334 MBIO
Biochemical Instrumentation Techniques

- Lab 8 -

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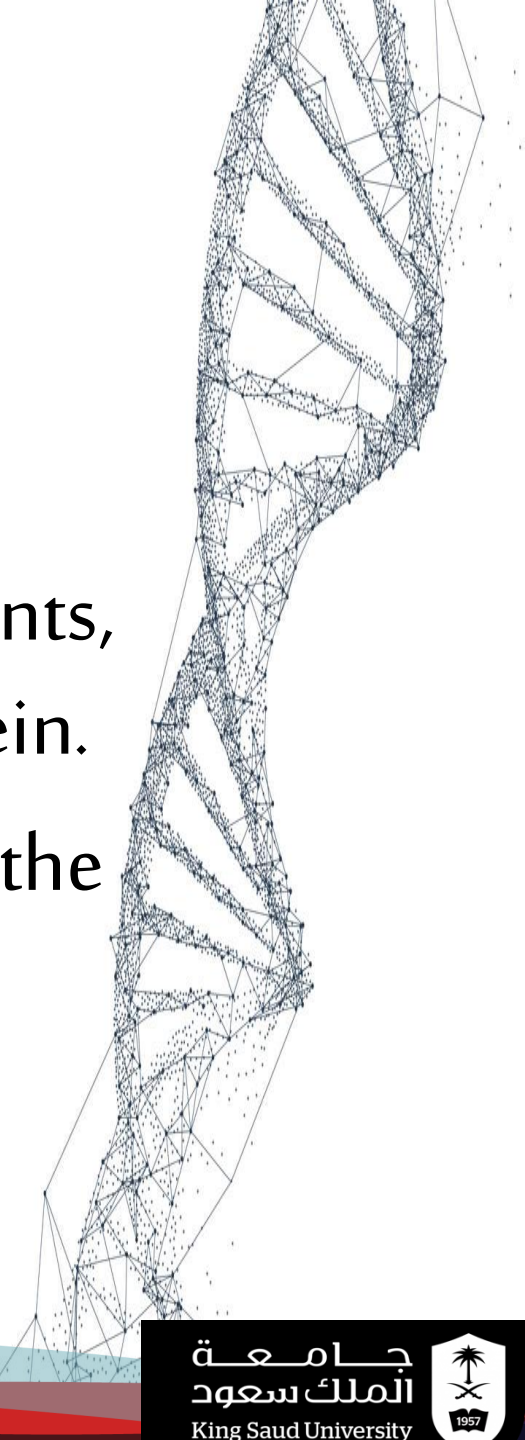
2025

Expression and Purification of Recombinant Proteins

Introduction

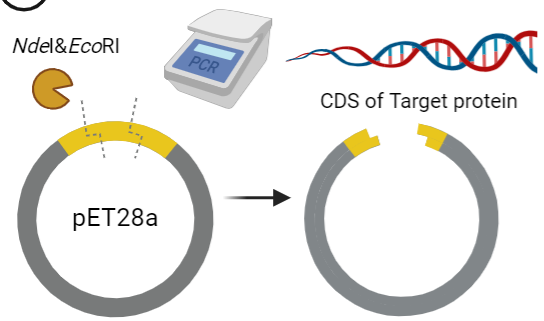
- **Protein extraction** is important for protein function and structure.
- Starting with **cellular lysis** ⟶ release the intracellular contents, it is followed by **purification** ⟶ isolate just that target protein.
- Various methods are employed for protein production using the unique features of **different expression systems**, from chemical to mechanical techniques.



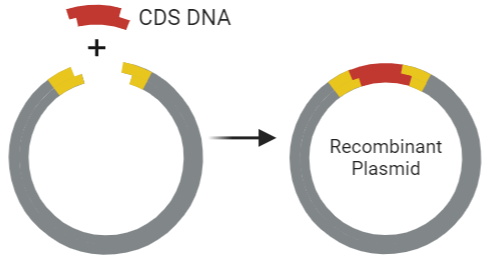
Workflow of Our Work So Far!

Plasmid construction

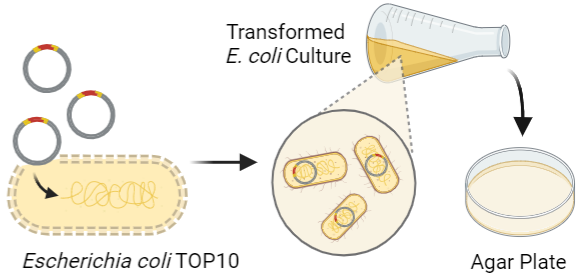
1 PCR and Backbone digestion



2 Gibson Assembly

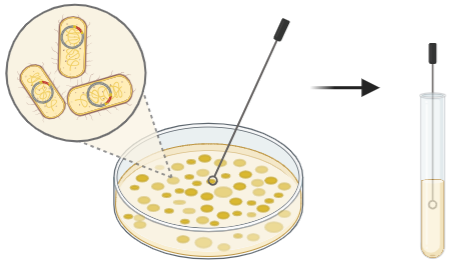


3 Bacterial Transformation



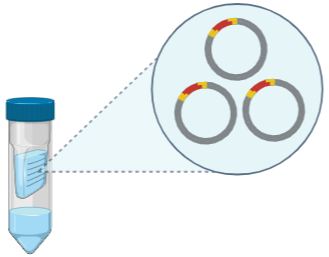
Clone Validation

4 Colony Inoculation



Transformed *E. coli* Plate and Screened by Kanamycin

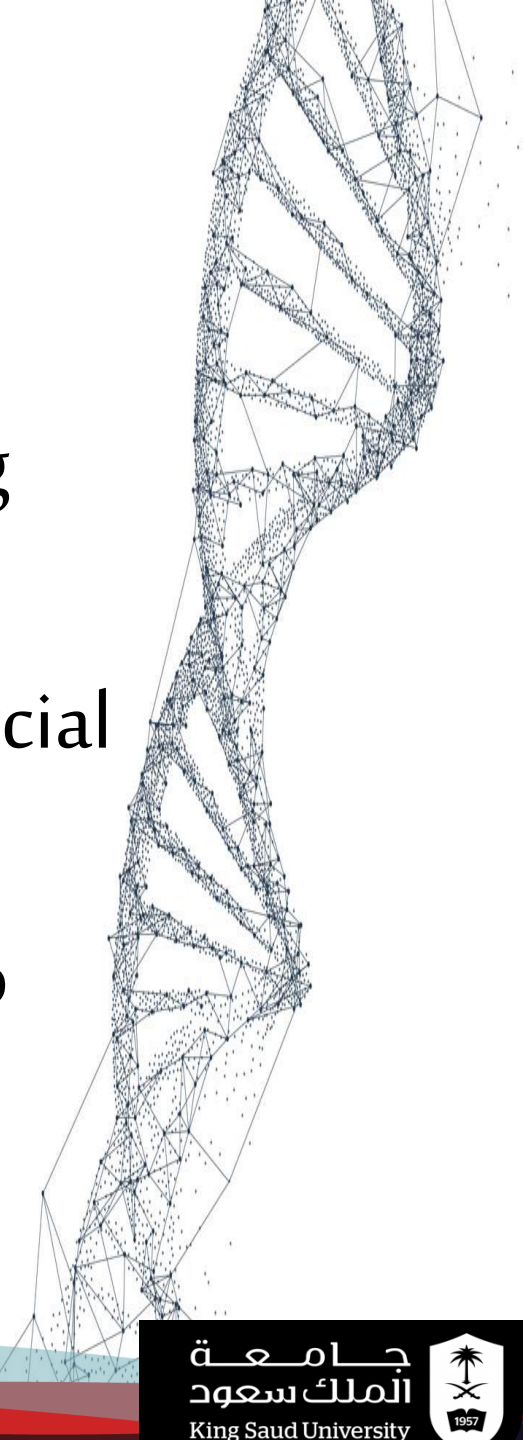
5 Plasmid Purification



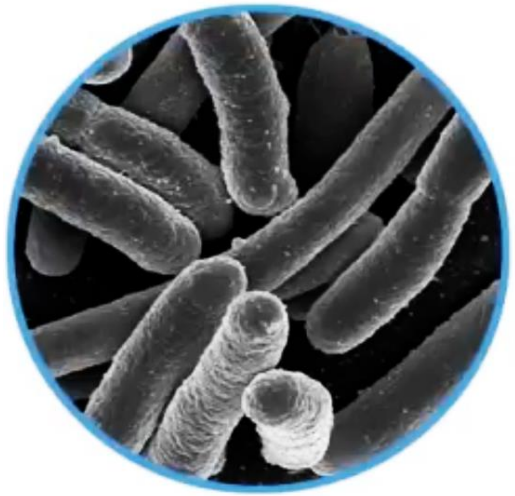
6 Sanger Sequencing Analysis

Protein Expression Systems

- **Expression systems:** is an overproduction of proteins by placing the gene encoding them under the control of a strong promoter.
- **Expression systems** are used in research and in the commercial production of enzymes or therapeutics.
- The most widely used for protein overproduction, both in lab and industrial scale, is the **prokaryotic system**.



