Protein Electrophoresis protocol

Ameer Effat M Elfarash

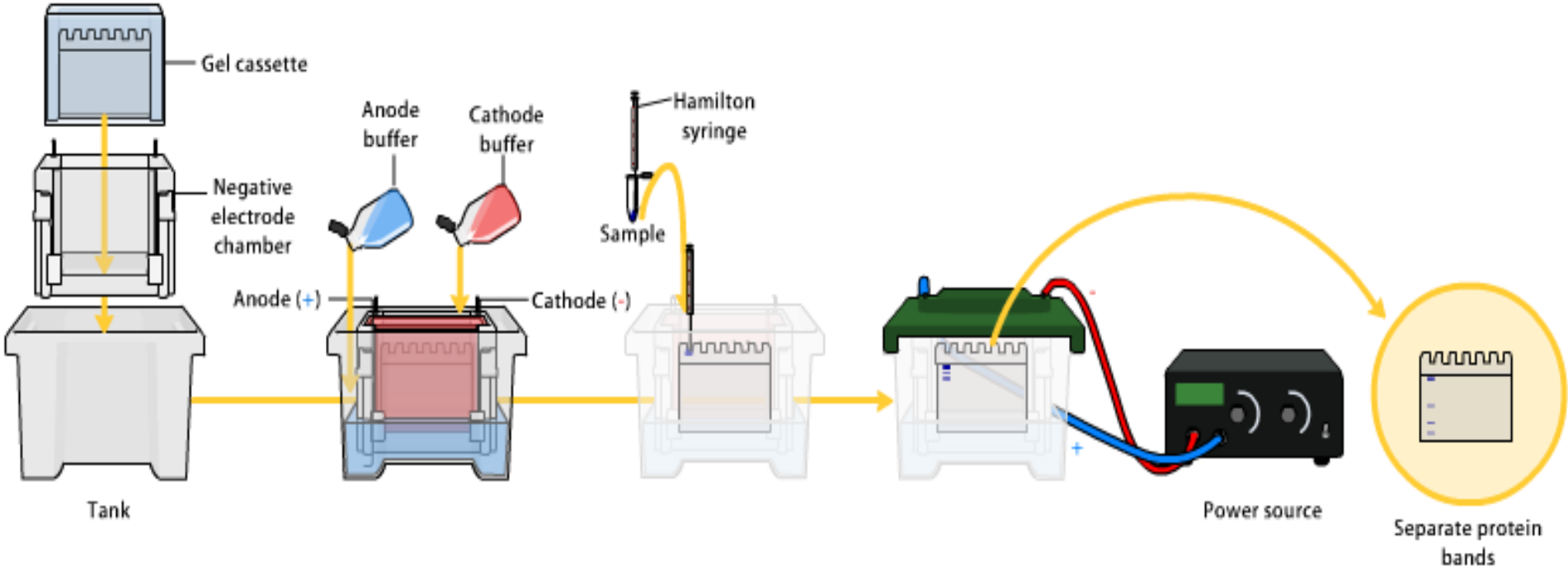
Dept. of Genetics
Fac. of Agriculture, Assiut Univ.

aelfarash@aun.edu.eg

SDS-PAGE purposes

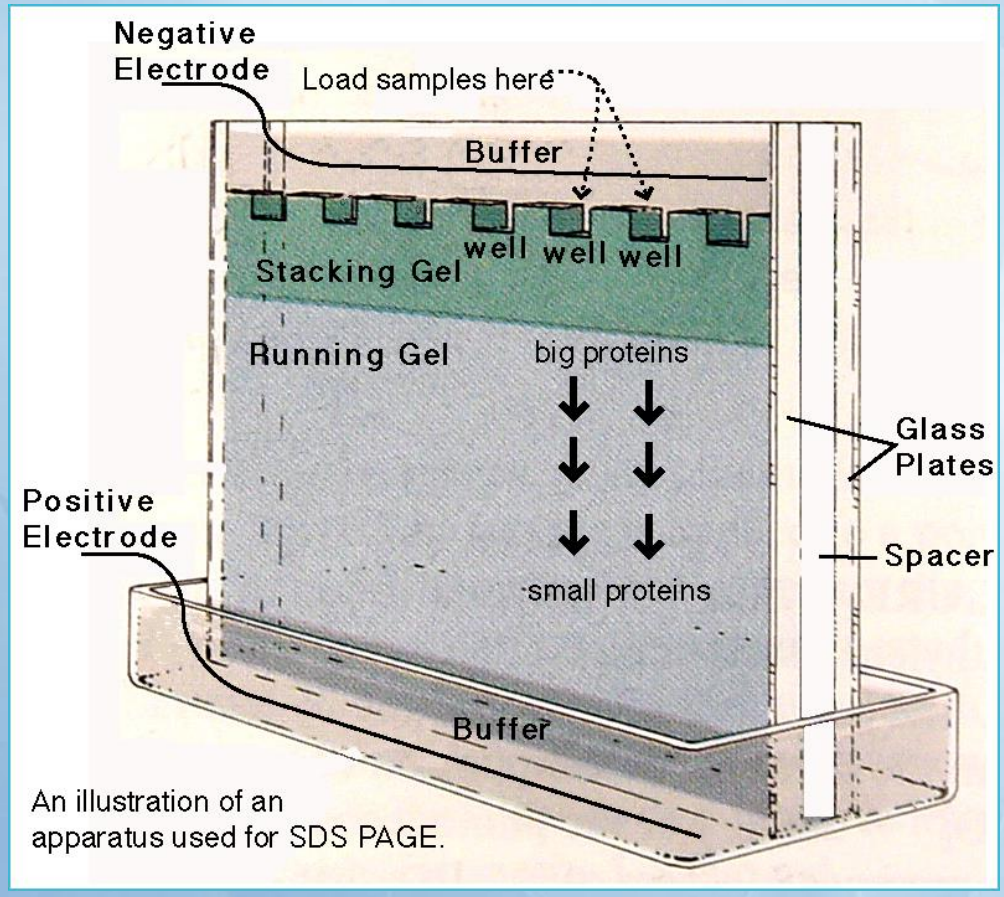
- To separate protein molecules on the basis of molecular weight
  - May then be electroblotted for immunoanalysis
- To determine the molecular weights of unknowns by comparison to standards

Procedure in Short



Principle

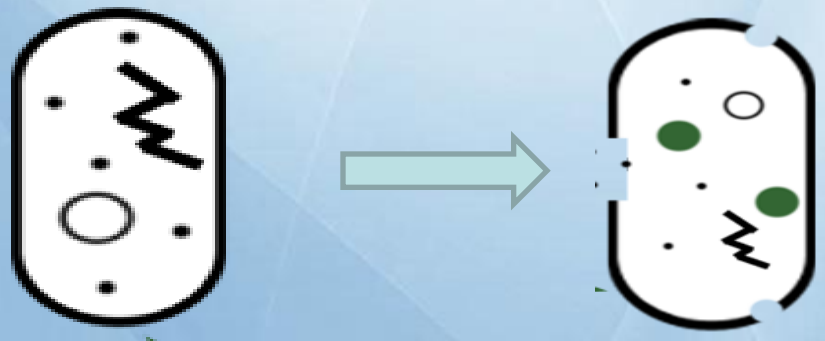
Proteins move in the electric field. Their relative speed depends on the charge, size, and shape of the protein



