Inoculation of liquid bacterial cultures

Introduction

In order to grow our *E.coli* we will use the "Inoculating a Liquid Bacterial Culture" protocol from Addgene.

For more information check the following link : [https://www.addgene.org/protocols/inoculate-bacterial-culture/]

Materials

- Materials
  - Liquid LB
  - LB agar plate with Bacteria
- Equipment
  - Pipette & tips
  - Falcon Tubes
  - Incubator

Procedure

Preparation of LB

1. Add the appropriate quantity of LB medium in a falcon tube.
2. Add the desired antibiotic at the correct concentration.

   The recomended working concentrations of antibiotics according to Addgene can be found in the table below:

Working concentrations of commonly used antibiotics according to Addgene

	A	B
1	**Commonly Used Antibiotics**	**Recommended Concentration**
2	Ampicillin	100 µg/mL
3	Bleocin	5 µg/mL
4	Carbenicillin	100 µg/mL
5	Chloramphenicol	25 µg/mL
6	Coumermycin	25 µg/mL
7	Gentamycin	10 µg/mL
8	Kanamycin	50 µg/mL
9	Spectinomycin	50 µg/mL
10	Tetracycline	10 µg/mL

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3. When ready to grow your culture, add liquid LB to a tube or flask and add the appropriate antibiotic to the correct concentration.

   Note: *If you intend to do a mini-prep you will usually want to start 2 ml in a falcon tube.*

4. Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.

5. Drop the tip or toothpick into the liquid LB + antibiotic and swirl.

6. Loosely cover the culture with sterile aluminum foil or a cap that is not air tight.

7. Incubate bacterial culture at 37°C for 12-18 hr in a shaking incubator.

   Note: *Some plasmids or strains require growth at 30°C. If so, you will likely need to grow for a longer time to get the correct density of bacteria since they will grow more slowly at lower temperatures.*

8. After incubation, check for growth, which is characterized by a cloudy haze in the media.

   Note: *Some protocols require bacteria to be in the log phase of growth. Check the instructions for your specific protocol and conduct an OD600 to measure the density of your culture if needed.*
   Note: *A good negative control is LB media + antibiotic without any bacteria inoculated. You should see no growth in this culture after overnight incubation.*
   Our Note : Since our bacteria are resistant to Chloramphenicol we will use this antibiotic and we want to have a concentration of 25 ug/ml.