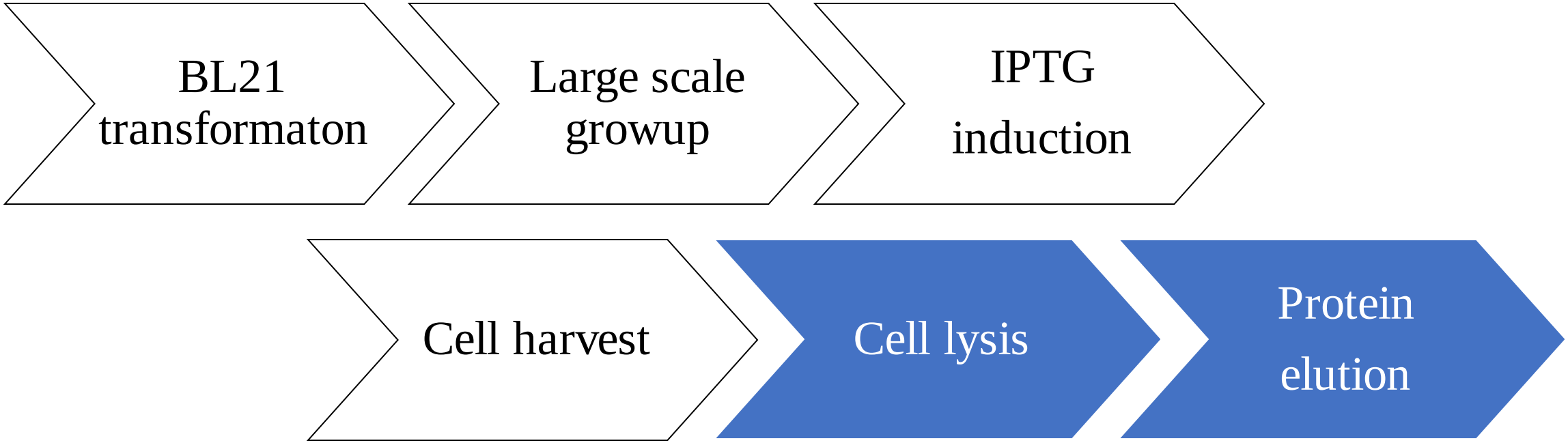
Exp 4.
Protein Purification and SDS PAGE (I)

