GV3101 Agrobacterium Competent Cells

Cat: C3620
Size: 10×100μL/20×100μL
Storage: Store at -70°C to avoid repeated freezing and thawing.

Genotype: *C58 (rif^R) Ti pMP90 (pTiC58DT-DNA) (gent^R /strep^R) Nopaline*

Introduction:

GV3101 strain is C58 type background, nuclear gene contains screening tag - Rifampicin resistance gene Rif, in order to facilitate the transformation operation, this strain carries carmine type Ti plasmid pMP90(pTiC58DT-DNA) without its own transport function, This plasmid contains the vir gene(vir gene is the necessary element for T-DNA insertion into the plant genome, the T-DNA transfer function of the pMP90(pTiC58DT-DNA) plasmid itself is damaged, but it can help the transfer of the transferred binary vector T-DNA smoothly). The pMP90(pTiC58DT-DNA) type Ti plasmid contains a screening tag: Strep and Gent confer resistance to streptin and gentamicin in GV3101 strain, which is suitable for transgenic plants such as Arabidax, tobacco, corn, potato, etc. The conversion efficiency can reach 10^3 cfu/μg by plant double pCAMBIA2301 plasmid detection, and the conversion efficiency does not change after 12 months of storage at -70°C.

Protocols(Using freeze-thaw method):

1. GV3101 Agrobacterium competent cells stored at -70°C were melted in ice water bath;
2. Under sterile conditions, 100ng-1μg plasmid DNA was added to the competent cells(it is best to do pre-experiment to determine the optimal amount of plasmid added before the first use), gently mixed, and left for 5 minutes in the ice water bath;
3. The centrifuge tube was quickly frozen in liquid nitrogen for 5min; (**Note:** Dry ice and anhydrous ethanol mixture can also be used instead of liquid nitrogen)
4. Then quickly place the centrifuge tube in a 37°C water bath for 5min without shaking the water;
5. Put the centrifuge tube back into the ice water bath for 5min;
6. Add 800μL of 2×YT or LB liquid medium without antibiotics under aseismic conditions and cultured at 28°C for 2-3h, and the bacteria recovered.
7. Centrifuge at 6000rpm for 1min to collect bacteria, leave about 100μL supernatant, gently blow the suspensory bacteria, take appropriate amount of bacteria solution, smear on the LB plate of corresponding antibiotics, and invert culture in the incubator at 28°C for 48-72h.

(When the plate only contains 50μg/mL Kan, it can be cultured at 28°C for 48h; When 50μg/mL Kan and 20μg/mL Rif were added to the plate at the same time, it needed to be cultured at 28°C for 60h; If the plate containing 50μg/mL Rif is used, it needs to be cultured at 28°C for 72-90h).

Notes:

1. The volume when adding the plasmid should not be greater than 1/10 of the volume of the receptive state; The conversion efficiency decreased sharply due to the impurity of the plasmid or the presence of organic matter pollution such as ethanol; When the plasmid is doubled, the conversion efficiency decreases by an order of magnitude.
2. The mixing of plasmids should be done gently, and the conversion of high concentrations of plasmids can correspondingly reduce the amount of bacteria ultimately used to coat the plate.
3. When the density of positive clones on the plate is too high, the growth of positive clones will slow down due to insufficient nutrition, and the colony will become smaller. In order to obtain a large colony, the amount of plasmids should be reduced.
4. Rifampicin concentration should not be higher than 25μg/mL, too high rifampicin concentration is not conducive to the growth of Agrobacterium, will reduce its growth rate and conversion efficiency.
5. The purpose of adding rifampicin to the medium is to prevent the growth of miscellaneous bacteria and screen agrobacterium; Adding Ti plasmid to screen antibiotics according to the resistance of the strains used can prevent the loss of Ti plasmid, but Ti plasmid screening of antibiotics is not conducive to the transgenic operation of Agrobacterium, so these antibiotics are generally not considered when cultivating Agrobacterium, and the probability of Ti plasmid loss is extremely low (can be ignored).
6. If the biochemical reagents produced by our company are not specially marked, they are basically non-aseptic packaging. If used in cell experiments, please pre-treat them in advance.
7. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
8. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
9. This product is for scientific research only. Do not use for medicine, clinical diagnosis or therapy, food or cosmetics. Do not store in ordinary residential areas.
10. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.