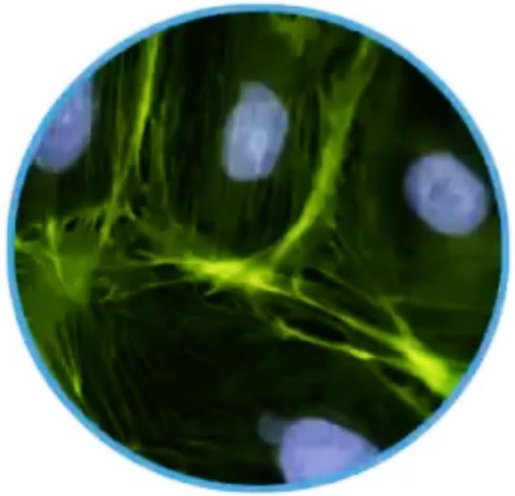
Multiple Host Systems Commonly Used for Protein Expression



E.coli cells

Yeast cells

Insect cells



Mammalian cells

General Considerations When Choosing the Expression system

Host compatibility: with cost, regulations, bioprocess optimization, and production capacity.

Protein origin: Mammalian cells suit human proteins; bacterial systems are better for bacterial proteins.

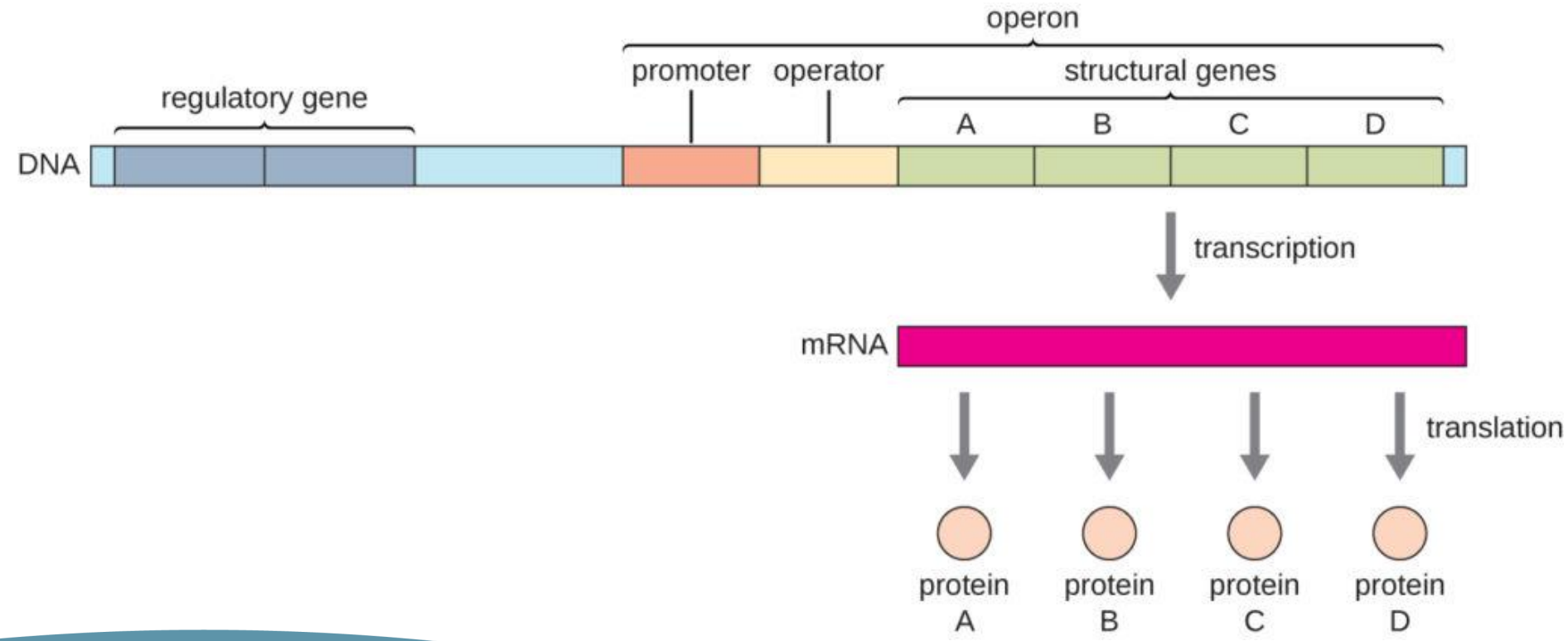
Post-translational modifications (PTMs): Eukaryotic systems are preferred for complex PTM requirements.

Production speed and yield: Bacterial and yeast systems offer fast growth and high yield, ideal for research and bulk production.

Protein folding: Eukaryotic cells are often better for proper folding of recombinant proteins, which may form insoluble aggregates in bacteria.

Prokaryotic Expression System

- This system is based primarily on the bacteria *E.coli*.
- The main components of prokaryotic gene regulation are:
  - Promoter
  - Operator
  - Operon
  - Activator
  - Inducer
  - Repressor

