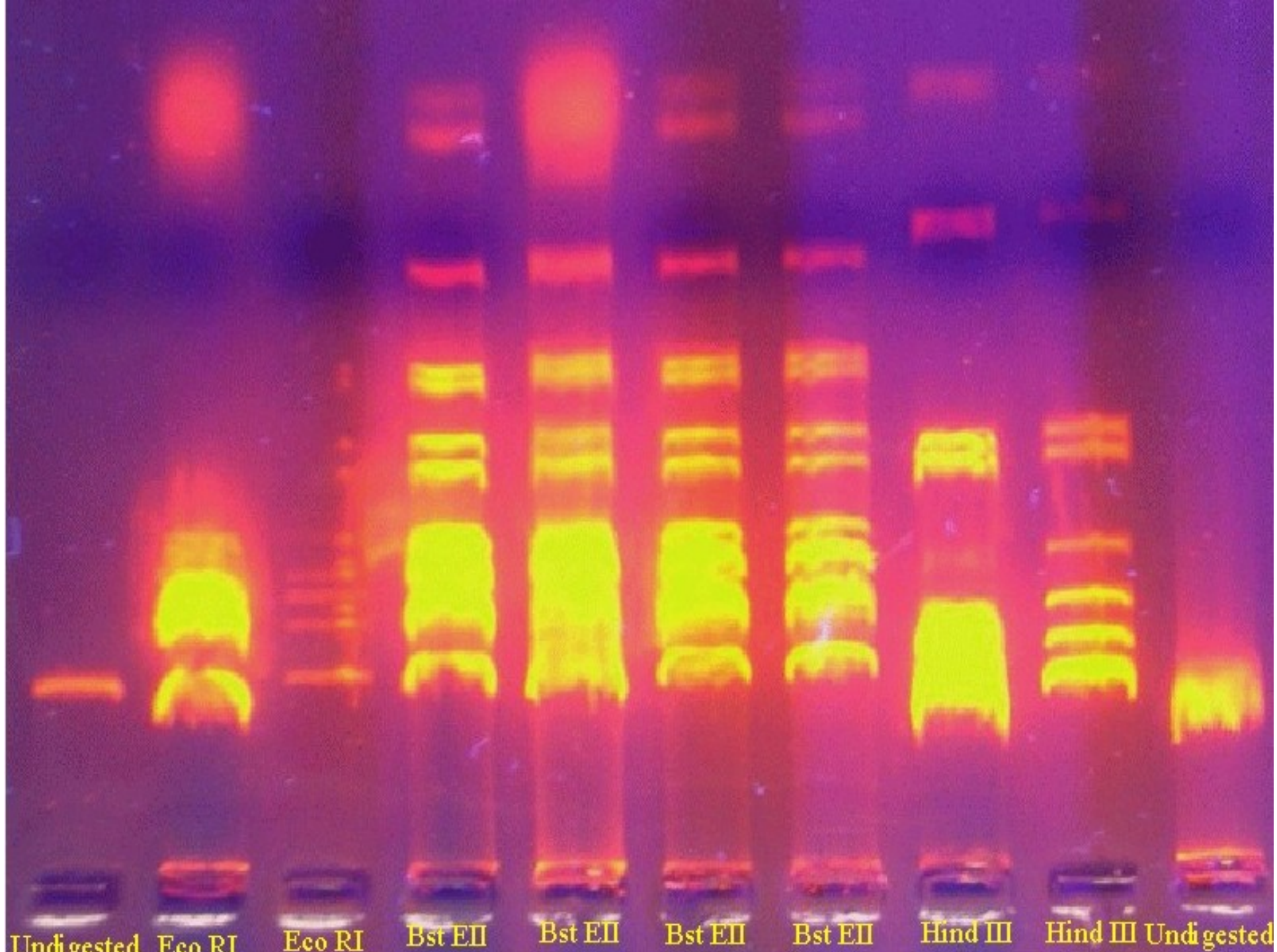


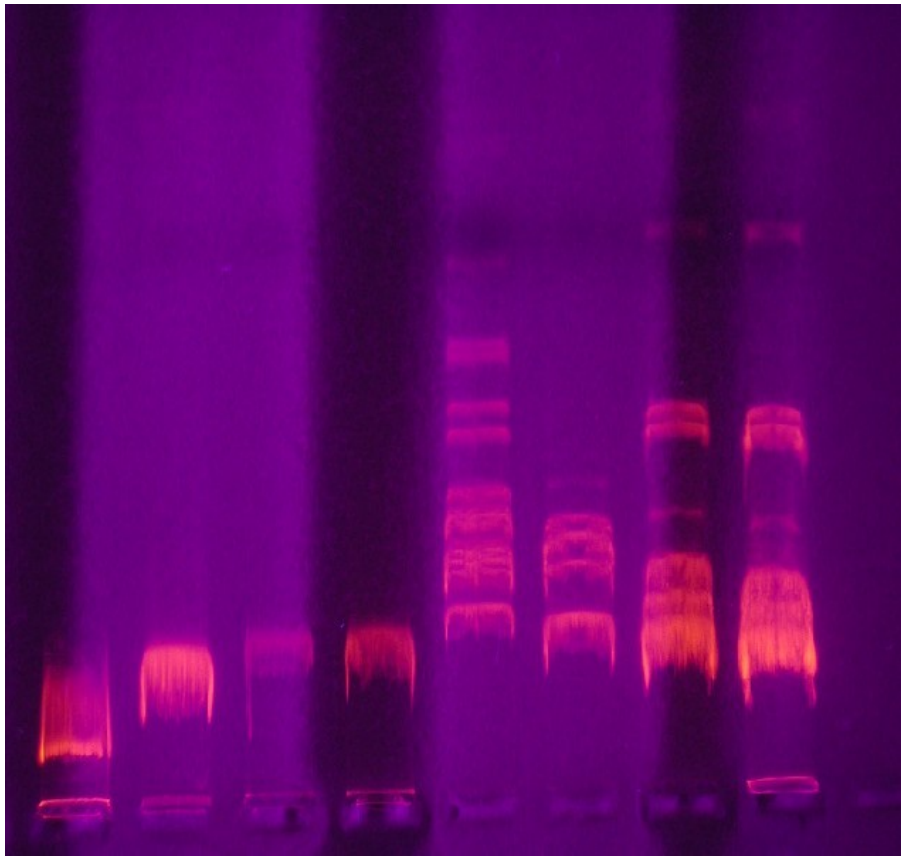

DNA of different sizes
Cathode
Power source
agarose gel
Anode

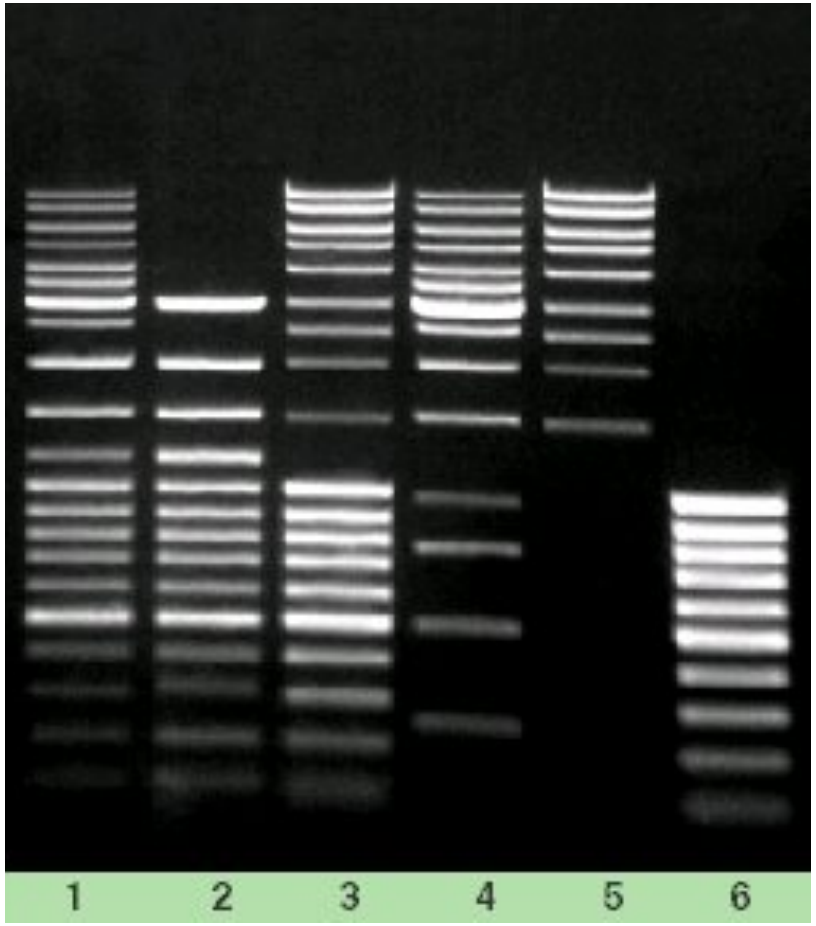

largest DNAs
smallest DNAs
agarose gel

