GV3101 Agrobacterium Electrocompetent Cells

Cat: C3660
Size: 10×50μL/20×50μL
Storage: Store at -70°C to avoid repeated freezing and thawing.

Product Parameters:
English name: GV3101 Electrocompetent cells

Genotype: *C58 (rif^R) Ti pMP90 (pTiC58DT-DNA) (Str^R /Gent^R), Nopaline typee*

Product Description:

GV3101 strain is Agrobacterium C58 type background, nuclear gene contains favorable fampicin resistance gene Rif. The strain also carries a disarmed Ti plasmid, which has a loss of self-transport function, in its plasmid. The GV3101 strain contains pMP90(pTiC58DT-DNA), a plasmid containing the vir gene, which is necessary for TDNA insertion into the plant genome. The T-DNA transfer function of the pMP90 plasmid was damaged. But AIDS in the transfer of T-DNA from the plant's binary expression vector). The pMP90(pTiC58DT-DNA) plasmid also contains Str and Gent resistance genes, conferring resistance to streptomycin and gentamicin in the GV3101 strain. Agrobacterium GV3101 is suitable for transgenic manipulation of plants such as Arabidopsis, tobacco, corn and potatoes. The GV3101 electrocompetent state is especially suitable for the transformation of large plasmids, and the transformation efficiency of pCAMBIA2301 plasmid is greater than 10^5 cfu/μg DNA.

Protocols:

1. Insert the electric cup with the electrode spacing of 0.1cm into the broken ice, compact the ice, and leave it in the ice for 5min to fully cool the electric cup. (Reuse method of electric cup: After each use, rinse it with plenty of tap water to remove bacterial liquid and DNA, wash it with distilled water 3 times, soak it in 75% ethanol for 30min, take out the cup, drain the liquid, put it in a super clean table to make the ethanol fully volatilize, cover it and put it in a dry place for use.)
2. Take -70°C stored agrobacterium sensibility state inserted into the ice for 5 minutes, to be melted, add 10ng-1μg plasmid DNA(elution or dissolved plasmid solution ions can not be too high, can be diluted with double steam water: It is best to do a pre-test to determine the optimal amount of added plasmids before the first use), gently mix the mixture with your finger at the bottom of the tube, and immediately insert it into the ice. Quickly transfer the mixture of the acceptor state and plasmid into the electric shock cup in a super-clean table with a sterile suction head, cover the cup, and keep the empty tube for use.
3. Start the electrocuter and set the shock parameters: C=25μF, PC=200ohm, V=2.4KV(Set the appropriate shock parameters for Agrobacterium according to different electrocuter). Wipe off the water on the outside of the cup with a paper towel, and quickly put the cup into the tank for

electric shock. After the shock was completed, the cup was quickly inserted into the ice, 700μL of antibiotic-free LB was added and transferred to the original retained competent empty tube at 28℃, 150-200rpm, and oscillated for 2-3h.

4. Centrifuge at 6000rpm for 1min to collect bacteria, keep about 200μL supernant to gently blow the heavy suspension bacteria block, take 100μL bacterial solution and smear it on LB or YEB plate containing corresponding antibiotics, and put it upside down in an incubator at 28℃ for 2-3 days(when the plate only contains 50μg/mL kan, it can be cultured at 28℃ for 48h; When 50μg/mL Kan and 20μg/mL Rif were added to the plate at the same time, it was required to be cultured at 28℃ for 60h; If the plate containing 50μg/mL Rif is used, it needs to be cultured at 28℃ for 72-90h).

Notes:

1. The volume of the added plasmid should not be greater than 1/10 of the volume of the competent state, and the conversion efficiency will be sharply reduced if the plasmid is impure or very large.
2. When there are too many colonies on the plate, the colonies are very small. To get a large colony, reduce the amount of plasmids or reduce the amount of coating, or transfer the colony to a new plate for growth.
3. The working concentration of rifampicin used should not be higher than 25μg/mL, too high a concentration of rifampicin will reduce the growth rate and conversion efficiency.
4. Rifampicin can prevent the growth of miscellaneous bacteria and screen for Agrobacterium; Adding streptomycin or gentamicin according to the resistance of the strain used can prevent the loss of Ti plasmid, but streptomycin is not conducive to the transgenic operation of agrobacterium, so the addition of streptomycin or gentamicin can not be considered in the general culture of agrobacterium, and the probability of Ti plasmid loss is extremely low (negligible).
5. 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.
6. Once it is prepared into a solution, please pack it separately and store it to avoid product failure caused by repeated freezing and thawing.
7. The product information is for reference only, if you have any questions, please call 400-968-6088 for consultation.
8. 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.
9. For your safety and health, please wear a lab coat and wear disposable gloves and a mask.