Problem: How to separate DNA fragments?

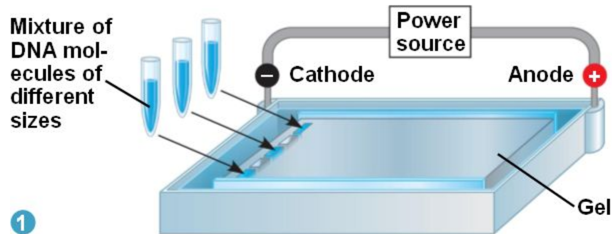
Gel Electrophoresis

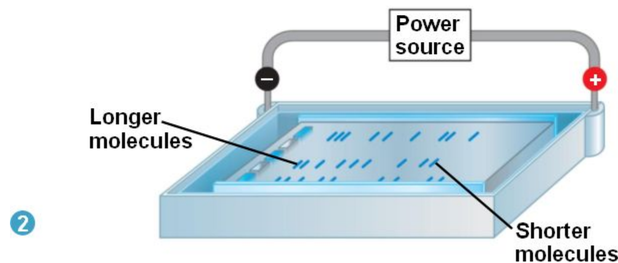
- Polyacrylamide gel electrophoresis
  - 20bp - 2000bp
- Agarose gel electrophoresis
  - 300bp - 40,000bp

Electrophoresis - Principle

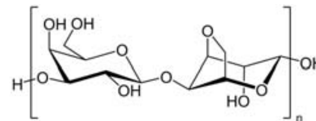
- (-) charged phosphate groups of DNA are attracted to a (+) electrode when a charge (potential) is applied.
  * What was the pH of the running buffer? Does it matter?
- DNA has evenly spaced charge (i.e., uniform charge density), thus it migrates according to **size**.

Electrophoresis - Principle

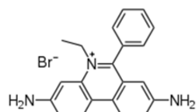


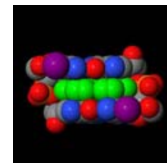


Agarose Gel Electrophoresis



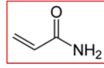
- Features
  - size separation
  - purification of DNA fragments
  - is relatively simple
  - is relatively rapid
  - fragments can be visualised using fluorescent intercalating agents such as ethidium bromide
  - main type is submersible, horizontal.

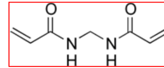


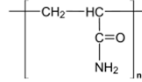


Polyacrylamide Gel Electrophoresis

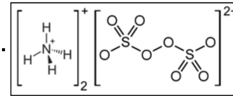
- Features



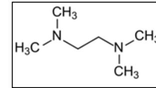




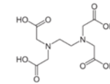
  - made up of two solutions, acrylamide and bis-acrylamide (cross linker) by polymerisation
  - polymerisation initiated by ammonium persulfate (**APS**). APS generates radicals.



  - TEM**ED** (Tetramethylethylenediamine, resemble **ED**TA) stabilizes free radicals and improves polymerization.



  - highly toxic (neurotoxin)
  - inhibited by presence of air, hence between glass plates
  - usually run vertical.



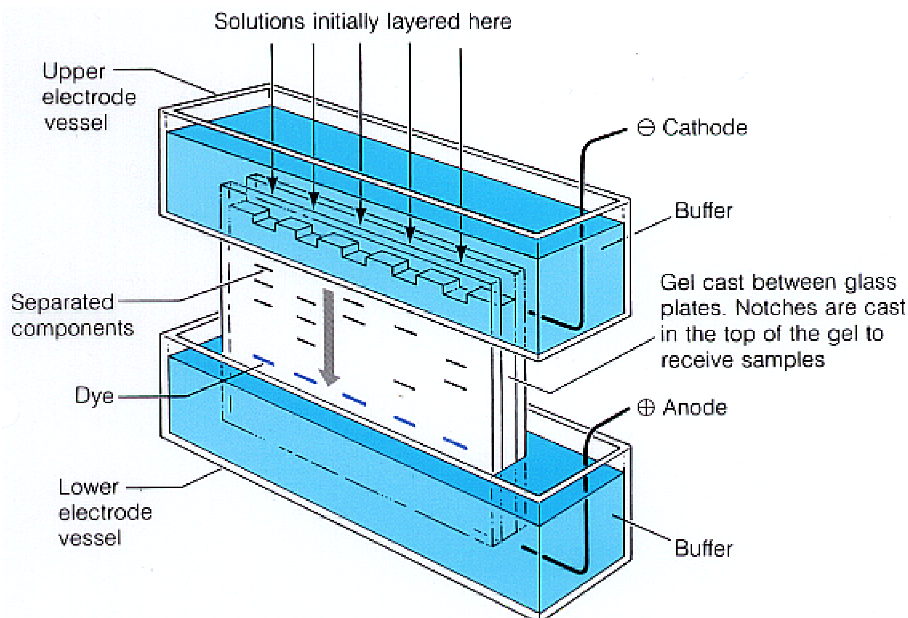
  - separation dependent upon - total concentration (3.5%-20%) and concentration of cross-linker.
    E.g. 3.5%: 100-1000 bp, 8%: 60-400 bp, 20%: 10-100 bp

