

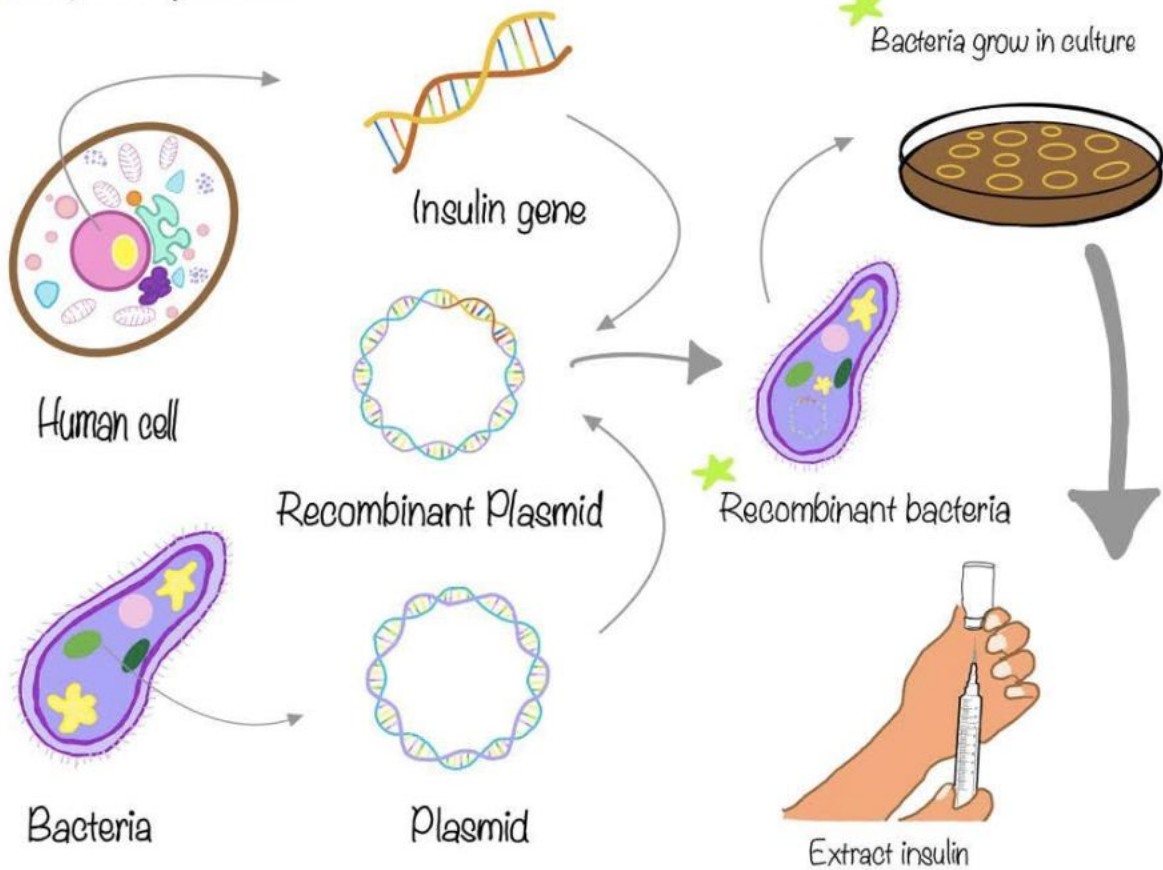
# **BCH 462- Biotechnology & Genetic engineering [Practical]**

