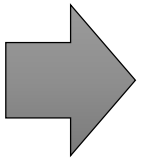
Biochemistry 4. Protein Purification

- **Objectives**

To purify the target protein from cloned genes from previous classes

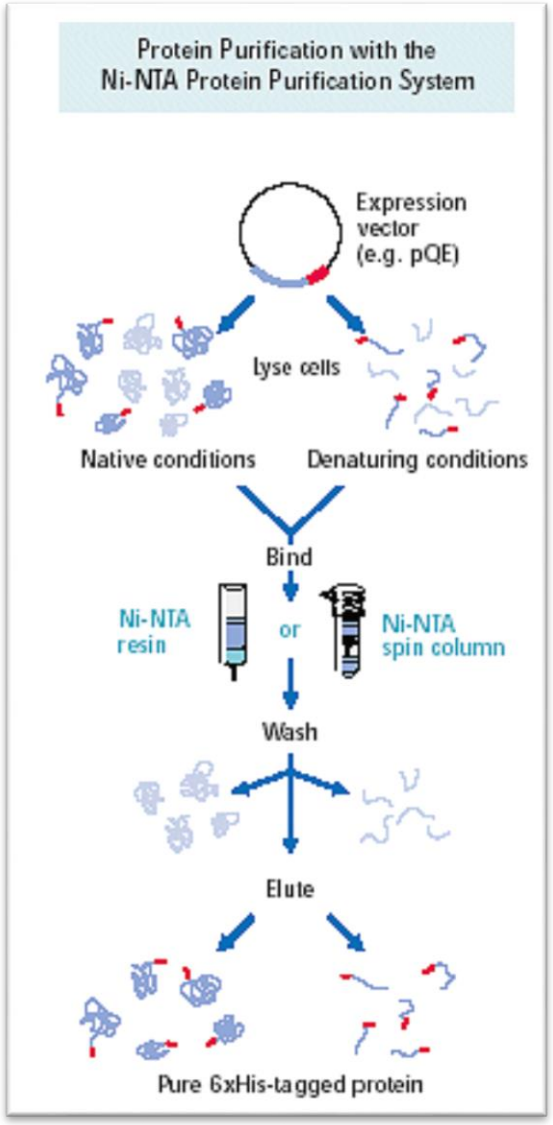
1. General Procedure of Protein Purification



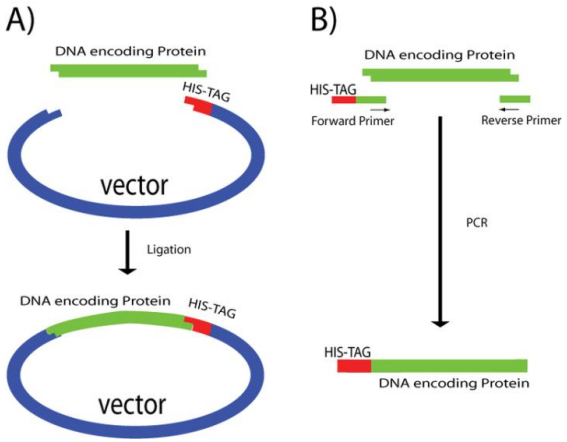
Overall Process

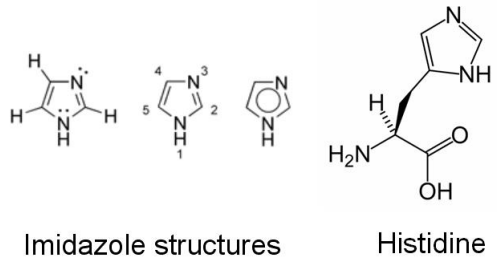
Polymerase Chain Reaction (PCR)	• Amplification of protein of interest (POI) gene
DNA purification	• Purification of POI genes by gel elution / restriction of amplified genes
Plasmid Ligation and Transformation	• Ligation of POI gene and expression vector / transformation into gene-expressing cells
Protein overexpression	• POI overexpression by cells
Protein purification	• Purification of POI genes by gel elution
Identification of POI	

