Preparation of electro-competent *E. coli* cells

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Note: To achieve high transformation rates preparation of competent *E. coli* requires that the **cells be kept ice cold at all times.** Failure to do this will result in inefficient competency and poor library construction. **Keep the cells and reagents to be added to cells on wet ice at all times.**

Removal of salts from the liquid surrounding the cells is also very important. It is critical to remove all liquid and to completely resuspend the cells between spins. Failure to do so will result in low competency and sparking in the electroporator.

Previously done by TAs:

Cultures of *E. coli* strain 43R are grown to mid log (OD_{600} of 0.4-0.7) in LB broth prepared without the addition of sodium chloride and grown at 37C while shaking at 200 rpm.

Preparation (one prep per table)

1. Rapidly cool cultures by placing them on wet ice and mixing for 5-10 minutes
2. Fill two 50 ml falcon tubes (clear plastic tubes with a conical bottom).
3. Balance and place in centrifuge. Spin 10 minutes at 2000g.
4. Pour off supernatant. Invert tube and tap on paper towels to remove the remaining liquid.
5. Add 1ml of distilled water and resuspend the cell pellet by gentle pipetting with a p1000. Remember to keep the tube on ice.
6. Combine the resuspended cells into one of the falcon tubes. Rinse the other falcon tube with the distilled water to remove the remaining cells and add this to the other tube. Fill the tube containing the cells to the top with distilled water. Invert several times to mix.
7. Balance tube with a neighboring table's tube and place in centrifuge. Spin 10 minutes at 2000g.
8. Pour off supernatant. Invert tube and tap on paper towels to remove the remaining liquid.
9. Add 1ml of distilled water and resuspend the cell pellet by gentle pipetting with a p1000.
10. Fill the tube containing the cells to the top with distilled water. Invert several times to mix.
11. Balance tube with a neighboring table's tube and place in centrifuge. Spin 10 minutes at 2000g.
12. Add 100ul of GYT broth to cells and resuspend the cell pellet by gentle pipetting with a p1000.
13. Remove 5 µl of cells and add to 495 µl of GYT broth. Mix and place in a cuvette. Measure the OD at 600 nm. The OD must be between 0.5 and 1.0. If the OD is above this dilute the stock of cells appropriately with GYT. Calculate the amount of volume needed using the $V_1*C_1 = V_2*C_2$ equation. If the OD is too low to cells must be centrifuged and resuspended in a smaller volume of liquid.

14. After the cells have been adjusted to the appropriate concentration aliquot cells in 35 µl volumes into pre-chilled and labeled eppendorf tubes and freeze on dry ice. Place these cells in the appropriate box in the -80 freezer.

Testing the competent cells

Note: the settings for the electroprator are as follows:
1.25 kV/mm, 25µf, 200ohms

1. Test one tube of cells by adding 1 ul of test plasmid to a thawed tube of competent cells. Note the concentration of test plasmid. Gently mix with a pipettor and. Add the mixture to a pre-chilled electroporation cuvette taking care to not introduce any air bubbles to the cuvette.
2. Gently tap the cuvette to remove any air bubbles and place in the electroporator. Hold both red buttons until the machine beeps.
3. Quickly add 436 µl of SOC media to cells and place at 37C for 30 minutes.
    a. Note the discharge time displayed. This value should be larger than 3.5 ms, shorter values may indicate sparking resulting from air bubbles or inefficient washing of cells.
4. Spread 10, 50, and 100 µl of the transformation on to LB plates with the appropriate antibiotic. Incubate overnight at 37C

Next Lab Calculating the transformation efficiency:

1. Calculate the transformation efficiency by counting the number of colonies on one of the plates that has between 30 and 300 colonies.
2. From this count calculate what the number of colonies in the **entire transformation** would be if you had plated it all out.(note that your transformation volume is: 35µl of competent cells + 1 µl of plasmid + 464 µl of SOC = 500 µl total volume). If you count the number of colonies on the plate with 50µl of the transformation plated on it you are effectively counting $1/10^{th}$ of the total number of cells transformed in the whole reaction (the 50 µl on this plate is $1/10^{th}$ of the total volume of 500µl).
3. The number you now have is the transformation efficiency per amount of plasmid added (____transformations/____ng of plasmid) Convert this ratio to ____transformations/ **1 ug of plasmid**

Here is a range of normal competency for *E. coli* cells to see how yours compare:

a. Natural competency of *E. coli*: 0-5 transformations/ug
b. Lab made heat shock competent cells: 10^4 - 10^7 transformations/ug
c. Professionally made heat shock competent cells: 10^6-10^8 transformations/ug
d. Lab made electrocompetent cells: 10^7-10^9 transformations/ug
e. Professionally made electrocompetent cells: 10^8-10^{10} transformations/ug

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Transformation of *E. coli* by Electroporation

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 (Chassy and Flickinger 1987; Chassy et al. 1988; Dower et al. 1988; please see the information panel on **ELECTROPORATION**). Alternatively, the cell suspension may be snap-frozen and stored at –70°C for up to 6 months before electroporation, without loss of transforming efficiency.

