Protocol

TD-P Revision 1.1

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GV3101 (pSoup-P19) Agrobacterium Chemically Competent Cells
Transformation Protocol

Introduction

GoldBio's GV3101 Agrobacterium chemically competent 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 in 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.

The pSoup plasmid present in these cells is required for the replication of pGreen, 62SK, and pGs2 series plasmids. The p19 protein is derived from tomato bush dwarf virus and improves the stability of heterologous gene transcripts by inhibiting RNA silencing of foreign genes and increasing the range of applications for the AGL-1 (pSoup-P19) system. These cells are resistant to rifampicin, carbenicillin, streptomycin and tetracycline, providing versatility in selection and antibiotic resistance.

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 transformation using GV3101 (pSoup) or GV3101 (pSoup-P19) *Agrobacterium* Chemically Competent Cells.

Materials

- GV3101 (pSoup) *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-115) **or** GV3101 (pSoup-P19) *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-125)
- pCAMBIA1391z Control DNA, 10 ng/µl
- *Agrobacterium* Recovery Medium
- Kanamycin (GoldBio Catalog # K-120)
- Rifampicin (GoldBio Catalog # R-120)
- Tetracycline (GoldBio Catalog # T-101)
- Yeast Extract Tryptone (YT) or LB Agar selection plates.
- Microcentrifuge tubes
- Shaker incubator
- Liquid nitrogen

Storage and Handling

- This product may be shipped on dry ice. GV3101 *Agrobacterium* Chemically Competent Cells should be stored at -80°C, pCAMBIA1391z Control DNA, 10 ng/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.
- Thaw GV3101 *Agrobacterium* Chemically Competent 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.

Note: Transformation efficiency is tested by using the pCAMBIA1391z control DNA supplied with the kit and using the protocol given below. Untransformed cells are tested for appropriate antibiotic sensitivity.

Method

Transformation protocol

Use this procedure to transform GV3101 (pSoup or pSoup-P19) *Agrobacterium* Chemically Competent Cells. Do not use these cells for electroporation.

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 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 (50 ng to 500 ng) of DNA to the chilled microcentrifuge tubes on ice.

   Note: It is important to use more DNA than in a typical bacterial transformation. We recommend using ~500 ng, if possible.

4. When the cells are thawed, add 50 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pCAMBIA1391z control, add 5 µl of (10 ng/µl) DNA to 50 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. Keep tubes on ice for 5 minutes, and then transfer to a dry ice/ethanol bath or liquid nitrogen for 5 minutes.

   Note that if you used a dry ice/ethanol bath you can place the frozen tubes on dry ice for up to 30 minutes prior to the 37°C heat shock.

   Note: For a dry ice/ethanol bath, we recommend adding ~200 ml EtOH to a used pipet tip container and slowing adding dry ice over the course of 10 minutes.

6. Incubate tubes in a 37°C water bath for 2 minutes (for dry ice/ethanol bath) or for 5 minutes (for liquid nitrogen).

7. Immediately add 950 µl of Recovery Media and gently pipette up and down three times to resuspend the cells.

8. Incubate at 30°C for 3 hours at 200 rpm in a shaking incubator.

9. Dilute the cells as appropriate, then spread 2 to 200 µl cells onto a selective plate. For the pCAMBIA1391z control, plate 200 µl of the diluted transformants onto a YT or LB plate containing 5 µg/ml rifampicin and 5 µg/ml tetracycline 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.

10. 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 µg/ml	Carb 100 µg/ml	Chlor 30 µg/ml	Chlor 100 µg/ml	Gent 30 µg/ml	Kan 50 µg/ml	Rif 5 µg/ml	Spec 50 µg/ml	Strep 50 µg/ml	Tet 5 µg/ml
GV3101	I	R	R	PR	R	S	R	S	R	S
GV3101 (pSoup)	I	R	R	PR	R	S	R	S	R	R
GV3101 (pSoup-P19)	I	R	R	PR	R	S	R	S	R	R

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 5 µl of (10 ng/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 the next day. If you count 500 colonies, the TE is calculated as follows:

Colonies = 500
µg of DNA = 0.05
Dilution = 100 µl/1000 = 0.1

TE = 500/0.05/0.1 = 1.0 x 10^5

Related Products

- GV3101 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-105)
- GV3101 (pSoup) Agrobacterium Chemically Competent Cells (GoldBio Catalog # CC-115)
- GV3101 (pSoup-P19) Agrobacterium Chemically Competent Cells (GoldBio Catalog # CC-125)
- Rifampicin (GoldBio Catalog # R-120)
- Kanamycin (GoldBio Catalog # K-120)
- Tetracycline (GoldBio Catalog # T-101)