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Protein expression using E. coli strain BL21DE3

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1 Works for me dx.doi.org/10.17504/protocols.io.7i4hkgw

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ABSTRACT

In this protocol will be explained how to express proteins using *E. coli* strain BL21DE3 using a plasmid with a T7 promoter. The promoter of T7 polymerase from *E. coli* BL21DE3 has to be induced with IPTG.

Day 1

16h

1 Prepare an overnight culture of the bacterium encoding for the protein you want to express.

Day 2

2 Prepare an Erlenmeyer flask containing LB medium and the right antibiotics

3 Inoculate the Erlenmeyer flask 1:100 from your overnight culture and grow the bacteria at 37°C and 120 rpm.

3h

4 Grow the culture to an OD600 of 0.6. This will usually take 2-3 hours.

30m

5 Take 1 ml of pre-induction sample and cold shock the culture for 30 minutes on ice. Keep the pre-induction sample in the fridge or freezer.

6 Add IPTG to a final maximum concentration of 1µM. IPTG is used because *E. coli* BL21DE3 has its T7 polymerase gene under an IPTG sensitive promoter and we have used plasmids with a T7 promoter in our protein expression experiments

18h

7 Grow the culture overnight (for 18hours) at 20°C and 120 rpm.

Day 3

8 Measure the OD600of a 10x diluted post-induction sample. Calculate the “real” optical density by multiplying this number by 10.

10m

9 Take 1 ml of post-induction sample and spin this, together with the pre-induction sample down for 10 minutes at 4700 g, using a tabletop centrifuge.

10 Resuspend the pellet of the pre-induction sample in 100µl of MQ. To correct for the difference in OD600 when you load the samples on a protein gel, resuspend the pellet of the post-induction sample in 100µl x ("real"OD600 post−induction sample)/(OD600pre−induction sample)

11 Prepare the samples to be loaded on a protein gel

12 Run both the pre-induction and post-induction sample on a protein gel