invitrogen
by Thermo Fisher Scientific

MAX Efficiency® DH10B™ Competent Cells

Cat. No. 18297010

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Component	Amount	Storage
DH10B™ Competent Cells	5 × 200 µL	–70°C (Do not store in liquid nitrogen)
pUC19 DNA (0.01 µg/mL)	100 µL	

Description

MAX Efficiency® DH10B™ Competent Cells are prepared by a patented modification of the procedure of Hanahan (1). The presence of the *mcr*A genotypic marker and the deletion of *mcr*BC and *mrr* makes this strain suitable for cloning DNA that contains methylcytosine and methyladenine (2,3,4). Therefore, genomic DNA, both prokaryotic and eukaryotic, can be cloned efficiently in DH10B™ (2,3,4,5). Plasmid rescue is also more efficiently performed with DH10B™ (6). MAX Efficiency® DH10B™ is resistant to the effects of ligase and ligase buffer and thus can tolerate the addition of small amounts of undiluted ligation reactions (see Note 4).

These cells are suitable for the construction of gene banks or for the generation of cDNA libraries using plasmid- derived vectors. The ϕ80d*lac*ZΔM15 marker provides α-complementation of the ß-galactosidase gene from pUC or similar vectors and therefore can be used for blue/white screening of colonies on bacterial plates containing X-gal or Bluo-gal.

Genotype

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ (*ara*, *leu*)7697 *gal*U *gal*K λ⁻ *rps*L(Str^R) *nup*G

For Research Use Only. Not for use in diagnostic procedures.

Transformation Procedure

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

1. Thaw competent cells on wet ice. Place required number of 17 × 100 mm polypropylene tubes (Fisher Cat. No. 1495911B; see Note 1) on wet ice.
2. Gently mix cells, then prepare 100 µL aliquots of competent cells in chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the –70°C freezer. Do not use liquid nitrogen.
4. To determine transformation efficiency, add 5 µL (50 pg) control DNA to one tube containing 100 µL competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
5. For DNA from ligation reactions (7), dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Add 1–5 µL of the dilution to the cells (1–10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix. For an alternative method of transforming DH10B™ with DNA from ligation reactions, see Note 4.
6. Incubate cells on ice for 30 minutes.
7. Heat-shock cells 45 seconds in a 42°C water bath; do not shake.
8. Place on ice for 2 minutes.
9. Add 0.9 ml of room temperature S.O.C. Medium (Cat. No. 15544034).
10. Shake at 225 rpm (37°C) for 1 hour.
11. Dilute the reaction containing the control plasmid DNA 1:100 with S.O.C. Medium. Spread 100 µL of this dilution on LB or YT plates with 100 µg/mL ampicillin.
12. Dilute experimental reactions as necessary and spread 100–200 µL of this dilution as described in Step 11.
13. Incubate overnight at 37°C.

Growth of Transformants for Plasmid Preparations

MAX Efficiency® DH10B™ Cells transformed with pUC-based plasmids should be grown at 37°C overnight in TB (9). A 100-mL growth in a 500-mL baffled shake flask will yield approximately 1 mg of pUC19 DNA.

Notes

1. Round-bottom 17 × 100 mm polypropylene tubes (e.g., Falcon® 352059 tubes) are required for optimal transformation efficiency. Microcentrifuge tubes (1.5-mL) can be used but the transformation efficiency will be reduced 3- to 10-fold.
2. For best results, each vial of cells should be thawed only once. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately 2-fold.
3. Media other than S.O.C. Medium can be used but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold (8).
4. Transformation efficiencies is about 10-fold lower for ligation of inserts to vectors than for intact control plasmids such as pUC19. Our data indicate that DH10B™ cells can tolerate addition of up to 1 µL (5–50 ng) of an undiluted ligation reaction (standard 20 µL ligation reaction using 1 unit of T4 DNA ligase and 1X ligation buffer) without a significant loss in transformation efficiency (approximately 2-fold). We have observed that the cells begin to saturate with 10–50 ng of DNA (7).
5. Generally, transformation efficiencies will be 10- to 100-fold lower for cDNA than for an intact control plasmid such as pUC19. The amount of cDNA used in a 100 µL transformation should be 1–5 ng in 5 µL or less.

6. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU in control plate}}{\text{pg pUC19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor}$$

For example, if 50 pg pUC19 yields 100 colonies when 100 µL of a 1:100 dilution is plated, then:

$$\text{CFU/}\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ mL}}{0.1 \text{ mL plated}} \times 10^2 = 2 \times 10^9$$

References

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Trademarks

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