## **Lab (3) Extraction and Determination of Bacterial Proteins**

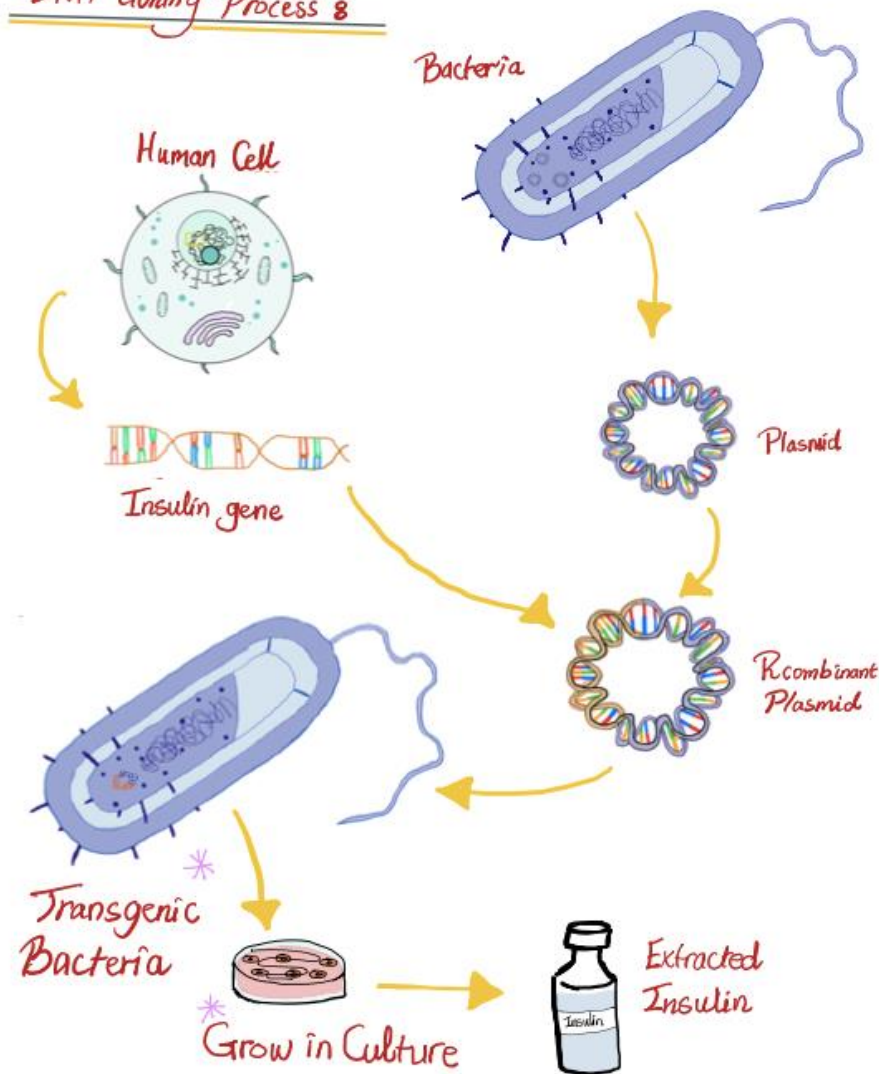
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★ Steps in the previous lab



### DNA Cloning Process 8



**Figure 1.** Schematic representation of recombinant insulin production

# Bacterial proteins

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- **Bacterial protein** is a protein which is either part of the bacterium structure or produced by bacterium as a part of its life cycle.
- Proteins are very essential for most of the bacterial metabolic functions as well as for cell integrity.
- **Studies on Bacterial proteins is beneficial because:**
  1. It can impact human health. Bacterial protein can be **Toxic**, causing illness or death in an organism which has been infected including humans.
  2. Used as a **model** to gather data about the proteins associated with larger organisms.

# Bacteria as biological factories

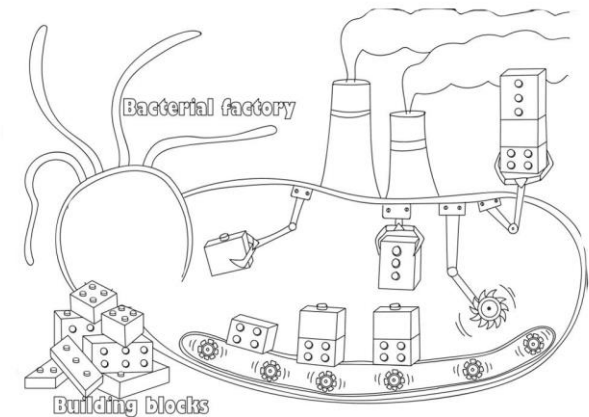
- **Bacteria** can produce **foreign** proteins from introduced gene using their own gene expression machinery.
- Scientists routinely **clone** the gene that encodes ‘their’ protein of interest and express large amounts of it in bacteria and other organisms.
- Many **medicines** and **drugs** are produced, for instance;

## A. Proteins hormones.

- I. **Insulin** (for treating diabetes).
- II. **Erythropoietin** (for treating anemia).
- III. **Growth hormones** (for treating growth disorders).

## B. Antibiotics, vaccines and enzymes.

- 💡 **Pause and Think** why scientist tend to lean toward protein therapy rather than gene therapy?



# Review

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Steps	Lab #1 [Plasmid Isolation]	Lab #2 [Transformation of Competent Cells]	Lab #3 [Estimation of Protein Concentration]
1	Growth of the bacterial culture.		
2	Harvesting of the bacteria by centrifugation.		
3	-Lysis of the bacteria -Purification of plasmid DNA.	-Using CaCl <sub>2</sub> solution and brief heat shock to transform the competent cells.	<b>-Lysis of the bacteria</b> <b>-Estimation of protein concentration using Bradford's method.</b>

# Practical Part

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# Practical part

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- **Aims:**

- Extraction of total bacterial proteins.
- Determination of bacterial proteins using **Bradford's assay**.

