Agrobacterium-mediated transformation of tobacco BY-2 cells and Arabidopsis cell suspensions, Prague, IEB ASCR

The transformation protocol of An (1985) as published with references in Petrasek et al. 2003 (Plant Physiology 131, 254-263).

Material and solutions:

1) 3-day-old (exponential) cell culture of tobacco BY-2 (30 ml in 100 ml erlenmayer flask) or *Arabidopsis thaliana* cell suspension.
2) Overnight culture of *Agrobacterium tumefaciens* strain carrying binary vector with your gene construct (C58C1, LBA1115, LBA1100 and others).
3) MS medium for BY-2 cells (3% [w/v] Sucrose, 4.3 g/l Murashige and Skoog salts SIGMA M5524, 100 mg/l inositol, 1 mg/l thiamin, 0.2 mg/l (1 μM) 2,4-dichlorophenoxyacetic acid, 200 mg/l KH_2PO_4, pH 5.8).
4) Acetosyringone stock solution (20 mM in ethanol)
5) Appropriate antibiotics stock solutions (depends on your constructs, only hygromycin and kanamycin is possible, not PPT in suspensions!!), sterile.
6) Plates with solidified MS medium (0.7% agar) for BY-2 cells containing 100 μg/ml Claforan (Cefotaxim) and appropriate selection antibiotics.
7) Sterile cell filtration device (Nalgene or similar) with 20 μm mesh filter, sterile petri dishes (6 cm diameter), sterile pipette tips.

Procedure:

1) Filter BY-2 cells and re-suspend them in the same volume of fresh MS medium.
2) Add acetosyringone stock solution (1 μl/1 ml). Using 10 or 5 ml pipette (with uncut tips!!!), suck in and out cca 20-times the cell suspension to help *Agrobacteium* to do its job by producing small lesions at the surface of BY-2 cells.
3) Transfer 2 ml of cell suspension into plates and add *Agrobacterium* (overnight culture), prepare at least four repeats for every construct. The concentration of *Agrobacterium* may range between 20 and 120 μl/2 ml. Doing this first time, start with at least three concentrations. Do not forget to have one plate dish without *Agrobacterium* for control. Wrap with parafilm and incubate 3 days at 27°C in darkness, without shaking. It is possible to check cells using inverted microscope. Successful progression of the transformation is characterized in that bacteria are attached to cell wall and medium is getting milky, but tobacco cells remain perfectly viable.
4) Wash cells three times in 50 ml of liquid medium containing high amount of Claforan (Cefotaxim, 500 μg/ml) to get rid of the excess of bacteria. Usually all cells from four replicates are mixed and washed together.
5) After the last washing step, add 2-3 ml of BY-2 medium with 500 μg/ml Cefotaxim and transfer this dense suspension onto plates with appropriate antibiotics (100 μg/ml kanamycin or 20 μg/ml hygromycin). Carefully spread cells over the surface of agar plate. This is the crucial step of the whole procedure, cells have to be spread in a layer that is dense enough to allow regeneration.
6) Wrap plates with parafilm and incubate in darkness at 27°C. Antibiotic-resistant colonies appeared after 3 to 4 weeks of incubation. Cell suspension can be established from these colonies. Make the suspension simply by transferring fresh small callus (several millimeters in dimensions) into the 1-2 ml of liquid medium, use multi well plates. Shake in darkness at 25°C (tobacco) or 23°C (Arabidopsis).

Original reference:

An G (1985) High efficiency transformation of cultured tobacco cells. Plant Physiol 79: 568-570