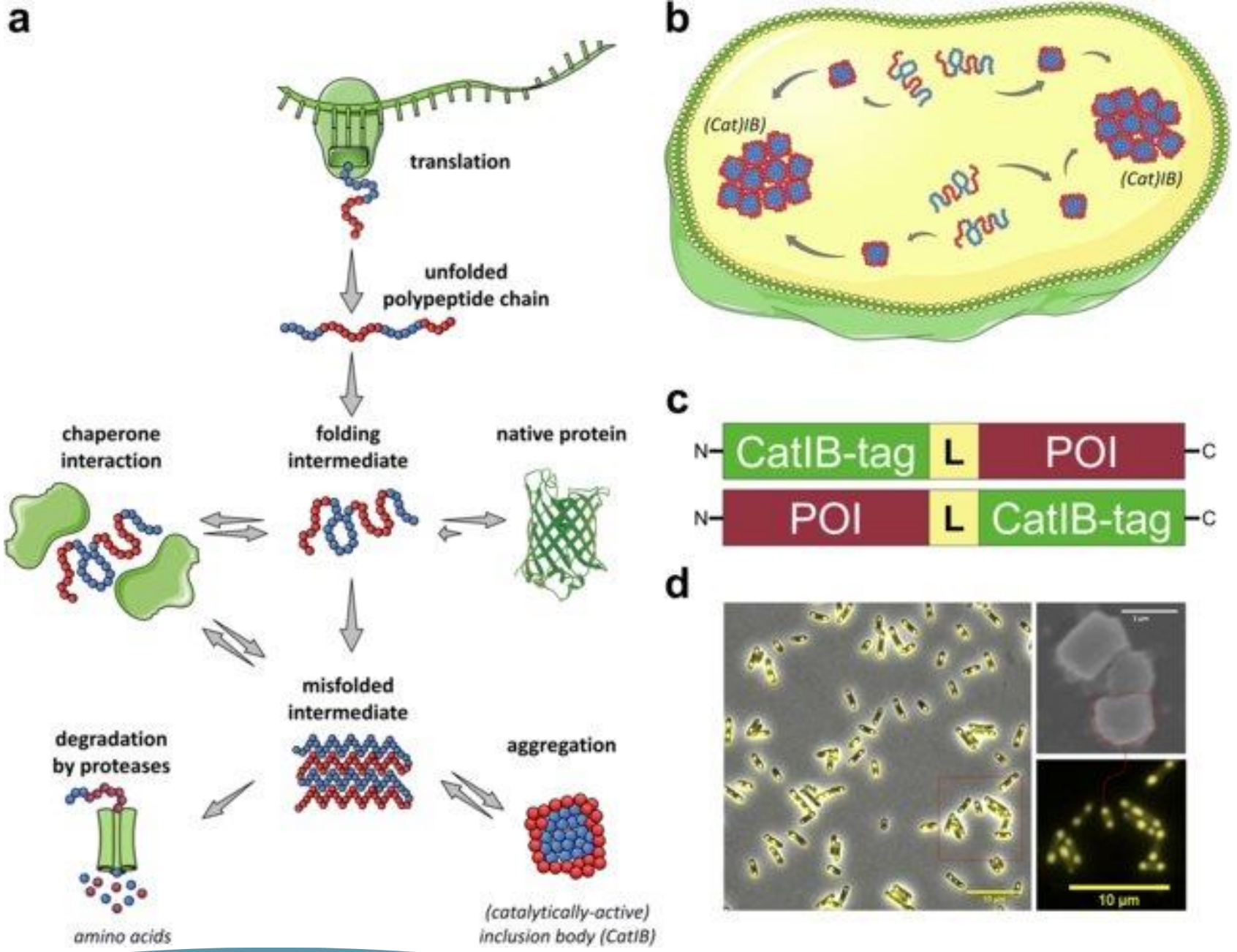


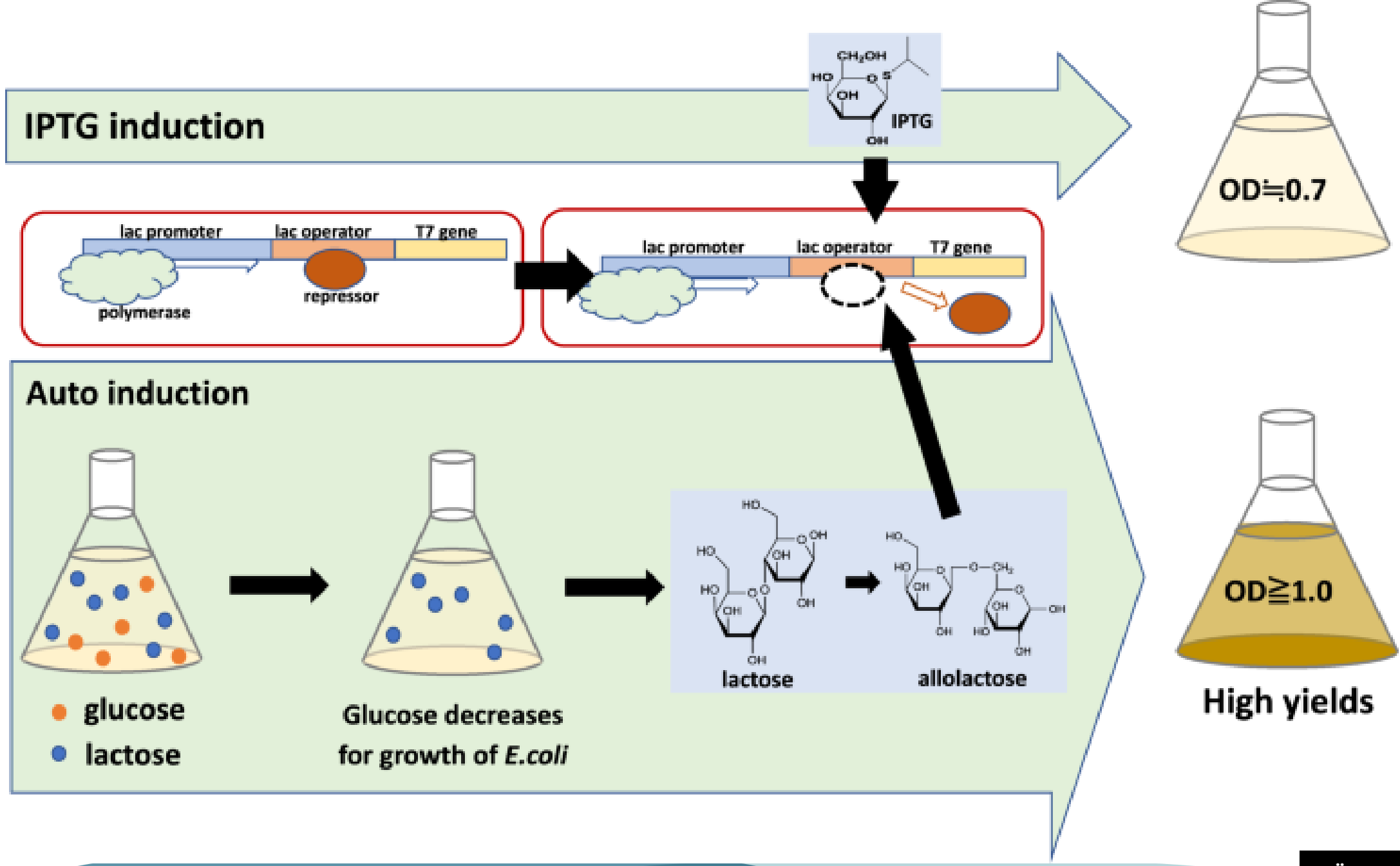
Prokaryotic gene regulation

Promoter	is like a "**start signal**" in DNA. It tells the cell **where** to begin making RNA.
Operator	is a short DNA segment located next to the promoter. It's like a "**gene switch**", in which a repressor/inducer can bind to block/activate RNA production.
Operon	a group of genes that are **controlled** together and turned on/off as a unit.
Activator	is a helper protein that **boosts gene activity**. It helps the machinery bind better to the promoter.
Inducer	is a small molecule that **turns genes on**. It either **activates** an activator or **removes** a repressor.
Repressor	is a protein that **turns genes off**.

Prokaryotic Expression System:

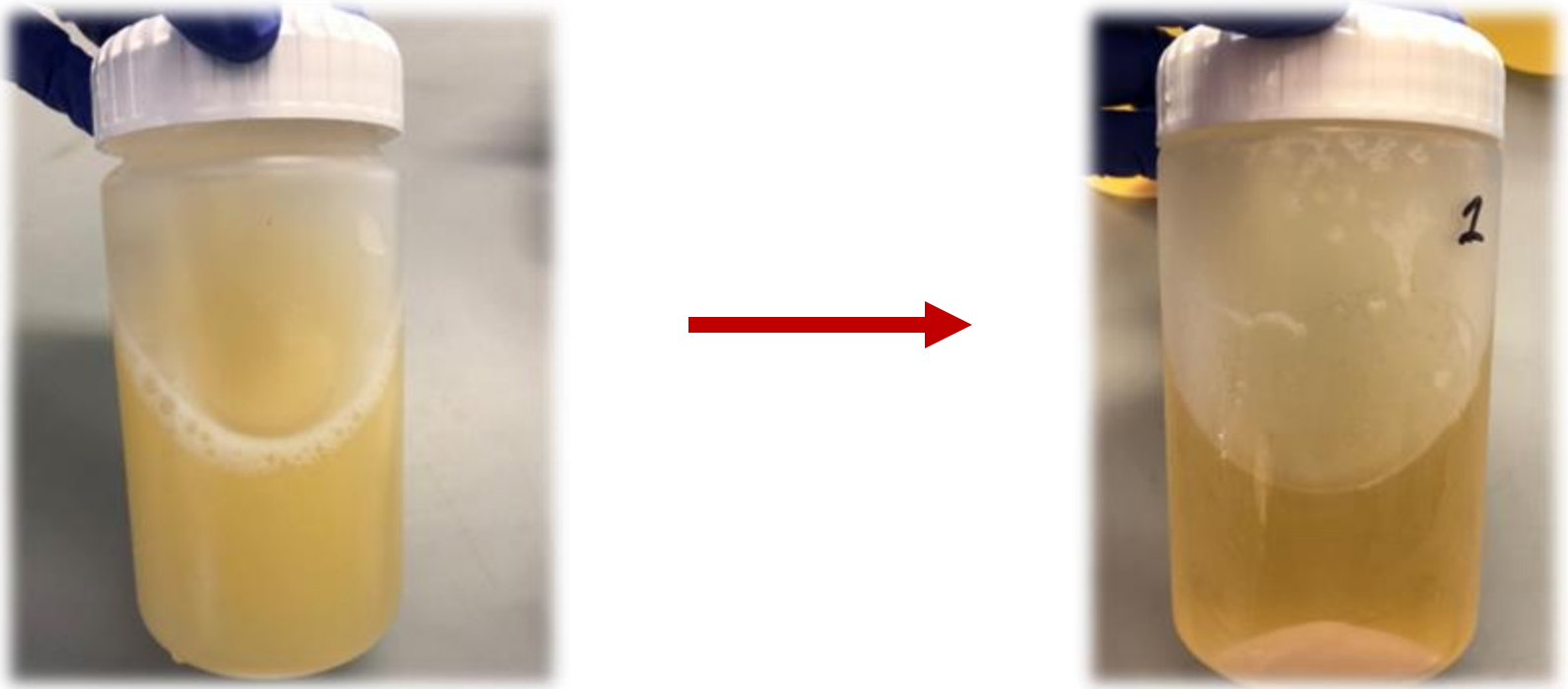
Pros	Cons
• **Fast Growth & High Yield:** Bacteria grow quickly, allowing rapid protein production in large amounts. • **Cost-Effective:** Culture media and maintenance are cheap compared to mammalian or insect systems. • **Simple Handling:** Easy to genetically manipulate, culture, and scale up. • **Well-Studied System:** *E. coli*, for example, has well-established protocols and tools available for expression.	• **Lack of PTMs:** Can't perform complex PTMs (like glycosylation) that are essential for the function of many eukaryotic proteins • **Protein Misfolding & Inclusion Bodies:** Some proteins don't fold correctly and end up as inclusion bodies, which are hard to purify and refold. • **Toxicity to Host:** Some proteins may be toxic to bacteria, reducing yield or killing the cells. • **Codon Usage Differences:** Human genes may need codon optimisation. • **Endotoxin Contamination:** *E. coli* produces harmful endotoxins that must be removed, especially for therapeutic use.




