Transformation of Tobacco BY-2 Cells with *Agrobacterium*

Preparation:

- Set up tobacco BY-2 cells. Three days before transformation, start new subculture. You will need 5 ml of culture for every transformation Shake at 25°C.

- Make MS liquid medium (about 80 ml for each transformation).

- Prepare agar plates:
  MS medium + 0.8% phytagar plus 500 μg/ml carbenicillin (1 per sample)
  MS agar + 500 μg/ml carbenicillin + 50 μg/ml kanamycin (up to 3 per sample)

- Agrobacterium strain containing binary vector. The day before doing the transformation, inoculate a large single colony of each Agrobacterium strain into 3 ml LB plus antibiotics in a test tube. (For most pBIN vectors in LBA4404, you will use 25 μg/ml streptomycin and 50 μg/ml kanamycin). Grow in roller drum at 28°C overnight.

Cocultivation:

1. Measure OD_{600} of each Agrobacterium strain. Dilute to OD_{600} = 1.0 in LB.

2. For each transformation, aliquot 5 ml BY-2 cells into a 100 mm Petri dish. Be sure to include a control (no bacteria).

3. Add 5 µl acetosyringone to BY-2 cells (stock is 20 mM in ethanol). Swirl gently to mix.

4. Add Agrobacteria carrying the binary vector. For each vector, set up two transformations using two different amounts of Agrobacteria, i.e., 25 µl and 100 µl. Swirl to mix.

5. Wrap plates with Parafilm and incubate at 25°C for two days.

Washing (2 days after setting up cocultivation):

7. Using a wide bore 10 ml pipette, transfer the BY-2 cells from each plate into a 15 ml centrifuge tube. Rinse the plate with an additional 5-7 ml MS, and add to the centrifuge tube.

8. Let the cells settle in the centrifuge tube.

9. Carefully remove the supernatant by aspiration.

10. Pour MS medium into the tube with the cells to 14-15 ml final volume. Mix by gentle inversion.

11. Pellet cells as in Step. 8.

12. Repeat steps 9-11 **two more times** using MS medium.

13. Repeat Steps 9-11 using MS medium plus 500 μg per ml carbenicillin.

14. Add MS/carbenicillin to 12 ml. Mix by gentle inversion.

Plating of cells on selective and non-selective medium:

15. Plate 1 ml of washed cells onto one 100 mm Petri dish containing MS plus carbenicillin (non selective) and 1 ml onto MS/carbenicillin/kanamycin plates (selective). Spread cells over agar surface by rocking and swirling the plate.

16. Leave plates open in the hood for 5-10 min until the liquid is absorbed; do not let the plates dry out.

17. Wrap the plate with parafilm and incubate at 25°C.

Screening transformants:

19. Calli should be visible on the selective plates in 10 to 14 days. For each transformation, pick 9 distinct microcalli using a sterile spatula. Transfer to a fresh plate containing MS/carbenicillin plus the plant selection marker.

20. Allow the calli to grow for 7 days, then score for growth and GFP production (fluorescence microscopy).

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updated on 02.06.23 by Andreas