**Positive control to check transformation efficiency**

Transform competent cells with 1 ng of a control plasmid containing an antibiotic resistance gene. Plate onto LB-agar plates containing the relevant antibiotic(s). Compare the number of colonies obtained with the control plasmid to the number obtained with the plasmid of interest to compare transformation efficiency.

**Negative control to check antibiotic activity**

Transform cells with 20 µl of TE. Plate at least 200 µl of the transformation mix on a single LB-agar plate containing the relevant antibiotic(s). An absence of colonies on the plates indicates that the antibiotic is active.

# Growth and Culture of Bacteria*

## Bacterial culture media and antibiotics

**Liquid media**

Liquid cultures of *E. coli* can generally be grown in LB (Luria-Bertani) medium. Please note that a number of different LB broths are commonly used. We recommend using the LB composition given in "Bacterial Culture Media and Buffers" (page 92) to obtain the highest yields of plasmid DNA.

**Sterilizing media**

Sterilize liquid or solid media by autoclaving, using a pressure and time period suitable for the type of medium, bottle size, and autoclave type.

Tip It is advisable to autoclave liquid medium in several small bottles rather than in one large vessel, to avoid possible contamination of an entire batch. Fill bottles only 3/4 full with medium and loosen the caps before autoclaving to avoid hot medium boiling over. Tighten caps once the media is cool (<40°C) to maintain sterility.

Tip Antibiotics and medium supplements such as amino acids are degraded by autoclaving. Antibiotics should be added to liquid medium immediately prior to use from stock solutions that have been filter-sterilized, distributed into aliquots, and stored in the dark at –20°C (see "Antibiotics", page 5).

** More extensive coverage of microbiological technique can be found in current manuals: see references 1 and 2, page 21.*

### Solid media

*E. coli* strains can generally be streaked and stored, for a short period of time, on LB plates containing 1.5% agar and the appropriate antibiotic(s).

### Preparation of LB-agar plates

Prepare LB medium according to the composition given in "Bacterial Culture Media and Buffers", page 92. Just before autoclaving, add 15 grams agar per liter and mix. After autoclaving, swirl the medium gently to distribute the melted agar evenly throughout the solution.

Tip Cool autoclaved agar medium to below 50°C (when you can hold it comfortably) before adding heat-sensitive antibiotics and nutrients. Mix thoroughly before pouring.

Tip Pour plates in a laminar-flow hood or on a cleaned bench surface next to a Bunsen burner. Use 30–35 ml medium per standard 90 mm petri dish.

Tip After pouring plates, any air bubbles may be removed by passing the flame of a Bunsen burner **briefly** over the surface.

Dry plates by removing the lids and standing the plates in a laminar-flow hood for 1 hour; with the covers slightly open in a 37°C incubator for 30 minutes; or left upside down with lids on at room temperature overnight.

Tip Store plates inverted at 4°C in a dark room or wrapped in aluminum foil to preserve light-sensitive antibiotics. Do not store for longer than 1 month as antibiotics may degrade. Label plates with the date and the antibiotic used.

### Antibiotics

Bacterial strains carrying plasmids or genes with antibiotic selection markers should always be cultured in liquid or on solid medium containing the appropriate selective agent. Lack of antibiotic selection can lead to loss of the plasmid carrying the genetic marker and potentially to selection of faster-growing mutants!

Tip Prepare stock solutions of antibiotics separately from batches of liquid or solid media, sterilize by filtration, aliquot, and store in the dark at –20°C. Recommended stock and working concentrations for commonly used antibiotics are shown in **Table 1**.

Tip Before adding antibiotics to freshly autoclaved medium, ensure that the medium has cooled to below 50°C.

**Table 1. Concentrations of commonly used antibiotics**

| Antibiotic | Stock solutions | | Working concentration (dilution) |
|---|---|---|---|
| | Concentration | Storage | |
| Ampicillin (sodium salt) | 50 mg/ml in water | –20°C | 100 µg/ml (1/500) |
| Chloramphenicol | 34 mg/ml in ethanol | –20°C | 170 µg/ml (1/200) |
| Kanamycin | 10 mg/ml in water | –20°C | 50 µg/ml (1/200) |
| Streptomycin | 10 mg/ml in water | –20°C | 50 µg/ml (1/200) |
| Tetracycline HCl | 5 mg/ml in ethanol | –20°C | 50 µg/ml (1/100) |
| Carbenicillin | 50 mg/ml in water | –20°C | 50 µg/ml (1/1000) |

## Storage of *E. coli* strains

There are different methods for storing *E. coli* strains depending on the desired storage time. Glycerol stocks and stab cultures enable long-term storage of bacteria, while agar plates can be used for short-term storage. When recovering a stored strain, it is advisable to check that the antibiotic markers have not been lost by streaking the strain onto an LB-agar plate containing the appropriate antibiotic(s).

