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Transformation of Arabidopsis Thaliana Protocol

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ABSTRACT

In this experiment, the flower immersion method was used to transfer the target gene into Arabidopsis thaliana using Agrobacterium mediated.

Experiment Summary

1 In this experiment, the flower immersion method was used to transfer the target gene into Arabidopsis thaliana using Agrobacterium mediated.

Main Reagents

2 YEB liquid medium, LB medium, 0.1 M CaCl2, 0.05 M MgSO4, flower immersion buffer (0.5XMS, 5% sucrose, 0. 03% Silwet L-77), Rif, Kan.

Main Equipment

3 Shaker, centrifuge, culture bowl, greenhouse, trays, plastic film.

Experimental Steps

4 1. Preparation and transformation of *Agrobacterium tumefaciens* receptor cells
(1) Pick a single colony of GV1301 in 10 ml of YEB or LB liquid medium and incubate with shaking at 28°C until late logarithmic phase.
(2) Add 0.5 ml of bacterial solution to 50 ml of fresh YEB or LB liquid medium and incubate at 28°C with shaking until OD_{600}=0.5.
(3) Transfer to 50 ml centrifuge tube and ice bath for 20 min.
(4) Transfer to a 50 ml centrifuge tube and ice bath for 20 min.
(5) Centrifuge at 4000 rpm for 10 min at 4°C and collect the organisms.
(6) Resuspend the precipitate with 8 ml of pre-cooled 0.1 M $CaCl_2$ and 0.05 M $MgSO_4$.
(7) Centrifuge at 4000 rpm for 10 min at 4°C and collect the bacteriophage.
(8) Take 200 ul of receptor cells, add 0.5 ug of plasmid and mix gently.
(9) Freeze in liquid nitrogen for 50 s, place on ice for 40 min, and heat-excite at 42°C for 90 s.
(10) Add 800 ul of LB medium and incubate for 1 hour at 28°C.
(11) Apply the appropriate amount of cells on the screening medium and blow dry the surface liquid on the ultra-clean table.
(12) Incubate at 28°C for two days.
2. Transformation of *Arabidopsis thaliana* by flower immersion
(1) Inoculate Agrobacterium clones containing expression vectors in 5 ml YEB medium (containing 100 ug/ml Rif, 100 ug/ml Kan) at 28°C, 200 rpm and incubate with shaking overnight.
(2) Transfer to 200 ml YEB medium at a ratio of 1:50 and incubate at 28°C, 200 rpm for 5 hours; centrifuge at 5000 X g for 15 min and collect the bacteriophage; resuspend in floral immersion buffer (0.5XMS, 5% sucrose, 0. 03% Silwet L-77) and adjust OD_{600} to 0.8.

(3) Place the Arabidopsis culture bowl upside down on a suitable size container containing flower soaking buffer, soak for 3-5 min, remove the culture bowl upside down in a tray, cover the tray with plastic film, remove the film after 24 hours, and continue the culture in the greenhouse.

Experimental Materials

5 Constructed expression vector, Arabidopsis thaliana.