Laboratory Method

Introduction to Bioanalytical Techniques

Wilson Huang

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Biomolecules

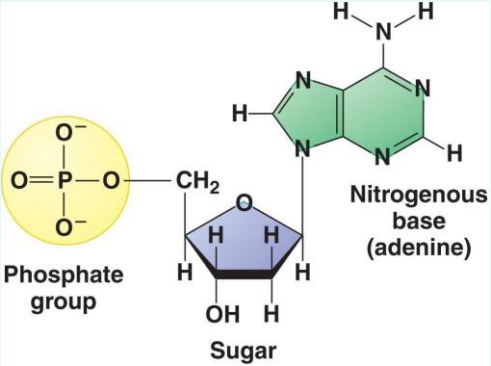
01

Objectives

Review the basics of what even is a DNA, RNA, and protein

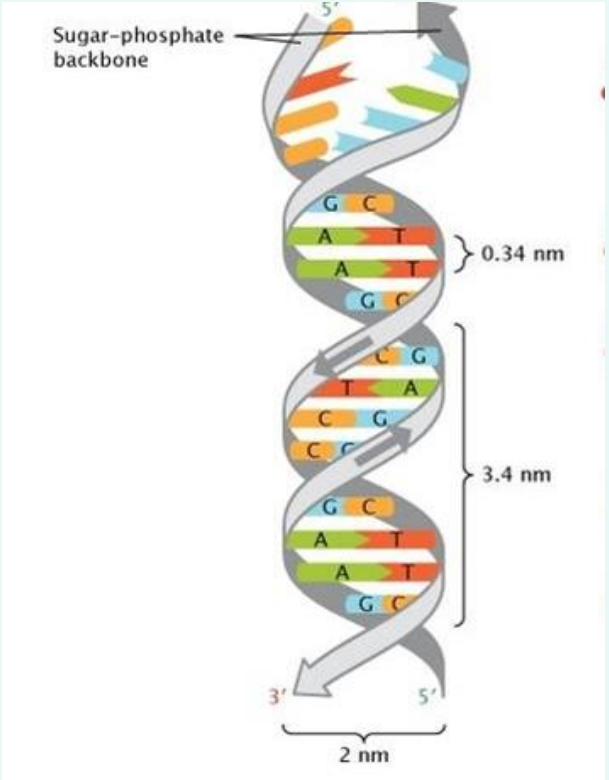
DNA - A sequence of nucleotides

Nucleotide Structure:



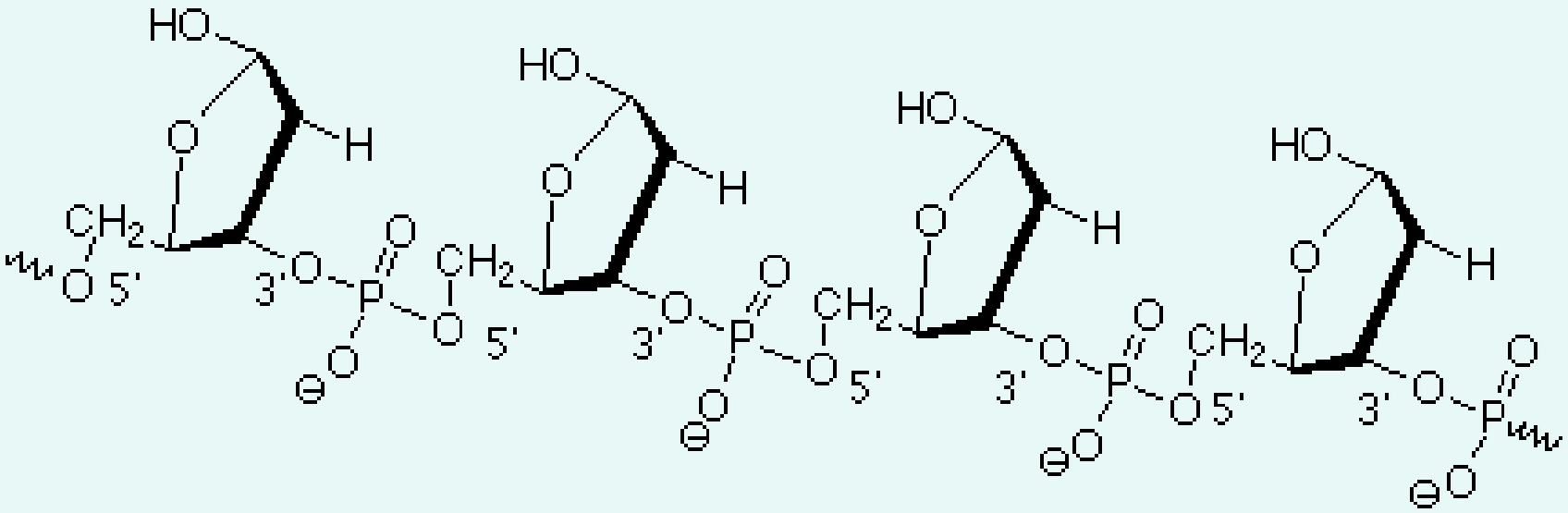
General Structure:

- Two helical chains coiled around the same axis
- The chains are anti-parallel
- Sugar-Phosphate backbone outside, bases inside



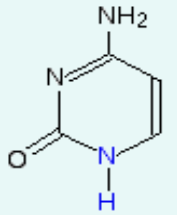
DNA Backbone

- **Phosphodiester Bond**
  - **Phosphate groups joins the 3’ carbon of one sugar molecule to the 5’ carbon of another sugar molecule**

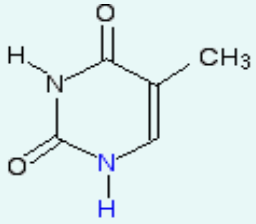


DNA Nitrogenous Base

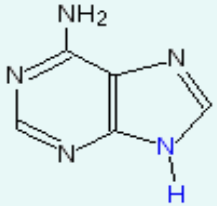
- Pyrimidines
  - Cytosine
  - Thymine
- Purines
  - Adenine
  - Guanine



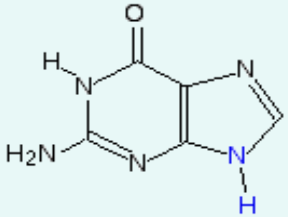
cytosine (C)



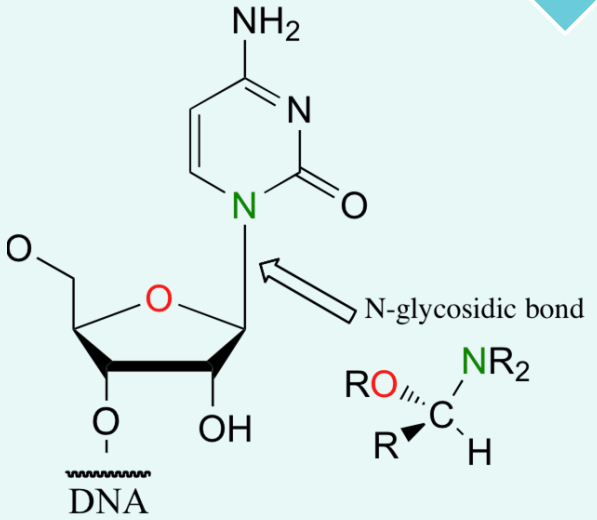
thymine (T)



adenine (A)



guanine (G)

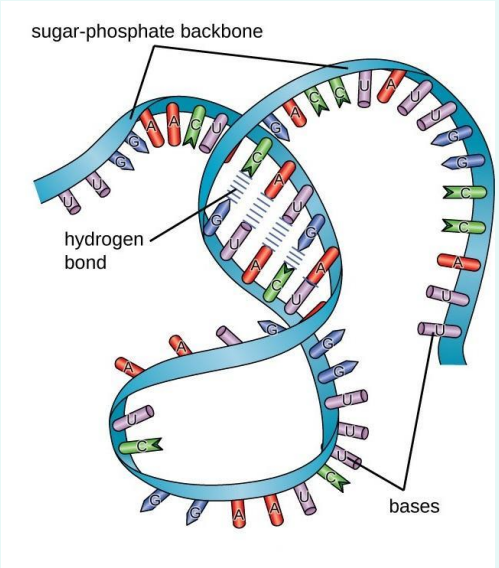


- N-glycosidic bond
  - Between the 1' carbon in pentose sugar and 9' nitrogen in purine bases and 1' nitrogen in pyrimidine bases

RNA

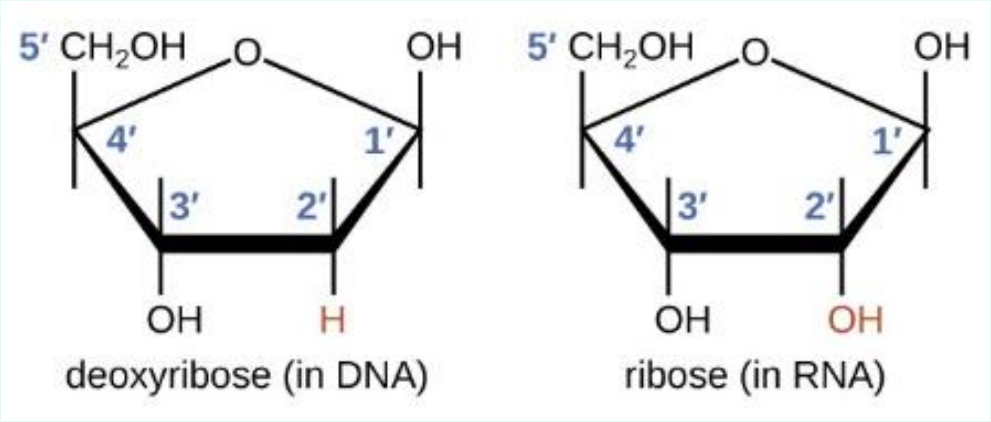
Nucleotide Structure:

- Same as DNA except:
  - Pentose sugar is ribose
  - Hydroxyl group at the 2’ carbon
- Has a different nitrogenous base



