Gels and their uses

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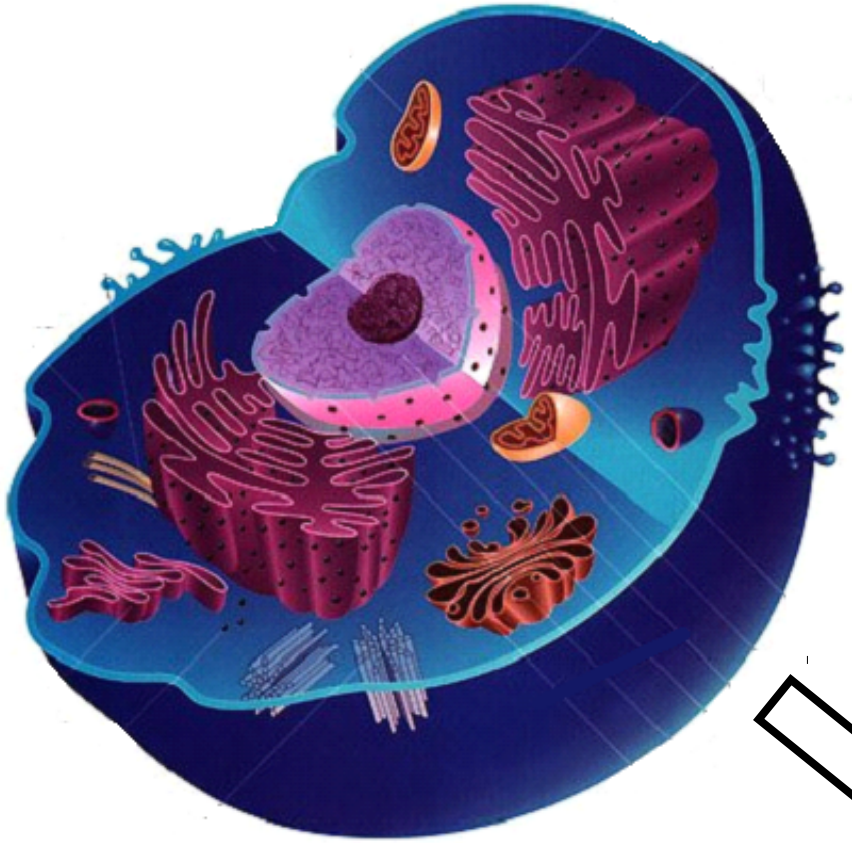
Pic: fathom.com

The problem:



100,000 proteins

We generally want to study just one or two

Separation techniques:

- Gel electrophoresis
- Filtration
- Differential precipitation
- Gel filtration
- Chromatography (generally HPLC)
- Affinity chromatography

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- Gel electrophoresis ******
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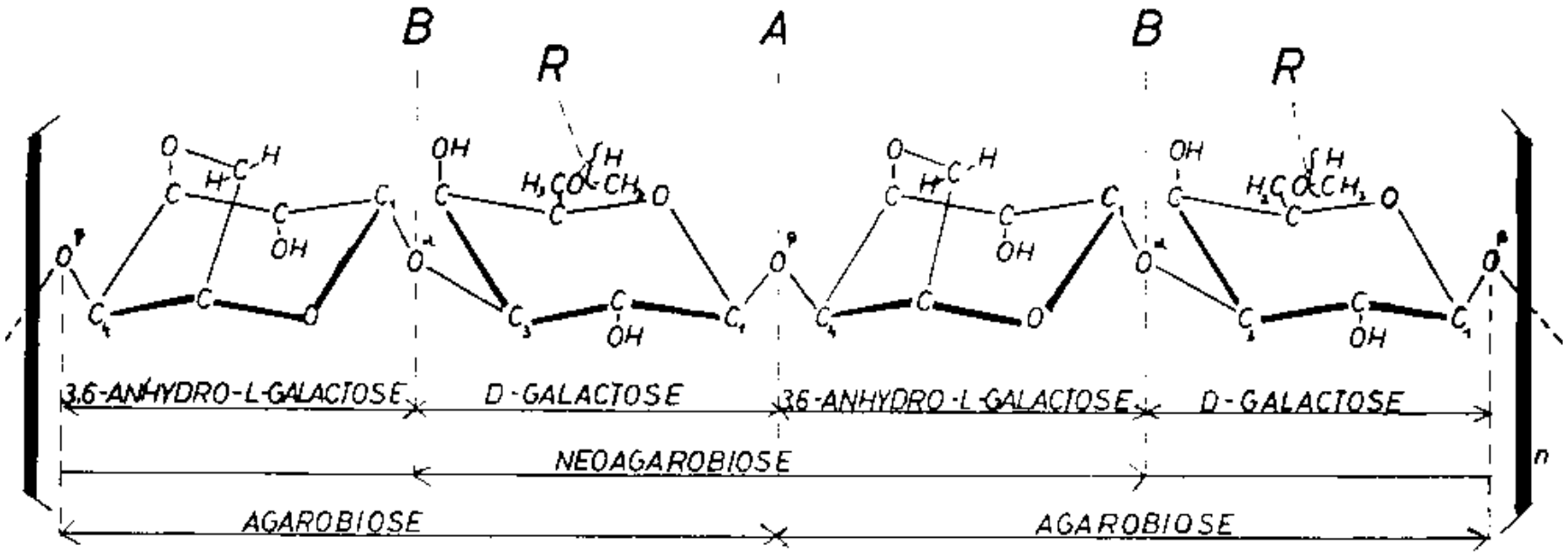
What is a gel?

- A **gel** (from the lat. *gelu*—freezing, cold, ice or *gelatus*—frozen, immobile) is a colloidal system in which a porous network of interconnected nanoparticles spans the volume of a liquid medium. In general, gels are apparently solid, jelly-like materials. Both by weight and volume, gels are mostly liquid in composition and thus exhibit densities similar to liquids, however have the structural coherence of a solid. An example of a common gel is edible gelatin.

From Wikipedia

Agarose gels



A LINK EASILY HYDROLYSED BY ACIDS.

B LINK EASILY HYDROLYSED BY ENZYMES.

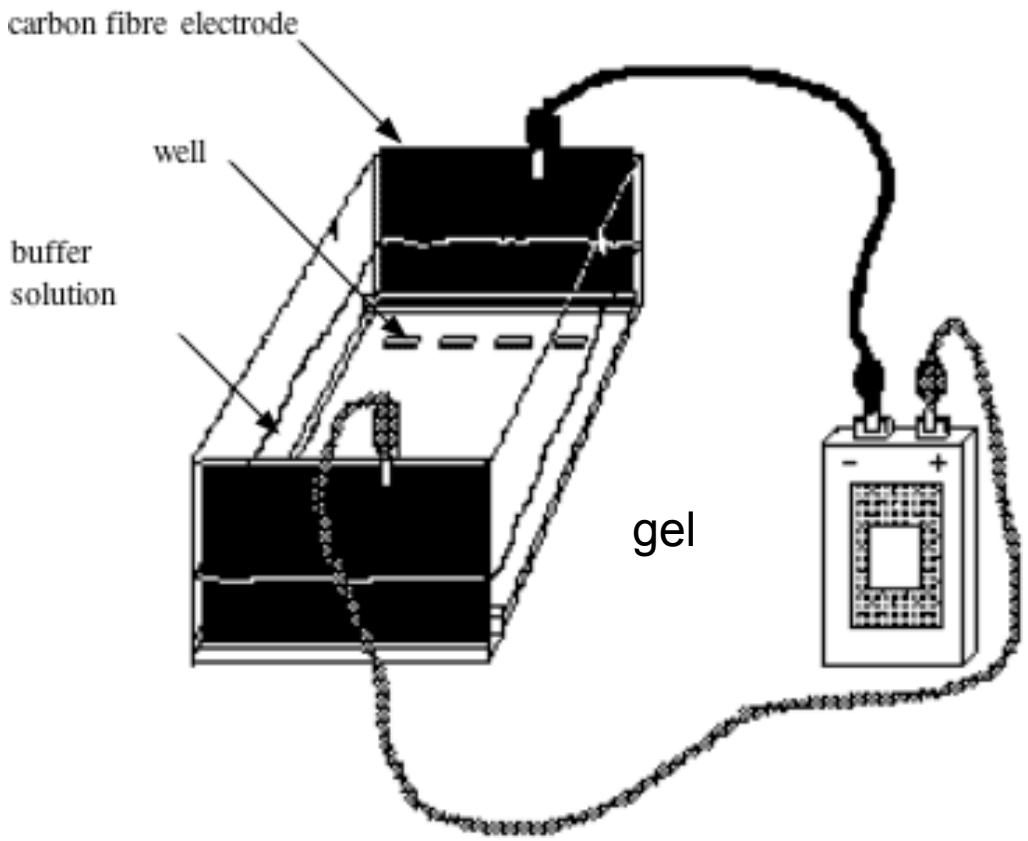
R SUBSTITUTION OF -H BY $-CH_3$ IS CONDITIONED BY THE SEAWEEDS USED.
GEL POINT IS INCREASED BY A HIGHER CONTENT OF $-CH_3$ GROUPS.

n POLYMERIZATION GRADE. IT CONDITIONS GEL STRENGTH.

Making an agarose gel:

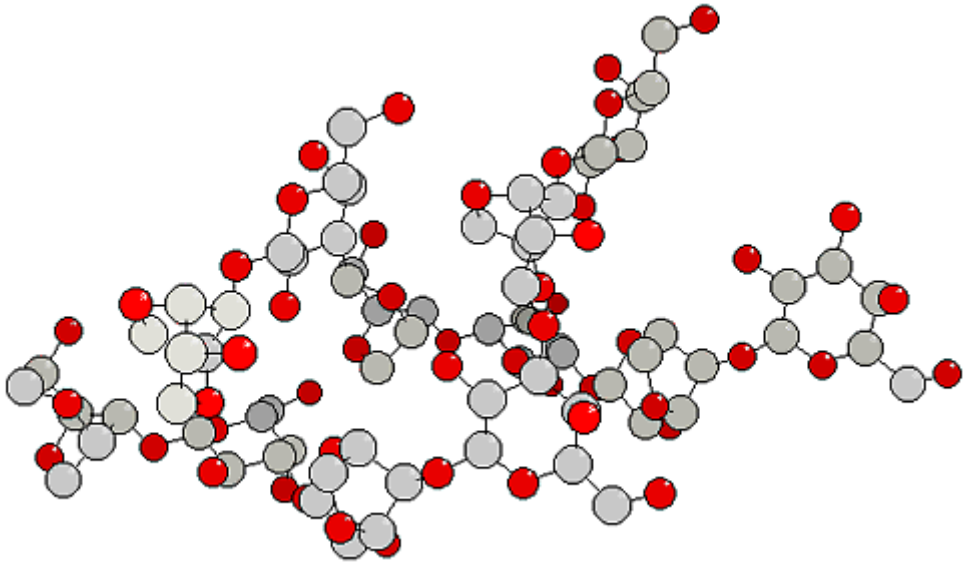
- Suspend agarose in a buffer
- Heat it (about 100 deg. C.): the agarose dissolves
- Let it cool

The basic idea of DNA agarose electrophoresis (more detail later)



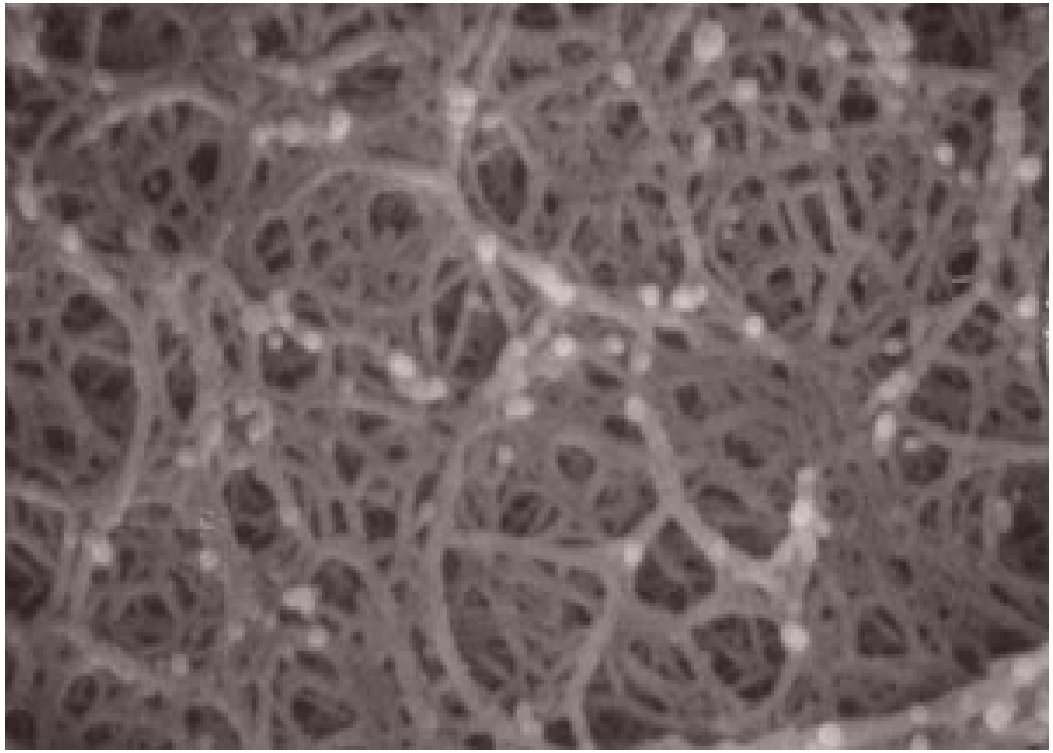
DNA is –vely charged, so seeks the anode

Properties of agarose gels

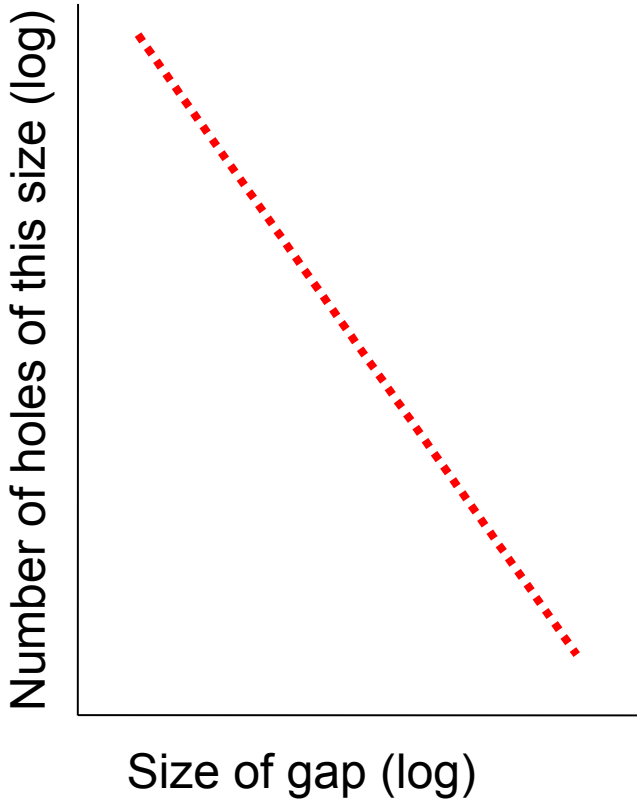


The molecules tangle to make a random network, that has some big holes and some small ones.

