

Protocol

Electrocompetent Cells (For Electroporation)

T.J. Magliery, 24 Sept. 2001

General Procedures:

Perform all steps in the cold room.

Wear gloves at all times!

Use good sterile procedure.

The total time from $OD_{600} = 0.5$ to dry ice (steps 7 to 16) should be under 1 h (ideally, 50 min).

You have to work fast!

Preparation of electrocompetent cells, large scale

1. Streak cells onto LB/agar with appropriate antibiotics and grow overnight.
2. Pick a single colony and inoculate 25 mL of sterile 2YT/antibiotics. Grow to saturation overnight.
3. In the meantime, prepare and autoclave 2 L of 2YT (in a 4 L flask), 2.5 L of 10 % glycerol in double-distilled H_2O , and four *clean* 500 mL GS3 tubes. Put the 2 L 2YT in the 37 °C shaker/incubator overnight to pre-warm and pre-aerate the medium.
4. In the morning, inoculate the 2 L of 2YT with all 25 mL of saturated culture, adding appropriate antibiotics. The cells should be grown at the appropriate temperature (30-37 °C) with vigorous shaking (250-300 rpm).
5. Monitor the growth of the cells by OD_{600nm} . You will want to stop the culture growth at or around $OD_{600} = 0.5$ for most cloning strains, because this is typically the middle of log-phase growth. For unusual strains, you should grow a culture and follow the growth to determine the middle of log phase. In general $OD_{600} = 0.5-0.65$ is good, 0.65-0.8 is ok and anything over this will yield poor cells. Remember that K12 *E. coli* typically double in 20 min at log phase. Hence, when you get to $OD_{600} = 0.25$, you probably only have about 30 min until the cells are ready. At $OD_{600} = 0.4$, you have about 5 min. Typically, it will take about 4 h to go from 25 mL of saturated culture in 2 L to $OD_{600} = 0.5$ at 37 °C.
6. While the cells are growing, pre-chill the 2.5 L of 10 % glycerol on ice in the cold room. Also pre-chill the GS3 tubes.
7. When the cells get to mid-log phase ($OD_{600} = 0.5$), plunge them into an ice-water bath in the cold room and swirl the culture for 5-10 minutes to stop the cell growth.
8. Transfer the 2 L of culture into 4 GS3 tubes. Spin the cells at 7000 rpm for 5 min, 4 °C.
9. Spill off the media and resuspend the pellets in a small amount (about 50 mL) of ice-cold 10 % glycerol by pipetting up and down. You should work quickly, but make sure there are no clumps of cells left.
10. Combine the pellets into two GS3 tubes and fill the tubes to the top (500 mL). Shake to mix.
11. Spin the cells down again, 7000 rpm, 5 min, 4 °C.
12. While the cells are spinning, crush some dry ice to a powder with a hammer. Pre-chill 10 sterile 1.5 mL eppendorf tubes on dry ice. Remember to label the tubes before you put them in dry ice.

13. Spill off the glycerol wash. Beware that the pellet will be much softer at this point. Resuspend the cells again in a small amount (50 μ L) of ice-cold 10 % glycerol. Fill the two GS3 tubes to the top (500 μ L) and shake to mix.
14. Spin the cells down again, 7000 rpm, 5 min, 4 $^{\circ}$ C.
15. Spill off as much glycerol wash as possible. The pellets will now be very fragile. Resuspend the pellets in the residual 10 % glycerol in the GS3 tubes. You should have about 1.2 mL of cells per tube (per L of original culture).
16. Transfer the cells to the eppendorf tubes on dry ice in 320 μ L aliquots. Return the tubes to dry ice to freeze solid, 5-10 min.
17. Store the cells at -80 $^{\circ}$ C. Cells should retain their efficiency for 2-3 months.