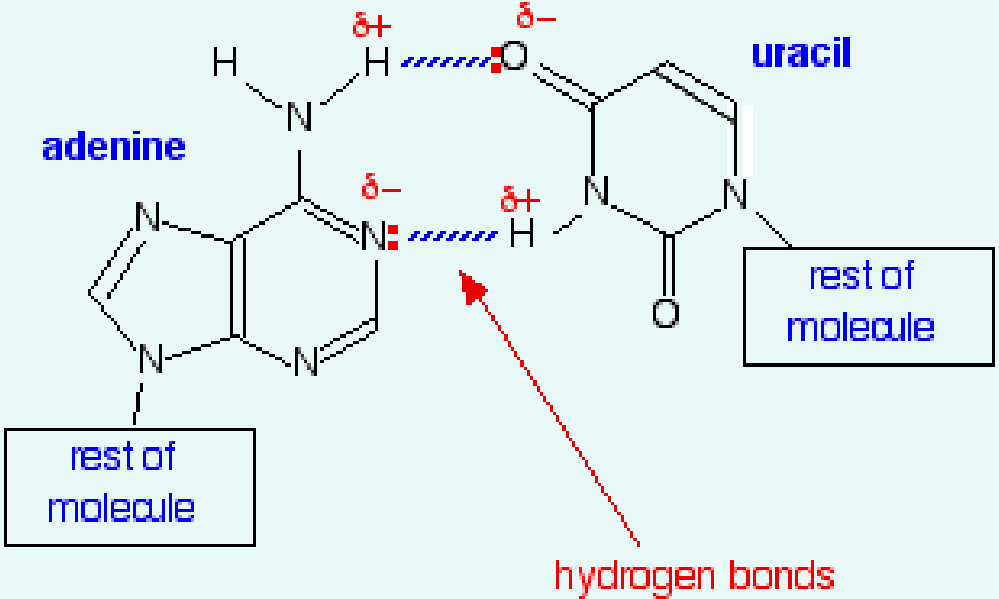
General Structure:

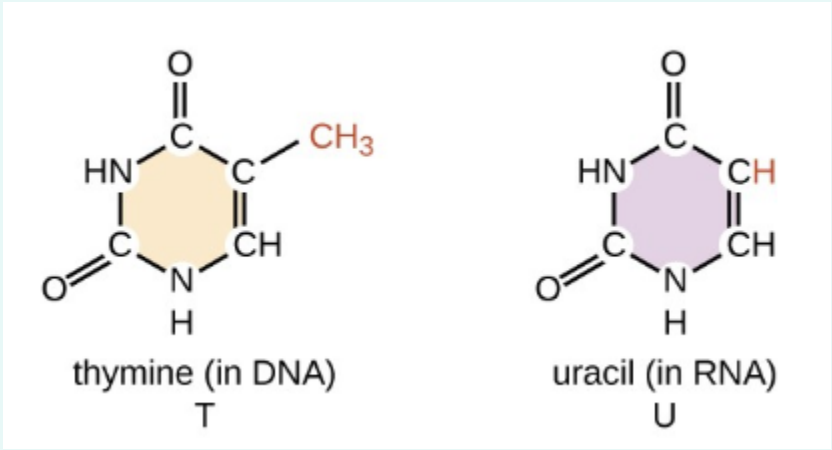
- Single stranded molecule
- Folds up in a variety of shapes



Uracil in RNA

- Uracil replaces Thymine
- Pyrimidine
- Binds to Adenine with 2 Hydrogen Bonds

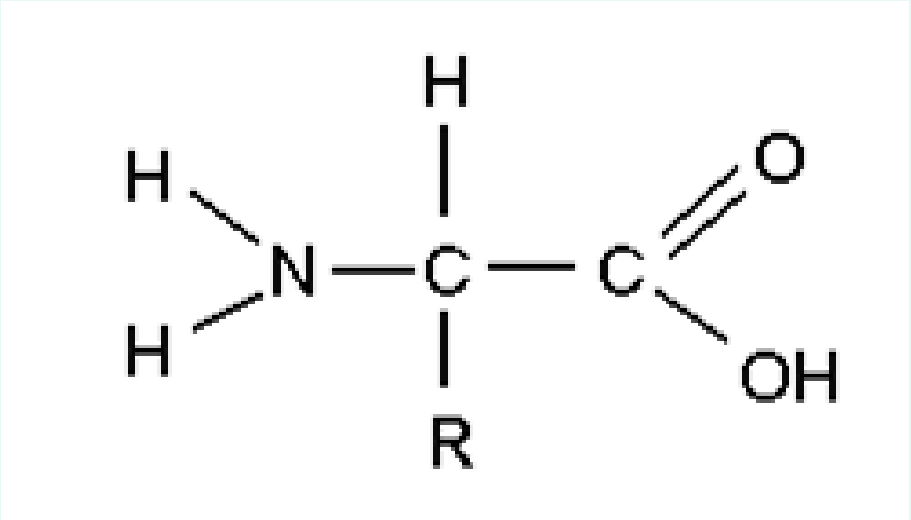




Proteins - Polymers of Amino Acids

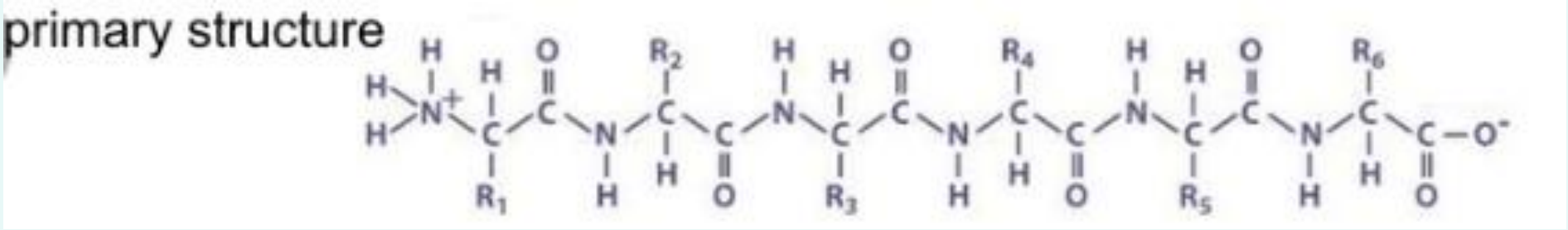
Amino Acid Structure:

- Amphoteric and Zwitterionic
  - Carboxyl Group
  - Amine Group
- R Group
- 20 amino acids
  - E.g. valine, raline, histidine



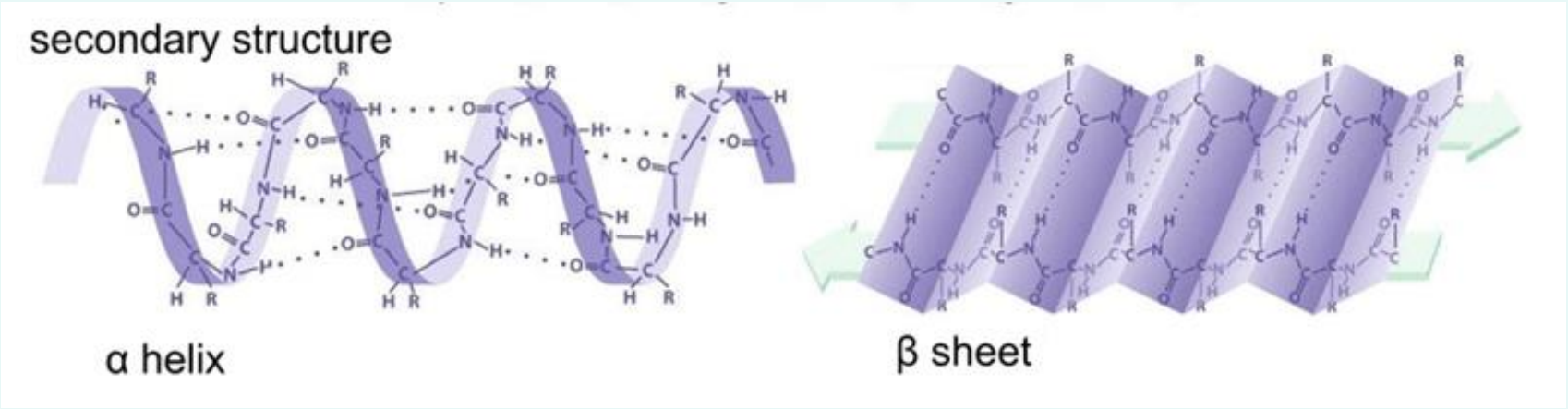
Primary Protein Structure

- Linear Sequence of Amino Acids
  - Peptide Bonds
- Dictated by Genetic Code



Secondary Protein Structure

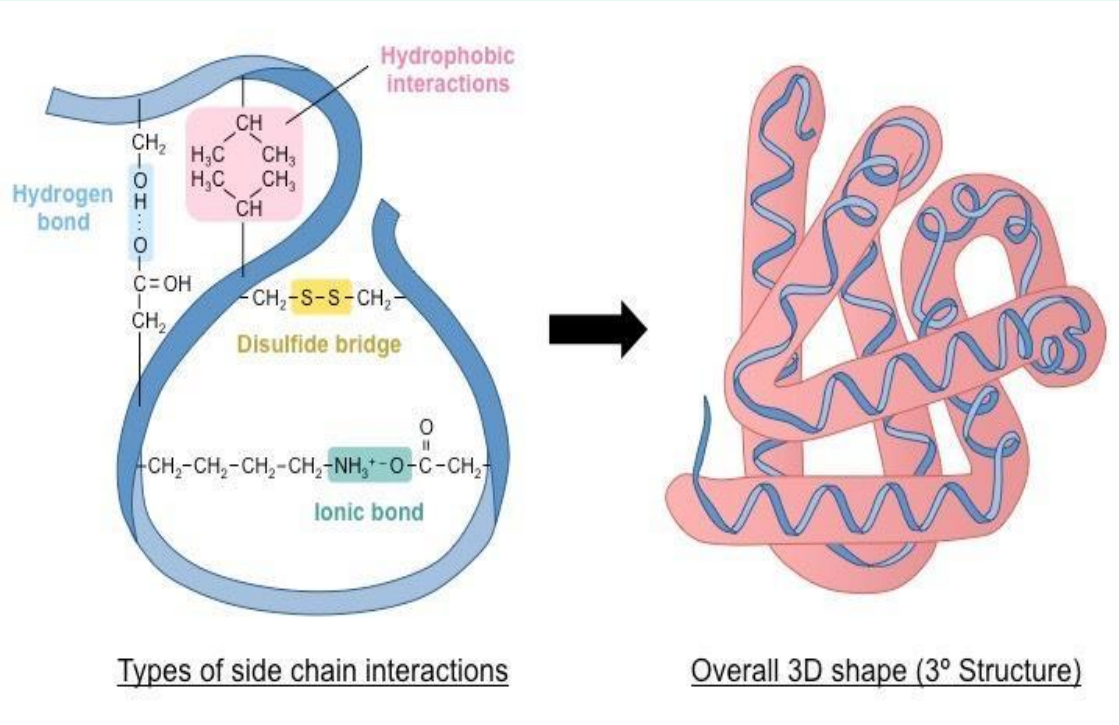
- Helices or Pleats in the Polypeptide
- Main types
  - Alpha Helix
  - Beta Pleats
- Form as a result of hydrogen bonding
  - Between repeating constituents of the polypeptide backbone



