Ci2350_HIS and Ci_MPB Protein Expression

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Notes: This protocol is intended for expression of Ci2350_his cloned in pET11a and Ci2350_MBP cloned in pET_blank (from Twist Bioscience) and sampling for subsequent SDS-PAGE, purification and quantification.

Materials

- Terrific Broth
    - Solution A
    - Solution B
- LB Medium
- Ampicillin
- Water
- IPTG
- Starting Cultures (from the previous day)
- Autoclaved 100ml flask with”cuts” on the side

Protein Expression: Step-by-step Procedure

1. Place the overnight culture on ice.
2. Pool together Ci2350_HIS culture tubes using a pipette in one tube, keep it on ice
3. Pool together Ci2350_MBP culture tubes using a pipette in a second tube, keep it on ice
4. Measure OD600 of the two starter cultures, you will likely have to dilute them 10x in LB medium (water) in the cuvette
5. Inoculation volume needed: for 100ml media (main culture) inoculate 0.5ml of starter culture with OD600=3 (For example, to inoculate 500 ml media 6.25 ml starter culture is needed if the OD is 1.2.)
6. Mix Solution A and B (for TB media) under sterile conditions
7. Add 100 µg/ml ampicillin to each individual flask
8. Inoculate flasks
9. Incubate at the chosen temperature and 170 rpm
    a. Predicted timepoint based on previous measurements should be ~3h
    b. Follow the culture, be aware you will need to dilute (10x) normally works ok, you can use TB medium to dilute it
    c. Induction should be done before OD600 actually reaches 1.5
10. OD600 reaches ~ 1.5, this will be T0 (uninduced, time of induction)
    a. Take the flask out of the incubator
    b. Take 1 sample for OD measurements (1 mL)
    c. Take 1 sample for SDS-PAGE analysis (1 mL)
11. Add IPTG at the chosen concentration and keep incubating the culture
12. Take samples for SDS-PAGE and OD600 measurements after 3h/4h
13. Stop the culture

Sampling: Step-by-step Procedure

You will need to recover two different samples: one for OD measurements and one for SDS-PAGE Analysis

For OD measurements:

1. First part of the culture should be enough 900µl and 100µl main culture (dilution 10x)
2. Second part of the culture you will probably need 950 µl and 50µl main culture

You can prepare in advance the cuvettes with , cut already pieces of parafilm that you will use to cover the cuvettes when mixing/inverting them before measuring.

SAMPLING:

1. Prepare and label 1.5ml tubes for SDS samples
2. Take out flasks from the incubator
3. Take the sample (by tilting the flask and not touching the walls by pipette)
4. Put the cultures back in the incubator