DH10Bac Competent Cells

Cat: C1480
Size: 10×100μL/ 20×100μL
Storage: Store at -70°C to avoid repeated freeze-thaw. 6 months. Not suitable for storage in liquid nitrogen.

Introduction:

Escherichia coli DH10Bac competent cells, suitable for homologous recombination strains in the Bac-to-Bac system of insect baculovirus, used to produce recombinant Bacmid. DH10Bac cells contain the parent Bacmid bMON14272 and helper plasmid pMON7124. The parental Bacmid contains a mini-F replicator, kanamycin resistance gene, att Tn7 locus, and lac Zα-complementary factor. Helper plasmids contain tetracycline resistance and the tns ABCD region, which provides the transposition protein required for mini-Tn7 to be inserted from the donor plasmid into the parental Bacmid target site. Donor plasmids such as pFastBac series (pFastBac1, pFastBacDual, etc.) contain Tn7R and Tn7L homologous recombination arms, and between Tn7R and Tn7L contain gentamicin resistance genes, insect virus polyhedrosomal gene promoters, polyclonal sites and SV40 virus PolyA tail signal. When the recombinant plasmid containing the target gene pFastBac is transformed into DH10Bac cells, recombinant Bacmid can be produced after the recombination, and the extracted and purified recombinant Bacmid can be transfected into insect cells Sf9 or Sf21 to package and produce insect virus. In addition, The product of the φ80dlacZΔM15 gene in DH10Bac cells can achieve α-complementing of β-galactosidase, which can be used for blue-white spot screening of recombinant Bacmid. Escherichia coli DH10Bac receptive cells were prepared by a special process and detected by pUC19 plasmid. The conversion efficiency was up to 10^7 cfu/μg.

Genotype: *F - MCRA Δ (MRR - hsdRMS - McRBC) phi 80 lacz Δ M15 Δ lacX74recA1endA1araD139 Δ (ara, leu) 7697 galugalk lambda - rpsLnupG/pMON14272 / pMON7124*

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 1-10ng recombinant plasmid to the competent cell suspension, gently rotate the centrifuge tube to mix the contents, and stand in the ice bath for 30min.
3. Place the centrifuge tube in a 42°C water bath for 90s, then quickly transfer the tube to the ice bath to cool the cells for 2 minutes without shaking the centrifuge tube during this process.
4. Add 900μL sterile SOC (without antibiotics) to each centrifuge tube, mix well, place at 37°C 200rpm, shake in a shaking table for 4h.
5. The SOC medium was diluted by 10 times gradient, such as 3 dilution gradients $10,^{-1}$ $10,^{-2}$ $10.^{-3}$

6. Take 100μL of each gradient culture for coating. After the liquid in the plate was completely absorbed, the plate was inverted and cultured at 37℃ for 24-48h.
7. 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 should not be frozen or thawed repeatedly, otherwise their 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 influence on 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)