Heat/chemical transformation (Inoue method)

Heat/chemical shock transformation method is a quick, economical method for transforming (inducing cell uptake of) self-propagating DNAs (plasmids). The Inoue method[1] starts by arresting culture growth in exponential phase, in which the cell wall is least developed, facilitating passage of DNA. The cells are stored in a transformation buffer, a solution of: the Good's buffer PIPES, an organic solvent DMSO to permeabilize the cell membranes, and monovalent potassium and divalent manganese and calcium cation salts to neutralize the divalent anionic charge of DNA phosphodiesters and LPS charge and disrupt protein–membrane/LPS interactions that occlude DNA binding. Cold temperature crystallizes the membrane, stabilizing pores called adhesion zones. The DNA to be transformed is added and incubated to give time for binding and penetrance of cells, facilitated by a heat shock that supposedly creates a temperature differential that induces flow that carries shielded DNA through adhesion zones. Recovery in non-selective medium allows expression of antibiotic resistance from the transformed DNA, necessary for survival of plated transformants on selective medium.

The Inoue method produces "ultra-competent" cells with a fairly standard procedure, simpler, less finicky, and more reproducible than its rival, the Hanahan method[3], yielding **1–3×10^8 CFU/µg DNA**.)

Based on the Inoue method[1] detailed in Sambrook & Russell's *Molecular Cloning* (3rd ed), Protocol 24[2].

Competent Cell Preparation

For *n* transformations of *V* volume:

1. Prepare Inoue transformation buffer (chilled to 4°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 Milli-Q H_2O. Adjust the pH of the solution to 6.7 with 5 M KOH, and then add H_2O to bring the final volume to 100 mL. Filter-sterilize through 0.22 µm filter [unclear why necessary]. Divide into 20 mL aliquots and store -20°C.

    b. Prepare Inoue transformation buffer by dissolving all of the solutes listed below in 800 mL Milli-Q H_2O, and then add 20 mL of 0.5 M PIPES (pH 6.7). Adjust volume to 1 L with Milli-Q H_2O.

	c_f, final conc.	
$MnCl_2 \cdot 4H_2O$	55 mM	10.88 g /L
$CaCl_2 \cdot 2H_2O$	15 mM	2.20 g /L
KCl	250 mM	18.65 g /L
$PIPES_{aq}$ (0.5 M, pH 6.7) (PIPES + KOH)	10 mM (10 mM; KOHpH=6.7)	20 mL /L (3.02 g /L)
Milli-Q H_2O	–	to 1 L

    c. Filter-sterilize through 0.22 µm filter. Aliquot and store at -20°C.
2. Grow seed culture: pick a fresh colony into 25 mL SOB or LB. Incubate at growth temperature shaking 250–300 rpm, 6–8 hr.
3. Inoculate one or more 12×*nv* mL volumes of SOB or LB with 0.5–1% of the seed culture. Incubate shaking 250–300 rpm refrigerated at 18°C (preferable) or room temperature (22°C). Use of a refrigerated incubator or incubator in a cold room is most consistent, though. Growth will be slow, 12–15 hr at 18°C.
4. Monitor OD_{600} until a culture reaches OD=0.55, at which point arrest growth by swirling the culture vessel in an ice bath until 1°C, 10 min depending on the culture volume. Discard overgrown cultures.
5. Harvest and wash cells: centrifuge 2500×*g* 10 min, 4°C. Decant medium and allow to drain inverted 2 min on paper towels. Aspirate residual liquid on walls/neck. Gently resuspend cells in 4× *nv* mL ice-cold Inoue buffer. Harvest cells as before, and resuspend in *nv* mL Inoue buffer.
    - *The original protocol uses a 250 mL culture washed in 80 mL buffer and resuspended in 20 mL.*
6. Add DMSO to 7% final (*nv*÷7.53). Mix the bacterial suspension by swirling and then incubate on ice for 10 min.

Transformation

1. When needed, remove a tube of competent cells from the -80° 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 min.
2. Transfer the cells to a sterile plastic culture tube.
3. 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.
    - *15 kb plasmid was shown to have 20% the transformation efficiency as 5–7 kb plasmids.*
4. Incubate 1°C or on ice for 30 min.
5. Heat shock in a 42°C circulating water bath, 90 s. Do not shake the tubes.
    - *The right temperature must be achieved at the correct rate, so results may vary depending on the tube and heat source, if not a circulating water bath.*
6. Rapidly return to 1°C for 1–2 min.
7. Recovery: Add 800 µL SOC medium to each tube. Incubate at growth temperature shaking for 45 min to allow the bacteria to recover and express the selection markers.
8. Spread/streak volume(s) of transformation recovery onto LB or SOB agar containing the appropriate antibiotic(s). Allow liquid to dry prior to incubating plates inverted at growth temperature.

7. Working quickly, dispense aliquots of the suspensions into chilled, sterile microcentrifuge tubes. Immediately flash-freeze the competent cells by immersing the tightly closed tubes in liquid nitrogen. Store the tubes at -80°C until needed.

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1. Inoue, Hiroaki, et al. “High Efficiency Transformation of Escherichia Coli with Plasmids.” *Gene*, vol. 96, no. 1, Jan. 1990, pp. 23–28. doi:10.1016/0378-1119(90)90336-P.
2. Sambrook, Joseph, and David W. Russell. “The Inoue Method for Preparation and Transformation of Competent *E. coli*: ‘Ultra-Competent’ Cells.” *Cold Spring Harbor Protocols*, vol. 2006, no. 1, June 2006, p. 1.112. doi:10.1101/pdb.prot3944.
3. Sambrook, Joseph, and David W. Russell. “The Hanahan Method for Preparation and Transformation of Competent *E. coli*: High-Efficiency Transformation.” *Cold Spring Harbor Protocols*, vol. 2006, no. 1, June 2006, p. 1.105. doi:10.1101/pdb.prot3942.