ATBC – Biomoleculen (BMBIO-T)

Moleculair and Biochemical Techniques

Lesson 1: Electrophoresis

HAN_UNIVERSITY OF APPLIED SCIENCES

Programm Moleculair and Biochemical Techniques

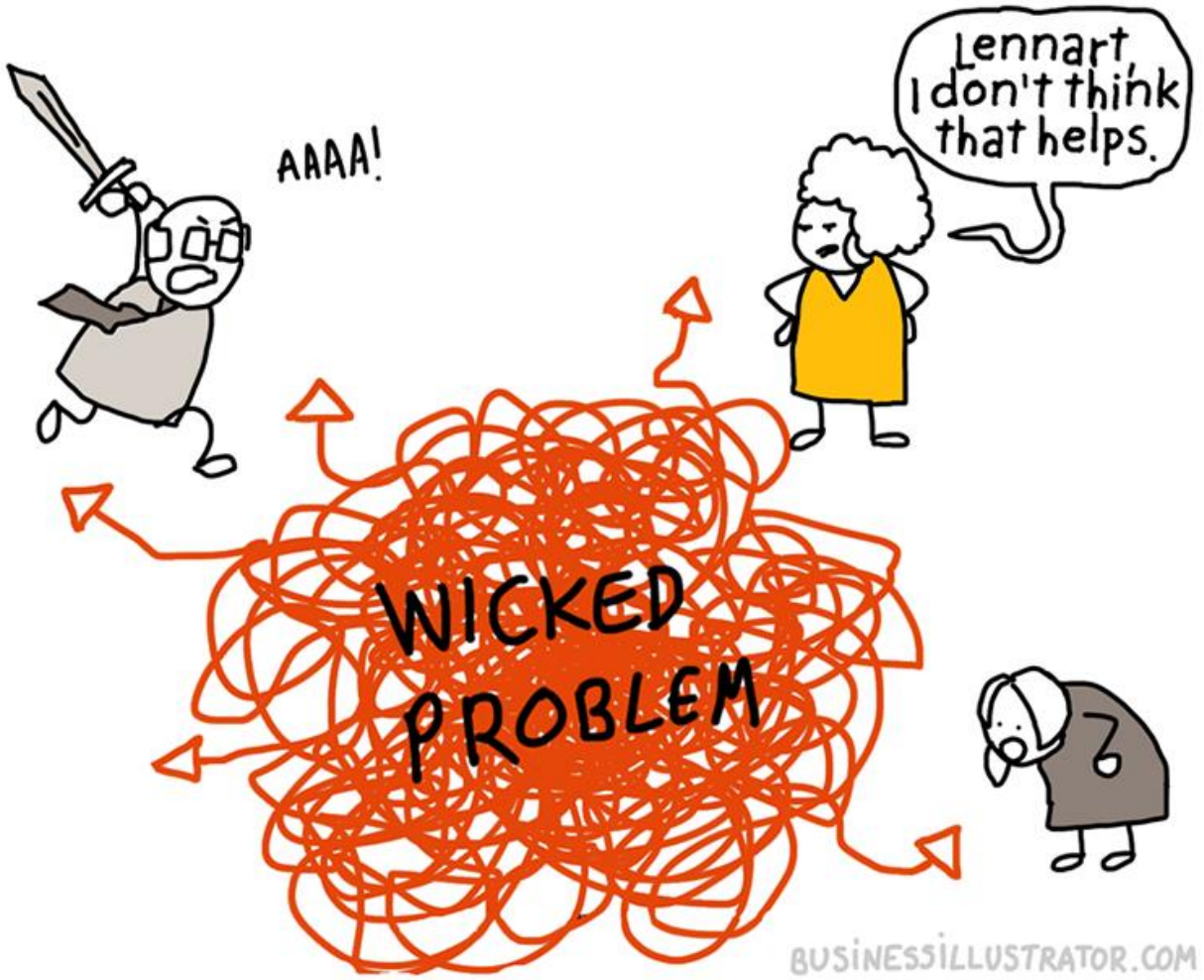
Lesson 1: Electrophoresis

Lesson 2: Centrifugation

Lesson 3: Fluorescence

→ Where and when will we use these techniques in the lab?

The benefit of knowledge in troubleshooting



Electrophoresis: what do you remember?

Lesson 1: Electrophoresis

Lesson 2: Centrifugation

Lesson 3: Fluorescence

In this lesson

Introduction Moleculair and Biochemical Techniques

Electrophoresis types

Gel electrophoresis:

- Matrix
- Electric field
- Buffer
- Charged biomolecules

Further analysis: blotting techniques

Learning goals lesson 1: Electrophoresis

After this lesson students:

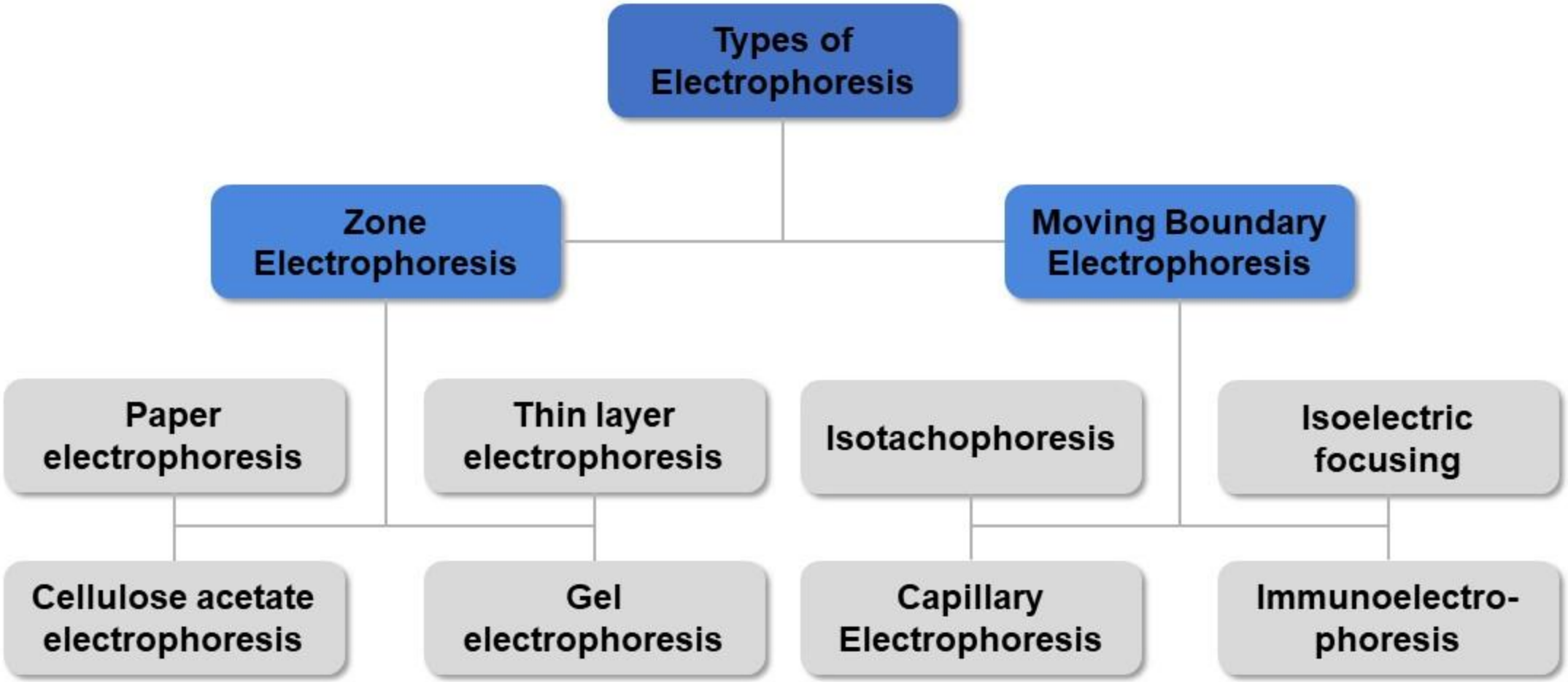
- Know the different types of electrophoresis: zone versus moving boundary and can name examples of both types (eg paper electrophoresis, gel electrophoresis, capillary electrophoresis and iso electric focusing).

- Know which components are needed for gel electrophoresis (matrix, electric field, buffer and charged molecules) and are able to explain which factors are important when setting up the experiment.

- Are able to argument how an experiment can be optimized, based on given formula's.