IPTG induction
IPTG
OD≒0.7
lac promoter
lac operator
T7 gene
polymerase
repressor
lac promoter
lac operator
T7 gene
Auto induction
glucose
lactose
Glucose decreases
for growth of E.coli
lactose
allolactose
OD≧1.0
High yields

Protein Extraction from Prokaryotic Cells

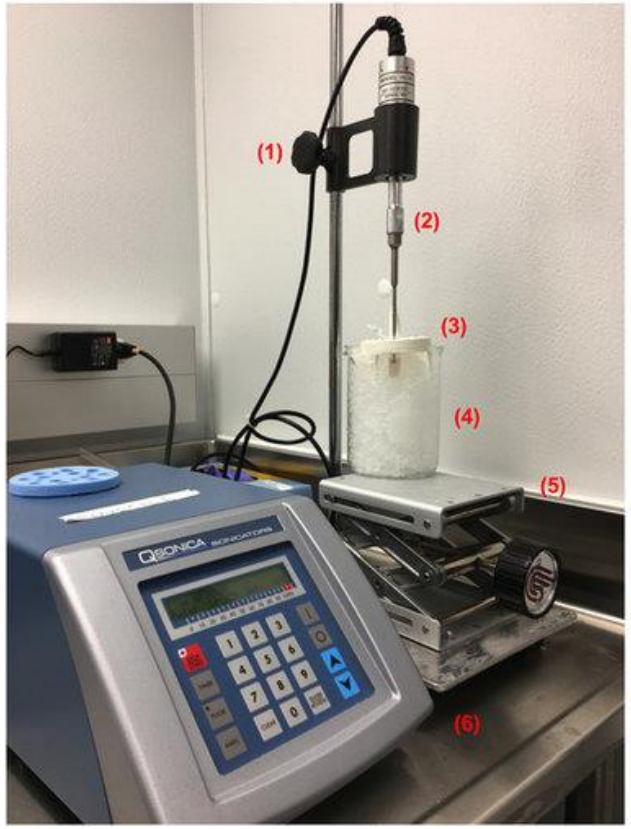
- Harvest the cells by centrifugation: 4000 *xg*, 20min.
- Flash freeze the pellet with liquid nitrogen and leave it overnight at -80 °C.



Protein Extraction from Prokaryotic Cells

- **PROTEINS LIKE TO BE COLD!!!!!!**
- Methods to lyse cells:
  - Freeze thaw
  - Enzymatic lysis with Lysozyme
  - Detergent (Triton X-100)
  - Sonication
- **Soluble protein** will be in **supernatant** after centrifugation
- Once outside of cells, protein will be susceptible to proteases. Therefore, **protease inhibitors** will be added to help prevent this.

Protein Extraction from Prokaryotic Cells



Sonication Equipment:

(1) Rod and Clamp

(2) Sonicator with Tip

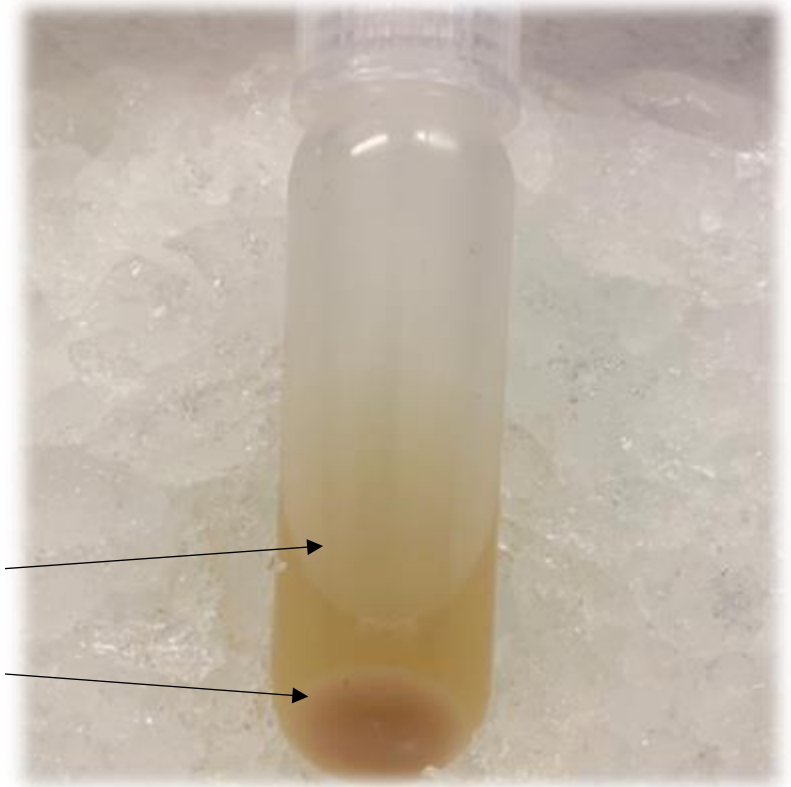
(3) Tube Holder

(4) 600 mL Beaker with Ice

(5) Adjustable Stage

(6) Sonicator Control Box

Protein Extraction from Prokaryotic Cells



Eukaryotic Expression System

- The problems associated with obtaining high yields of native recombinant proteins from genes cloned in *E. coli* have led to the development of expression for other organisms, such as:

- **Yeast**
- **Insect cells** (e.g., baculovirus expression)
- **Mammalian cells** (e.g., human embryonic kidney (HEK), and Chinese hamster ovary (CHO) cells).

Eukaryotic Expression System

- Eukaryotes **do not have operons**.
- Eukaryotic systems use **strong promoters**, but the type depends on the host cell
- **Signal peptide** should be optimised for better expression and yield.
- Eukaryotes genes are organised into **two segments**:

- **Intros** ⟶ non-coding sections

- **Exons** ⟶ coding sections

Eukaryotic Expression System:

Pros	Cons
• **PTMs:** Can perform complex PTMs (like glycosylation, phosphorylation) • **Native Protein Folding:** help produce proteins in their biologically active and native conformations • **Suitable for Human Proteins:** Especially mammalian systems, which closely mimic the human cellular environment. • **Versatile Hosts:** Multiple systems available (yeast, insect, mammalian), each with different strengths for various protein types. • **Scalable:** an be adapted for small-scale lab work or large-scale industrial production.	• **Higher Cost:** expensive media, equipment, and maintenance compared to bacterial systems. • **Slower Growth:** grow slower than bacteria, leading to longer production times. • **Complex Handling:** Requires sterile conditions and more expertise in cell culture techniques. • **Lower Yields:** may produce lower amounts of protein compared to bacteria. • **Risk of Contamination:** Especially in mammalian systems, contamination with viruses or other pathogens is a concern.

