The Inoue Method for Preparation and Transformation of Competent E. Coli : "Ultra-Competent" Cells

This protocol reproducibly generates competent cultures of E. coli that yield 1 x 10^8 to 3 x 10^8 transformed colonies/ug of plasmid DNA. The protocol works optimally when the bacterial culture is grown at 18 ℃.

Buffers and Solutions

DMSO, Inoue transformation buffer (please see Step 1)

Chilled to 0 ℃ before use.

SOB and SOC medium

Method

1.1 Prepare Inoue transformation buffer (chilled to 0 ℃ 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 H_2O (Milli-Q). Adjust the pH of the solution to 6.7 with 5 M KOH, and then add pure H_2O to bring the final volume to 100 ml. Sterilize the solution by filtration through a disposable prerinsed Nalgene filter (0.45-µm pore size). Divide into aliquots and store frozen at -20 ℃.

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
$MnCl_2 \cdot 4H_2O$	10.88 g	55 mM
$CaCl_2 \cdot 2H_2O$	2.20 g	15 mM
KCl	18.65 g	250 mM
PIPES (0.5 M, pH 6.7)	10 ml	10 mM
H_2O	to 1 liter	

c. Sterilize Inoue transformation buffer by filtration through a prerinsed 0.45-µm Nalgene filter. Divide into aliquots and store at -20 ℃.

1.2 Prepare SOB and SOC medium

20 g Tryptone

5 g Yeast extract

0.6 g NaCl

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.) Sterilize by autoclaving for 20 minutes at 121 ^oC. 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.Sterilize by using a0.22um filter). SOC medium is identical to SOB medium, except that it contains 20 mM glucose. After the SOB medium has been autoclaved, allow it to cool to 60 ℃ 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.)

2. Pipet 1 ul competent cells to 1000ul LB medium and transform 200 ul onto agar plate in the afternoon. Next morning, pick a single bacterial colony (2-3 mm in diameter) from a plate that has

been incubated for 16-20 hours at 37℃.Transfer the colony into 5 ml of SOB medium (LB may be used instead) in a 15 ml tube. Incubate the culture for 6-8 hours at 37℃ with vigorous shaking (250-300 rpm).

3. At about 9 o'clock in the evening, use this starter culture to inoculate two 500 ml flasks, each containing 100 ml of SOB. The first flask receives 200 ul of starter culture, the second receives 400 ul. Incubate all two flasks overnight at 18℃ with moderate shaking.

4. The following morning, read the OD_{600} of all two cultures. Continue to monitor the OD every 45 minutes.

5. When the OD_{600} of the cultures reaches 0.25-0.5, transfer the culture vessel to an ice-water bath for 10 minutes.

6. Harvest the cells by centrifugation at 4000 rpm for 10 minutes at 4℃.

7. Pour off the medium and pipet out remanent SOB medium.

8. Resuspend the cells gently in 16 ml of ice-cold Inoue transformation buffer. (**NOTE**: Don’t use pipette to resuspend the cells, you can shake it gently in the ice.

9. Harvest the cells by centrifugation at 4000 rpm for 10 minutes at 4℃.

10. Pour off the medium and pipet out remanent medium.

11. Resuspend the cells gently in 4 ml (the volume depend on the $OD_{600,}$ when it is 0.25, 4 ml is ok.) of ice-cold Inoue transformation buffer.

12. Add 0.3 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 100ul/tube. Immediately snap-freeze the competent cells by immersing the tightly closed tubes in a bath of liquid nitrogen. Store the tubes at -70℃ until needed.