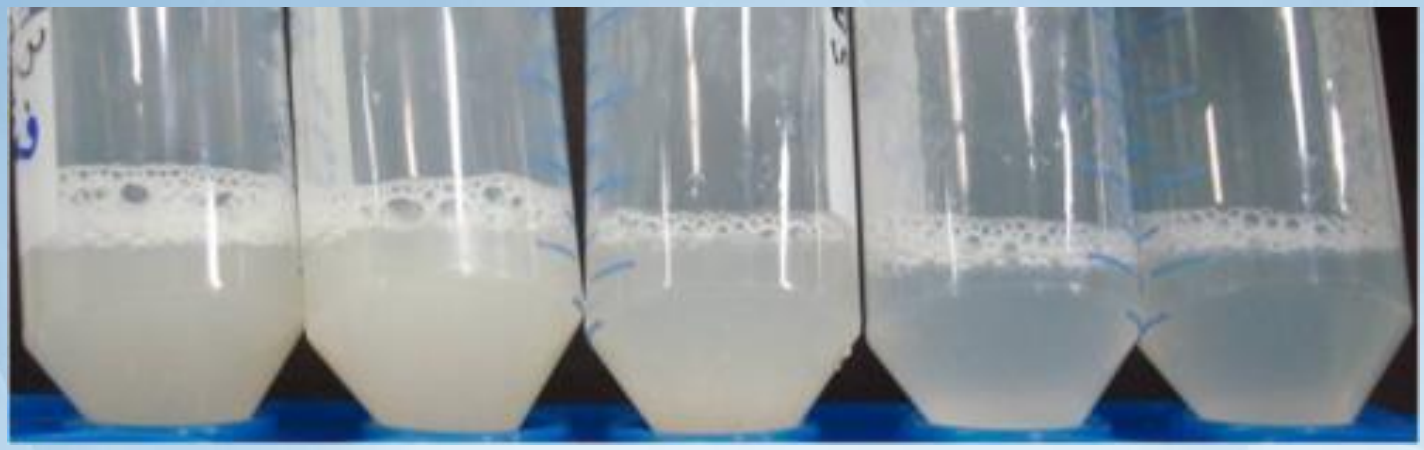
An illustration of an apparatus used for SDS PAGE.

Preparation of cell extract

- Lyse cells in a lysis buffer containing inhibitors of proteases.
  - Chemical (Lysis buffer)
  - Mechanical
- Centrifuge lysate to remove membranous cellular debris.
- Determine protein concentration in μg/μl of the lysate.



Loading buffer

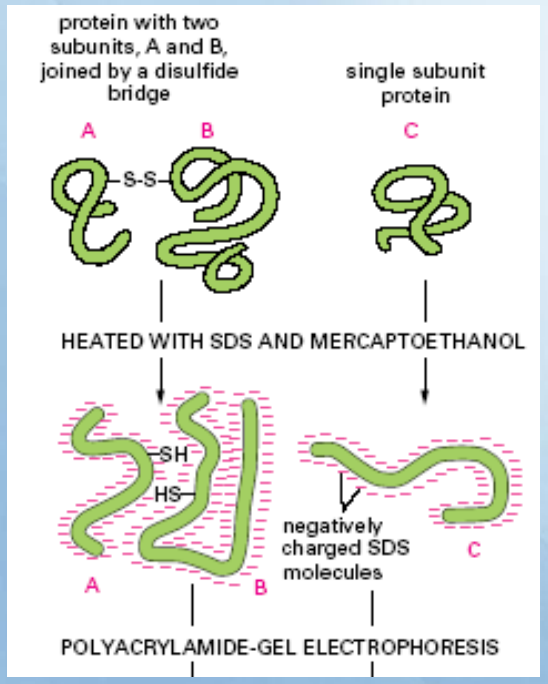


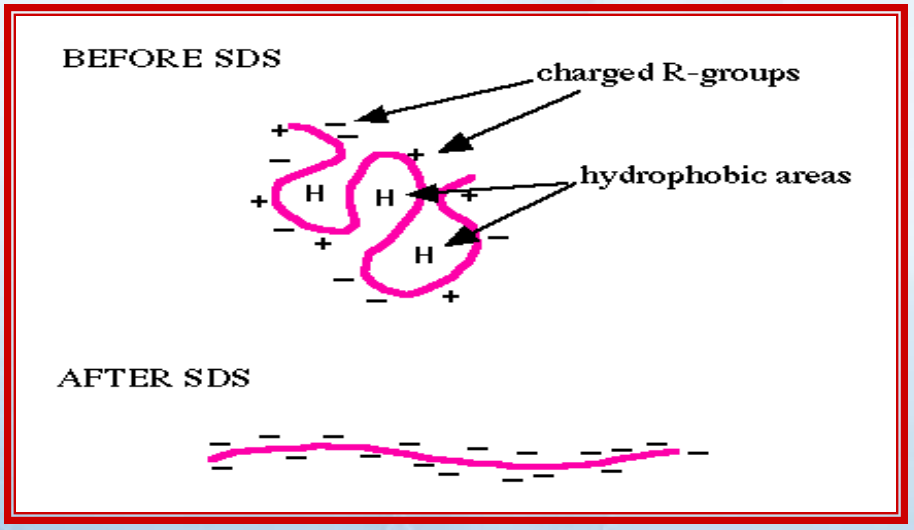
- Major components of the sample loading "buffer"
  - SDS
  - DTT (or β -mercaptoethanol)
  - Tracking dye
  - Glycerol

Function Of SDS

 # Solubilizes and denatures proteins
 # Negative charge to proteins

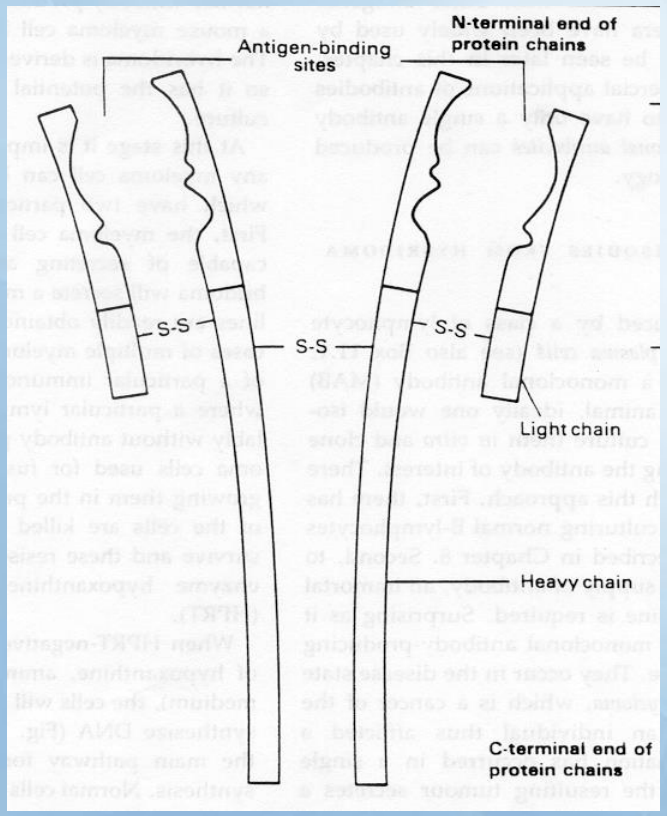
One SDS binds with every two Aas. Thus, each protein has a similar charge-to-mass ratio.



