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Growing overnight bacterial culture in 96WP (Cabreiro Lab)

Forked from Growing overnight bacterial culture in 96WP

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Behavioural Genomics

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SUBMIT TO PLOS ONE

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 in 96-well plate format from frozen stock plates. The following parameters need to be mentioned for the specific type of bacterial strains inoculated:

Parameters	Example
1. Name/s of the bacterial strain	OP50
2. Growth temperature	37C
3. Incubation time	16-18hrs
4. Antibiotic resistance (if any)	None
5. Rpm of the shaking incubator (if needed)	200-220 rpm if required

PROTOCOL CITATION

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FORK NOTE

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PARENT PROTOCOLS

In steps of
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1 Obtain LB Broth from the Media kitchen

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

2 Wipe the work area with 70% ethanol and create a sterile environment on the laboratory bench by using a bunsen or gas burner. Work under the hood if you have a large number of plates. Book the hood in advance in that case.

3 Label the inoculation plates and the lids with the corresponding frozen library plate ID

4 Add 200µl liquid LB to each well of the 96-well plate using a multichannel pipette and a sterile reservoir.
Details of the plates used:
Name: ThermoFisher Scientific Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, Non-Treated
Catalogue number: 268200

5 Take the frozen library plates out of the freezer just before doing the inoculation to avoid the wells thawing completely (We want to avoid repetitive freeze thawing of the bacterial strains). You can also use dry ice to take all the plates out of the freezer at once. In that case, just before inoculation take the respective plate off the dry ice and leave on bench top for 2mins

6 Position the plates so that well A1 of every plate is at the top left. You can also put sticker on the replicator to mark A1 and H12, to avoid confusion and contamination

7 To use the replicator, strap some laboratory/masking tape around your index and middle finger together with the adhesive part on the outer side. Use the stickiness of the tape to pick up the replicator by gently pressing on the surface

8 Carefully lower the sterile replicator into the frozen library plate, making sure to touch the surface or puncture into the frozen wells to obtain sufficient bacterial cells

9 Move the replicator immediately over to the inoculation plate containing LB and Kanamycin and dip the replicator into the liquid (try to swirl the replicator slightly while inside the wells to ensure the bacteria mixes with the liquid)

10 Wash the replicator with ethanol and then with water, and place inside an autoclavable box to be sterilised for later use (use a new replicator for every inoculation plate to avoid contamination)

11 Cover the inoculation plates with plastic lids and wrap in a damp tissue.

12 Place the bacterial cultures in an airtight box (to prevent evaporation from the wells) and incubate overnight at 37°C (no shaking).

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