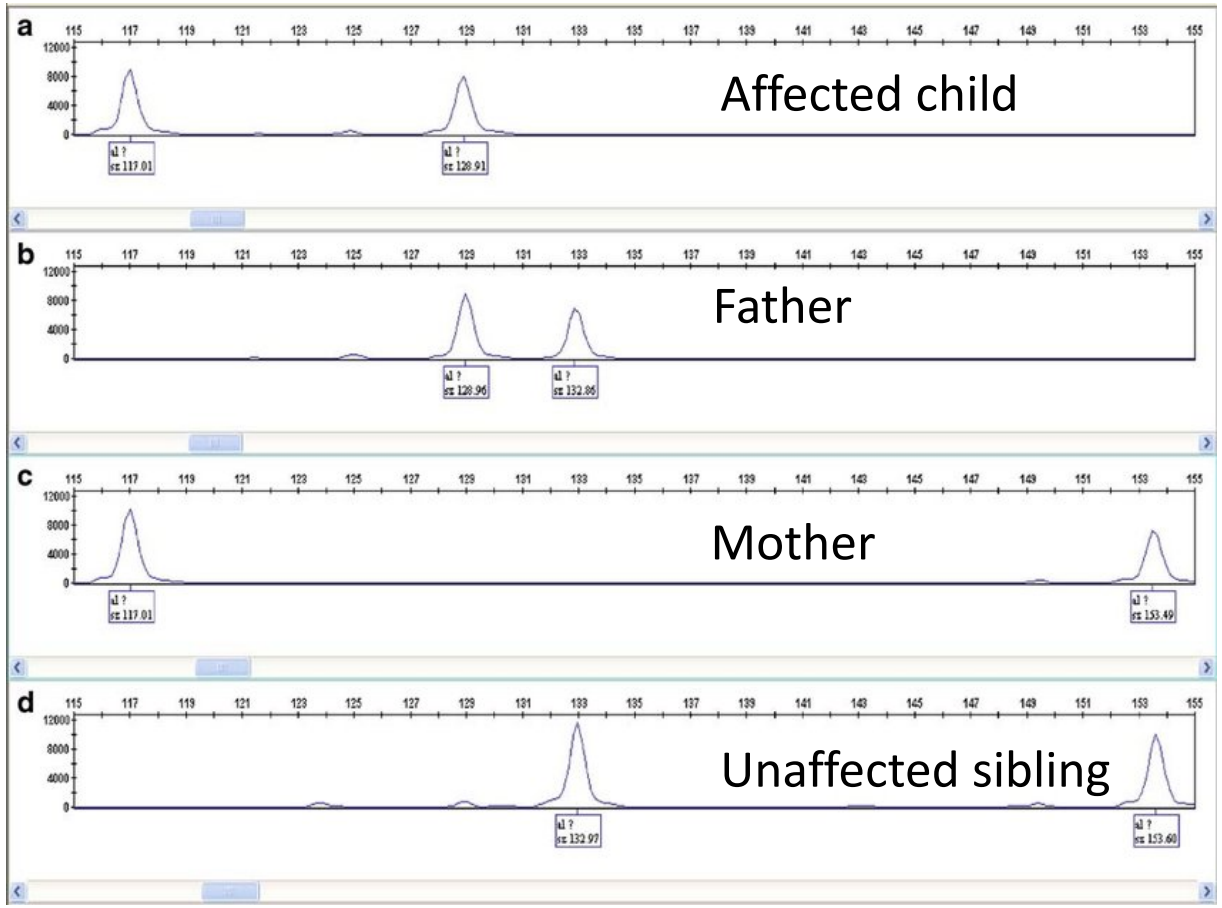
- Are aware of the possible follow up experiment after gel electrophoresis: Southern blot for DNA, Nothern blot for RNA and Western blot for Proteins

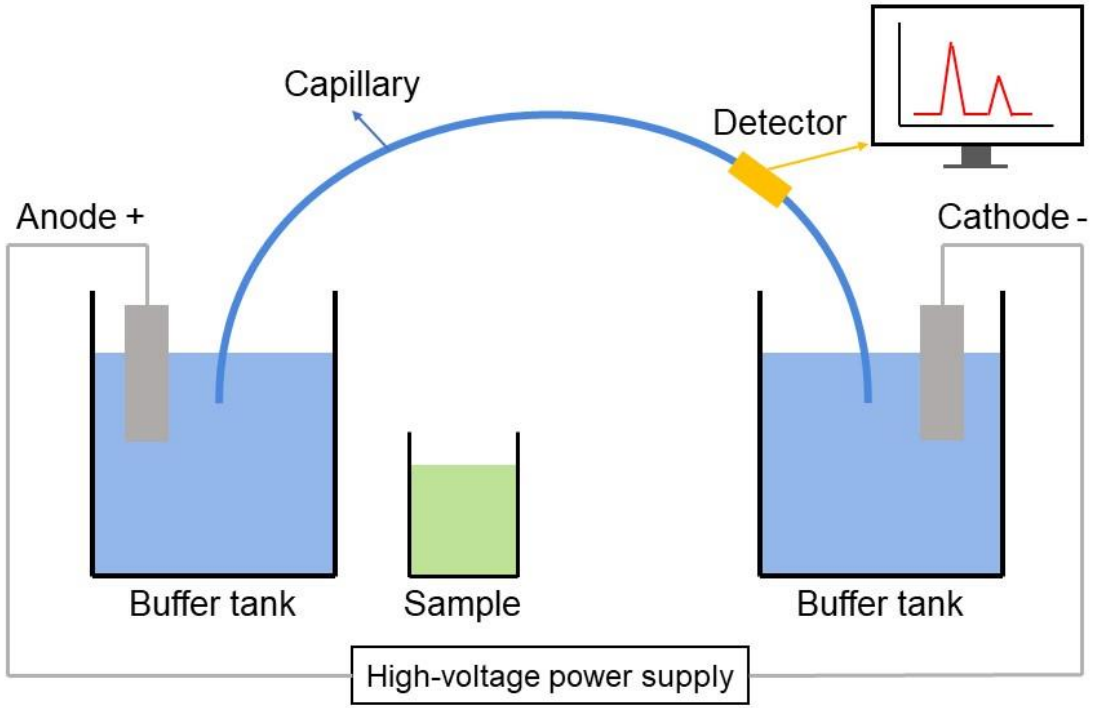
There is more than gel electrophoresis only



STEMART. (n.d.). *Electrophoresis Technology*. Retrieved January 17, 2025, from https://www.ste-mart.com/electrophoresis-technology.htm

