Protein Expression

Aim

To express recombinant protein of interest with T7 promoter in BL21(DE3) cells through IPTG induction.

Procedure

1. Transform expression plasmid into BL21(DE3). Plate on antibiotic selection plates and incubate overnight at 37 ◦C.

2. Resuspend a single colony in 10 ml liquid culture with relevant selection antibiotic.

3. Incubate at 37 ◦C, 180 rpm until OD600 reaches 0.6.

4. Induce with IPTG of a final concentration of 0.1 mM, 0.5 mM and 1 mM for 3 to 5 hours at 37 ◦C. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. If using 500 mM IPTG stock: 1 mM final concentration: add 20 µl IPTG stock 0.5 mM final concentration: add 10 µl IPTG stock 0.1 mM final concentration: add 2 µl IPTG stock

5. At designated time points, remove all cell suspension and add into Falcon tube (pipette up and down well to assure good suspension).

6. Centrifuge at 4000 rpm, 15 min. Discard supernatant.

Note!

Liquid cultures grow best when the liquid makes up 10-15 % of the total flask volume so 10 ml can be grown in either 50 ml or 100 ml E-flasks. When adding the IPTG you should pipette up and down a bit to ensure that the small volumes are ejected properly. For this you will need to enter into the E-flask quite deeply with the pipette so wipe the pipette with 70 % ethanol beforehand to avoid contamination.

Lab protocol

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iGEM Stockholm

Sources

This protocol is modified from New England BioLabs and Gold Biotechnology. Original protocols can be found on the following links: New England BioLabs: https://www.neb.com/protocols/1/01/01/protein-expression-using-bl21de3-c2527 (retrieved 04.10.2016)
GoldBiotechnology: https://www.goldbio.com/documents/1062/IPTG+Protein+Induction+%26+Extraction+Protocol2.pdf (retrieved 04.10.2016)