The Inoue Method for Preparation of Competent *E. coli*

Reference: *Molecular Cloning* 1.112

Pick a single bacterial colony (DH5α) and transfer the colony into 4 ml of LB broth. Incubate the culture overnight at 37°C with vigorous shaking.

Early in the morning, inoculate 125 ml of LB with 0.2, 0.4, 1 or 2 ml of the starter culture. Incubate the culture 24 hr at 18°C with moderate shaking.

The first thing in the following morning is to put a frozen Inoue transformation buffer* (50 ml) on ice in order to thaw it.

When the OD_{600} of the culture reaches 0.55, transfer the Erlenmeyer to an ice-water bath for 10 minutes.

Harvest the cells by centrifugation at 2,500g for 10 minutes at 4°C.

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 walls of the centrifuge bottle or trapped in its neck.

Resuspend the cells gently in 40 ml of ice-cold Inoue transformation buffer.

Harvest the cells by centrifugation at 2,500g for 10 minutes at 4°C.

Remove the supernatant using the procedure described above.

Resuspend the cells gently in 10 ml of ice-cold Inoue transformation buffer.

Add 750 μl of DMSO. Mix the bacterial suspension by swirling and then store it in ice for 10 minutes.

Working quickly, dispense aliquots (50 μl each) of the suspensions into chilled, sterile microfuge tubes (about 200 microfuge tubes are needed). Immediately snap-freeze the competent cells by immersing the tightly closed tubes in a bath of liquid nitrogen. Store the microfuge tubes at –70°C.

* Inoue transformation buffer
PIPES 0.5 M (pH 6.7) (Sigma # P1851, not disodium salt)
PIPES 7.55g in 40 ml of pure H_2O

Adjust the pH to 6.7 with 5 M KOH or HCl.
Add pure H_2O to 50 ml.

Store PIPES in –20 °C.

$MnCl_2.4H_2O$	5.44g
$CaCl_2.2H_2O$	1.10 g
KCl	9.33 g
PIPES (0.5 M, pH 6.7)	10 ml
H_2O	to 500 ml

Filter through a prerinsed 0.45-µm Nalgene filter. Store at –20 °C.