Moving boundery electrophoresis – example Capillary electrophoresis

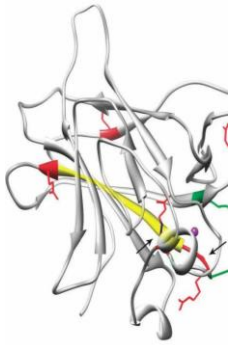


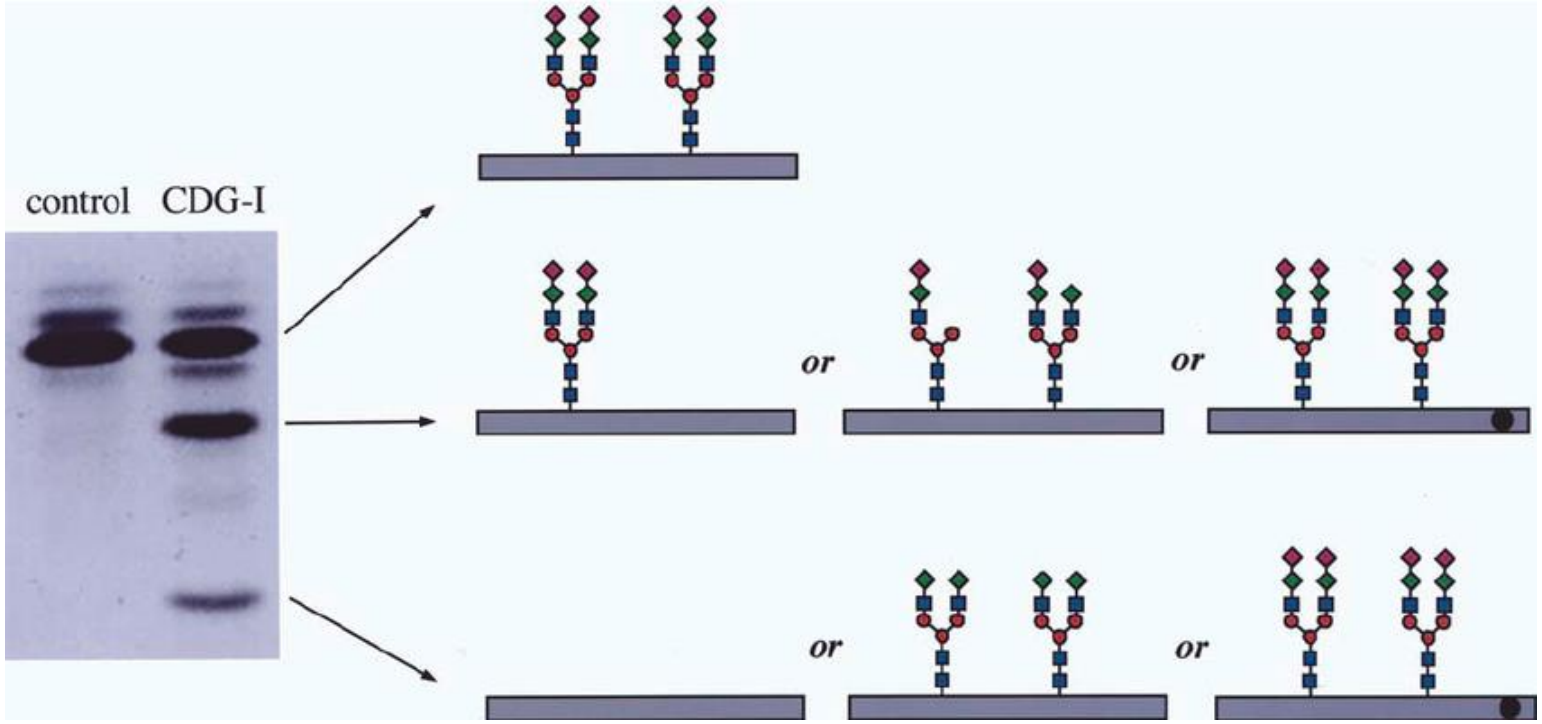


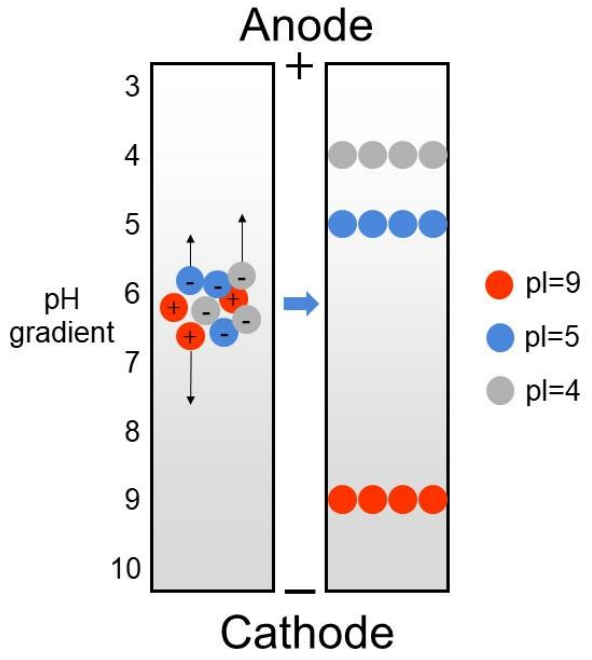
STEMART. (n.d.). *Electrophoresis Technology*. Retrieved January 17, 2025, from https://www.ste-mart.com/electrophoresis-technology.htm

Bick, S.L., Bick, D.P., Wells, B.E. *et al.* Preimplantation HLA haplotyping using tri-, tetra-, and pentanucleotide short tandem repeats for HLA matching. *J Assist Reprod Genet* **25**, 323–331 (2008). https://doi.org/10.1007/s10815-008-9233-2

Moving boundery electrophoresis – example Iso Electric Focussing



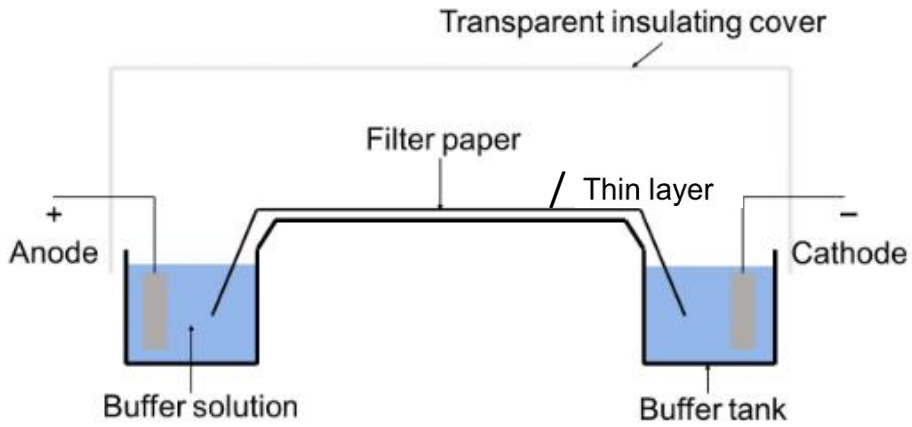




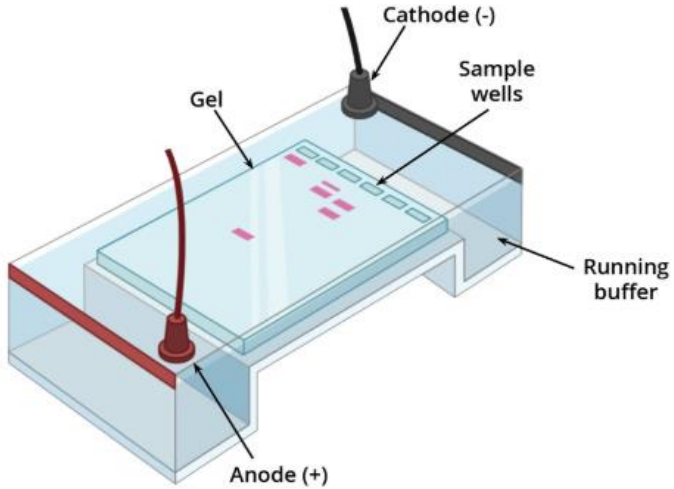
STEMART. (n.d.). *Electrophoresis Technology*. Retrieved January 17, 2025, from https://www.ste-mart.com/electrophoresis-technology.htm

Marquardt, T., Denecke, J. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. *Eur J Pediatr* **162**, 359–379 (2003). https://doi.org/10.1007/s00431-002-1136-0

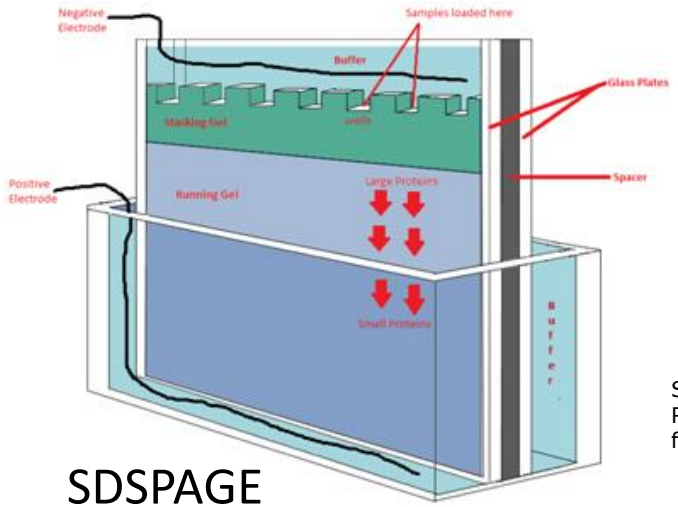
Zone electrophoresis – examples



Paper electrophoresis
Thin layer electrophoresis



Agarosegel electrophoresis

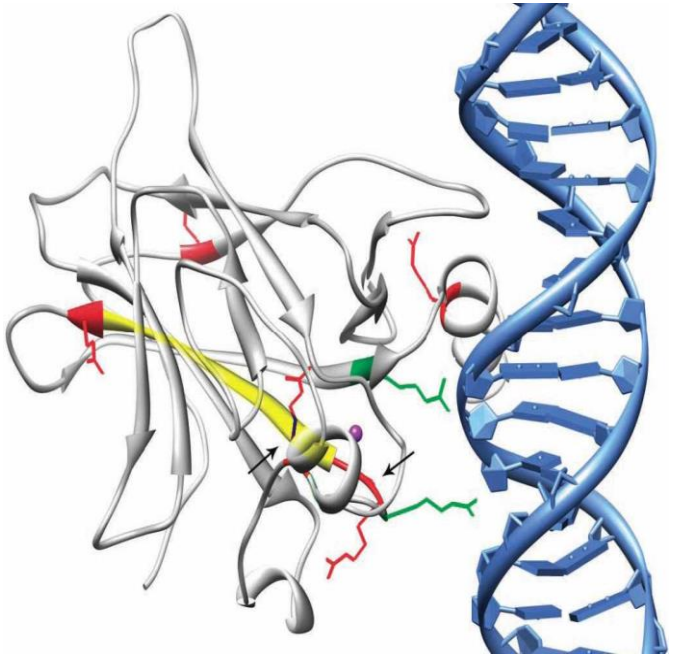


SDSPAGE

STEMART. (n.d.). *Electrophoresis Technology*. Retrieved January 17, 2025, from https://www.ste-mart.com/electrophoresis-technology.htm

Skendrick Laboratories. (n.d.). *How does 1D gel electrophoresis work?* Retrieved January 17, 2025, from https://kendricklabs.com/1d-method/

Electrophoresis: separating molecules based on a property under the influence of an electric field



Necessary for (gel) electrophoresis:

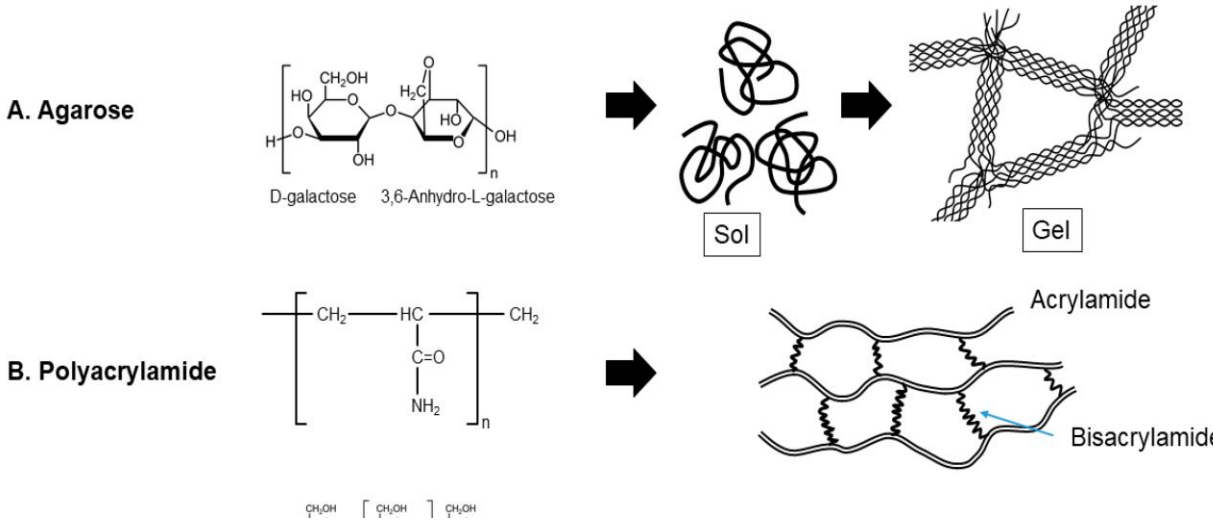
- ✓ Matrix: polyacrylamide (SDSpage) or agarose
- ✓ Electric field
- ✓ Buffer, electrolyte
- ✓ Charged biomolecules

Charged molecules depends on:

- ➢ pH value
- ➢ Size
- ➢ Effective charge – other bound ions can shield the charge of the biomolecule!

