Preparation of Electro-Competent Cells

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This is in my opinion the best protocol for electro-competent bacteria. These bacteria are especially good for big plasmids over 15 kb.

Prepare first:

- 2 liter of LB without NaCl (10 g tryptone, 5 g yeast extract)
- 250 ml of 8.7 % v/v glycerol autoclaved/sterile
- 2 liter of milliQ water autoclaved/sterile
- 250 ml cylinder closed with aluminum foil containing autoclaved/sterile
- 4x 2 L Erlenmeyer flasks autoclaved/sterile
- 4x 250 ml centrifuge tubes for Beckam rotor J14 autoclaved/sterile
- 5 ml pipet tips autoclaved/sterile
- 1 ml pipet tips autoclaved/sterile
- 200 µl pipet tips autoclaved/sterile
- At least 500x 0.5 ml eppendorf tubes autoclaved/sterile
- Required: Centrifuge Beckam with rotor J14 or similar

Work very sterile because we do not use any antibiotics!

1. Reserve 15 ml LB in a small bottle at 4 °C
2. Inoculate 2 L LB Medium with a starter culture and mix well
3. Spread to 4x 2 L Erlenmeyer flasks
4. Incubate at 37 °C shaking 200 rpm
5. Measure OD 600 of all four flasks every 45 min
6. If the first flask reaches OD 0.40 put all flasks for 15 min on an ice-water bath
7. Pellet (1.) at 3500 G for 20 min at 4 °C
8. Discard supernatant and resuspend in 6x 150 ml ice cold water
9. Pellet (2.) at 5000 G for 20 min at 4 °C
10. Discard supernatant and resuspend in 6x 150 ml ice cold water
11. Pellet (3.) at 5000 G for 20 min at 4 °C
12. Discard supernatant and resuspend in 2x 150 ml ice cold water
    Attention: 3 buckets are now pooled in one!

13. Pellet (4.) at 5000 G for 20 min at 4 °C
14. Discard supernatant and resuspend in 2x 100 ml ice cold 8.7 % glycerol
    Attention: use now 8.7 % glycerol, not water!
15. Pellet (5.) at 5000 G for 20 min at 4 °C
16. Discard supernatant and resuspend in 2x 2.5 ml ice cold 8.7 % glycerol
17. Pool both buckets in one 50 ml tube
18. Make 50 µl aliquots in 0.5 ml eppendorf tubes
19. Freeze and store at -80 °C

Commented Protocol:

1. Reserve 15 ml LB in a small bottle at 4 °C

We will need LB without bacteria as a blank sample to measure the OD tomorrow.

2. Inoculate 2 L LB Medium with a starter culture and mix well

As a starter culture you can use an overnight grown 3 ml culture which was picked from a single colony. I prefer to use the Starter Glycerol Stocks.
If possible use also for the starter cultures LB free of NaCl.

3. Spread to 4x 2 L Erlenmeyer flasks

Each Erlenmeyer should be filled with not more than 500 ml. To grow bacteria flasks should be never filled with more than a quarter of it's volume or the aeration is limiting.

4. Incubate at 37 °C shaking 200 rpm

5. Measure OD 600 of all four flasks every 45 min

Use the 15 ml LB from the day before to blank. Measure all 45 min till the desired OD is reached.

6. If the first flask reaches OD 0.40 put all flasks for 15 min on an ice-water bath

This is the step which limits quality most. Bacteria are most competent at OD 0.4-0.5 and 0.9. Because it is very difficult to catch them at OD 0.9 every protocol uses OD 0.4-0.5. If the bacteria are over OD 0.5 the competence will be reduced.

7. Pellet (1.) at 3500 G for 20 min at 4 °C

Never let the bacteria warm up again! If you can, work in a cold room on ice. The quality of the competent cells will compensate for the uncomfortable time.

From now on it is not necessary to worry about sterility so much. If you get a contamination, it will result in one or two colonies on a plate, so nothing dramatic.

If the medium does not fit all in the 6 buckets, spin down several times, accumulating the pellet in each bucket.

8. Discard supernatant and resuspend in 6x 150 ml ice cold water

Use the 250ml cylinder to measure 150 ml take out 5 ml with the pipett and resuspend the pellet first in this small volume. Then add the rest.

9. Pellet (2.) at 5000 G for 20 min at 4 °C

10. Discard supernatant and resuspend in 6x 150 ml ice cold water

11. Pellet (3.) at 5000 G for 20 min at 4 °C

12. Discard supernatant and resuspend in 2x 150 ml ice cold water

Attention: 3 buckets are now pooled in one!

13. Pellet (4.) at 5000 G for 20 min at 4 °C

14. Discard supernatant and resuspend in 2x 100 ml ice cold 8.7 % glycerol

Attention: use now 8.7 % glycerol, not water!

15. Pellet (5.) at 5000 G for 20 min at 4 °C

16. Discard supernatant and resuspend in 2x 2.5 ml ice cold 8.7 % glycerol

17. Pool both buckets in one 50 ml tube

Measure the OD of a 1:100 dilution. The dilution should have a OD_{600} of 0.6. If it is higher, you can dilute the suspension.

18. Make 50 µl aliquots in 0.5 ml eppendorf tubes

Cool the eppis! I put the eppis on ice and fill them fast with a multi-pipet. Be fast and let a colleague help you. One fills the tubes the other one closes them. In this protocol you should not use liquid nitrogen because it is reported to reduce efficiency 4-5 times.

19. Freeze and store at -80 °C

In -80 °C the cells will stay good at least half a year. Test them after production and retest them if you are not sure if they are still OK. See the transformation protocol for details. At best you can reach 0.5-1.0 x 10^9 col / µg plasmid.

Known Issues:

- Work fast, clean and cold - you will get good cells. The more practice you get the better the cells will be. If you are not happy with the results, just repeat it and they will be good.
- If the pulse times are low, the washing was not sufficient.
- Works for: DB3.1, DH5alpha, NGR234

References and Comments:

This is a very basic protocol. I got to know this protocol in the Bisseling Lab and did it many times. It works well.

How to cite this page in publications:

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