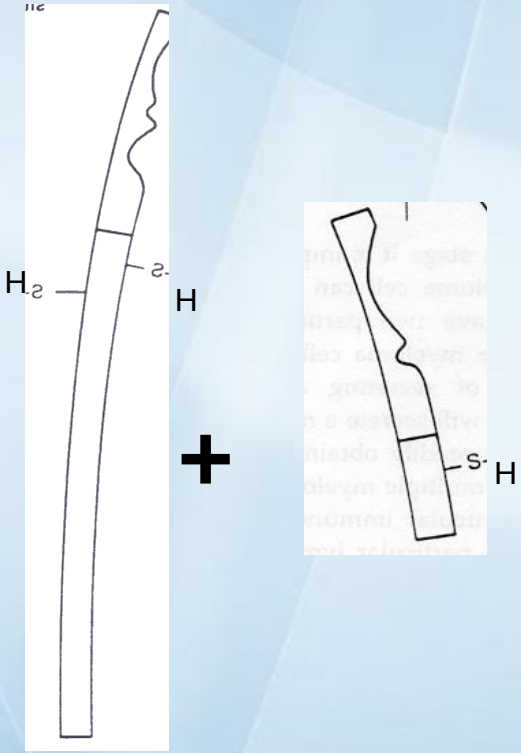


Function of DTT (or β -mercaptoethanol)

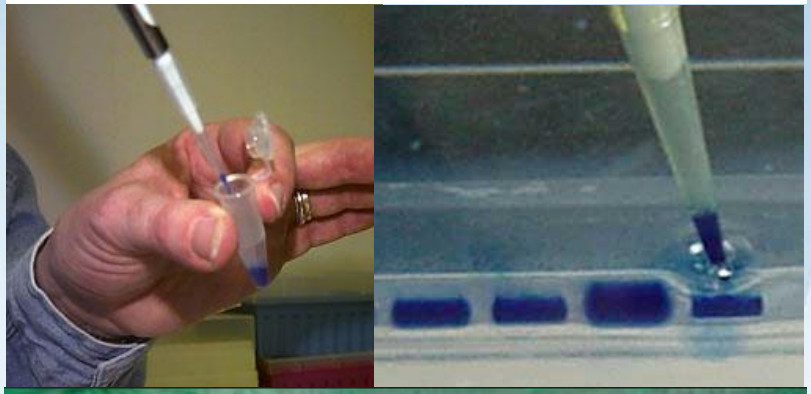
Causes disulfide bonded peptides to become independent



