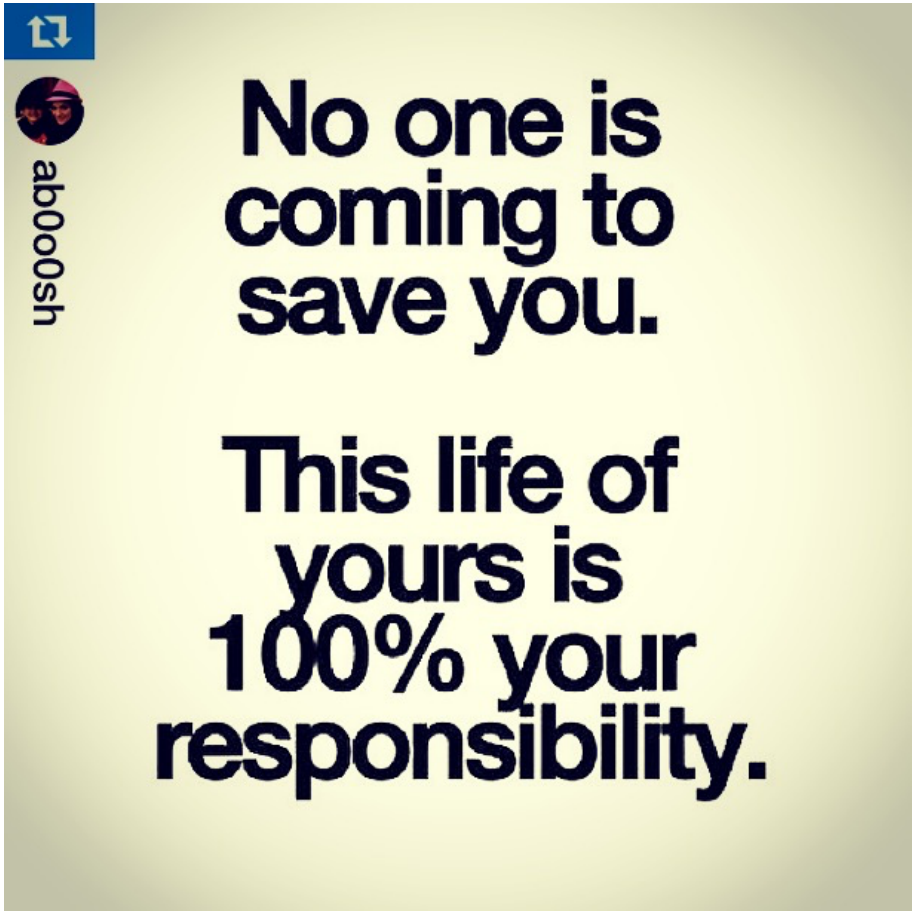
Lecture 5.2

Molecular techniques

II. DNA quality and quantity

Course 485

Lessons for life



AIMS

- Learn the fundamentals of analyzing the quality and quantity of DNA.

- Understand why one needs to know:
    - The quality of DNA
    - The quantity of DNA

- Learn analyzing DNA qualitatively using gel electrophoresis.

AIMS

- Learn the types of electrophoresis methods.

- Learn how using charge of DNA to separate the sizes.

- Learn what DNA staining method you should use and when.

- Learn the standard quantitative methods.

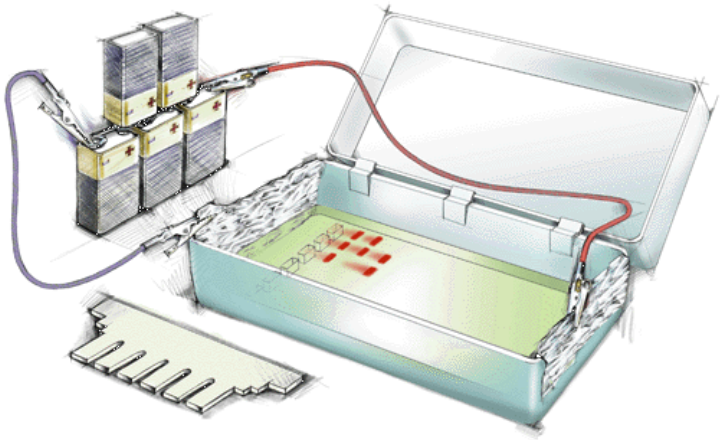
DNA analysis – a first step

Nucleic Acid (DNA)

Qualitative Analysis

Size - Quality

Gel Electrophoresis



Quantitative Analysis

Purity - Yield

Spectrophotometry

1. Qualitative Analysis

- One standard method to test the quality of a DNA sample is to run the sample on a gel.

- This method allows you to visualize the quality of DNA.
    - Large amount?
    - Small amount?
    - Big size (genomic)?
    - Small size (degraded)?

- The intensity of the band may give you a hint about the quantity but this is not precise.

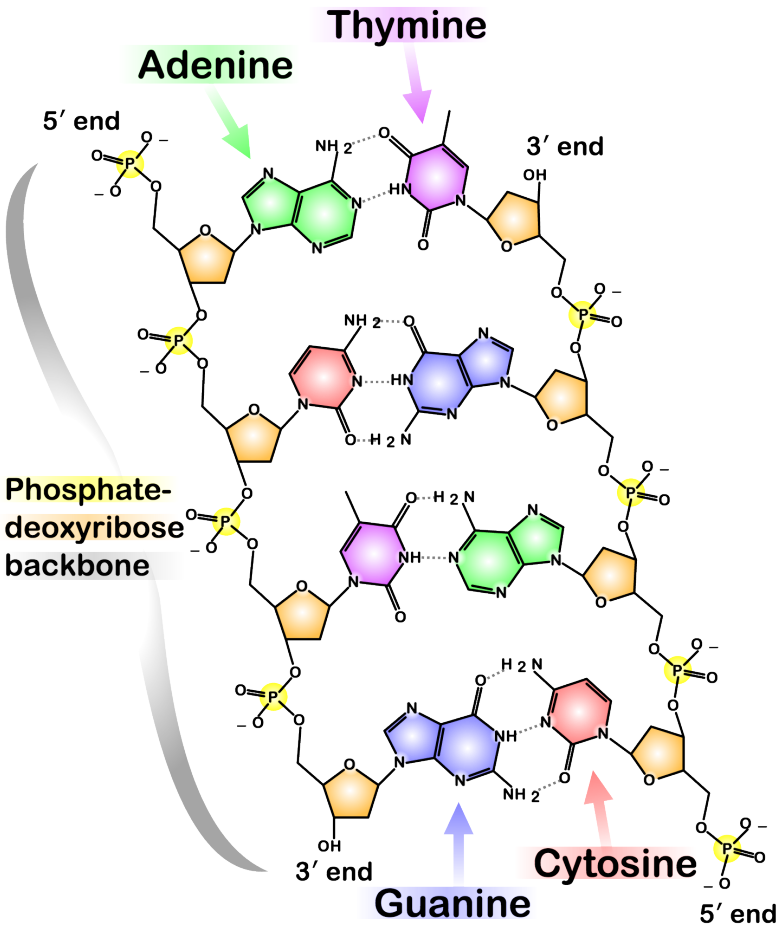
DNA charge

What is the net charge of DNA?

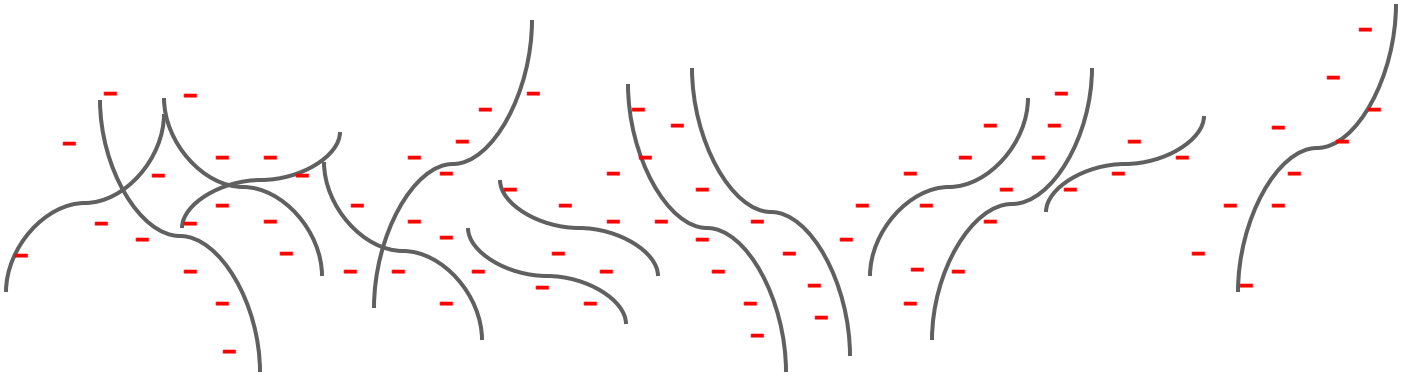
DNA charge

It is negative charge because of the phosphate in the backbone.