Gel electrophoresis: what **matrix** do you use?

Agarose	Polyacrylamide
Poly saccharide extracted from sea weed	Cross-linked polymer of acrylamide
Gel casted horizontally	Gel casted vertically
Non-toxic	Potent neuro toxic
Separate large molecules	Separate small molecules
Commonly used for DNA separations	Commonly used for DNA or protein separations
Staining before or pouring the gel	Staining after pouring the gel



Arakawa, T., Nakagawa, M., Sakuma, C., Tomioka, Y., Kurosawa, Y., & Akuta, T. (2024). Polysaccharide as a Separation Medium for Gel Electrophoresis. *Polysaccharides*, *5*(3), 380-398. https://doi.org/10.3390/polysaccharides5030024

This course we will use agarose gel for DNA separation and Polyacrylamide (SDSPAGE) for protein separation

HAN_UNIVERSITY OF APPLIED SCIENCES

Agarose Gel electrophoresis: separate DNA fragments by size

Matrix is a network of polymers with pores
→ Size of the pores depends on the concentration of the used gel

Percent Agarose Gel (w/v)	DNA Size Resolution(kb = 1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

Table 1: Correct Agarose Gel Concentration for Resolving DNA Fragments

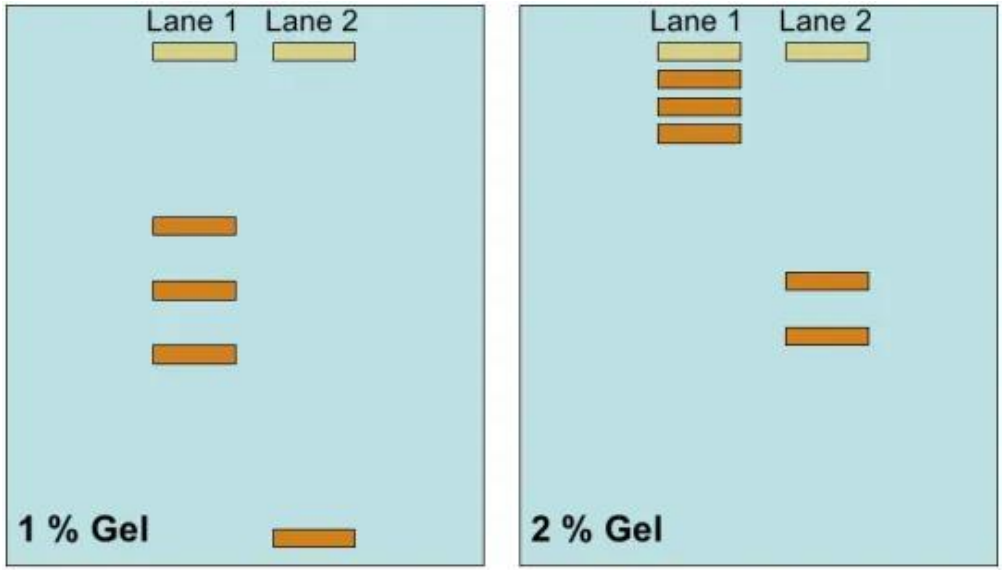
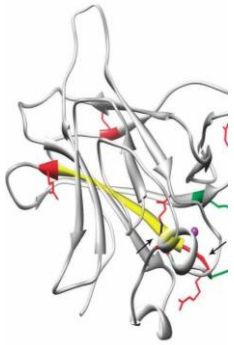


Figure 1: Comparing Resolution of 1 % Gel and 2 % Agarose Gel.

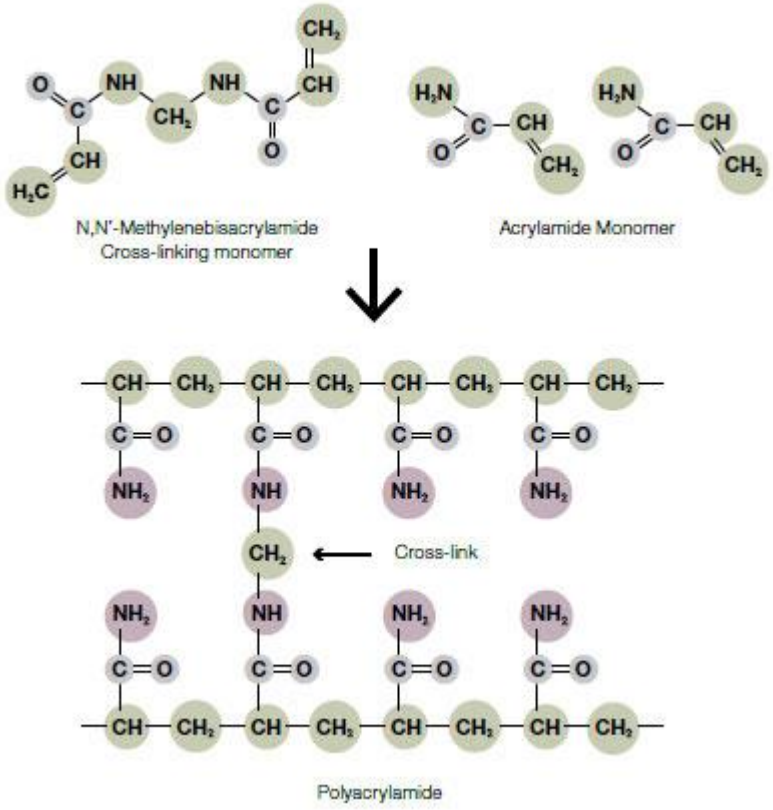
Lane 1 shows the separation of nicked, supercoiled and linear DNA of a 6 kb fragment. Lane 2 shows separation of 425 bp and 450 bp PCR fragments. The 1 % gel could not resolve the two PCR fragments. They appear as a single band. Gel purification would not eliminate either band. The PCR fragment would a produce a mixed sequencing result.

agctsequencing. (February 14,2012). *DNA sequencing and Fragment analysis*. Retrieved January 17, 2025, from https://agctsequencing.wordpress.com/2012/02/14/dna-fragments-resolve-better-on-correct-percent-agarose-gel/

Polyacrylamide Gel Electrophoresis (PAGE): separate proteins by size



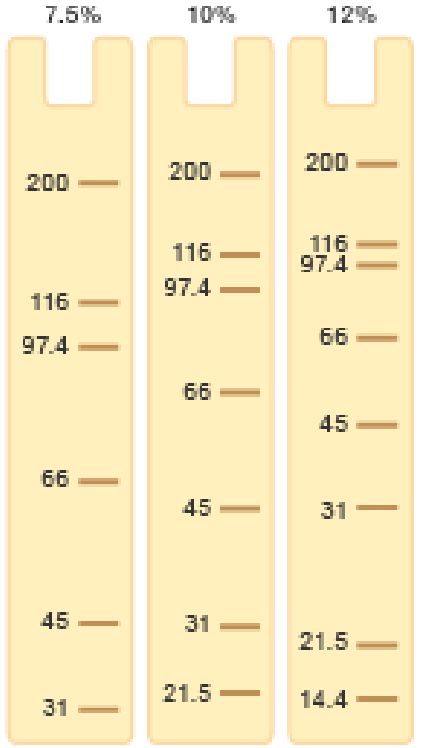
Matrix is a network of polymers with pores
→ Size of the pores depends on the concentration of the used gel



$$\%T = \frac{\text{g acrylamide} + \text{g cross-linker}}{\text{Total volume, ml}} \times 100$$

$$\%C = \frac{\text{g cross-linker}}{\text{g acrylamide} + \text{g cross-linker}} \times 100$$

