

EHA105 (pSoup-P19) *Agrobacterium* ElectroCompetent Cells Transformation Protocol

Introduction

GoldBio's EHA105 *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. This strain 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.

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.

The EHA105 strain is useful for developing transgenic plants from rice, tobacco and other plants. Here, we present a detailed protocol for transformation using EHA105 (pSoup) or EHA105 (pSoup-P19) *Agrobacterium* Electrocompetent Cells.

Materials

- EHA105 (pSoup) *Agrobacterium* ElectroCompetent cells (GoldBio Catalog # CC-235) or EHA105 (pSoup) *Agrobacterium* ElectroCompetent cells (GoldBio Catalog # CC-255)
 - **EHA105 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)
- Tetracycline (GoldBio Catalog # T-101)
- 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. EHA105 *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.
- Thaw EHA105 *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. Untransformed cells are tested for appropriate antibiotic sensitivity.

Method

Transformation Protocol

Use this procedure to transform EHA105 (pSoup or pSoup-P19) *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 wet 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 the 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.

- Pipette 26 μ l 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

- Resistance only. Capacitance off.
 - Capacitance timing is therefore off.
 - Resistance timing R5 which equals 129.
 - Voltage: set at 1.66 to 1.90 kV
 - milliseconds should be about 5 msec after pulse
 - Cuvette – 1 mm
- Immediately add 976 μ l 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.
 - Incubate at 30°C for 3 hours at 200 rpm in a shaker incubator.
 - Dilute the cells as appropriate, then spread 2 to 200 μ l cells onto a selective plate. For the pCAMBIA1391z control, plate 50 μ l of the diluted transformants onto a YT or LB plate containing 5 μ g/ml rifampicin and 5 μ g/ml tetracycline to select for the *Agrobacterium* as well as and 50 μ g/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 μ l, 20 μ l and 200 μ l onto separate plates for each transformation. For the 2 or 20 μ l plates, add 200 μ l of recovery media to help spread. This helps to save time if transformation efficiencies are either very low or very high.

- 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
EHA 105	R	R/S	R	N/A	R/S	S	R	S	R	S
EHA 105 (pSoup)	R	R/S	R	N/A	R/S	S	R	S	R	R
EHA 105 (pSoup-P19)	R	R/S	R	N/A	R/S	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.

Calculation of Transformation Efficiency

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

TE = Colonies/µg/Plated

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 10⁷

Associated Products

- EHA 105 *Agrobacterium* Electrocompetent Cells (GoldBio Catalog # CC-225)
- EHA 105 (pSoup) *Agrobacterium* Electrocompetent Cells (GoldBio Catalog # CC-235)
- EHA 105 (pSoup-P19) *Agrobacterium* Electrocompetent Cells (GoldBio Catalog # CC-245)
- Kanamycin (GoldBio Catalog # K-120)
- Rifampicin (GoldBio Catalog # R-120)
- Tetracycline (GoldBio Catalog # T-101)