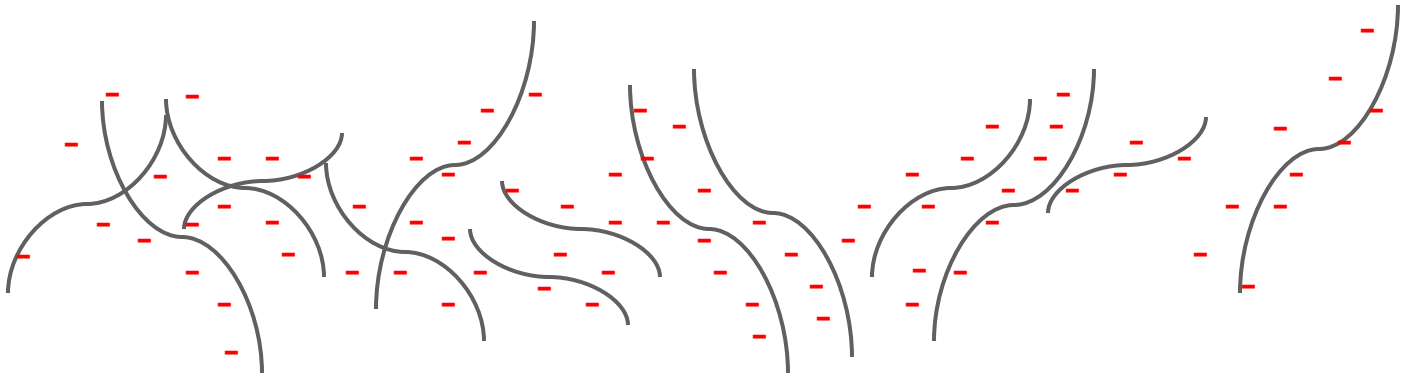
So if placed in an aqueous ionic solution and electric field, **DNA will move from the negative electrode to the positive one.**



DNA charge



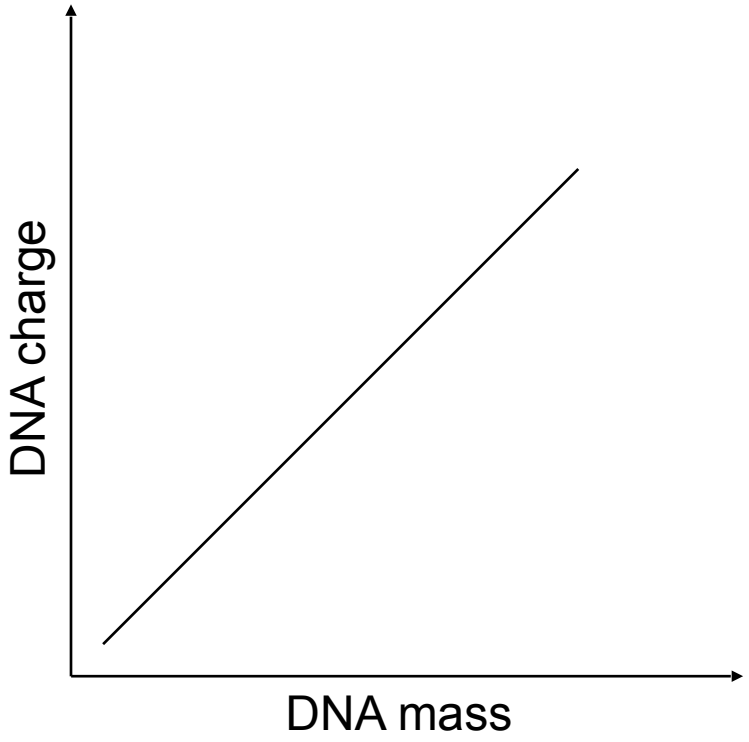
All DNA molecules will move from - → +
BUT
Do all DNA molecules have the same amount of (-) charge?



DNA mass to charge ratio

The bigger the DNA molecule the more charge it has! But it is still negative charge.

How can we separate molecules?



A gel matrix

- How can we make DNA molecules move according to their size?
- We can separate molecules based on their size while all being negatively charged by allowing the molecules to move in a gel matrix.

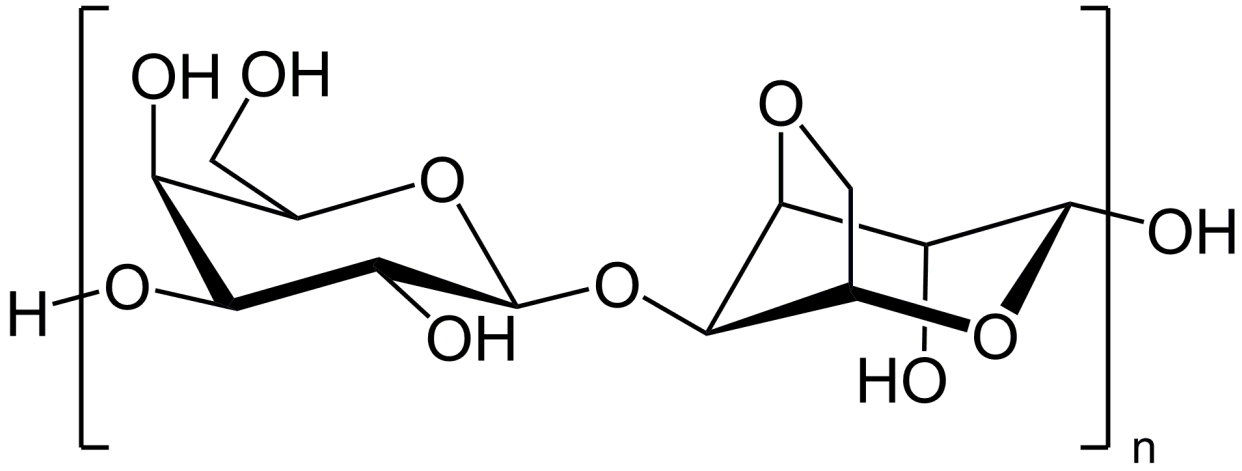
A gel matrix

- **Types of gelatinous mediums:**
  - Agarose
  - Polyacrylamide
  - Cellulose acetate and starch