Tertiary Protein Structure

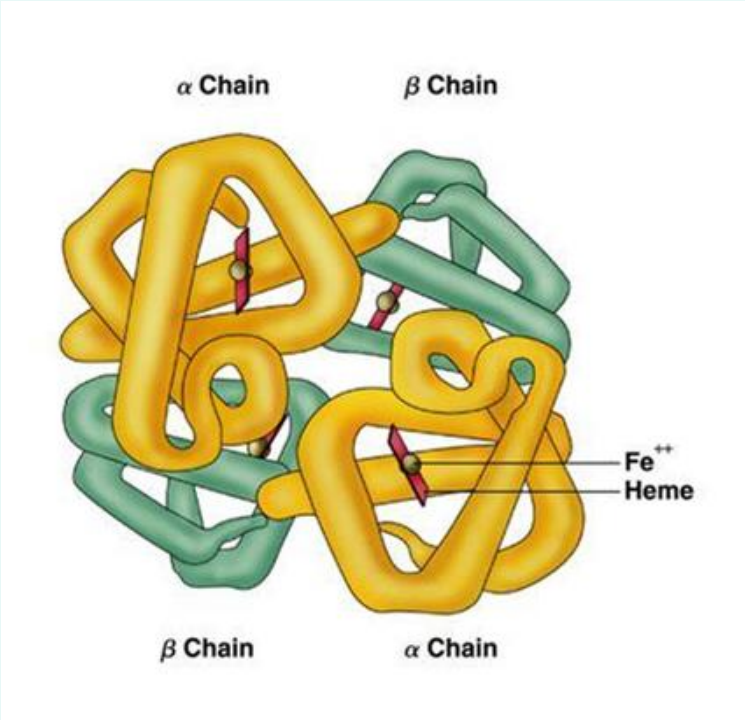
Fold in specific ways due to interactions between R groups

- Ionic Bond
- Hydrogen Bond
- Hydrophobic Interactions
- Disulfide Bridge



Quaternary Protein Structure

- Two or more polypeptide aggregate
  - Forms 1 function protein
- E.g. Hemoglobin - Globular Protein
- E.g. Collagen - Fibrous Protein



02

Objectives

Cover basics experiments to run the cloning cycle

The Cloning Cycle

Transformation and Cultures

Plasmid DNA replication

Restriction Digest

Cut the DNA at specific parts

Ligation

Joining together different DNA fragments

DNA Isolation

Purify out the plasmid DNA

Gel Electrophoresis & Purification

Separate DNA fragments

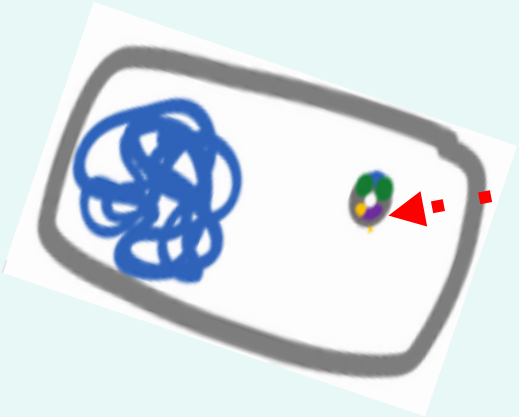
Retransform & Colony PCR

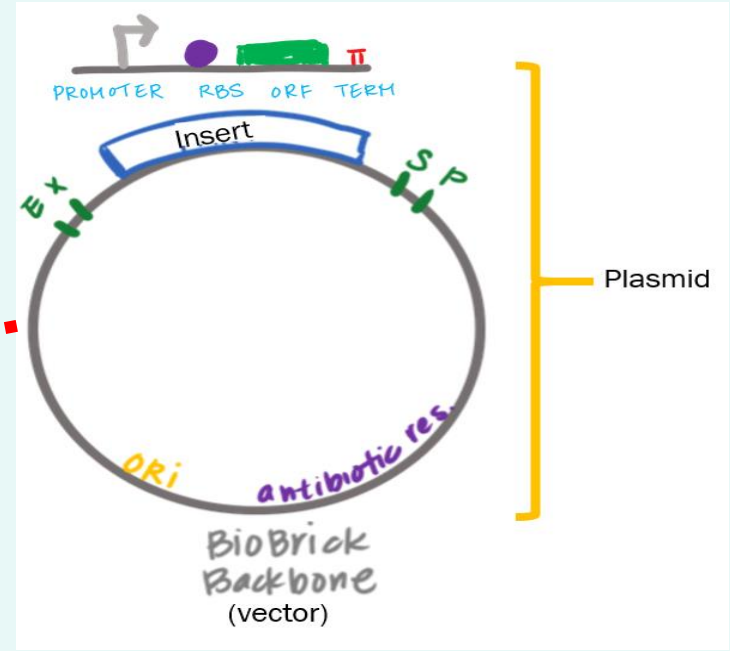
Replicate DNA and check

Transformation Principle

Deliver plasmids into bacteria

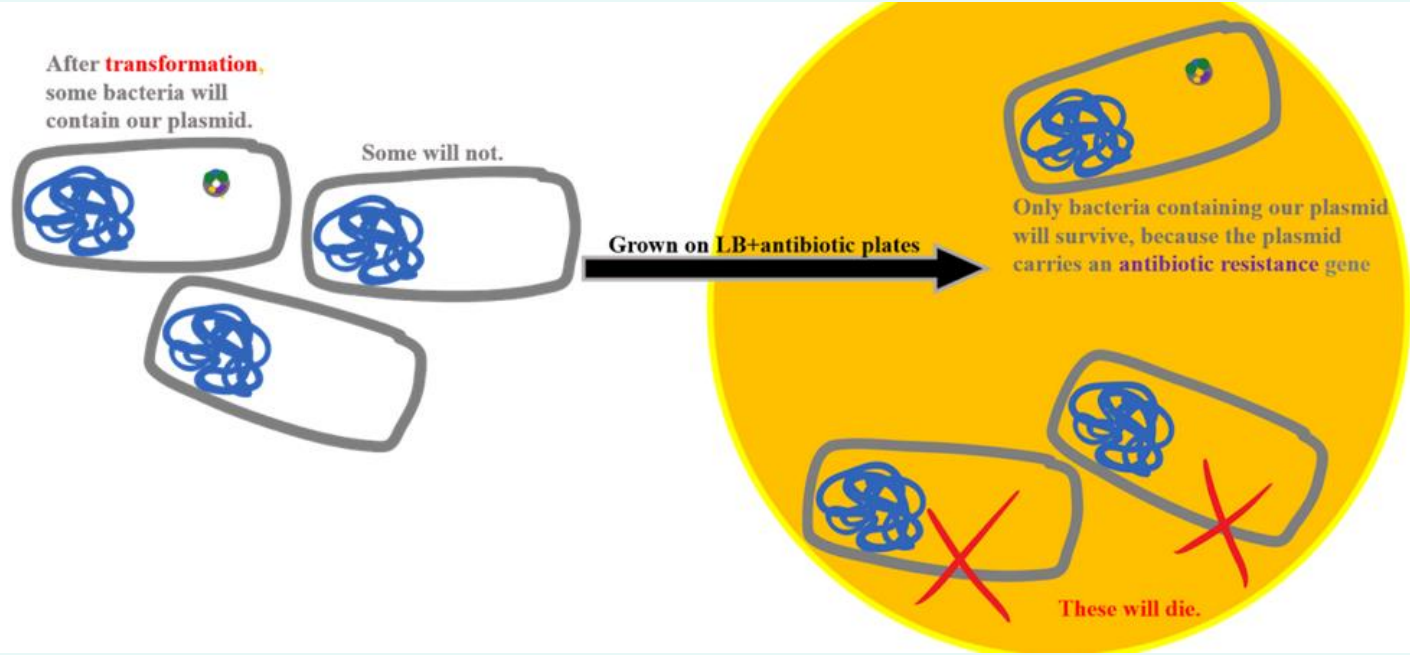
- To replicate the plasmid
- To make proteins





Transformation Principle

Antibiotic Resistance selects for the bacteria containing out plasmid



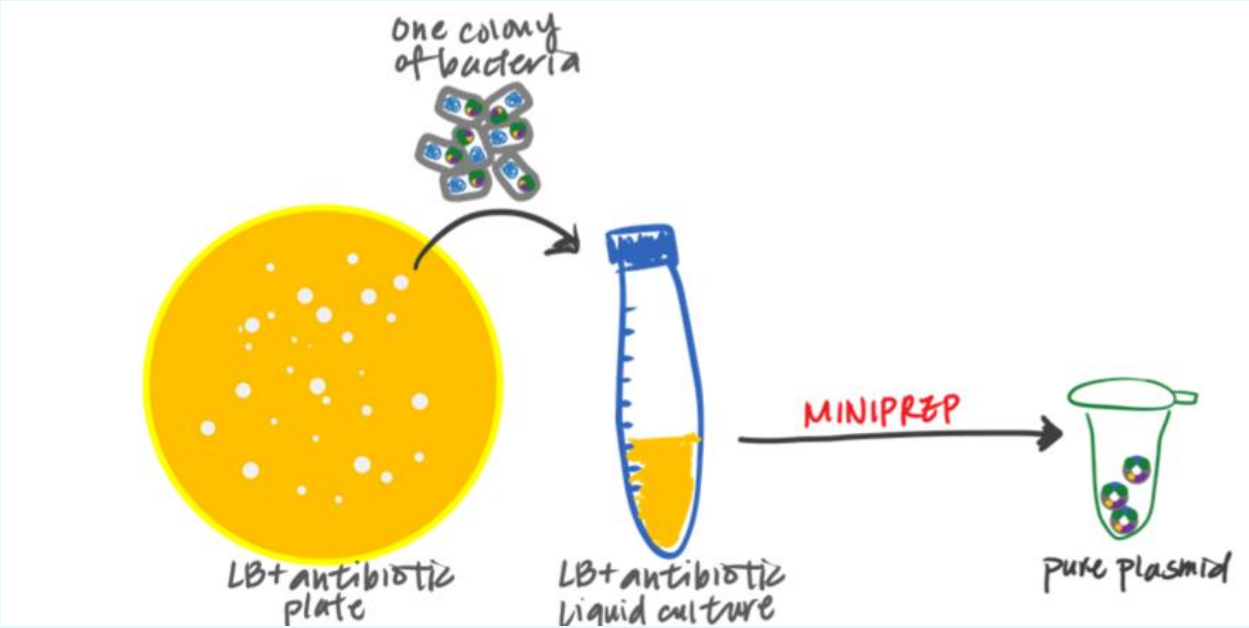
Transformation Protocol

1. Cells made competent before use, usually by treatment with $CaCl_2$
    - Makes bacterial cell membrane more permeable
2. Incubate competent cells & DNA on ice
    - Foreign DNA mixed together with bacterial cells
3. Heat shock at 42 °C for 30-45s
    - Transiently opens up pores in cell membrane so foreign DNA can enter
4. Brief incubation on ice
    - Closes pores in cell membrane
5. Plate bacterial cells on LB + an antibiotic that only allows growth of *transformed* cells