### Protocol 3. Preparation of glycerol stocks

Protocol 3

*E. coli* strains can be stored for many years at –70°C in medium containing 15% glycerol.

Prepare glycerol stocks of bacteria as follows:

1. Add 0.15 ml glycerol (100%) to a 2 ml screw-cap vial and sterilize by autoclaving.

Tip Vials of sterilized glycerol can be prepared in batches and stored at room temperature until required.

2. Add 0.85 ml of a logarithmic-phase *E. coli* culture (see "Growth of *E. coli* cultures", page 7) to the vial of pre-sterilized glycerol.
3. Vortex the vial vigorously to ensure even mixing of the bacterial culture and the glycerol.
4. Freeze in an dry ice–ethanol bath or liquid nitrogen and store at –70°C.

Tip Avoid repeated thawing and re-freezing of glycerol stocks as this can reduce the viability of the bacteria.

Tip For precious strains, storage of two stock vials is recommended.

### Protocol 4. Preparation of stab cultures

Protocol 4

*E. coli* strains can also be stored for up to 1 year as stabs in soft agar. Stab cultures can be used to transport or send bacterial strains to other labs.

Prepare stab cultures as follows:

1. Prepare and autoclave 0.7% LB agar (standard LB medium containing 7 g/liter agar) as described in "Bacterial Culture Media and Buffers", page 92.
2. Cool the LB agar to below 50°C (when you can hold it comfortably) and add the appropriate antibiotic(s). While still liquid, add 1 ml agar to a 2 ml screw-cap vial under sterile conditions, then leave to solidify.

Tip Vials of agar can be prepared in batches and stored at room temperature until required.

3. Using a sterile straight wire, pick a single colony from a freshly grown plate and stab it deep down into the soft agar several times.
4. Incubate the vial at 37°C for 8–12 h leaving the cap slightly loose.
5. Seal the vial tightly and store in the dark, preferably at 4°C.

## Growth of *E. coli* cultures

**Figure 1** shows the sequence of steps necessary to go from a stored stock of bacteria to a liquid culture for plasmid isolation. Bacterial stocks should always be streaked onto selective plates prior to use, to check that they give rise to healthy colonies carrying the appropriate antibiotic resistance. Stocks can potentially contain mutants arising from the cultures used to prepare them, or can deteriorate during storage.

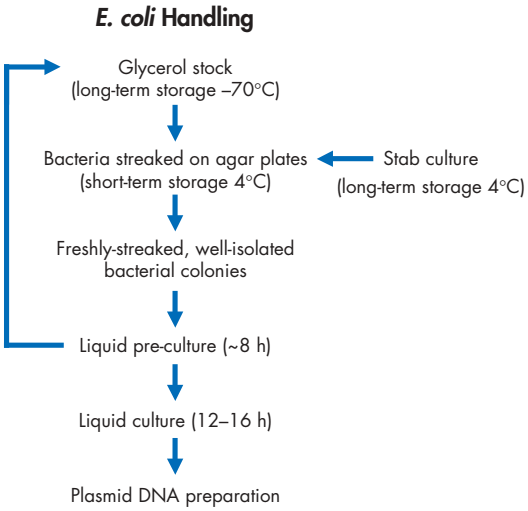


***Figure 1.*** *Essential steps for storage and handling of* E. coli.

### Protocol 5. Recovery of single colonies from stored cultures

Protocol 5

Plates of streaked bacteria can be sealed with Parafilm® and stored upside-down at 4°C for several weeks.

Bacteria should always be streaked onto plates containing the appropriate antibiotic to ensure that selective markers are not lost.