By differentiation

Stem cell culture

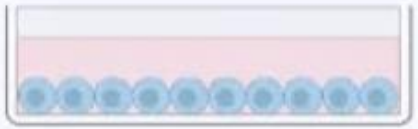
- Undifferentiated cells
- Able to differentiate into other cell types

Differentiated cell culture

- Differentiated cells
- Lost ability to further differentiate

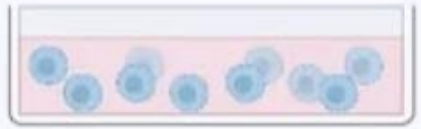
By adhesion

Adherent



- Cells are attached to solid surface
- Grown as monolayers

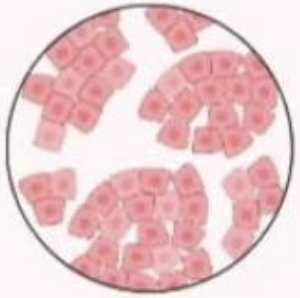
Suspension



- Cells are free-floating in liquid medium

By cell type / morphological structure

Epithelial



- Squamous, columnar or cuboidal-shaped

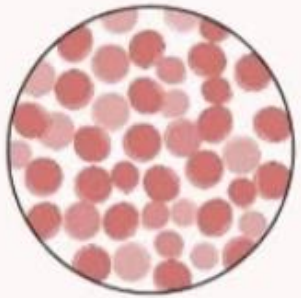
Endothelial

- Rounded outline
- Elongated

Fibroblast-like

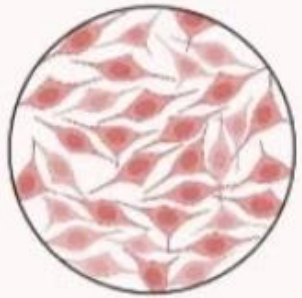
- Spindle-shaped
- Elongated

Lymphoblast-like



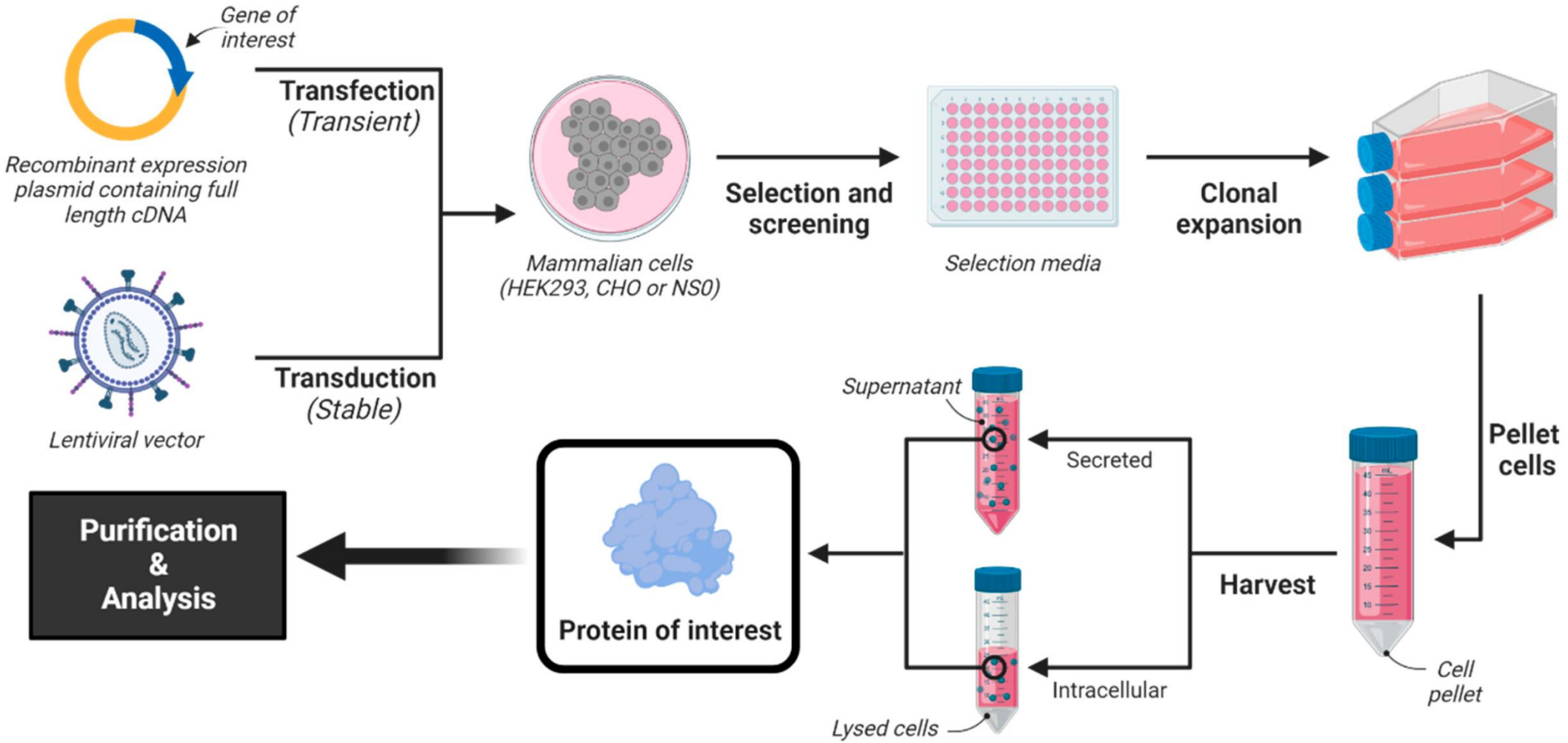
- Spherical-shaped
- Cells range in size

