JM110 Competent Cells

Cat: C1460
Size: 20×100μL
Storage: Store at -70℃ to avoid repeated freezing and thawing. 6 months. Not suitable for storage in liquid nitrogen.

Introduction:

JM110 competent cells produced by our company are the competent cells obtained by special processing of Escherichia coli JM110 strain, which can be used for chemical transformation of DNA. For strains belonging to dam- and dcm-, the influence of dam, dcm methylation was excluded. The conversion efficiency of pUC19 plasmid was up to 10^6 cfu/μg. After the transformation of JM110 by pEGFP-N1, the XbaI site was demethylated. The extracted plasmid could be incised by XbaI enzyme, and the linearized plasmid size was 4733bp.

Genotype: *rpsL (Str^r) thrleuthi - 1 lacygalkgaltaratonatsxdamdcmsupe44 Δ (lac - proAB)/F ′ [traD36proABlacI^{q}Z Δ M15]*

Protocols: (The following operations are carried out according to the standard of sterile conditions)

1. Take the competent cells and place them in an ice bath. If necessary, the freshly melted cell suspension can be divided into a sterile pre-cooled centrifuge tube and placed in the ice bath.
2. Add the target DNA to the receptive cell suspension, gently rotate the centrifuge tube to mix the contents, and let it rest in the ice bath for 30min.
3. Place the centrifuge tube in a 42℃ water bath for 60s, then quickly transfer the tube to the ice bath and allow the cells to cool for 2min without shaking the centrifuge tube.
4. 500μL sterile SOC or LB medium(without antibiotics) was added to each centrifuge tube, mixed and placed at 150rpm at 37℃ for shaking culture for 60min. The purpose was to express related resistance marker genes on the plasmid and resuscitate the bacteria.
5. Under aseptic conditions, appropriate amount of bacterial solution was added to LB solid medium plate containing corresponding antibiotics, and the cells were evenly coated with sterile bacterial coater or glass beads. After the liquid in the plate was completely absorbed, the plate was inverted and cultured at 37℃ for 12-16h.
6. Keep the remaining bacterial solution in the refrigerator at 4℃, and decide whether to stay or leave according to the growth of bacterial colonies on the plate.

Notes:

1. The competent cells should be kept at -70℃, and cannot be frozen or thawed repeatedly, otherwise its conversion efficiency will be reduced.

2. During the experiment, aseptic operation should be strictly carried out to prevent contamination of other DNA or miscellaneous bacteria, so as to avoid affecting future screening and identification.
3. During the conversion, the conversion efficiency is proportional to the concentration of foreign DNA within a certain range, but when the amount of foreign DNA added is too large or too large, the conversion efficiency will be reduced. The volume of DNA during transformation should be less than one-tenth of the volume of competent cells.
4. Calculation of conversion rate: Conversion rate = total number of colonies produced/total amount of paving DNA.
5. In case the conversion experiment is unsuccessful, part of the junction product can be retained for re-conversion to minimize the loss.

Related Products:

I1020 IPTG solution(50mg/mL)
A1170 Ampicillin storage Solution(100mg/mL)
K1030 Kanamycin(100mg/mL)
L1015 LB solid medium(dry powder)
L1020 SOC Liquid medium(dry powder)
X1010 X-gal(20mg/mL)