Protocol

Transformation of *Escherichia coli* by Electroporation

Michael R. Green and Joseph Sambrook

Preparing electrocompetent bacteria is considerably easier than preparing cells for transformation by chemical methods. Bacteria are simply grown to mid-log phase, chilled, centrifuged, washed extensively with ice-cold buffer or H_2O to reduce the ionic strength of the cell suspension, and then suspended in an ice-cold buffer containing 10% glycerol. DNA may be introduced immediately into the bacteria by exposing them to a short high-voltage electrical discharge. Alternatively, the cell suspension may be snap-frozen and stored at −70°C for up to 6 mo before electroporation, without loss of transforming efficiency.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Escherichia coli strain (e.g., DH5α or commercially available derivatives Electromax DH5α [Life Technologies], Turbo Electrocompetent *E. coli* [NEB] or Electromax DH10B [Life Technologies]), grown as colonies on a fresh agar plate

Glycerol (10% v/v) (molecular biology grade), ice cold

GYT medium <R>

Chill to ice cold.

LB (Luria-Bertani) liquid medium <R>

Prewarm to 37°C.

Plasmid DNA

Ideally, the DNA to be electroporated should be resuspended at a concentration of 1–10 µg/mL in H_2O (pH 8.0). For maximum efficiency of transformation, ligation mixtures should be desalted by microcolumn purification (Schlaak et al. 2005) or ultrafiltration although Microcon/Centricon cartridges (Millipore). However, for routine cloning or subcloning, small volumes of ligation mixtures that have been diluted twofold to fivefold with H_2O can simply be added to the electroporation cell. For the construction of libraries, where high efficiency is required and cotransformants are undesirable, total DNA concentrations of <10 ng/mL are recommended (Dower et al. 1988). For routine transformation of E. coli *with a superhelical plasmid, 10–50 pg of DNA is adequate. When subcloning into a plasmid, up to 25 ng of DNA diluted from the ligation mixture can be used.*

Pure H_2O

Milli-Q or equivalent, sterilized by filtration through prerinsed 0.45-µm filters. Store at 4°C.

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SOB medium <R>

Prepare SOB agar plates containing 20 mM $MgSO_4$ and the appropriate antibiotic. Standard SOB contains 10 mM $MgSO_4$.

SOC medium <R>

Approximately 1 mL of this medium is needed for each transformation reaction.

Equipment

Centrifuge bottles, sterile, chilled on ice
Electroporation device and cuvettes fitted with electrodes spaced 0.1 cm apart
Ice-water bath
Liquid nitrogen
Microcentrifuge tubes (0.5 mL), chilled on ice
Pasteur pipette attached to a vacuum line
Polypropylene tubes (17 × 100 mm or 15 × 150 mm)
Rotating wheel in a warm (37°C) room
Shaking incubator (37°C)
Sorvall R-6 centrifuge with appropriate rotor precooled to 4°C
Spectrophotometer
Ultra microcuvettes to read optical density at 600 nm

METHOD

All steps in this protocol should be performed aseptically.

Preparation of Cells

1. Inoculate a single colony of *E. coli* from a fresh agar plate into a flask containing 50 mL of LB medium. Incubate the culture overnight at 37°C with vigorous aeration (250 rpm in a rotary shaker).
2. Inoculate 500 mL of prewarmed LB medium in a 2-L flask with 25 mL of the overnight bacterial culture. Incubate the flasks at 37°C with agitation (300 cycles/min in a rotary shaker). Measure the OD_{600} of the growing bacterial cultures every 20 min.

   Many researchers grow cultures for electroporation to an OD_{600} of 0.6–0.8. However, in our hands, optimum results (>10^9 transformants/μg of plasmid) are obtained when the OD_{600} of the culture is 0.35–0.4. This density is usually achieved after ~2.5 h of incubation. To ensure that the culture does not grow to a higher density, measure the OD_{600} of the culture every 20 min. Plot a graph of the data so that the time when the OD_{600} of the culture approaches 0.4 can be predicted with some accuracy. Begin to harvest the culture when the OD_{600} reaches 0.35.

3. As the OD_{600} of the cultures reaches 0.4, rapidly transfer the flask to an ice-water bath for 15–30 min. Swirl the culture occasionally to ensure that cooling occurs evenly. In preparation for the next step, place the centrifuge bottles in an ice-water bath.

   For maximum efficiency of transformation, it is crucial that the temperature of the bacteria not increase above 4°C at any stage in the protocol.