Liquid Cultures

Prepare Bacterial Liquid Cultures

- Add LB, antibiotic (1000x), and one single bacterial colony into a conical tube
  - Pick single bacterial colony from agar plate using pipette tip and pipette and release the tip
- Incubate at shaking incubator 37°C (loosened cap with tape, slanted) - overnight



DNA Isolation - Principle

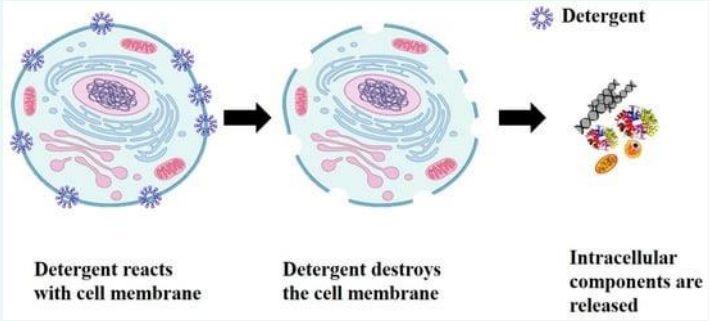
Commercial kits use "columns" that have a *silica* membrane

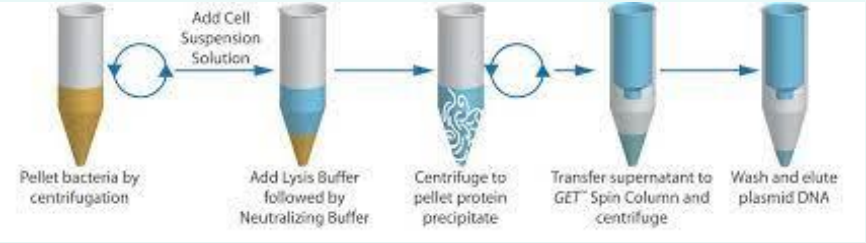
- **DNA binds to silica under *high* salt conditions**
- **Use this property to isolate DNA**

DNA Isolation - Protocol

Overview of Steps:

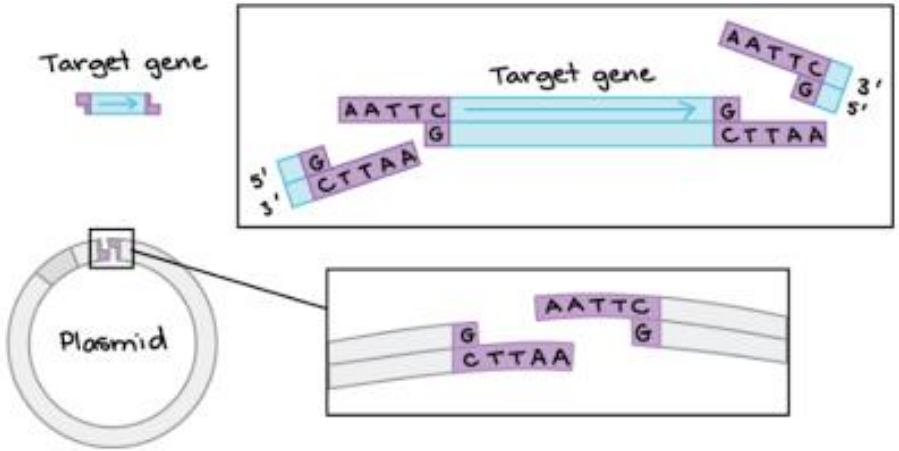
1. Grow cells in liquid media & collect cells
2. Resuspend cells (Buffer PD1)
3. Lyse cells (Buffer PD2)
- *SDS disrupts lipid membrane*
- *Alkaline solution denatures proteins and DNA*
1. Neutralize cells (Buffer PD3)
- *Neutralize alkaline conditions (plasmids can renature)*
- *Contain high salt*
1. Centrifuge
- *Spin down large cell debris*
- *Plasmid DNA small enough to stay in supernatant*
1. Bind plasmid DNA to silica membrane in column
2. Wash
3. Elute plasmid DNA (with *low-salt* buffer) into clean tube