Protein purification

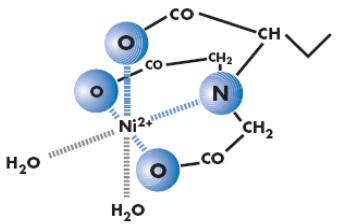


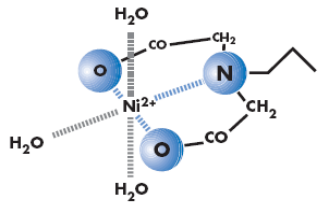
His-tag affinity purification

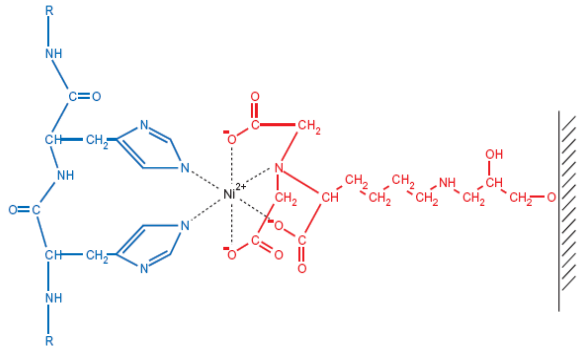




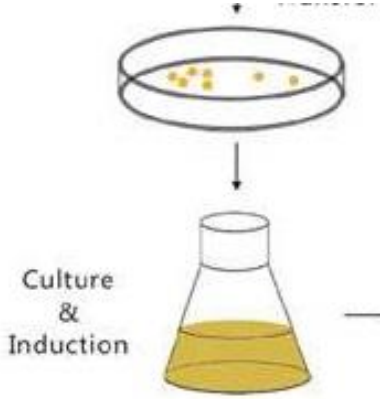
Ni-NTA or IDA







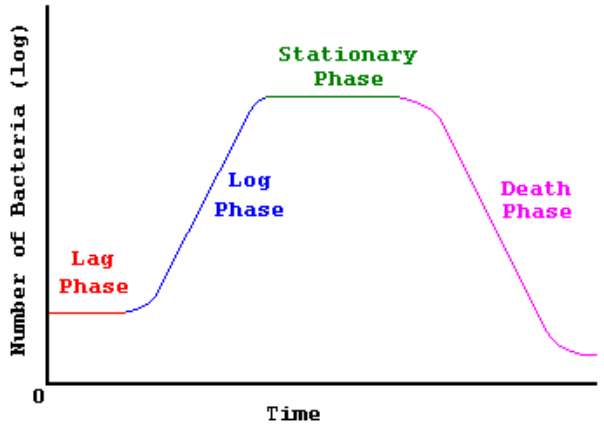
GFP expression from colony



PROCEDURE

- Pick single colony from plate and grow it in 20ml LB medium containing Ampicilin at 37°C for overnight
- inoculate the starter culture into fresh LB medium (200 mL) and incubate at 37 °C until O.D reaches A_{600} = 0.6
- IPTG was added to 1 mM and incubate the culture for overnight
- Cells were harvested by centrifugation at 6000 rpm for 1min.
- Freeze the cells at -20 or -70°C

Bacteria growth curve.



1. **Lag phase** : bacteria adapt themselves to growth conditions , synthesis of RNA, enzymes and other molecules occurs
2. **Exponential phase(log phase)** : cell doubling.
3. **Stationary phase** : growth-limiting factor ;this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids(newly formed cells per time = dying cells per time)
4. **Death phase** : bacteria run out of nutrients and die

Reagents and Apparatus

- Lysis buffer(50 mM Tris pH 8.0, 500 mM NaCl, 5 mM imidazole, Lysozyme)
- Washing buffer(50 mM Tris pH 8.0, 500 mM NaCl, 50 mM imidazole)
- Elution buffer(50 mM Tris pH 8.0, 500 mM NaCl, 250 mM imidazole)
- NTA or IDA resin
- Centrifuge
- 15 ml conical tube, 50 ml conical tube, 1.5 mL Eppendorf tube, rack
- Sonicator

Protein Purification

PROCEDURE

• Cell pellet is resuspended with **lysis buffer**.(50 mM Tris, 500 mM NaCl, 5 mM Imidazole, lysozyme, beta-mercaptoethanol, pH 8.0; 20 mL cell culture volume in 1 mL lysis buffer)
• Sonication to break the cell membrane.
• Centrifuge lysate at 13000 rpm for 10 min.
• Equilibrate the **Ni-IDA column(100 µL)** with 5x volume of **EQ buffer** (50 mM Tris, 500 mM NaCl, 5 mM Imidazole)
• Load cleared **lysate** containing GFP onto the pre-equilibrated Ni-IDA spin column.
• Wash the Ni-IDA column twice with **WASH buffer** (50 mM Tris, 500 mM NaCl, 50 mM Imidazole, pH 8.0)
• Elute the protein twice with 100 µL **Elution buffer** (50 mM Tris, 500 mM NaCl, 250 mM Imidazole, pH 8.0)
• Collect the eluate.