Growth of Bacteria in Liquid Media

Introduction

The Growth of E. coli ON Liquid Media protocol is used to prepare 2 mL cultures of BL21 or DH5α or TOP10 *E. coli* for innoculating expression cultures or isolating plasmid DNA, respectively. This technique allows to add types of liquid media and any additionally added supplements, depending on the purpose of the experiment.

Reference:
1. Tuttle, A. R., Trahan, N. D., & Son, M. S. (2021). Growth and Maintenance of Escherichia coli Laboratory Strains. Current protocols, 1(1), e20. https://doi.org/10.1002/cpz1.20

Materials

- LB broth: 2ml
- Bacterial single colonies on solid medium
- (Antibiotics)

Procedure

Culture

1. Using aseptic techniques, carefully transfer a 2-ml aliquot of LB broth into a capped sterile glass culture tube.
2. Supplement the LB broth with antibiotics, if appropriate.

   The same antibiotic is required, if more than one strain is to be inoculated and cultured.
3. With a sterile 2 ul tip, touch a single, well-isolated colony on the solid medium plate.
4. Inoculate the LB broth in the glass culture tube by resuspending the picked colony into the liquid medium.
5. Loosen the cap, incubate the test tube in a 37°C/200–250 rpm incubator with aeration for **12–16 h(**overnight).
6. take 100 ul sample added with 100 ul LB broth to measure OD600 (LB as blank, dilute first if >1.0).
7. Record the values.
8. Collect cells: 11,000 × g, 5 min, 4°C, remove the supernatant (or retain supernatant depending on downstream needs).
9. Proceed with subsequent protocols: plasmid extraction / glycerol stock / fluorescence measurement.