Competent Cells - Starter Glycerol Stocks

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This protocol is for scientists who have to make competent cells many times per year. The glycerol stocks will allow you to start with little preparation.

Prepare first:

- 500 ml LB medium (10 g NaCl, 10 g trypton, 5 g yeast-extract in 1 L water)
- 25 ml glycerol solution (65% glycerol, 0,1 M MgSO4, 0,025 M Tris/HCl pH8)
- 1x 1 L Erlenmeyer flask autoclaved/sterile
- 3x 10-50 ml flasks autoclaved/sterile
- Agar plates without antibiotics (1 L LB + 16 g agar)
- 2x 250 ml centrifuge tubes for Beckam rotor J14 autoclaved/sterile
- At least 50 eppendorf tubes autoclaved/sterile
- Required: Centrifuge Beckam with rotor J14 or similar

1. Spread bacteria on a LB plate free of antibiotics
2. Incubate overnight at 37 °C.
3. In the morning pick three times one single colony and inoculate 3 ml LB
4. Incubate at 37 °C shaking (200 rpm)
5. In the evening inoculate 250 ml of LB medium in a 1L Erlenmeyer using one of the cultures
6. Incubate at 37 °C shaking (200 rpm) over night
7. In the morning spin down at 4000 rpm for 10 min at 4 °C
8. Resuspend in 25 ml cold LB
9. Add 25 ml glycerol solution and mix well
10. Store in 1 ml aliquots at -80 °C

Commented Protocol:

1. Spread bacteria on a LB plate free of antibiotics

Make three dilution strikes and work very sterile. We need to pick single colonies in the next step.

2. Incubate overnight at 37 °C.

3. In the morning pick three times one single colony and inoculate 3 ml LB

It is important to get nice looking big (fast growing) colonies. This will be the starting material of all your stocks, if the colonies now are not perfectly healthy things down the road will be tricky for a long time. Here we use the sterile 10-50 ml flasks.

4. Incubate at 37 °C shaking (200 rpm)

5. In the evening inoculate 250 ml of LB medium in a 1L Erlenmeyer using one of the cultures

Use the one growing fastest of the three. We prepared three cultures to be able to choose now.

6. Incubate at 37 °C shaking (200 rpm) over night

7. In the morning spin down at 4000 rpm for 10 min at 4 °C

8. Resuspend in 25 ml cold LB

9. Add 25 ml glycerol solution and mix well

10. Store in 1 ml aliquots at -80 °C

This is now a concentrated glycerol stock ready to use. It allows you to inoculate cultures with equal amounts, what allows to predict the time till they reach a certain OD.

Known Issues:

- Work very sterile! We can not use antibiotics in competent bacteria and any contamination will be propagated in the next step. You will only do this once in a long time, take your time and work as good as possible!

References and Comments:

I started to make competent cells this way when I had to make competent cells for the whole lab. It really saved some time.

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