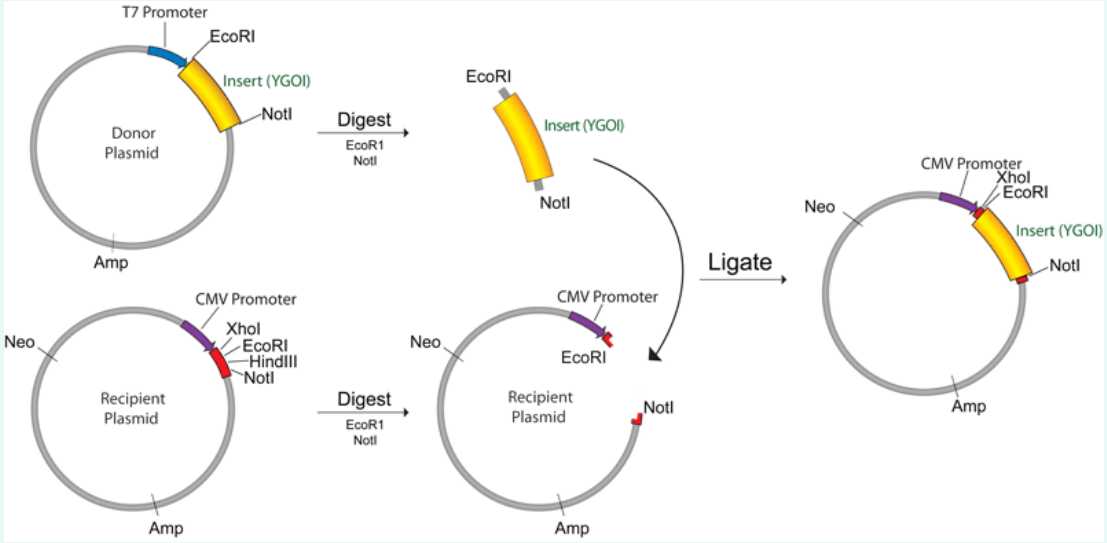
Restriction Digest Principle

Restriction enzymes cut at specific sequences on the plasmid



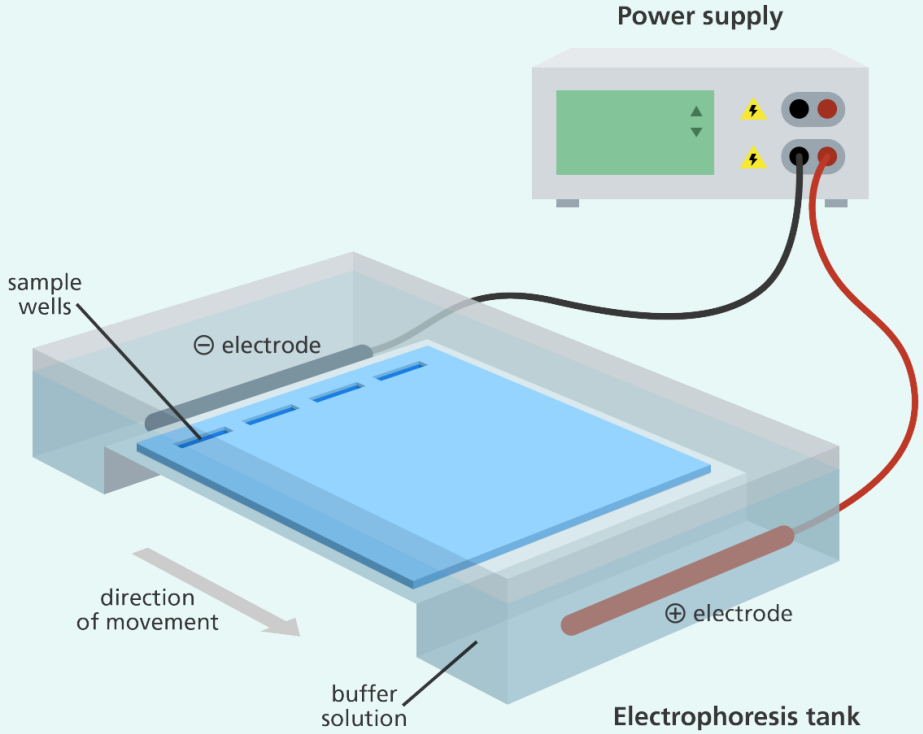
Restriction Digest Protocol

Add water, buffer, DNA, and enzymes and incubate at 37°C for 1-4 hours



Gel Electrophoresis Principle

Separate the different fragments of DNA after cutting

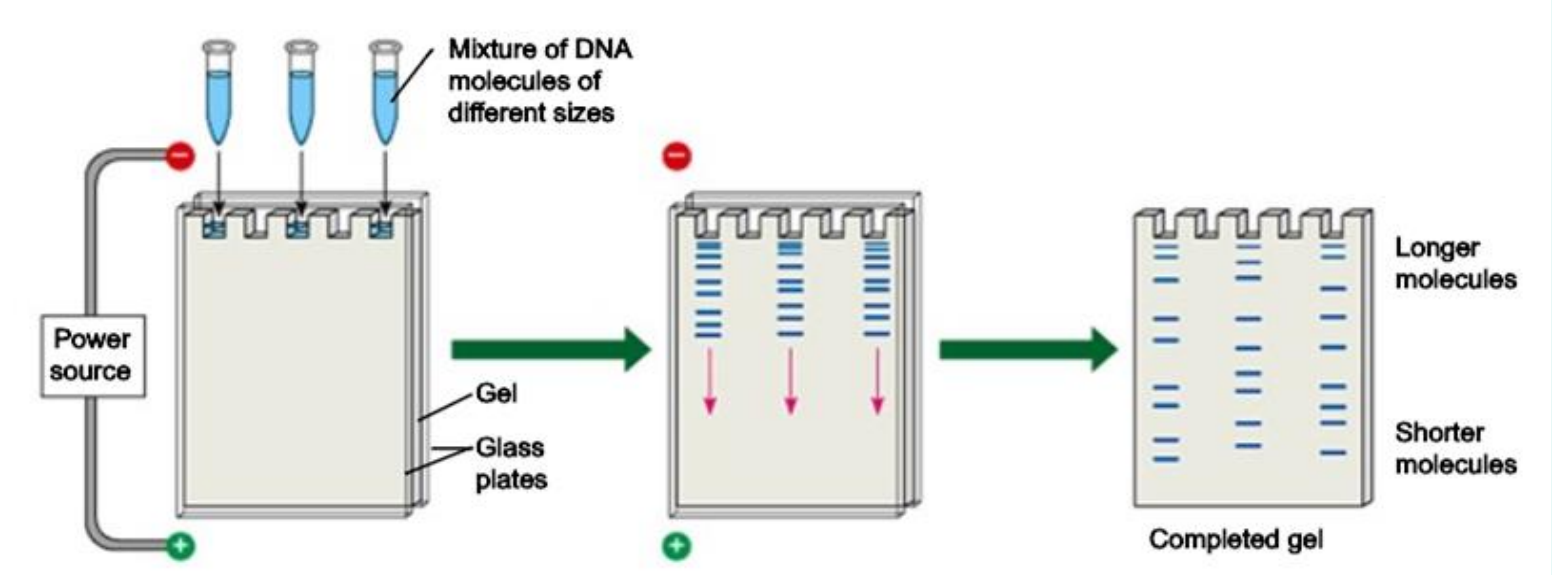


Gel Electrophoresis Principle

Gel apparatus connected to a positive and negative electrode

- What is the charge of DNA?
- Which electrode will it move toward?

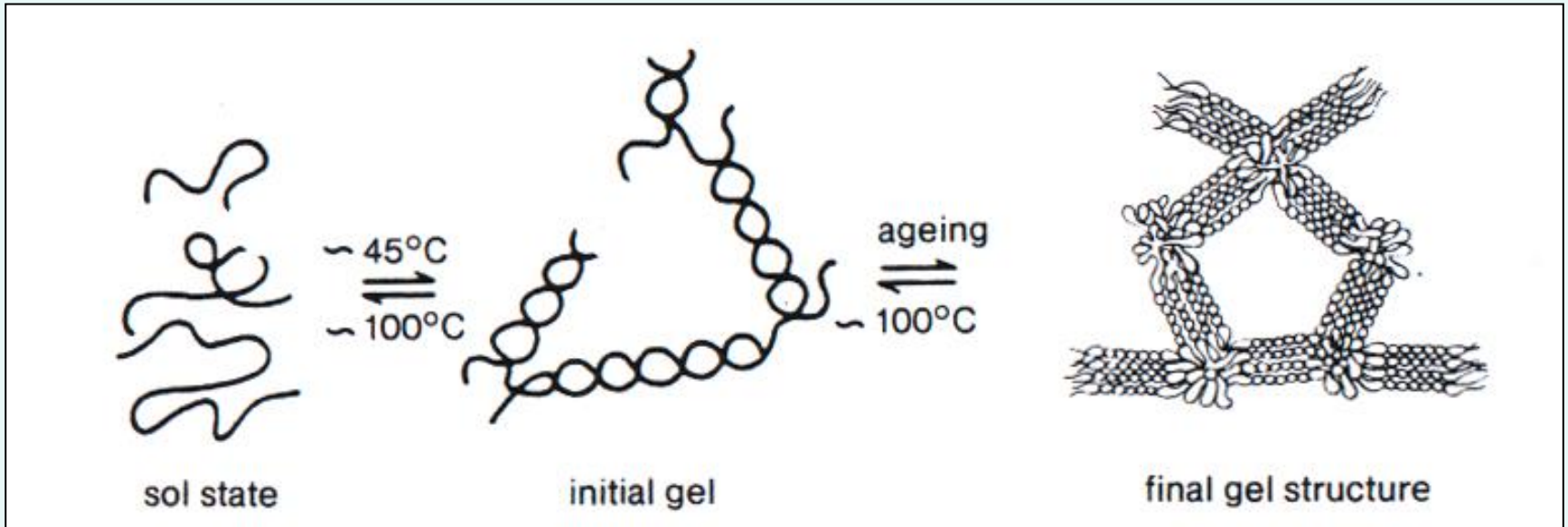
Smaller pieces of DNA will travel faster



Gel Electrophoresis Principle

Agarose Gel - Porous Material

- **Solid state**: single-stranded molecules
- **Boiled**: molecules form helices with each other
- **Cooled**: jello-like final product



Gel Electrophoresis Principle

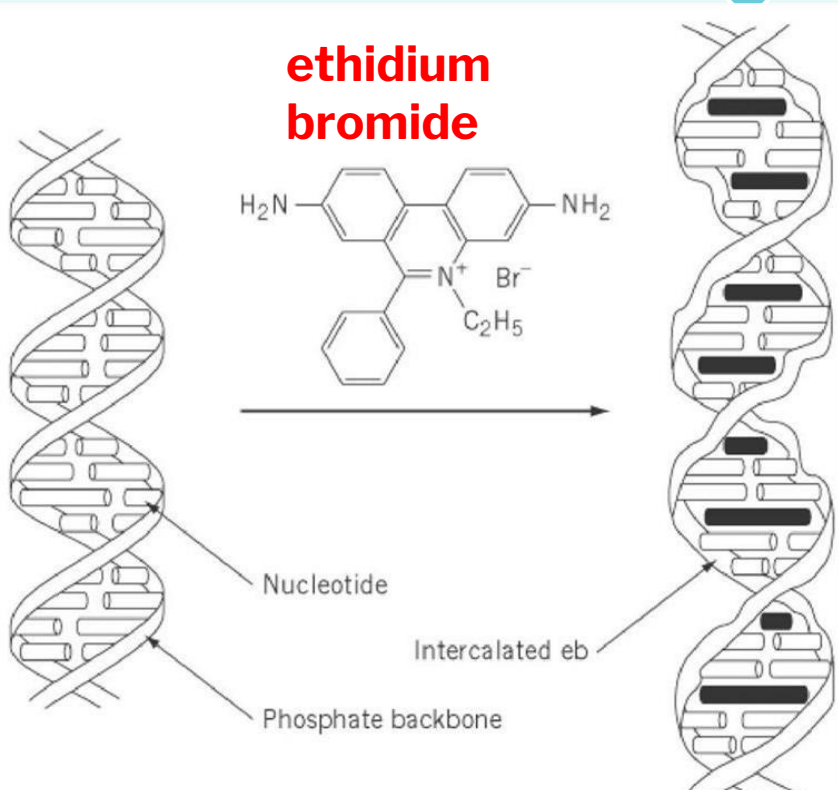
DNA Dye for Agarose Gels - Allows us to visualize DNA bands

Conventional dye: ethidium bromide

- Works by inserting itself into DNA
- Causes mutations in DNA
- Carcinogenic

Safer alternatives now

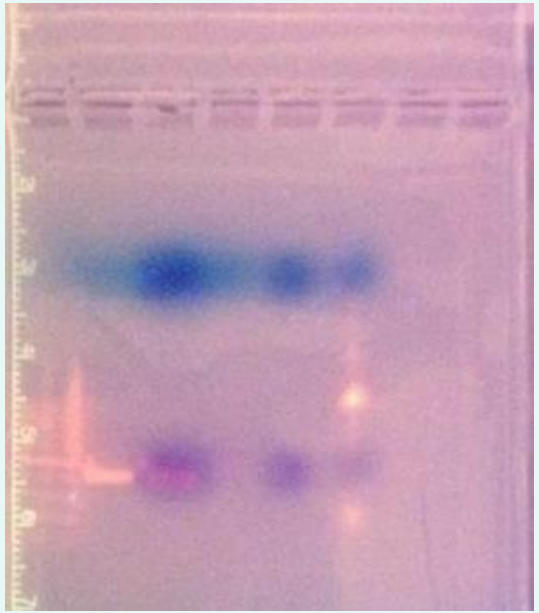
- We use ***SeeingSafe***
- Binds to DNA, but not toxic
- Visualize bands under UV light