Neuronal



- Round, pyramidal, or spindle-shaped

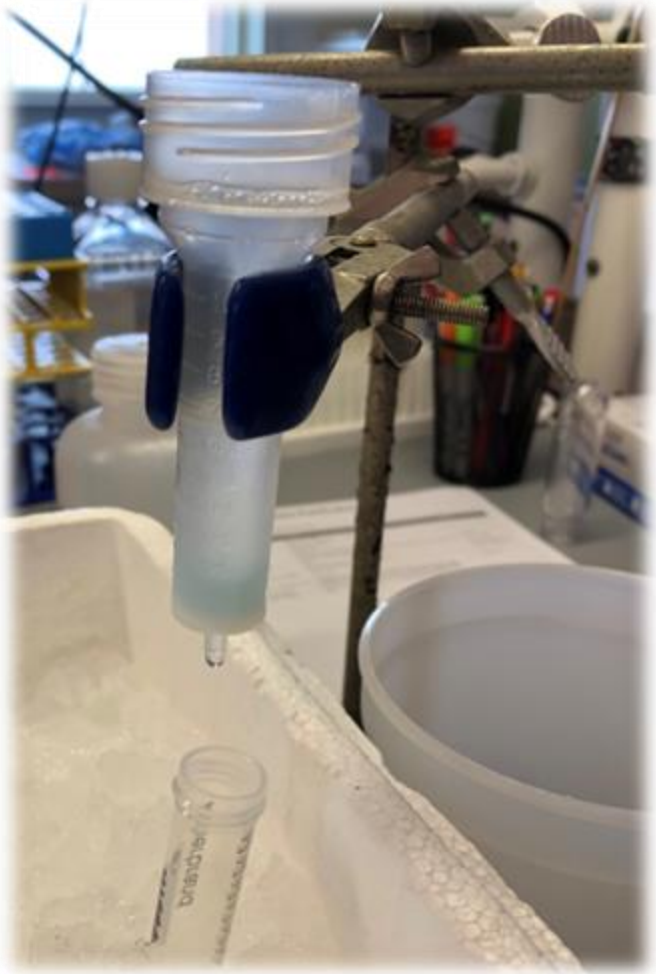
Mammalian Expression System



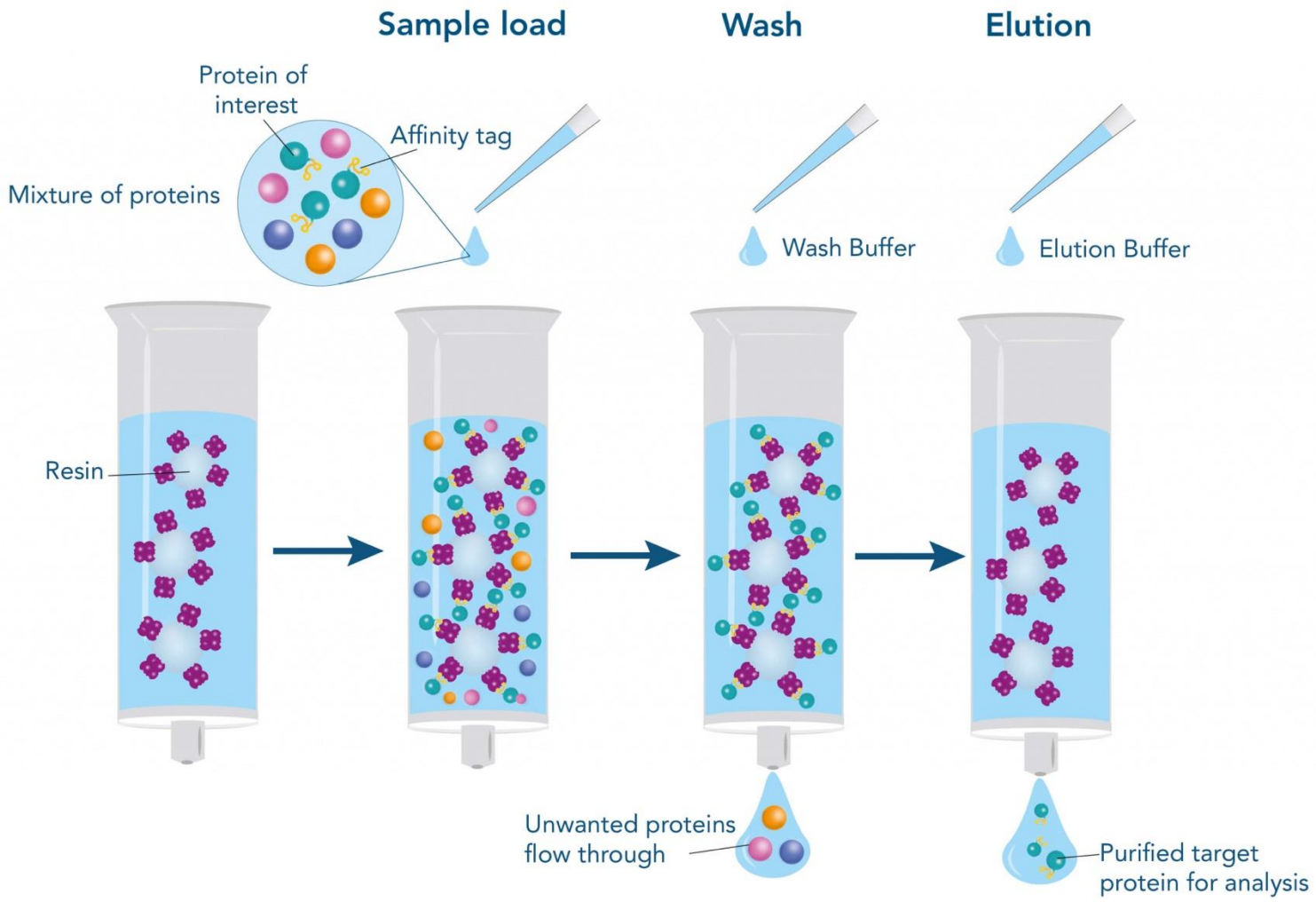
Protein Purification

Affinity chromatography

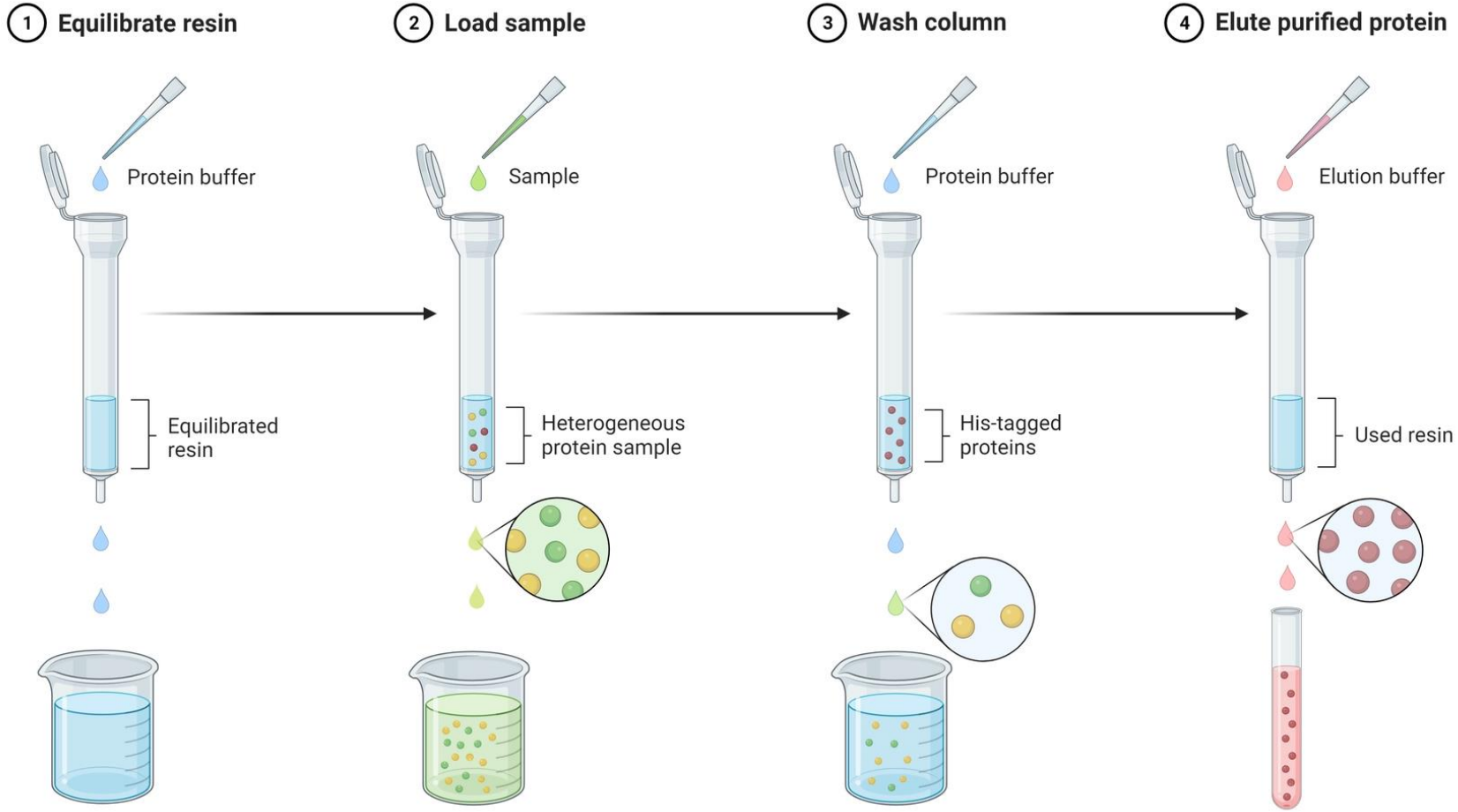
- Protein purification depends on the type of **tag used**.
- Each tag binds to a specific resin or purification system.
- Some tags improve protein solubility and stability (e.g., Fc-tag).

