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Electrocompetent *E. coli*

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Alba Balletbó[1]

[1]Wageningen University

iGEM Wageningen 2019

Alba Balletbó

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

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Materials

MATERIALS

SOC Media

Glycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G5516

LB medium **Catalog #**/

1 Grow 5 mL culture of LB w/o salts overnight

2 Pre-cool centrifuge to 4ºC.

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 4ºC.

6 Remove supernatant and add 5 mL + 45 mL of 10% glycerol.

7 Centrifuge for 10 minutes at 2000g and 4ºC.

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 4ºC.

11 Remove supernatant and resuspend in 1 mL ice-cold 10% glycerol, pool fractions.

12 Centrifuge for 5 minutes at 1500g and 4ºC.

13 Remove supernatant and add 400 µL 10% glycerol (=800 µL suspension).

14 Aliquot 40µL in pre-cooled eppendorfs and flash freeze with N_2(l). Store -80ºC.