Transformation of plasmids into a bacterial host

In the event of ELECTRIC SHOCK:
▷ **Seek immediate medical attention, even if symptoms are mild or absent**

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Recombinant DNA can be stably maintained in bacterial hosts as extrachromosomal plasmids, enabling clonal amplification (“molecular cloning”) and, in some cases, expression of encoded transgenes.
Various non-pathogenic *Escherichia coli* strains have been engineered to optimize plasmid propagation or facilitate high-level protein expression. These strains can be rendered competent for DNA uptake through different transformation methods.
One widely used approach involves heat shock, where transformation efficiency is greatly enhanced in the presence of divalent cations (Inoue et al., 1990) such as $CaCl_2$ (*Alternative A*) or $MnCl_2$ (*Alternative B* and *Alternative C*), which neutralize the negative charges on DNA and cell surfaces. However, some *E. coli* strains with thicker cell walls may be less amenable to this method.
For a fast but less efficient approach, TSS transformation (Chung et al., 1989) (*Alternative D*) offers a convenient option, especially for wild-type *E. coli* strains that are sensitive to $CaCl_2$.
Electroporation (*Alternative E*) typically yields transformation efficiencies of 1×10^{9}–1×10^{10} cfu/µg DNA—significantly higher than chemical methods (1×10^{6}–1×10^{8} cfu/µg). A high-voltage pulse transiently permeabilizes the membrane, allowing DNA uptake. To avoid arcing, cells must be prepared in a low ionic-strength solution, which also makes it more sensitive to residual salts or impurities in the DNA preparation.
While freshly prepared competent cells yield the highest efficiencies, they can be prepared in bulk and stored at −80 °C in glycerol or DMSO with minimal loss of competence.

This is a bench card. Full protocol available online.

Procedures

›› ***Preparation of* E. coli *in logarithmic growth phase***

(1.) Inoculate a single colony into 1–5 mL LB medium. Grow overnight at 37 °C.

> ***Critical:*** Add antibiotics if needed. For example, to maintain BL21(DE3)pLysS, which carries a plasmid encoding T7 lysozyme, supplement with chloramphenicol. Many laboratory background strains of *E. coli* do not harbor antibiotic resistance markers. In that case, sterilize glassware and plastics used for growth.

(2.) Inoculate 1.0 vol LB or S. O. B. medium with 5 mL of the overnight culture per liter of final volume. The starting optical density (OD600) should be around 0.025 mL^{-1}.

(3.) Grow the culture at 18 °C, shaking at 150–200 rpm, until it reaches an OD600 of 0.4–0.6 mL^{-1}.

> ***Critical:*** Although growth at 37 °C for 2–3 h is faster, cold growth for at least three doublings is key for high competence. Do not allow the culture to overgrow! Harvest during log phase. Diluting stationary-phase cells is ineffective.

(4.) Chill the culture on ice for 10 min. Pellet the cells in a refrigerated centrifuge at 1 500–2 000 × *g* for 5–10 min. Carefully decant the supernatant.

A › ***Chemically competent* E. coli *K-12 and B strains for heat shock transformation***

(1.) *Optional:* Resuspend the cells in 0.5 vol ice-cold, sterile 100 mM $MgCl_2$ solution.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend the cells in 0.5 vol ice-cold, sterile 50 mM $CaCl_2$ solution. Chill for 15 min on ice.

(4.) Collect by centrifugation as above. Discard the supernatant.

(5.) Resuspend in 0.07 vol ice-cold, sterile 50 mM $CaCl_2$ 15% glycerol solution.

B › *Highly efficient chemically competent* E. coli *K-12 and B strains for heat shock transformation*

- ☐ R0185 RF1, 200 mL
- ☐ R0186 RF2, 200 mL

(1.) Resuspend the cells in 0.3 vol ice-cold RF1. Chill the suspension for 15 min on ice.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend in 0.08 vol ice-cold RF2. Chill for 15 min on ice.