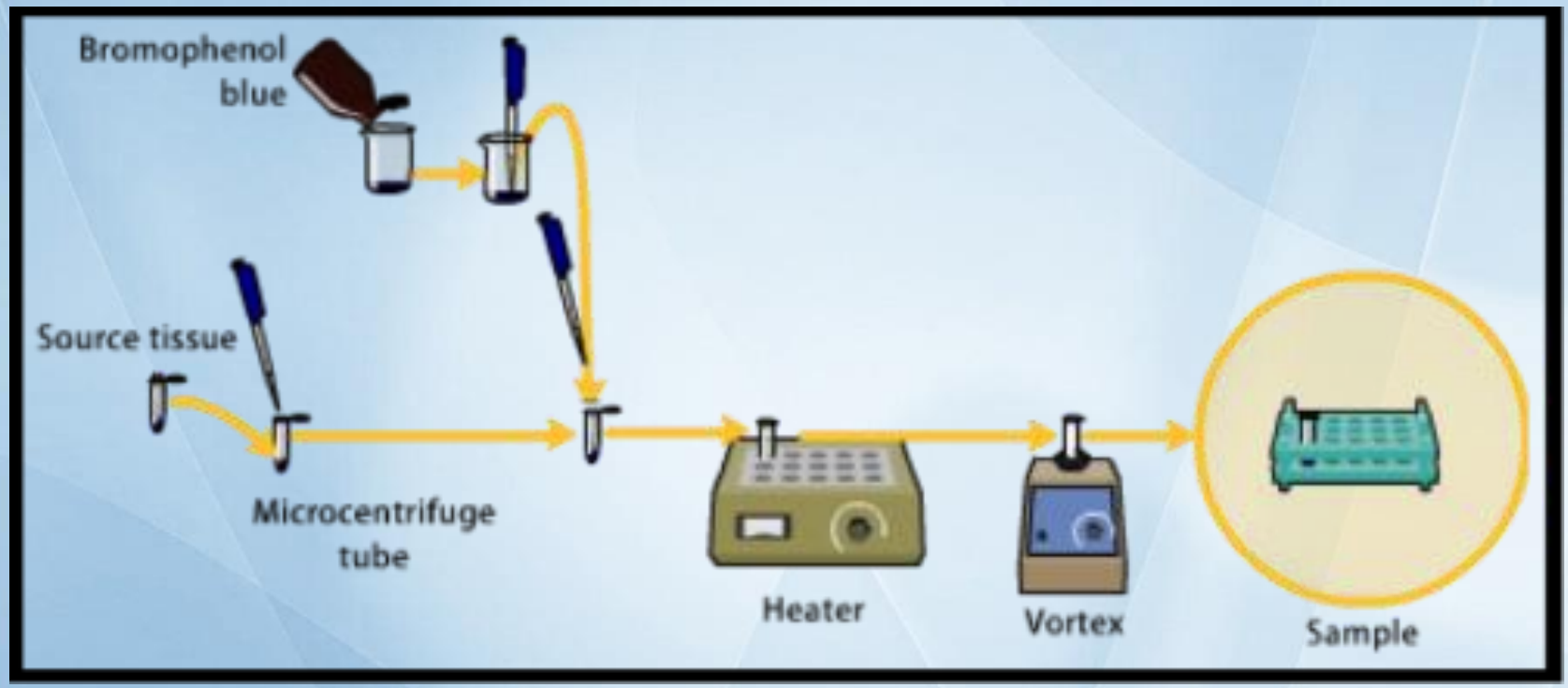
Heat
Excess DTT



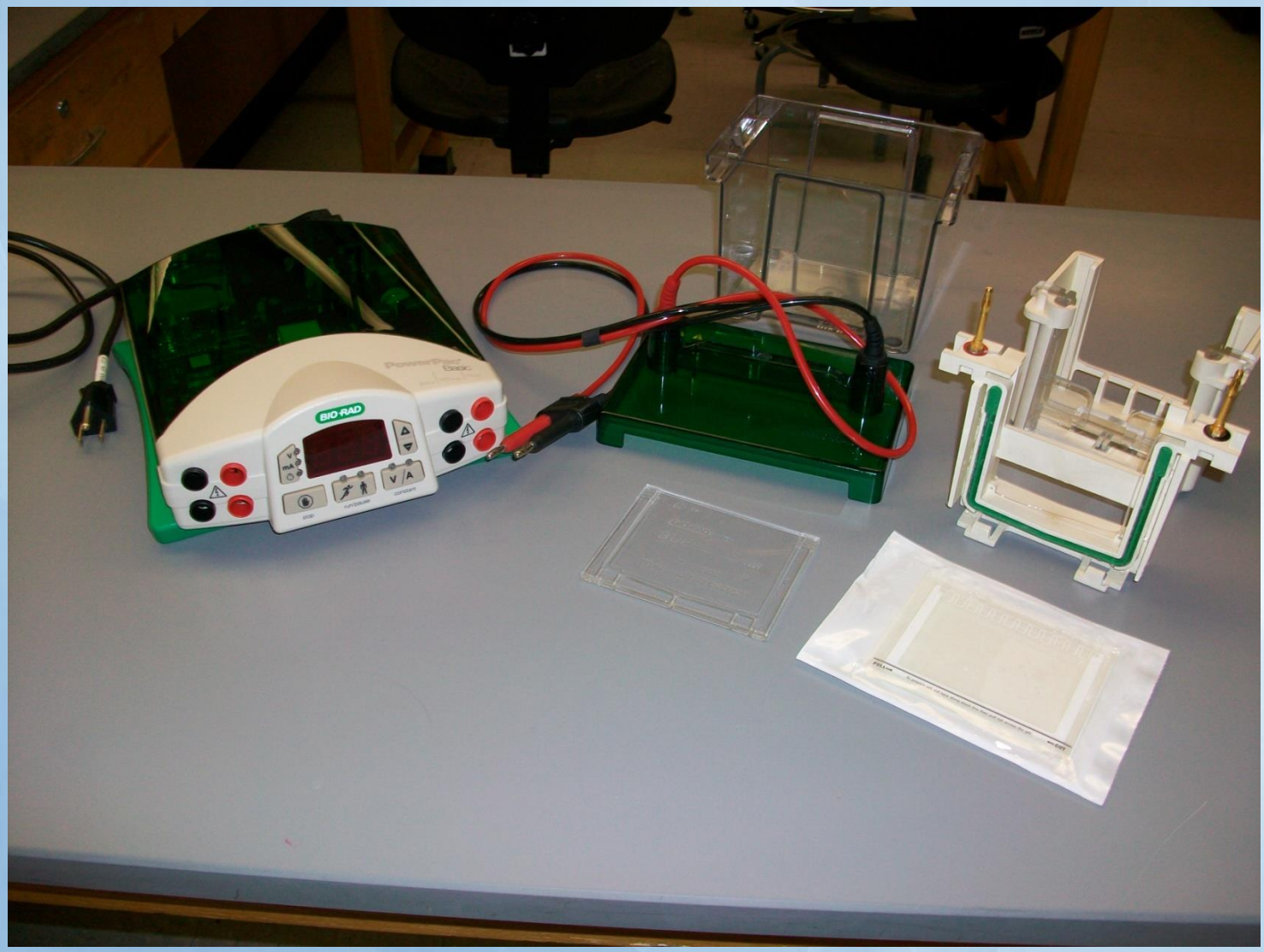
- Dye is included to monitor migration during PAGE
  - Bromphenol blue



- Glycerol is included to make sample denser than running buffer
  - minimizes diffusion during loading


Bromophenol blue
Source tissue
Microcentrifuge tube
Heater
Vortex
Sample

Equipment for Electrophoresis



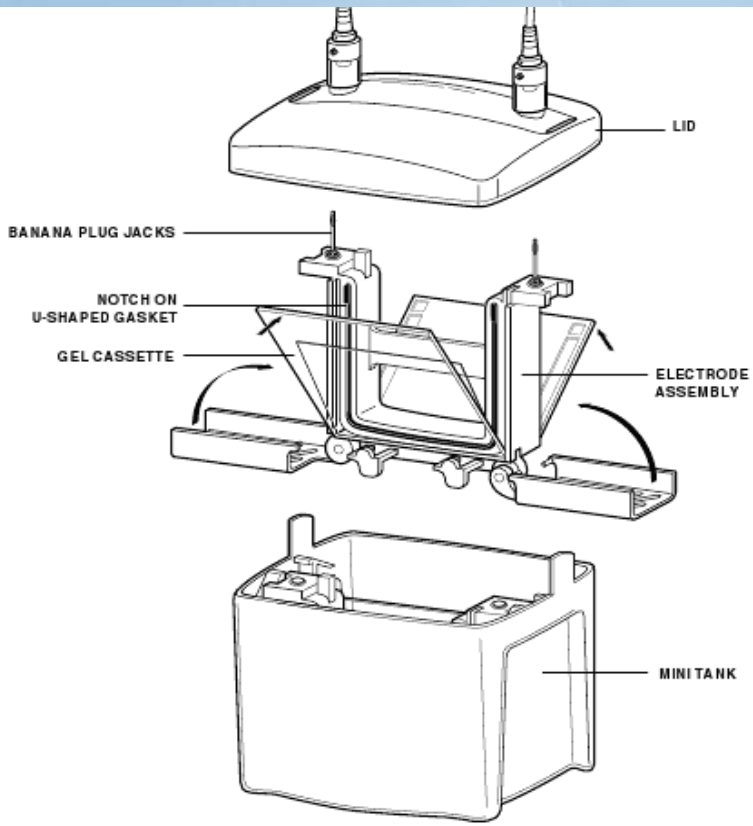


Fig. 1. Assembling the Mini-PROTEAN Tetra cell.

