Nov 07, 2018

Growing an overnight bacteria culture

DOI
dx.doi.org/10.17504/protocols.io.vbne2me

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Protocol Citation: Katy M Monteith 2018. Growing an overnight bacteria culture. **protocols.io https://dx.doi.org/10.17504/protocols.io.vbne2me**

Protocol status: Working
We use this protocol and it's working

Created: November 06, 2018

Last Modified: November 07, 2018

Protocol Integer ID: 17486

Keywords: overnight bacteria culture, overnight culture of bacteria, overnight bacteria culture this protocol, bacterial growth, lawn of bacterial growth, overnight culture, larger volume of lb broth, bacteria, liquid media broth, lb broth, lb, liquid, culture

Abstract

This protocol describes how to grow an overnight culture of bacteria in LB (Luria-Bertani) liquid media broth.

The overnight culture can be used directly, reinoculated into a larger volume of LB broth or plated to acquire either a lawn of bacterial growth or single isolated colonies.

Guidelines

Growing an overnight bacteria culture can be done by either-
1. Inoculating media with a single isolated colony of bacteria collected from a plate
2. Inoculating media by adding a frozen glycerol-bacteria suspension aliquot grown from a single isolated colony

Troubleshooting

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 start

Before starting this protocol users will need to have prepared LB broth. If instruction is required for making LB broth please see the protocol 'LB (Luria-Bertani) liquid medium' by the same author.

How much LB broth you inoculate will depend on what you plan to do with the bacteria culture the next day?
If only requiring a small volume of bacteria and a low optical density (i.e. OD <5) then only a small volume of LB broth (i.e. 10 mL) will be required as you will either dilute down the bacterial suspension or spin down and pellet the bacteria before re-suspension to achieve the desired optical density.
However, if requiring a large volume of bacteria and/or a high optical density (i.e. OD <25), the following day you will need to re-inoculate the culture into a larger volume of LB broth, allow this to reach exponential growth phase and then spin down and pellet the bacteria, before re-suspension to reach the required O.D. If planning on re-inoculating the culture then the overnight culture volume should be 10% of the total volume you plan to re-inoculate (i.e. inoculate 100 mL LB broth if planning to re-inoculate 1 L the next day).

1 Pour appropriate volume of LB broth into your selected autoclaved conical container.

Note

For efficient bacterial growth in media, the media should not come above the widest area of the conical shaped flask/tube. Therefore, it is important to chose an appropriate sized flask/tube depending on how much media you plan to inoculate. For a 10 mL inoculation, a 50 mL falcon tube is recommended.

2 Open and add the frozen aliquot of bacteria to the LB broth **OR** using an inoculation loop, carefully select your isolated colony from the media plate and shake the loop in media until the fragment has come loose and is visibly floating in media.

3 Loosely seal the container, either using tape to secure the loosely fitted lid of a tube or tinfoil to cover a flask

4 Place the tube/flask in an orbital shaker at ~140rpm at the optimal growing temperature of your bacterial species (this will likely be 30 or 37° C). Leave the liquid culture to grow overnight for approximately 12-14 hrs.

Note

Before the bacteria culture is used it is prudent to check the optical density using an Absorbance Microplate reader. If using the bacteria directly then it is important to assure the culture is in the exponential growth phase (OD600 ~0.6-0.8). If reinoculating the bacteria into a larger volume of LB broth the growth phase of the bacteria is less important but if reinoculated during the lag or stationary phase of growth then the reinoculated culture will take longer to reach the exponential growth phase.