Protocol

TD-P Revision 7.3

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GV3101 *Agrobacterium* Electrocompetent Cells
Transformation Protocol

Introduction

GoldBio's GV3101 Agrobacterium Electrocompetent Cells allow you to obtain high transformation efficiency in applications such as gDNA or cDNA library construction. Our GV3101 strain harbors the C58 chromosomal backbone containing rifampicin resistance and the Ti plasmid pmp90 (pTiC58DT-DNA) harboring the gentamicin resistance.

A functional T-DNA binary system can be built using our GV3101 strains as the T-DNA region has been deleted from the Ti plasmid and instead has a binary vector containing the missing T-region. The binary system makes possible to transfer genetic material into a host plant's genome. Our system is often used for Agrobacterium-mediated transformation in mono and dicotyledonous species such as Arabidopsis thaliana, tobacco, potato, soybeans and corn. Here, we present a detailed protocol for electroporation using GV3101 *Agrobacterium* Electrocompetent Cells.

Materials

- GV3101 *Agrobacterium* Electrocompetent Cells (GoldBio Catalog # CC-207)
- pCAMBIA1391z Control DNA, 500 pg/µl
- *Agrobacterium* Recovery Medium
- Gentamicin sulfate (GoldBio Catalog # G-400)
- Kanamycin (GoldBio Catalog # K-120)
- Yeast Extract Tryptone (YT) or LB Agar selection plates.
- Sterile electroporation cuvettes
- Microcentrifuge tubes
- Electroporator
- Shaker incubator

Storage and Handling

- This product may be shipped on dry ice. GV3101 *Agrobacterium* Electrocompetent Cells should be stored at -80°C, pCAMBIA1391z Control DNA, 500 pg/µl, 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.

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- Thaw GV3101 *Agrobacterium* Electrocompetent Cells and pCAMBIA1391z Control DNA on ice and mix by gently tapping the tube. After thawing, these products should be kept on ice before use. These products can be refrozen for storage, but the transformation efficiency may decrease.

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 ≥2 x 10^8 cfu/µg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

Method

Transformation protocol

Use this procedure to transform GV3101 *Agrobacterium* Electrocompetent Cells. Do not use these cells for chemical transformation.

Note: Handle the competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.

Note: Thaw competent cells on ice and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.

1. Place sterile cuvettes and microcentrifuge tubes on ice.

2. Remove competent cells from the -80°C freezer and thaw completely on ice (10-15 minutes).

3. Aliquot 1 µl (10 pg-1 ng) of DNA to the chilled microcentrifuge tubes on ice.

Note: DNA amounts above 1 ng will give decreasing transformation efficiencies.

4. When the cells are thawed, add 25 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pCAMBIA1391z control, add 1 µl of (500 pg/µl) DNA to 25 µl 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 µl of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. For electroporation settings, check the manufacturer's handbooks. Below is an example of electroporation settings for *Agrobacterium*.

Example: Electroporation settings for BTX electroporator

a) **Resistance only. Capacitance off.**
b) **Capacitance timing is therefore off.**
c) **Resistance timing R5 which equals 129.**
d) **Voltage: set at 1.66 to 1.90 kV**

e) **milliseconds should be about 5 msec after pulse**
f) **Cuvette – 1 mm**

6. Immediately add 976 µl of Recovery Media to the cuvette, gently pipette up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.

7. Incubate at 30°C for 3 hours at 200 rpm in a shaker incubator.

8. Dilute the cells as appropriate, then spread 2 to 200 µl cells onto a pre-warmed selective plate. For the pCAMBIA1391z control, plate 50 µl of the diluted transformants onto a YT or LB plate containing 5 µg/ml rifampicin to select for the *Agrobacterium* as well as and 50 µg/ml kanamycin to select for the pCAMBIA control. Use a sterilized spreader or autoclaved plating beads to spread evenly.

Note: Dry plates for at least 25 minutes in a biohood for best results.

Note: For best results, we recommend spreading 2 µl, 20 µl and 200 µl onto separate plates for each transformation. For the 2 or 20 µl plates, add 200 µl of recovery media to help spread. This helps to save time if transformation efficiencies are either very low or very high.

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)

	Antibiotic Selection									
Competent cells	Amp 100 µg/ml	Carb 100 µg/ml	Chlor 30 µg/ml	Chlor 100 µg/ml	Gent 30 µg/ml	Kan 50 µg/ml	Rif 25 µg/ml	Spec 50 µg/ml	Strep 50 µg/ml	Tet 50 µg/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	S	R	S	R	S
C58C1	R	R	R	N/A	R/S	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.

Calculations

Transformation efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1 µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/Dilution

Where:
Colonies = the number of colonies counted
µg = amount of DNA transformed in µg
Dilution = total dilution of the DNA before plating

Example:
Transform 1 µl of (500 pg/µl) pCAMBIA1391z control plasmid into 25 µl of cells, add 975 µl of Recovery Medium. Recover for 3 hours and plate 100 µl. Count the colonies on the plate in two days. If you count 500 colonies, the TE is calculated as follows:

Colonies = 500
µg of DNA in 10 pg = 0.0005
Dilution = 100/1000 = 0.1

TE = 500/0.0005/0.1 = 1 x 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)
- Gentamicin sulfate (GoldBio Catalog # G-400)
- Kanamycin (GoldBio Catalog # K-120)