Plating bacteria to isolate a single colony

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ABSTRACT

This protocol decribes how to plate bacteria in order to isolate a single colony of bacteria. Once this protocol is completed the isolated single colony can be used directly for experimental purposes or used to build up a freezer stock of the bacterial species.

For further information on building up a freezer stock, continue onto the protocol 'Building up a freezer stock of bacteria' by the same author on completion of this protocol.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

For efficient bacterial growth in liquid media, the media should not come above the widest area of the conical shaped flask/tube. Chose a flask/tube wisely depending on the volume of media which you plan to inoculate. For a 10 mL inoculation, a 50 mL falcon tube is recommended.

SAFETY WARNINGS

1. All work involving bacteria should be carried out in a hazard group specific Microbiology Safety Cabinet.
2. Individuals carrying out this protocol should always wear appropriate PPE, i.e. a lab coat and nitrile gloves.
3. Where appropriate, all other Health & Safety requirements relating to the bacterial species used should be followed.

BEFORE STARTING

Before starting this protocol users will need to have prepared LB broth and LB agar plates (or alternatively a genus specific media plate, i.e. Pseudomonas isolation media). If instruction is required for making LB broth and/or LB agar please see the protocols 'LB (Luria-Bertani) liquid medium' and 'LB (Luria-Bertani) agar' by the same author.

1 Take previously grown bacterial colony- either frozen aliquot of bacteria in glycerol suspension or lawn of bacteria on agar media plate.

2 Using a sterile inoculation loop, collect a small fragment of bacteria and inoculate in 10 mL LB broth in a 50 mL falcon tube. Shake loop in media until the fragment has come loose and is visibly floating in media.

3 To allow aerobic conditions for growth, loosely replace falcon tube lid (use tape to secure) and incubate in an orbital shaker set to 30/37 °C (depending on the optimal growth temperature for species) at 140 rpm for approximately 12-14 hrs.

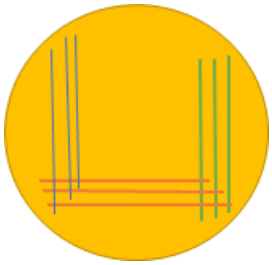
4 The following day check media for bacterial growth. The media should be cloudy. If unsure growth has occurred then check optical density of media using Absorbance Microplate Reader (an O.D. 0.6-0.8 is ideal as the bacteria will be in an exponential growth phase).

5 Prepare an LB agar plate and bring to room temperature before use. Alternatively a genus specific media plate can be used, i.e. Pseudomonas isolation media. This will reduce the risk of plate contamination with a different bacterial species.

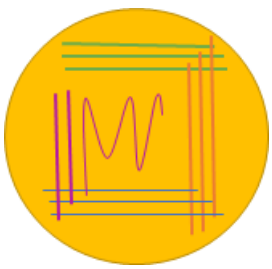
6 Using a sterile inoculation loop, dip into the overnight media culture and streak three discrete lines across the bottom of the LB agar plate.

7 Take a new sterile inoculation loop and dip it into sterile LB broth. Streak three discrete lines at an adjacent angle from the initial three bacteria lines along the side of the LB agar plate. The first LB broth line should draw across all three bacteria lines, the second should draw across two bacteria lines and the third should draw across one bacteria line.

8 Turn the LB agar plate 90° so the three LB broth lines are at the bottom of the plate. Take a new sterile inoculation loop and, this time not dipping the loop into media prior, streak three discrete 'clean' lines at an adjacent angle from the LB broth lines along the side of the LB agar plate. Again, the first line should draw across all three LB broth lines, the second should draw across two LB broth lines and the third should draw across one LB broth line.



9 Turn the LB agar plate 90° so the three 'clean' lines are at the bottom of the plate. Repeat step 8, streaking three discrete 'clean' lines at an adjacent angle from the previously streaked 'clean' lines using a new sterile inoculation loop. The third line drawn can be streaked into the centre of the plate to spread out the possible growth area of bacterial colonies. Ensure the last three 'clean' lines do not make contact with the original bacteria drawn lines to reduce the risk of lawn growth across plate.



10 Place the plates, inverted, into an incubator set at 30/37 °C and leave for approximately 24 hrs.

11 The next day check plate for bacterial growth. Should see multiple isolated colonies (typically ~1-2 mm diameter) spread out across the plate. If there is not enough growth then consider leaving the plate for a further 12-24 hrs or alternatively repeat above process but dip inoculation loop into LB broth for 3rd and 4th set of streaks instead of using a 'clean' loop. This will spread the bacteria over the plate more. If there is too much growth and the plate appears as a lawn of bacteria instead of isolated colonies then consider diluting overnight bacteria liquid culture prior to streaking (i.e. add an additional 40 mL sterile LB broth for a 1:4 dilution before streaking).

12 Select an isolated colony and using a sterile inoculation loop carefully (ensuring you do not touch any other colony) collect and inoculate into 10 mL LB broth. Again, incubate with aerobic conditions in an orbital shaker at 30/37 °C, 140 rpm for approximately 12-14 hrs. Here the amount of LB broth you choose to inoculate will depend on what you plan to do next with your bacteria. If you are building up frozen stocks then 10 mL should be plenty to then split into 100-200 µL individual glycerol suspended aliquots. If you are planning to re-inoculate your culture the next day then your overnight culture should be 10% of the final inoculate volume (e.g. for a final volume of 1 litre then an overnight culture of 100 mL is required).

13 After 12-14 hrs check the optical density of liquid culture is optimal (between 0.6-0.8) before carrying on to make frozen stocks/experimental procedure