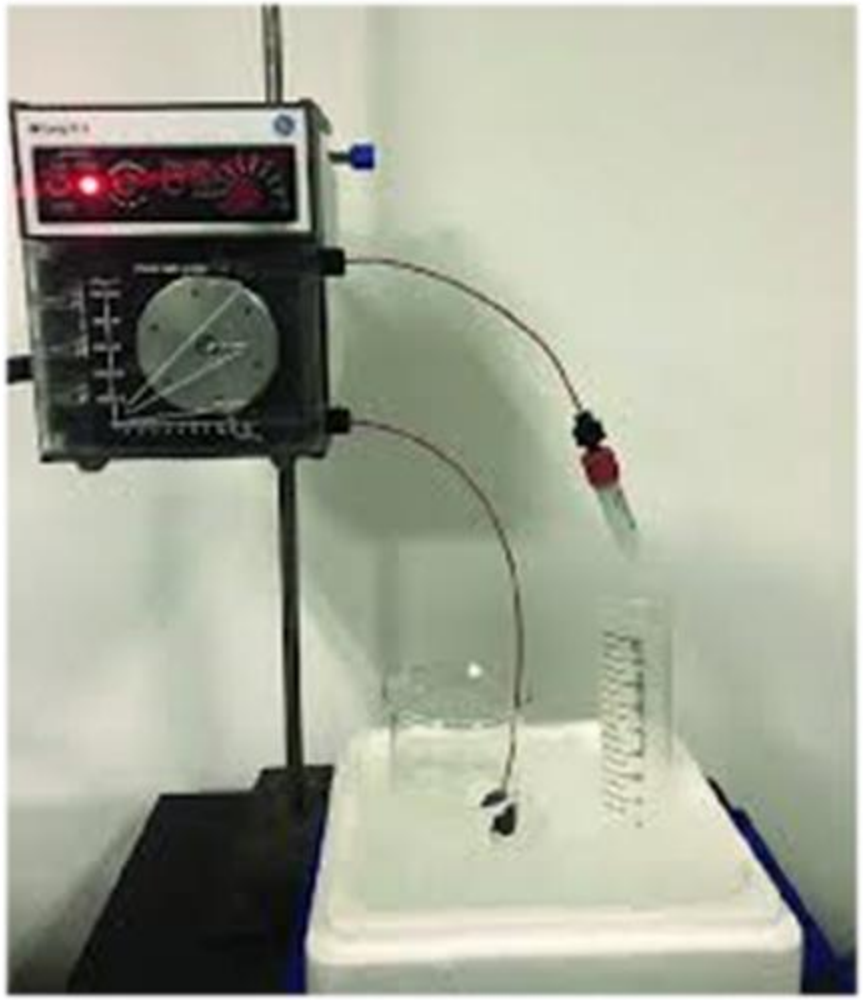


Affinity chromatography

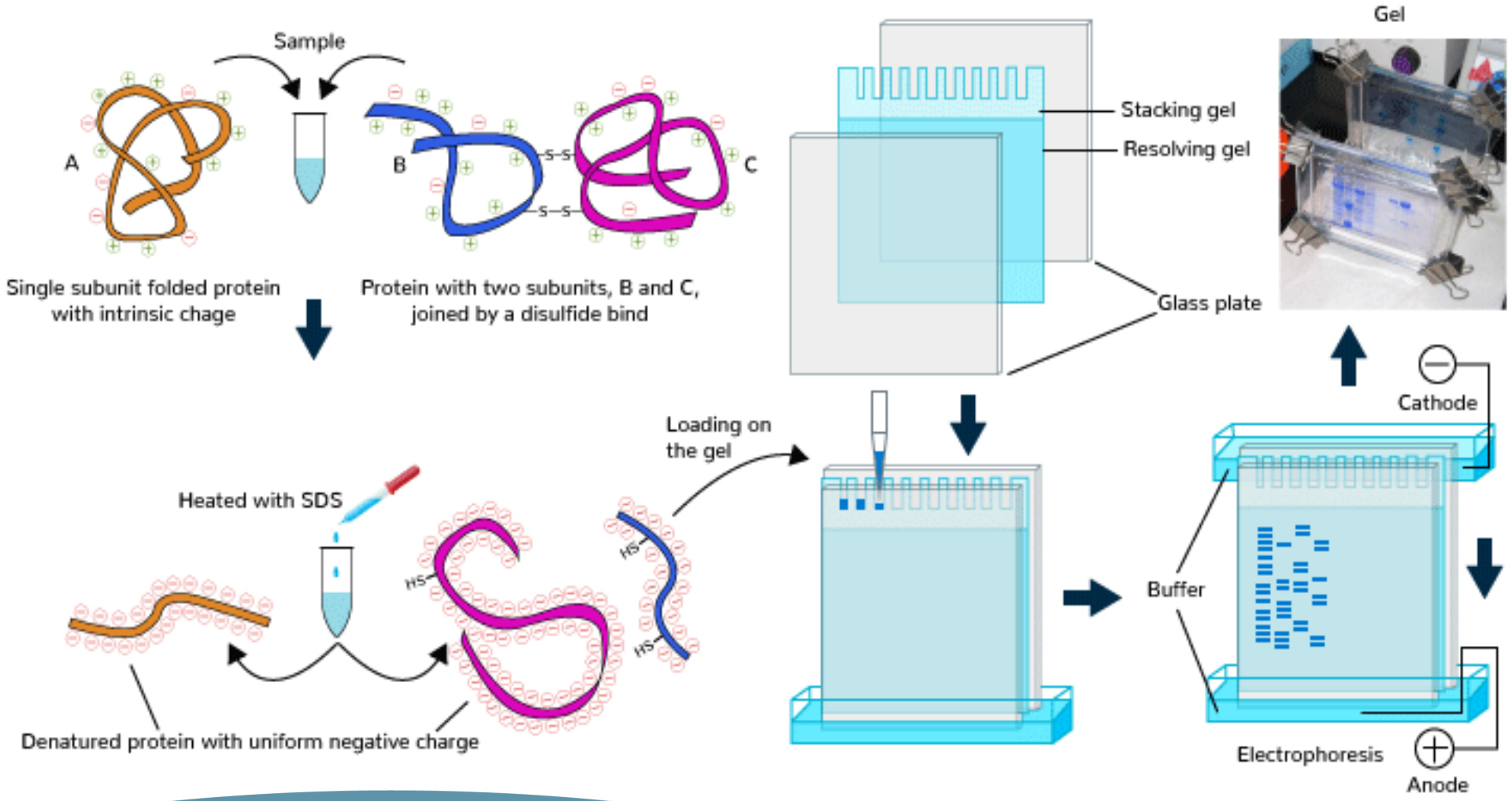


Affinity Chromatography





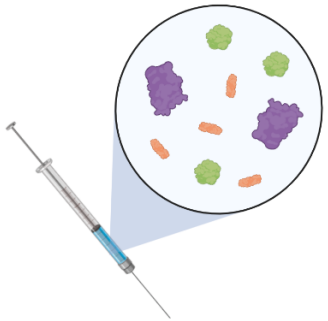
SDS-Page Analysis



Size Exclusion Chromatography (SEC)

