Protocol

Easy Transformation of *Escherichia coli*: Nanoparticle-Mediated Transformation

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In this protocol, a colloidal solution containing mineral nanofibers is mixed with *Escherichia coli* and plasmid DNA and plated immediately on the appropriate selective plates. Sliding frictional forces, created when the bacteria are spread with a polystyrene stir stick across the surface of the agar, may result in penetration of the bacteria by the mineral fibers with its adherent cargo of DNA. The number of transformants increases during the first 60 sec of exposure to the mineral fiber:DNA complex and then reaches a plateau of $\sim 1 \times 10^6$ to 2×10^6 transformants/milligrams of DNA. Although not highly efficient, the method is very simple.

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

E. coli culture (e.g., TOP10 cells or another robust strain) in log phase

HEPES (1 M, pH 7.4)

KCl (1 M)

Plasmid DNA

A standard preparation of a plasmid DNA should be stored in aliquots and used to measure the competence of batches of chemically treated E. coli. pUC19, which is commonly used for this purpose, is available from New England Biolabs.

Sepiolite

Spanish sepiolite with a mean fiber length of 2 mm and a mean diameter of 20 nm is available from Kremer Pigmente GmbH & Co, KG, Germany (catalog no. 58945)

SOB, SOC, or LB agar plates (1%–2%) containing the appropriate antibiotic

LB (Luria-Bertani) liquid medium <R>

SOB medium <R>

SOC medium <R>

Equipment

Incubator (37°C)

Polystyrene stick (available from Sarstedt, Germany)

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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.

METHOD

See Box 1 for descriptions of essential controls.

1. Autoclave a suspension of 0.01% sepiolite in 200 mM KCl and 5 mM HEPES (pH 7.4).
2. Briefly centrifuge 500 mL of a log-phase culture of *E. coli*.
3. Resuspend the bacterial pellet in 100 mL of sepiolite suspension, and add 50 ng of plasmid DNA. Wait for 60 sec and then use a polystyrene stick to spread the resulting suspension on a 1%–2% agar plate (at least 2 d old) containing the appropriate antibiotic. Spreading should be continued for ~30 sec after the liquid has soaked into the agar, when the frictional resistance to spreading increases.
4. Incubate the plate overnight at 37°C.

DISCUSSION

Yoshida's group in Miyazaki published a series of papers confirming and extending the observation by Appel et al. (1988) that transformation of *E. coli* by plasmids can be accomplished using mineral nanofibers (Yoshida et al. 2001, 2002, 2007; Yoshida and Sato 2009 [and references therein]). Previously, an impediment to the wider adoption of this method was the nature of the mineral fibers used as the transfection agents: Both Appel et al. (1988) and Yoshida's group used chrysotile, a form of asbestos. More recently, however, Yoshida's observations were confirmed (Wilharm et al. 2010) using a suspension of noncarcinogenic fibers (Spanish sepiolite) instead of the potentially dangerous chrysotile whisker used by Yoshida and his coworkers. The basic protocol outlined by Wilharm et al. (2010) is above.

RECIPES

LB (Luria-Bertani) Liquid Medium

Reagent	Amount to add
H ₂ O	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 H₂O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle.

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

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/cm²) 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 H₂O, 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 H₂O.) 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 H₂O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle. Just before use, add 5 mL of a sterile solution of 2 M MgCl₂. (This solution is made by dissolving 19 g of MgCl₂ in 90 mL of deionized H₂O. Adjust the volume of the solution to 100 mL with deionized H₂O and sterilize by autoclaving for 20 min at 15 psi [1.05 kg/cm²] 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 H₂O, 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 H₂O.) 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 H₂O. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle. Just before use, add 5 mL of a sterile solution of 2 M MgCl₂. (This solution is made by dissolving 19 g of MgCl₂ in 90 mL of deionized H₂O. Adjust the volume of the solution to 100 mL with deionized H₂O and sterilize by autoclaving for 20 min at 15 psi [1.05 kg/cm²] 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 H₂O. After the sugar has dissolved, adjust the volume of the solution to 100 mL with deionized H₂O and sterilize by passing it through a 0.22-μm filter.)

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