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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 plan 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 (genes responsible for *vir* gene induction and T-DNA transfer), 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. Kit Contents

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

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

＊2 : SOC media :

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 the storage temperature is not maintained evenly, the efficiency of transformation will decrease. You may determine if such a problem will arise by testing the efficiency of transformation of stored cells by using the included pRI 900 DNA.
Do not preserve with liquid nitrogen.

IV. Protocol

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

1. Thaw tubes of *Agrobacterium tumefaciens* LBA4404 Electro-Cells on ice.
2. Add 1 μl (1 ng) of binary vector plasmid DNA to 20 μl of competent cells on ice in a 1.5 ml tube, and mix gently.
   * If you store left over competent cells, flash freeze 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 competent 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 media*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 on LB agar plates with 50 μg/ml kanamycin*3 and 100 μg/ml streptomycin.*4 Incubate for up to 48 hours at 30℃.

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

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., (1983) *Nature*, **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., (1982) *Plasmid*, **7**, 15-29.
3) S. Wen-jun and B. G. Forde (1989) *Nucleic Acids Research*, **17** (20), 8385

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)

Phone : +81-77-543-7247 Fax : +81-77-543-9254

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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