4. Transfer the culture to ice-cold centrifuge bottles. Harvest the cells by centrifugation at 1000*g* for 15 min at 4°C. Decant the supernatant and resuspend the cell pellet in 500 mL of ice-cold pure H_2O by gentle up and down pipetting.
5. Harvest the cells by centrifugation at 1000*g* for 20 min at 4°C. Decant the supernatant and resuspend the cell pellet in 250 mL of ice-cold 10% glycerol.
6. Harvest the cells by centrifugation at 1000*g* for 20 min at 4°C. Decant the supernatant and resuspend the pellet in 10 mL of ice-cold 10% glycerol. Mix by gentle up and down pipetting.

   Take care when decanting because the bacterial pellets lose adherence in 10% glycerol.

7. Harvest cells by centrifugation at 1000*g* for 20 min at 4°C. As soon as the centrifuge stops, carefully decant the supernatant and use a Pasteur pipette attached to a vacuum line to remove any remaining drops of buffer. Resuspend the pellet in 1 mL of ice-cold GYT medium.

   This is best done by gentle swirling rather than pipetting or vortexing.

8. Measure the OD_{600} of a 1:100 dilution of the cell suspension. Dilute the cell suspension to a concentration of 2×10^{10} to 3×10^{10} cells/mL (1.0 $OD_{600} = \sim 2.5 \times 10^8$ cells/mL) with ice-cold GYT medium.

9. Dispense 40 µL aliquots of the cell suspension into sterile, ice-cold 0.5-mL microcentrifuge tubes, drop them into a bath of liquid nitrogen before transfer to a –80°C freezer. Withdraw two of the microcentrifuge tubes and measure the transformation efficiency of the preparation using 10 and 50 pg of superhelical plasmid DNA, as described below. If everything has worked to plan, the efficiency of transformation of the preparation should be $\sim 10^9$ colonies/mg of plasmid DNA and the number of transformants should be proportional to DNA concentration.

   Closed-circular, superhelical plasmid DNA transforms E. coli *far more efficiently than linearized plasmid DNA, which is degraded rapidly (Conley and Saunders 1984).*

Electroporation

10. To use frozen electrocompetent cells, remove an appropriate number of aliquots from the –80°C freezer. Store the tubes at room temperature until the bacterial suspensions are just thawed and then transfer the tubes to an ice bath.

11. Transfer 25 µL of the suspension to an ice-cold microcentrifuge tube. Add 1 µL of the DNA preparation.

    For construction of cDNA libraries, where high efficiency is required and cotransformants are undesirable, total DNA concentrations of <10 ng/mL are recommended (Dower et al. 1988). For routine transformation of E. coli *with a superhelical plasmid, 10–50 pg of DNA is adequate. When subcloning into a plasmid, up to 25 ng of DNA diluted from the ligation mixture can be used.*

12. Using a micropipette, transfer 20 µL of the bacterial/DNA suspension between the bosses of the electroporation chamber (0.1-cm gap). With practice, this can be done with the electroporation chamber in ice.

    It is best to wedge the pipette tip along one of the clear walls of the cuvette and to pipette the cells at one end of the cuvette channel. Tap the cuvette to move the suspension into the channel. Make sure no bubbles of air are present and that the liquid at the bottom of the cuvette forms a complete bridge between the two electrodes. Cap the cuvette.

    Include all of the appropriate positive and negative controls (see Box 1).

13. Following the manufacturer's instructions, deliver the appropriate electrical pulse to the cells.

    The presence of ions in the electroporation cuvette increases the conductivity of the solution and causes the electrical current to arc or skip through the solution of cells and DNA. Arcing is usually manifest by the generation of a popping sound in the cuvette during the electrical pulse. The uneven transfer of the charge through the cuvette drastically reduces the efficiency of transformation. Arcing increases at higher temperatures and occurs with solutions having an electrical conductance >5 mEq (e.g., 10 mM salt or 20 mM Mg^{2+} solutions). If arcing occurs in the presence of DNA but not in its absence, remove ions from the DNA preparation as described in Materials.

14. As quickly as possible after the pulse, remove the electroporation cuvette and add 1 mL of SOC medium at room temperature.

    Some investigators believe that the addition of medium at room temperature provides a heat shock that increases the efficiency of transformation.

15. Transfer the cells to a 17 × 100-mm or 17 × 150-mm polypropylene tube and incubate the cultures with gentle rotation for 1 h at 37°C.

16. Mix different volumes of the transformation mixture with 200 µL aliquots of prewarmed sterile medium. Plate each aliquot onto an SOB agar medium containing 20 mM $MgSO_4$ and the appropriate antibiotic.

