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Growing overnight culture of OP50 as worm food

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Behavioural Genomics

Bonnie Evans

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 protocol is specifically for making a liquid bacterial culture of OP50 and the following parameters are specific for this bacterial strain:

1. Name of the bacterial strain: E.coli (OP50)
2. Growth temperature: 37C
3. Incubation time: 16-18hrs (Overnight)
4. Rpm of the shaking incubator: 200-220 rpm

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Growing overnight culture of OP50 as worm food, Priota Islam

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1 Obtain 100mL LB Agar from the media kitchen. Heat in the microwave in 20s bursts until liquid.

2 Label 2 x 90mm Petri dishes with 'LB Agar' and the date. Using a stripette, dispense 35mL LB agar into each of them. Leave to set on bench.

3 Streak OP50 onto LB Agar and grow overnight at 37C.

Streaking and Isolating Bacteria on a LB Agar Plate
by Priota Islam,
Imperial College London

PREVIEW RUN

3.1 Obtain an LB agar plate with appropriate antibiotic if any.

3.2 Label the bottom of the plate with the strain name and the date. It is also a good idea to add the antibiotic resistance (if any) and your initials.

3.3 Sterilize your lab bench by spraying it down with 70% ethanol and wiping it down with a paper towel. Maintain sterility by working near a flame or bunsen burner.

3.4 Find the bacteria of interest from the bacterial stock database and obtain the appropriate glycerol stock from the -80C freezer (Freezer 6- Bacterial Stock box)

3.5 Using a sterile pipette tip touch the top of the glycerol stock to obtain the bacteria on the tip

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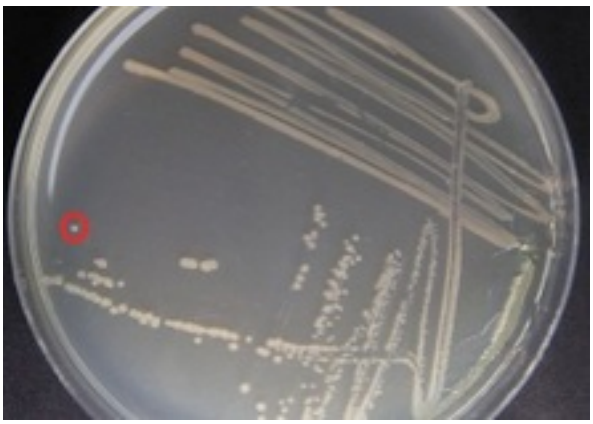
3.6 Gently spread the bacteria over a section of the plate, as shown in the diagram below, to create streak #1
Using a fresh, sterile pipette tip, drag through streak #1 and spread the bacteria over a second section of the plate, to create streak #2
Using a third sterile pipette tip, drag through streak #2 and spread the bacteria over the last section of the plate, to create streak #3

The repeated streaking is done to dilute the bacteria and obtain single colonies.



3.7 Incubate the plate (lid side down) with the newly plated bacteria overnight (generally 12-18 hours) at the appropriate growth temperature for the particular strain. In case of antibiotic resistant plasmids, incubation should not exceed the recommended duration as it poses risk of the bacteria metabolising the antibiotic.

3.8 Following adequate incubation, single colonies should be visible. A single colony should look like a white dot growing on the solid medium. This dot is composed of millions of genetically

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identical bacteria that arose from a single bacterium. If the bacterial growth is too dense and you do not see single colonies, re-streak onto a new agar plate to obtain single colonies.

3.9 Store the streaked plates at 4C (lid side down to avoid condensation falling on the single colonies). The streaked plates can be used for up to a month to make liquid cultures.

4 The next day, check for individual colonies on OP50 plate. Parafilm the plate and store in 4C fridge for up to a month.

5 Obtain 1L LB Broth from the media kitchen

LB Broth contents:
4gNaCl
4 g Tryptone
2 g Yeast Extract dH2O to 400 mL

6 Obtain 2 x 2L sterile Erlenmeyer flask from the glassware room

7 In the microbial hood, add 500mL liquid LB to each flask

8 Using a sterile pipette tip, select a single colony from your plate. Drop pipette tip into the flask.

9 Re-cover the flask with the sterile aluminium foil

10 Incubate the bacterial cultures at the 37C 200rpm for 16-18 hours.

Transport inoculated cultures to and from incubator in plastic box labelled 'Dirty only'.

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

12 Measure the optical density of the overnight culture.

Pipette culture into cuvettes in the microbial hood as there is a risk of aerosol production. Use specified box to transport cuvettes to lab.

Optical density measurement for Bacterial solution (Worm Food)
by Priota Islam,
Imperial College London

PREVIEW RUN

12.1 Pre-measurement

1. Clean the disposable cuvettes with ethanol and lint free paper
2. Aliquot small amount of LB broth into a falcon tube (For Blank)
3. Fill about 1ml of the LB broth into a clean cuvette
4. Mix/Vortex the tube containing the stock bacteria to achieve a homogenized solution
5. Take three aliquots from the same tube in three different cuvettes

12.2 Actual measurement

1. Switch on the spectrophotometer
2. Press the bottom left button to select the OD program
3. Increase the wavelength to 600nm
4. Put the blank cuvette first and press the button showing blank
5. Put the sample cuvette 1 and press the button showing sample
6. Take a note of the OD reading
7. Place the second sample and repeat
8. After taking readings for all three samples take the average
9. Record the OD as OD600 = 1.35

12.3 Post measurement

1. Discard the cuvettes
2. Close the lid to the spectrophotometer
3. Switch it OFF

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13 Adjust OD600 of the culture to 1.0 using LB.

Amount of LB (mL) to add = (OD600 x 500mL) - 500mL

14 Aliquot culture into 50mL Falcon tubes and store in 4C fridge.

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