

Protocol:

Bacterial Culture for Expression

Bacterial Cultures: There are typically two reasons for culturing bacteria in our lab. First is to purify DNA and the other to express protein. The volume of culture depends on the plasmid purification method and the final yield you wish to achieve. Please refer to any manufacture's protocols for specifics. Below is a GENERAL guideline that can change depending on the volume of culture you need.

<u>Antibiotic</u>	<u>Stock Concentration</u>	<u>Storage</u>	<u>Working Conc (dilution)</u>
Ampicillin (Sodium Salt)	50 mg/ml in water (500X)	-20°C	100µg/ml (2 µl of stock/ml)
Chloramphenicol	34 mg/ml in EtOH (200X)	-20°C	170 µg/ml (5 µl of stock/ml)
Kanamycin	25 mg/ml in water (500x)	-20°C	50 µg/ml (2 µl of stock/ml)
Streptomycin	10 mg/ml in water (200X)	-20°C	50 µg/ml (5 µl of stock/ml)
Tetracycline HCl	5 mg/ml in EtOH (100X)	-20°C	50 µg/ml (10 µl of stock/ml)

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 60oC 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.

Culturing Cells – Starting your culture should almost always be done from an isolated colony from a freshly streaked plate. In a pinch you can use from glycerol stock 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°C incubator and store the new plate wrapped in parafilm in the fridge (4°C).

Bacterial Strains –

- DH5alpha: Not appropriate for protein expression, good for plasmid purification or storage.
- BL21 – good basic cells for protein expression, is deficient (reduced not gone) in proteases.
- XJ Autolysis™ *E. coli* strains are a new alternative for bacterial transformation and lysis. These strains are efficiently lysed following arabinose-induced expression of the bacteriophage λ endolysin protein, coupled to a single freeze-thaw cycle.

XJa Autolysis™ (E. coli, K-strain JM109) Grows quite well, especially when media is supplemented with 1 mM magnesium This strain lyses very easily. Suitable for general screening, but proteases may degrade small or otherwise unstable recombinant proteins. XJb Autolysis™ (E. coli, B-strain BL21) A very robust strain, reaching higher OD's than E. coli K-strains. XJb lysis efficiency is 10-20 % lower than XJa. For optimal lysis, more care needs to be taken when selecting the lysis buffer. However, even very low concentrations of a detergent improve lysis significantly XJb is ideal for recombinant protein expression.

IMPORTANT NOTES: (DE3) for pET. These plasmids are under the control of the strong bacteriophage T7 transcription. These constructs MUST be expressed in a bacteria that contain the T7 RNA Polymerase gene, gamma DE3 lysogen (DE3). **pET28 use KAN**

For non autolysis cells (XJa or XJb strains) - DO NOT INCLUDE Arabinose.

Starting Volumes – LB Media with appropriate antibiotic

- 50 ml culture - use 1 ml of 10 ml starter culture
- 250 ml culture - use 5 ml of 10 ml starter culture
- 1 and 1.5 L cultures use 20-40 ml of starter culture.

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1) Starter Culture - Transfer each colony to 10 ml of media and incubate at 37°C (shaking) for 3 to 5 hours (adjust volumes as above). For larger starter culture volumes, use one colony in the larger volume of media and incubate for 4-6 hours.

- Use room temp LB with antibiotic to seed the media.
- While not optimal, this can be started from a frozen glycerol stock.
- Incubate the culture with agitation at 37°C until OD600 0.55-0.6. DO NOT LET GET BEYOND this OD for strong expression. If the bacteria grow too dense, the cells will inhibit the protein expression. Alternative to grow the starter overnight at a high density is not the best choice but is used by some labs.
- Once the culture has reached the correct OD - Save one ml of culture, centrifuge down at max speed for 5 min. in a microfuge tube. Discard supernatant and freeze pellet at -20oC.
- Either place the culture at 4°C (for overnight – no longer) or continue with the procedure (expansion of starter culture). NOTE what you did in the lab book. Some cultures and constructs can tolerate overnights at low temps others do not.

2) Expansion of Starter Culture-

- Expand the culture by adding the starter culture larger volume LB with antibiotic (room temp) and incubate for 1-4 hours until culture density reaches 0.5-0.6 OD600. Top the flask with cotton or a culture flask cap to allow culture to oxygenate without getting environmental contamination. Do NOT seal with parafilm or aluminum foil.

3) Protein Expression Induction.

OPTION 1 ROOM TEMP (20°C) INDUCTION.

Induce Expression (see note below) – After culture has reached OD 0.5-0.6, cool down to room temperature by placing in 'fridge or placing in iced water bath.

- induce expression by adding IPTG to a final concentration of 0.1 to 1.0 mM. IPTG is a frozen solution in the -20oC freezer.
- Induce overnight (12-18 hours) at room (20oC) temp. with shaking.
 - Refreeze unused IPTG.
 - Note: IF using XJa or XJb autolysis cells. You must add L-Arabinose/MnCl when adding IPTG. Add Autolysis protocol to your experiment if using this strain of bacteria
 - Note – for very difficult expression proteins you may need to express at 16°C. In that case, induce for 24-48 hours.

OPTION 2 37°C INDUCTION

- Induce Expression (see note below) – After culture has reached OD 0.5-0.6 induce expression by adding IPTG to a final concentration of 0.5 mM. IPTG is a frozen solution in the -20oC freezer.
- Induce for 3 to 4 hours at 37oC with shaking.
- Refreeze unused IPTG.
- Note: IF using XJa or XJb autolysis cells. You must add L-Arabinose/MnCl You must add L-Arabinose/MnCl when adding IPTG. Add Autolysis protocol to your experiment if using this strain of bacteria

4) Collect Cells – Centrifuge the cells at 3,500 x g for 20 min.

- - Resuspend cells in ICE COLD PBS and re-centrifuge in an appropriate sized tube.
- - Remove the supernatant and freeze pellet for later processing.

5) Cell Lysis - Lyse cells using appropriate protocol (autolysis or other)