Because *E. coli* cells are small, they require very high field strengths (12.5–15 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 (~2×10^{10}/ml) contained in specially designed cuvettes fitted with closely spaced electrodes. Electroporation is temperature-dependent and is best carried out at 0–4°C. The efficiency of transformation drops as much as 100-fold when electroporation is carried out 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) 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.

The method outlined in this protocol works well with most strains of *E. coli* and with plasmids <15 kb in size. However, substantial variation in the efficiency of transformation between strains of *E. coli* has been reported (e.g., please see Elvin and Bingham 1991; Miller and Nickoloff 1995), and given what is known about the mechanism of uptake of DNA by electroporation, it would be reasonable to expect that very large plasmids would transform *E. coli* with reduced efficiency. As is the case with chemical transformation, linear plasmid DNAs introduced into *E. coli* by a pulsed electrical discharge transform very inefficiently — from 10- to 1000-fold less effi-

ciently than the corresponding closed circular DNA — perhaps because the exposed termini o linear DNA are susceptible to attack by intracellular nucleases.

The application of a sharp pulse of electricity is thought to cause dimpling of membranes followed by formation of transient hydrophobic pores whose diameter fluctuates from a minimum of 2 nm to a maximum of several nm. Some of the larger hydrophobic pores are converted to hydrophilic pores because the energy needed to create and maintain a hydrophilic pore is reduced as the transmembrane voltage is increased (Weaver 1993). Reclosing of pores seems to be a stochastic process that can be delayed by keeping the cells at low temperature. While the pores remain open, DNA molecules can easily pass from the medium into the cytoplasm (please see Figure 1-18).

The transmembrane voltage required for formation of large hydrophobic pores varies in direct proportion to the diameter of the target cell. Most manufacturers of electroporation machines provide literature describing the approximate voltages required for transfection of specific cell types in their particular apparatus. Three important parameters of the pulse affect the efficiency of electroporation:

- *Length* of pulse is determined mainly by the value of the capacitor and the conductivity of the medium. Most commercial electroporation machines use capacitative discharge to produce controlled pulses.
- *Field strength* varies in direct proportion to the applied voltage and in inverse proportion to the distance between the electrodes. Most manufacturers provide cuvettes of various sizes to suit the task at hand and recommend that the cuvettes be used only once. However, many investigators, in an effort to reduce costs, wash and re-use the cuvettes several times. The wisdom of this practice is a topic of ongoing debate (e.g., please see Hengen 1995).
- *Shape* is determined by the design of the electroporation device. The wave form produced by most commercial machines is simply the exponential decay pattern of a discharging capacitor (Dower et al. 1988).

For most commonly used strains of *E. coli*, maximum rates of transformation are achieved after a single electrical pulse with a field strength of 12.5–15 kV cm^{-1} and a length of 4.5–5.5 milliseconds. Under these conditions, ~50% of the cells survive.

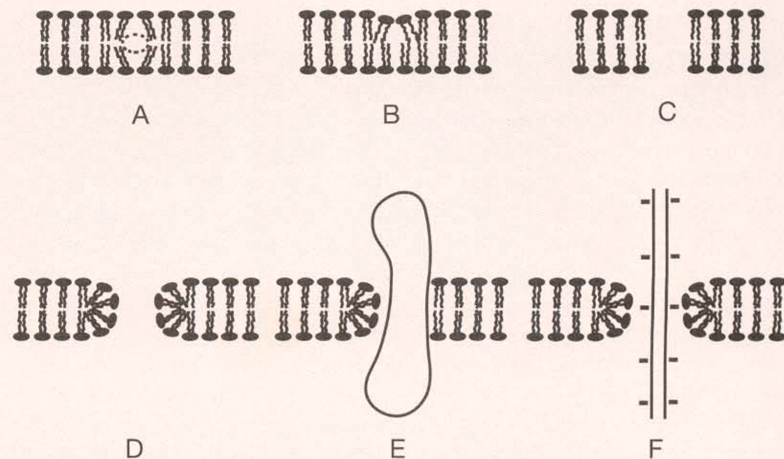

FIGURE 1-18 Changes in the Membrane during Electroporation

Drawings of hypothetical structures for transient and metastable membrane conformations believed to be relevant to electroporation. (*A*) Fredd volume fluctuation; (*B*) aqueous protrusion or "dimple"; (*C,D*) hydrophobic pores usually regarded as the "primary pores" through which ions and molecules pass; (*E*) composite pore with "foot in the door" charged macromolecule inserted into a hydrophilic pore. The transient aqueous pore model assumes that transitions from A→B→C or D occur with increasing frequency as U is increased. Type F may form by entry of a tethered macromolecule, while the transmembrane voltage is significantly elevated, and then persist after U has decayed to a small value through pore conduction. It is emphasized that these hypothetical structures have not been directly observed and that support for them derives from the interpretation of a variety of experiments involving electrical, optical, mechanical, and molecular transport behavior. (Reprinted, with permission, from Weaver 1993 [copyright Wiley-Liss, Inc.].)