Stbl4 Competent Cells

Cat: C1570

Size: 20×100μL

Storage: Store at -70℃ for 6 months to avoid repeated freezing and thawing. Not suitable for storage in liquid nitrogen.

Introduction:

Stbl4 strain is derived from Stbl2 strain (Stbl2 is a JM109-derived strain), which is used to clone unstable sequences(such as repeat sequences, retroviral sequences, etc.) and methylated DNA sequences, and is especially suitable for constructing recombinant retroviral or lentiviral plasmids. It can be used for the construction and amplification of large plasmids, and is suitable for constructing and augmenting plasmid cDNA libraries. Different from Stbl2 strain, Stbl4 strain can be screened for blue and white spots by α-complementary principle in the presence of IPTG and X-gal. The competent cells were prepared by special technology, and the conversion efficiency of pUC19 plasmid was greater than 10^8 cfu/μg.

Genotype: *mcrAΔ (mcrBC-hsdRMS-mrr)recA1endA1gyrA96gal-thi-1supE44 lambda-Rela1δ (lac-proAB)/F 'Proab + lacIqZΔM15 Tn10 (Tet^R)*

Strain Resistance: Sensitive to ampicillin, kanamycin, magnificycin; Resistant to tetracycline.

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

1. Put the competent cells in an ice bath to melt. After the cells are just melted, add plasmid DNA or 5-10μL of the connection product to the cells, dial the bottom of the tube with your finger, and gently mix;
2. Place in ice water bath for 15-30min, do not shake;
3. Heat at 42℃ for 60s, do not shake;
4. Place in ice bath for 2min, do not shake;
5. Add 500μL sterile SOC or LB medium;
6. placed in a shaking table at 37℃, 150-200rpm shock resuscitation culture for 60min;
7. Take 50-100μL bacterial solution and spread it on LB plate containing resistance. After the liquid was drained, the plate was turned upside down and cultured at 37℃ for 12-16h.

(When cloning unstable fragments, in order to reduce the recombination error rate, it is best to use 30℃ culture conditions for resuscitation culture and coating culture, and it takes about 24h for plate culture at 30℃.)

(Plate scribing separation method: After the resuscitation culture, centrifuge at 12000rpm for 30s, discard the supernatant, leave about 100μL of liquid, gently blow the bacterial mass with 200μL suction head, take 10μL of the suspended bacterial liquid into more drops on the resistant LB plate, tilt the suction head, and use the side of the suction head to line the liquid dripping on the plate back

and forth. Overnight culture at 37°C. This method results in more and larger monoclonal colonies.)

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 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)