The distribution of gaps is fractal (scale free)



220,000x



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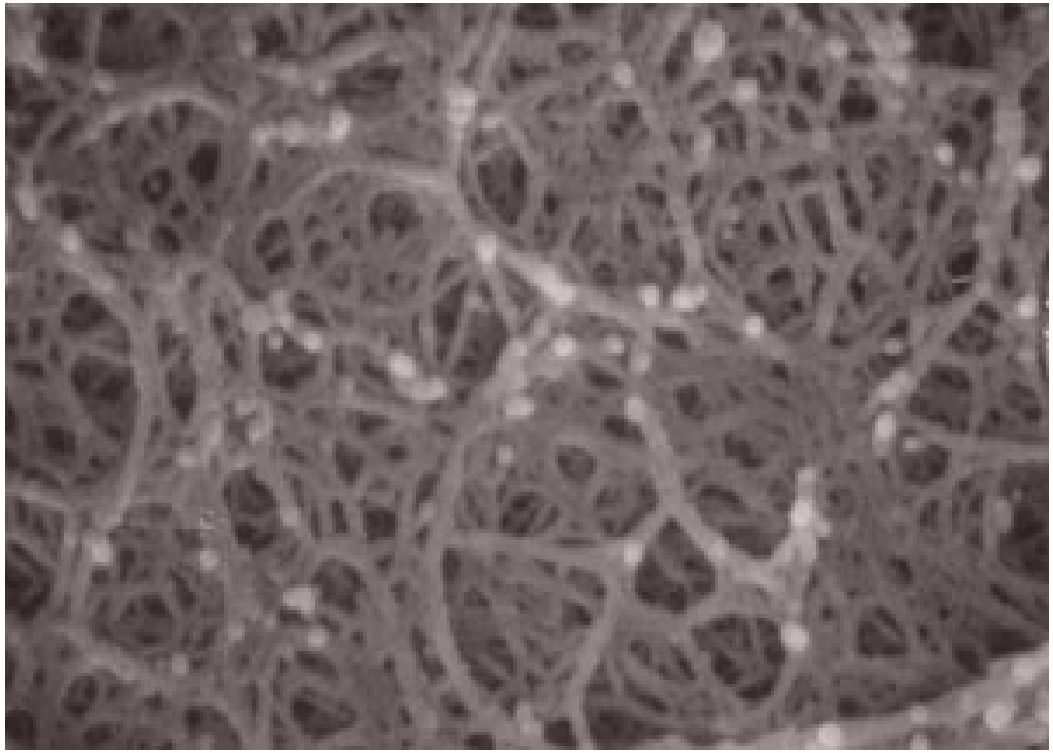

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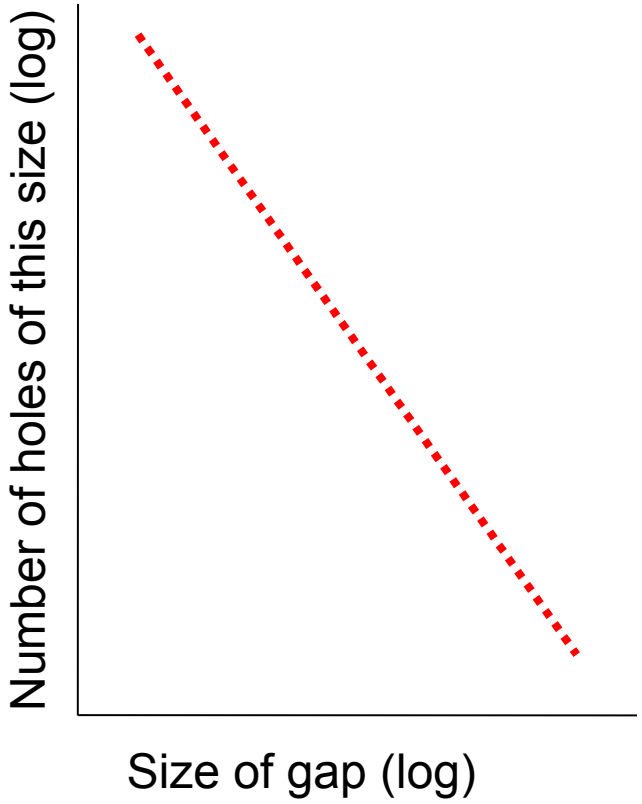


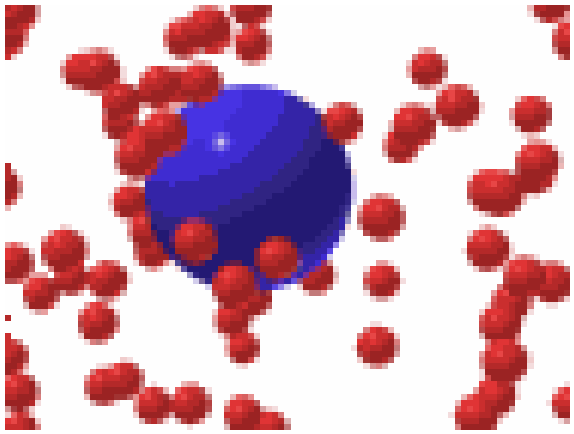
$P[X>x] \approx x^{-k}$

How do large molecules get through?

- Obviously, they cannot get through passages smaller than themselves

- They can get through passages larger than themselves.

Brownian Motion

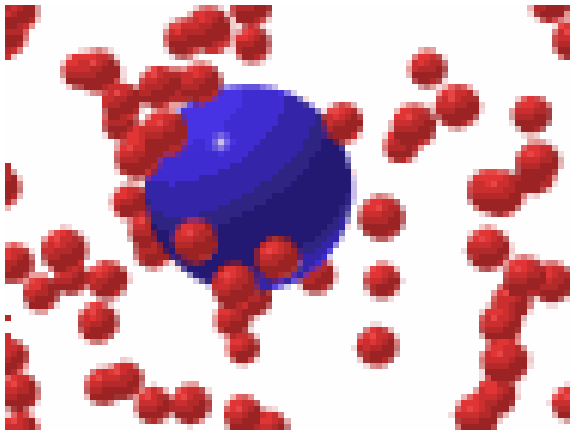


$$\langle E \rangle = \frac{3kT}{2}$$

so…. (using $E=0.5mv^2$)

$$v = \sqrt{\frac{3kT}{m}}$$

Brownian Motion

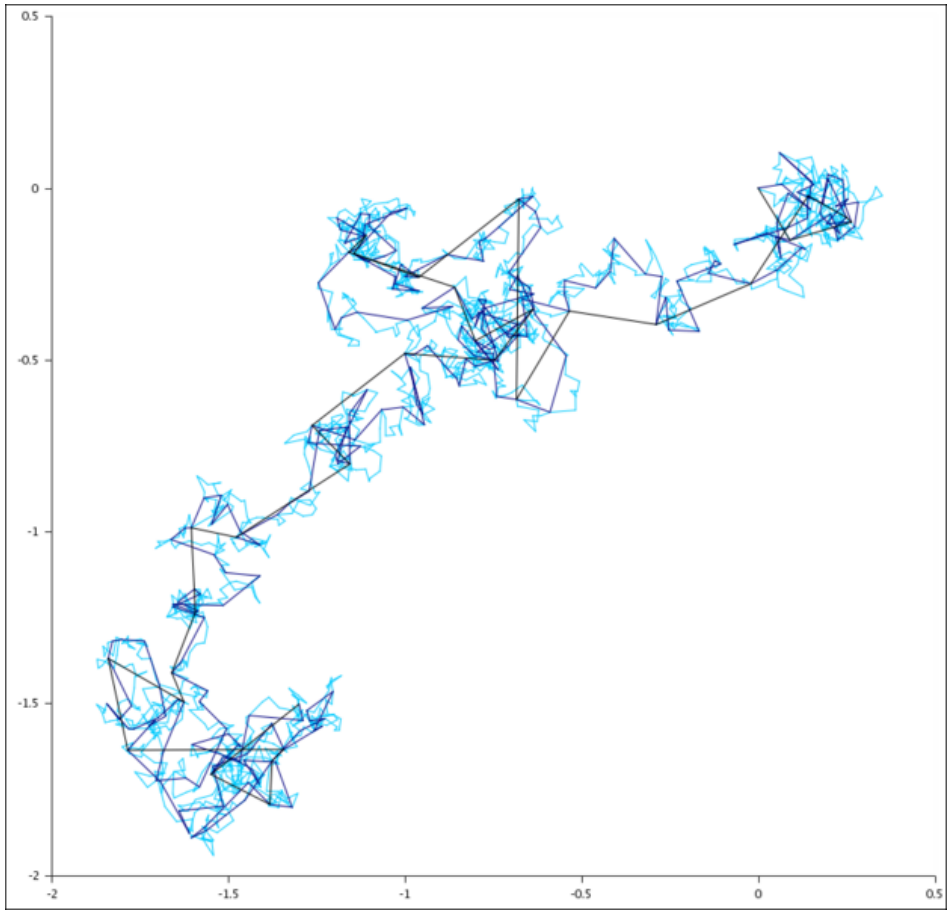


$$\langle E \rangle = \frac{3kT}{2}$$

so…..

$$v = \sqrt{\frac{3kT}{m}}$$

Large particles move more slowly



Simulated random walks

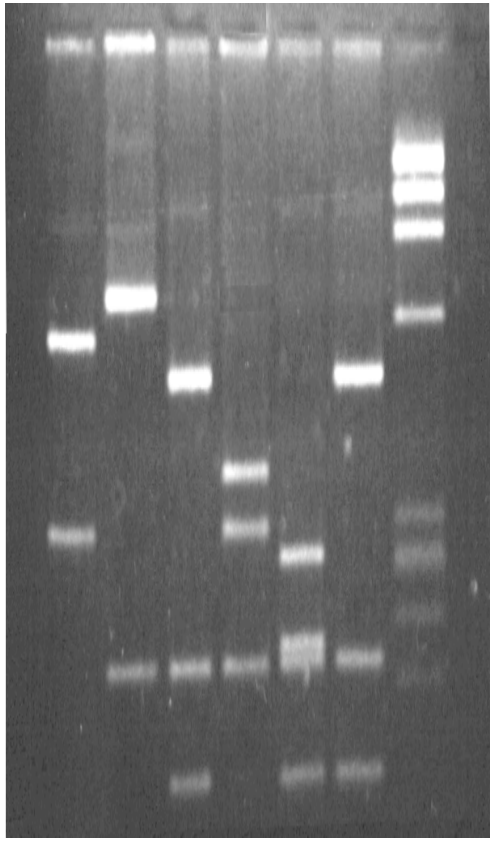
On average, the distance moved in time t;

$$d = \sqrt{t\,v}$$

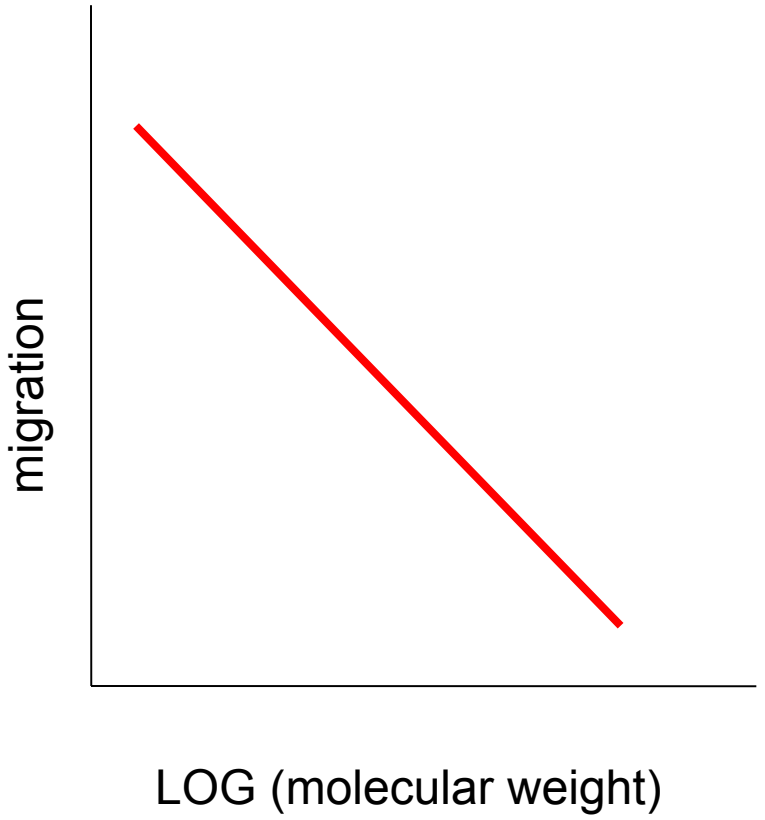
or the time to go distance d,

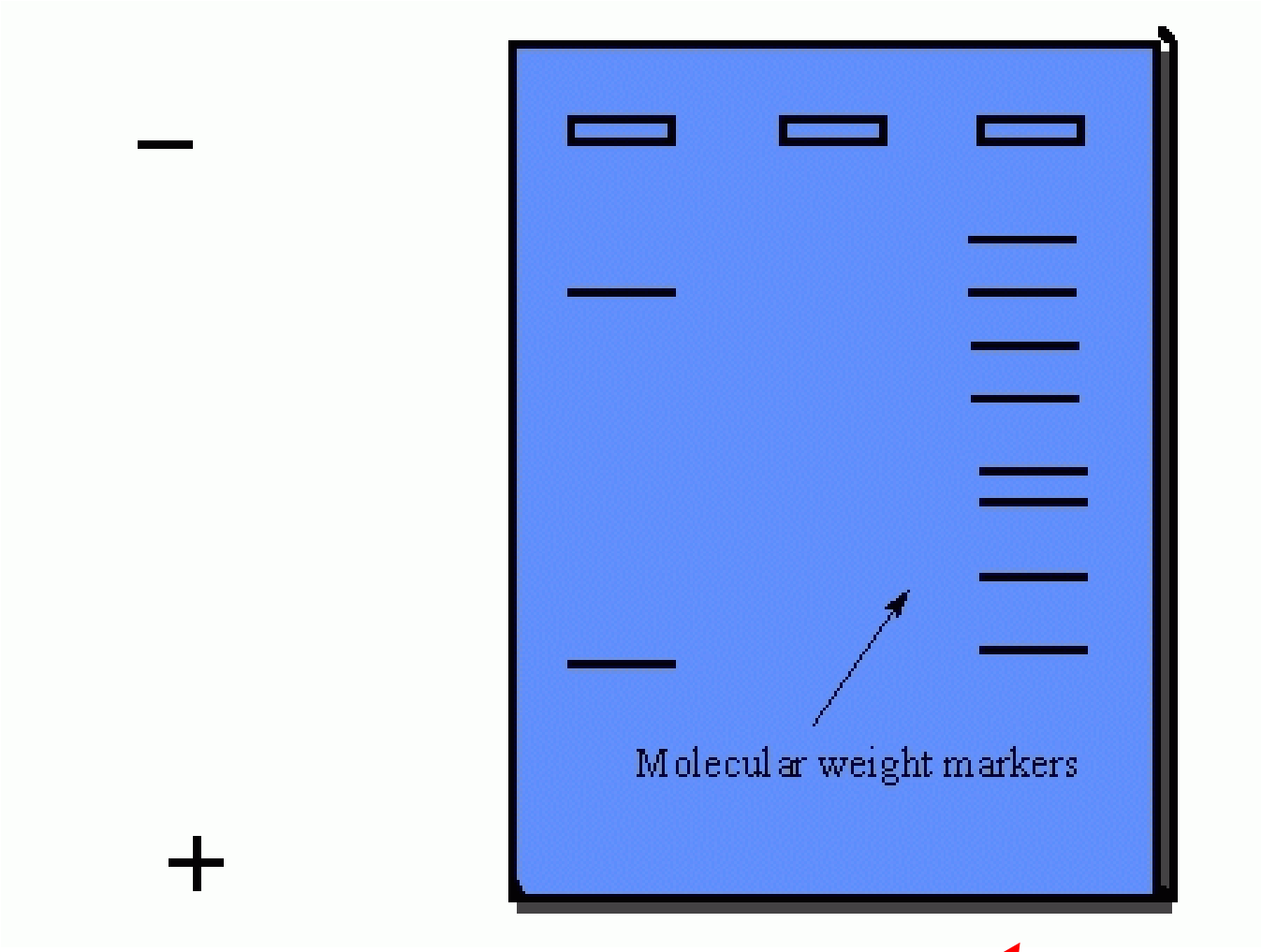
$$t = d^2/v$$

On a log/lin graph:



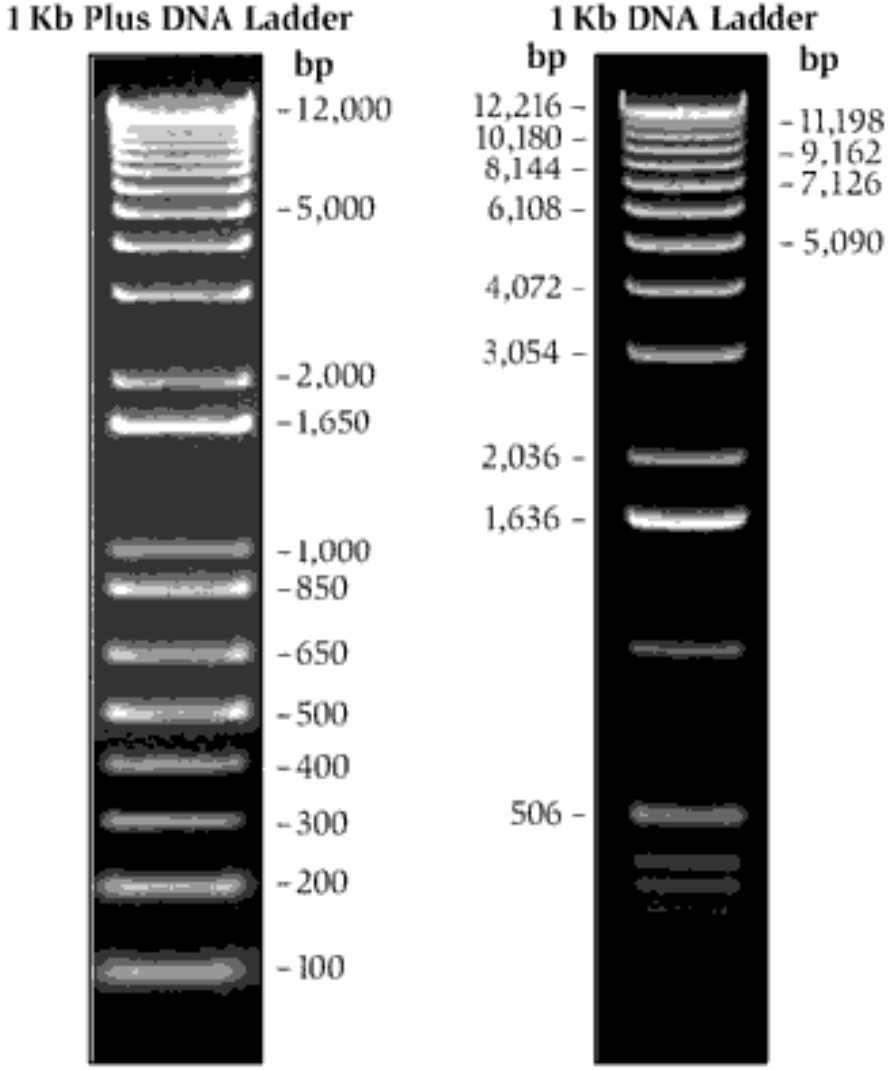
Distance of migration





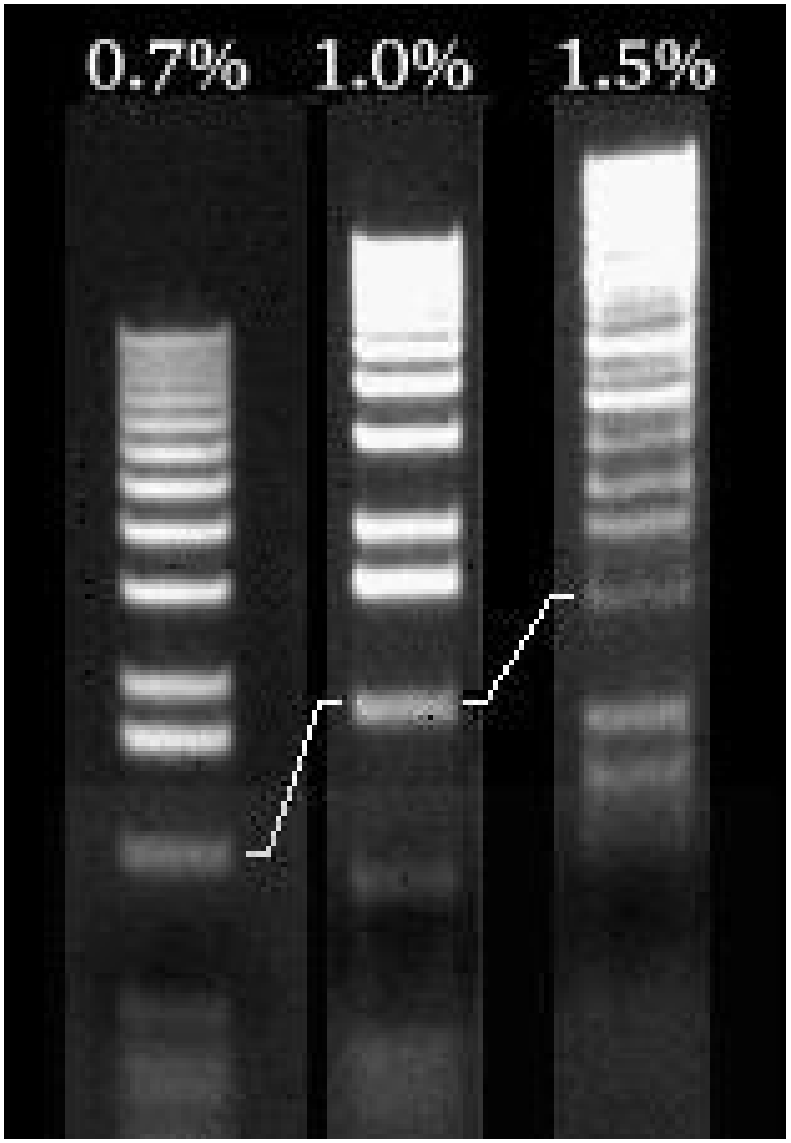
Calibrate your graph with these

Comparison between 1 Kb Plus DNA Ladder and 1 Kb DNA Ladder. 0.9% agarose gel stained with ethidium bromide



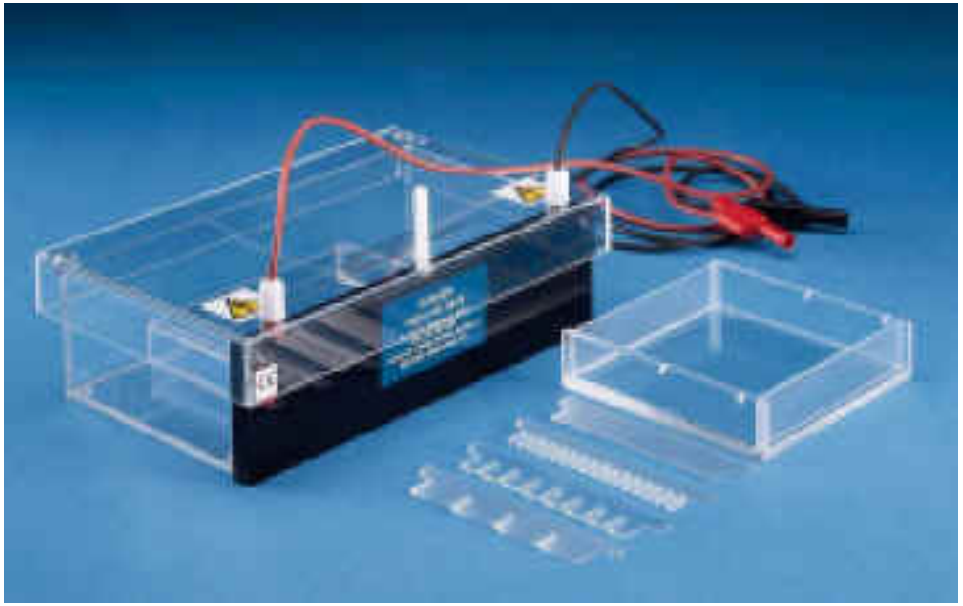
0.9 µg/lane

0.9 µg/lane



Higher concentrations of agarose facilitate separation of small DNAs, while low agarose concentrations allow resolution of larger DNAs.

Real-world apparatus

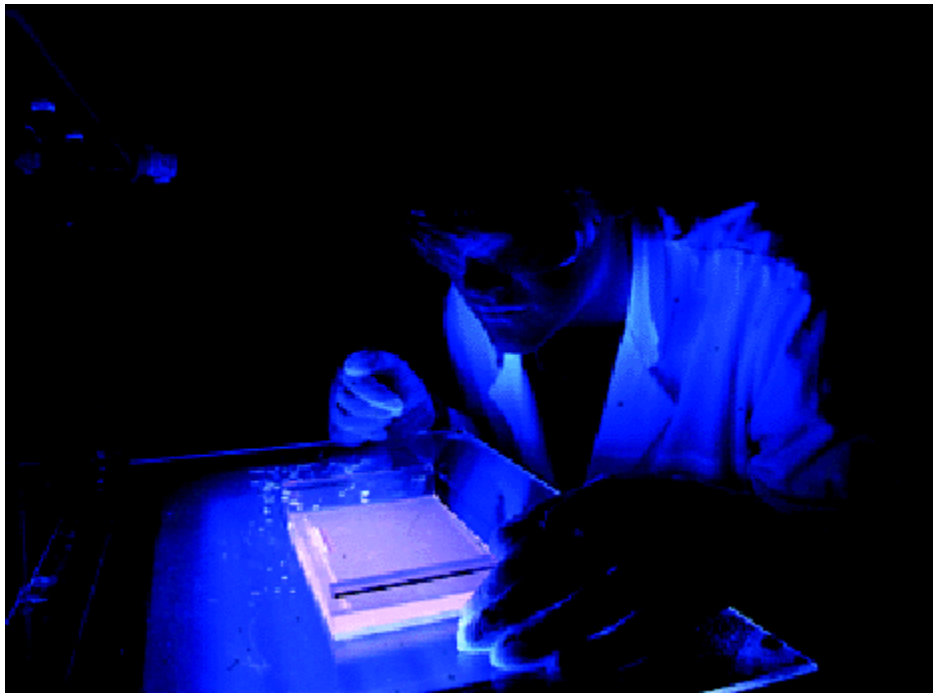


Detecting your DNA

Stain with ethidium bromide.

View under uv light.

You can use gels to purify

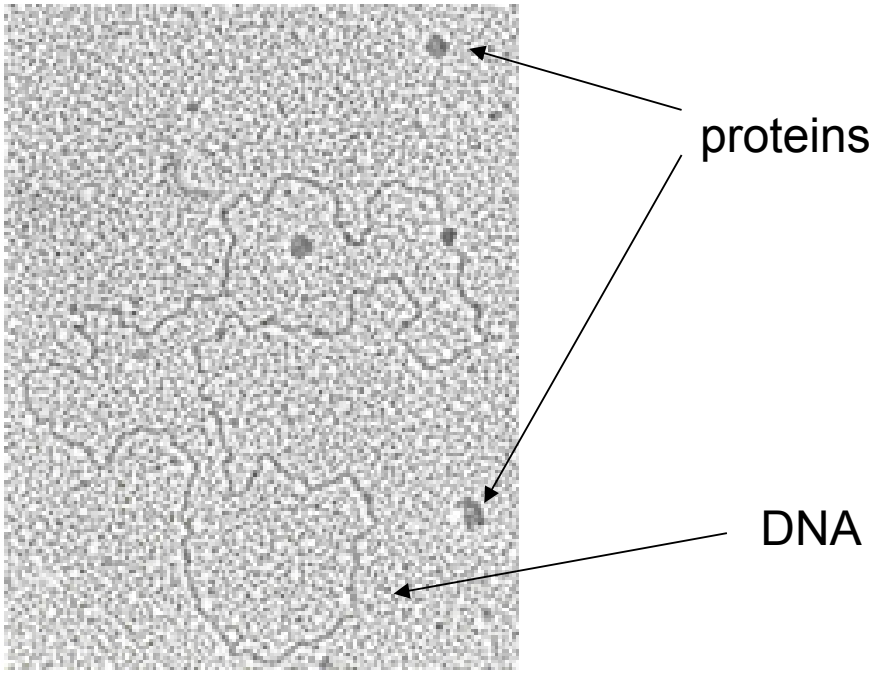


- Physically cut out the band you want (scalpel).
- Melt the agar into a recovery buffer
- Clean agarose away (spin columns).

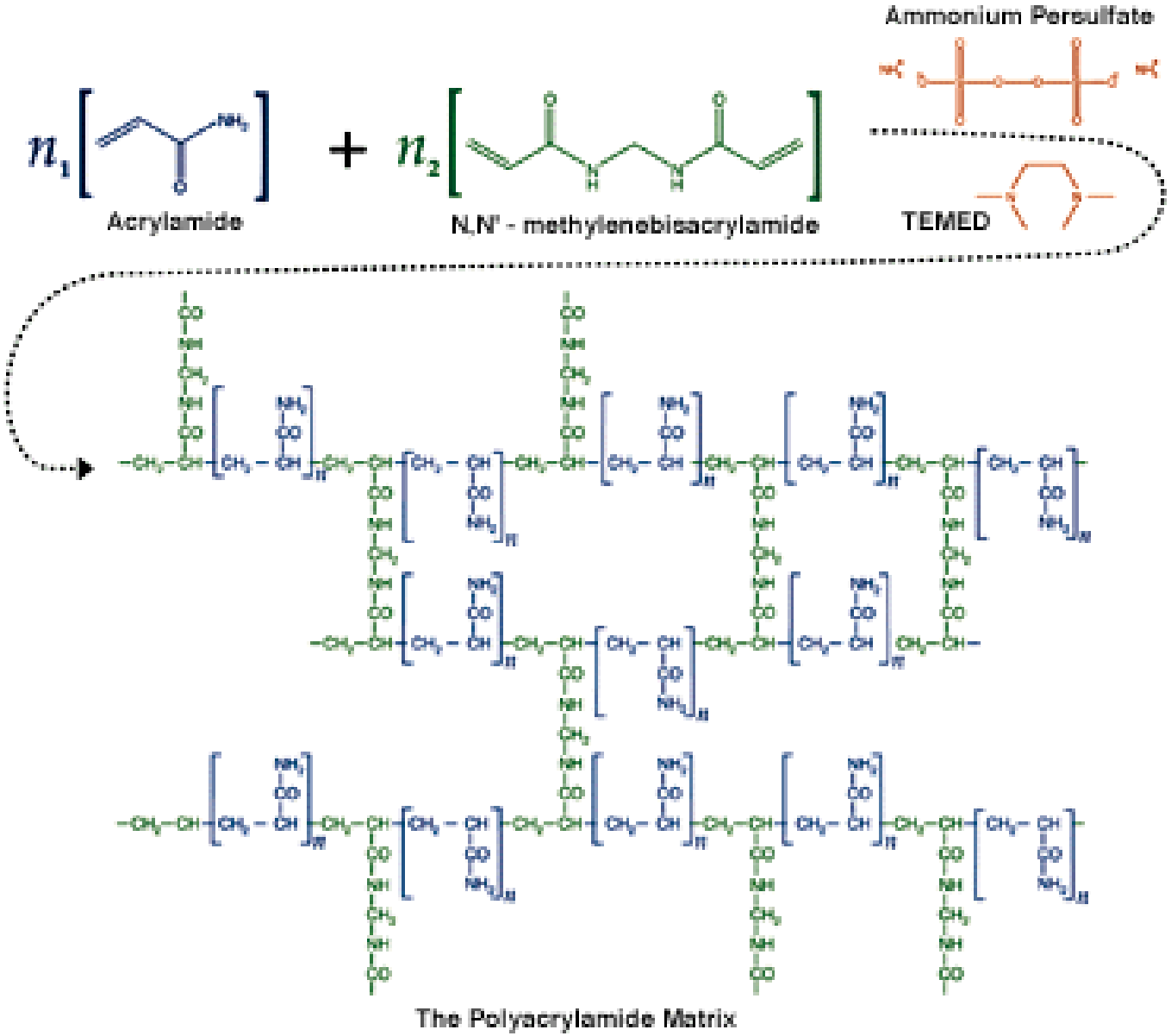
RNA can be treated the same way.