%T = Total monomer concentration
%C = Crosslinker concentration

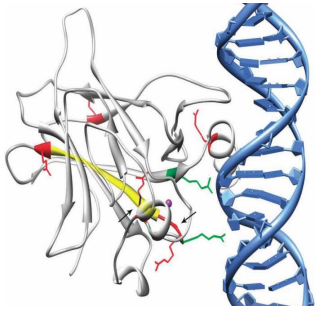


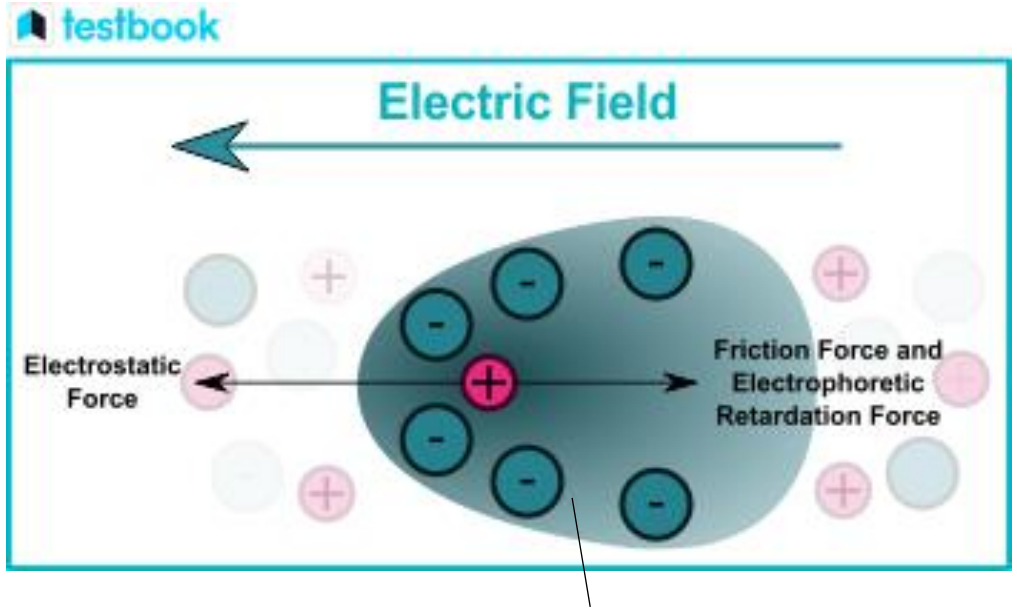
This course: % T = 40%
Acrylamide:Crosslinker = 29:1 → %C = 3.33%

Bio-rad. (n.d.) *Introduction to polyacrylamide gels*. Retrieved January 17, 2025, from https://www.bio-rad.com/en-nl/applications-technologies/introduction-polyacrylamide-gels?ID=LUSPBRM5B

HAN_UNIVERSITY OF APPLIED SCIENCES

Particles in an **electric field** are exposed to an electric force





Anode +

Cathode -

Negatively charged biomolecule

Electrostatic force

$F_e = E \cdot Q$

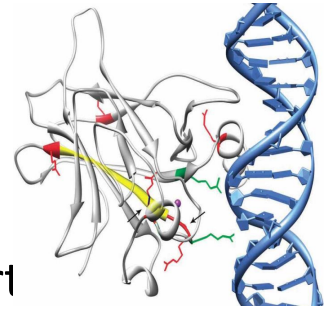
Friction force

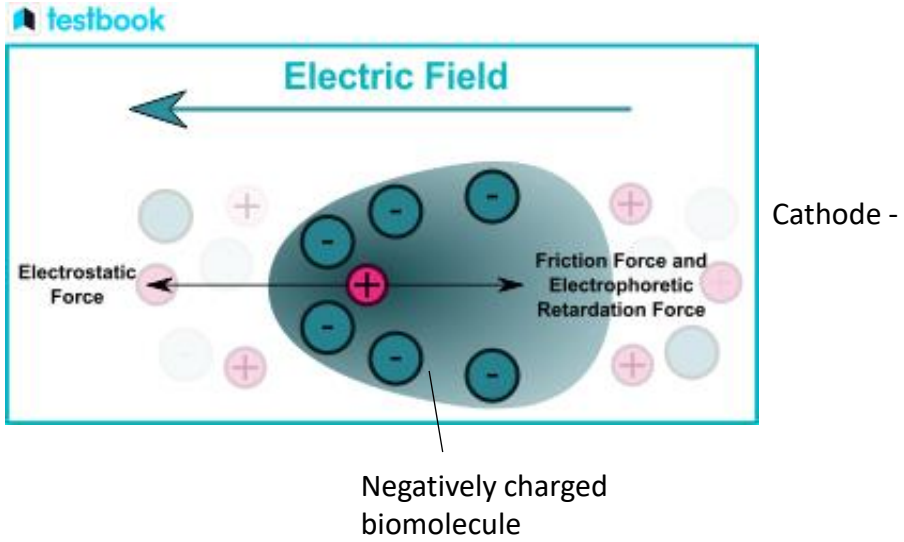
$F_f = f \cdot v$

E = electric field strenght
Q = charge of biomolecule
f = friction coefficient
v = migration velocity

Testbook.com. (2024, April). *Electrophoresis – A comprehensive Guide on DNA analysis*. Retrieved January 17, 2025, from https://testbook.com/chemistry/electrophoresis-technique-used-for-dna-analysis

Particles in an **electric field** are exposed to an electric force





Motion starts with <u>acceleration</u>, after short time there will be an <u>uniformly motion</u>:

$F_e = F_f$

So: $Q \cdot E = f \cdot v$ Or: $v = Q \cdot E / f$

For a spherical partical:

$f = 6 \cdot \pi \cdot \eta \cdot r_p$

So migration velocity:

$v = EQ / 6\pi\eta r$

Mobility:

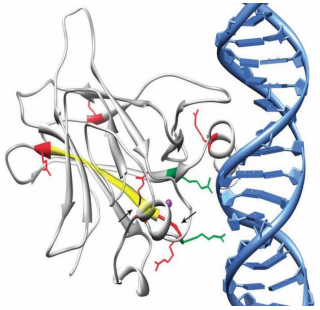
$\mu = v/E \rightarrow = Q/ 6\pi\eta r$

E = electric field strenght
Q = charge of biomolecule
f = friction coefficient
v = migration velocity

η = viscosity solution
r_p = radius particle

Testbook.com. (2024, April). *Electrophoresis – A comprehensive Guide on DNA analysis*. Retrieved January 17, 2025, from https://testbook.com/chemistry/electrophoresis-technique-used-for-dna-analysis

Migration velocity: $v = EQ / 6\pi\eta r$ (m/s)



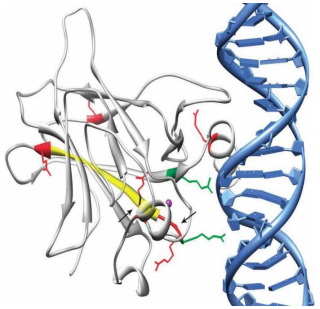
Migration velocity depends on:

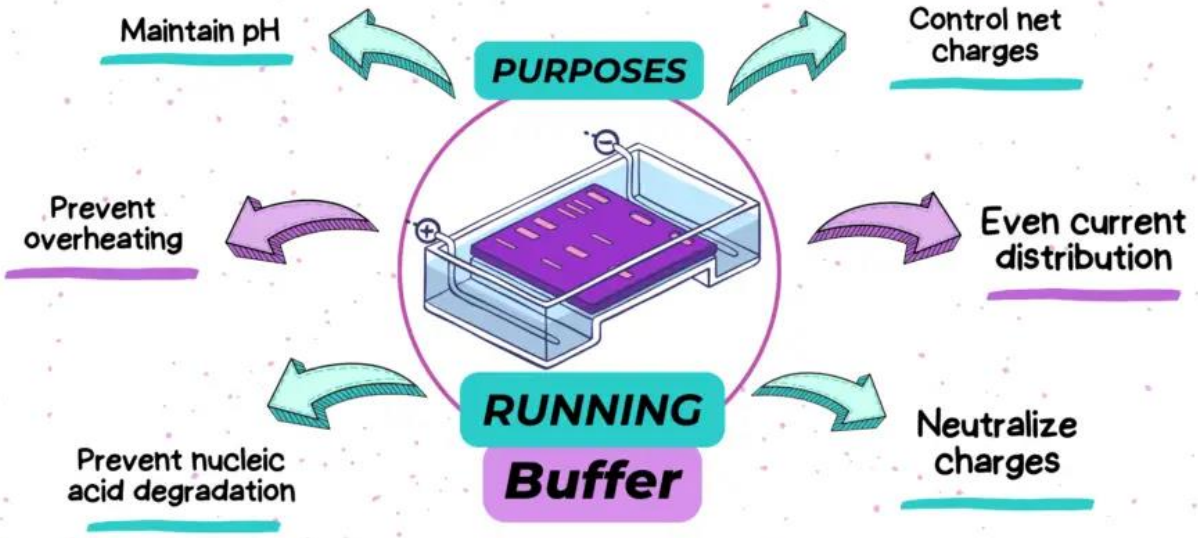
✓ Charge of biomolecule	Q (C)
✓ Electric field strenght	E (V/m)
✓ Installed electric potential difference	U (V)
✓ Distance between electrodes	x (mm)
✓ Viscosity of solution	η
✓ Size of particle	r (mm)
✓ Friction coefficient	f (N.s/m)

How to use this knowledge in troubleshooting?

Mix of 2 DNA fragments sizes 300bp and 320bp show up as 1 band on agarosegel
Which variable can I adjust to make them separate?

What is the function of a **running buffer** in electrophoresis?

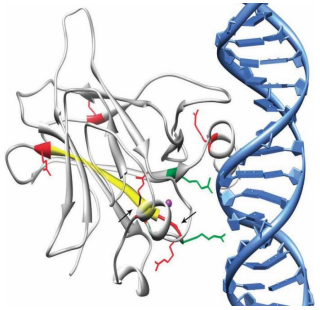




What will happen when water is used to pour or run the gel?
What will happen when a (to) low concentration buffer is used to pour or run the gel?