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BOX 1. ESSENTIAL CONTROLS FOR BACTERIAL TRANSFORMATION

In every experiment, it is essential to include positive controls to measure the efficiency of transformation, and negative controls to eliminate the possibility of contamination and to identify the potential causes of failure.

Negative Controls

An aliquot of competent cells to which no DNA is added should be carried through the transformation experiment. The entire aliquot should be plated on a single agar plate containing the appropriate antibiotic used to select transformants. No bacterial colonies should grow on this plate or on a selective plate that received no bacteria at all. If any are detected, the following possibilities should be considered.

- The competent cells are contaminated with an antibiotic-resistant strain of bacteria during the experiment. Perhaps one of the solutions/reagents used in the transformation protocol is contaminated.
- The selective plates are defective. Perhaps the antibiotic was omitted altogether from the plates or was added to agar that was too hot.
- The selective plates are contaminated with an antibiotic-resistant strain of bacteria. In this case, colonies usually appear both on the surface of the medium and in the agar.

Positive Controls

An aliquot of competent cells should be transformed with a known amount of a standard preparation of circular superhelical plasmid DNA. This control provides a measure of the efficiency of transformation and allows a standard for comparison with previous transformation experiments.

When transforming with a superhelical plasmid, where transformants can be expected in abundance, a small volume of the transformed culture can be streaked with a sterile loop onto an agar plate (or a segment of a plate) containing the appropriate antibiotics. However, if only small numbers of transformants are expected, it is best to spread 200-µL aliquots of the bacterial suspension on each of five plates. We do not recommend plating a concentrated suspension of the bacterial culture on a single plate because the large number of dead cells resulting from electroporation may inhibit the growth of rare transformants.

17. Store the plates at room temperature until the liquid has been absorbed.
18. Invert the plates and incubate them at 37°C. Transformed colonies should appear in 12–16 h. Count the colonies and calculate the transformation efficiency (number of transformants/mg of DNA).

DISCUSSION

Because *E. coli* cells are small, they require very high field strengths (12.5–18 kV cm^{-1}) for electroporation compared to those used to introduce DNA into eukaryotic cells (Dower et al. 1988; Smith et al. 1990). Optimal efficiency is achieved using small volumes of a dense slurry of bacteria ($\sim 2 \times 10^{10}$/mL) contained in specially designed cuvettes fitted with closely spaced (0.1 cm) electrodes. To prevent arcing, the conductivity of the solution must be low. In practice, this means that the bacterial slurry must be extensively washed to remove salts. To achieve maximal transformation efficiency (e.g., for construction of cDNA libraries), the DNA preparation used for electroporation should also be desalted by microcolumn purification (Schlaak et al. 2005) or ultrafiltration although Microcon/Centricon cartridges (Millipore). However, for routine cloning or subcloning, small volumes of ligation mixtures that have been diluted twofold to fivefold with H_2O can simply be added to the electroporation cell.

Many variables affect transformation efficiency: the genotype of the *E. coli* strain (Elvin and Bingham 1991; Miller and Nickoloff 1995), the state of growth of the culture at the time of harvest, the temperature during harvesting and preparation of the bacterial cells, the topology of the DNA (linear or closed circular), and the timing, intensity, and shape of electrical pulse.

Most of the *E. coli* strains used for transformation carry a deletion in *recA*, a gene required for homologous recombination. The RecA protein helps to initiate ssDNA strand invasion of dsDNA and

causes the further unwinding that allows branch migration. *recA*$^-$ strains of *E. coli* are highly competent for transformation (Kurnit 1989) and in such strains, plasmids carrying repeated DNA sequences are stabilized. The method outlined in this protocol works well with *E. coli* DH5α, a *recA*$^-$ strain, and even better with some its commercially available derivatives (e.g., Electromax DH5α-E; Life Technologies; Turbo Electrocompetent *E. coli*, NEB; Electromax DH10B, Life Technologies). As is the case with chemical transformation, higher efficiencies of transformation are achievable with commercially produced electrocompetent cells rather than homemade preparations. However, highly efficient electrocompetent strains are required only in demanding circumstances (e.g., for construction of cDNA libraries). For most routine purposes, DH5α is perfectly adequate.

