MCLAB
Molecular Cloning Laboratories

User Manual

Version 2.1

Product name: JM109 Competent *E. coli*

Cat #: JM-100, JM-196

Description:

High stability, high efficiency, no heat shock necessary. For Research Use Only.

Application:

- Ideal for sub-cloning, single strained DNA, high quality plasmid preparation, and library construction
- JM109 is a K strain bacterium that provides minimized recombination and aids in plasmid stability which results in high quality plasmid DNA preparation. The strain carries the hsdR17 genotype, which prevents cleavage of heter-ologous DNA by an endogenous endonuclease. JM109 strain supports Alpha-Comple-mentation for blue/white screening for recombinant plasmids. The presence of the F′ factor also allows growth of bacterio-phage M13 vectors for rescue of single stranded DNA. Transform efficiency is >1.0 x 10^9 cfu/µg with pUC18 control DNA.

Recommended storage condition:

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

Recommended reaction conditions:

- Mix DNA with competent cell
- Let stand on bench for 5 minutes
- Direct plate

Genotype:

F′ (traD36, proAB+, lacIq, lacZ-M15),endA1, recA1, hsdR17, (rk-, mk+), mcrA, supE44, e-gyrA96, relA1, -(lac-proAB)

Protocol:

1. Remove the competent cells from -80°C and place directly in ice. Thaw the cells for 5 to 10 min.
2. Gently mix cells by tapping tube.
3. Add 1-50 ng of DNA [or 1 µL control DNA] into 50 µL competent cells. Swirl the pipettor tip through the cells while dispensing DNA. Gently tap tube to mix.
4. Place the tubes on ice for 30 min.
5. Heat-shock the cells for 45 sec. in a 42°C water bath. Do not shake.
6. Add 450 µL of room temperature SOC medium to each transformation reaction.
7. Incubate at 37°C for one hour, with shaking (225 to 250 rpm).
8. Spread on LB agar plates containing appropriate antibiotic (e.g., 100 µg/mL ampicillin for control pUC19).
9. Incubate the plates at 37°C overnight (12 to 16 hours).