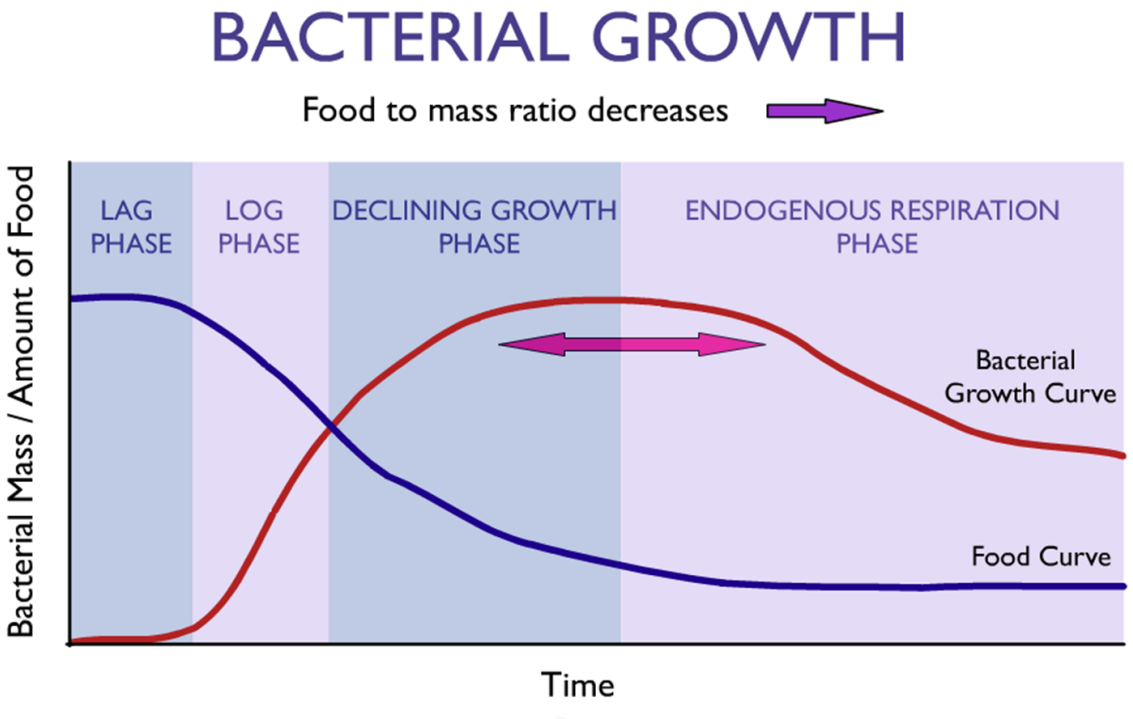
Protein purification



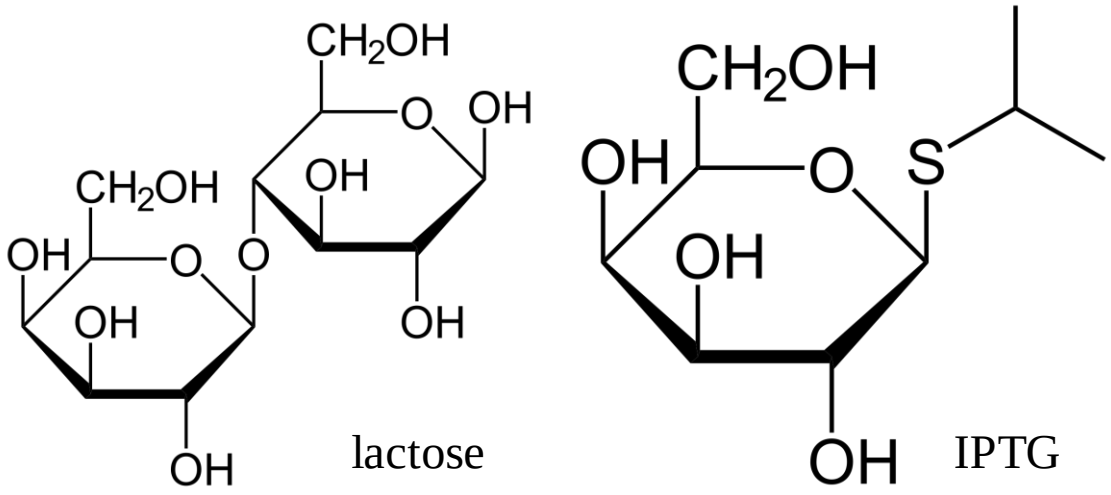
GFP expression from colony

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 until O.D reaches A_{600}= 0.6
- IPTG was added to 1 mMand incubate the culture for overnight
- Cells were harvested by centrifugation at 6000 rpm for 1min.
- Freeze the cells at -20 or -70°C