Cultures grown in the laboratory for electroporation are harvested in early log phase, chilled rapidly and processed at 0°C–4°C as described in the protocol. Electroporation is temperature dependent and is best carried out at 0°C–4°C. The efficiency of transformation drops as much as 100-fold when electroporation is performed at room temperature. The highest *efficiency* of transformation (colonies/μg input plasmid DNA) is obtained when the concentration of input DNA is high (1–10 μg/mL), when the DNA is in a closed-circular conformation and when the length and intensity of the electrical pulse are such that only 30%–50% of the cells survive the procedure. Under these conditions, as many as 80% of the surviving cells may be transformed. Higher *frequencies* of transformation (colonies/molecule input DNA) are obtained when the DNA concentration is low (~10 pg/mL). Most of the transformants then result from the introduction of a single plasmid molecule into an individual cell. High concentrations of DNA, on the other hand, favor the formation of cotransformants in which more than one plasmid molecule becomes established in transformed cells (Dower et al. 1988). This is highly undesirable in some circumstances, for example, when generating cDNA libraries in plasmid vectors.

Electroporators commonly used for transformation generate a waveform pulse of electricity that decays exponentially as the capacitor discharges. The length of the pulse (the time constant) can be adjusted by changing the resistance. The field strength applied to the sample is inversely proportional to the length of the gap across which the pulse travels in the cuvette. So if a pulse of 200 V is applied to a cuvette with a 0.1-cm gap, the field strength will be 2000 V/cm. Efficient electroporation of *E. coli* requires field strengths of 12.5–15 kV/cm (Dower et al. 1988). The conditions for transformation by *E. coli* vary between makes of electroporators but typically would be 1.8 kV, 25 μF, and 200 Ω.

The smaller the gap between the electrodes, the higher the chances of arcing. Cuvettes with a larger gap are easier to load but require a greater volume of electrocompetent bacteria. Most investigators use cuvettes with a gap of 0.1 cm.

Electroporation is typically about 10-fold more efficient than chemical transformation. Efficiencies of $\geq 10^{10}$ transformants per milligram of DNA can be achieved using commercial preparations of electrocompetent *E. coli* and optimal quantities of DNA. As in the case with chemical transformation, the *efficiency* of transformation is reported to decrease as the size of the DNA increases (Leonardo and Sedivy 1990; Siguret et al. 1994). However, the *frequency* of transformation remains constant with plasmids up to at least 30 kb in size (Donahue and Bloom 1998). Electroporation is more efficient than chemical transformation with plasmids of all sizes.

For more information, see Chassy et al. (1988), Dower et al. (1988), and Miller et al. (1988).

RECIPES

GYT Medium

Reagent	Quantity (for 100 mL)	Final concentration
Glycerol	10 mL	10% (v/v)
Yeast extract	0.125 g	0.125% (w/v)
Tryptone	0.25 g	0.25% (w/v)

Sterilize the medium by passing it through a prerinsed 0.22-μm filter. Store in 2.5-mL aliquots at 4°C.

LB (Luria-Bertani) Liquid Medium

Reagent	Amount to add
H_2O	950 mL
Tryptone	10 g
NaCl	10 g
Yeast extract	5 g

Combine the reagents and shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL). Adjust the final volume of the solution to 1 L with H_2O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle.

For solid medium, see the recipe entitled "Media containing agar or agarose."

Media Containing Agar or Agarose

Prepare liquid media according to the recipe given. Just before autoclaving, add one of the following:

Bacto agar (for plates)	15 g/L
Bacto agar (for top agar)	7 g/L
Agarose (for plates)	15 g/L
Agarose (for top agarose)	7 g/L

Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle. When the medium is removed from the autoclave, swirl it gently to distribute the melted agar or agarose evenly throughout the solution. *Be careful!* The fluid may be superheated and may boil over when swirled. Before adding thermolabile substances (e.g., antibiotics), allow the medium to cool to 50°C–60°C, and mix the medium by swirling to avoid producing air bubbles.

Before pouring the plates, set up a color code (e.g., two red stripes for LB-ampicillin plates; one black stripe for LB plates, etc.), and mark the edges of the plates with the appropriate colored markers. Pour plates directly from the flask; allow ~30–35 mL of medium per 90-mm plate. To remove bubbles from the medium in the plate, flame the surface of the medium with a Bunsen burner before the agar or agarose hardens. When the medium has hardened completely, invert the plates and store them at 4°C until needed.

The plates should be removed from storage 1–2 h before they are used. If the plates are fresh, they will "sweat" when incubated at 37°C. When this condensation drops on the agar/agarose surface, it allows bacterial colonies or bacteriophage plaques to spread and increases the chances of cross-contamination. This problem can be avoided by wiping off the condensation from the lids of the plates and then incubating the plates for several hours at 37°C in an inverted position before they are used. Alternatively, remove the liquid by shaking the lid with a single, quick motion. To minimize the possibility of contamination, hold the open plate in an inverted position while removing the liquid from the lid.

