

Growing bacterial cultures

1. Pick a single colony from an LB agar plate using a pipet tip and drop the pipet tip in a tube containing 1-5 mL LB medium with the appropriate selective antibiotic (most likely ampicillin). Alternatively, if working from a glycerol stock, stab the stock a few times with a pipet tip and drop the tip into the culture tube containing LB medium.
2. For minipreps (or other small culture volumes), incubate the “starter culture” overnight (12-16 hr) at 37°C with shaking (~220 rpm). This starter culture is all you will need to proceed with minipreps the next day. See instructions in Qiagen handbook for harvesting bacterial cells in preparation for minipreps.

If using your starter culture to inoculate for maxipreps or other larger cultures, you can incubate for as few as 8-10 hr during the day so that you can begin the larger culture late in the day and let it grow overnight. For greater assurance of a turbid culture, you can grow the starter culture overnight, as for minipreps.

3. To expand the starter culture to a larger culture, dilute it 1/500 to 1/1000 in LB medium containing antibiotic. For example, to make a 150 mL culture for a Maxiprep, add between 150 and 300 μ L of the starter culture to 150 mL of LB medium containing antibiotic and grow for 12-16 hrs with shaking. However, if the starter culture lacks visible turbidity, the entire volume of the starter culture can be added to the larger culture for overnight growth.

Selective antibiotics

- For virtually all vectors in lab, if not all, antibiotic resistance for bacterial culture is against ampicillin. Ampicillin stock (25 mg/mL) is stored in the -20°C freezer.
- Ampicillin should be added to cool LB broth (i.e., well after autoclaving) at a working concentration of 100 μ g/mL.

Minipreps and Maxipreps: For complete details on minipreps and maxipreps, including additional details on growth conditions, maximum recommended culture volumes, and harvesting of bacteria, see handbooks/instruction inserts from Qiagen.