Protein electrophoresis

- Problem 1: proteins are more compact than nucleic acids. They all go through agarose gels too quickly.

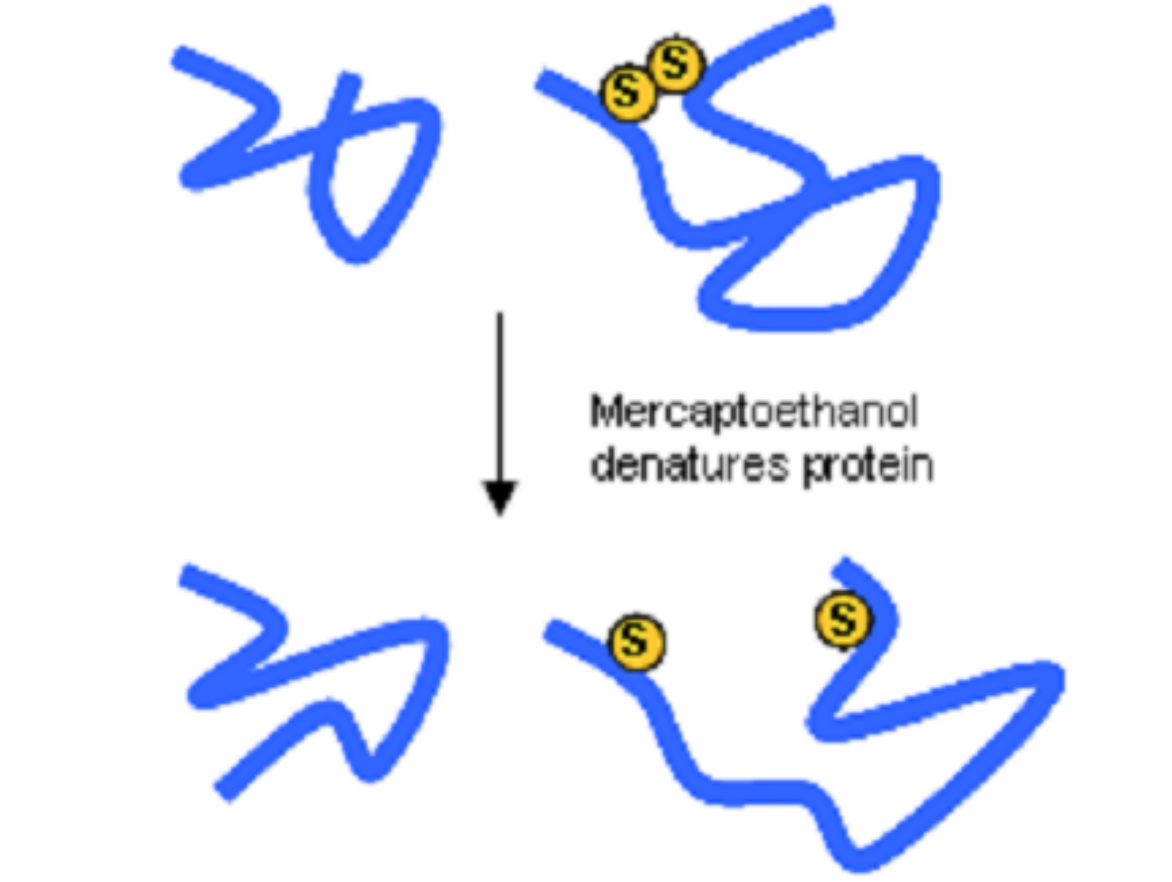


Polyacrylamide

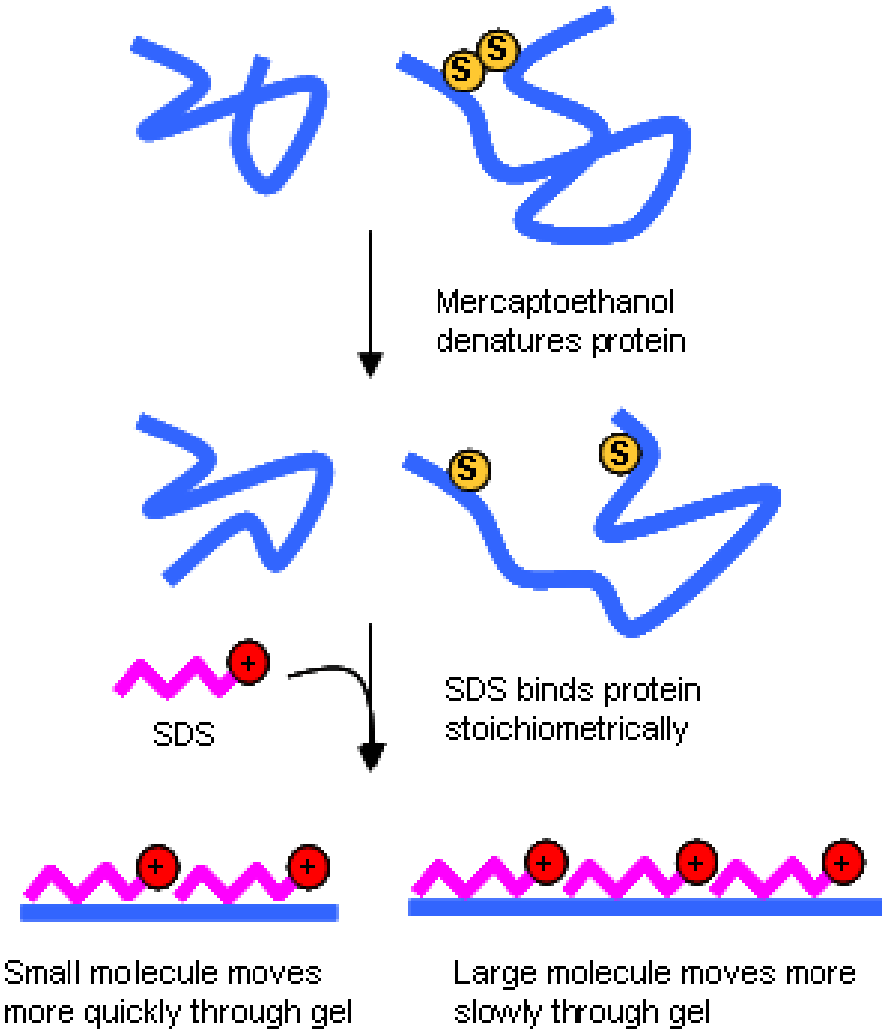


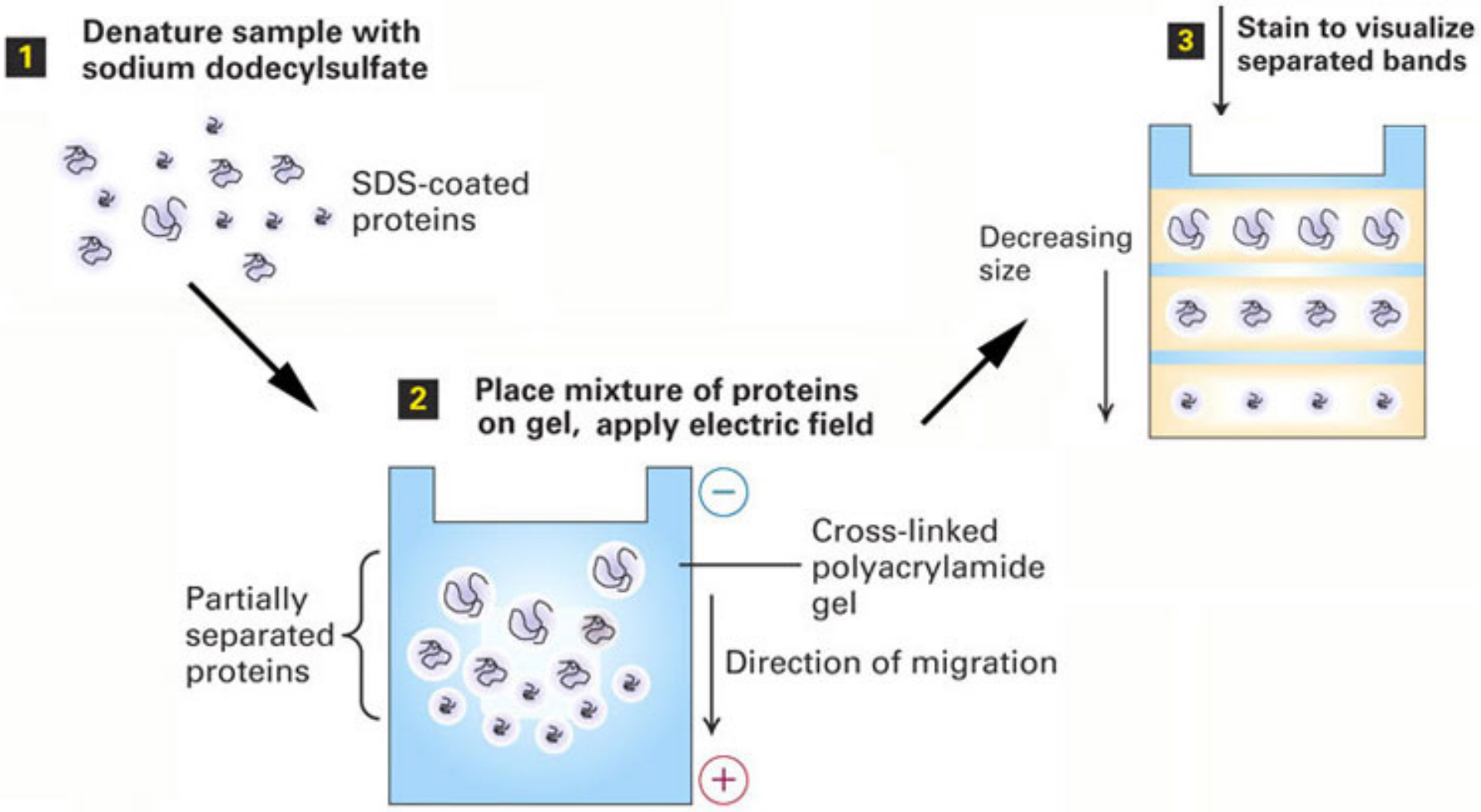
Problem 2: proteins can have S-S bonds

- Solution: reducing agents



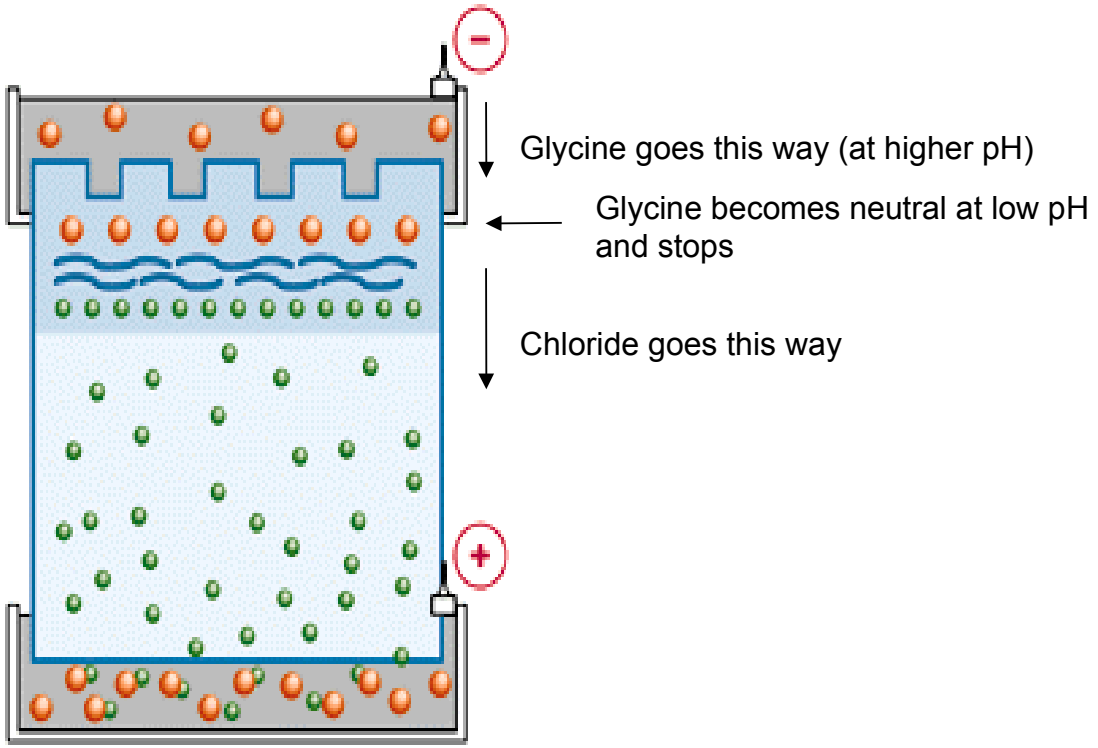
- Problem 3: unlike nucleic acids, proteins do not have a constant charge/mass ratio.




1
Denature sample with sodium dodecylsulfate
SDS-coated proteins
2
Place mixture of proteins on gel, apply electric field
Partially separated proteins
Cross-linked polyacrylamide gel
Direction of migration
3
Stain to visualize separated bands
Decreasing size

Problem 4: proteins are usually dilute, so large volumes have to be added

Solution: stacking gel (low conc^{n} of acrylamide, more acid, Cl^{-} ions).