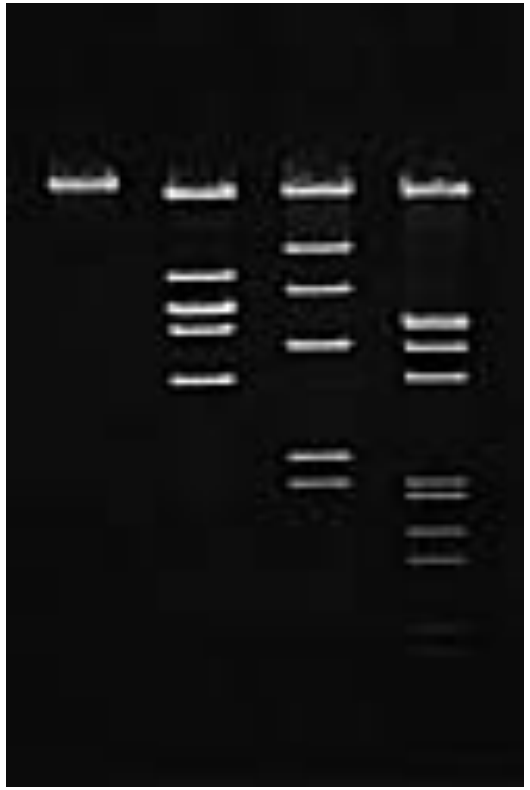

wells
samples
unknown size
distance
DNA ladders
fragments of known size


wells
samples
unknown size
DNA ladders
fragments of known size


Undigested
Eco RI
Eco RI
Bst EII
Bst EII
Bst EII
Bst EII
Hind III
