

## LBA4404 *Agrobacterium* ElectroCompetent Cells Transformation Protocol

### Introduction

LBA4404 *Agrobacterium* ElectroCompetent cells are optimized for the highest transformation efficiencies. The cells are ideal for applications requiring high transformation efficiencies, such as cDNA and gDNA library construction. The LBA4404 strain is useful for developing transgenic plants from tomatoes, tobacco and other plants.

This strain contains a rifampicin resistance gene (*rif*) and streptomycin resistance. In addition, LB4404 strain contains an octoprine-type Ti plasmid pAL4404 without self-transport function, which contains the *vir* gene.

### Materials

- LBA4404 *Agrobacterium* ElectroCompetent cells (GoldBio Catalog # [CC-220](#))
- pCAMBIA1391z Control DNA, 500 pg/μl
- *Agrobacterium* Recovery Medium
- Kanamycin (GoldBio Catalog # [K-120](#))
- Rifampicin (GoldBio Catalog # [R-120](#))
- Yeast Extract Tryptone (YT) Agar selection plates
- Sterile electroporation cuvettes
- Microcentrifuge tubes
- Electroporator
- Shaker incubator

### Storage and Handling

- This product is shipped on dry ice. LBA4404 *Agrobacterium* Electrocompetent Cells should be stored at -80°C, pCAMBIA1391z Control DNA should be stored at -20°C and recovery medium should be stored at 4°C immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Do not mix cells by pipetting or vortexing.
- Thaw LBA4404 *Agrobacterium* Electrocompetent Cells and pCAMBIA1391z Control DNA on ice and mix by gentle tapping. After thawing, these products should be kept on ice before use. Transform cells immediately after thawing.

**Note:** Transformation efficiency is tested by using the pCAMBIA1391z control DNA supplied with the kit and using the protocol given below. Transformation efficiency should be  $\geq 1 \times 10^7$  cfu/ $\mu$ g pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

**Note:** A high-voltage electroporation apparatus, capable of generating field strengths of 16 kV/cm, is required.

**Note:** Reagents needed for one reaction: 25  $\mu$ l of LBA4404 electrocompetent cells, 1  $\mu$ l of pCAMBIA1391z (500  $\mu$ g/ $\mu$ l) and recovery medium.

## Method

### Transformation Protocol

Use this procedure to transform LBA4404 *Agrobacterium* ElectroCompetent cells. Do not use these cells for chemical transformation.

1. Place sterile cuvettes and microcentrifuge tubes on ice.
2. Remove competent cells from the -80°C freezer and thaw completely on wet ice (10-15 minutes).
3. Aliquot 1  $\mu$ l (10  $\mu$ g -1  $\mu$ g) of DNA into the chilled microcentrifuge tubes on ice.
4. When the cells are thawed, add 25  $\mu$ l of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pCAMBIA1391z control, add 1  $\mu$ l of (500  $\mu$ g/ $\mu$ l) DNA to the 25  $\mu$ l of cells on ice. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
5. Pipette 26  $\mu$ l of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well and then electroporate.

**Note:** Use Electroporation settings below:

Mode Exponential protocol

Voltage (V): 1,800 V

Capacitance 25  $\mu$ FD

Resistance 200 Ohms

Cuvette 1 mm.

6. Immediately add 976  $\mu$ l of *Agrobacterium* Recovery Medium or any other medium of choice to the cuvette, pipette up and down three times to re-suspend the cells. Transfer the cells and Recovery Medium to a tube.
7. Incubate tubes at 30°C for 3 hours at 200 RPM.
8. Dilute the cells as appropriate then spread 20-200  $\mu$ l cells onto a pre-warmed selective plate. For the pCAMBIA1391z control, plate 50  $\mu$ l of diluted transformants onto a YT plate containing 15  $\mu$ g/ml rifampicin and 50  $\mu$ g/ml kanamycin. Use sterilized spreader or autoclaved plating beads to spread evenly.
9. Incubate the plates for 2-3 days at 30°C.

Table 1. Antibiotic Disc Sensitivity for GoldBio's *Agrobacterium* Strains (using standard BD antibiotic discs)

Competent cells	Antibiotic Selection									
	Amp 100 $\mu$ g/ml	Carb 100 $\mu$ g/ml	Chlor 30 $\mu$ g/ml	Chlor 100 $\mu$ g/ml	Gent 30 $\mu$ g/ml	Kan 50 $\mu$ g/ml	Rif 25 $\mu$ g/ml	Spec 50 $\mu$ g/ml	Strep 50 $\mu$ g/ml	Tet 50 $\mu$ g/ml
GV3101	I	R	R	PR	R	S	R	S	R	S
EHA 105	R	R/S	R	N/A	R/S	S	R	S	R	S
LBA 4404	S	S	S	N/A	S	S	R	S	R	S
AGL-1	R	R	R	N/A	R	S	R	S	R	S
C58C1	R	R	R	N/A	R	S	R	S	R	S

S = Sensitive

R = Resistant

R/S= intermediate zones using standard discs.

I= growth in inhibitory zone with standard disc. "Opaque", not clear zone of inhibition.

### Calculation of Transformation Efficiency

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1 $\mu$ g of plasmid into a given volume of competent cells.

$$TE = \text{Colonies}/\mu\text{g}/\text{Plated}$$

Colonies = the number of colonies counted

$\mu$ g = amount of DNA transformed in  $\mu$ g

Dilution = total dilution of the DNA before plating

*Example:*

*Transform 1  $\mu$ l of (500 pg/ $\mu$ l) pCAMBIA1391z control plasmid into 25  $\mu$ l of cells, add 974  $\mu$ l of Recovery Medium.*

*Recover for 3 hours and plate 100  $\mu$ l. Count the colonies on the plate in two days.*

*If you count 500 colonies, the TE is calculated as follows:*

*Colonies = 500*

*$\mu$ g of DNA = 0.0005*

*Dilution = 100/1000 = 0.1*

*TE = 500/.0005/.1 =  $1 \times 10^7$*

## Associated Products

- GV3101 *Agrobacterium* Electrocompetent Cells (GoldBio Catalog # [CC-207](#))
- AGL-1 *Agrobacterium* Electrocompetent Cells (GoldBio Catalog # [CC-208](#))
- LBA4404 *Agrobacterium* ElectroCompetent Cells (GoldBio Catalog # [CC-220](#))
- C58C1 *Agrobacterium* ElectroCompetent Cells (GoldBio Catalog # [CC-240](#))
- EHA 105 *Agrobacterium* Electrocompetent Cells (GoldBio Catalog # [CC-225](#))
- Kanamycin (GoldBio Catalog # [K-120](#))
- Rifampicin (GoldBio Catalog # [R-120](#))