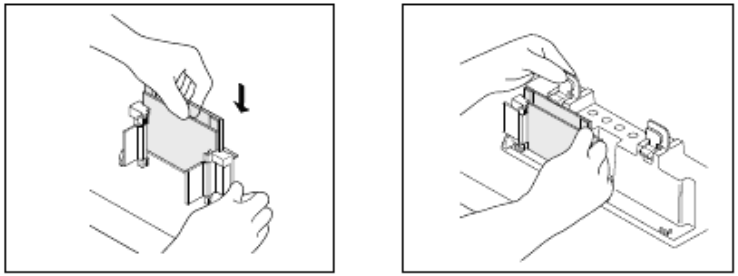
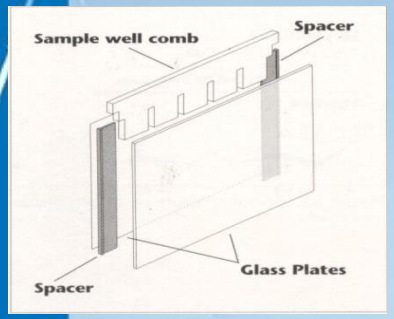
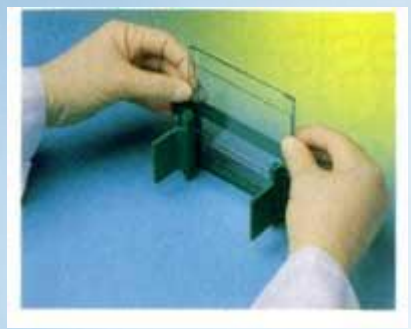


Fig. 2. Assembling the Mini-PROTEAN Tetra cell casting frame and casting stand.

Step by Step Instructions on how to assemble the polyacrylamide gel apparatus



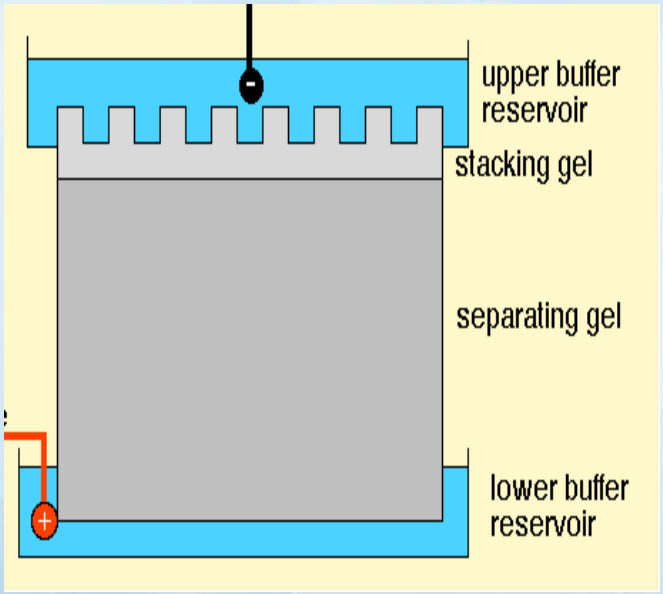


SDS-PAGE gel system

- **Stacking** (concentrating) gel
  - **4%** acrylamide (36:1, acryl/bis)
  - **125 mM** Tris-H^+Cl^-, **pH 6.8**, 0.1% SDS

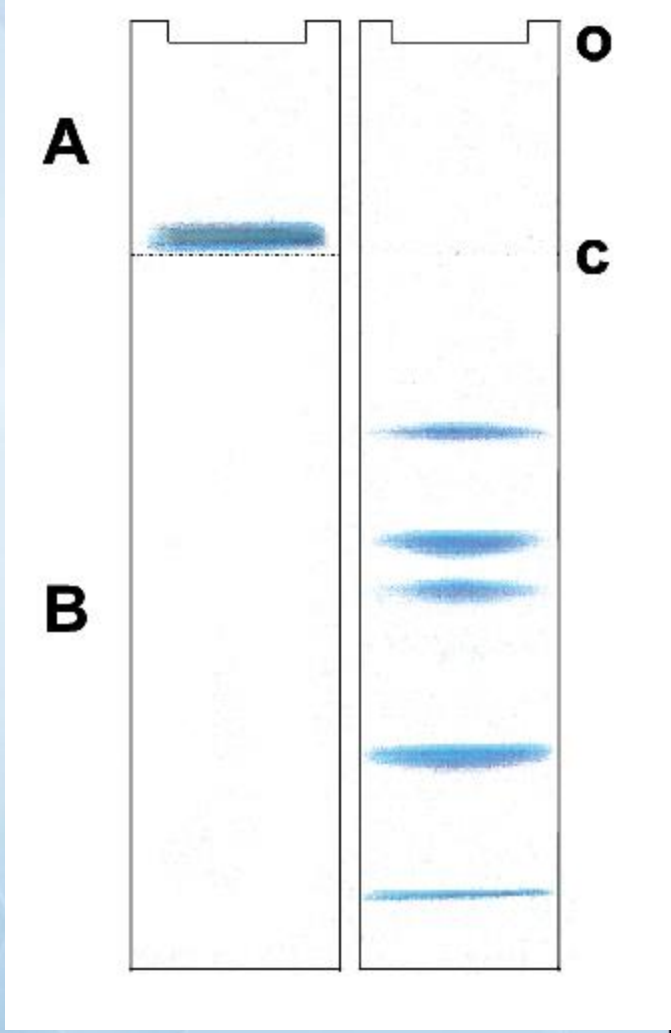
- **Resolving** (separating) gel
  - **8-15%** acrylamide (36:1, acryl/bis)
  - **425 mM** Tris-H^+Cl^-, **pH 8.8**, 0.1% SDS

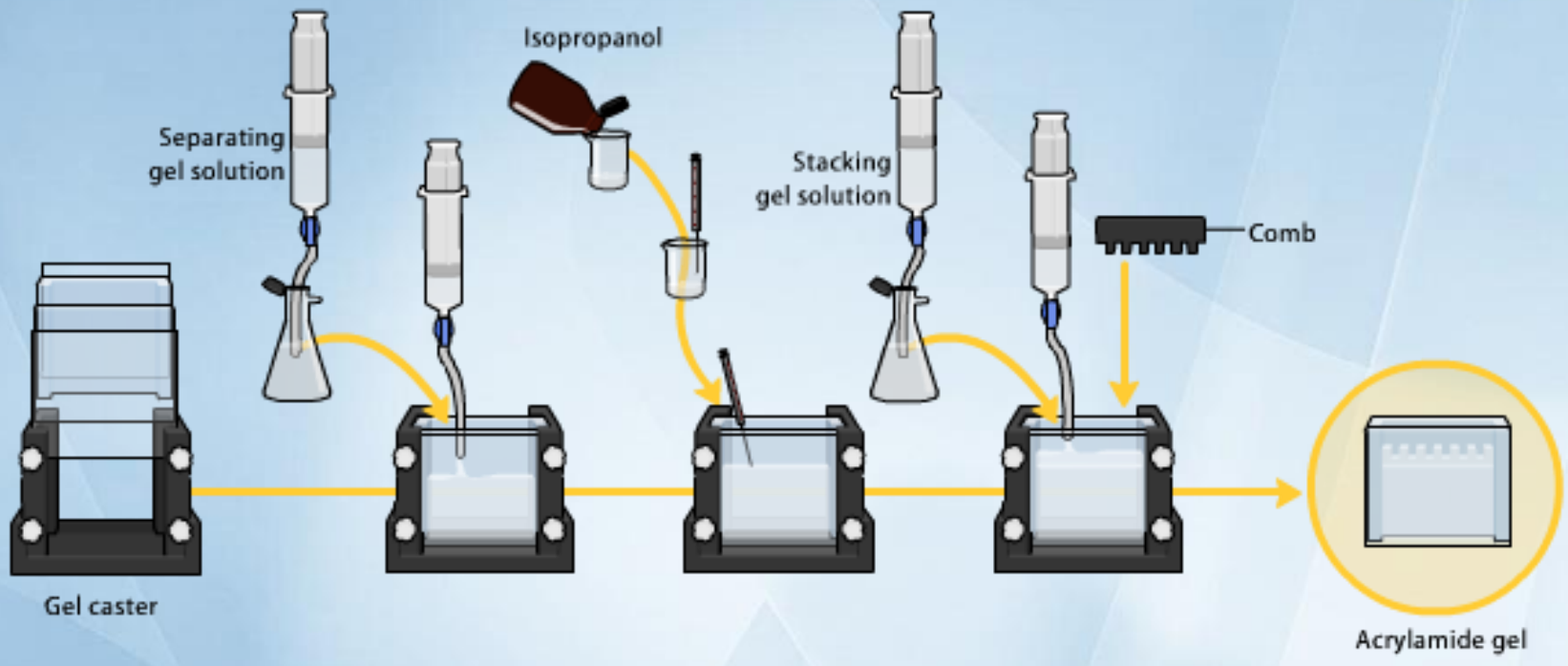
- **Running buffer**
  - 25 mM Tris base; **192 mM glycine**, pH 8.3; 1% SDS



