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Building up a freezer stock of bacteria

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We use this protocol and it's working

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Abstract

This protocol describes how to build up a freezer stock of a given bacterial strain.

Following this protocol will result in individual, glycercol-suspended aliquots of genetically similar bacteria originating from a clone of cells forming an isolated colony, derived from a single precursor cell.

Using an isolated colony to build up a freezer stock of a bacterial strain ensures the individual aliquots are genetically similar and not contaminated with another bacterial strain. For full instructions, users must follow the steps in 'How to isolate a single bacterial colony' by the same author before commensing with the current protocol.

Guidelines

It is important to ensure that all frozen aliquots of a bacterial strain originated from the same isolated colony. This ensures that the bacteria is genetically similar for the duration of an experiment where several aliquots must be grown up on different days or similarly between different experiments.

Materials

MATERIALS

Glycerol A.R **Scientific Laboratory Supplies Ltd** **Catalog #**CHE2068

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 should be followed.

Before start

Before starting this protocol users should follow the instructions in the protocol 'Plating bacteria to isolate a single colony' in order to obtain a single colony isolate of a bacterial strain.

1 The previous day an isolated colony will have been selected from a growth media plate and inoculated into LB broth for overnight growth as described in '**Plating bacteria to isolate a single colony**'. The next day using an Absorbance Microplate Reader check to optical density (OD), at a wavelength of measurement of 600, ensuring an OD of ~0.6-0.8.

Note

Under OD 0.6 the bacteria are still in the lag phase and past an OD 0.8 the bacteria are reaching the stationary phase of growth. It is optimal to use bacteria during the exponential growth phase which is generally 0.6-0.8.

2 Once at the appropriate OD, 100% glycerol should be added to the culture for a final concentration of 20-25% glycerol. i.e. to a 10 mL culture add 2.5 mL of 100% glycerol

Note

Pipetting glycerol can be a slow process, be patient and if necessary snip ~4mm of the end of the pipette tip to make uptake of the viscous solution easier.

3 Vortex the glycerol/bacterial suspension thoroughly for at least 1 min until well combined and then separate the suspension into 100-200 µL aliquots

4 Place in a sealed, labelled bag within a sealed, labelled container and store at -70/-80 °C

Note

Freezer stocks should be checked periodically to ensure the bacteria are still viable and stocks should be replaced ideally every 6-12 months. Once your freezer stock starts to run low you can select a frozen aliquot and repeat the procedures detailed within 'Plating bacteria to isolate a single colony' and those described here.