

## Bacterial Culture Protocol for Plasmid DNA



**Bacterial Cultures:** There are typically two reasons for culturing bacteria in our lab. First is to purify DNA and the other to express protein. These are two different means. So if you are making protein and not plasmid DNA you are in the wrong place. The volume of culture depends on the plasmid purification method and the final yield you wish to achieve. Please refer to any manufacturer's protocols for specifics. Below is a GENERAL guideline that can change depending on the volume of culture you need.

**Points of Interest:** Unless noted, you can assume most of our plasmids are mid to high copy. That means the bacteria will typically have between 50 to 500 copies of the plasmid in each cell. Use this guide when deciding on your purification cultures. It is important to make certain you are using an appropriate host strain of bacteria for DNA propagation. Strains that are good for protein expression typically are problematic for DNA purification and maintenance. Strains like DH5 $\alpha$  are commonly used as they do not recombine the DNA. There are pros and cons for using other cell strains and info can be found in the Qiagen plasmid purification book (see the link on our web page). *You should read appendix C!*

To maintain cells that only carry your plasmid, an antibiotic should be included in all phases. Ampicillin acts to damage the membranes of *E. coli* by inhibiting the crosslinking of the bacterial membrane. Another commonly used antibiotic is kanamycin. This drug works by blocking protein synthesis at the mRNA level. It is important to remember that the antibiotic will "break down" (usually a hydrolysis of the compound) above 60 $^{\circ}$ C or if left at room temp for several days. We typically keep concentrated antibiotic in the freezer. While it is not proper to re-freeze, we find little problem re-freezing unused antibiotic.

Antibiotic	Stock Concentration	Storage	Working Conc (dilution)
Ampicillin (Sodium Salt)	50 mg/ml in water (500X)	-20 $^{\circ}$ C	100 $\mu$ g/ml (2 $\mu$ l of stock/ml)
Chloramphenicol	34 mg/ml in EtOH (200X)	-20 $^{\circ}$ C	170 $\mu$ g/ml (5 $\mu$ l of stock/ml)
Kanamycin	25 mg/ml in water (500x)	-20 $^{\circ}$ C	50 $\mu$ g/ml (2 $\mu$ l of stock/ml)
Streptomycin	10 mg/ml in water (200X)	-20 $^{\circ}$ C	50 $\mu$ g/ml (5 $\mu$ l of stock/ml)
Tetracycline HCl	5 mg/ml in EtOH (100X)	-20 $^{\circ}$ C	50 $\mu$ g/ml (10 $\mu$ l of stock/ml)

**Culturing Cells** - Starting your culture should always be done from an isolated colony from a freshly streaked plate. Do not go directly from a glycerol stock into your starter culture. It may be tempting but you can lose your plasmid this way. Don't trust the plates that have been around for too long (a month or so). They may look good but are likely dead or contaminated with a mold or fungus or some other nasty critter. If the plates are old, either transform a new set of cells or chip of a bit of frozen glycerol stock from the top of the tube with a pipet tip (do NOT let the frozen cell thaw) and spread on an LB Agar plate with antibiotic. Culture overnight in the 37 $^{\circ}$ C incubator and store the new plate wrapped in parafilm in the fridge (4 $^{\circ}$ C).

**Materials (autoclave all media and glassware before using)**

**Antibiotics** - Both the powder form and the concentrated stocks are stored in freezer in KH 303 or Hagen 102. Look for frozen stocks first. Re-freeze unused antibiotic.

**LB Broth** - 10 g/L tryptone, 5 g/L yeast extract, 10 g/l NaCl. OR use the pre-mixed powder as directed. Autoclave, but do not fill the flask more than 30% of capacity. For 1 liter cultures, use 2 liter flasks

**LB Plates with Antibiotic** - prepared plates are stored at 4 $^{\circ}$ C covered with aluminum foil. We commonly use 60x15 mm plates. Fisherbrand Cat 08-757-13A. These hold about 10 ml. The large plates hold about 50 ml.

**LB Agar** - LB medium containing 15 g of Agar OR use pre-mixed powder as described on the bottle. Add antibiotics when the agar is cool to the touch (~55 $^{\circ}$ C) so you do not destroy the antibiotic.

These protocols are for culturing bacteria for plasmid DNA purification NOT protein expression.

Culture protocol for 30 - 50 ml cultures	Culture protocol for 500 - 1000 ml cultures
<ul style="list-style-type: none"> <li>Select an isolated colony and transfer using a pipet tip into 5 ml of LB broth with antibiotic. Use a loosely capped test tube or a tube that has a cotton plug.</li> <li>Culture with shaking or rotating for 8 hours.</li> <li>Transfer 0.1 ml (35 ml cultures) or .2 ml (100 ml culture) for your starter culture into LB broth with antibiotic.</li> <li>Incubate culture for 12 - 16 hours max.</li> <li>Harvest cells in 50 ml conical tubes at 5000 rpm for 15 min.</li> <li>Decant supernate, turn tube upside down on paper towels to remove as much of the liquid as possible.</li> <li>Weigh and record the pellet mass and culture volume on the tube and freeze at -20<math>^{\circ}</math>C if not continuing. (Should be about 3g/liter)</li> </ul>	<ul style="list-style-type: none"> <li>Select an isolated colony and transfer using a pipet tip into 20 ml of LB broth with antibiotic. Use a small flask.</li> <li>Culture with shaking for 8 hours.</li> <li>Transfer 1 ml (500 ml cultures) or 2 ml (1 l culture) for your starter culture into LB broth with antibiotic.</li> <li>Incubate culture for 12 - 16 hours max.</li> <li>Harvest cells in 500 ml bottles at 6000 rpm for 15 min.</li> <li>Decant supernate, turn tube upside down on paper towels to remove as much of the liquid as possible.</li> <li>Weigh and record the pellet mass and culture volume on the tube and freeze at -20<math>^{\circ}</math>C if not continuing. (Should be about 3g/liter)</li> </ul>