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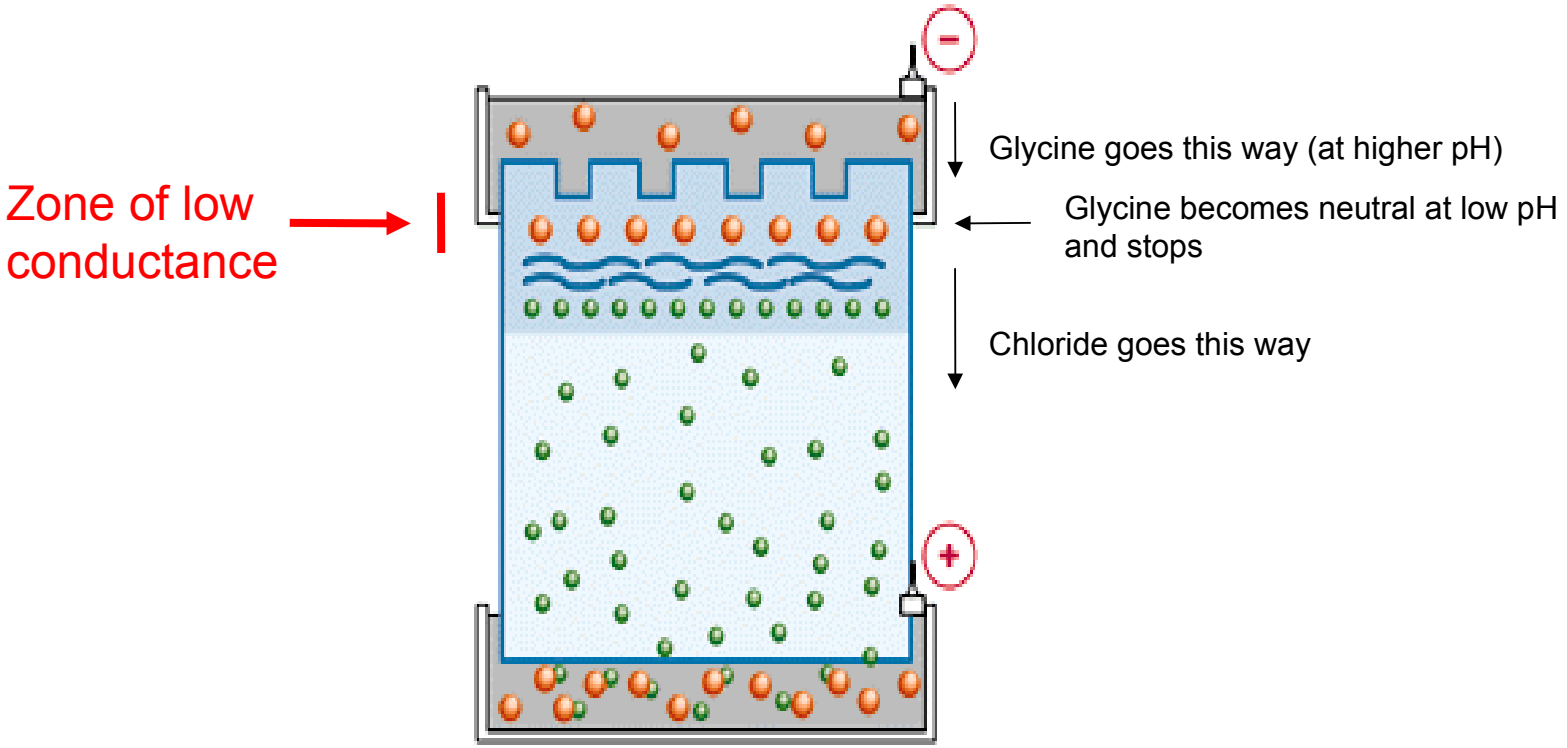

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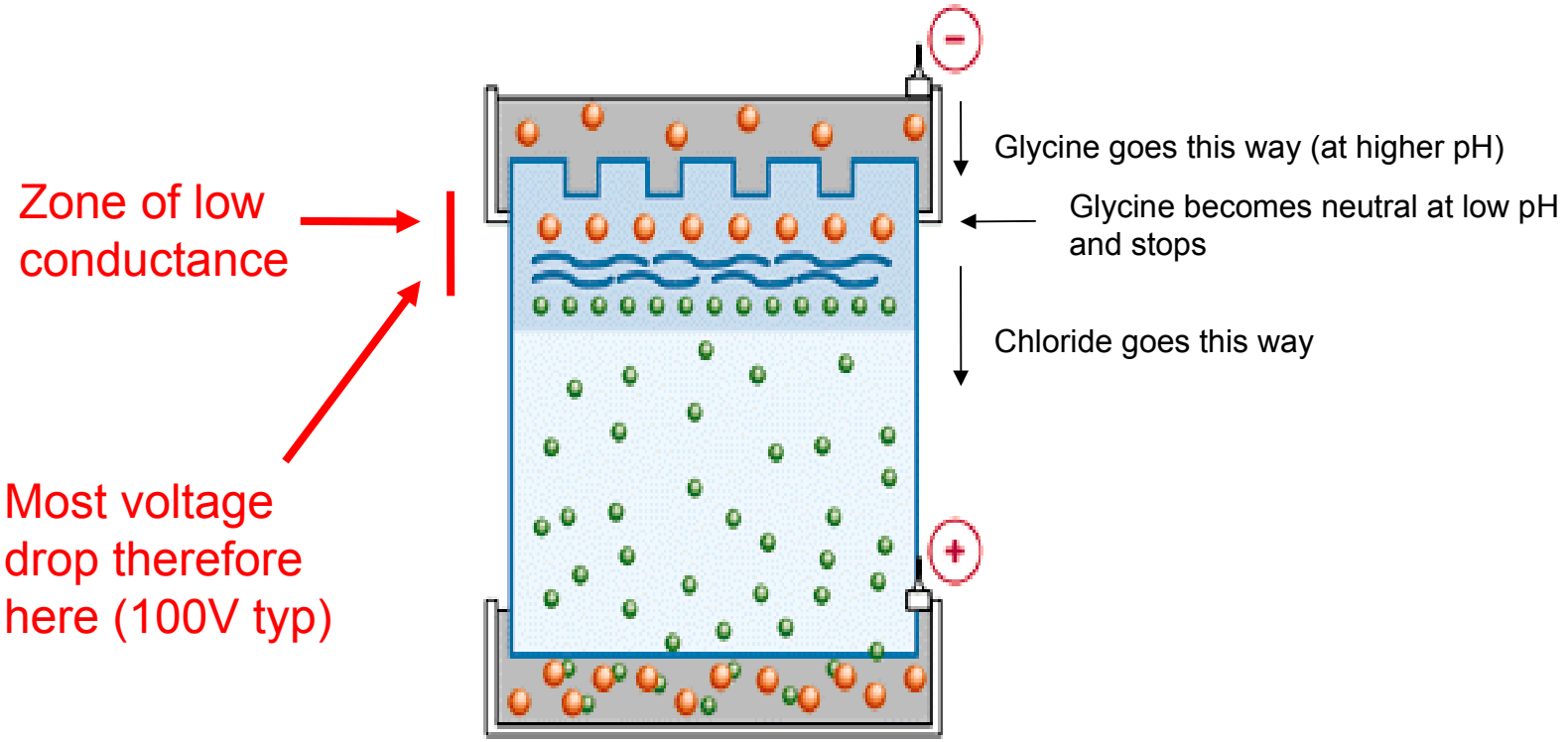
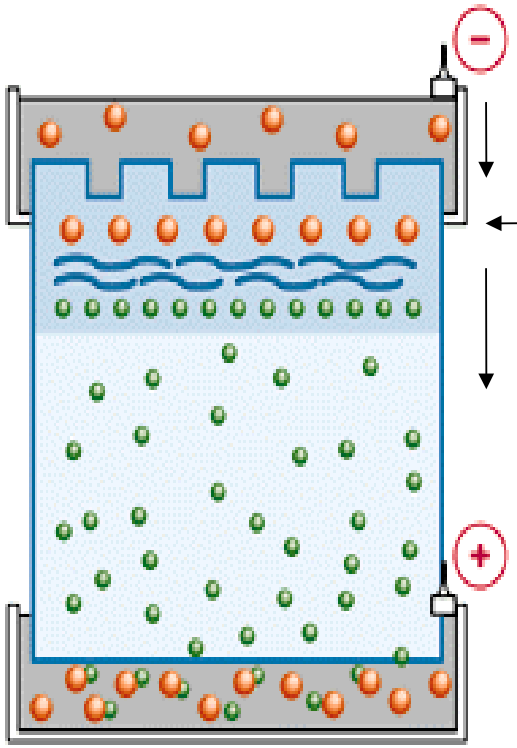


Problem 4: proteins are usually dilute, so large volumes have to be added

Solution: stacking gel (low conc^{n} of acrylamide, more acid, Cl^{-} ions).

Proteins go fast, but have to slow a lot when they reach chloride ions (high conductance, low voltage)



Glycine goes this way (at higher pH)

Glycine becomes neutral at low pH and stops

Chloride goes this way

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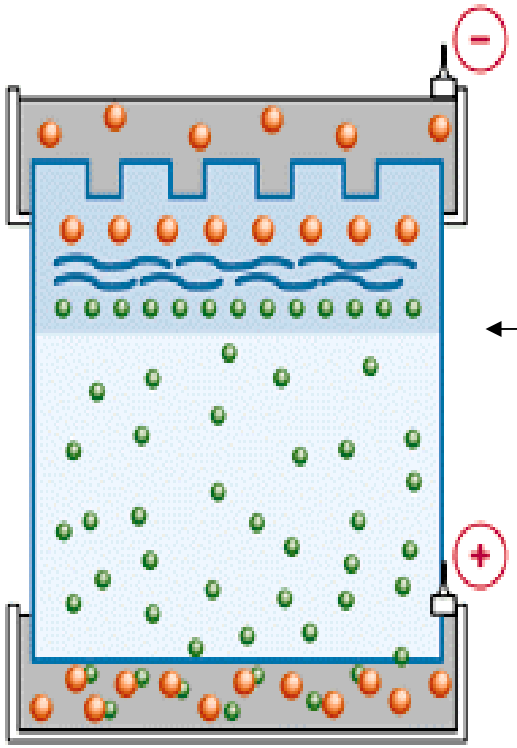
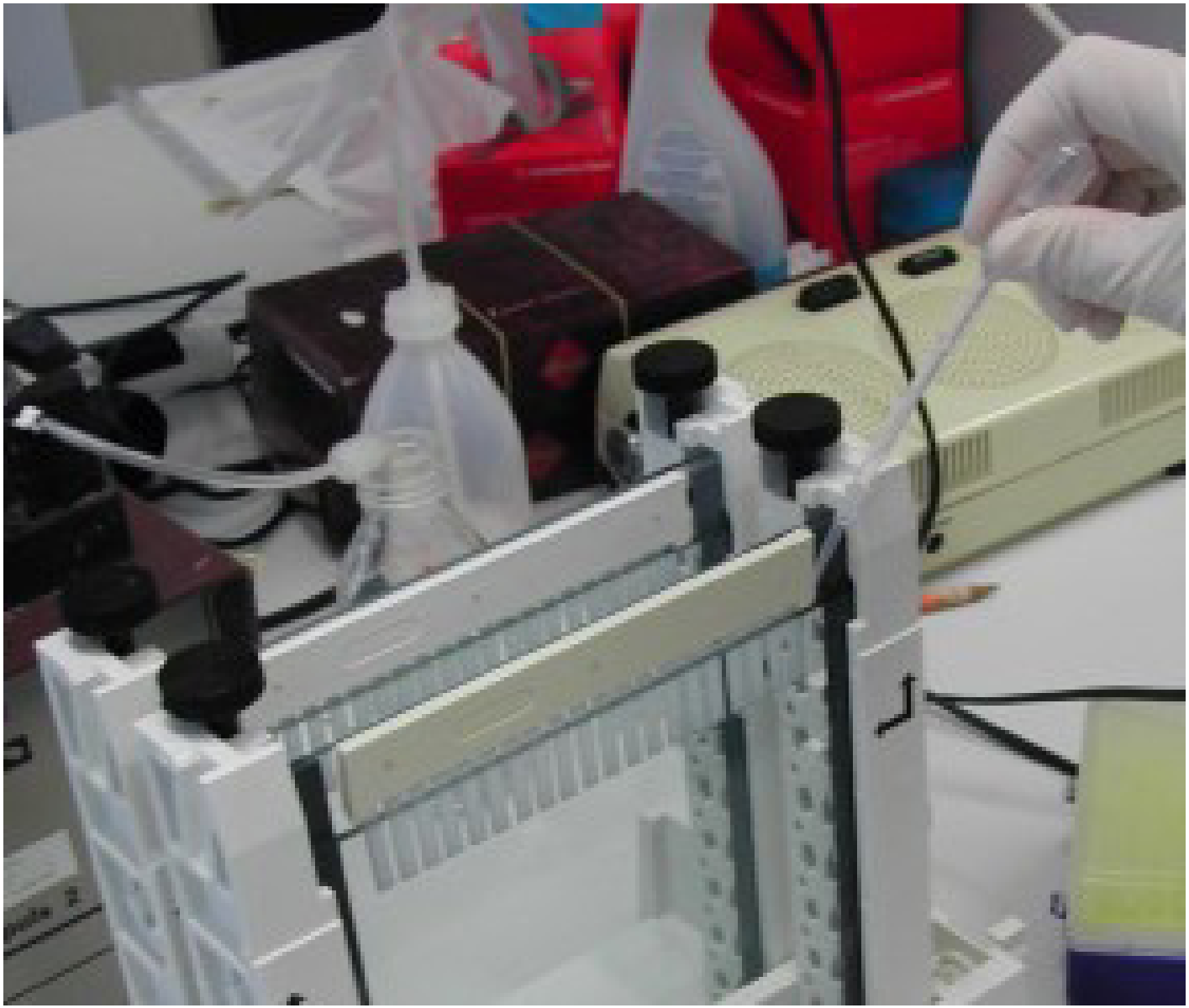
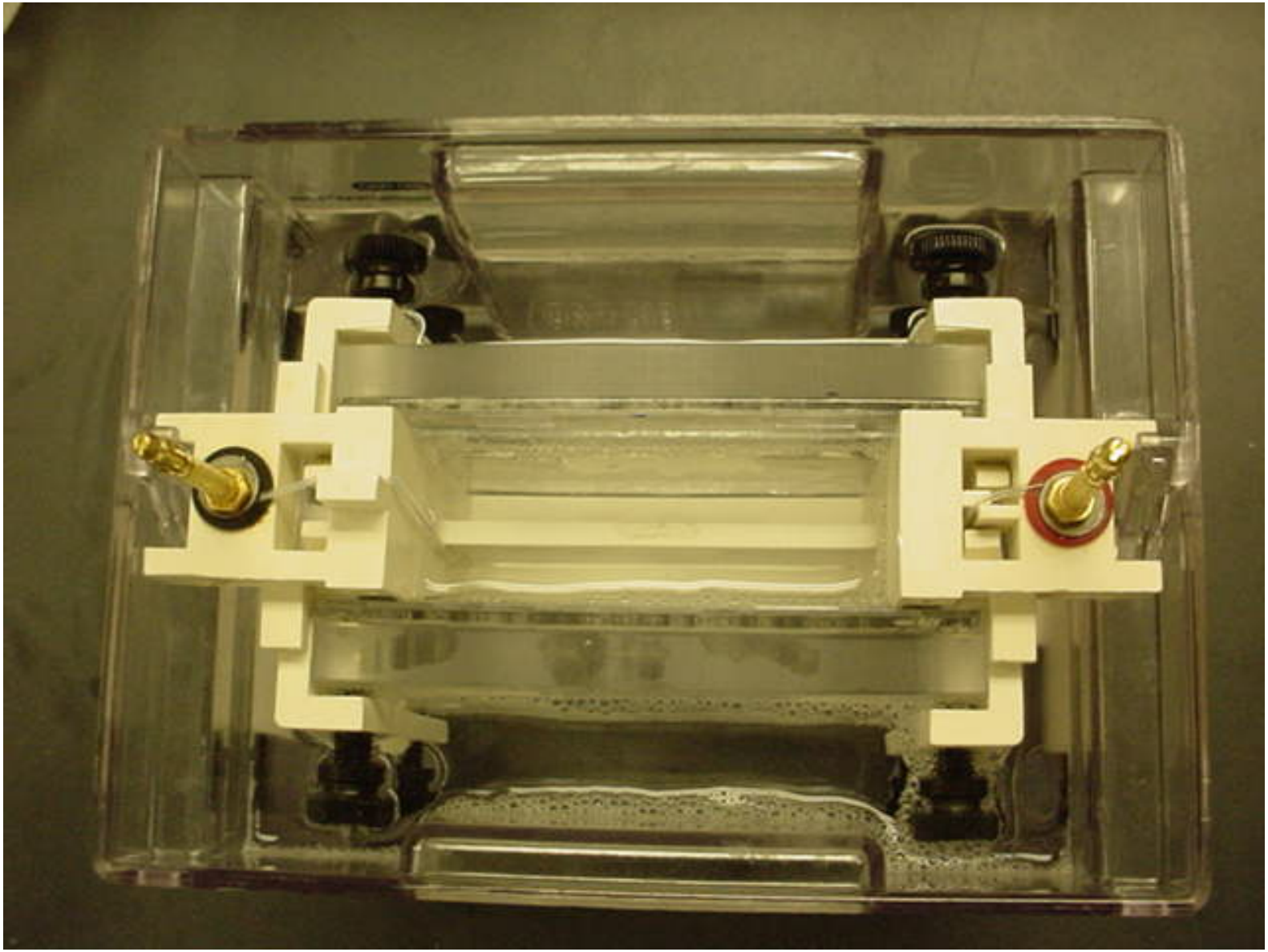
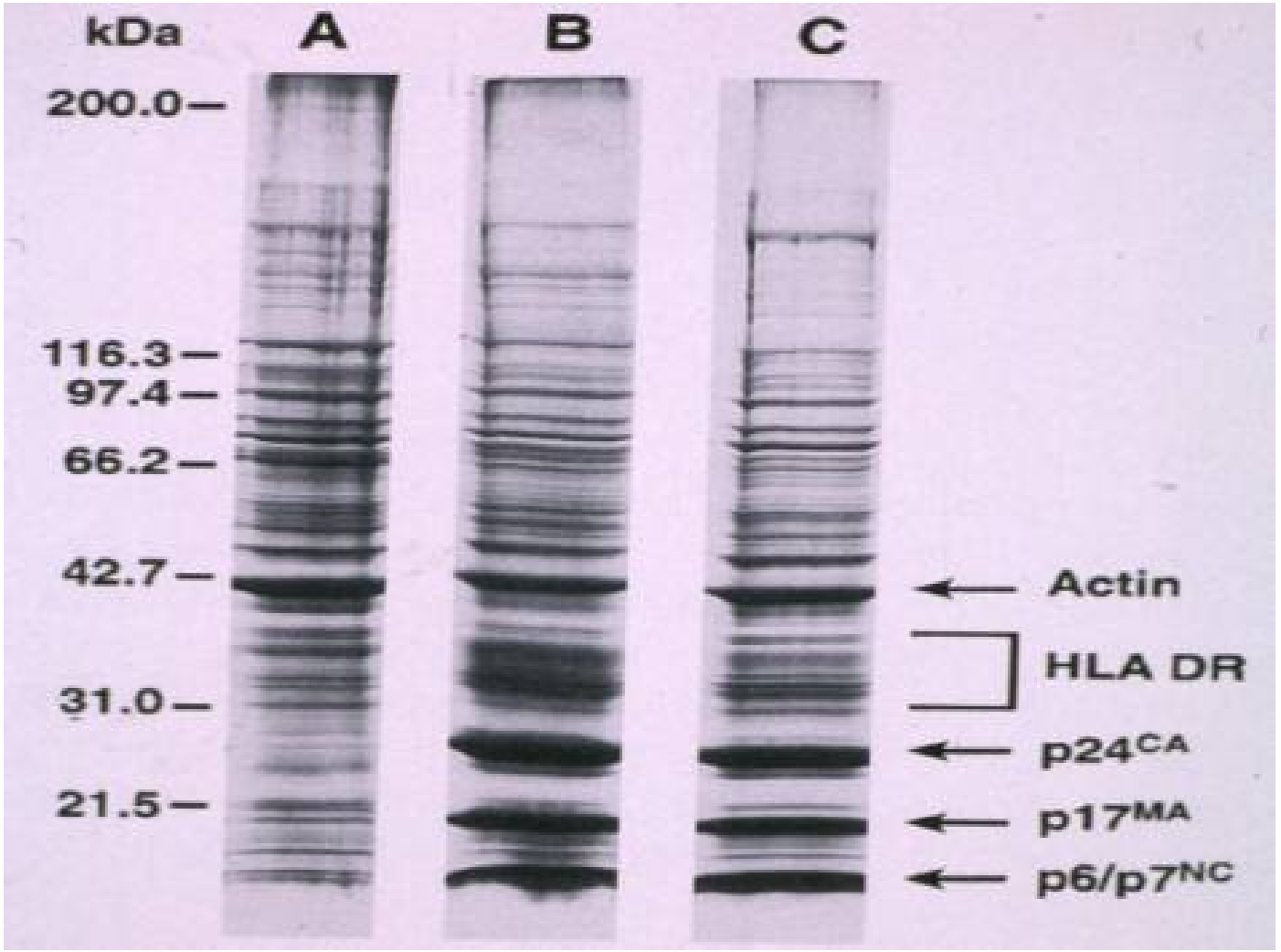
Solution: stacking gel (low conc^{n} of acrylamide, more acid, Cl^{-} ions).