Gel Electrophoresis Principle

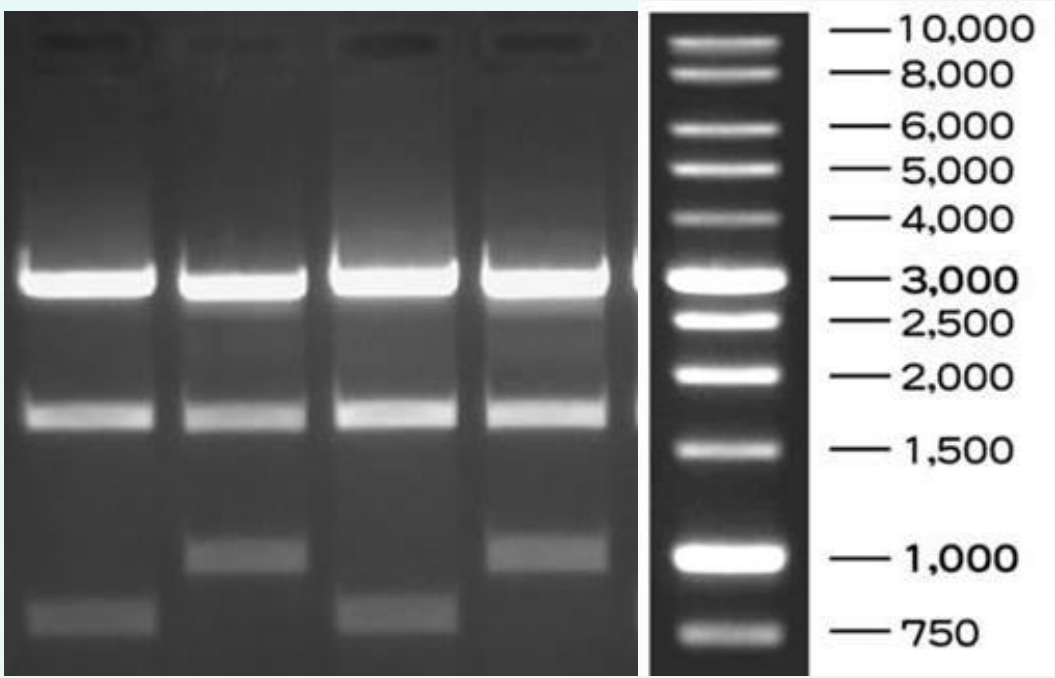
DNA Loading Dye

- Weighs down DNA in wells
- Visible, to track progress



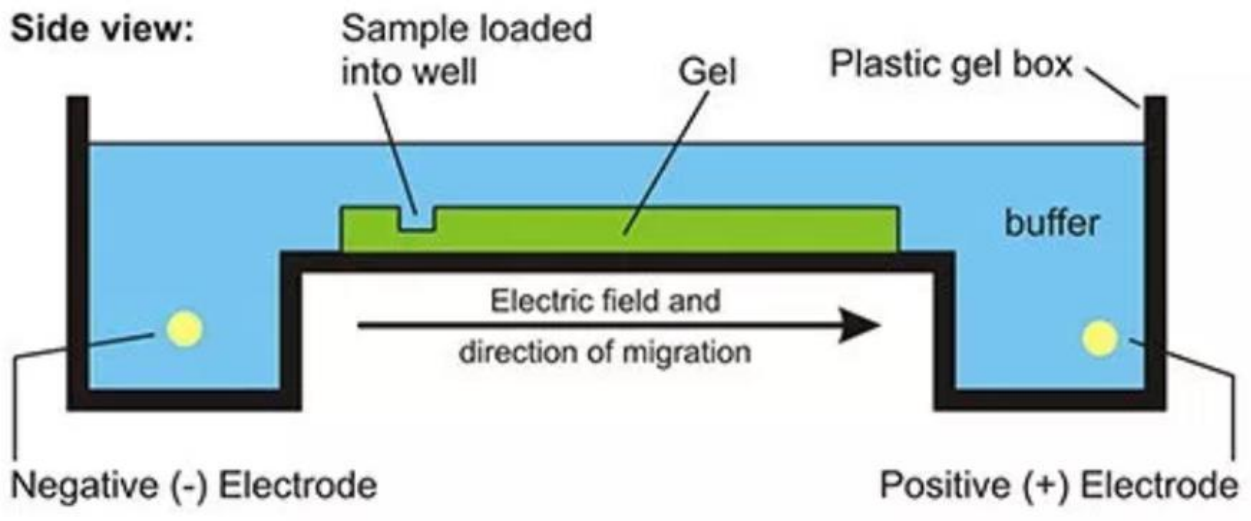
DNA Ladder (Geneaid 1kb)

- Mimics known DNA sizes

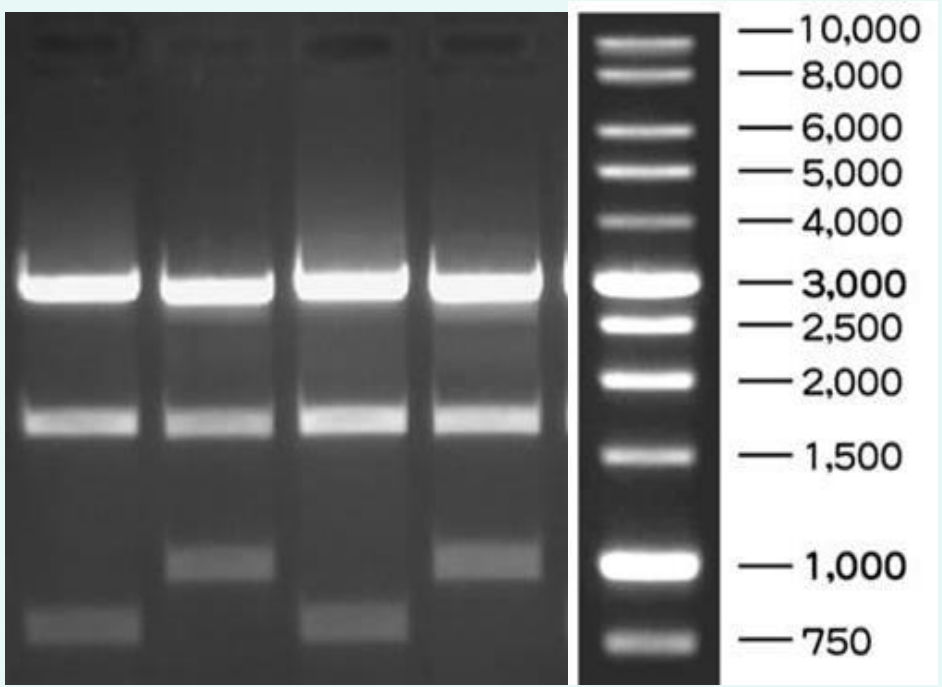


Gel Electrophoresis Protocol

1. Set up tank: fill with 1x TAE buffer
2. Prepare DNA samples
   - Mix **6x DNA loading dye** with **2uL of DNA**
   - How much distilled water do you add?
3. Load samples into wells
4. Run gel at 100V for ~20 min.

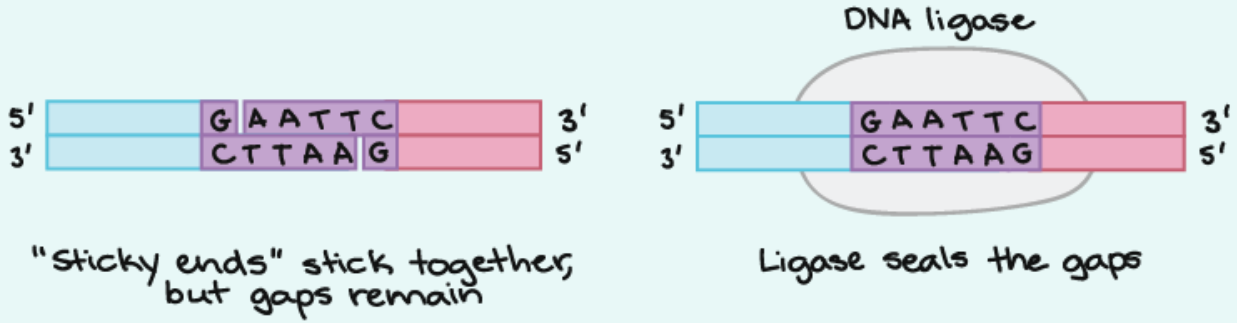


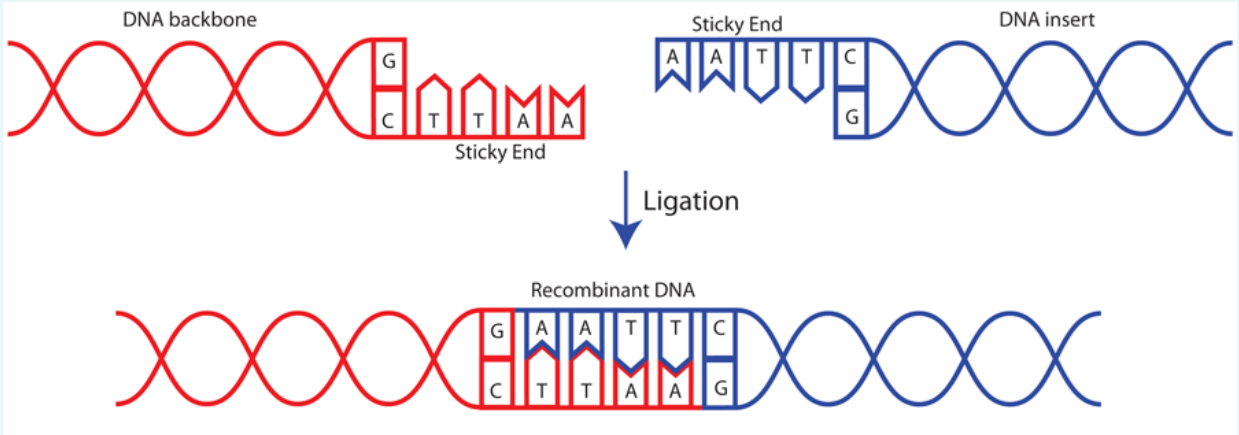
Gel Cutting and Purification



Ligase Principle

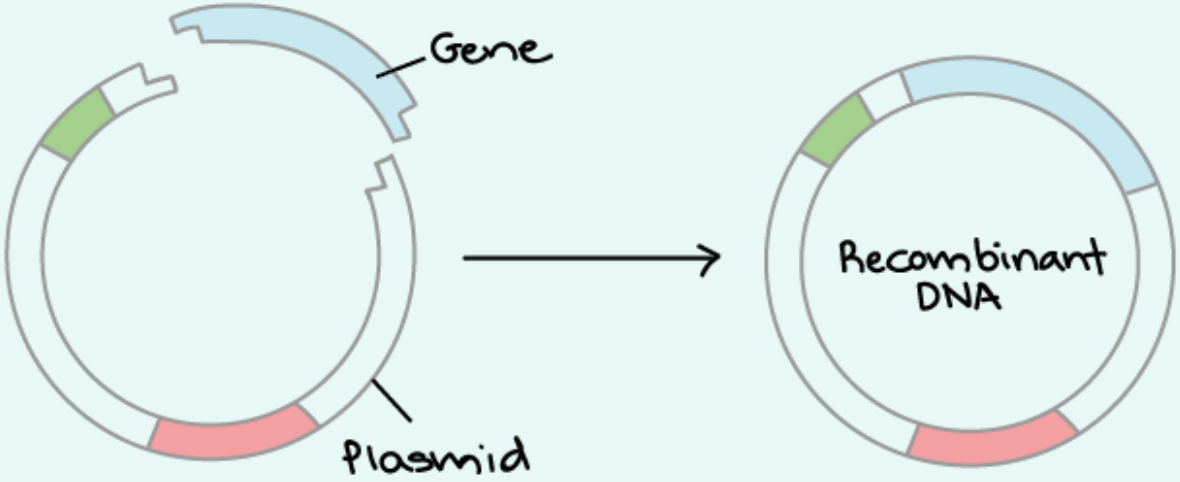
DNA ligase enzymes join together the ends of DNA strands





