MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0
Revision Date: 09/21/2015

Product name: DH5-α Competent *E. coli*

Cat #: DA-100, DA-196, DA-144A*

Description:

DH5α is the most frequently used *E. coli* strain for routine cloning applications. In addition to supporting blue/white screening, *rec*A1 and *end*A1 mutations in DH5α increase insert stability and improve the quality of plasmid DNA prepared from minipreps.

Genotype:

F^- ϕ80*lac*Z ΔM15Δ (*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *gal*$^-$ *pho*A *sup*E44 λ$^-$ *thi*$^-$1 *gyr*A96 *rel*A1

Transformation Protocol:

A stock pUC19 solution (0.01 µg/ml) is provided as a control to determine the transformation efficiency. To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.

1. Thaw required number of tubes containing 100 µl competent cells on ice.
2. To determine the transformation efficiency, add 5 µl (50 pg) pUC19 control DNA to one tube containing 100 µl competent cells. Gently tap the tube to mix.
3. For DNA from ligation reaction, add 1-5 µl (1 to 10 ng DNA) of the ligation reaction directly to the competent cells. Gently tap the tube to mix.
4. Incubate cells on ice to 15 minutes. Heat-shock cells for 45 seconds in a 42°C water bath; do not shake.
5. Place on ice for 2 minutes.
6. Add 0.9 ml room temperature S.O.C. Medium.
7. Shake at 225 rpm (37°C) for 1 hour.
8. Dilute the reaction containing the control plasmid DNA 1:100 with S.O.C. Medium. Spread 100 µl of this dilution on LB plates with 100 µg/ml ampicillin.
9. Dilute the experimental reactions if necessary and spread 100 to 200 µl of this dilution as described in Step 8.
10. Incubate overnight at 37°C.

5 Minute Transformation Protocol:

1. Thaw a tube of DH5α Competent *E. coli* cells on ice.
2. Add 1-5 µl containing 1 ng-100 ng of plasmid DNA to the tubes containing 100 µl competent cells. Carefully flick the tube 4-5 times to mix cells.
3. Place the mixture on ice for 2 minutes.
4. Heat shock at exactly 42°C for exactly 30 seconds.
5. Place on ice for 2 minutes.
6. Pipette 950 µl of room temperature S.O.C into the mixture. Immediately spread 50-100 µl onto a selection plate and incubate overnight at 37-42°C.