Inoculation and growth

To prepare the perfect bacterial culture for your plasmid prep, follow the steps below.

1. Prepare a starter culture by inoculating a single colony from a freshly streaked selective plate (2) into 2–10 ml LB medium containing the appropriate antibiotic (1). Grow at 37°C for ~8 hours (logarithmic growth phase, see page 18) with vigorous shaking (~300 rpm).

   Do not inoculate directly from glycerol stocks, agar stabs, or plates that have been stored for a long time, as this may lead to loss or mutation of the plasmid.

   It is often convenient to grow the starter culture during the day so that the larger culture can be grown overnight for harvesting the following morning.

2. Dilute the starter culture 1/500 to 1/1000 into a larger volume of selective LB medium, as indicated in the appropriate plasmid purification protocol.

   Use a flask of at least 4 times the volume of culture to ensure sufficient aeration (see Figure 1).

   Do not use a larger culture volume than recommended in the protocol, as this will result in inefficient lysis and reduce the quality of the preparation.

3. Grow the culture at 37°C with vigorous shaking (~300 rpm) for 12–16 hours (see next section).
Harvest the bacterial culture 12–16 hours after inoculation. This corresponds to the transition from logarithmic into stationary growth phase (see Figure 2), when cell density is high (3–4 x 10^9 cells per ml) and RNA content of cells is low. Harvesting too early may result in lower than expected yields of plasmid DNA due to a lower cell density. Harvesting too late may result in low plasmid quality and yield due to DNA degradation from overaging of the culture.

Growth of cultures is dependent on factors such as host strain, plasmid insert and copy number, and culture medium. To determine the optimal harvesting time for a particular system, monitor the cell density and the growth of the culture by measuring the OD_{600} (see next section).

Harvest the bacterial culture by centrifugation at 6000 x g for 15 min at 4°C. Remove all traces of supernatant by inverting the open centrifuge tube until all of the medium has been drained. The cells are now ready for the lysis procedure, as indicated in the appropriate plasmid purification protocol.

The procedure may be stopped at this point and continued later by freezing the cell pellets obtained by centrifugation. The frozen cell pellets may be stored at –20°C for several weeks.

**E. coli growth curve**

The growth curve of an E. coli culture can be divided into several distinct phases (Figure 2). The first, **lag phase**, occurs directly after dilution of the starter culture into fresh medium. During this phase, cell division is slow as the bacteria adapt to the fresh medium. The bacteria then start to divide more rapidly and the culture enters **logarithmic (log) phase** (4–5 hours after dilution), during which the number of cells increases exponentially. As the available nutrients in the medium are used up and released metabolites inhibit bacterial growth, the culture becomes saturated and enters **stationary phase** (~16 hours after dilution), during which cell density remains constant. Eventually the culture enters the **phase of decline** as cells start to lyse, the number of viable bacteria falls, and DNA becomes partly degraded.
The growth curve of a bacterial culture can be monitored photometrically by reading the optical density at 600 nm (Figure 2). Note however that photometric measurements of cell density can vary between different spectrophotometers due to variable distance between the sample and the detector.

**Tip**

Calibrate your spectrophotometer by determining the number of cells per ml giving a particular OD<sub>600</sub> reading. Plate serial dilutions of a culture on LB agar plates in the absence of antibiotics, count the colonies, and calculate the number of cells per ml in the original culture. This is then set in relation to the measured OD<sub>600</sub> value.

High OD<sub>600</sub> readings should be calculated by diluting the sample in culture medium to enable photometric measurement in the linear range between 0.1–0.5 OD<sub>600</sub>.

Another way of estimating the amount of cell harvest is to assess the pellet wet weight. Typically a 1-liter, overnight, shaker culture of E. coli containing a cell density of 3–4 x 10<sup>9</sup> cells per ml corresponds to a pellet wet weight of approximately 3 grams.

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