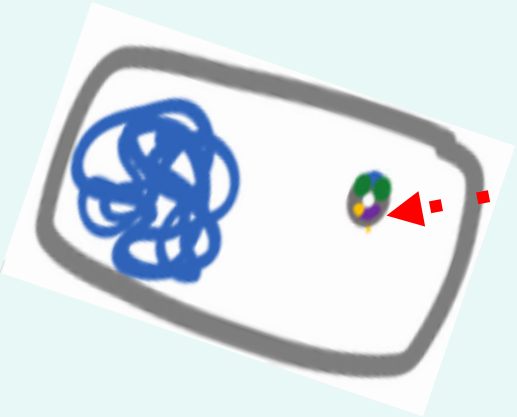
Ligation Protocol

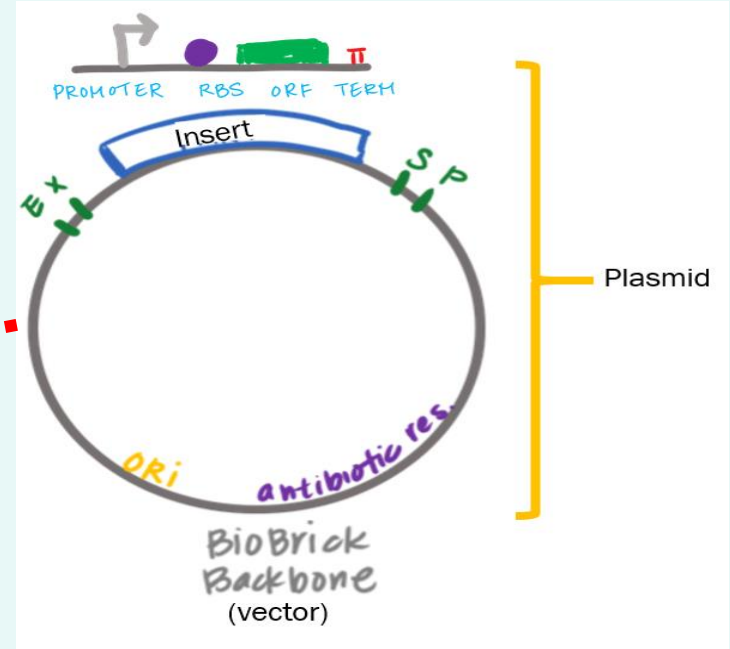
1. Calculate molar ratio of insert and vector
2. Add the vector, insert, ligase buffer, ligase, and water into a tube
3. Incubate in room temperature for 1-3 hours



Retransform

Deliver the new plasmid into bacteria



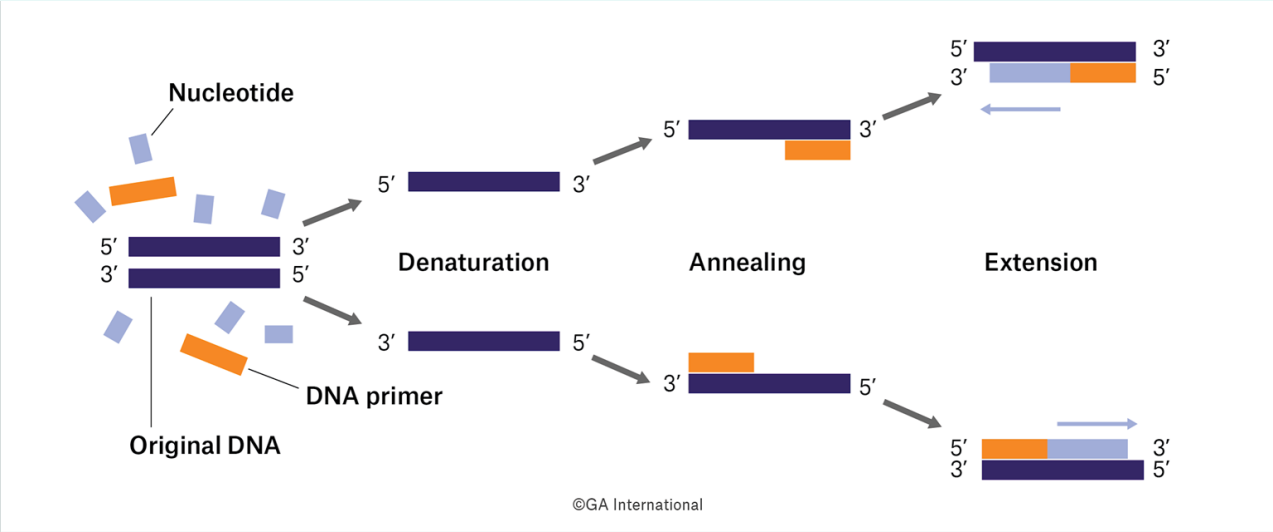


3 in 1 Colony PCR

1. Select a colony using a pipette tip
    a. Dip colony in PCR tube for subsequent analysis
        i. Check if the DNA product is right
    b. Re-streak colonies
        i. New bacteria colonies with desired DNA inside
    c. Dispense tip into liquid culture
        i. Can miniprep for more DNA samples

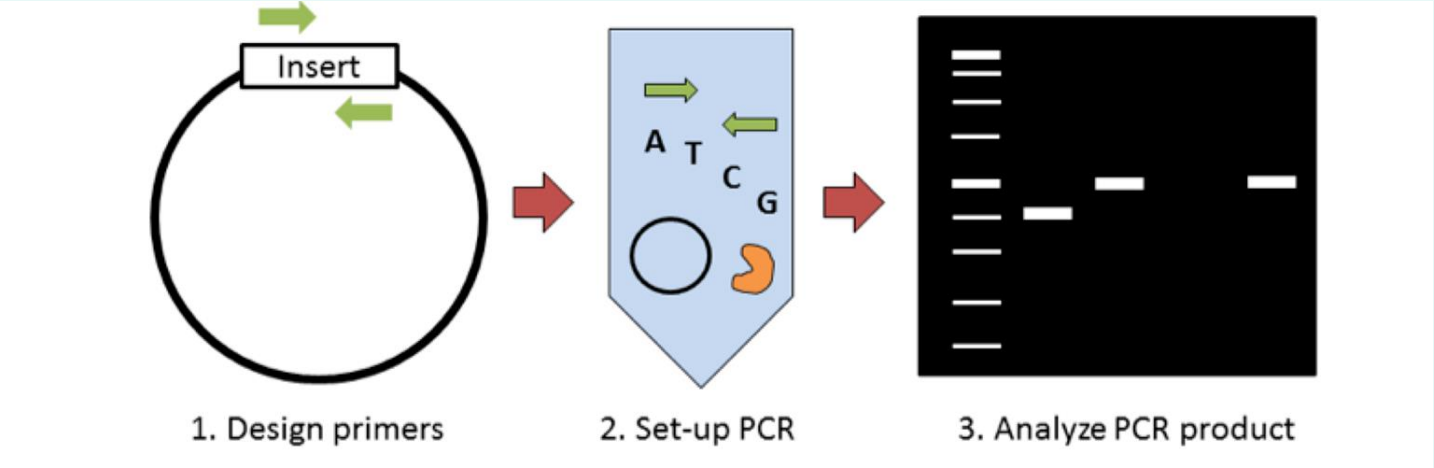
PCR - Principle

1. Denaturation
2. Annealing
3. Extension



PCR - Protocol

1. Add water, 1 bacterial colony, forward primer, reverse primer, Taq DNA Polymerase
2. Run heat cycles of 95°C (denature), 55°C (annealing), 72°C (elongation) on a thermocycler
3. Run on gel to check if the DNA product matches the expected size



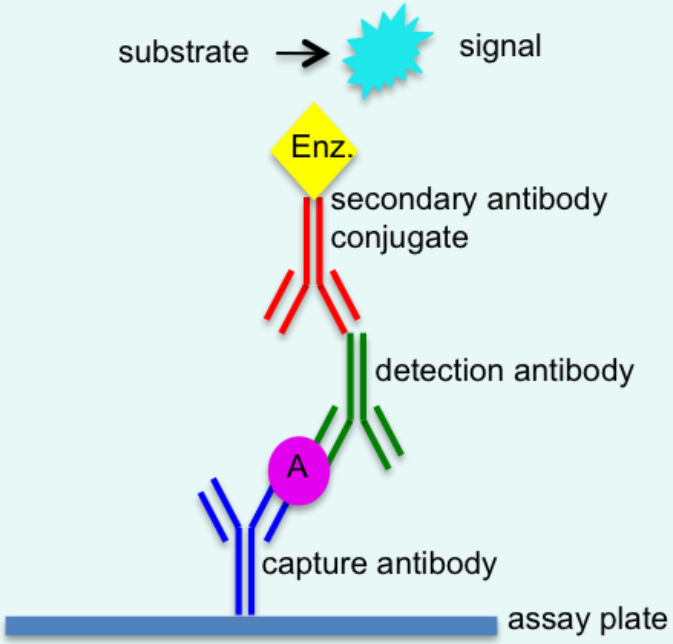
03

Objectives

Learn how to analyze proteins using ELISA and Western Blot

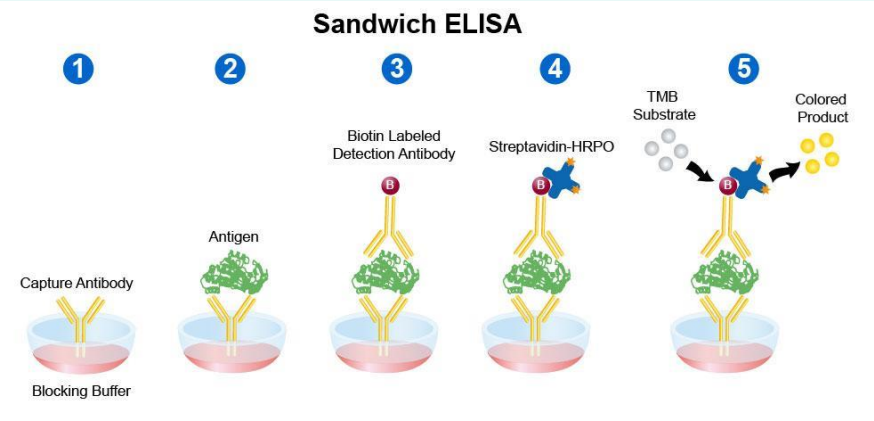
Sandwich ELISA Principle

- Selectively capture proteins using antibodies
- Detecting and secondary detecting antibodies amplify the signal that is produced (usually change in color)



