Expression of proteins in *E.coli* with pBAT vectors

A general protocol for producing protein in *E.coli* using T7*lac* promoter based expression vectors (or any other vector which is conrolled by *lac* repressor).

Protocol

1. Start o/n culture of your construct in BL21(DE3) strain. Use always cells from a fresh plate, never from directly from glycerol stock. If possible plate the cells on an LB plate the day before. If you have an unstable plasmid, you might wish to skip the overnight liquid culture and start expression directly from the plate. Prepare 10 ml for each liter you plan to express the next day + some extra. Grow the cells in LB supplemented with ampicillin (100 μg/ml) at 37°C. If you have plasmid encoding the rare Arg-tRNA (pUBS-520) in the cells, add also 25 μg/ml of kanamycin.

2. Put your LBs into 37°C to have them warm next morning.

3. In the morning pour 1 liter of LB or 2TY into 2 l shaker flask. Add the antibiotics as above and then 10 ml of the overnight culture. Or if you start directly from a plate, make a smaller starter culture with few colonies and add that to the final volume after an hour or two.

4. Grow at 37°C until OD_{600} is 0.7 - 1.0 (usually 3 - 4.5 hours)

5. Add 1 ml of 400 mM isopropyl-β-thiogalactopyranoside (IPTG) into each flask to induce the expression. If you wish to do the expression at lowered temperatue (which can increase the solubility of your protein), transfer the flasks to desired temperature and allow sufficient time for cooling before induction.

6. Express the protein for 3 hours at 37°C. At lower temperatures the expression can be continued longer — 4 hours at 25°C and 20 hours at 15°C.

7. Spin the cells down 5000 rpm 10-15 minutes.

8. Carefully pour supernatant back to flask and add Virkon to decontaminate them. Let the bottles stand upside down to remove the last drops of media.

9. Add 20 ml of your lysis buffer to one bottle and vortex to resuspend the cells. Pour the suspension to next bottle and vortex again. Resuspend all the cells this way. In the end add few milliliters of fresh buffer to the first bottle, collect residual cell supension by pipetting and do the same for the rest of the bottles. Combine all and either freeze at -20°C or proceed with lysis — French press or sonication.

Solutions

LB media

5 g Yeast extract
10 g Bacto tryptone
10 g NaCl
H_20 to 1 l, autoclave and store at room temperature

2YT media

10 g Yeast extract
16 g Bacto tryptone
5 g NaCl
H_20 to 1 l, autoclave and store at room temperature

100 mg/ml ampicillin stock (1000x)

1 g of ampicillin
Sterile H_20 to 10 ml
Filter sterilise with 0.2 μm filter
Store in aliquotes at -20°C

25 mg/ml kanamycin stock (1000x)

250 mg kanamycin
Sterile H_20 to 10 ml
Filter sterilise with 0.2 μm filter
Store in aliquotes at -20°C

400 mM IPTG stock (1000x)

953 mg IPTG
Sterile H_20 to 10 ml
Filter sterilise with 0.2 μm filter
Store in aliquotes at -20°C

Written by: Marko Hyvönen
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