Cat. # 9115

For Research Use

TaKaRa

Agrobacterium tumefaciens LBA4404 Electro-Cells

Product Manual

v202003Da

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I. Description

Agrobacterium tumefaciens (*Rhizobium radiobactor*) can transfer T-DNA (transfer DNA) which is part of its own Ti plasmid into host plant cells, and insert this DNA into the plant chromosomal DNA. The inserted genes on T-DNA are expressed, then the cells are transformed into tumor cells called Crown gall. By utilizing this gene-transfer mechanism, the binary vector method was invented for plant transformation[1)]. In this system, the pathogenic genes of T-DNA in Ti plasmid are replaced with selective marker genes and the exogenous target gene, to transfer the target gene into plant chromosomal DNA by means of *Agrobacterium*-mediated gene transfer.
Agrobacterium tumefaciens strain LBA4404 and the binary vector method was invented by Dr. P. J. J. Hooykaas at Leiden University in the Netherlands.
Agrobacterium tumefaciens has pAL4404 plasmid, which only contains the T-DNA *vir* region, which contains genes responsible for gene induction and transfer of T-DNA, and is a widely used strain for plant transformation.
This product contains competent cells for transformation by electroporation[3)]. Using *A. tumefaciens* and the binary vector method, you can transform various plants for infection (transfection) experiments.

II. Components

Agrobacterium tumefaciens LBA4404 Electro-Cells	40 μl x 5
pRI 900 DNA ＊1 (1 ng/μl)	10 μl
SOC Medium ＊2	1 ml x 10

＊1 pRI 900 DNA : pRI 910 DNA (Cat. #3261) without a multicloning site.

＊2 SOC Medium :

2%	Tryptone
0.5%	Yeast extract
10 mM	NaCl
2.5 mM	KCl
10 mM	$MgSO_4$
10 mM	$MgCl_2$
20 mM	Glucose

III. Storage -80℃

Note : Store at -80℃. If it is not stored at -80℃, transformation efficiency may decrease. In this case, we recommend confirming the transformation efficiency with the supplied pRI 900 DNA prior to using it in an application. Do not preserve with liquid nitrogen.

IV. Protocol

Example of transformation protocol (For Bio-Rad Gene Pulser II and cuvette).

1. Thaw tubes of *Agrobacterium tumefaciens* LBA4404 Electro-Cells on ice.
2. Add 1 μl (1 ng) of binary vector DNA to 20 μl of the electro-cells in a 1.5 ml tube on ice, and mix gently.
   ＊ If you store remaining electro-cells, freeze quickly them with dry ice/ethanol or in dry ice, then store at -80℃. The transformation efficiency will decrease.
3. Chill a 0.1 cm electroporation cuvette (Bio-Rad) on ice.
4. Set the Gene Pulser II to 25 μF, 200 Ω, and 2 - 2.5 kV.＊1
5. Transfer the electro-cells and DNA prepared in step 2 into electroporation cuvettes, and tap to collect the mixture at the bottom. Put the cuvette in the Gene Pulser II, and pulse.
6. Take cuvette out, add 1ml of SOC Medium＊2 and transfer to a 14 ml round bottom tube (Falcon tubes, etc).
7. Incubate for 1 hour at 30℃, shaking at 100 rpm. Plate 50 - 100 μl of cells*3 on LB agar plates with 50 μg/ml kanamycin*4 and 100 μg/ml streptomycin. Incubate for 48 hours at 30℃.

＊1 Conditions depend on the cuvette type, and electroporator system. Set to 2.5 kV when using MicroPulser.
＊2 You can use different medium, but transformation efficiency might decrease.
＊3 We recommend using original and 10-fold diluted suspension with SOC Medium for seeding.
＊4 When using a binary vector plasmid that does not have kanamycin resistance, use an the appropriate antibiotic.

V. Quality

According to IV. Protocol, the transformation efficiency was tested with 1 ng of pRI 900 DNA, and the colonies were selected in plates containing kanamycin and streptomycin. We obtained the efficiency > 5 x 10^6 colonies/μg·pRI 900 DNA.

VI. References

1) A Hoekema, P R Hirsch, P J J Hooykaas, and R A Schilperoort. *Nature*. (1983) **303**: 179-180.
2) G Ooms, P J J Hooykaas, R J M V Veen, P V Beelen, T J G Regensburg-Tuink, R A Schilperoort. *Plasmid*. (1982) **7**: 15-29.
3) S Wen-jun and B G Forde. *Nucleic Acids Research*. (1989)**17** (20): 8385.

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VII. Related Products

pRI 909 DNA (Cat. #3260)
pRI 910 DNA (Cat. #3261)
pRI 101-AN DNA (Cat. #3262)
pRI 101-ON DNA (Cat. #3263)
pRI 201-AN DNA (Cat. #3264)
pRI 201-ON DNA (Cat. #3265)
pRI 201-AN-GUS DNA (Cat. #3266)*
pRI 201-ON-GUS DNA (Cat. #3267)*

* Not available in all geographic locations. Check for availability in your area.