Protocol

Auxo-Agro™ LBA4404 Electrocompetent Cells

Transformation Protocol

Introduction

GoldBio's new Auxo-Agro™ competent cells are methionine auxotrophic strains of *Agrobacterium* which reduce overgrowth during the infection process while increasing plant transformation efficiency.

Our LBA4404 strain of Agrobacterium tumefaciens can be used in genetic transformation of tomato, tobacco and other plants. After transformation, antibiotics are commonly used to remove Agrobacterium. However, antibiotics have been shown to adversely affect plant germination and tissue growth. Goldbio Auxo-Agro™ LBA4404 is a LBA4404ΔMet1 Agrobacterium tumefaciens cell line that is methionine dependent, which both minimizes the use of antibiotics and simplifies the transformation procedure. Goldbio Auxo-Agro™ auxotrophic strains eliminate the need for antibiotics. Instead, the removal of methionine from the culture media for the Auxo-Agro™ line of Agrobacterium automatically induces Agrobacterium cell death.

GoldBio's LBA4404 Agrobacterium chemically competent cells allow you to obtain high transformation efficiency in applications such as gDNA or cDNA library construction. Our LBA4404 strain harbors a rifampicin resistance (rif) gene. Furthermore, LBA4404 has an octoprine-type Ti plasmid pAL4404 without self-transport function, containing the vir genes.

Materials

- Auxo-Agro™ LBA4404 Electrocompetent Cells (GoldBio Catalog # CC-267)
    - **Strain was generated, and primary clone supplied by Dr. Wayne Parrott under license from his institution.**
- pCAMBIA1391z Control DNA, 10 ng/µl
- *Agrobacterium* Recovery Medium
- Kanamycin (GoldBio Catalog # K-120)
- Rifampicin (GoldBio Catalog # R-120)
- Yeast Extract Tryptone (YT), or LB Agar selection plates.
- Microcentrifuge tubes
- Shaker incubator
- Sterile electroporation cuvettes
- Microcentrifuge tubes
- Electroporator

Storage and Handling

- This product will be shipped on dry ice. LBA4404 *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 LBA4404 *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 will decrease substantially.

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 Auxo-Agro™ LBA4404 *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.

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 974 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.

   Note: GoldBio recommends growing LBA4404 transformants in YEM media, as they can be sensitive to culture conditions and may not grow as well in other media types.

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	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.

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 10^7

Associated Products

- Auxo-Agro™ LBA4404 Chemically Cells (GoldBio Catalog # CC-168)
- Auxo-Agro™ LBA4404 Chemically Competent Cells (GoldBio Catalog # CC-167)
- Auxo-Agro™ LBA4404 Electrocompetent Cells (GoldBio Catalog # CC-267)
- Competent Cell Recovery Media (GoldBio Catalog # CC-300)
- Rifampicin (GoldBio Catalog # R-120)
- Kanamycin (GoldBio Catalog # K-120)
- YEM Media