- Additional points to study:

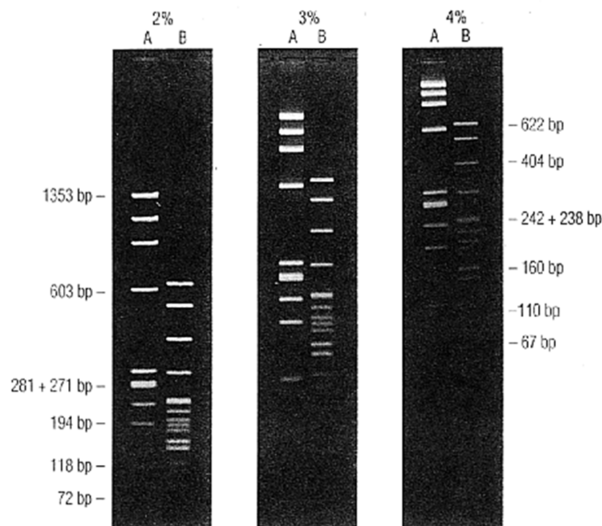
❑ Compare mw of aa and bp

❑ What is required in the sample loading buffer?

Polyacrylamide Gel Electrophoresis



Polyacrylamide Gel Electrophoresis



Migration parameters

- Molecular size of the DNA
  - Migration inversely proportional to $\log_{10}$MW
- Matrix concentration
  - Molecular sieve effect. Increase concentration decrease larger molecules separating
- Buffers
  - Tris-acetate, Tris-borate or Tris-phosphate, pH~8. Usually Tris -acetate for agarose and Tris-borate for polyacrylamide. + EDTA – chelates metals and inhibit DNAase.
- Conformation of DNA
  * supercoiled DNA (e.g. plasmid from bacteria) run faster than relaxed (e.g., nicked DNA).
- Applied current - best resolution at 5 V/cm

Protein Expression & Purification:

Protein Expression and Purification

- ***Why?***
  - Obtain pure (clean) protein
  - **Protein research**: Understanding protein structure and function requires the study of individual proteins.
    - E.g, biochemical properties, crystallography, protein-protein interactions.
  - **Protein Drugs**:
    - e.g., Insulin, Growth Hormone, Erythropoietin (EPO), Interferon, Herceptin

Protein Expression and Purification

- ***How?***

1. Express recombinant protein in host cells.

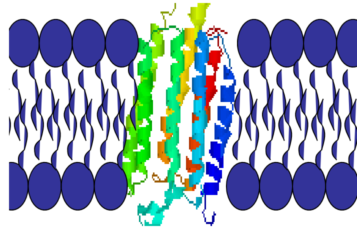
2. Separate/Purify protein.

3. Identify/Analyze purified protein.

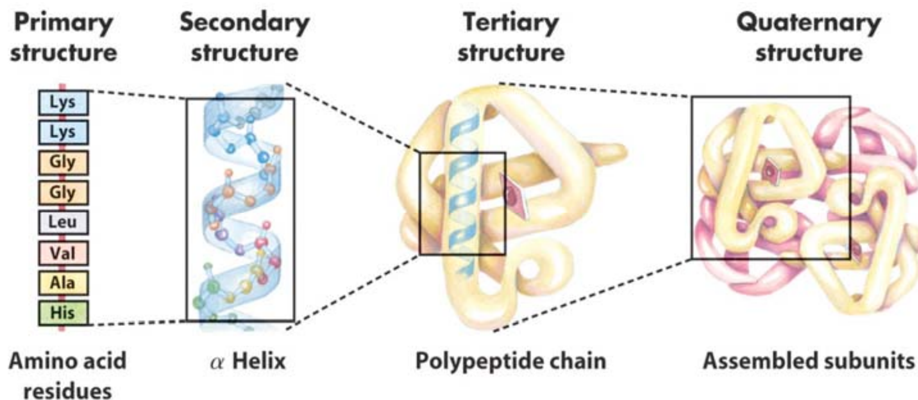
Some considerations

- How soluble and how stable?
  E.g.,Is it membrane or water soluble?
- Are specific chaperones or co-factors or post-translational modifications?
- Is it single or multi-subunit?
- How much protein is needed?