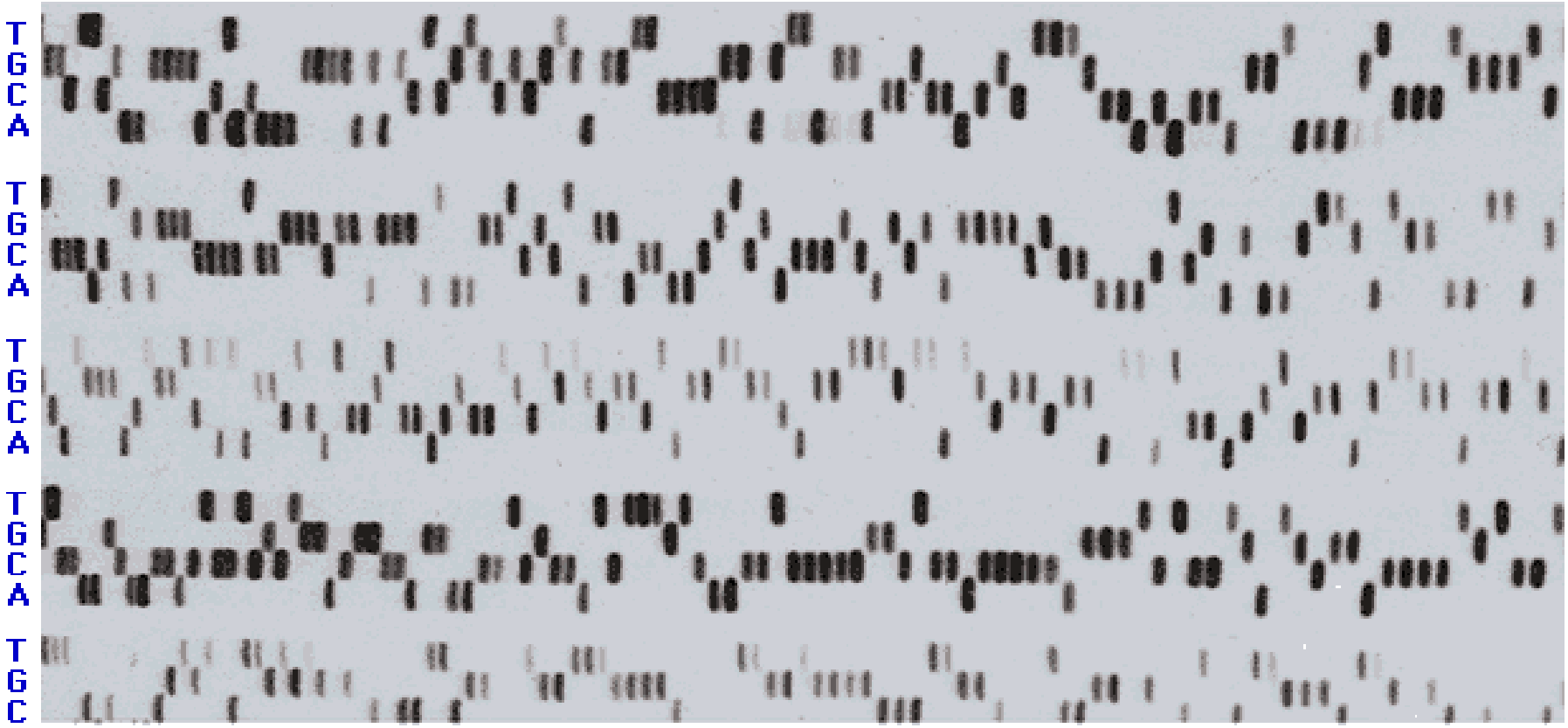
Here, pH rises, glycine becomes charged, and conductivity rises: proteins are seived by gel.






kDa
A
B
C
200.0
116.3
97.4
66.2
42.7
31.0
21.5
Actin
HLA DR
p24CA
p17MA
p6/p7NC

PAGE can also be used for short pieces of DNA

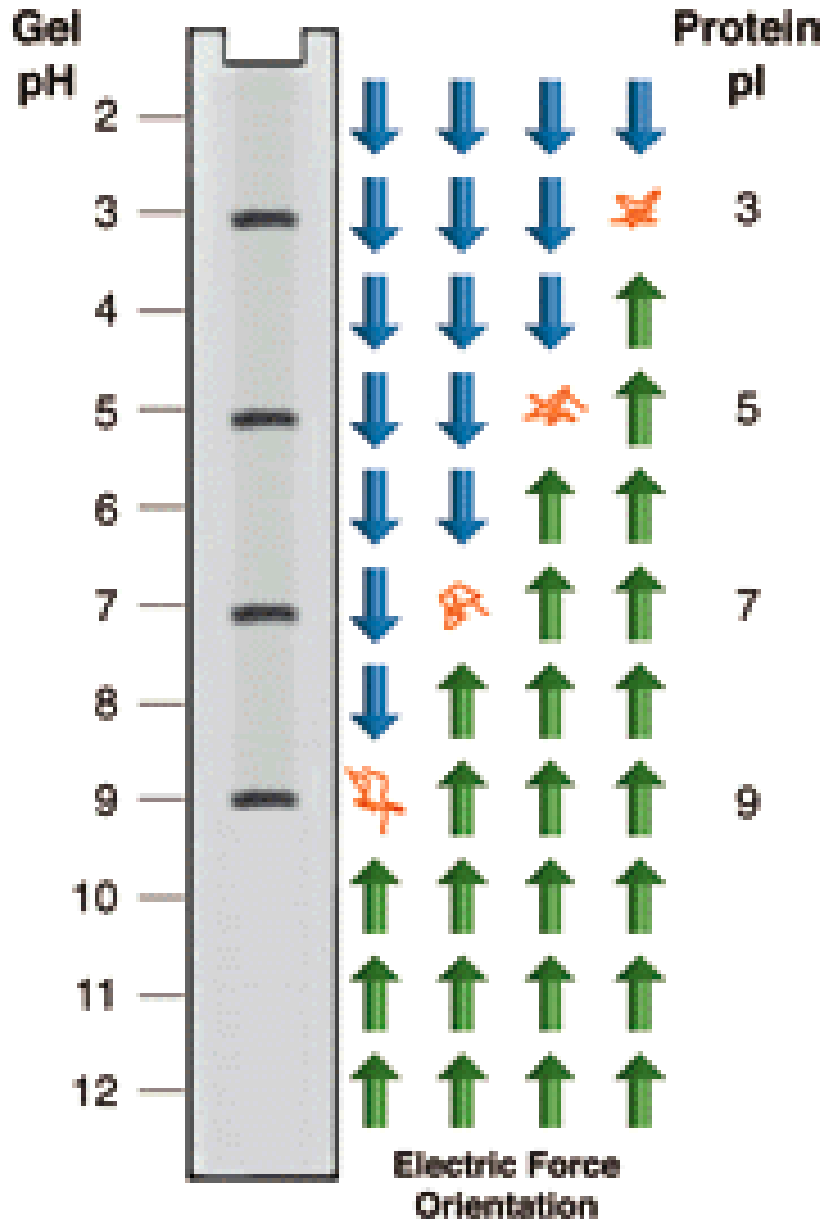


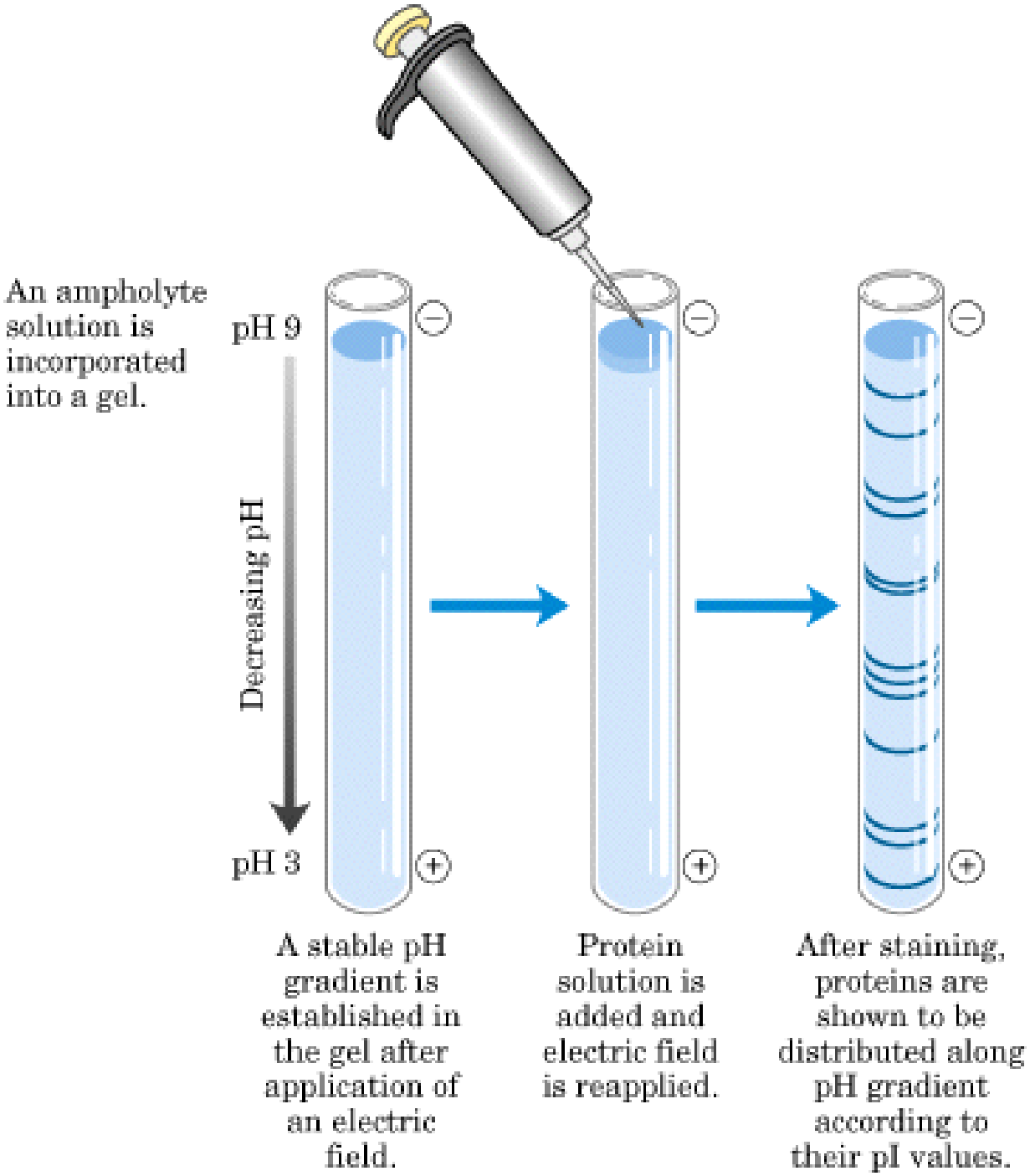
Isoelectric Focusing

- The pI of a protein is the pH at which it carries no net charge.
- (more acid than the pI, and the basic groups become NH_3^+ and the protein becomes positive: less acid than the pI, and the COOH groups become COO^- and the protein becomes negative).

