

Technic : Preculture

Goal : Start bacterial growth in liquid medium in a small volume before diluting it in a larger volume. This step enables faster bacterial growth in a larger volume.

Procedure :

1. Pick a single colony from a freshly streaked plate with a sterile loop or pipette tip.
2. Inoculate 5 mL LB + appropriate antibiotic (5  $\mu$ L Ampicillin to 5 mL LB for 100  $\mu$ g/mL) in a 15 mL culture tube.
3. Incubate overnight (~12–16 h) at 37°C with shaking (200–250 rpm) until turbid (monitor OD<sub>600</sub>; preculture OD ~1–3 typical).

Reagents and Solutions :

- LB medium
- Antibiotic

Equipment :

- Shaking incubator (37°C)
- Sterile 15 mL tubes or flasks, sterile loop/pipette tips

Estimated time :

~10 min

overnight growth 12–16 h

Trouble shooting :

- There are several ways to sample a single colony; we used 2 of them:
  - Colony recovery with an oese
  - Recovery of a colony using a micropipette cone

Unlike oese recovery, in the second case the cone is left in the culture medium, so you need to be sure not to contaminate the culture. To do this, use autoclaved cones and wear gloves.