Protocol

Creation Date: 5/25/2016
Revision Date: 12/18/2018

Tobacco Plasmid Transformation

Introduction

Biolistic transformation is a widely used method for introducing genes into plants that are resistant to *Agrobacterium*-mediated transformation. This technique promotes gene transfer through direct delivery, resulting in stably transformed plants. Usually, the vector to be introduced contains a selectable marker gene that can confer resistance to different antibiotics, including spectinomycin, an aminocyclitol antibiotic, that is encoded by the *aadA* gene and mutated *rpsE* gene. Here, we present a protocol that describes the biolistic transformation of Tobacco plastids using spectinomycin as the selection marker.

Materials

- 4-6 week old tobacco plants
- DNA
- Tungsten particles
- RMOP Medium
- Murashige and Skoog (MS) medium, pH 5.8
- Sucrose
- Spectinomycin (GoldBio Catalog # S-140)
- Selective shoot regeneration medium
- Plant agar (GoldBio Catalog # P1001.0100)
- Magnetic stir plate/stir bar
- Microcarriers for biolistic gun
- Biolistic gun
- Spermidine

Method

1. Asceptically grow tobacco (*N. tabacum*) plants on agar-solidified (5 g/L) MS medium containing sucrose (30 g/L).

2. Place leaves (3-5 cm) from 4-6 week-old tobacco plants abaxial side up on Revised Medium for Organogenesis of Nicotiana plumbaginifolia (RMOP) medium prior to bombardment.

3. Coat tungsten microprojectiles (1 µm) with DNA.
    a. Mix sterilized tungsten particles (in water) with DNA (in water) in solution.

b. Add 2.5M $CaCl_2$ and 0.1M free-base spermidine to precipitate DNA onto particles.
c. Vortex continuously to achieve uniform coating and avoid aggregation of particles.
d. Collect the particles by centrifuging for 30 minutes at 13,000 x g, and load onto the gun.

Note: Optimal amount of DNA is 5 µg per mg of particles.

4. Bombard leaves with DNA coated tungsten particles using a gene or biolistic gun.

5. Identify transplastomic clones as green shoots regenerating on leached leaf sections on RMOP medium containing 500 mg/L of spectinomycin.

6. Illuminate spectinomycin-resistant shoots with UV light and transfer shoots emitting green light to spectinomycin-free MS medium (with 3% sucrose) on which fluorescent or nonfluorescent sectors can form.

7. Excise and transfer fluorescent sectors to selective shoot regeneration medium (500 mg/L spectinomycin).

8. Test regenerated shoots for uniform transformation using Southern blot analysis.

Associated Products

- Spectinomycin (GoldBio Catalog # S-140)
- Plant agar (GoldBio Catalog # P1001.0100)

References

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