How to use this:

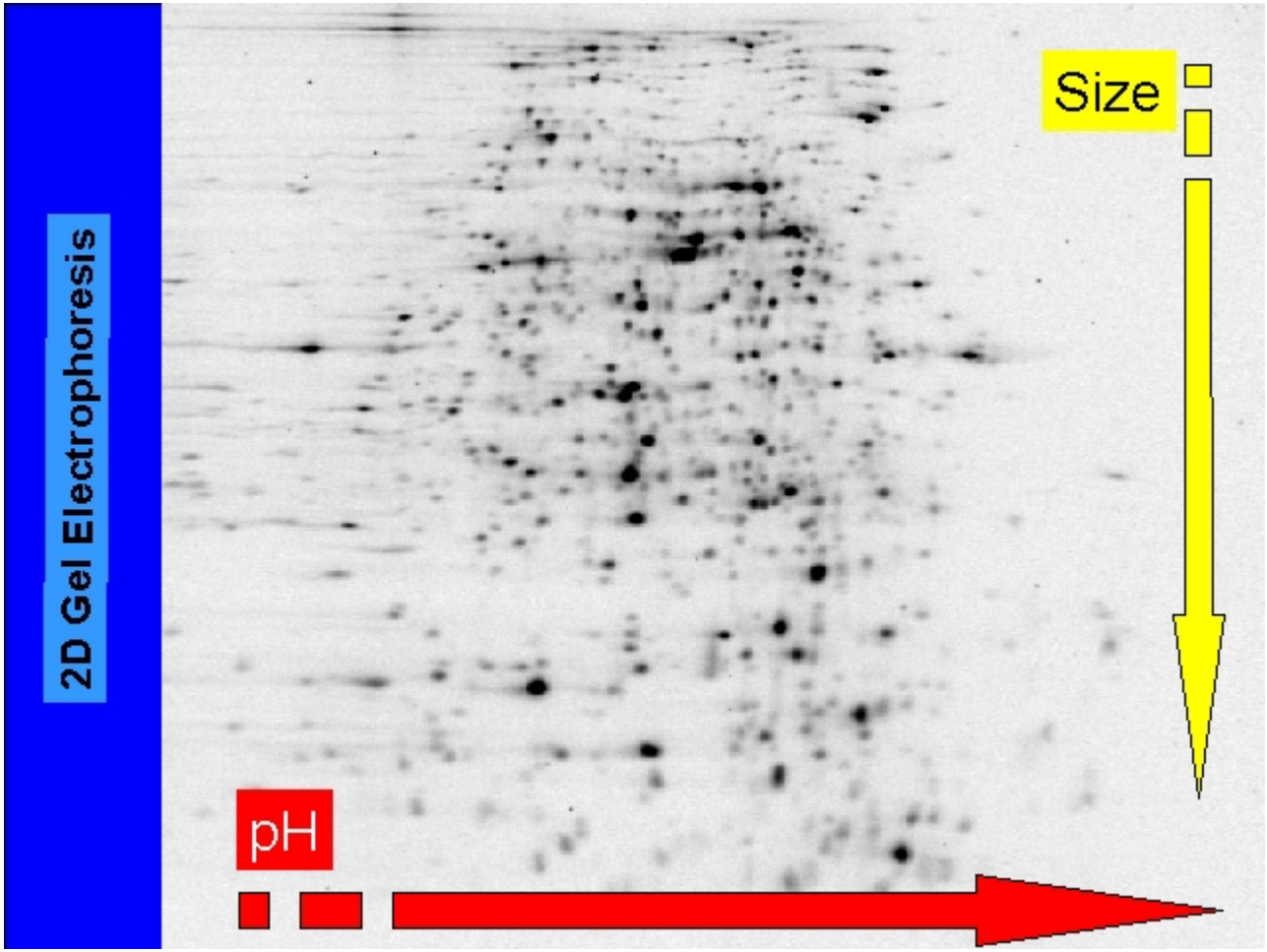
- Make a gel with a gradient of pH
- Apply an electric field, positive to alkaline end.
- Proteins negatively charged will be pulled towards the positve electrode until they meet the part of the gel where pH=pI.
- Proteins positively charged will be pulled towards the negative electrode until they meet the part of the gel where pH-pI

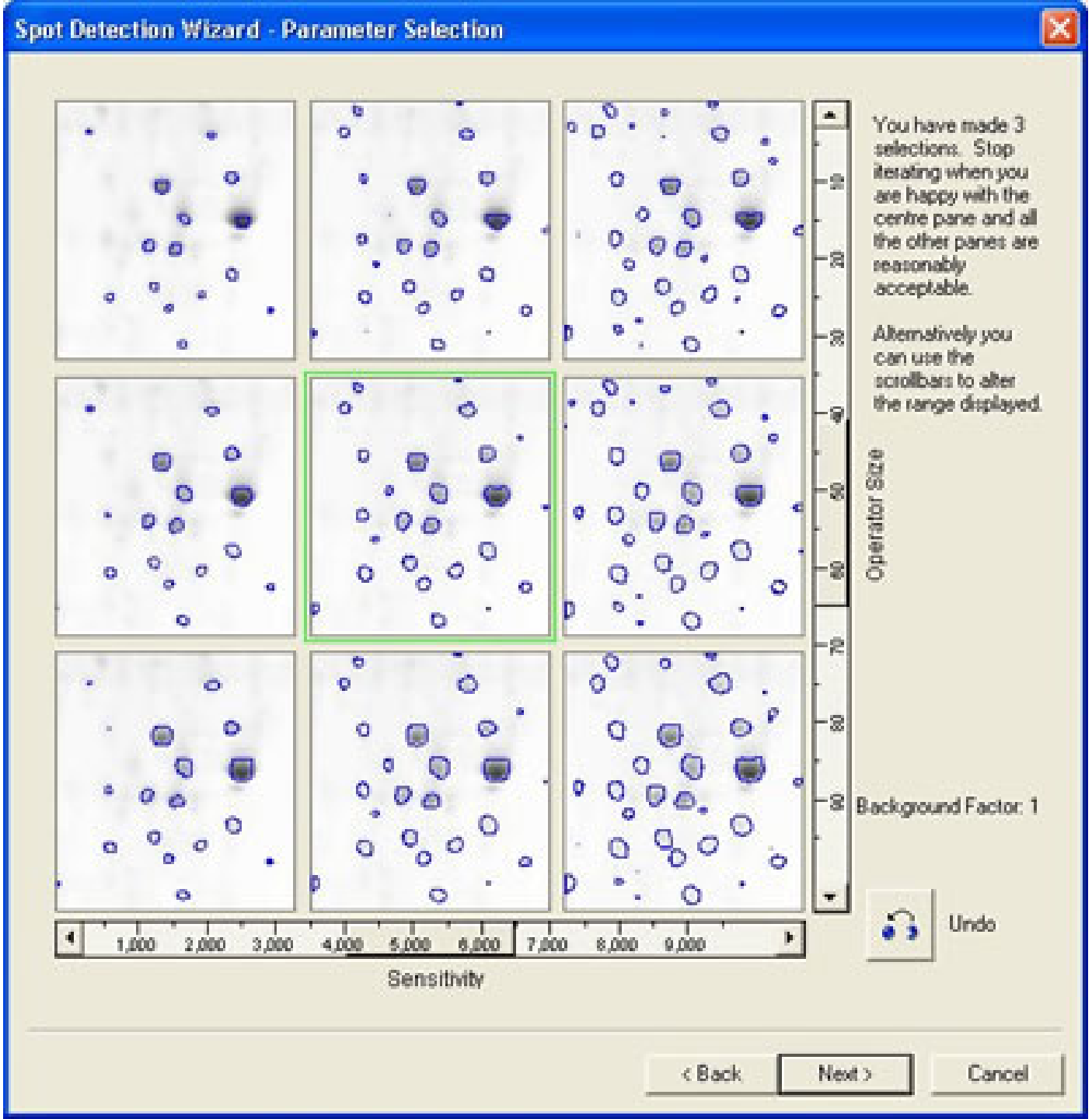

Gel
pH
2
3
4
5
6
7
8
9
10
11
12
Protein
pI
3
5
7
9
Electric Force
Orientation


An ampholyte solution is incorporated into a gel.
pH 9
Decreasing pH
pH 3
A stable pH gradient is established in the gel after application of an electric field.
Protein solution is added and electric field is reapplied.
After staining, proteins are shown to be distributed along pH gradient according to their pI values.

2D electrophoresis

- Make a rod gel and conduct isoelectric focussing.
- Lay the rod on top of a 2D SDS gel
- Run the gel.




Spot Detection Wizard - Parameter Selection
You have made 3 selections. Stop iterating when you are happy with the centre pane and all the other panes are reasonably acceptable.
Alternatively you can use the scrollbars to alter the range displayed.
Operator Size
Background Factor: 1
Undo
1,000
2,000
3,000
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7,000
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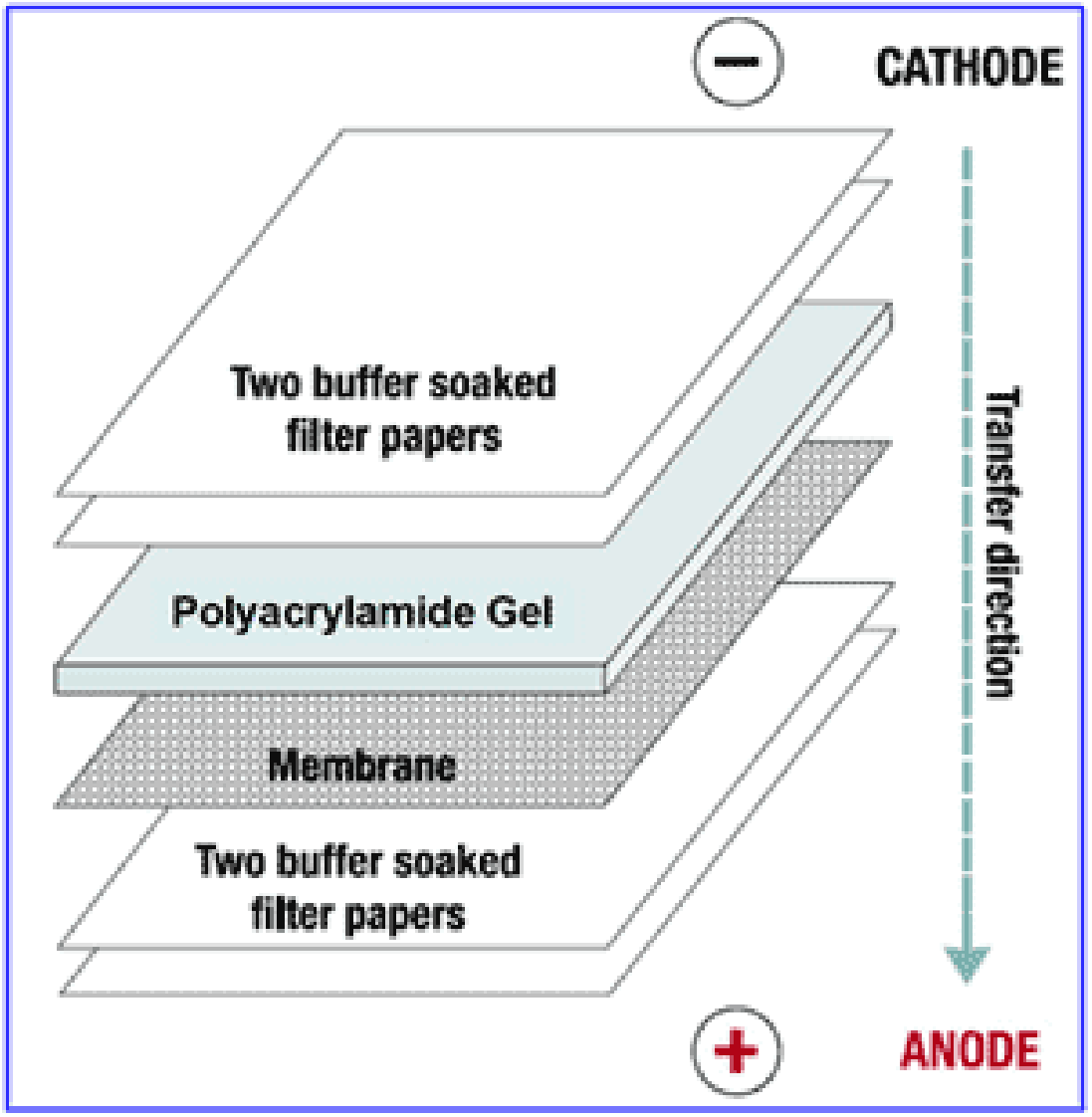
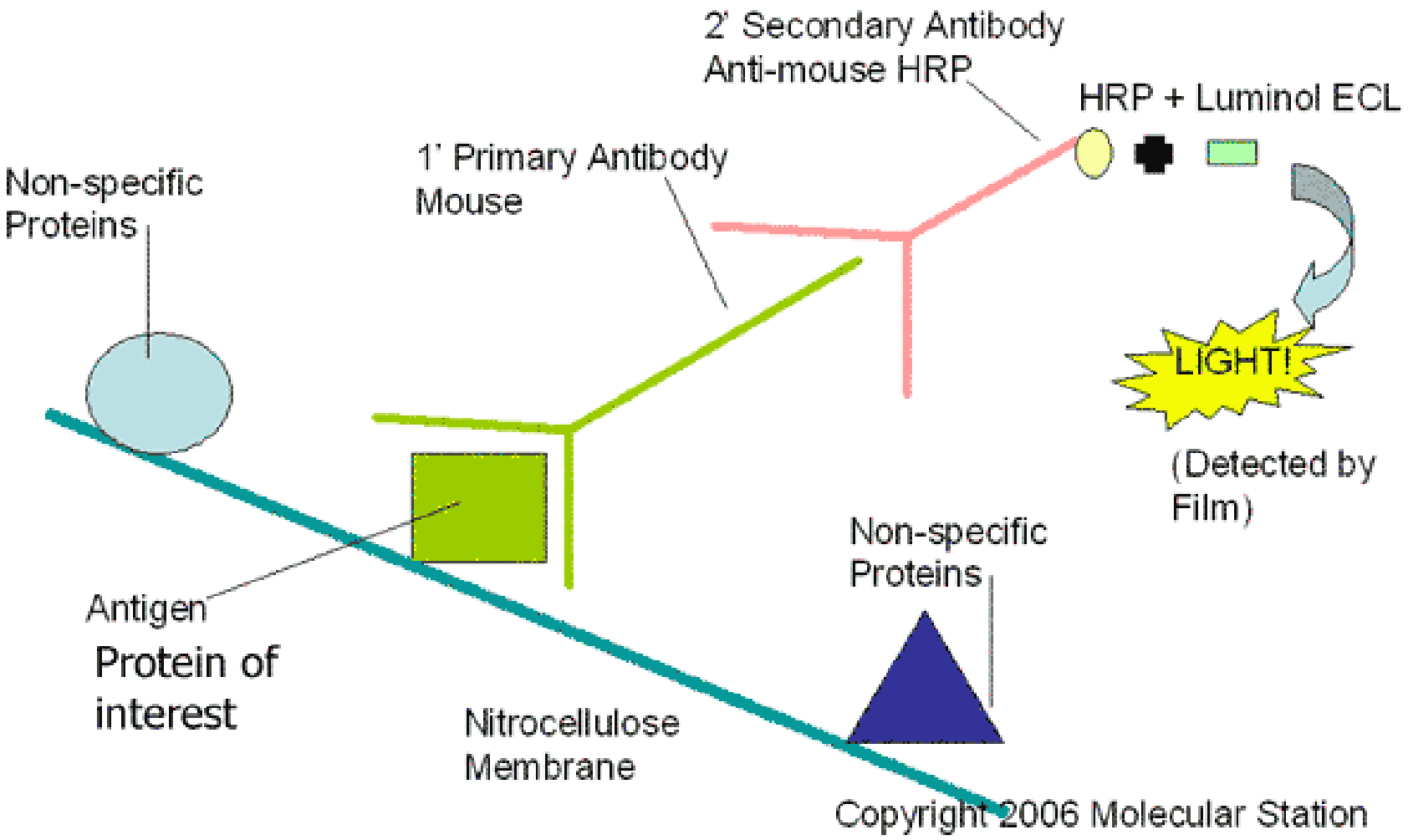
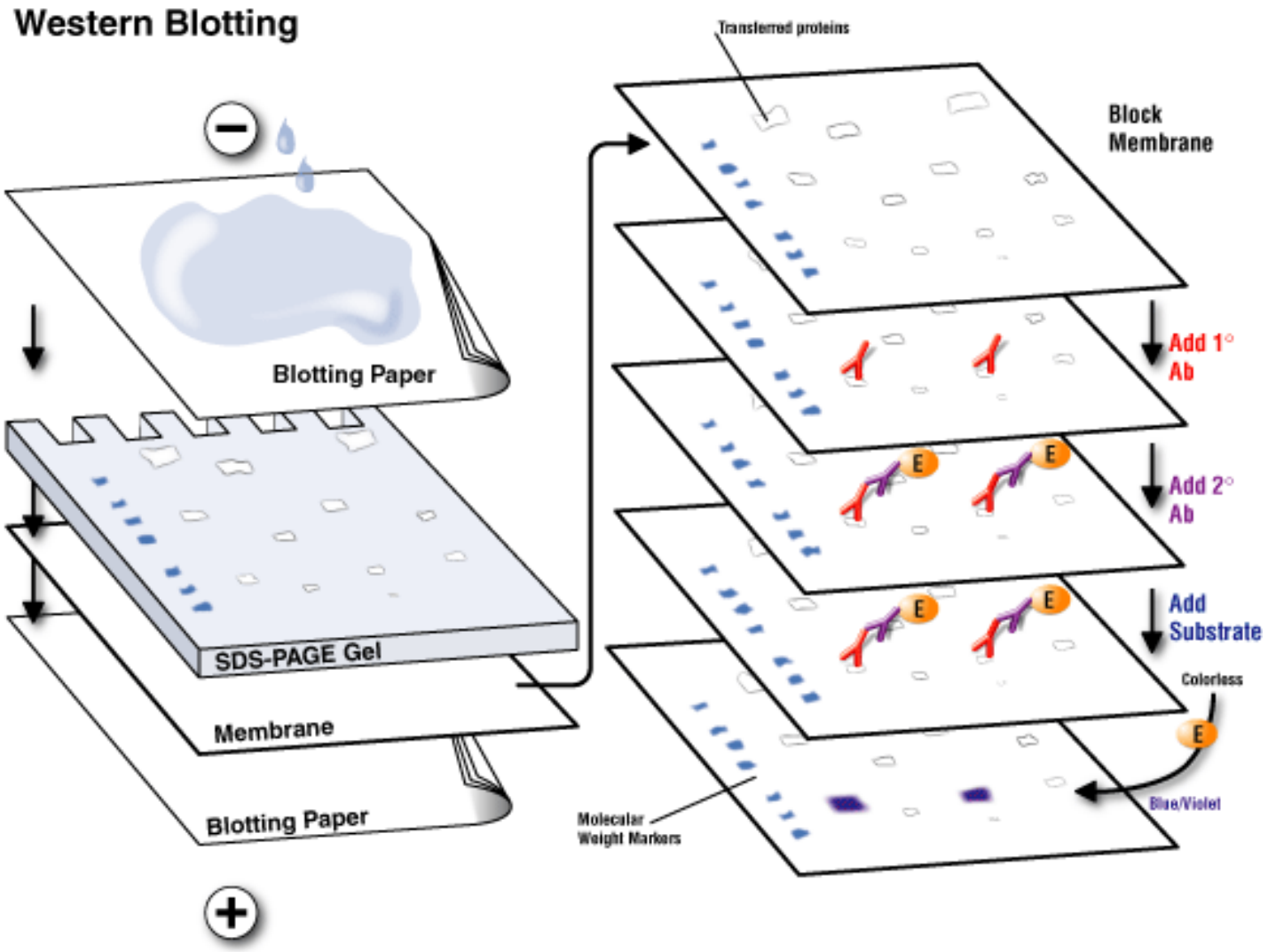
Sensitivity
< Back
Next >
Cancel