- **Principle:**

**Isolation of bacterial proteins involves several steps:**

1. Growth and induction of bacterial cultures.
2. Lysis of cells in a suitable buffer which achieved by sonication ( 20 kHz) for 30–60s.
3. DNase and RNase treatment for the removal of the nucleic acids.
4. Determine the protein concentration using suitable method (**Bradford's assay**).
5. Passage of the extract through an affinity resin and finally elution of proteins.

# Practical part

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- **Sonication** refers to the process of applying sound energy to agitate particles in a liquid.
- Ultrasonic frequencies (>20 kHz) are usually used, so the process is also known as **ultrasonication**.
- The method uses pulsed, high frequency sound waves to **agitate** and **lyse cells**.

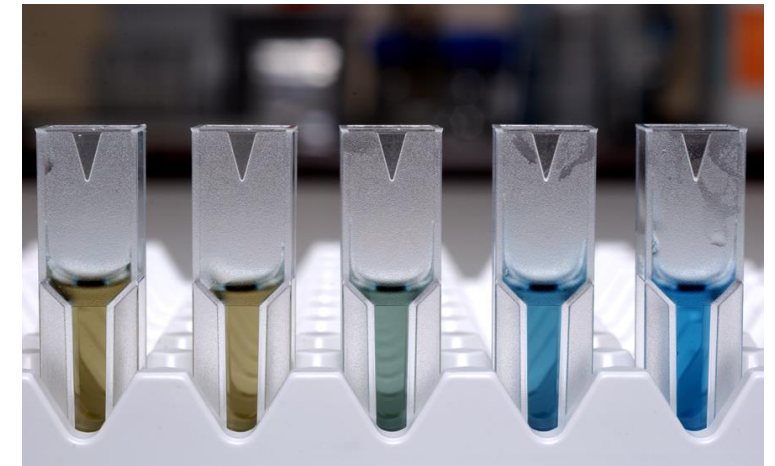
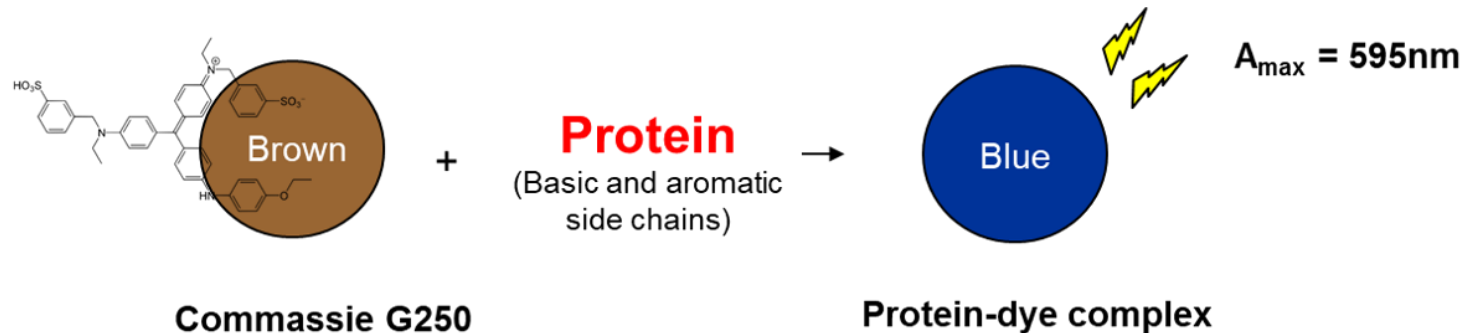




# Practical part

## ■ Principle:

- Bradford method is used to determine the protein concentration, using **standard curve of concentrations**.
- The Bradford protein assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 (*reddish brown*) shifts from 465 to **595** nm when binding to protein occurs (*blue form*).
- The intensity of the colored product is linearly proportional to the concentration of protein present in the solution.



# Practical part

## Results:

Test tube	Distilled water [ $\mu$ l]	Stock BSA solution (62.5mg/L) [ $\mu$ l]	Sample [ $\mu$ l]	Protein concentration [mg/l]
Blank	200	-		
A	180	20		
B	150	50		
C	100	100		
D	50	150		
E	-	200		
F (Unknown soluble proteins)	-	-	200	?
F'	100	-	100	?
G (Unknown insoluble proteins)			200	?
G'	100		100	?

