



Sep 22, 2019

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1

Works for me

[dx.doi.org/10.17504/protocols.io.7jrhm6](https://doi.org/10.17504/protocols.io.7jrhm6)

Alba Balletbó



MATERIALS

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SOC Media

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Glycerol

G5516

[Sigma Aldrich](#)

LB medium

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- 1 Grow 5 mL culture of LB w/o salts overnight
- 2 Pre-cool centrifuge to 4°C.
- 3 Inoculate 400 mL LB w/o salts with 5 ml pre-culture and incubate for 3 hours to OD600 0.5-1.0.
- 4 Transfer culture volume to 8 Greiner tubes of 50 mL and leave on ice for 15 minutes.
- 5 Centrifuge for 10 minutes at 2000g and 4°C.
- 6 Remove supernatant and add 5 mL + 45 mL of 10% glycerol.
- 7 Centrifuge for 10 minutes at 2000g and 4°C.
- 8 Remove supernatant and add 5mL + 45mL of 10% glycerol.
- 9 Hold on ice for 10 minutes.

- 10 Centrifuge for 10 minutes at 2000g and 4°C.
- 11 Remove supernatant and resuspend in 1 mL ice-cold 10% glycerol, pool fractions.
- 12 Centrifuge for 5 minutes at 1500g and 4°C.
- 13 Remove supernatant and add 400 µL 10% glycerol (=800 µL suspension).
- 14 Aliquot 40µL in pre-cooled eppendorfs and flash freeze with N₂(l). Store -80°C.



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