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Making electro-competent E. coli cells and transformation of them V.1

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We use this protocol and it's working

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Abstract

This protocol describes a method to make*Escherichia coli*cells electro-competent. The method involves one overnight culturing step, followed by several hours of culturing during the day and 1.5 - 2 hours of preparation of electro-competent cells.

The protocol also describes how to do the transformation (electroporation) afterwards.

Guidelines

- The reason for resuspending the cells in 5 ml glycerol solution first before adding the remainder of the 50 ml is that it is easier to resuspend in a smaller volume than in a completely filled tube.
- Competent cells can be stored for 6 to 12 months at -80°C
- Work sterile during the whole procedure (next to bunsen burner or in flow cabinet)
- After transformation, the cells are spread onto a selective plate. To assure that single colonies appear, two plates can be used instead: one to spread 1/10 of the cells onto, and another one to spread 9/10 of the cells onto. If the transformation was very efficient, the single colonies will appear on the 1/10 plate and a lawn will appear on the 9/10 plate. In the case of a less efficient transformation, single colonies will appear on both plates or only on the 9/10 plate (and nothing on the 1/10 plate).

Materials

MATERIALS

- Glycerol **Catalog #**G5516
- conical tubes, 50ml
- Electroporation System Gene Pulser XCell **Bio-Rad Laboratories**
- NanoDrop spectrophotometer **Thermo Fisher Scientific Catalog #**ND-1000
- Shaker incubator
- Ice
- Centrifuge
- Thermomixer C or R **Eppendorf Catalog #**5382000015 / Z605271
- Liquid nitrogen
- Liquid LB medium
- Escherichia coli

This needs some work and investigation if we can specify a specific medium with the ingredient list there.. or we do it here

For making competent cells:
- 500 ml LB w/o salt (per liter: 10 g tryptone, 5 g yeast extract)
- 1 l ice-cold 10% v/v glycerol (per liter: 100 ml glycerol)

For transformation:
- SOC medium, 0.5 ml per transformation: (per liter: 20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 0,186 g KCl – adjust to pH 7 with NaOH – autoclave – add 5 ml of sterile 2M $MgCl_2$(which is 19 g $MgCl_2$in 100 ml) and 4,5 ml of sterile 80% w/v glucose)
- Selective agar plates, depending on the plasmid that will be transformed into *E. coli*

For making competent cells:
- Centrifuge for disposable 50 ml tubes that can be cooled to 4°C
- Shaking incubator
- Spectrophotometer

For transformation:
- Thermomixer or water bath at 37°C
- Electroporation machine

For making competent cells:
- 1 sterile 50 ml tube for culturing (Corning mini bioreactor centrifuge tube 50 ml, #431720)
- 8 sterile 50 ml tubes (generic ones, not the ones for culturing)

- 20-30 sterile 1.5 ml eppendorf tubes that don't pop open when submerged in N_2(l)
- 1 sterile 1 L Erlenmeyer flask

For transformations:
- Sterile cooled electroporation cuvette with a 2 mm gap
- Sterile 1 ml syringe
- Sterile needle (BD Microlance; 21G 1.5″- Nr. 2; 0,8 × 40 mm; REF 304432; or a thicker needle)

Troubleshooting

Safety warnings

- Be careful when using needles.
- Avoid skin contact with N_2(l), it can cause cold burns.
- All materials that came into contact with transformed *E. coli*should be autoclaved before being disposed of.

Day 1

1 **Inoculate** 5 mL LB w/o salt in 50ml tube + 2 µL E.coli + 37 °C + 12:00:00 Overnight

Original:
Inoculate 5 ml LB w/o saltin a 50 ml culturing tube withE. coliand incubate overnight at 37°C / 250 rpm

Day 2

2 400 mL LB/ w/o salt in 1 Liter erlenmeyer flask + overnight culture
[M] 0.2 Genome copies per ml OD600

Original:
Next day, first thing in the morning, inoculate 400 ml LB w/o salt in a 1 l Erlenmeyer flask with enough of the overnight culture to reach OD600 of 0.2 and incubate for ~3 hours to OD600 0.5-1.0.

3 03:00:00
37 °C
[M] 0.5 Genome copies per ml OD600 <between>
[M] 1.0 Genome copies per ml OD600

4

0 °C ice 00:15:00

Original:

Transfer culture volume to 8 Greiner tubes of 50 ml and cool on ice for 15 minutes.

5 Centrifuge for 00:10:00 2000 g 4 °C

6 Discard supernatant and add 5 mL of [M] 10 % volume glycerol , resuspend the pellet in it and then add 45 mL of [M] 10 % volume glycerol , shake by hand a few times to mix.

7 Centrifuge for 10 minutes at 2000g and 4°C.

8 Discard supernatant and add 5ml of 10% glycerol, resuspend the pellet in it and then add 45ml of 10% glycerol, shake by hand a few times to mix.

9 Put on ice for 10 minutes.

10 Centrifuge for 10 minutes at 2000g and 4°C.

11 Discard supernatant and resuspend the pellet in 1 ml ice-cold 10% glycerol, pool the contents of all 8 tubes into one tube.

12 Centrifuge for 5 minutes at 1500g and 4°C.

13 Discard supernatant and add 400 µl 10% glycerol (this will result in ~800 µl suspension).

14 Make aliquots of 40µl in pre-cooled eppendorf tubes and flash freeze with N2(l). Store at -80°C.