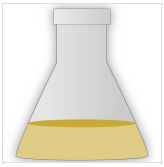

Aug 16, 2021

Inoculating a Liquid Bacterial Culture

Forked from Inoculating a Liquid Bacterial Culture

Addgene The Nonprofit Plasmid Repository[1], Joe A Kaczmarski[2]

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ABSTRACT

This protocol is for inoculating a liquid bacterial culture. To see the full abstract and additional resources, visit https://www.addgene.org/protocols/inoculate-bacterial-culture/.

DOI

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EXTERNAL LINK

https://www.addgene.org/protocols/inoculate-bacterial-culture/

PROTOCOL CITATION

Addgene The Nonprofit Plasmid Repository, Joe A Kaczmarski 2021. Inoculating a Liquid Bacterial Culture. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bxdmpi46

FORK NOTE

Edited slightly from original AddGene protocol.

FORK FROM

Forked from Inoculating a Liquid Bacterial Culture, Addgene The Nonprofit Plasmid Repository

IMAGE ATTRIBUTION

https://www.maxpixel.net/Science-Liquid-Biology-Chemistry-Apparatus-Flask-157197

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GUIDELINES

Tips and FAQ

- **What is the difference between high copy and low copy plasmids?**
  The copy number refers to the number of copies of an individual plasmid within a single bacterial cell. Large plasmids usually have a low copy number (approximately one or two copies per cell) and they need to grow for longer periods of time (approximately **18:00:00** - **30:00:00**). On the other hand, smaller plasmids can be present in large numbers, 50 or more per cell and have a high copy number. High copy number plasmids should only need to be grown for **12:00:00** - **16:00:00** on average. Certain features of a plasmid may render it low copy regardless of plasmid size. See the plasmid's info page to determine if your plasmid is high or low copy.

  image.png

- **I didn't get any growth after overnight incubation. What went wrong?**
  Try growing the culture for more time. Some bacterial cultures grow more slowly. Also, bacteria incubated at **30 °C** rather than **37 °C** often require longer incubation times.
  Double-check that the antibiotic in your LB media matches the antibiotic resistance on your plasmid.
  If the bacteria on your LB agar plates are not fresh, you should streak your bacteria onto a new LB agar plate before growing in liquid culture.
  More aeration may help to increase the density of the culture. Normally cultures shake at 150 - 250 rpm, increase this to 350 - 400 rpm to obtain a higher cell density.

1 If you haven't already, prepare autoclaved liquid LB. For example, to make **400 mL** of LB, weigh out the following into a **500 mL** glass bottle:

- **4 g** NaCl
- **4 g** Tryptone
- **2 g** Yeast Extract
- add dH_2O to **400 mL**

Note, if your lab has pre-mixed LB agar powder, use the suggested amount, instead of the other dry ingredients above.

Loosely close the cap on the bottle (do NOT close all the way or the bottle may explode!) and then loosely cover the entire top of the bottle with aluminium foil. Autoclave (media setting) and allow to cool to room temperature. Now screw on the top of the bottle and store the LB at **Room temperature** .

2 When ready to grow your culture, add liquid LB to a sterile tube or flask and add the appropriate antibiotic to the correct concentration (see table below in step 9).

Note, if you intend to do a mini-prep you will usually want to start **2 mL** in a falcon tube, but for larger preps you might want to use as much as a liter of LB in a **2 L** Erlenmeyer flask.

3 Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.

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4 Drop the tip or toothpick into the liquid LB + antibiotic and swirl your tube or flask.

> In some cases (e.g. ON cultures for minipreps) it is best to not leave the tip/toothpick in the media. Instead, just keep tip on pipette swirl a few times in the media (or pipette up and down a few times) and then remove the tip along with the pipette.

5 Loosely cover the culture with sterile aluminium foil or a cap that is not airtight.

6 Incubate bacterial culture at **37 °C** for **12:00:00** - **18:00:00** in a shaking incubator (ideally on ~25° angle) at ~ 250 rpm.

> Note, some plasmids or strains require growth at **30 °C** . If so, you will likely need to grow for a longer time to get the correct density of bacteria since they will grow more slowly at lower temperatures.

7 After incubation, check for growth, which is characterized by a cloudy haze in the media (see image below).

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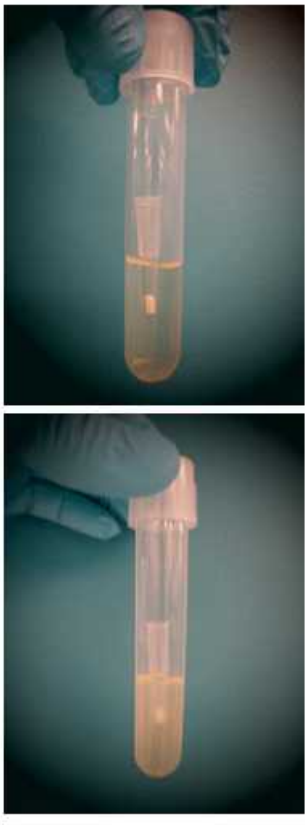

Figure 1: Media without growth (top) and with growth (bottom)

Notes:

- Some protocols require bacteria to be in the log phase of growth. Check the instructions for your specific protocol and conduct an OD600 to measure the density of your culture if needed.
- A good negative control is LB media + antibiotic without any bacteria inoculated. You should see no growth in this culture after overnight incubation.

8 *(Optional)* For long term storage of the bacteria, you can proceed with Creating a Glycerol Stock.

9 You can now isolate your plasmid DNA from the bacterial culture by isolating your plasmid DNA.

Antibiotic Concentrations

image.png

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