Sea weed and Agarose

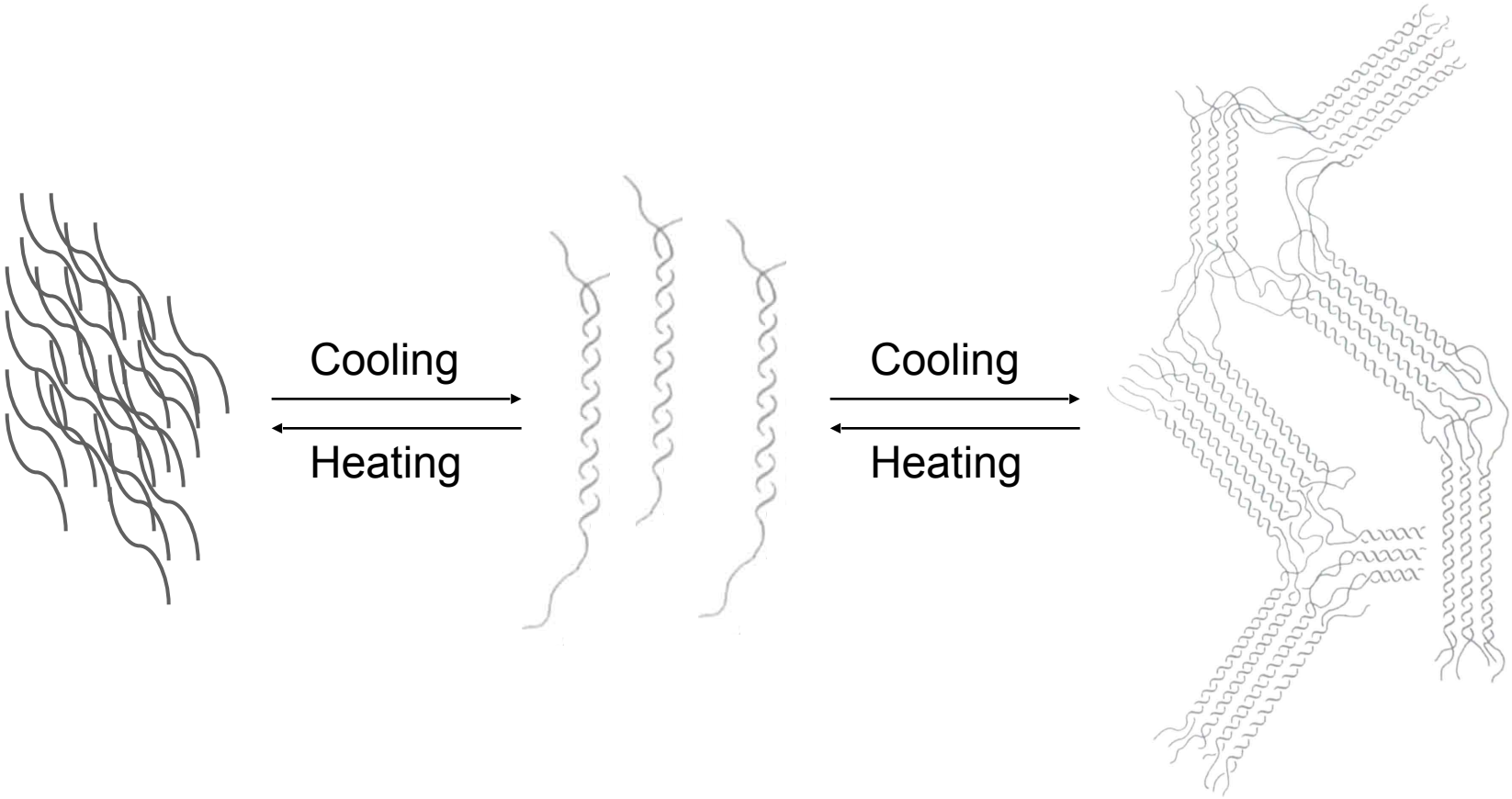
Agarose gel

- **Agarose**: a gel that forms a three dimensional matrix with pores that differ in size.



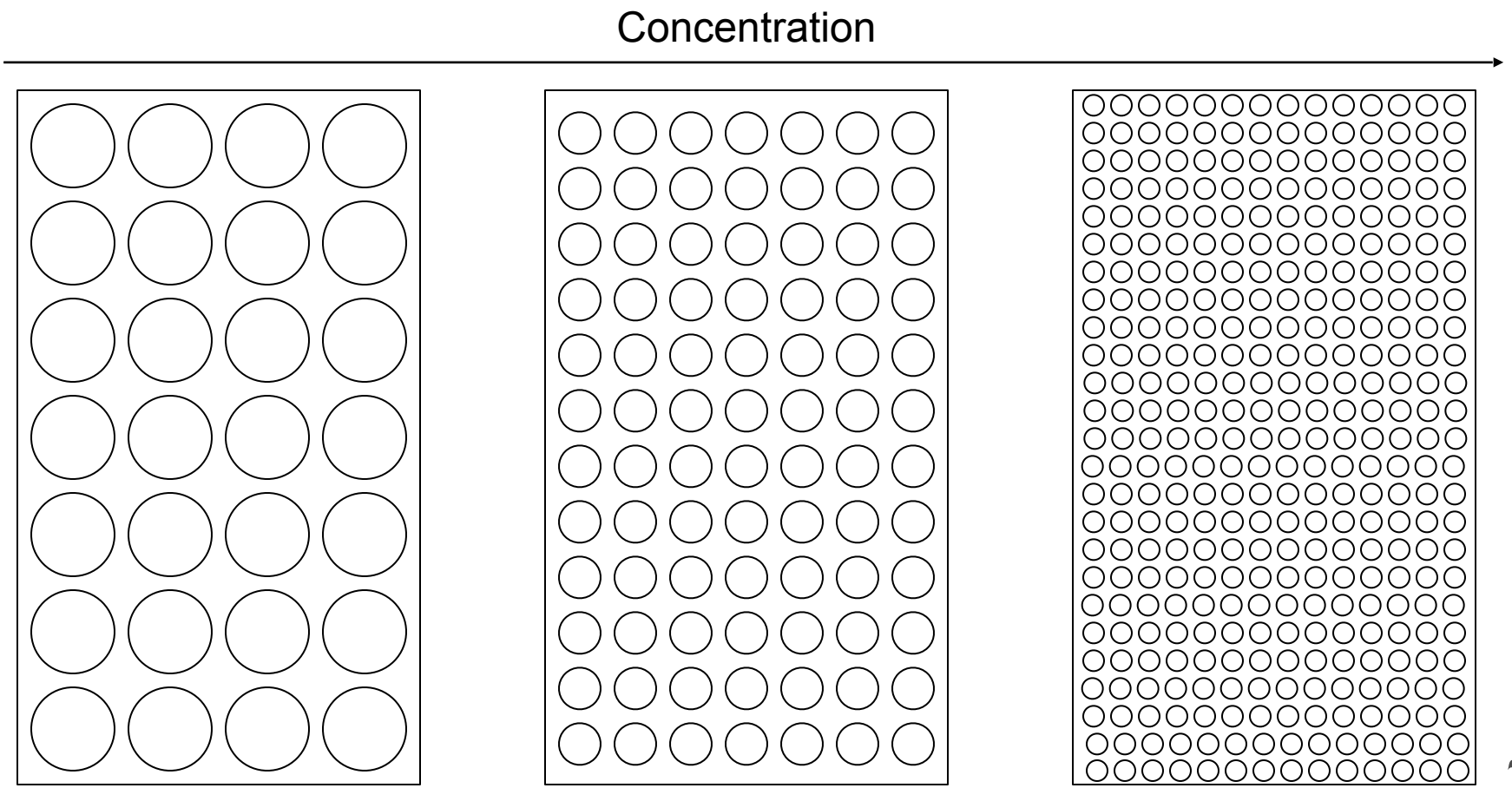
Agarose gel

- When agarose solution is heated, it is in liquid form and as it cools down the matrix gets formed.



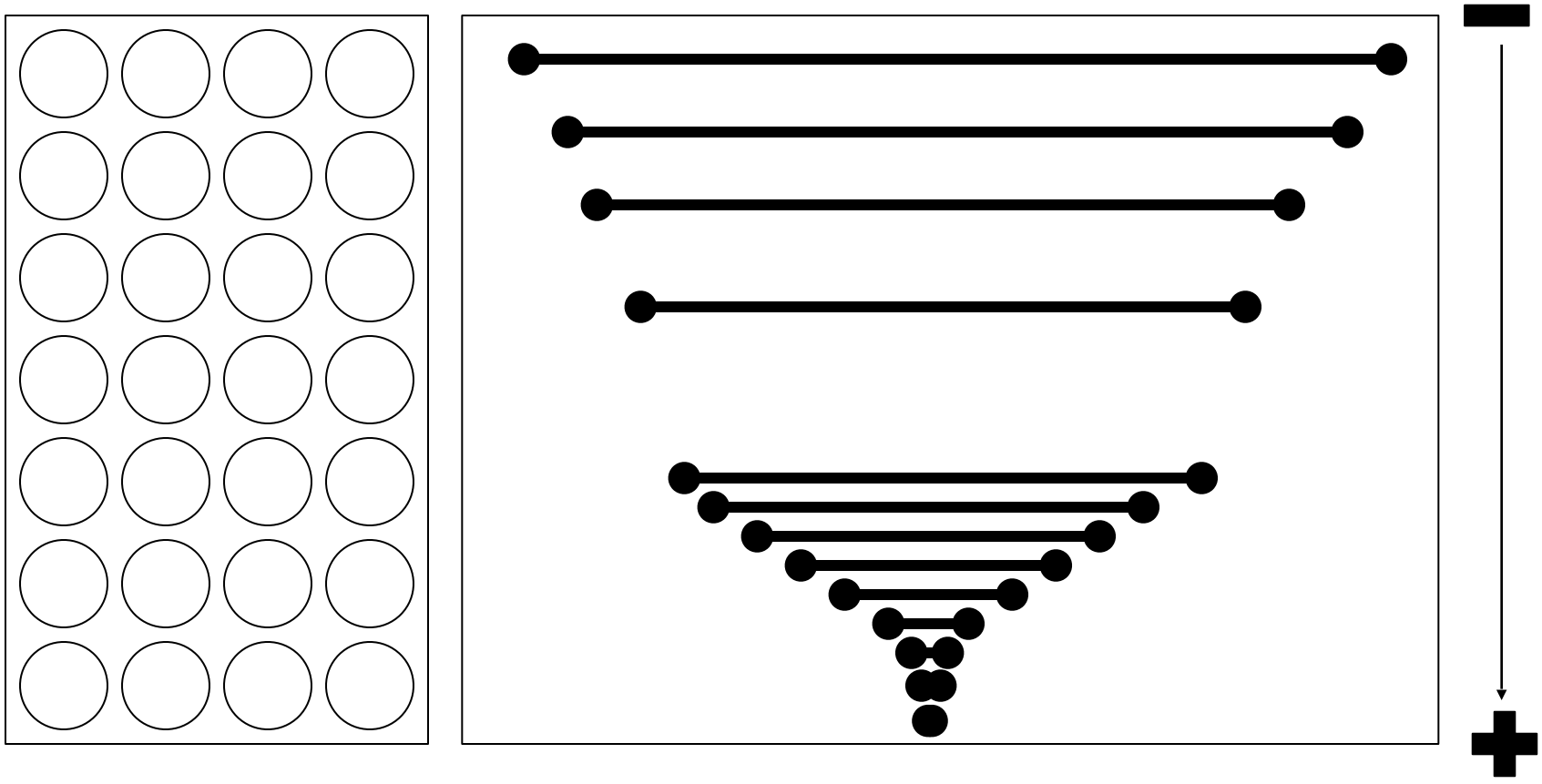
Agarose concentration

As we increase the concentration of agarose (g/ml) the pores gets smaller!



