MBP protein purification

-streak out strain on 1-2 LB-agar plates containing appropriate antibiotics and incubate overnight

-next day scrape off bacteria into 1L of LB+appropriate antibiotics+**0.2% glucose** to an OD600 of 0.1 (can use LB to wash plate to get more bacteria off). **Glucose is crucial as it represses expression of the bacterial amylase gene. If amylase is present it will breakdown the maltose on the beads and your protein will come out in the unbound fraction.**

-grow at 37ºC to an OD600 of 0.5-0.7, then induce with 1mM IPTG, and incubate with shaking at 30ºC for 3-4 hours (I find 30 as opposed to 37 or 16 works best with MBP fusions) (Make fresh 100 mM IPTG stock-25 mg/ml)

-harvest the cells by centrifugation at 4000xg, 15min, 4ºC. Resuspend in 50 ml PBS to wash, then repellet by centrifuging at 4000xg, 15min. Can either freeze pellet down to purify at a later date or proceed with purification.

-Buffers required for purification:

column buffer (20mM Tris-HCl, 200mM NaCl, 1mM EDTA, pH 7.4)

elution buffer (=column buffer+10mM maltose (3.6 mg/ml maltose))

-resuspend 1L bacterial pellet in 40ml column buffer+1 tablet COMPLETE protease inhibitor cocktail

-lyse by 3-4 passages through Stansted machine

-**take 100 µl lysate sample**

-clarify extract by centrifugation at 30K, 4ºC for 30 min. transfer extract to fresh tubes and spin 30k, 4ºC, 20 min (while spinning equilibrate MBP beads)

--**take 100 µl soluble sample**

-take 20ml of bead-ethanol slurry and transfer to a 50ml Falcon tube (this corresponds to about 15ml packed beads. Fill tube with column buffer. Centrifuge in Eppendorf 5810 at 4k for 5min. Remove supernatant, discard, and repeat wash 3 times. Remove supernatant and add clarified extract to beads. Incubate rotating at 4ºC overnight.

-the next day transfer slurry to a column and allow beads to settle

-let cleared lysate drain, keeping it as the unbound fraction (take **100 µl sample** for SDS-PAGE analysis) **Never let column run dry**

-wash with 12 volumes column buffer (180ml) keeping wash (take **100 µl sample** for SDS-PAGE analysis)

-elute with 25-30 ml elution buffer, taking 2ml fractions

-run every 2nd fraction on an SDS-PAGE gel along with lysate, sol, wash, and unbound fractions.

-pool fractions containing protein-MBP fusion

-regenerate beads by washing:

water: 3 column volumes,

0.1% SDS: 3 column volumes

water: 1 column volume

column buffer:3 column volumes

Can use resin up to 5 times