Protocol

TD-P Revision 5.0

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EHA105 *Agrobacterium* Chemically Competent Cells

Transformation Protocol

Introduction

GoldBio's EHA105 *Agrobacterium* Chemically Competent Cells are optimized for the highest transformation efficiencies which is ideal for applications such as cDNA or gDNA library construction. The EHA105 strain is useful for transgenic operations of tomatoes, tobacco and other plants. EHA105 contains a rifampicin resistance gene (rif). Our EHA105 strain is a non-virulent version of the super-virulent A. tumefaciens A281. The native T-DNA is deleted (pTiBo542dT-DNA) but the strain is still capable of moving an alternate T-DNA in trans to the plant host using the vir genes remaining on this helper plasmid.

Materials

- EHA105 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-108)
  - **Strain was generated, and primary clone supplied by Dr. Elizabeth Hood.**
- 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
- Microcentrifuge tubes
- Shaker incubator
- Liquid nitrogen

Storage and Handling

- This product may be shipped on dry ice. EHA105 *Agrobacterium* Chemically Competent 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.
- Thaw EHA105 *Agrobacterium* Chemically Competent Cells and pCAMBIA1391z Control DNA on ice and mix by gentle vortexing. 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. Transformation efficiency should be ≥1 x 10^5

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cfu/µg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

Method

Transformation protocol

Use this procedure to transform EHA105 *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 (10 pg-1 µg) of DNA into the chilled microcentrifuge tubes on ice.
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 1 µl of (500 pg/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 liquid nitrogen for 5 minutes.
6. Incubate tubes for additional 5 minutes in a 37°C water bath.
7. Immediately add 950 µl of *Agrobacterium* Recovery Medium or any other medium of choice, 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 20-200 µl cells onto a prewarmed selective plate. For the pCAMBIA1391z control, plate 100 µl of the diluted transformants onto a YT plate containing 15 µg/ml rifampicin and 50 µg/ml kanamycin. 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 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	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.

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 the next day. If you count 500 colonies, the TE is calculated as follows:

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

TE = 500/0.0005/0.1 = 1.0 x 10^7

Related Products

- AGL-1 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-106)
- GV3101 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-105)
- LBA4404 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-107)
- EHA 105 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-108)
- C58C1 Agrobacterium Chemically Competent Cells (GoldBio Catalog # CC-109)
- Rifampicin (GoldBio Catalog # R-120)
- Kanamycin (GoldBio Catalog # K-120)