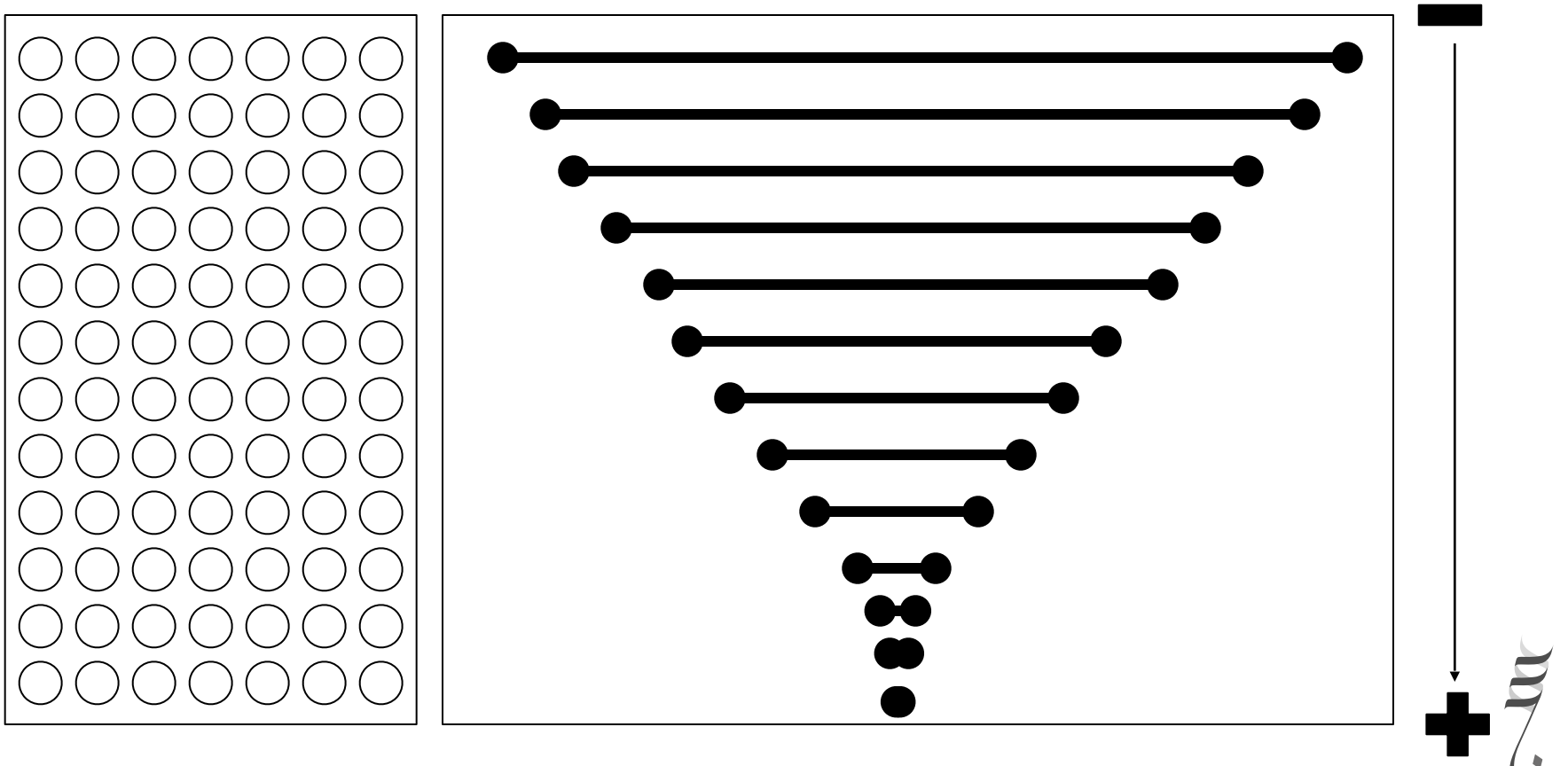
Agarose concentration

Low concentration gel separates large DNA molecules more clearly.
Why?



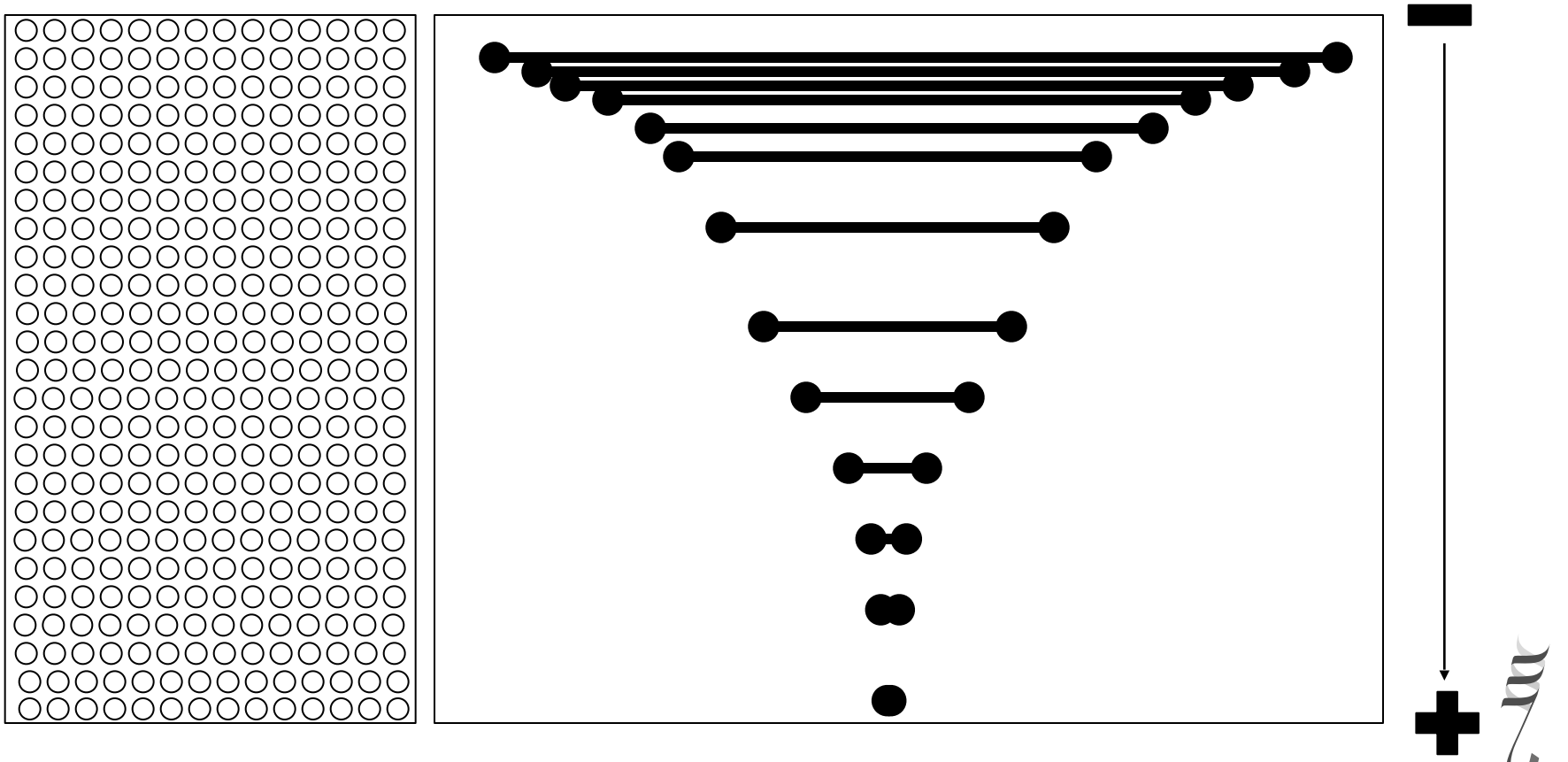
Agarose concentration

Intermediate concentration gel separates DNA molecules almost uniformly.
Why?



Agarose concentration

High concentration gel separates small DNA molecules more clearly.
Why?



Electrophoresis

• **Electrophoresis**: the migration of charged particles through a gelatinous medium under the influence of an electric field.

