Inoculating a Liquid Bacterial Culture

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1 Works for me

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ABSTRACT

Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab. LB agar plates are frequently used to isolate individual (clonal) colonies of bacteria carrying a specific plasmid. However, a liquid culture is capable of supporting a higher density of bacteria and is used to grow up sufficient numbers of bacteria necessary to isolate enough plasmid DNA for experimental use. The following protocol is for inoculating an overnight culture of liquid LB with bacteria.

This is a general protocol for making a liquid bacterial culture and the following parameters need to be mentioned for a specific type of bacterial strain:
1. Name of the bacterial strain
2. Growth temperature
3. Incubation time
4. Antibiotic resistance (if any)
5. Rpm of the shaking incubator

1 Obtain LB Broth from the Media kitchen
LB Broth contents:
- 4 g NaCl
- 4 g Tryptone
- 2 g Yeast Extract
- dH_2O to 400 mL

2 Add liquid LB to a tube or flask and add the appropriate antibiotic (if required) to the correct concentration (see table below).

Antibiotic Concentrations

Commonly Used Antibiotics	Recommended Concentration
Ampicillin	100 µg/mL
Bleocin	5 µg/mL
Carbenicillin	100 µg/mL
Chloramphenicol	25 µg/mL
Coumermycin	25 µg/mL
Gentamycin	10 µg/mL
Kanamycin	50 µg/mL
Spectinomycin	50 µg/mL
Tetracycline	10 µg/mL

Antibiotic concentrations

Note: If you intend to do a mini-prep you will usually want to start 2 mL in a falcon tube, but for larger preps you might want to use as much as a litre of LB in a 2 L Erlenmeyer flask.

3 Using a sterile inoculation loop, select a single colony from your bacteria streaked LB agar plate.

4 Dip the inoculation loop into the liquid LB and swirl.
Discard the inoculation loop.

5 Loosely cover the culture with sterile aluminium foil or a cap that is not air tight as bacteria needs air.

6 Incubate the bacterial culture at the required growth temperature overnight (i.e. 12-18 hrs in general) in a shaking incubator.

7 After incubation, check for growth, which is characterized by a cloudy haze in the media.
Measure the optical density of the bacterial culture at 600nm wavelength using a spectrophotometer.
Record the OD600 three times and calculate average, use LB Broth as Blank.