Geneticeducation.co.in. (2023, November). *7purposes of a running buffer in electrophoresis*. Retrieved January 17, 2025, from https://geneticeducation.co.in/7-purposes-of-a-running-buffer-in-electrophoresis/

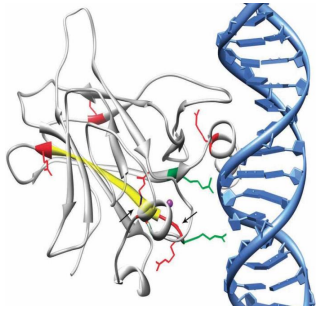
Electrophoresis buffers



Buffer	pH	Properties
Barbitone	Around 8.0	Serum protein separation Poor resolution Weak buffer
Phosphate	Around 7.0	Enzyme separation Low buffering capacity High conductivity
Tris-borateEDTA (TBE)	Around 8.0	Nucleic acid separation Good resolution High buffering capacity Low conductivity
Tris-acetateEDTA (TAE)	**Around 8.0**	**Nucleic acid separation Good resolution High buffering capacity Low conductivity**
Tris-glycine	**>8.0**	**Protein separation High buffering capacity Low conductivity**

This course:
TAE for agarosegel electrophoresis
Tris-glycine for SDSPAGE

The effect of **heat** in electrophoresis



Power → Heat development

Joules first Law: 'the joule effect'

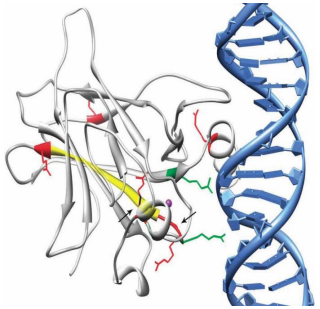
$Q = I^2.R.t$

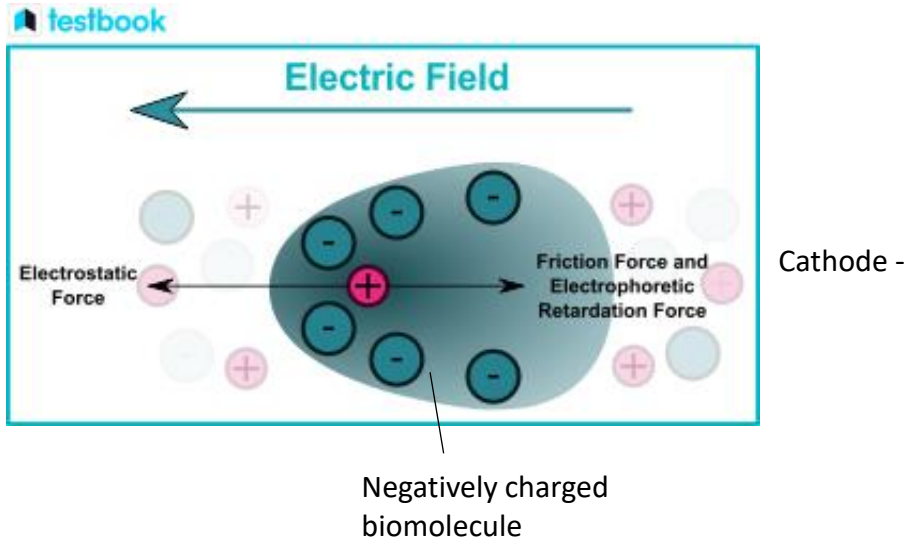
Q = heat expressed (Joule)
I = Electrical current (Ampere)
R = Resistance (Ohms)
t = time (seconds)

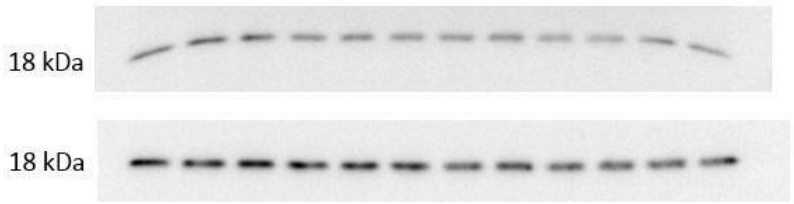
- Solution evaporates → buffer concentration changes, pH and ion concentration changes
- Diffusion of bands
- Viscosity changes → mobility changes
- Proteins denaturates

Increasing voltage to speed up your experiment has its limit!

Electro-endosmosis: smiling and frowning







Matrix may have a negative charge at the surface

⬇

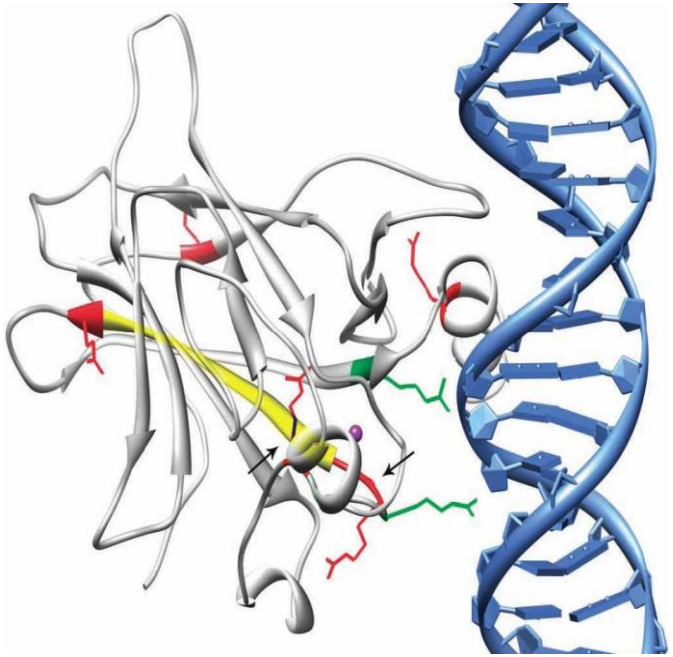
+ charged buffer ions will move to the - pole (counter current)

⬇

Weakly charged biomolecules are hindered, looks like these molecules move to the - pole

Do not add your ladder in the outside lanes

Electrophoresis: separating molecules based on a property under the influence of an electric field



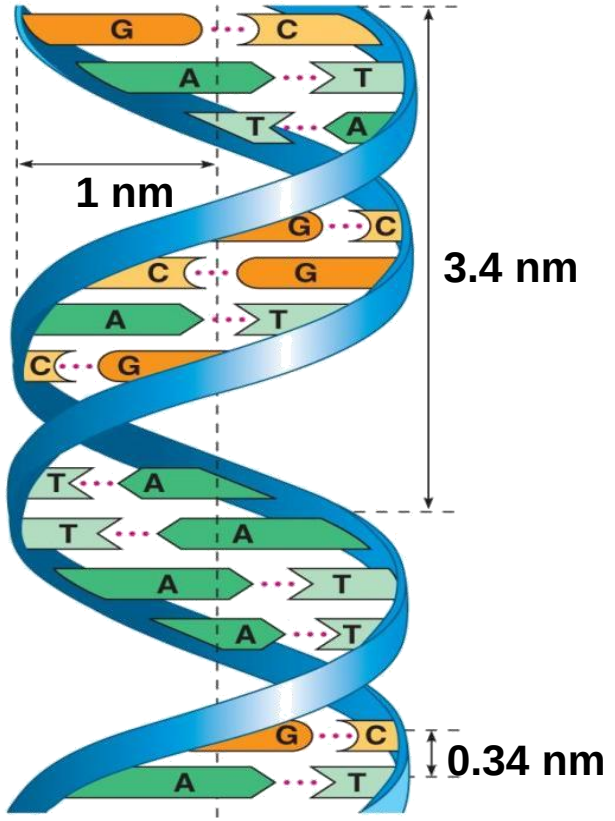
Necessary for (gel) electrophoresis:

- ✓ Matrix: polyacrylamide (SDSpage) or agarose
- ✓ Electric field
- ✓ Buffer, electrolyte
- ✓ **Charged biomolecules**

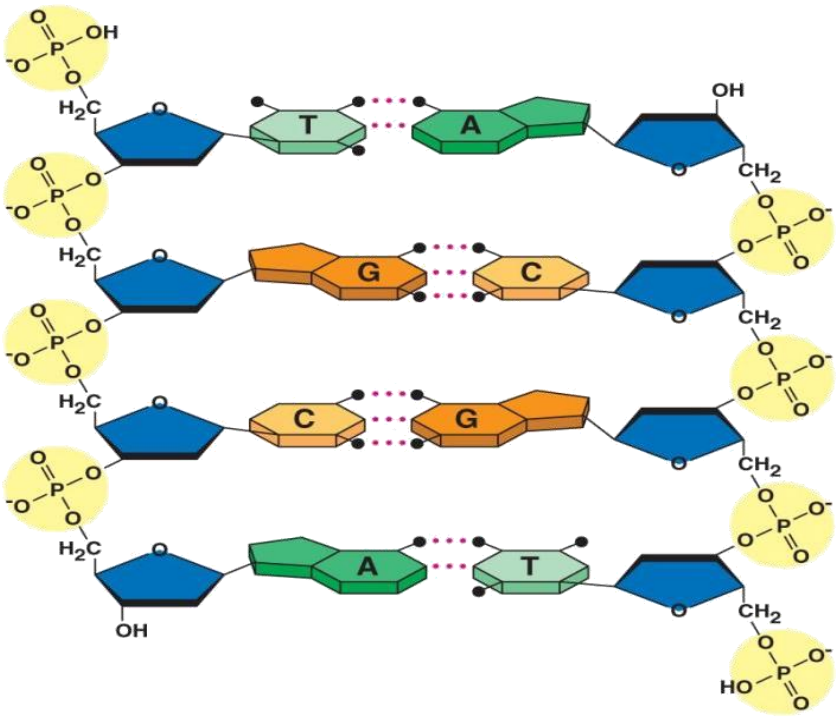
Charged molecules depends on:

- ➢ pH value
- ➢ Size
- ➢ Effective charge – other bound ions can shield the charge of the biomolecule!