Why use a discontinuous gel and buffer system for SDS-PAGE?

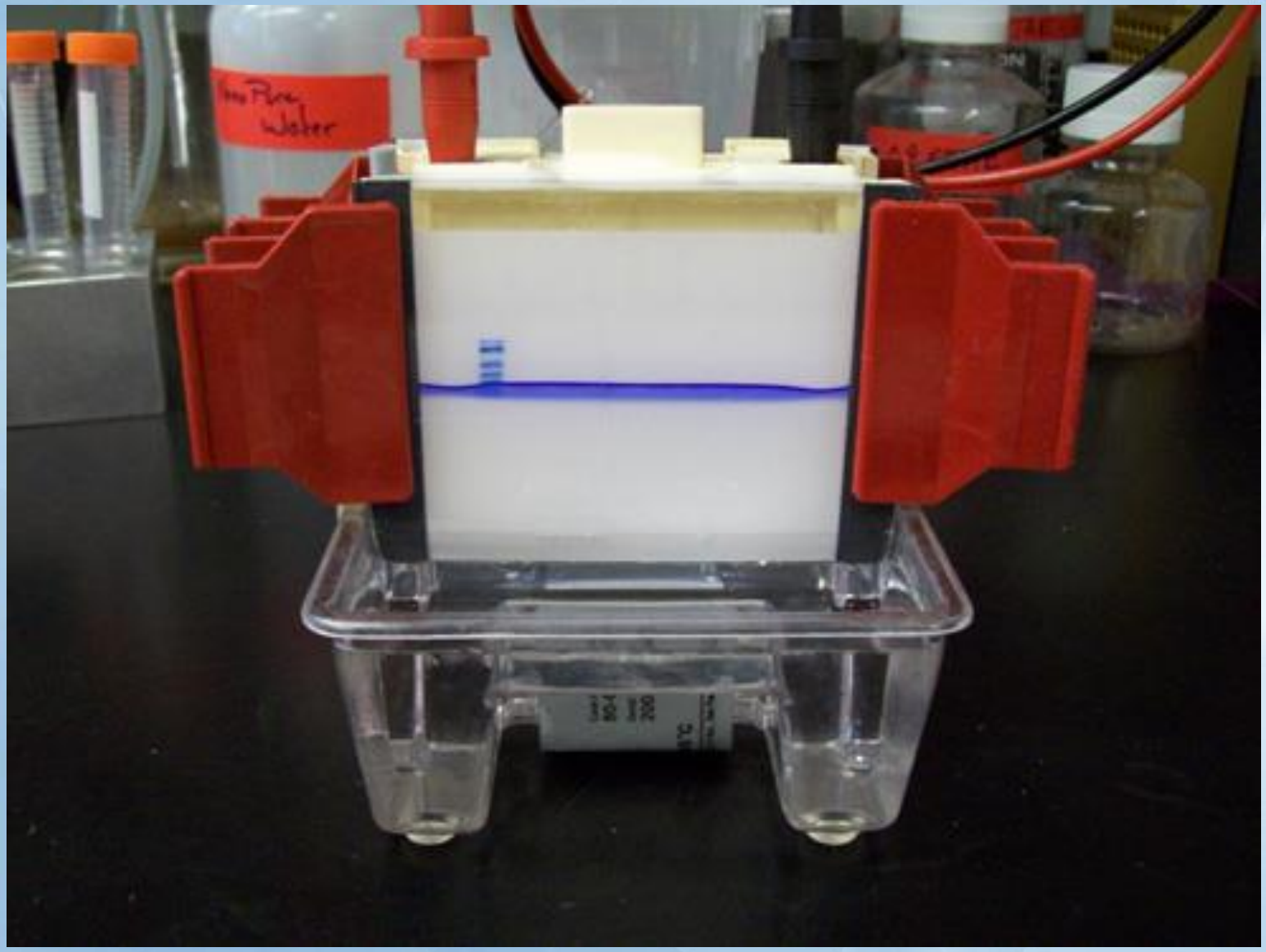
- Purpose of the stacking gel: to concentrate all the proteins in the sample into a thin band at the top of the resolving gel
- Purpose of the resolving gel: to separate the proteins on the basis of size.




Isopropanol
Separating
gel solution
Stacking
gel solution
Comb
Gel caster
Acrylamide gel


