Expression of His Tagged Proteins

from T7 Expression Vectors

The general design of this protocol:

Uses two liters of expressed protein culture.
Employs Ampicillin at 1:1000 as the Transformation and Induction antibiotic.
Uses 4 ml Ni-NTA resin, (8 ml Ni-NTA slurry) for Ni-NTA Affinity Chromatography.
Finalizes the purification process with FPLC Column Chromatography using a column equilibrated to 100 mM KCl.

Some steps require a lot of activity. Read the entire protocol to understand this method.

Transformation

In the mid to late afternoon, thaw E. coli DE3 competent cells.

Pipet 50 ul DE3 competent cells into 1.5 ml microcentrifuge tube.

Pipet 20 ng of selected DNA into the 50 ul DE3 competent cells, and gently mix with pipettor.

Incubate on ice 30 minutes. Heat shock at 42C for 90 seconds. Incubate on ice again two minutes.

Add 1 ml LB. Incubate at 37C, shaking at 250 rpm for one hour.

Spin at 13,000 rpm for 90 seconds in table-top microfuge. Pour off supernatent. Add 100 ul room temperature LB, and resuspend pellet with pipettor.

LB/Ampicillin plates will have 1:1000 Ampicillin at 100 mg/ml.

Plate 90 ul of the resuspended cells on a room temperature LB/Ampicillin plate, and the remaining resuspended cells on another plate.

Incubate plates overnight at 37C.

First thing in the morning, pick colonies and make 4 ml mini cultures:

Add 20 ul Ampicillin (100 mg/ml) to 20 ml LB.

Pipet 4 ml of the LB/amp into each of four Falcon 14 ml round bottom tubes.

Select one colony from the overnight plates, and using your preferred sterile technique, pick the colony from the plate, and place it in a 4 ml LB/amp tube. Repeat adding a single colony for each of the three other LB/amp tubes.

Incubate at 37C, shaking at 250 rpm for 8 – 12 hours.

Of the 4 ml cultures, select one that has successful E. coli growth.

Pipet 2 ml the 4 ml culture into each of two 2 ml microcentrifuge tubes, and spin at 13,000 rpm in a table top microfuge for 90 seconds.

Decant liquid, and resuspend pellet with 1 ml 1 X PBS. Repeat PBS wash.

Thoroughly resuspend pellet with 1.5 ml LB/amp.

Induction and Expression

Have two Fernbach flasks with 1 L LB each, autoclaved and cooled down. Add 1 ml of 100 mg/ml ampicillin per liter of LB.

Take an aliquot of the LB/amp and use it to blank the spectrophotometer at OD600.

Complete the two 1 L LB/amp flasks by adding 750 ul each of the resuspended E. coli.

Cover top of flasks with aluminum foil. Incubate at 37C, shaking at 250 rpm.

Periodically read the OD600 of the cultures. The cultures will grow faster over time, so reading the OD600 more frequently as time goes by is necessary.

When the OD600 reaches 0.5 – 0.6, it is time to turn the temperature down to 18C, and induce with 250 ul/L 0.4 M IPTG. It takes ~3.5 hours for the large cultures to reach 0.5 – 0.6 OD600. Express overnight.

Next day we will complete the purification.