SOB Medium

Per liter: To 950 mL of deionized H_2O, add:

Tryptone	20 g
Yeast extract	5 g
NaCl	0.5 g

Shake until the solutes have dissolved. Add 10 mL of a 250 mM solution of KCl. (This solution is made by dissolving 1.86 g of KCl in 100 mL of deionized H_2O.) Adjust the pH of the medium to 7.0 with 5 N NaOH (~0.2 mL). Adjust the volume of the solution to 1 L with deionized H_2O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle. Just before use, add 5 mL of a sterile solution of 2 M $MgCl_2$. (This solution is made by dissolving 19 g of $MgCl_2$ in 90 mL of deionized H_2O. Adjust the volume of the solution to 100 mL with deionized H_2O and sterilize by autoclaving for 20 min at 15 psi [1.05 kg/cm^2] on liquid cycle.)

For solid medium, please see the recipe entitled "Media containing agar or agarose." <R>

SOC Medium

Per liter: To 950 mL of deionized H_2O, add:

Tryptone	20 g
Yeast extract	5 g
NaCl	0.5 g

SOC medium is identical to SOB medium, except that it contains 20 mM glucose. To prepare SOB medium, combine the above ingredients and shake until the solutes have dissolved. Add 10 mL of a 250 mM solution of KCl. (This solution is made by dissolving 1.86 g of KCl in 100 mL of deionized H_2O.) Adjust the pH of the medium to 7.0 with 5 N NaOH (~0.2 mL). Adjust the volume of the solution to 1 L with deionized H_2O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle. Just before use, add 5 mL of a sterile solution of 2 M $MgCl_2$. (This solution is made by dissolving 19 g of $MgCl_2$ in 90 mL of deionized H_2O. Adjust the volume of the solution to 100 mL with deionized H_2O and sterilize by autoclaving for 20 min at 15 psi [1.05 kg/cm^2] on liquid cycle.)

After the SOB medium has been autoclaved, allow it to cool to 60°C or less. Add 20 mL of a sterile 1 M solution of glucose. (This solution is made by dissolving 18 g of glucose in 90 mL of deionized H_2O. After the sugar has dissolved, adjust the volume of the solution to 100 mL with deionized H_2O and sterilize by passing it through a 0.22-µm filter.)

REFERENCES

Chassy BM, Mercenier A, Flickinger JL. 1988. Transformation of bacteria by electroporation. *Trends Biotechnol* **6:** 303–309.

Conley EC, Saunders JR. 1984. Recombination-dependent recircularization of linearized pBR322 plasmid DNA following transformation of *Escherichia coli*. *Mol Gen Genet* **194:** 211–218.

Donahue RA, Bloom FR. 1998. Transformation efficiency of *E. coli* electroporated with large plasmid DNA. *Focus (Life Technologies)* **20:** 77–78.

Dower WJ, Miller JF, Ragsdale CW. 1988. High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Res* **16:** 6127–6145.

Elvin S, Bingham AH. 1991. Electroporation-induced transformation of *Escherichia coli*: Evaluation of a square waveform pulse. *Lett Appl Microbiol* **12:** 39–42.

Kurnit DM. 1989. *Escherichia coli* recA deletion strains that are highly competent for transformation and for in vivo phage packaging. *Gene* **82:** 313–315.

Leonardo ED, Sedivy JM. 1990. A new vector for cloning large eukaryotic DNA segments in *Escherichia coli*. *Biotechnology* **8:** 841–844.

Miller EM, Nickoloff JA. 1995. *Escherichia coli* electrotransformation. *Methods Mol Biol* **47:** 105–113.

Miller JF, Dower WJ, Tompkins LS. 1988. High-voltage electroporation of bacteria: Genetic transformation of *Campylobacter jejuni* with plasmid DNA. *Proc Natl Acad Sci* **85:** 856–860.

Schlaak C, Hoffmann P, May K, Weimann A. 2005. Desalting minimal amounts of DNA for electroporation in *E. coli*: A comparison of different physical methods. *Biotechnol Lett* **27:** 1003–1005.

Siguret V, Ribba AS, Cherel G, Meyer D, Pietu G. 1994. Effect of plasmid size on transformation efficiency by electroporation of *Escherichia coli* DH5α. *BioTechniques* **16:** 422–426.

Smith M, Jessee J, Landers T, Jordan J. 1990. High efficiency bacterial electroporation 1×10^{10} *E. coli* transformants per microgram. *Focus (Life Technologies)* **12:** 38–40.

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