To obtain isolated colonies, streak an agar plate as follows:

1. Flame a wire loop, and cool on a spare sterile agar plate.
2. Using the wire loop, streak an inoculum of bacteria (from a glycerol stock, stab culture, or single colony on another plate) across one corner of a fresh agar plate, as shown in **Figure 2**.
3. Flame and cool the wire loop again. Pass it through the first streak and then streak again across a fresh corner of the plate.
4. Repeat again to form a pattern as in **Figure 2**.
5. Incubate the plate upside down at 37°C for 12–24 h until colonies develop.
6. Inoculate liquid cultures from a healthy, well-isolated colony, picked from a freshly streaked selective plate. This will ensure that cells growing in the culture are all descended from a single founder cell, and have the same genetic makeup.

Tip Culture volumes >10 ml should not be inoculated directly from a plate, but with a pre-culture of 2–5 ml diluted 1/500 to 1/1000.

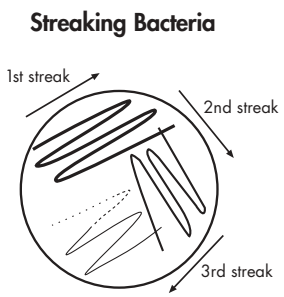


***Figure 2.*** *Streaking bacteria on agar plates.*

### *E. coli* growth curve

The growth curve of an *E. coli* culture can be divided into distinct phases (Figure 3). **Lag phase** occurs after dilution of the starter culture into fresh medium. Cell division is slow as the bacteria adapt to the fresh medium. After 4–5 hours the culture enters **logarithmic (log) phase,** where bacteria grow exponentially. Cells enter **stationary phase** (~16 hours) when the available nutrients are used up. The cell density remains constant in this phase. Eventually the culture enters the **phase of decline,** where cells start to lyse, the number of viable bacteria falls, and DNA becomes partly degraded.

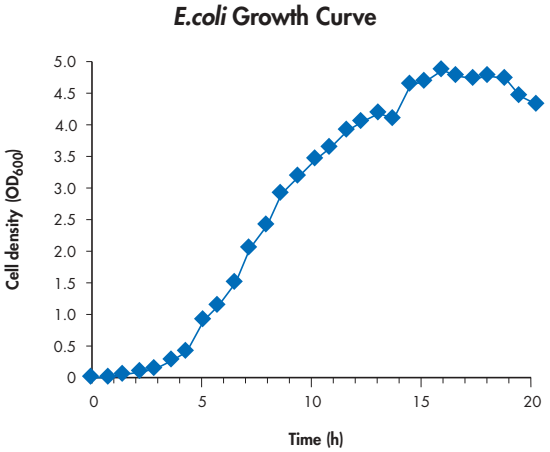


*Figure 3. Growth curve of* E. coli *in LB medium. Host strain: DH5α™; plasmid: pUC21. High $OD_{600}$ readings were calculated by diluting the sample to enable photometric measurement in the linear range of 0.1–0.5 $OD_{600}$*

### Measuring cell density

The growth curve of a bacterial culture can be monitored photometrically by reading the optical density at 600 nm (Figure 3). Note however that photometric measurements of cell density can vary between different spectrophotometers.

Tip Calibrate your spectrophotometer by determining the number of cells per milliliter giving a particular $OD_{600}$ reading. Plate serial dilutions of a culture on LB agar plates and calculate the number of cells per milliliter in the original culture. This is then set in relation to the measured $OD_{600}$ value.

Tip High $OD_{600}$ readings should be calculated by diluting the sample in culture medium to enable measurement in the linear range of 0.1–0.5 $OD_{600}$.

Tip Another way of estimating the amount of cell harvest is to assess the pellet wet weight. Typically a 1 liter, overnight culture of *E. coli* within a cell density of 3–4 x $10^9$ cells per milliliter corresponds to a pellet wet weight of approximately 3 grams.

## Protocol 6. Preparation of bacteria for plasmid preps



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

1. Prepare a starter culture by inoculating a single colony from a freshly grown selective plate into 2–10 ml LB medium containing the appropriate antibiotic. Grow at 37°C for ~8 h with vigorous shaking (~300 rpm).

Tip 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.

Tip Use a flask of at least 5 times the volume of culture to ensure sufficient aeration.

Tip Do not use a larger culture volume than recommended in the protocol, as use of too many cells 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 h.

Tip Growth for 12–16 h corresponds to the transition from logarithmic into stationary growth phase (see Figure 3), when cell density is high (3–4 x $10^9$ cells per ml) and RNA content of cells is low.

Tip 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 previous section).

4. Harvest the bacterial culture by centrifugation at 6000 x *g* for 15 min at 4°C. Remove all traces of the supernatant. The cells are now ready for the lysis procedure.

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