Membrane or Water Soluble?



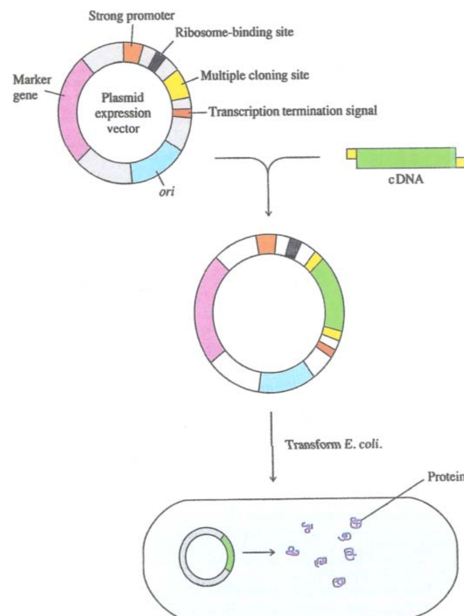
Single or Multi-subunit(s)?
Chaperones/Co-factors/PTMs?

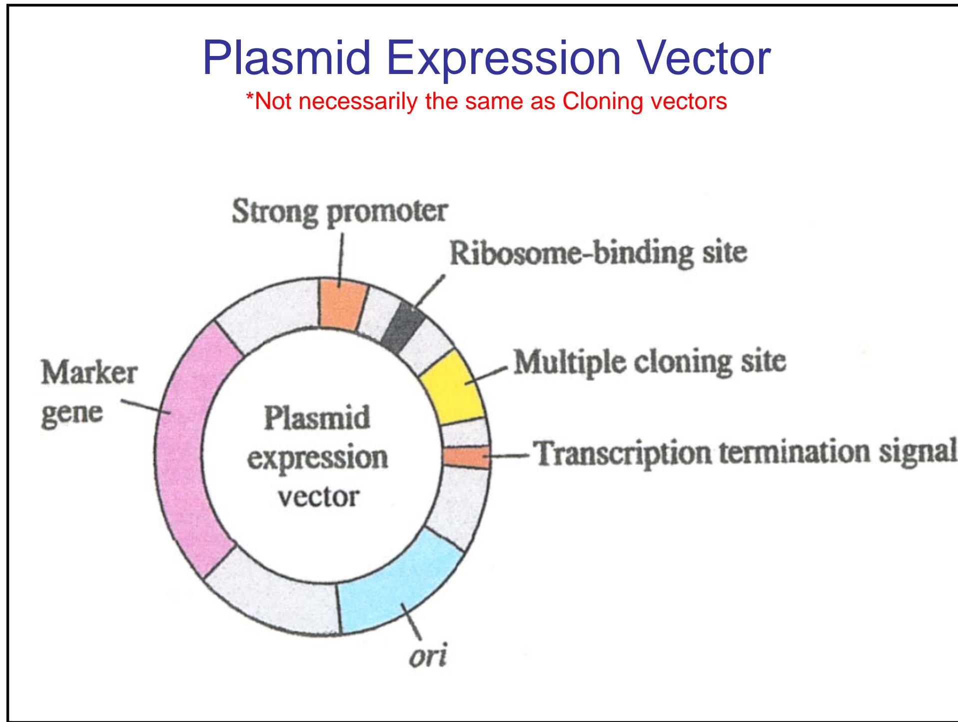


Expression systems

- E. coli
  - Large amounts of protein
  - May not be good for eukaryotic proteins that need special chaperones, have PTMs, etc.
- Yeast
  - For bacterial or eukaryotic proteins
  - Large amounts of protein
- Mammalian cells
  - Slow growth, most expensive but can be necessary for some “difficult” proteins.
- Insect cells
  - Good for many mammalian proteins, but easier to work with than mammalian cells.

Recombinant Protein Expression




Plasmid Expression Vector
*Not necessarily the same as Cloning vectors
Strong promoter
Ribosome-binding site
Multiple cloning site
Transcription termination signal
Marker gene
Plasmid expression vector
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