C › *Simple and efficient chemically competent* E. coli *K-12 and B strains for heat shock transformation*

- ☐ R0187 Simple & efficient buffer, 200 mL

(1.) Resuspend in 0.3 vol ice-cold SEB. Chill the suspension for 10 min on ice.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend in 0.04 vol ice-cold SEB. While swirling the tube, add 0.006 vol DMSO in a drop-wise manner. Chill for 10 min on ice.

D › *TSS-competent* E. coli *cells*

- ☐ R0188 2 × Transformation & storage medium, 50 mL

(1.) Resuspend in 0.025 vol cold LB medium.

(2.) Add an equal volume of ice-cold 2 × TSS solution. Mix gently by pipetting.

> ***Critical:*** Do not exceed 30 min incubation on ice. Longer exposure may lower transformation efficiency, possibly due to membrane recovery or PEG toxicity.

E › *Electrocompetent* E. coli

(1.) Resuspend in 0.5 vol ice-cold, sterile, reagent-grade water.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend in 0.2 vol ice-cold, sterile, reagent-grade water.

(4.) Collect by centrifugation as above. Discard the supernatant.

(5.) Resuspend in 0.008 vol ice-cold, sterile 10% glycerol.

›› *Frozen aliquots for long-term storage*

(1.) Pre-chill all containers that will be used for storage.

> ***Critical:*** Keep materials on ice and proceed quickly. The shorter this process, the better.

(2.) Dispense into 50–200 µL aliquots.

(3.) Freeze aliquots in liquid nitrogen or in an ethanol/dry ice bath.

(4.) Store at −80 °C for up to two years. Freeze-thawing will significantly decrease transformation efficiency.

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›› ***Preparing the transformation mixture***

(1.) Thaw cells on ice. Do not warm the tube in your hands, as even brief warming reduces competence. Homogenize the suspension by gently flicking the tube.

(2.) *Optional:* If necessary, dispense the thawed suspension into additional pre-chilled tubes.

(3.) To 20–50 µL suspension, add 0.1–100 ng plasmid DNA. The added material should not exceed 0.2 vol of the total volume. Mix the contents by gently flicking the tube.

A,B,C › ***Transformation of chemically competent bacteria***

(1.) Incubate the mixture for 5–30 min on ice.

(2.) Place the tubes in a water or sand bath of 42 °C for 30–45 s with no shaking.

(3.) Place the tubes on ice for 2 min.

(4.) Add 200–500 µL pre-warmed SOC or LB medium.

D › ***Transformation of TSS competent bacteria***

(1.) Vortex the suspension. Incubate for 20–30 min on ice.

Critical: Longer incubation time will reduce transformation efficiency.

E › ***Transformation of electrocompetent bacteria***

(1.) Pre-chill the cuvette for electroporation on ice.

(2.) Pulse with a field strength of 7.5–10 kV/cm, which corresponds to 1.5–2.5 kV for a 0.2 cm cuvette.

> ***Quality assurance:*** A successful pulse yields a time constant between 4–8 ms. If arcing occurs, discard both sample and cuvette.

> ***Safety:*** Turn off the pulse apparatus according to the manufacturer's instructions to fully discharge the capacitors!

(3.) Add 1 mL pre-warmed SOC or LB medium to the cuvette. Gently pipette up and down to mix. Transfer the suspension to a clean tube for recovery.

(4.) *Optional:* Rinse the cuvette with 70% ethanol. It can be reused after thorough drying.

›› ***Selection of transformed clones***

(1.) Incubate the transformed suspension in non-selective growth medium for at least 45–60 min at 37 °C. 60 min

(2.) Spread 0.1–1.0 vol of the recovered suspension onto a selective LB agar plate. Allow the plate to dry and place it upside-down at 37 °C in an incubator. Colonies will be visible after 12–18 h.

List of references

C. Chung, S. Niemela, and R. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **86**(7), 2172—2175 (1989).

H. Inoue, H. Nojima, and H. Okayama, *Gene* **96**(1), 23—28 (1990).