Hind III
Undigested

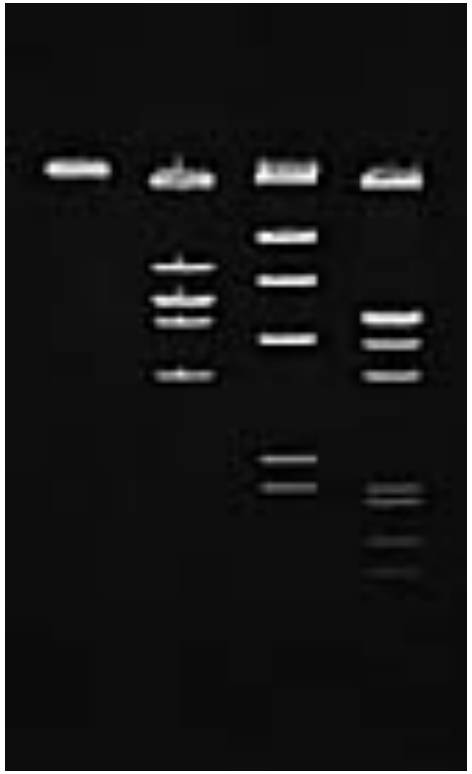



1
2
3
4
5
6



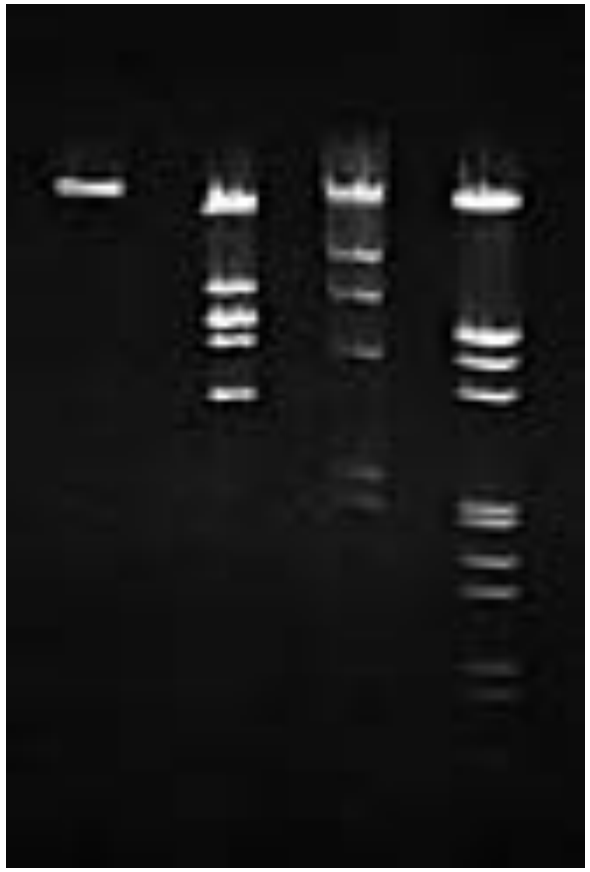
0.8% agarose mini-gel in 1X TBE buffer run at 60 Volts for 2 hours.

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.