

Overexpression test

DNA for gene of interest has already been transformed into a cell type optimized for protein production from a plasmid (BL21 DE3, Rosetta)

Recommended to test 4 colonies

Day 1

1. Prepare overnight culture of 5 mL LB + 5 μ L antibiotic(s)
2. Inoculate with one colony from plate
3. Grow at 37°C with shaking for 12- 16 hours

Day 2

1. Obtain sterile flasks with 30 mL LB
2. Add 30 μ L antibiotic and 300 μ L O/N culture
3. Incubate cells 37°C
4. Make glycerol stocks from overnight cultures: Add 150 μ L sterile 100% glycerol and 850 μ L of O/N culture to cryo vial, store at -80°C
5. Let cells grow until OD 600nm is between 0.6 and 0.8 (about 3 hours)
6. Take gel sample: Spin down 0.5 mL cells, decant sup. Resuspend pellet in 100 μ L H₂O, add 34 μ L of 4X SDS
7. Induce cells to overexpress protein with 0.4 mM IPTG (for 28 mL, add 11.2 μ L of 1 M IPTG)
8. Put in shaker at 37°C for 4 hours
9. Take gel sample after 4 hours, bleach and discard culture, run gel to analyze overexpression