Protein Production and Purification

Introduction

This protocol purifies and yields the CMB proteins that were the hallmark of our project.

Reagents

- IPTG
- Tris-HCl buffer
- PBS
- Urea
- Beads bound with MCC
- SDS-PAGE gel
- Bacterial cellulose samples

Equipment

- Centrifuge
- Incubator cabinet
- OD600 spectrophotometer
- Chromatography column

Procedure

1. Transform plasmid containing insert into *E. coli* BL21 (DE3) and grow overnight at 37°C in a shaking incubator cabinet. (*Protocol: Electrocompetent BL21 Cell Transformation*)
2. Protein production is induced with IPTG for 4 hours of growth (or until OD600 reaches 0.5).
3. Centrifuge protein to collect cells and remove supernatant.
4. Resuspend in Tris-HCl buffer containing RNAase and DNAase to lyse cells by sonication on ice. Repeat this step four times.
5. Collect insoluble fraction through centrifugation (30 minutes at 12,000 g and 4°C).
6. Wash insoluble protein fraction with Tris-HCl for resuspension.
7. CBMs are purified by cellulose affinity. Beads bound with MCC are used for purification of CBMs.
    a. CBM solution is run through a column with beads bound with MCC. Due to the affinity of CBMs for cellulose, the CBMs should attach to the beads in the column.
8. To release the CBMs, 3 additions of 6 M urea are used to outcompete the binding of CBMs to cellulose. Urea is a strong denaturant used to unfold the CBM bound to the cellulose beads to elute through the column and collect into a separate tube.
9. To remove urea from the protein solution, a dialysis tube is used with a large volume of Phosphate-buffered saline (PBS) or Tris-HCl.
10. Purified CBM solution is run on an SDS-PAGE gel to confirm the expected size of the purified protein to make sure nothing else was bound by the cellulose in the column.

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11. Nanodrop CBM solution to measure the total amount of purified CBM.

Selection and Quantification

1. To the purified CBM, three additions of 1.0 g pre-weighed/pre-dried bacterial cellulose are used to remove the free CBMs in solution.
    a. Centrifuge and remove cellulose after every addition.
    b. After the third addition, discard solution with unattached CBMs.
2. Using the cellulose with bound purified CBMs, we can begin the protein quantification process. The following steps are proceeded alongside a control tube with no CBM added.
3. The protein concentration was analyzed by a colorimetric method using MicroBCA protein assay kit with Bovine Serum Albumin standards.
    a. The MicroBCA Protein Assay Kit helps measure the total protein concentration of dilute protein solutions.
4. Spin down the desired amount of cellulose bound with CBMs through centrifugation. Resuspend the resulting pellet in 1 mL Tris-HCl buffer. Centrifuge again and remove the supernatant.
5. Resuspend pellet again in 1 mL Tris-HCl and incubate sample for 30 minutes at 60°C.
6. Protein concentration was determined colorimetrically using a spectrometer absorbance machine.
7. The free CBM concentration was determined by subtracting by the protein bound concentration (which we found through the colorimetric assay) from the total CBM added to the tube (Nanodrop results after purification of the CBM solution).