Sarstedt
DO NOT


WARNING


Water

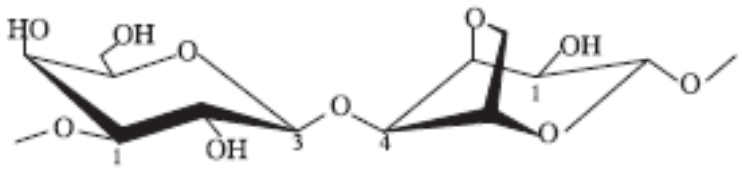

202 kd
133 kd
71 kd
41.8 kd
30.6 kd
17.8 kd
6.9 kd

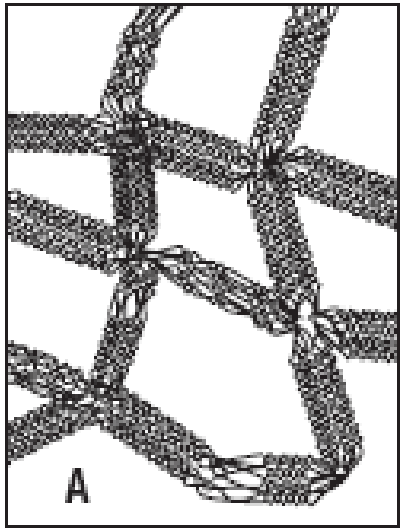
Gels

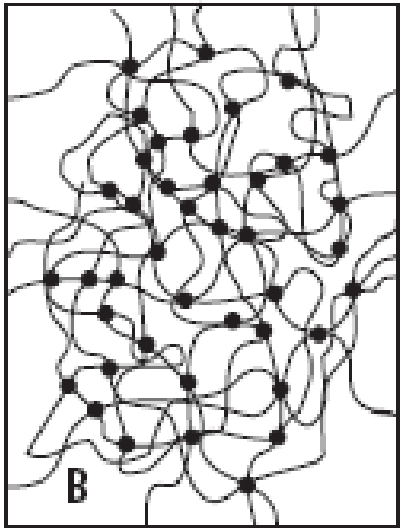
acrylamide

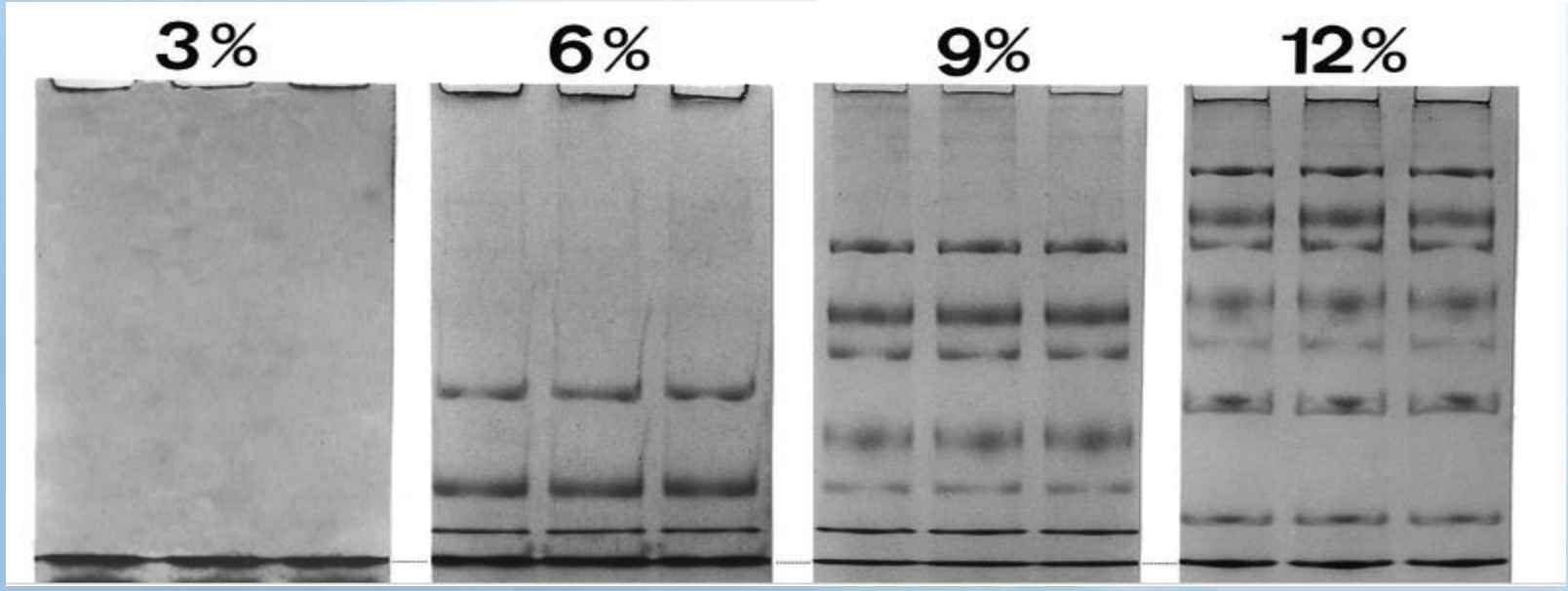
$$H_2C = CH\text{-}\overset{\overset{O}{\|}}{C}\text{-}NH_2$$

agarose









Protein visualization on gels

Common stains:

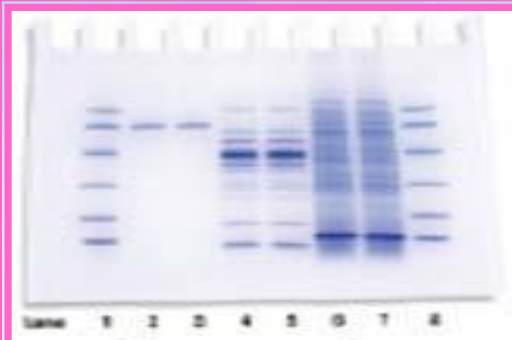
- **Coomassie Blue** in a fixative solution. Stain from a few hours to overnight. Destaining 4-12 hrs.

  * It provides a reasonably permanent record

- **Silver stain.** complex process, excellent, long-lasting record, sensitive.

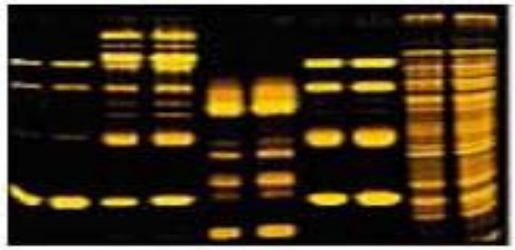
- **SYPRO (fluorescent)** staining is similar to Coomassie Blue in complexity, except the Destaining takes about 30 min.

