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Creating Bacterial Glycerol Stocks for Long-term Storage of Plasmids [↗](#)

Addgene The Nonprofit Plasmid Repository¹

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Works for me

[dx.doi.org/10.17504/protocols.io.4g5gty6](https://doi.org/10.17504/protocols.io.4g5gty6)



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ABSTRACT

This protocol is for creating bacterial glycerol stocks for long-term storage of plasmids. To see the full abstract and other resources, visit the [Addgene protocol page](#).

EXTERNAL LINK

<https://www.addgene.org/protocols/create-glycerol-stock/>

GUIDELINES

Tips and FAQ

- The optimal concentration of long-term glycerol storage is unknown. Most labs store bacteria in 15-25% glycerol.
- You can prepare the glycerol stock the same time you prepare your plasmid DNA. In the morning, when you retrieve your liquid bacterial culture, take **500 µl** of culture to make your glycerol stock before you begin your plasmid mini-prep.
- Try not to freeze/thaw your glycerol stock too many times. Placing the glycerol stock on dry ice while streaking onto LB agar will prevent it from thawing completely and will improve the shelf life.
- It is very important that you shake the glycerol before freezing (5-6 times). Make sure that you see one uniform solution, and there are no layers present.
- Be sure to label both the lid and the tube of a glycerol stock before you place the sample at **-80 °C**. Frozen tubes are hard to write on and samples stored for long periods at **-80 °C** can lose labels stuck to tube!

- 1 Follow the steps for [Inoculating an Overnight Liquid Culture](#).
- 2 After you have bacterial growth, add **500 µl** of the overnight culture to **500 µl** of 50% glycerol in a **2 ml** screw top tube or cryovial and gently mix.



Notes:

- Make the 50% glycerol solution by diluting 100% glycerol in dH₂O.
- Snap top tubes are not recommended as they can open unexpectedly at **-80 °C**.

- 3 Freeze the glycerol stock tube at -80°C . The stock is now stable for years, as long as it is kept at -80°C . Subsequent freeze and thaw cycles reduce shelf life.
- 4 To recover bacteria from your glycerol stock, open the tube and use a sterile loop, toothpick or pipette tip to scrape some of the frozen bacteria off of the top. Do not let the glycerol stock unthaw! [Streak the bacteria onto an LB agar plate.](#)
- 5 Grow your bacteria overnight at the appropriate temperature. Growth conditions, including copy number and growth temperature, can be found on your plasmid's information page. The next day you will be able to start an overnight culture for plasmid DNA prep the following day.



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