Transformation of *Agrobacterium* strains by Electroporation

This protocol is based on Mattanovich et al., 1989 (*Nucleic Acids Research* 17, 6747) with some modifications.

Materials and Equipment:

- Appropriate antibiotics
- LB liquid and solid media (YEP or YM media can be used too)
- 1 L or 2.5 L culture flask (or 250 mL flask for a small scale)
- 500 mL sterile centrifuge bottles (or 50 mL centrifuge tubes for a small scale)
- Sterile deionized water (should be cold)
- Sterile 10% glycerol in deionized water (should be cold)
- Centrifuge with temperature control
- Ice bucket with ice
- 1.5 mL tubes
- Liquid nitrogen and container
- 15 mL culture tubes
- 50 mL culture tubes
- Bio-Rad MicroPulser or Gene Pulser
- 2 mm electroporation cuvettes

Protocol:

Agrobacterium *competent cell preparation*

1. From a frozen glycerol stock, streak a solid medium with appropriate antibiotics, seal with parafilm, and incubate in a 28-30 °C incubator for 2-3 days.
2. Pick a single colony and inoculate a 5 mL seed culture in a 50 mL culture tube. Grow overnight in a shaking incubator at 28-30 °C and 200-250 rpm.
3. Add 5 mL overnight seed culture into 500 mL LB medium in a 2.5 L flask.
4. Incubate in a shaking incubator until OD_{600} reaches ~ 0.5 (this can take up to several hours).
5. Chill the bacterial culture on ice for 15 min (prechill centrifuge bottles on ice).
6. Harvest *Agrobacterium* cells by centrifugation at 4,000 xg for 10 min at 4 °C.
7. Carefully remove supernatant and wash *Agrobacterium* cells with an equal volume (500 mL) of cold deionized water and centrifuge for 10 min at 4,000 xg.
8. Repeat Step 7, three more times.

9. Wash *Agrobacterium* cells with 1/10th volume of 10% cold glycerol (50 mL).
10. Resuspend in 1/100th volume of 10% cold glycerol (5 mL).
11. Add 40 µL aliquots into 1.5 mL tubes and flash freeze in liquid nitrogen and store at -80 °C.

Agrobacterium *transformation by electroporation*

1. Thaw competent cells on ice.
2. Turn on the electroporator (e.g., Bio-Rad MicroPulser) and select preset "Agr" from the menu (push the "へ" button three times).
3. Add 1 µL of plasmid DNA (10-100 ng) into each tube.
4. Transfer cells and DNA into a 2 mm electroporation cuvette and gently tap the cuvette.
5. Place the cuvette in the chamber slide and push the slide into the chamber until both sides of the cuvette are in contact with the base of the chamber.
6. Press the "Pulse" button once.
7. Remove the cuvette from the chamber and add 1 mL of LB broth into the cuvette and carefully transfer *Agrobacterium* cells into a 15 mL culture tube.
8. Incubate the cells for 1-2 h at 28-30 °C, shaking at 200-250 rpm.
9. Spread 100 µL of cells on LB agar plate with appropriate antibiotics.
10. Incubate 2-3 days at 28-30 °C.

Bio-Rad MicroPulser electroporator

2 mm Electroporation cuvettes