  * It fades with time after a few hours

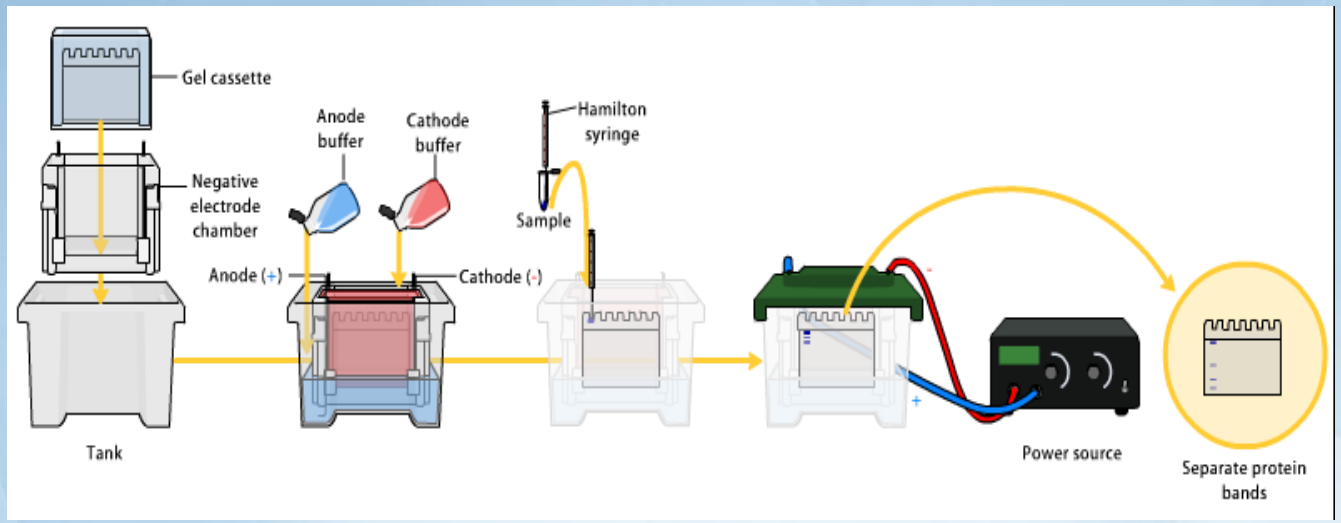


Coomassie

Silver

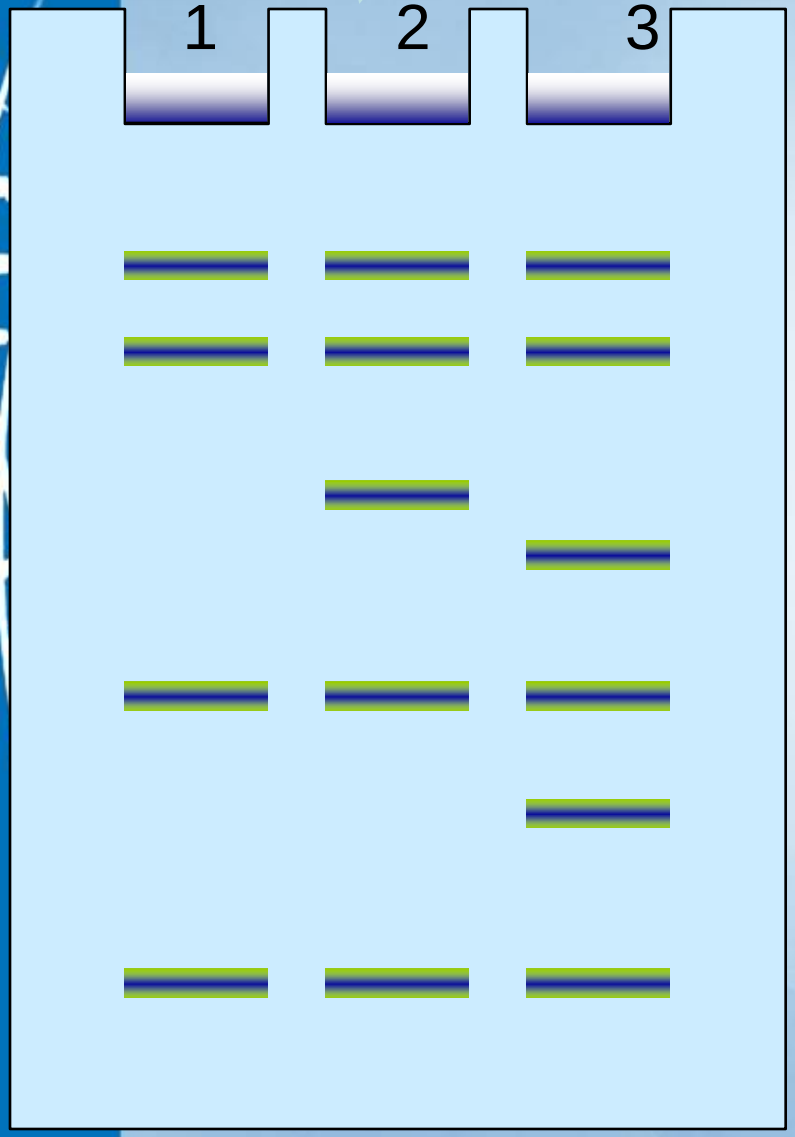


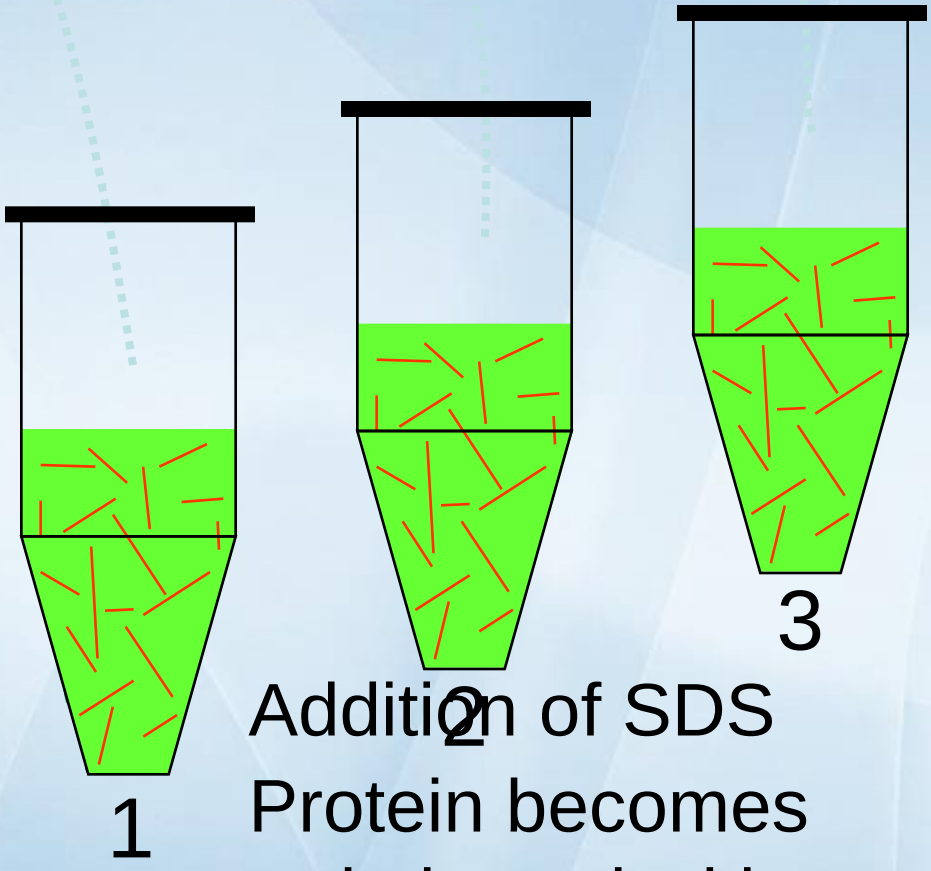
SYPRO orange


Gel cassette
Negative electrode chamber
Anode buffer
Cathode buffer
Hamilton syringe
Sample
Anode (+)
Cathode (-)
Tank
Power source
Separate protein bands

Animation

SDS-PAGE





Addition of SDS
Protein becomes rod-shaped with uniform charge distribution