

Sample Protocol

Protocol
1.24

The Inoue Method for Preparation and Transformation of Competent *E. Coli*: “Ultra-competent” Cells

This method to prepare optimally competent *E. coli* differs from other procedures in that the bacterial culture is grown at 18°C rather than the conventional 37°C. Incubating large-scale cultures at 18°C can be a challenge. One solution is to move the bacterial shaker into a cold room. However, if this is impossible, the cultures may be grown at 20–23°C with little loss of transforming efficiency.

MATERIALS

CAUTION: Please see Appendix 4 for appropriate handling of materials marked with <!\>.

Reagents and Solutions

Please see Appendix 1 for components of stock solutions, buffers, and reagents. Dilute stock solutions to the appropriate concentrations.

DMSO <!\>
Inoue transformation buffer

Vectors and Hosts

Plasmid DNA for transformation
Strain of *E. coli* suitable for transformation

Media and Antibiotics

LB or SOB medium
SOB agar plates containing 20 mM MgSO₄ and the appropriate antibiotic
Standard SOB contains 10 mM MgSO₄.
SOB or LB agar plates
SOC medium

Centrifuges/Rotors/Tubes

Sorvall SLC-1500 rotor (4°C) and centrifuge bottles

Additional Items

Circulating water bath (42°C)
Freezer (–70°C)
Ice-water bath
Liquid nitrogen bath
Nalgene filters (0.45-μm pore size)
Sterile microfuge tubes
Sterile 17 × 100-mm polypropylene tubes, chilled in ice
Vacuum aspirator

Additional Information

Inoue H., Nojima H., and Okayama H. 1990. High efficiency transformation of *Escherichia coli* with plasmids. *Gene* **96**: 23–28.

METHOD

1. Prepare Inoue transformation buffer (chilled to 0°C before use).
 - a. Prepare 0.5 M PIPES (pH 6.7) (piperazine-1,2-bis[2-ethanesulfonic acid]) by dissolving 15.1 g of PIPES in 80 ml of pure H₂O (Milli-Q or its equivalent). Adjust the pH of the solution to 6.7 with 5 M KOH and then add pure H₂O to bring the final volume to 100 ml. Sterilize the solution by filtration through a disposable prerinsed Nalgene filter (0.45- μ m pore size). Divide into aliquots and store frozen at -20°C.
 - b. Prepare Inoue transformation buffer by dissolving all of the solutes listed below in 800 ml of pure H₂O and then add 20 ml of 0.5 M PIPES (pH 6.7). Adjust the volume of the Inoue transformation buffer to 1 liter with pure H₂O.

Reagent	Amount per Liter	Final Concentration
MnCl ₂ ·4H ₂ O	10.88 g	55 mM
CaCl ₂ ·2H ₂ O	2.20 g	15 mM
KCl	18.65 g	250 mM
PIPES (0.5 M, pH 6.7)	20 ml	10 mM
H ₂ O	to 1 liter	

- c. Sterilize Inoue transformation buffer by filtration through a prerinsed 0.45- μ m Nalgene filter. Divide into aliquots and store at -20°C.
2. Pick a single bacterial colony (2–3 mm in diameter) from a plate that has been inoculated with a strain of *E. coli* suitable for transformation and incubated for 16–20 hours at 37°C. Transfer the colony into 25 ml of SOB medium (LB may be used instead) in a 250-ml flask. Incubate the culture for 6–8 hours at 37°C with vigorous shaking (250–300 rpm).
3. Use this starter culture to inoculate three 1-liter flasks, each containing 250 ml of SOB. The first flask receives 10 ml of starter culture, the second receives 4 ml, and the third receives 2 ml. Incubate all three flasks for 14 hours at 18–22°C with moderate shaking.
4. Read the OD₆₀₀ of all three cultures. Continue to monitor the OD every 45 minutes.
5. When the OD₆₀₀ of one of the cultures reaches exactly 0.55, transfer the culture vessel to an ice-water bath for 10 minutes. Discard the two other cultures.
6. Harvest the cells by centrifugation at 2500g (3900 rpm in a Sorvall SLC-1500 rotor) for 10 minutes at 4°C.
7. Pour off the medium and store the open centrifuge bottle on a stack of paper towels for 2 minutes. Use a vacuum aspirator to remove any drops of remaining medium adhering to the walls of the centrifuge bottle or trapped in its neck.
8. Resuspend the cells gently in 80 ml of ice-cold Inoue transformation buffer.
9. Harvest the cells by centrifugation at 2500g (3900 rpm in a Sorvall SLC-1500 rotor) for 10 minutes at 4°C.
10. Pour off the medium and store the open centrifuge tube on a stack of paper towels for 2 minutes. Use a vacuum aspirator to remove any drops of remaining medium adhering to the walls of the centrifuge tube or trapped in its neck.
11. Resuspend the cells gently in 20 ml of ice-cold Inoue transformation buffer.
12. Add 1.5 ml of DMSO. Mix the bacterial suspension by swirling and then store it in ice for 10 minutes.
13. Working quickly, dispense aliquots of the suspensions into chilled, sterile microfuge tubes. Immediately snap-freeze the competent cells by immersing the tightly closed tubes in a bath of liquid nitrogen. Store the tubes at -70°C until needed.

14. When needed, remove a tube of competent cells from the -70°C freezer. Thaw the cells by holding the tube in the palm of the hand. Just as the cells thaw, transfer the tube to an ice bath. Store the cells on ice for 10 minutes.
15. Use a chilled, sterile pipette tip to transfer the competent cells to chilled, sterile 17 x 100-mm polypropylene tubes in an ice bath. Store the cells on ice.

Include all of the appropriate positive and negative controls.

16. Add the transforming DNA (up to 25 ng per 50 μl of competent cells) in a volume not exceeding 5% of that of the competent cells. Swirl the tubes gently several times to mix their contents. Set up at least two control tubes for each transformation experiment, including a tube of competent bacteria that receives a known amount of a standard preparation of superhelical plasmid DNA and a tube of cells that receives no plasmid DNA at all. Store the tubes on ice for 30 minutes.
17. Transfer the tubes to a rack placed in a preheated 42°C circulating water bath. Store the tubes in the rack for exactly 90 seconds. Do not shake the tubes.
18. Rapidly transfer the tubes to an ice bath. Allow the cells to cool for 1–2 minutes.
19. Add 800 μl of SOC medium to each tube. Warm the cultures to 37°C in a water bath and then transfer the tubes to a shaking incubator set at 37°C . Incubate the cultures for 45 minutes to allow the bacteria to recover and to express the antibiotic resistance marker encoded by the plasmid.
20. Transfer the appropriate volume (up to 200 μl per 90-mm plate) of transformed competent cells onto agar SOB medium containing 20 mM MgSO_4 and the appropriate antibiotic.
21. Store the plates at room temperature until the liquid has been absorbed.
22. Invert the plates and incubate them at 37°C . Transformed colonies should appear in 12–16 hours.