MCLAB
Molecular Cloning Laboratories

User Manual

Version 2.0
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Product name: DH10B Competent *E. coli*

Cat #: DH10-100, DH10-196

Description:

The DH10B chemically competent *E. coli* has a transformation efficiency of 1 x 10^9 cfu/µg for supercoiled DNA pUC19, is suitable for high-efficiency cloning and plasmid propagation. It is stable replication of high-copy number plasmids and has high efficiently transformation for DNA containing methylcytosine & methyladenine (*i.e.* genomic DNA for genomic libraries).

Genotype

F$^-$ *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC) ϕ80d*lac*ZΔM15 *lac*X74 *end*A1 *rec*A1 *ara*D139Δ(*ara*-*leu*)7697 *gal*U *gal*K *rps*L *nup*G λ$^-$

Transformation Protocol

A stock pUC19 solution (0.01 µg/ml) is provided as a control plasmid 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 tube to mix.
3. For DNA experimental, add 1-5 µl to the cells (1 to 10 ng DNA). Gently tap tubes to mix.
4. Incubate the cells on ice to 15 minutes.
5. Heat-shock cells for 45 seconds in a 42°C water bath.
6. Place on ice for 2 minutes.
7. Add 0.9 ml room temperature S.O.C. Medium.
8. Shake at 225 rpm (37°C) for 1 hour.
9. 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.
10. Dilute the experimental reactions if necessary and spread 100 to 200 µl of this dilution as described in Step 9.
11. Incubate overnight at 37°C.

5 Minute Transformation Protocol

1. Thaw a tube of DH10B competent *E. coli* cells on ice.
2. Add 1-5 µl containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA.
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°C.