• **Separating macromolecules (nucleic acids or amino acids) based on:**

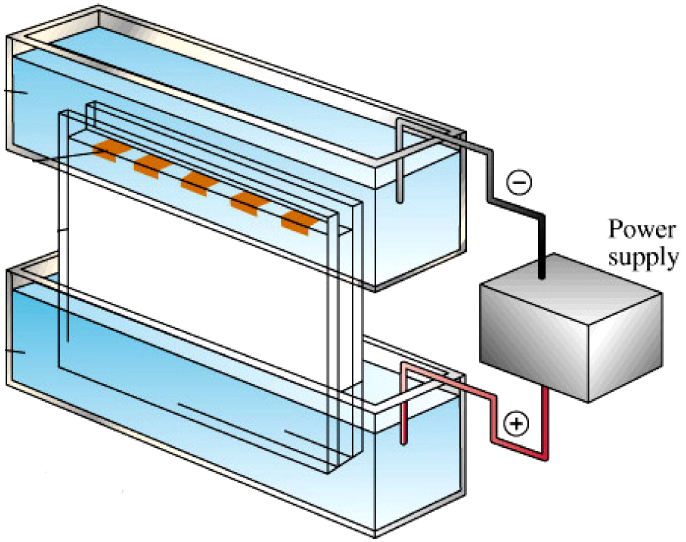
- Size
- Net electric charge
- Physical properties

Electrophoresis

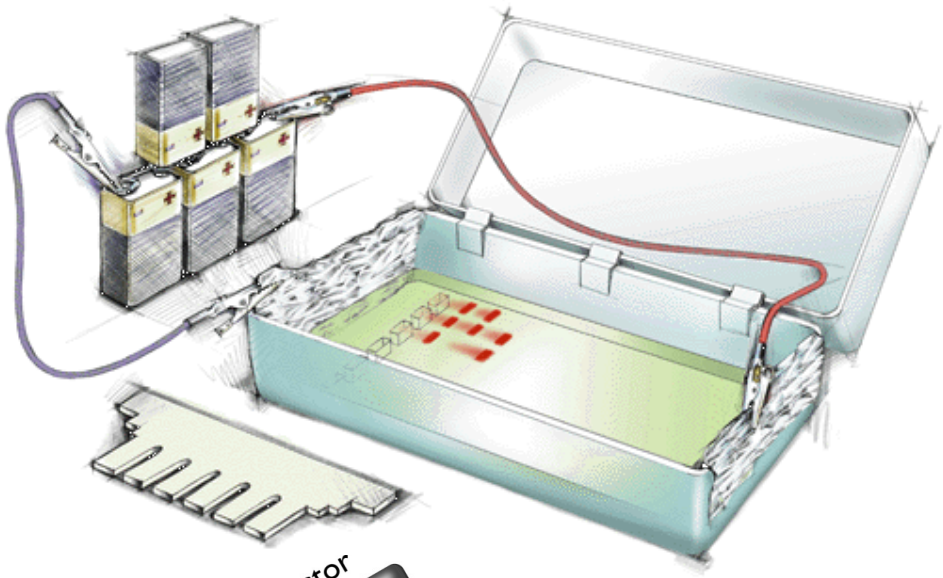
- **The migration rate of molecules through an electric field depends on:**
  - The strength of the electric field
  - The density (percentage) of the gelatinous matrix (gel)
  - The size and shape of the molecule (thus net charge)
  - The ionic strength and temperature of the buffer

Electrophoresis apparatus

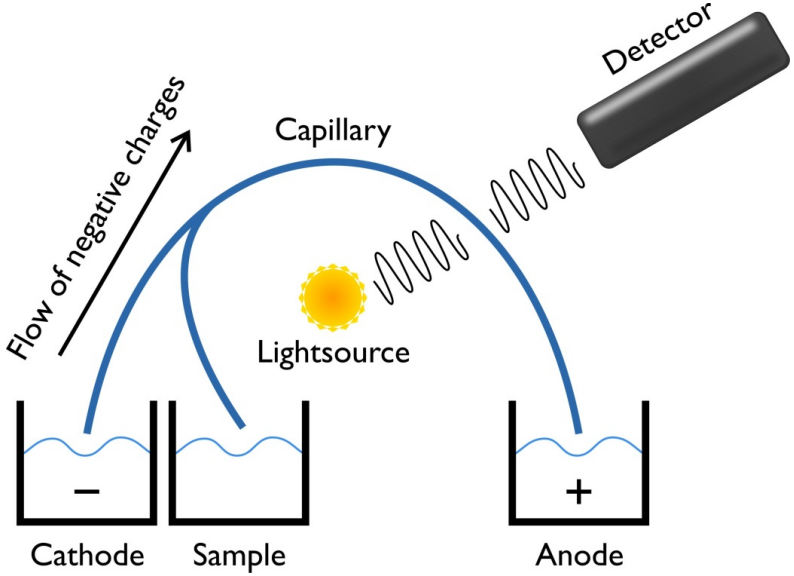
Vertical apparatus



Horizontal apparatus



Capillary electrophoresis



DNA (band) size estimation

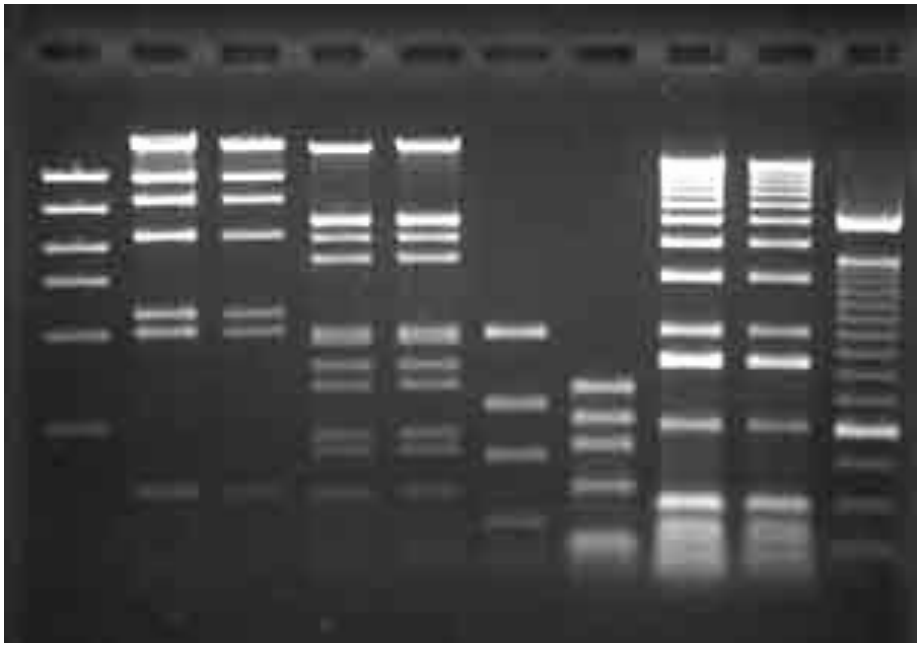
How do we determine the size of DNA molecule?

- Use the relative mobility (R_f) of the DNA molecule in the gel (Distance travel vs. size)

- Compare your DNA sample to a standard of known sizes (DNA ladder).

DNA (band) size estimation

- R_f = distance of the DNA band/ distance of the tracking dye

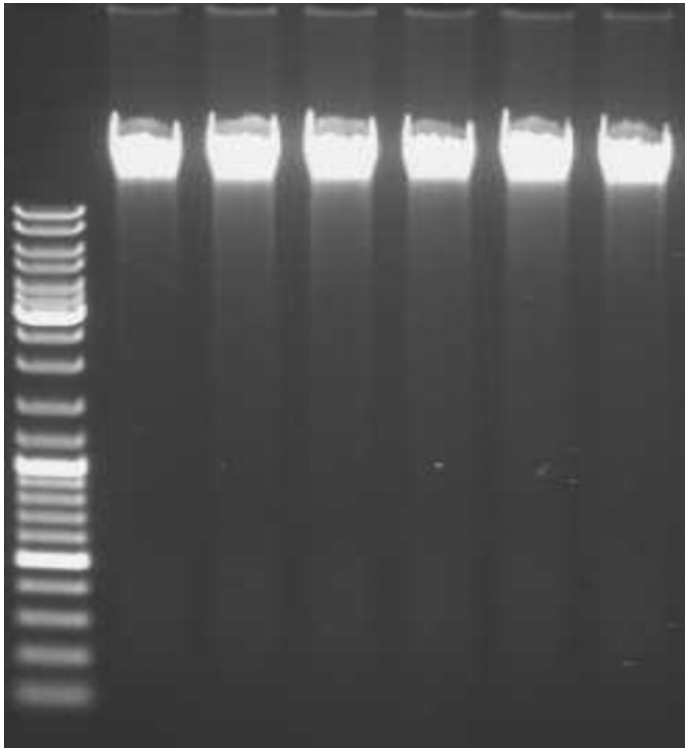


Qualitative Analysis

- Analyzing DNA quality using gel electrophoresis.

