MCLAB
Molecular Cloning Laboratories

Manual

Version 2.0

Product name: MC1061 Competent *E. coli*

Cat #: MC1061-100, MC1061-196

Description:

- Chemically competent ***E. coli*** cells with high stability suitable for high efficiency transformation. For research use only.
- Parent of DH10B/TOP10 and derived strains.

Application:

- For cloning, sub-cloning and phage display, but cannot be used for reinfection.
- Transform efficiency is >1.0x10^8 cfu/μg with pUC19 control DNA.

Genotype:

F$^-$ Δ(*ara-leu*)*7697* [*araD139*]$_{B/r}$ Δ(*codB-lacI*)3 *galK16 galE15* λ$^-$ e14$^-$ *mcrA0 relA1 rpsL150*(Str^R) *spoT1 mcrB1 hsdR2*(r$^-$m$^+$)

Recommended storage condition:

This product should be stored at -80°C. Thaw on ice only before use. Do not re-freeze.

Protocol:

A stock pUC19 DNA solution (10 pg/μl) 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. Remove competent cells from -80°C and thaw competent cells on ice. Place required number of autoclaved 1.5 ml microcentrifuge tubes on ice.
2. Gently mix cells, then aliquot 50-100 μl of competent cells into chilled microcentrifuge tubes.
3. To determine transformation efficiency adds 5 μl (500 pg) control pUC19 plasmid DNA to one tube containing 50-100 μl competent cells.
4. For DNA from ligation reaction, add 1-3 μl of the ligation reaction directly to the competent cells. Gently tap the tube to mix.
5. Incubate the cells on ice for 15 minutes.
6. Heat-shock the cells for 45 seconds in a 42°C water bath.
7. Place on ice for 2 minutes.
8. Add 0.9-0.95 ml room temperature S.O.C Medium.
9. Shake at 225 rpm for 0.5-1 hour at 37°C.
10. Dilute the reaction containing the control pUC19 plasmid DNA 1:100 with S.O.C medium. Spread 100 μl of the diluted on LB plates with 100 μg/ml ampicillin.
11. Dilute experimental reactions as necessary and spread 100-200 μl of this dilution as described in Step 11.
12. Incubate overnight at 37°C.

Notes:

1. MC1061 Competent Cells may be refrozen. Subsequent freeze-thaw cycles will reduce transformation efficiency by approximately 2-fold.
2. Do not use EDTA to stop ligation reactions. Instead, freeze ligations to store for later use. The

combination of DNA, ligase, and ligase buffer can inhibit transformation. Optimal results are obtained with T4 DNA Ligase and 5X ligase reaction buffer. If desired, ligation can be concentrated by ethanol precipitation prior to transformation. Dissolve dry pellet in filter sterilized TE buffer. tRNA can be used as a carrier.