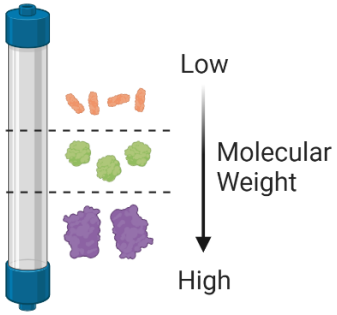
1 Injection

Heterogeneous protein sample is loaded in a FPLC system



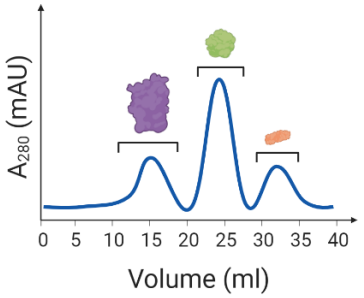
2 Elution

Proteins are separated by molecular weight



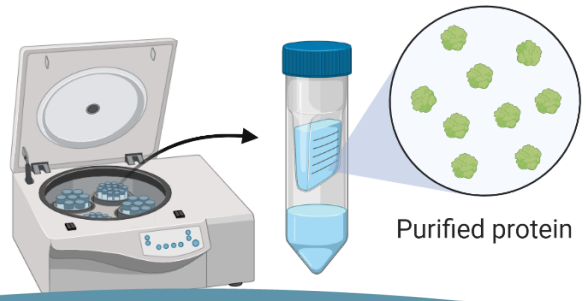
3 A_{280} Measurement

Absorbance is measured for all the eluted fractions and plot into a chromatogram



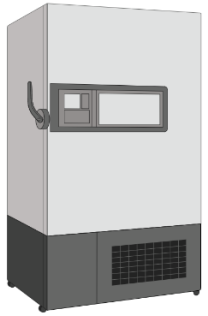
4 Concentration

Fractions of interest are mixed and concentrated

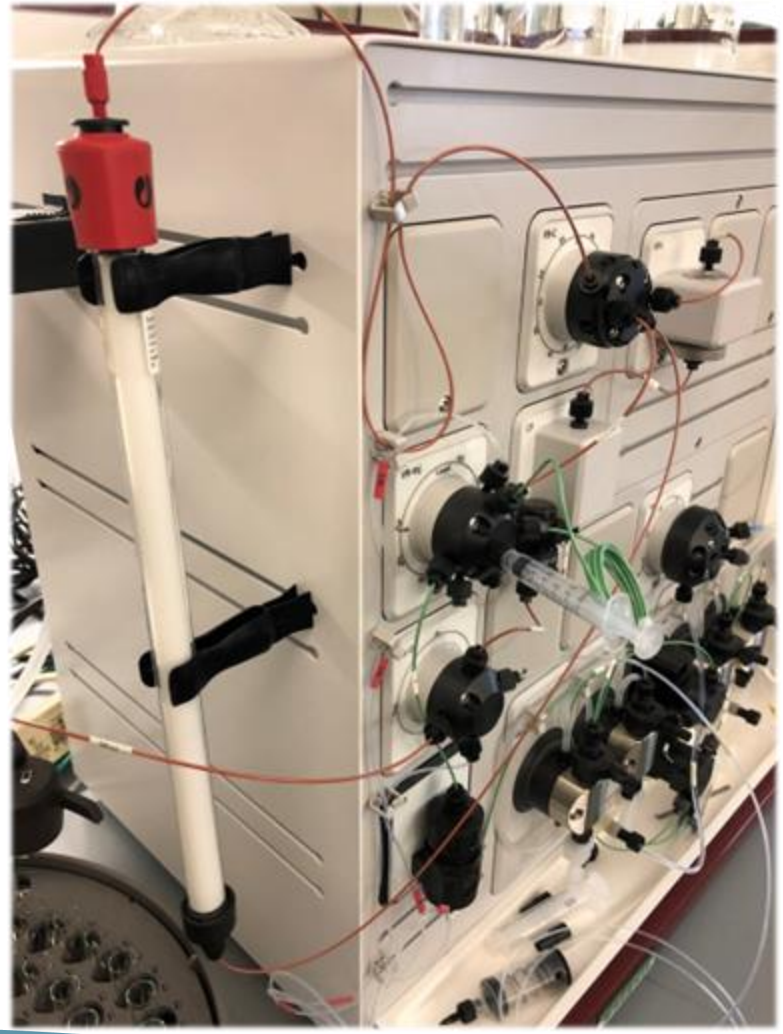


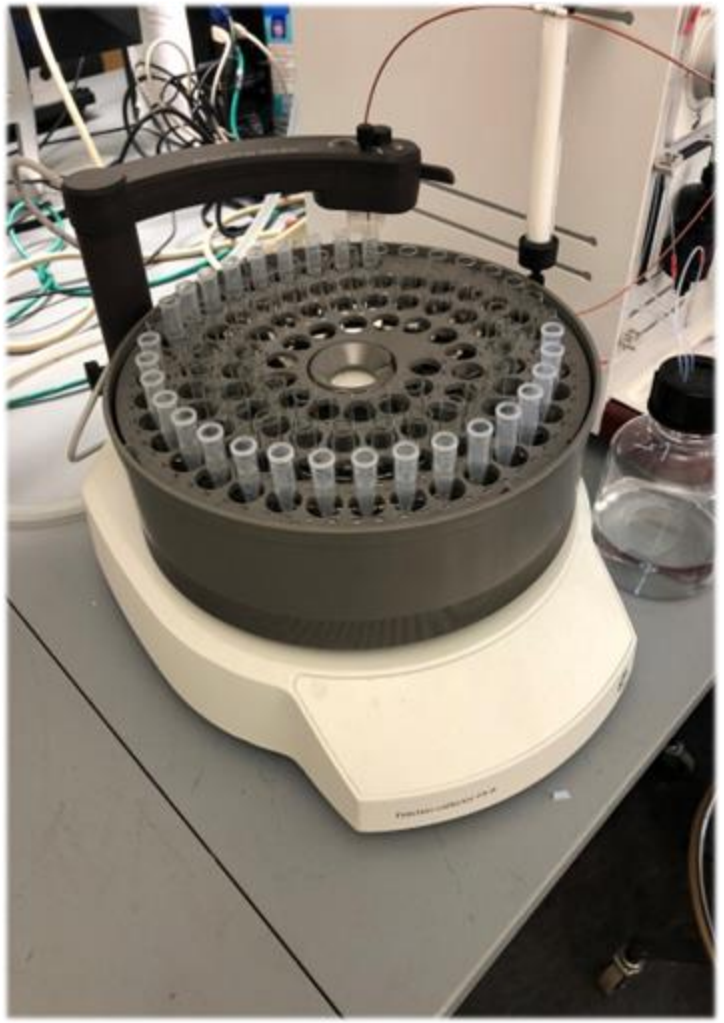
5 Storage

Purified proteins are stored at -80°C



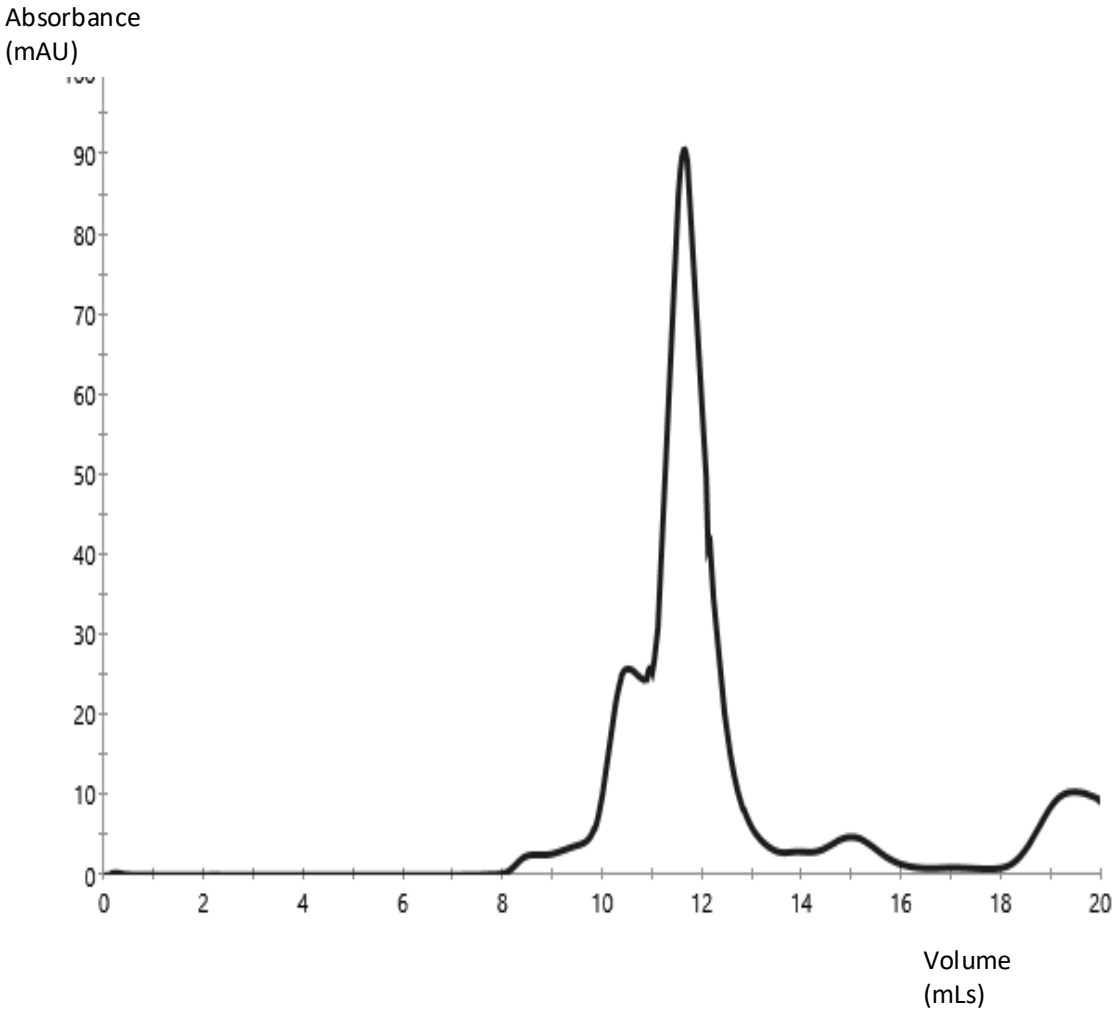
Size Exclusion Chromatography (SEC)





Size Exclusion Chromatography (SEC)

- As protein **travels down** the column, the UV monitor tracks the 280nm absorbance.
- The computer software graphs the absorbance over the volume in mLs.
- **A narrow high peak** means <u>good purification</u>!



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