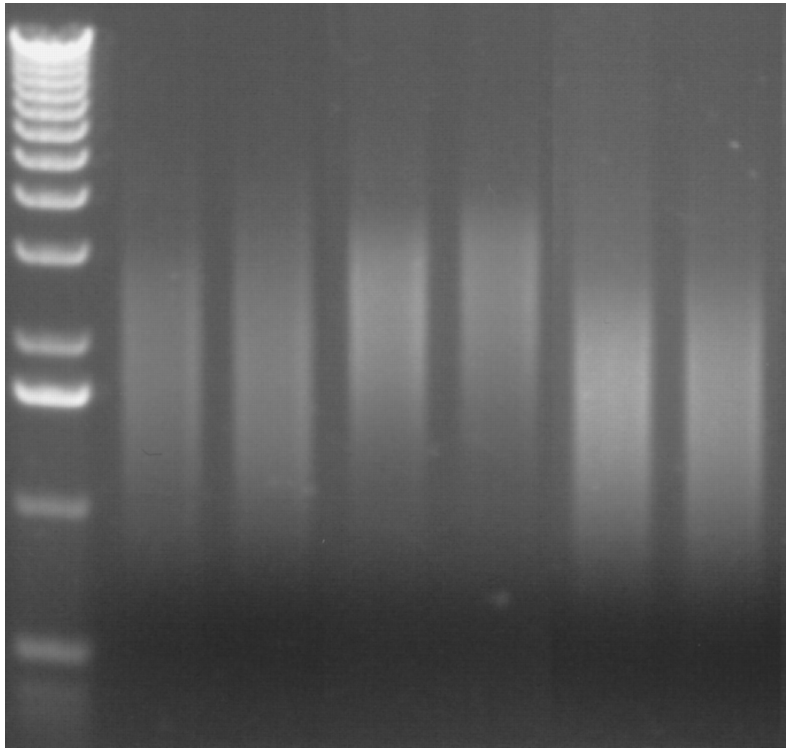
- Up in the gel (large in size)
- Down in the gel (small in size)

Genomic DNA

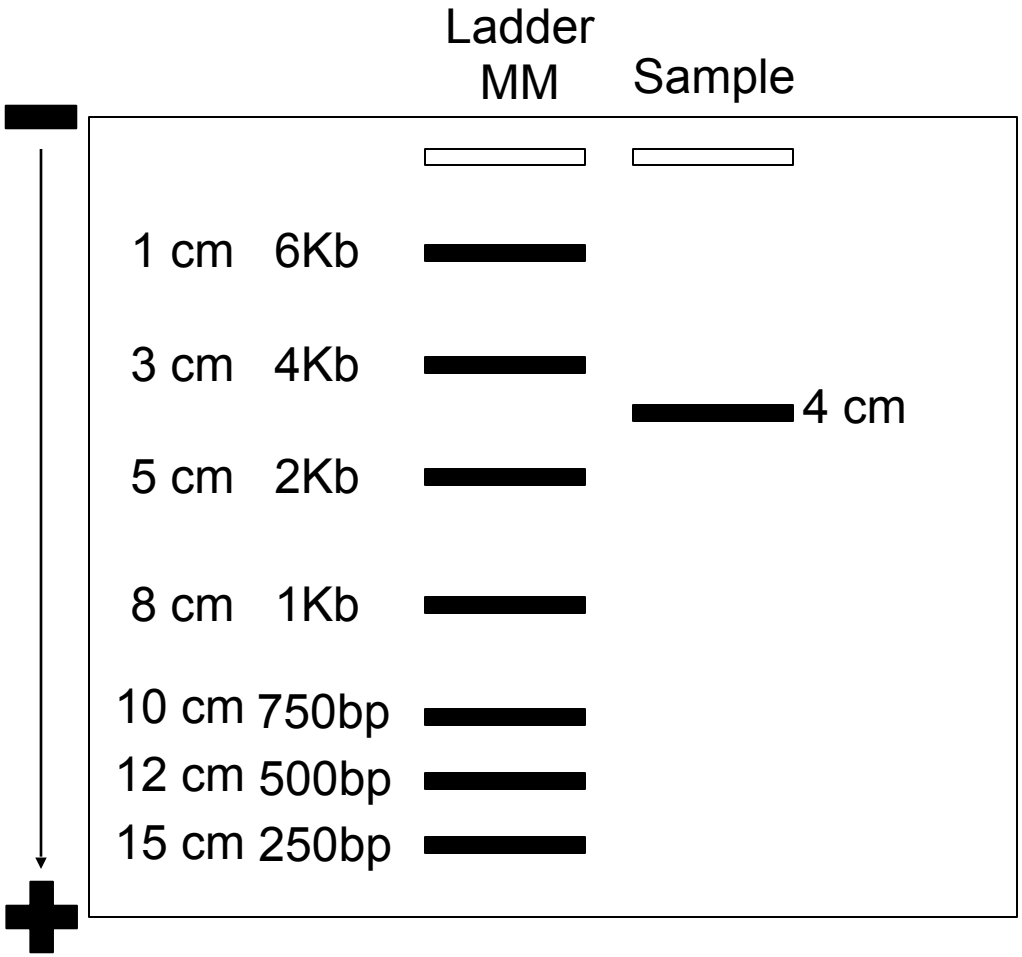


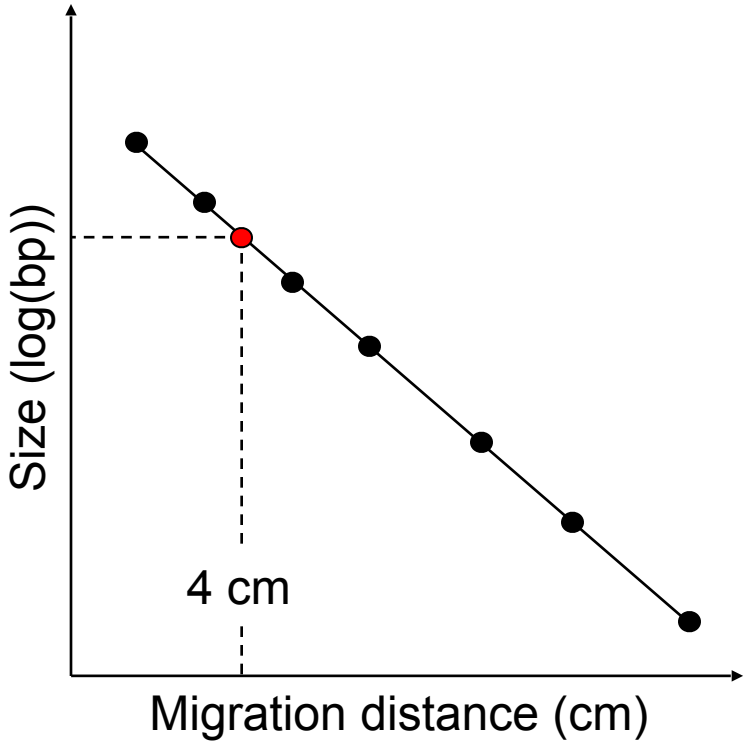
- Intense color (high quantity)
- Down in the gel (low quantity)

Degraded low quality DNA



DNA (band) size estimation



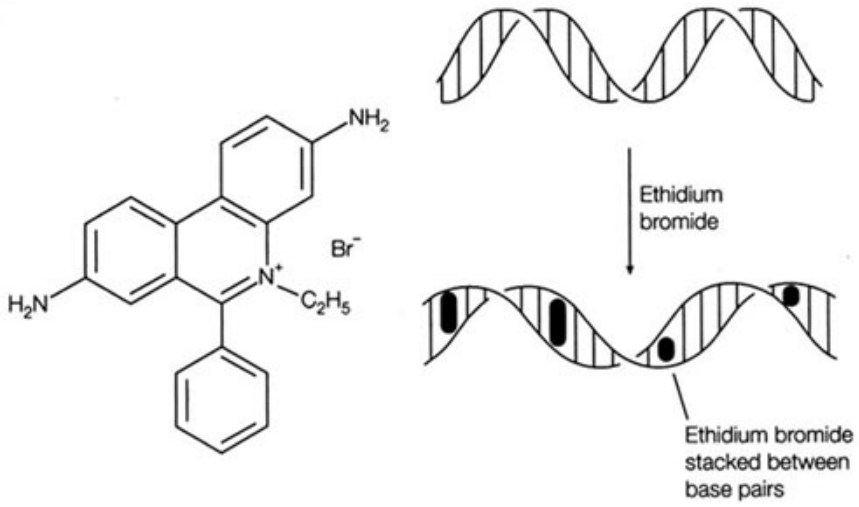


How to visualize?

