

Testing for expression of proteins in *E.coli*

Protocol on how to do the first expression test for a new construct in *E.coli*. Includes also preparation of glycerol stock for future storage and testing for solubility.

Protocol

1. Transform the expression plasmid to appropriate strain(s) and plate on LB plates with corresponding antibiotics. Transform the the same cells also with plasmid without insert to the same strain(s) as a negative control.
2. Grow overnight at 37°C.
3. Prepare 2 ml of LB or 2xYT with antibiotics and inoculate a single colony of each construct and control into own tubes. If you wish to test the effect of several different expresison temperatures, add an extra 1 ml of media for each temperature. So for 37°C and 15°C expression, start a 3 ml culture.
4. Grow at 37°C with shaking (200-220 rpm) until clearly turbid (usually 3 - 4.5 hours)
5. Take 900 μ l of the culture using sterile pipette and mix with 100 μ l of 80 % sterile glycerol in sterile screw cap tube (prepare a box full of the tubes in advance by aliquoting the glycerol and autoclaving the whole lot). Mix well and place in -*)°C freezer. This is your glycerol stock for long-term storage and for startering new plates for large scale expression.
6. If you wish to test for solubility at different temperature than 37°C, split the culture into separate tubes, 1000 μ l each. Place the tubes in the desired expression temperature (say 37°C and 15°C) for 30 minutes to allow them to equilibrate.
7. Add 1 μ l of 400 mM isopropyl- β -thiogalactopyranoside (IPTG) to the cultures to induce expression.
8. Express the protein for 3 hours at 37°C. At lower temperatures the expresion can be continued longer—4 hours at 25°C and 20 hours (over-night) at 15°C.
9. Transfer 500 μ l the induced cultures to eppendorf tubes and spin 1 min at maximum speed.
10. Carefully remove all the supernatant by suction.
11. Add 1/10 of the culture volume of BugBuster reagent (Novagen) along with 3-5 ul of Lysonase per 1 ml. Lysonase is a mixture of lysozyme and DNase which help with lysing the cells and breaking down genomic DNA. Vortex well to resuspend the cell pellet. Incubate 10-15 minutes at room temperature.

*3-5 ul lysonase per ml of BugBuster, NOT per ml of E.coli culture

12. Spin the lysate in a microcentrifuge for 3 min at maximum speed.
13. Take the supernatant to a clean tube (this is your soluble cell fraction) and resuspend the pellet to same volume of 1/5 diluted BugBuster. Spin again 3 min.
14. Discard the supernatant and resuspend the pellet well in the same volume again.
15. Run the soluble fraction (supernatant from step 13 and pellet fractions on an SDS-PAGE gel along with the relevant negative and positive controls.

Solutions

LB media

5 g Yeast extract
10 g Bacto tryptone
10 g NaCl
Distilled H₂O to 1 l, autoclave in suitable aliquotes and store at room temperature

2 x YT media

10 g Yeast extract
16 g Bacto tryptone
5 g NaCl
H₂O to 1 l, autoclave and store at room temperature

100 mg/ml ampicillin stock (1000x)

1 g of ampicillin
Sterile H₂O to 10 ml
Filter sterilise with 0.2 μ m filter
Store in aliquotes at -20°C

25 mg/ml kanamycin stock (1000x)

250 mg kanamycin
Sterile H₂O to 10 ml
Filter sterilise with 0.2 μ m filter
Store in aliquotes at -20°C

400 mM IPTG stock (1000x)

953 mg IPTG
Sterile H₂O to 10 ml
Filter sterilise with 0.2 μ m filter
Store in aliquotes at -20°C

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