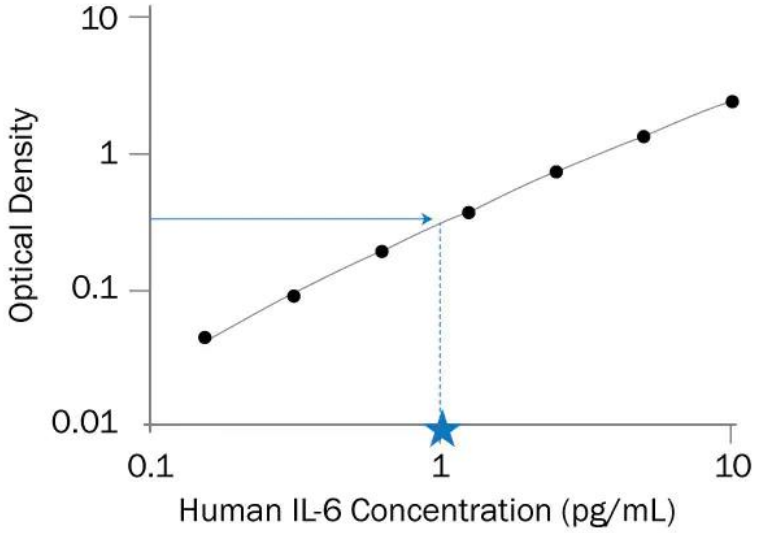
Sandwich ELISA Protocol

1. Coat wells with capture antibody and incubate overnight
2. Wash out coating solution and add milk to block protein-binding sites not from antibodies
3. Wash samples with PBS and add lowering dilutions of protein samples and standard solutions with known concentrations
4. Add detecting antibody and secondary detecting antibody conjugated with HRP (Horseradish Peroxidase) enzyme
5. Add TMB substrate to yield blue color



Sandwich ELISA Data Analysis

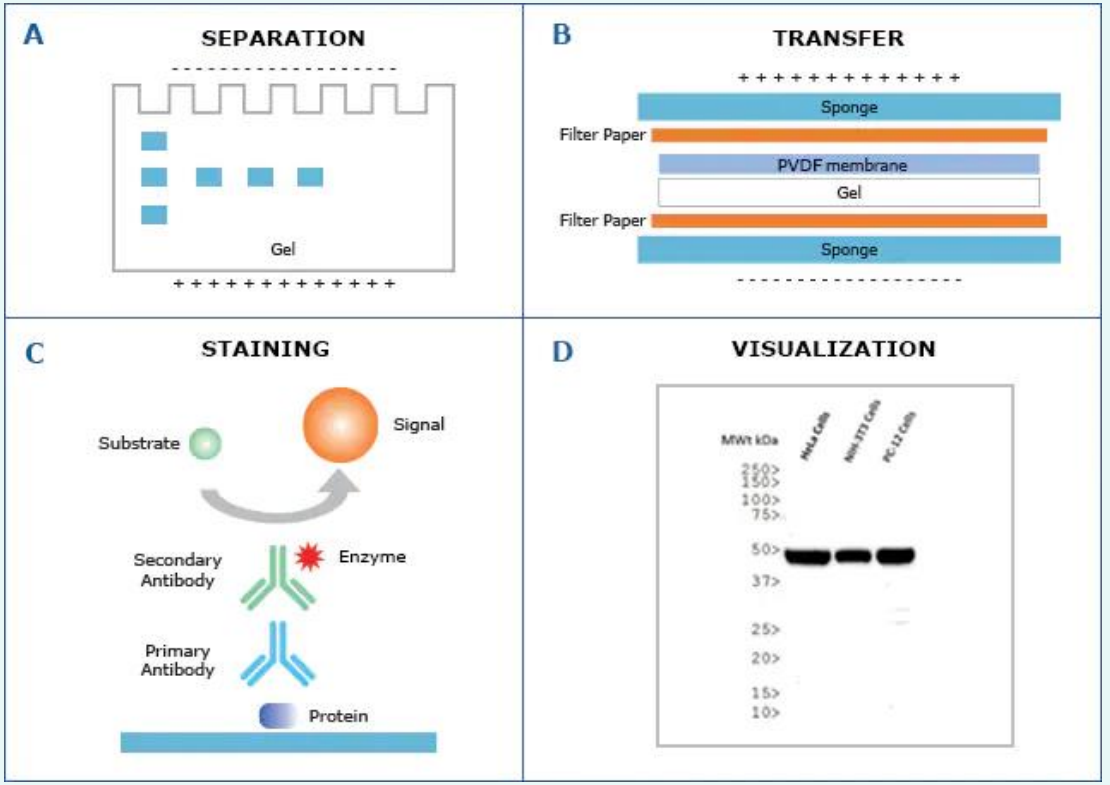
1. Plot a curve of the optical density (measurement of color in wells) over the known concentration of standards
2. Then use the equation of the curve to solve for the concentration of the protein sample (plug in the optical density measurements)



(pg/mL)	O.D.	Average	Corrected
0	0.051	0.059	-
	0.067		
0.156	0.101	0.103	0.044
	0.105		
0.313	0.148	0.149	0.090
	0.149		
0.625	0.246	0.251	0.192
	0.255		
1.25	0.431	0.432	0.373
	0.433		
2.5	0.798	0.804	0.745
	0.809		
5	1.407	1.418	1.359
	1.429		
10	2.485	2.498	2.439
	2.510		

Western Blot Principle and Protocol

A. Protein gel electrophoresis can separate proteins by size
B. Transfer proteins onto a membrane - porous for antibody staining
C. Same concept of ELISA, a signal is produced
D. The signal on the membranes can be visualized and imaged



04

Objectives

Understanding techniques used in clinical practice

Next Generation DNA Sequencing Principle

Used for highly accurate DNA sequencing of clinical samples:

- Can be used to determine mutations - cancer and other diseases

Different than traditional sequencing because of sample library preparation and more advanced PCR steps

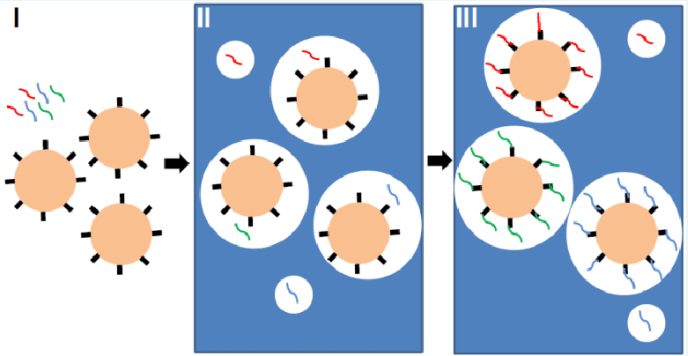
Next Generation DNA Sequencing Principle

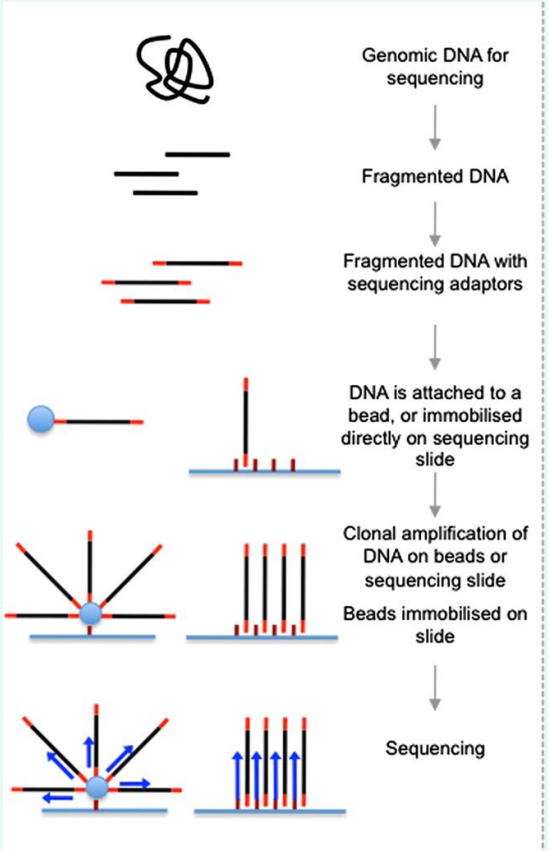
Sample Library Preparation:

- Templates are digested, ligated, elongated
- Adapters are added (to attach to beads)
- Amplified

Emulsion PCR:

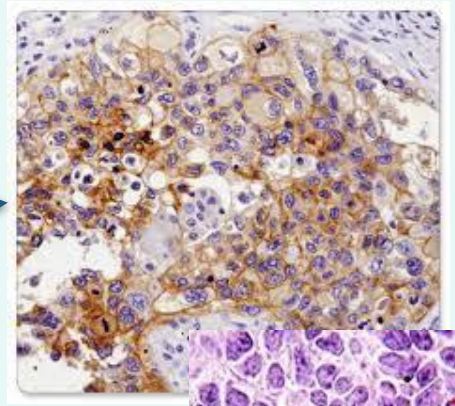
- Magnetic beads with library of DNA are amplified in oil droplets - DNA enrichment



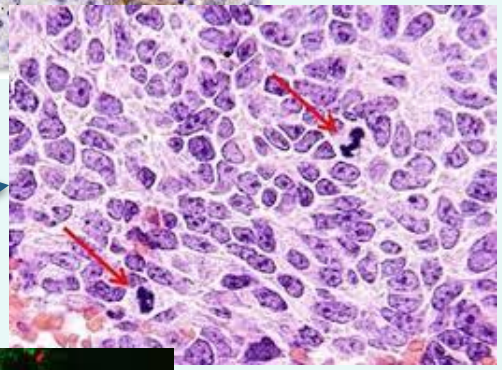


Different types of Staining

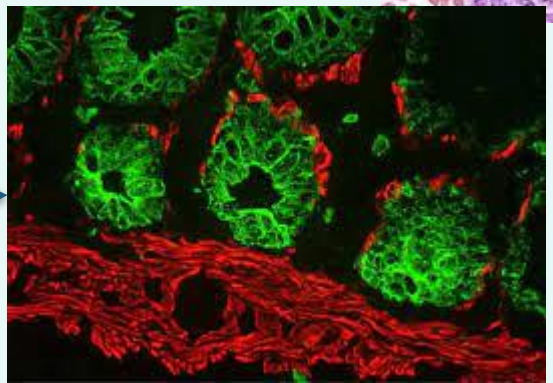
IHC (Immunohistochemistry)
- Detect protein biomarkers



H&E Staining
- Hematoxylin - stains for nuclei
- Eosin - cytoplasm



Immunofluorescence Staining
- Detect protein biomarkers with fluorescence



Thanks!

Do you have any questions?

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