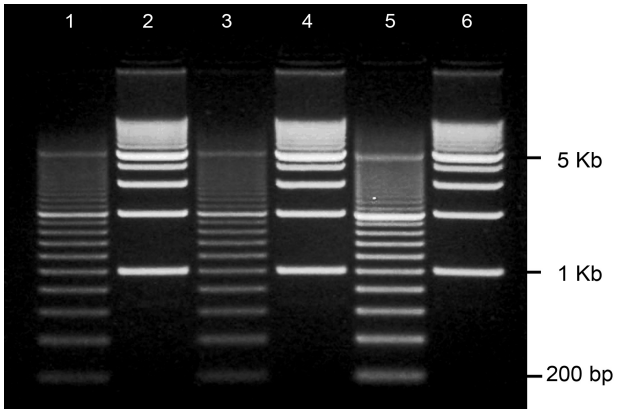
- Staining of DNA on gel:
    - Ethidium bromide.
    - Silver staining.
- Fluorescent dyes for capillary electrophoreses:
    - Labeling PCR products with:
        - FAM
        - NED
        - VIC
        - etc.

Ethidium Bromide

- EtBr gets intercalated between the bases.
- It is a mutagen and a carcinogen (careful!!!).
- You can see the DNA bands in a gel if you stain the gel after or before running your sample.
- Detection is achieved when using UV light.

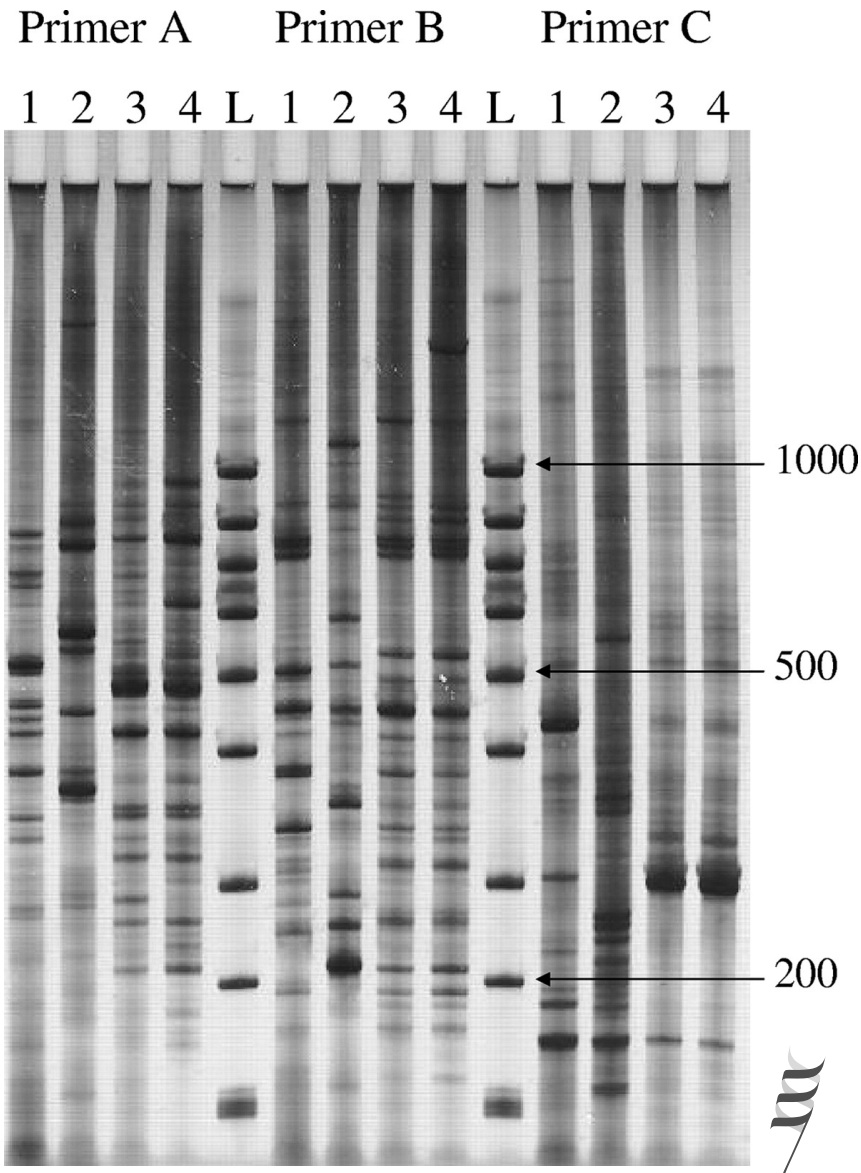


Ethidium Bromide



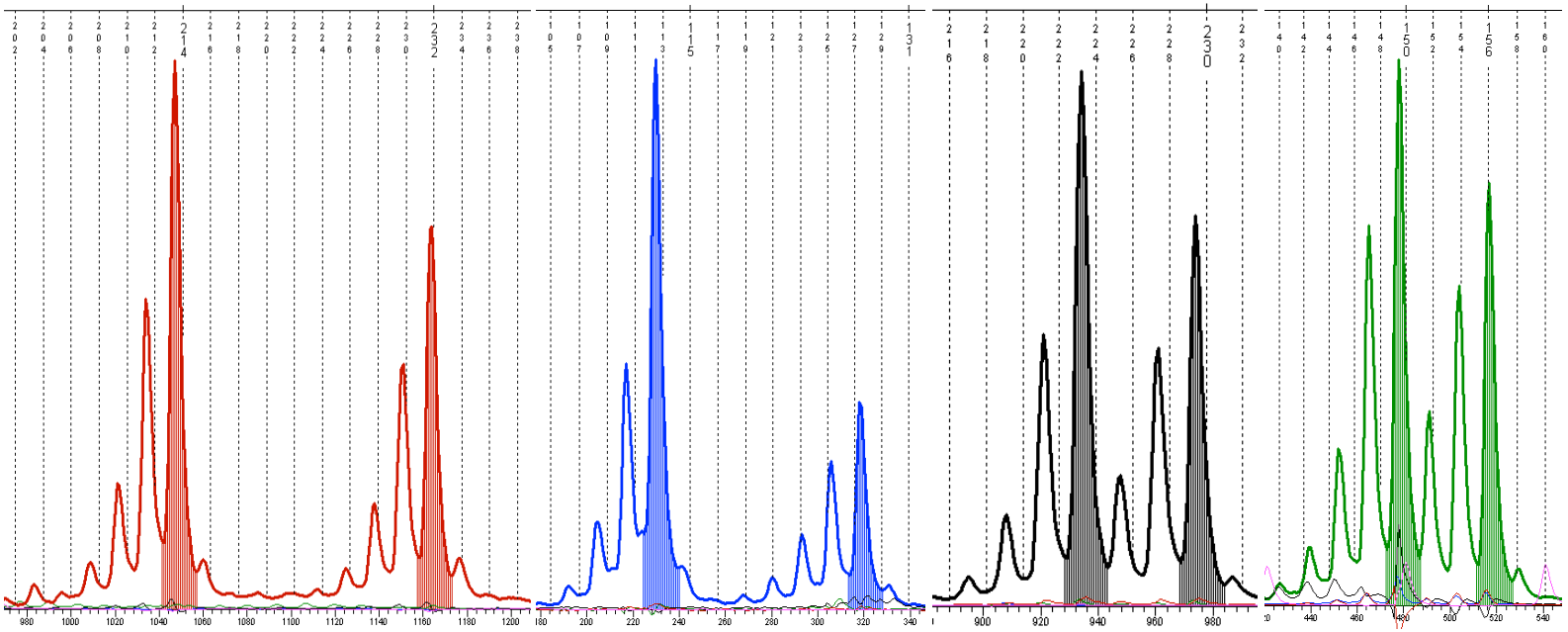
Silver staining

- Done mostly on polyacrylamide gels.
- Higher sensitivity than ethidium bromide but takes longer steps.
- **Silver staining stain DNA, RNA, and proteins.**
- **This makes its specificity low!**



Fluorescent dyes for capillary

- Attach a fluorescent dye to your primer.
- Your amplified DNA fragment will have a fluorescent dye that can be detected by the capillary machine.
- Can detect small differences in size.