Testing the efficiency of the cells

1. Thaw an aliquot of cells on ice. Pre-chill an electroporation cuvette and a sterile eppendorf tube on ice at the same time.
2. Combine 40 μ L of cells with plasmid DNA in the eppendorf tube. Use commercially available pBR322 or pUC18. Dilute the DNA so that you can add 1 ng of plasmid DNA in 1-2 μ L. Keep everything on ice. Re-freeze the remainder of the 320 μ L aliquot.
3. Shake down the cells into the cuvette and tap the cuvette on the benchtop to remove any bubbles. Make sure that the cell suspension is in contact with both plates of the cuvette. Wipe the outside of the cuvette dry and shock the cells. You will typically want the following settings for a 0.2 cm cuvette for *E. coli*: 2.5 kV, 25 μ F, 200 Ω . For 0.1 cm cuvettes, use 1.7 kV. Note that this is a higher field strength, and that you must have very clean (i.e., salt-free) DNA for this. These settings should yield a time constant of about 4.7 ms. Anything below 4.0 ms is poor and indicates high conductance for the cells or DNA.
4. Immediately quench the cells in 1 mL 2YT and pipette slowly to mix. Ideally, the 2YT should be pre-warmed to 37 $^{\circ}$ C.
5. Outgrow the cells for 1-2 h at 30 $^{\circ}$ C in a polypropylene culture tube. You can do this for 1 h at 37 $^{\circ}$ C instead, but you will get better results at the lower temperature.
6. Plate the appropriate amount of cells (see below) on LB/agar with the appropriate antibiotics. For pBR322 and pUC18, use LB/agar/Amp plates with 100 μ g mL⁻¹ Amp. The plate should be pre-warmed and dried at 37 $^{\circ}$ C.
7. Grow the plate overnight and count the colonies. For good cloning strains like DH10B, TOP10 and DH5 α , you can expect transformation efficiencies in the neighborhood of 10⁹ cfu μ g⁻¹. Thus, when you transformed 1 ng of plasmid DNA, you should have about 10⁶ cfu in the 1 mL quench. If you plated 1 μ L of this, there would be 1,000 colonies on the plate. For poorer strains like JM109 or, worse, BL21, expect transformation efficiencies in the neighborhood of 10⁶ cfu μ g⁻¹. Here, if you plated 100 μ L of the 1 mL quench, you would expect 100 colonies.

The formula for transformation efficiency is:

$$\text{TE [cfu } \mu\text{g}^{-1}] = \frac{(\text{colonies on plate})(\text{volume of quench in } \mu\text{L})}{(\text{volume of quench plated in } \mu\text{L})(\mu\text{g of plasmid})}$$

Transformation of cells

In general you will follow the above procedure for normal transformation. For cloning, 40 μL of cells is adequate to generous, depending upon how much DNA you have. For libraries, you want to use something like 320 μL of cells per μg of plasmid DNA. Typically, you will plate 200 μL of the 1 mL quench for cloning, or the whole thing for libraries.

The salinity of the DNA solution will have a major impact on the transformation efficiency. For example, you can only use 1-1.5 μL of a ligation with 40 μL of cells, or you will cause arcing (“sparking”). It is worth noting that, for cloning purposes or transforming a homogeneous plasmid, you should go ahead and plate the quench even if you got arcing. I’ve often been surprised by the fact that a few colonies will grow even after an arc. For larger quantities of DNA (1 μg and above), the DNA itself will add substantially to the ionic strength of the solution. Therefore, you **MUST** make sure that the DNA is essentially salt-free, typically by ethanol precipitation (2.5 vol absolute ethanol and 0.1 vol 3 M sodium acetate pH 5) and two sequential 70 % ethanol washes.

General notes

The most important factors for high-quality competent cells are: starting with a fresh, single colony; clean, sterile tubes and glassware; keeping the cells cold at all times during the prep; and completing the prep in less than 1 hour. The most important factors for successful transformation are purity of the DNA and low salinity of the solutions.

If the DNA quantity is small, and you need to ethanol precipitate the DNA to reduce salinity, *E. coli* tRNA carrier (perhaps 1 μg) is a good choice that won’t reduce your transformation efficiency.