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Growth of *E. coli* in liquid medium

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Abstract

We describe the procedure for inoculating overnight (starter) cultures of *E. coli* from a single colony, along with considerations for growing larger cultures. We also include two methods for monitoring the number of cells per unit volume (density) of liquid cultures, using a spectrophotometer, and a hemacytometer or “count slide”.

BASIC PROTOCOL 1

GROWING AN OVERNIGHT CULTURE

Small freshly saturated cultures of *E. coli* are called *overnights*. To make an overnight, remove the cap from a sterile 16- or 18-mm culture tube. Working quickly to minimize contact of the tube with the possibly contaminated air, use a sterile pipet to transfer 5 ml of liquid medium into the tube. Inoculate the liquid with a single bacterial colony by touching a sterile inoculating loop, needle, toothpick, or applicator stick to the colony, making certain that some of the cells have been transferred, and then dipping the inoculating loop, needle, or tip of the stick or toothpick into the liquid and shaking it a bit. At any point in the above steps, you may pass the open mouth and first few centimeters of the top of the tube through an open flame such as that of a bunsen burner to try to heat kill any live organisms that may have fallen in from the air. Replace the tube's cap, and place the tube on a roller drum at 60 rpm, 37°C. Grow until the culture is freshly saturated (at a density of $1-2 \times 10^9$ cells/ml, which typically takes at least 6 hr).

BASIC PROTOCOL 2

GROWING LARGER CULTURES

Larger cultures are generally inoculated with overnight cultures diluted 1:100. Use an Erlenmeyer or baffled flask whose volume is at least 5 times the volume of the culture. Grow the culture at 37°C with vigorous agitation (if using a shaker with an orbital diameter of 1.5 cm or higher, aim for at least 250 rpm) to ensure proper aeration. If it is necessary to grow a culture without shaking (for example, if the strain is temperature-sensitive for growth and no low-temperature shaker is available), then, to ensure that the cells get adequate aeration, grow the culture in an Erlenmeyer flask whose volume is at least 20 times that of the culture.

BASIC PROTOCOL 3

MONITORING NUMBERS OF CELLS IN LIQUID

With a Count Slide—Take a clean count slide (or hemacytometer) and cover it with a clean cover slip. Dip a 0.1- or 1-ml glass pipet or tip of a pipeting device into the culture medium and allow a small drop of liquid to form on the end of the pipet. Touch this lightly to one of the two Vs on the slide (shown in Figure 1) where the tip of the V is covered by the cover slip. The liquid will quickly spread under the cover slip. Put the slide on the stage of a phase-contrast microscope set to 400×, and focus on the cells. Each cell in a small square is equivalent to 2×10^7 cells/ml (see Fig. 1, right panel).

With a Spectrophotometer—The concentration of cells in a culture can also be determined with a spectrophotometer by measuring the amount of 600-nm light scattered by the culture. The level of absorbance (A) at 600 nm will depend on the distance between the cuvette and the detector and will vary among spectrophotometers, often by a factor of 2. It is thus wise to calibrate each instrument by recording the OD_{600} (sometimes expressed as A_{600}) of a culture that contains a known number of cells determined by some other method, such as observation on a count slide or titring for viable colonies (*unit 1.3*).

If the culture is visibly turbid, also measure a *10-fold dilution* of it. For a culture grown in rich medium, a good rule of thumb is that each 0.1 OD unit is roughly equivalent to 1×10^8 cells/ml.

Calculate the number of cells/ml from whichever suspension (the undiluted or the diluted) has an $OD_{600} < 1$.

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Figure 1.

Monitoring cells per unit volume via a hemacytometer or count slide. Left panel shows the slide from above. When in use, a cover slip covers the tip of both V shaped indentations at the center of the slide. Center panel shows the grid at 400X magnification. Right panel shows one of the grid squares. One cell per little square corresponds to 2×10^7 cells per ml. By eye, there are ~12 intact cells within the 5 X 5 square grid, so the culture is at 2.4×10^8 cells/ml.