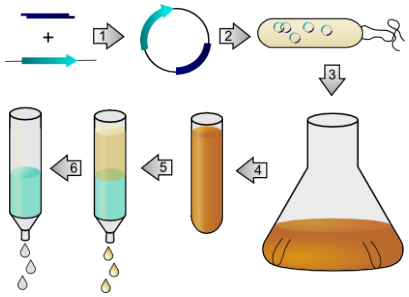
Biochemistry Lab

Expression and His-Tag Purification

Lecture Notes Handout

Expression and Purification of His Tag MDH

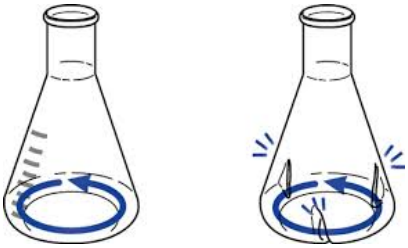


- Expression – use bacteria to make protein
- Lysis – break open bacteria cells
- Purification – use the His Tag
- Characterize – how do you know you have MDH?

Expression

Start with clone (resistance?) from plate or frozen glycerol stock

- Pick a colony and place in starter culture
- Incubate overnight (o/n) OR limit OD
  - OD – Optical Density – diffusion of light through media (not absorbanc)
  - Some cells stop expressing well if OD is > 0.7 units



Expansion

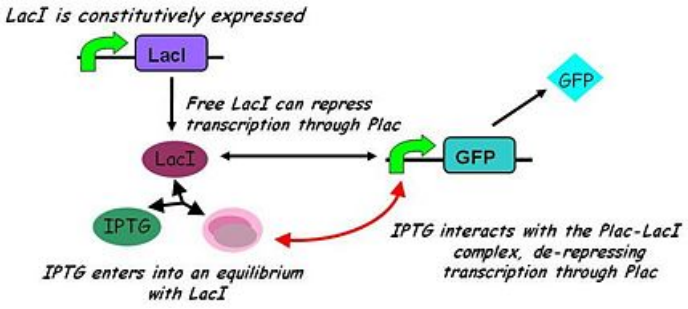
Transfer portion of starter (small initial culture) to larger flask

- Never use more than ½ of total volume – aeration issues
- Baffle flask if possible, cap with cotton or open flask cap
- Orbital shaking while growing to keep media oxygenated and cells from sinking to bottom for healthy growth

Induction

Allow cells to grow to moderate density before inducing e. coli to express protein

- 0.4-0.6 OD before starting culture
- Cells grow exponentially! Growth starts slowly and speeds up fast at the end. DON'T overgrow! Impacts health of cells and expression levels.
- Some plasmids are leaky – may need to add glucose to decrease expression until IPTG is added



Cell Harvesting

- Induce for 3-4 hours at 37oC or 12-18 hours for 20oC (room temp) to 16oC.
- Carefully balance tubes and centrifuge cells for pellet – can be frozen for later processing

Cell Lysis

We will use a novel approach – autolysis

- XJ autolysis cells (BL21 strain) - with second plasmid cobacteriophage endolysin protein under arabinose containing gene for bacteriophage gamma endolysin
  - Endolysin – hydrolytic enzyme degrading cell wall
  - Allows cell to be damage after freeze-thaw
- Addition of PMSF to inhibit released proteases
- DNAse A and sonication reduce DNA viscosity
- Cenrifuge membrane from soluble portion = lysate

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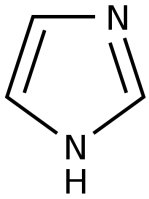
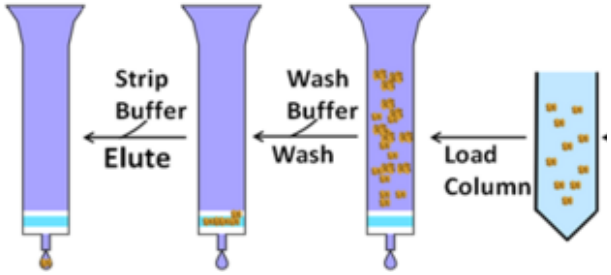
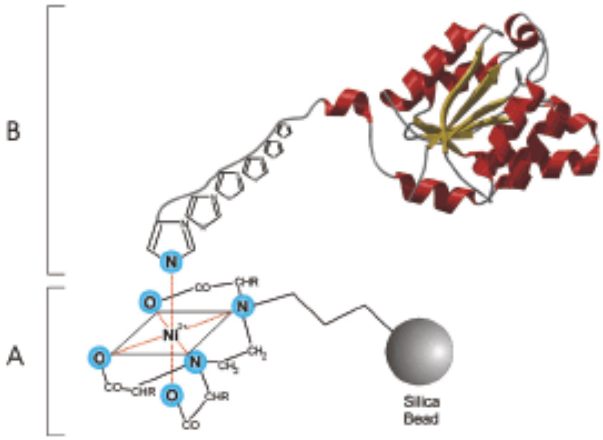
• Purification

Nickle-Agarose (or some other solid support) beads



- Mix lysate with beads to allow maximum binding
- Load onto column, allow beads to settle and re-load lysate through
- Inclusion of low concentration of Imidazole to reduce weak Ni+2 binding.
- Wash poorly bound proteins (~10 column volumes)
- Elute (remove from beads) with imidazole





Characterization

Protein Concentration

Enzyme Activity (specific activity)

- Demonstrates purity and active enzyme

SDS PAGE Analysis

- Demonstrates purity
- Protein should migrate to
- correct size/location on gel