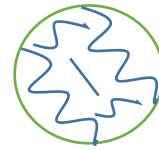
10/15/16 Quynh Nguyen

PROTEIN PURIFICATION/ OVEREXPRESSION PROTOCOL

I. Day 1

- Streak plate from glycerol stock - Incubate @ 37°C, overnight (from 16 – 18 hrs)



- Prepare one flask of 50mL LB Broth and one (or more) big flask of 1L LB Broth

II. Day 2

- Add corresponding anti-biotic to the LB (about 100uL antib. – 100mL LB)

**Antibody concentration: Carb: 100ug/uL ; Amp: 100ug/uL ; Kan: 50ug/mL*

**Aliquot antib. stock: Carb: 1g/ 10mL*

- Inoculate 1 colony in 50mL LB + antib. overnight (16- 18 hrs) - Shaking @ 37°C, 225 rpm

III. Day 3

- Add 10mL of previous day culture in 1L of (LB + antib.)

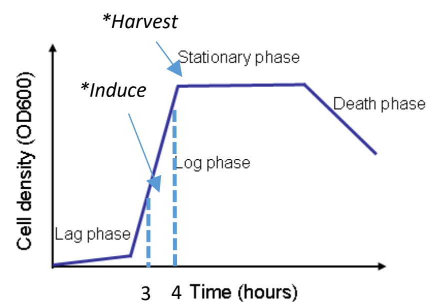
- Shake @ 37°C, 225rpm for 3- 4 hrs.

- Take 1mL of culture out as sample to run SSD-PAGE gel afterwards

- Take 1mL of culture to check for OD (Use 1mL of original LB as control)

- Add **1mM** IPTG (Isopropyl β-D-1-thiogalactopyranoside – bind to Lac repressor and induce DNA translation & RNA transcription into protein of interest) to shaking LB culture when OD (Optical Density) @ 600nm = 0.5 -0.8

1mL IPTG / L of LB (IPTG 1mM/ 1L – For storage 1M)



- Shake at 37C for 3 hr OR Shake at 18C overnight

- Take 1mL of culture out as sample to run SSD-PAGE gel afterwards

- Aliquot into bottles & balance in pairs with scale

- Centrifuge @ 4C, 3500rpm for 15 – 20 mins

- Discard supernatant, suspend pallets in 4 – 6mL of 1X TBS buffer

(Use 4-6 mL of TBS for first bottle, suspend pallet, then pass the same mixture to next bottle for suspension, etc.)

- Add product into conical tubes – This can be stored @ -20C

IV. Day 4

- Run SDS-PAGE gel to confirm successful induction

  + Centrifuge the pre-induce and post-induce 1mL culture sample @ 4C, 13,000 rpm

- Column Purification (Fusion Protein)

  + Thaw conical tube by dipping in 37C water bath for 30s, then leave @RT till almost finish thawing, then set on ice

  + Sonicate tube on ice (setting #7, "continuous") – 10 times, 30s ea. , resting 15 s in between

  ***Rest more if needed, to prevent warming up sample

  + Split sample into small centrig. tube – balance in pairs, centrifuge @4C, 13000 rpm for 40'

  + Collect supernatant in conical tube – this can be stored at 4C || Keep pallets, if needed

  * If supernatant appears cloudy, pipette into 2mL tubes & centrig @4C, 13000 rpm for 20' , then combine.

+ Filter supernatant through filter syringe with 20um filter (depend on viscosity – liquid will be thinner after filtration) ---→ This is one source of protein for running column.

********* Purify protein from centrifuged pallets

+ In bottle containing pallets, add 10mL of 10% TRITON-X and mix well

+ Balance in pairs - Centrifuge @4C, 13 000 rpm for 15'; Save supernatant in a new conical tube (#SUPE1)

+ In bottle containing pallets, add 10mL of 1% TRITON-X and mix well

+ Save supernatant in a new conical tube (#SUPE2)

+ In bottle containing pallets, add 10mL of 1% TRITON-X and mix well

+ Save supernatant in a new conical tube (#SUPE3)

+ In bottle containing pallets, add 5mL of 6M Urea Tris-HCL buffer, then shake/ stir/ rotate @ 4C, overnight

+ Dialyze final product till pure – Buffer exchange

****** Protein fusion

- Everything should be cold and stay on ice;

-Always add liquid to the column from the side, not straight into the beads

- If use new/ foreign column, run x2 with 0.1M Glycine ***(pH 3.5 - 15mL – finish in 20' like normal elution)*** *with 30mL TBS in between: Glycine → TBS → Glycine → TBS → Test to ensure pH 8.0*

- Prep column with 10mL of **cold** 1X TBS, let drip gradually

- Add supernatant into column, let drip; Once done dripping, repeat (add the dripped liquid back to the column)

- May repeat again, if needed.

- Then wash the column with 40mL of TBS (wash TBS volume should be ~ 10-20 times column's vol, our short column is 3mL) – test to ensure pH 8.0

- While waiting, prepare a set of 15 Eppendorf 1.7mL tube, numbered, & add 25uL of 1M Tris (ph 8.5 to neutralize sample from elution later)

- Elute the protein bound in column by adding 0.1M Glycine to the column.

1mL / time/ tube – cover column with palm and apply pressure to push liquid out of column into prepared tubes.

+ **Time sensitive process – Only have 20' for elution**

- After elution, wash again with 40mL TBS – test to ensure pH 8.0

V. Day 5

- Add the 15mL of eluted protein (15 tubes) to the protein concentrate conical tube (pick pore size of tube according to size of protein).

- Centrifuge at RT, @ 3000rpm, in 30 min cycle, and check in between → until we get about 2mL protein.

- Aliquot in 1.6 Eppendorf tube, 200 mL protein/ tube – LABEL neatly

- Pipette one tube of around 30 – 50mL protein for BCA & SDS-PAGE

- Do BCA to find protein concentration

- Run SDS-PAGE to confirm protein purity