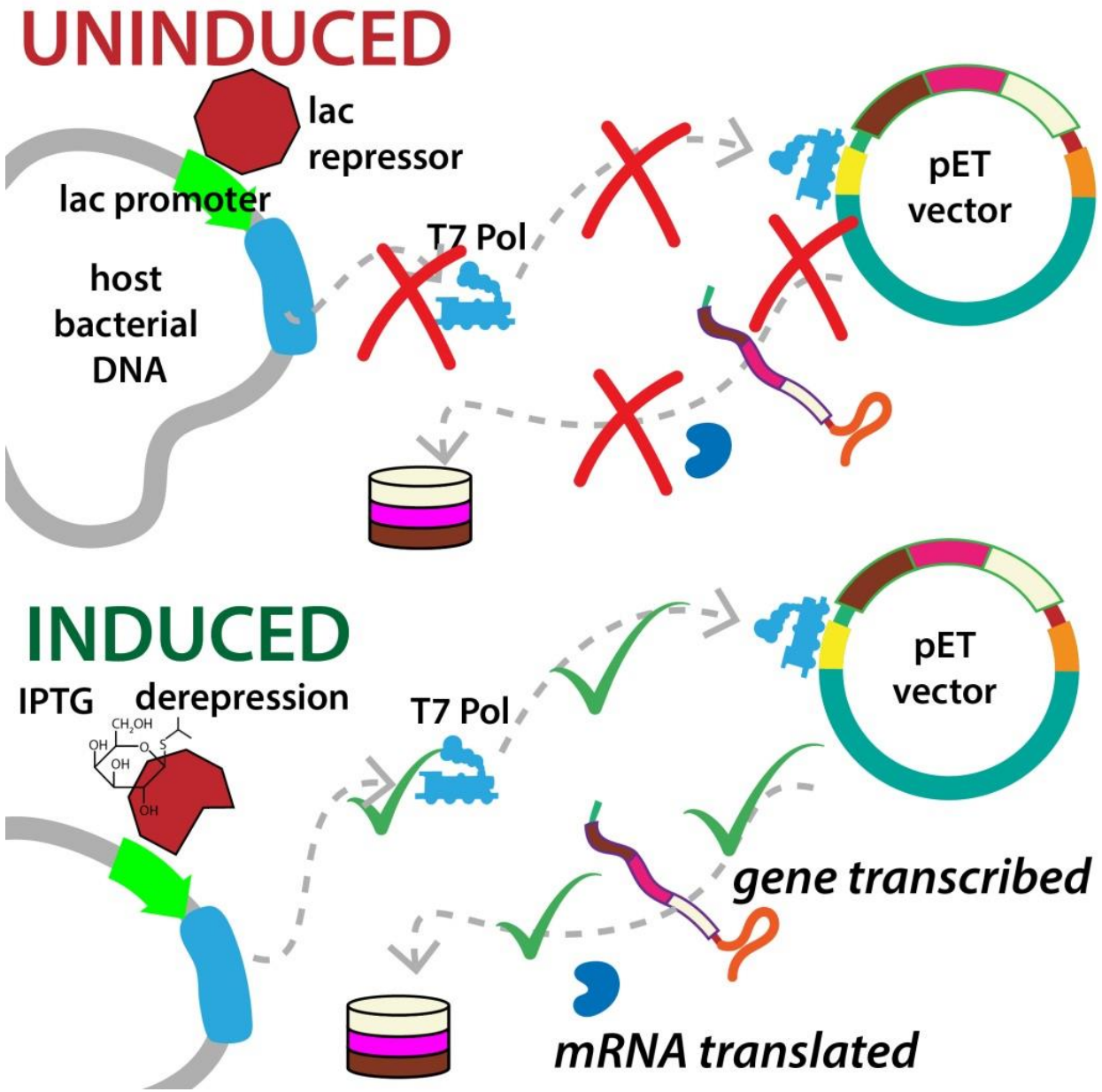


IPTG induction



After IPTG binds to the lac repressor :

- The gene encoding T7 Pol (host bacterial DNA) is translated
- T7 Pol transcripts the pET vector (DOI)
- mRNA is translated to protein (POI; GFP)



Protein Purification

Reagents and Apparatus

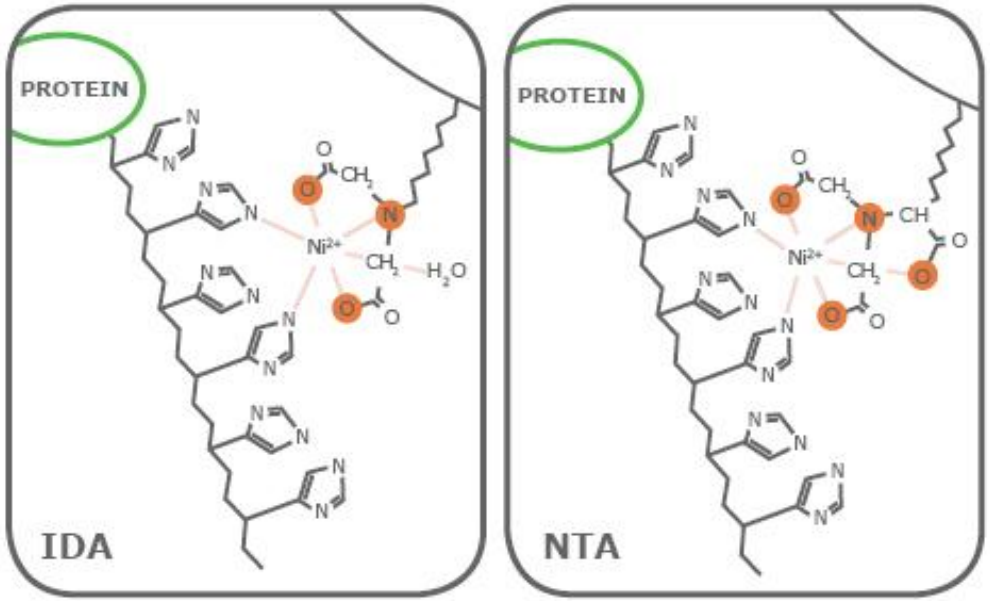
- Lysis buffer (50 mM Tris pH 8.0, 500 mM NaCl, 5 mM imidazole, Lysozyme)
- Washing buffer (50 mM Tris pH 8.0, 500 mM NaCl, 50 mM imidazole)
- Elution buffer (50 mM Tris pH 8.0, 500 mM NaCl, 250 mM imidazole)
- NTA or IDA resin
- Centrifuge
- 15 ml conical tube, 50 ml conical tube, 1.5 mL Eppendorftube,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

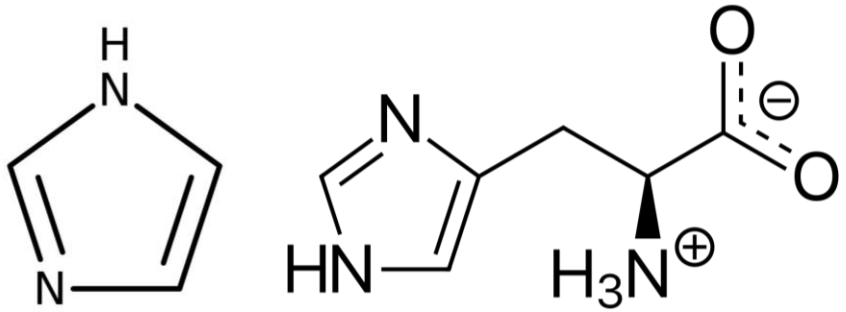
Protein Purification

PROCEDURE

- Cell pellet is resuspendedwith **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 mMTris, 500 mMNaCl, 5 mMImidazole)
- Load cleared **lysate**containing GFP onto the pre-equilibrated Ni-IDA spin column.
- Wash the Ni-IDA column twice with **WASH buffer** (50 mMTris, 500 mMNaCl, 50 mMImidazole, pH 8.0)
- Elute the protein twice with 100 µL **Elution buffer** (50 mMTris, 500 mMNaCl, 250 mMImidazole, pH 8.0)
- Collect the eluate.

Affinity column chromatography





imidazole

Histidine