Protocol Name: Glycerol Stock Preserves

Category: General

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Source(s): Addgene (2016) Creating Bacterial Glycerol Stocks For Long-Term Storage of Plasmids https://www.addgene.org/protocols/create-glycerol-stock/ (Accessed: 01/07/2018)

Time Required: 1 hour preparation day before, 30 minutes on the day

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Additional Notes: Timing may be dependent upon growth rate of bacteria

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Materials:

15ml LB broth (per species)

1x 50ml falcon tube (per species)

10ml 50% glycerol

Sterile 1.5ml Eppendorf tubes

Liquid Nitrogen (Optional)

100-1000µl pipette and appropriate sterile tips

Procedure:

1) The day prior to producing the stock preserves, inoculate 15ml LB in a 50ml falcon tube with a single colony of the desired species from an agar plate.
    a. Incubate at appropriate temperature and rotations per minute for 12-16 hours
2) Observe falcon tube to determine if there has been substantial bacterial growth
3) Rock the falcon tube to resuspend any sediment cells
4) Add 500µl of overnight culture to 10 (or less) 1.5ml Eppendorf tubes
5) Add 500µl of 50% glycerol to the Eppendorf tubes containing the overnight culture
6) (Optional, dependent upon bacteria) Taking appropriate safety measures, freeze the Eppendorf tubes in liquid nitrogen for 10 seconds then retrieve
7) Store the Eppendorf tubes at -80°c
8) To retrieve bacteria from a stock, use a sterile loop or pipette tip to scrape some bacteria off the top. The object can then be used to plate bacteria or inoculate liquid culture as normal
    a. Aim to not thaw the stock as this will reduce the lifespan of the stock if refrozen