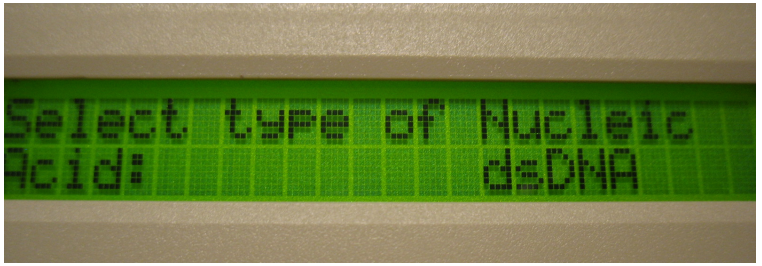
2. Quantitative Analysis

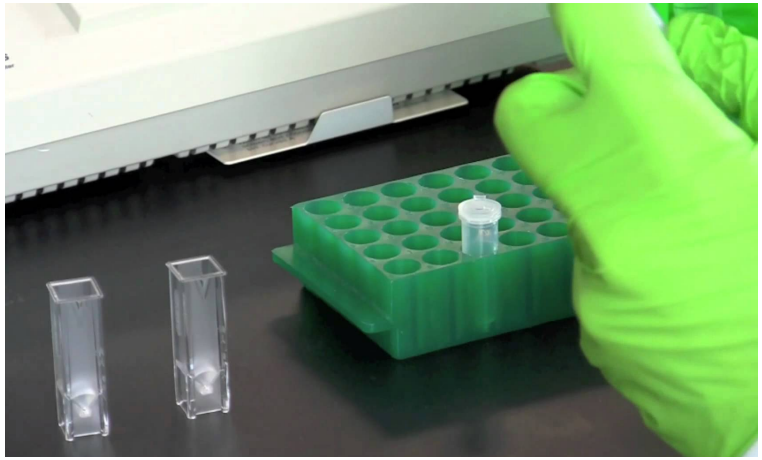
Spectrophotometry

- Quantifying DNA using spectrophotometer measures the absorbance of light by your sample.
- At a specific wavelength (260nm – 280nm).
- The spectrophotometer is designed to give you the quantity of DNA and can shed light on the purity of your sample.
- Simple procedure and does not take a lot of time.
- Spectrophotometers have different detection limits.

2. Quantitative Analysis

Spectrophotometry

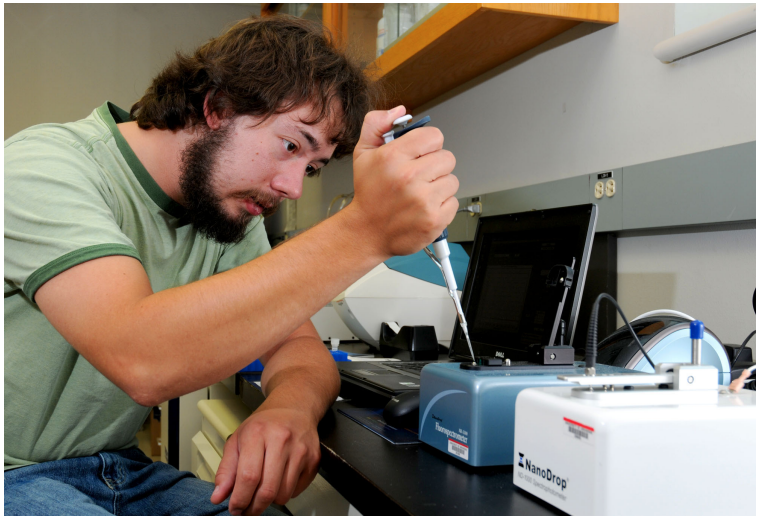


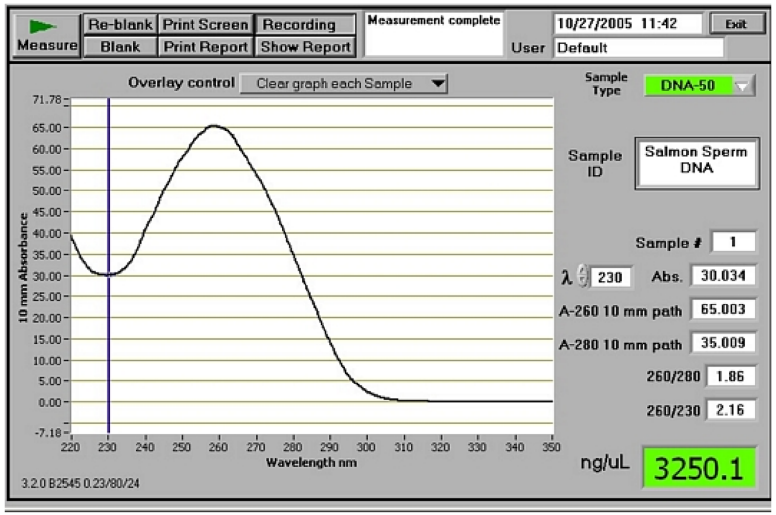


Quantitative Analysis

- The **Nanodrop** is a spectrophotometer that uses the DNA absorbance of light as a measure of its quantity.

- Requires small amount of sample (1-2 ul).
- Detect quantities in the nanogram level.
- Fast and easy to perform.





To study

Mutagen

VIC

FAM

agarose

DNA charge

Relative mobility

Polyacrylamide

Carcinogen

Capillary electrophoresis

UV light

Relative mobility

Spectrophotometer

Silver staining

Ethidium bromide

NED

Gel electrophoresis

Fluorescent dyes

Nanodrop

Expectations

- You know why DNA moves in an electric field.
- You know why to use a gel matrix for DNA separation.
- You know why you need to stain DNA on a gel and how.
- You know capillary electrophoresis and why you use fluorescent labeled primers.

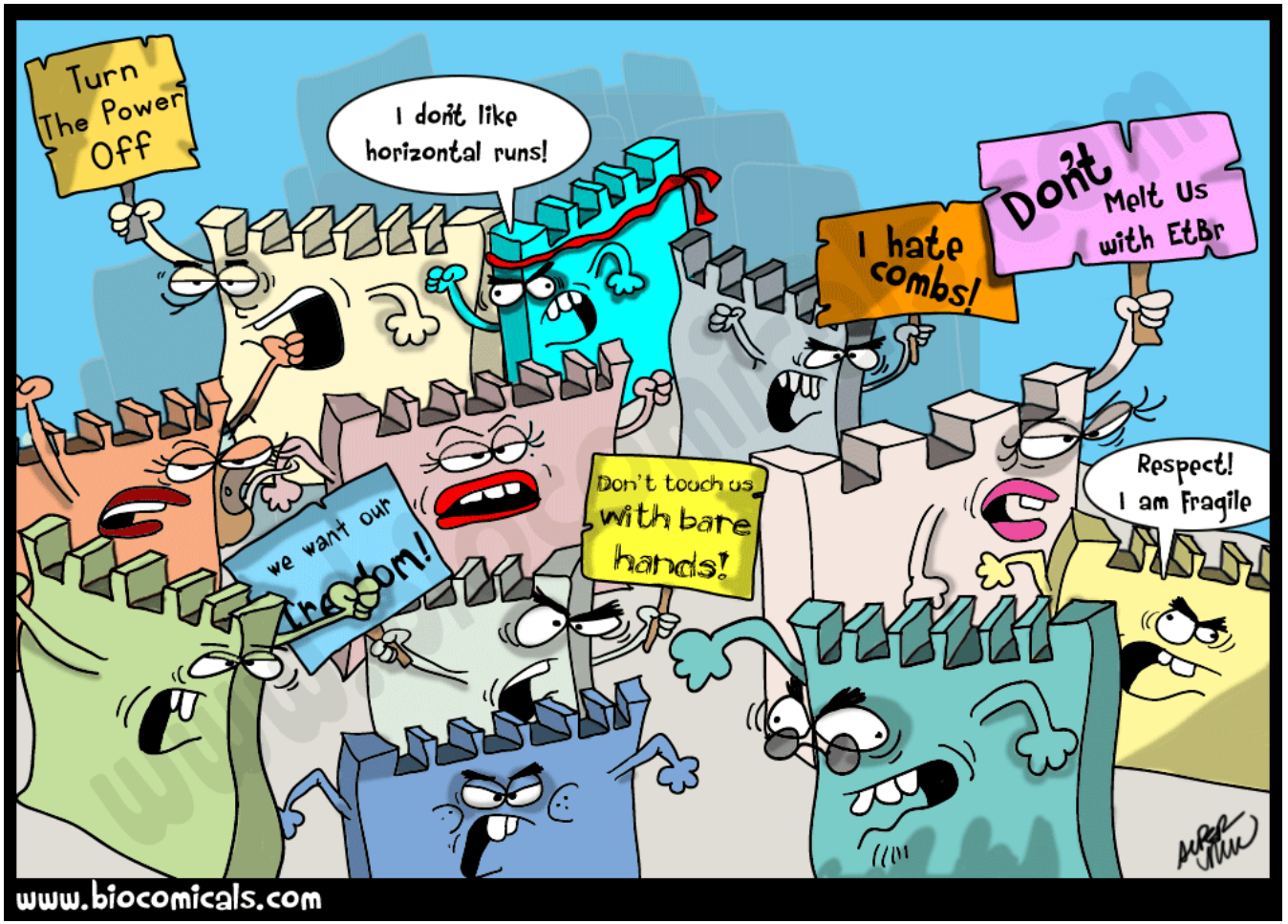
Expectations

- You know the qualitative methods you can run to check the size and quality of your DNA sample.

- You know some of the quantitative methods you can use to assess the yield and purity of your DNA sample.

- You know when to use a specific method of DNA quantification and why.

For a smile


Turn The Power Off
I don't like horizontal runs!
I hate combs!
Don't Melt Us with EtBr
we want our freedom!
Don't touch us with bare hands!
Respect! I am Fragile
www.biocomicals.com