IMPACT Purification of EF-G (Prashant Khade)

This protocol is for the purification of EF-G on 15mL chitin column. It uses 500 mL of culture per 15mL column. Each column is good for 4 uses then should be replaced.

Cell culture and protein expression:

- Inoculate 5mL LB/Amp from frozen cell stock.
  - Incubate 37^{o}C shaking overnight (>12hr)
- Inoculate 500mL LB/Amp with 2mL of saturated starter culture.
  - Incubate on 37^{o}C shaker until OD_{600} = 0.6 (3-4 hr)
  - Induce by adding IPTG to final concentration of 0.5mM (60mg for each 500mL culture)
  - Incubate 15^{o}C shaking overnight (18-20 hrs)
- Pellet cells by centrifuging 5,000xg 30min. 4^{o}C
- Store pellet at -20^{o}C or continue to affinity purification.

Affinity purification:

- Prepare the chitin column.
  - Use 15 mL chitin beads for 500 mL of cell culture.
  - Equilibrate with 10 column volumes (CV) (150mL) of column buffer before using.
- Prepare crude cell extract.
  - Resuspend cell pellet in 50mL cold cell lysis buffer
  - Lyse cells by sonication (8sec on 8sec off for 1min. at 60%. Repeat 3X with 1min rest between each)
  - Save 40µL of total cell extract for gel analysis
  - Centrifuge 20,000xg 30min 4^{o}C (will need to split into 2 oak ridge tubes)
  - Save 40µL of cleared cell extract for gel analysis.
- Load cleared cell extract onto column (All steps has to done at 4^{o}C).
  - Flow rate ~0.5-1mL/min.
  - Save 40µL of flow-through for gel analysis.
- Wash the column with 10CV (150mL) of column buffer (flow rate ~2mL/min)
- Quickly flush column with 3CV (45mL) of cleavage buffer
- Incubate column for 16hours at 4^{o}C.
- Elute column with cleavage buffer
  - Collect ~20 x 1.5mL fractions
- Run SDS-PAGE (5% stacking, 10% separating) of fractions, total cell lysate, cleared lysate, and flow through (load at least 10µL from each fraction)
- Pull all the fraction together which has EF-G(20-30 mL).
- Concentrate fractions containing EF-G in concentrator (spin speed and time depends upon concentrator used. EF-G is ~77kDa so use a MW cutoff no larger than 30kDa)
  - Concentrate initial fractions down to 5-7mL.

- Dialysis at 4 ^{o}C; 2 X 1L using dialysis buffer(at least 2hr each time, spectrum bag cut off 6-8000 Da.)
- Final conc. of EF-G yield a protein concentration of ~50µM (1-2 mL Volume)
- Determine protein concentration by Bradford assay.
- Aliquot store at -20^{o}C

Column Regeneration:

- Wash column with 3CV (60mL) of 0.3M NaOH
- Stop flow, let soak 30min.
- Wash with 7CV (140mL) of 0.3M NaOH
- Wash with 20CV (400mL) of water
- Wash with 5CV (100mL) of column buffer with 0.02% Sodium Azide
- Store column at 4^{o}C. Each column should be used no more than 4 times!

Buffers:

Cell Lysis Buffer

20mM K+ Hepes;pH 7.6	(1M)	2mL
500mM NaCL	(5M)	10mL
1mM EDTA	(0.5M)	200µL
0.1% Triton X-100	(100%)	100µL
Final Volume		100mL

Column Buffer

20mM K+ Hepes;pH 7.6	(1M)	8mL
500mM NaCl	(5M)	40mL
1mM EDTA	(0.5M)	0.8mL
Final Volume		400mL

Clevage Buffer

20mM K+ Hepes;pH 7.6	(1M)	2mL
500mM NaCL	(5M)	10mL
1mM EDTA	(0.5M)	200µL
50mM DDT	(1M)	5mL
Final Volume		100mL

Dialysis Buffer

20mM K+ Hepes;pH 7.6	(1M)	20mL
200mM NaCL	(5M)	40mL
Glycerol		500mL
Final Volume		1000mL

Example of purification gel: (does not have total extract, cleared extract or flow through lanes)