Western Blotting




2' Secondary Antibody
Anti-mouse HRP
HRP + Luminol ECL
1' Primary Antibody
Mouse
Non-specific
Proteins
LIGHT!
(Detected by
Film)
Non-specific
Proteins
Antigen
Protein of
interest
Nitrocellulose
Membrane
Copyright 2006 Molecular Station

Blotting

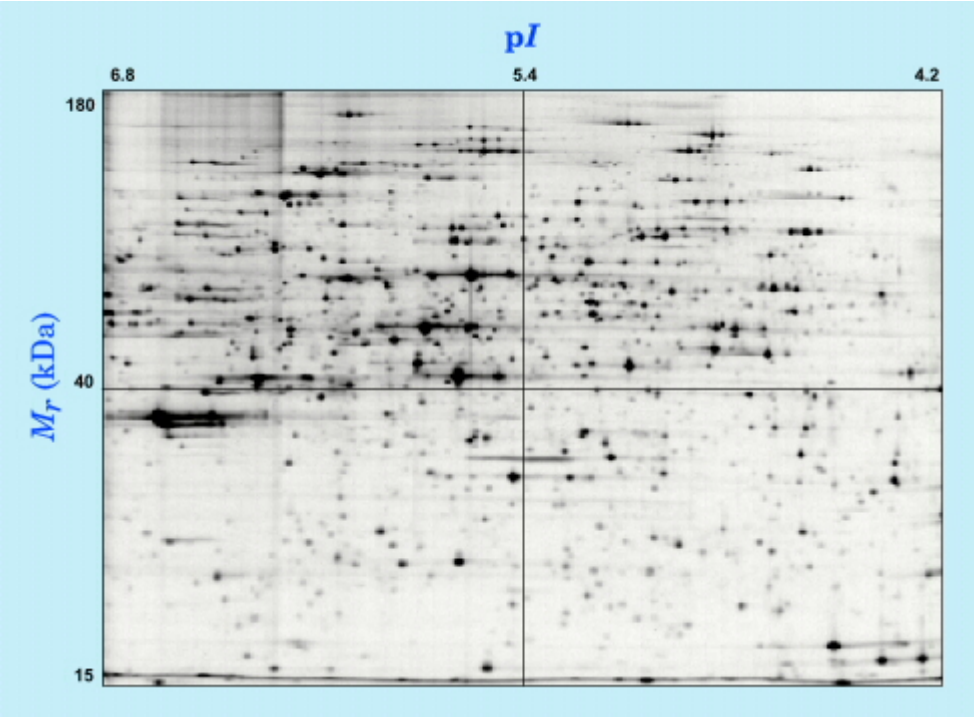

Western Blotting
Transferred proteins
Block Membrane
Add 1° Ab
Add 2° Ab
Add Substrate
Colorless
Blue/Violet
Blotting Paper
SDS-PAGE Gel
Membrane
Blotting Paper
Molecular Weight Markers
E

There are databases:

Yeast 2D Map

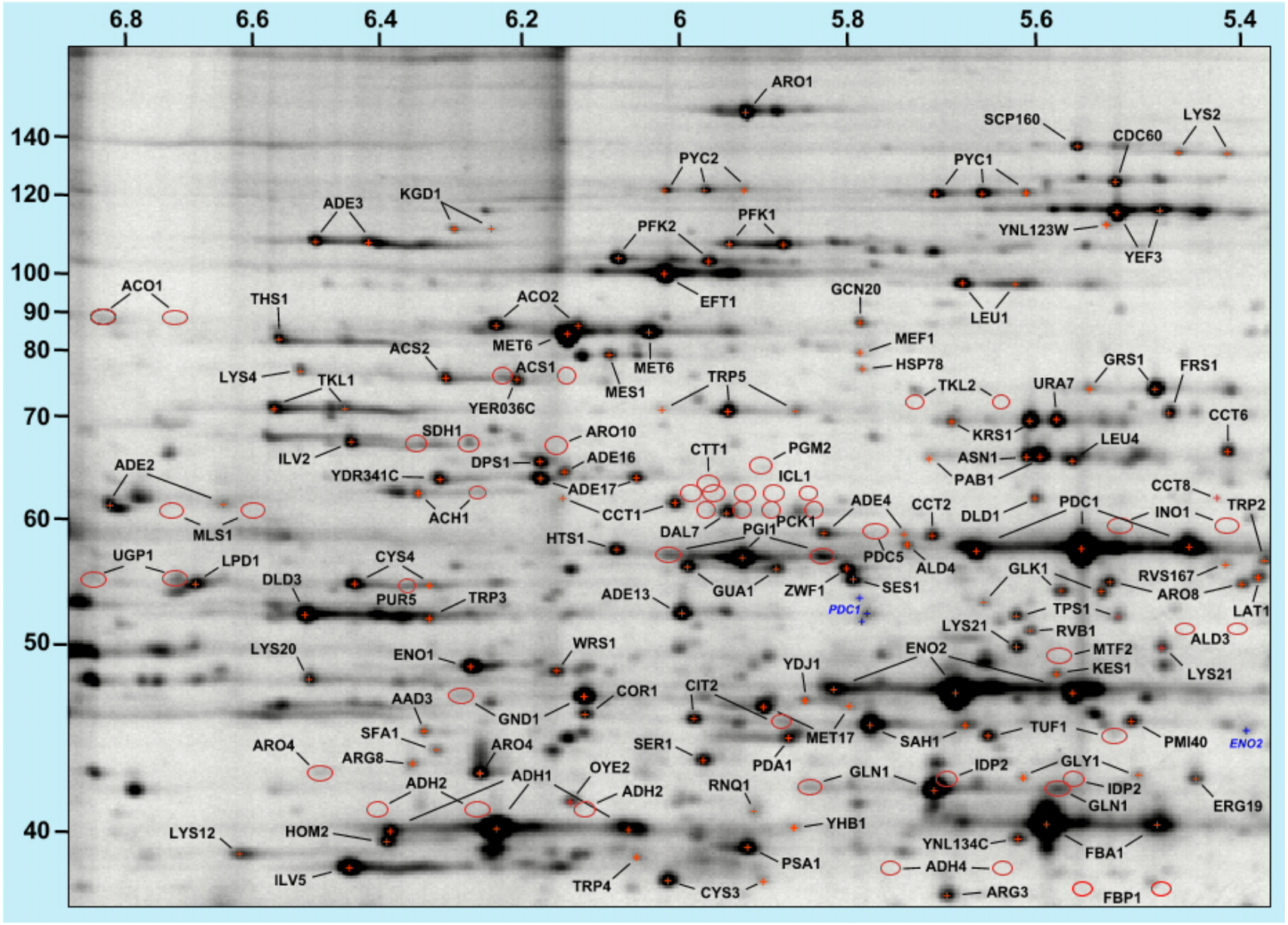
The pattern shown corresponds to the soluble proteins synthesized by strain S288C exponentially growing on glucose. First dimension was carried out using ampholytes. Second dimension was run on 11% slab gel.

Click on one of the four parts of this map, and you will obtain a zoom of it:



http://www.ibgc.u-bordeaux2.fr/YPM/carte.htm

Click on a spot to get information about it (blue crosses correspond to protein fragments)



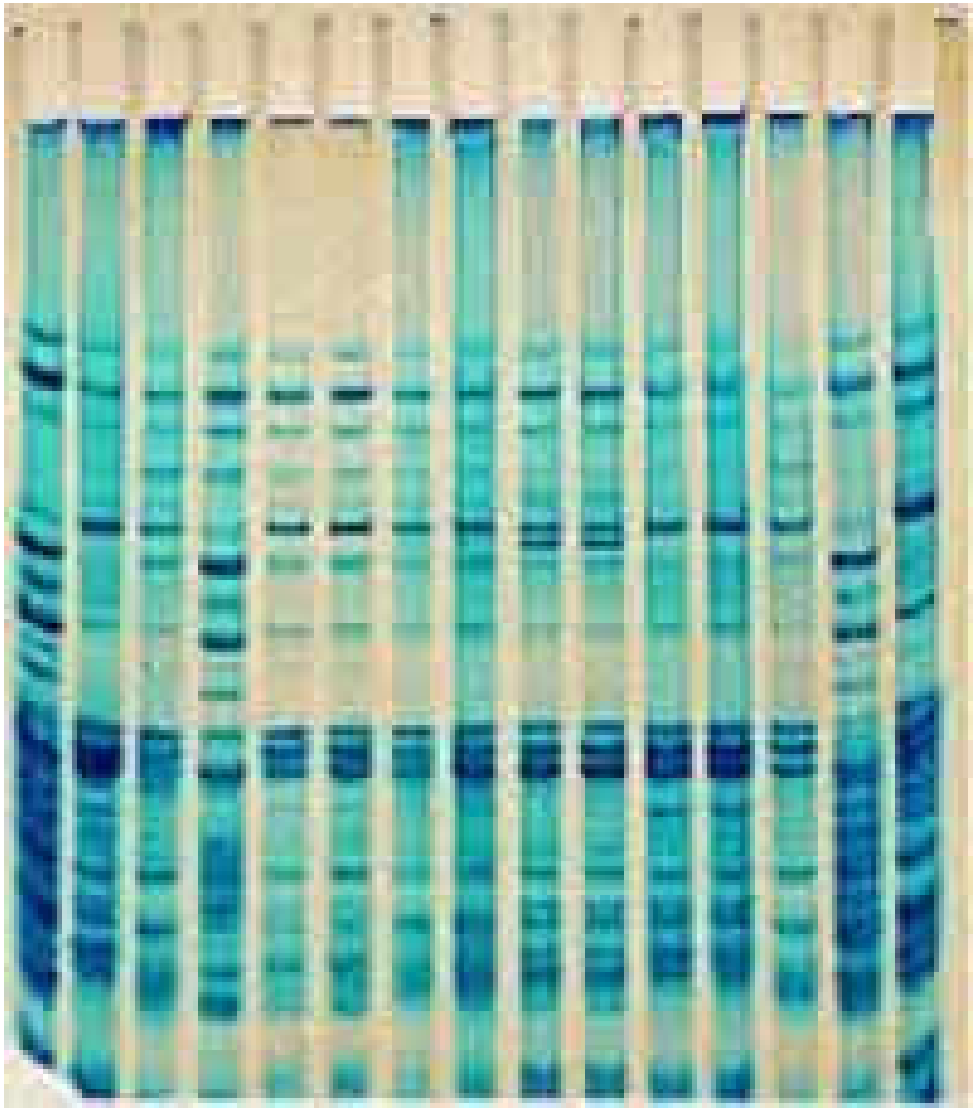
Spot report

Spot number :	B48C95
Gene :	MET6
CBI / CAI :	0.77 / 0.66
Protein :	homocysteine methyltransferase
SWISSPROT :	P05694
SGD :	S000000893
Acetylation :	non acetylated
NH2 aa removed :	1
Apparent pI :	6.15
Calculated pI :	6.01
Apparent Mr :	84100
Calculated Mr :	85727
Localisation :	cyt
Identification method :	sequencing, aa ratio, MS
Physiol. behaviour :	

Home | Entire Map | Gene List

Problems:

- **Anomalous MW:** (eg tubulin – usually caused by high endogenous charge or non-stoichiometric binding of SDS)
- **Poor focus of bands:** (usually because you messed up the stacking gel or buffer in some way, or tried to re-use a gel)
- **Smiles**: either you managed to compress the middle of the gel (thinner, lower conductance so higher voltage) or you got it hot.
- **Precipitation as the sample goes in**: You have KCl in your sample (it makes SDS precipitate)



Problems: PAGE

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- **Precipitation as the sample goes in**: You have KCl in your sample (it makes SDS precipitate)
- **Bands all clustered together**: wrong concentration of acrylamide

Problems: Western Blots

- **No protein on blot:** wrong polarity, buffer or time. Try staining the gel afterwards to see if you didn't move them, or if they went right through.
- **High background on probed blot**: insufficient blocking, or too much antibody. First do a titration of secondary only, having run the primary in the gel and blotted it. The titrate the primary on the real samples in the gel.

Electrophoresis sniglets

- **Multiple agarasms**: the need to pour a gel many times, because it keeps leaking (usually into the draw below the bench) before it has set.

- **Retrophoresis**: running a gel backwards due to mis-connection of the electrodes or a mis-set polarity switch on the power lack.

- **Broadbanding**: failure of focus (usually a problem with the stacking gel)

- **Aeroblot:** a blot that has failed due to bubbles being trapped between layers

Good luck !!!