Agarose Gel electrophoresis: DNA is negatively **charged**



(a) Key features of DNA structure

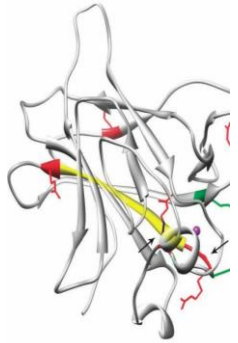


(b) Partial chemical structure

Since all DNA molecules have the same amount of charge per mass we are able to seperate DNA fragments based on size only

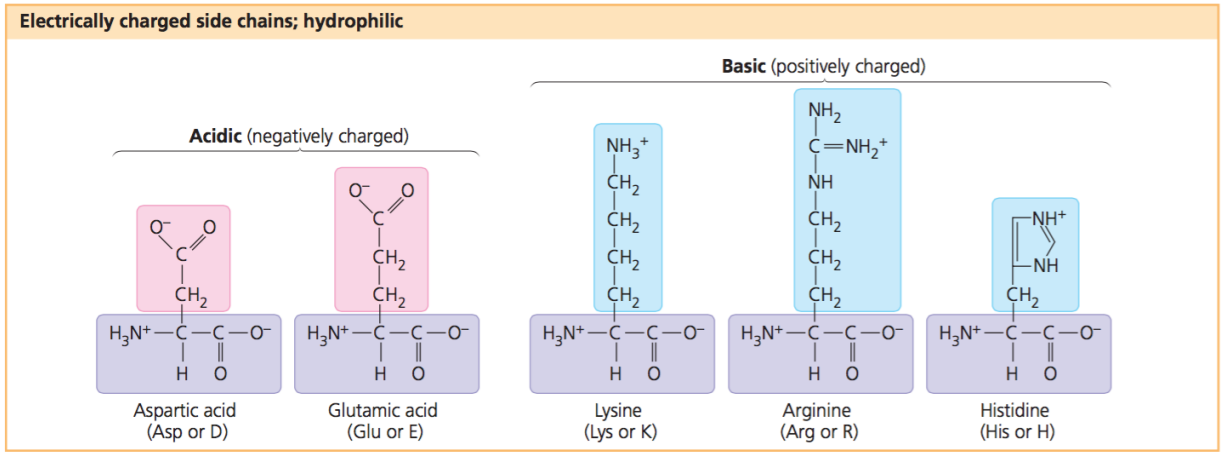
Campbell, N.A. et al. (2013). *Biology*. (10th edition). San Francisco: Pearson/Benjamin Cummings. Figure 16.7

SDSPAGE: the **charge** of proteins is variable



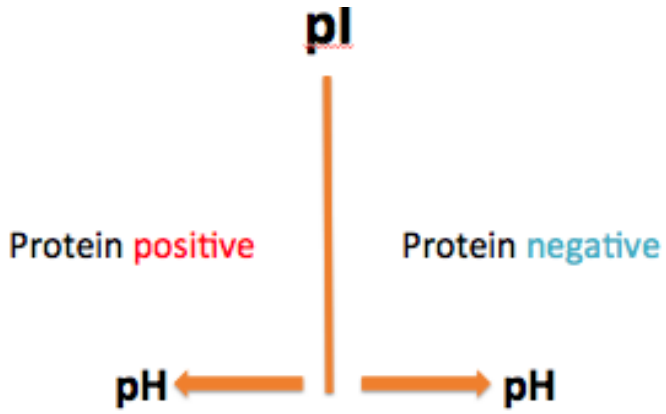
The charge of a protein depends on:

1) Charged side chains



Campbell, N.A. et al. (2013). *Biology*. (10th edition). San Francisco: Pearson/Benjamin Cummings. Figure 5.14

2) Iso electric point/ pH

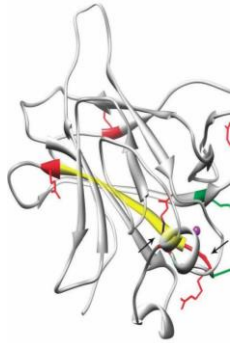


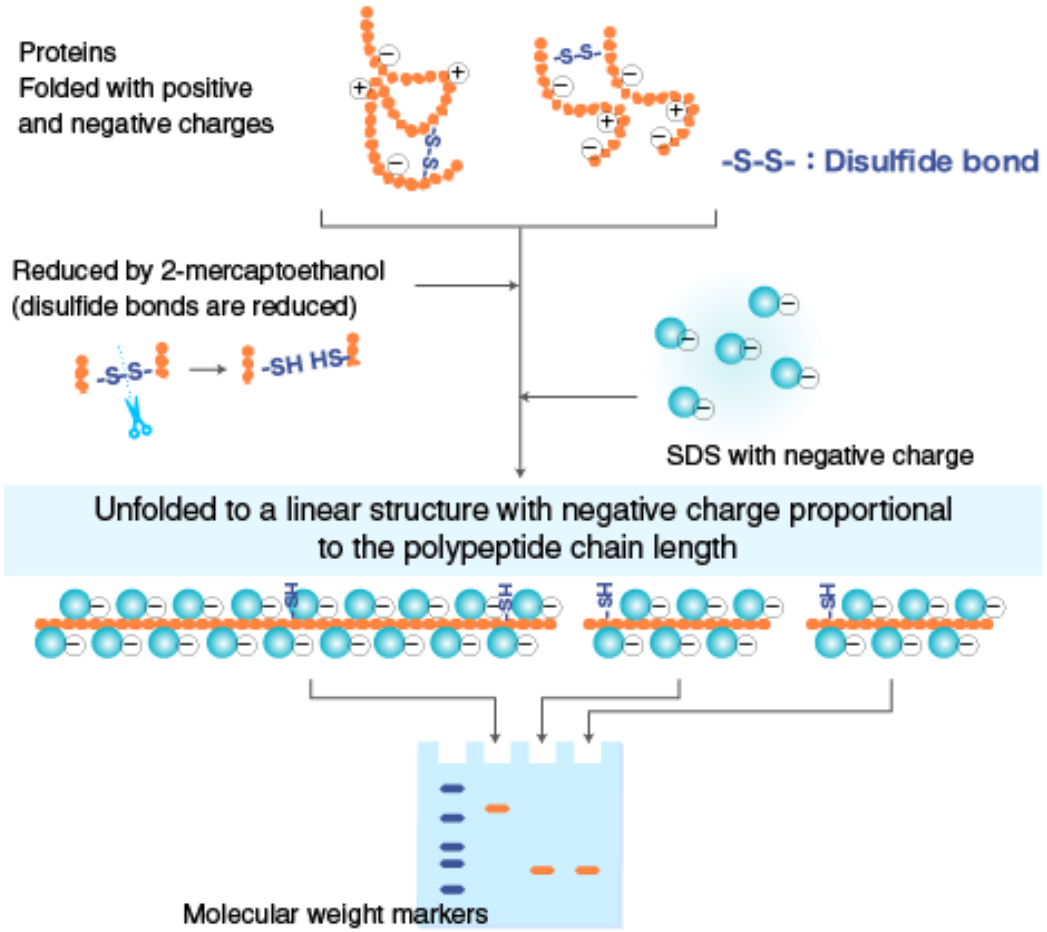
AND: folding has an effect on migration velocity as well!

How to make sure we separate on size only, and not on other properties?

HAN_UNIVERSITY OF APPLIED SCIENCES

SDSPAGE: difference in charge is masked by SDS





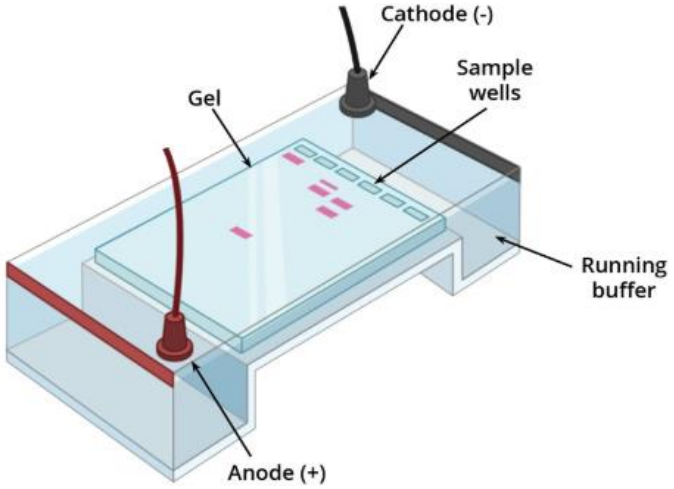
Disrupt hydrofobic and H bonds with ureum

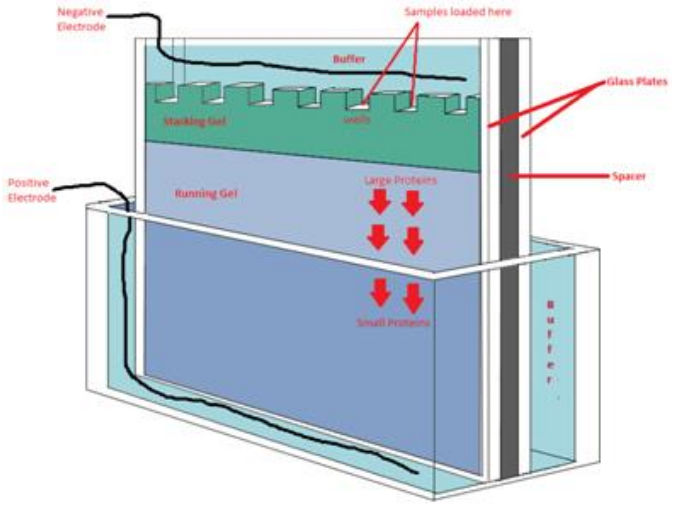
Disrupt disulfide bonds with β-mercapthoethanol

Mask difference in charge with Sodiumdodecylsulfaat (SDS)

MBL Life science. (n.d.). *SDS-PAGE*. Retrieved January 17, 2025, from https://ruo.mbl.co.jp/bio/e/support/method/sds-page.html/

Gel electrophoresis: further analysis on a blot





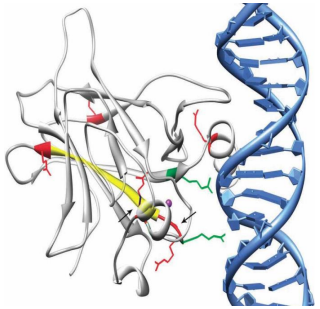
Agarosegel electrophoresis

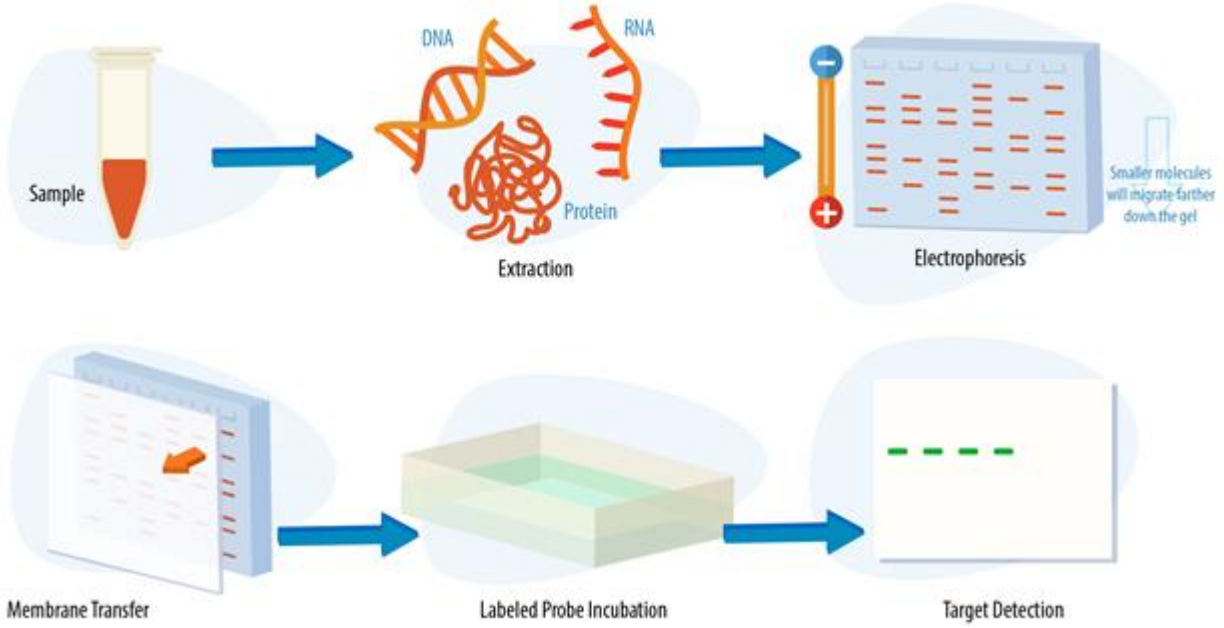
SDSPAGE

⬇

Blotting techniques

Blotting techniques





Separation by size

⬇

Transfer biomolecules to a solid support

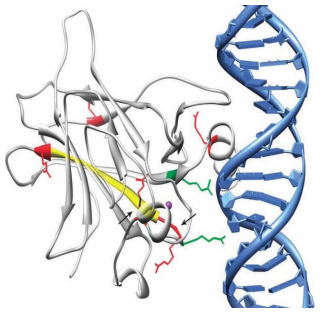
⬇

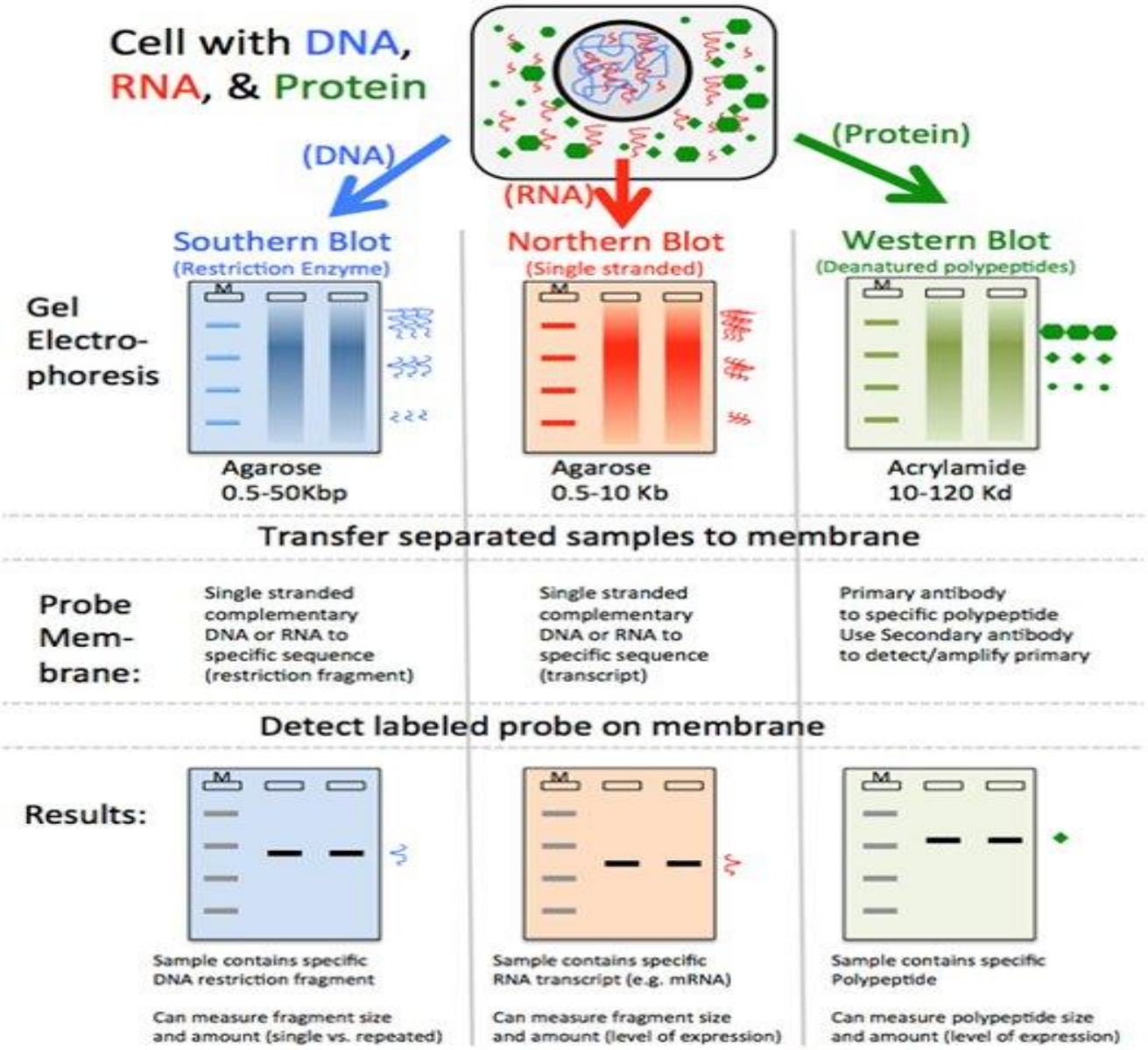
Mark target molecule specifically

Lab Manager. (2019, September). *Blotting techniques*. Retrieved January 17, 2025, from https://www.labmanager.com/southern-vs-northern-vs-western-blotting-techniques-854

What could be the aim of a Blot after gel electrophesis?

Blotting techniques





Biology exams 4U. (n.d.). *Southern blotting principle procedure and application*. Retrieved January 17, 2025, from https://www.biologyexams4u.com/2013/12/southern-blotting-principle-procedure.html#.WKGON9IzWUk

Learning goals lesson 1: Electrophoresis

After this lesson students:

- Know the different types of electrophoresis: zone versus moving boundary and can name examples of both types (eg paper electrophoresis, gel electrophoresis, capillary electrophoresis and iso electric focusing).

- Know which components are needed for gel electrophoresis (matrix, electric field, buffer and charged molecules) and is able to explain which factors are important when setting up the experiment.

- Are able to argument how an experiment can be optimized, based on given formula's.

- Are aware of the possible follow up experiment after gel electrophoresis: Southern blot for DNA, Nothern blot for RNA and Western blot for Proteins