solution (250 mg/mL). Pour the medium into sterile Petri dishes or into sterile plant containers and let it solidify at room temperature.

16. Selective root regeneration medium: Dissolve 4.6 g of MS salts and 10 g of sucrose in 1 L of ddH_2O, add 4 g of agar, adjust the pH to 5.7 with KOH, and autoclave. Cool the medium down to 50°C in a water bath. Then add 10 mL of 100X B5 vitamin stock solution, 2 mL of kanamycin stock solution (25 mg/mL), and 2 mL of carbenicillin stock solution (250 mg/mL). Pour the medium into sterile plant containers and let it solidify at room temperature.
17. Germination medium: Dissolve 4.6 g of MS salts (Sigma) in 1 L of agar and adjust the pH to 5.7 with KOH. Autoclave and cool down to 50°C. Pour the medium into sterile plant containers and let it solidify at room temperature.

3. Methods

1. Sterilize tomato seeds for 30 s in ethanol, for 20 min in 1.5% NaOCl, 0.1% Tween-20, and then wash three times in sterile water. The seeds are then seeded on sterile germination medium and are incubated at room temperature in the dark. After 3–5 d, most seeds have germinated. About 20–30 seeds are incubated in one sterile plant container (7 × 10 cm). For one transformation experiment, 50–60 seedlings are needed.
2. After germination, the sterile plant containers are transferred to a plant growth chamber with a temperature of 25°C and with a photoperiod of 16 h light and 8 h dark. After 8 d, the cotelydons have grown to the maximal size and can be used for transformation.
3. Thirty-six hours before the harvest of the cotyledons, the *Agrobacterium* strain containing the plasmid to be tranferred into the tomato genome, e.g., pBin19, is inoculated in 5 mL Min A medium. For pBin19, add 50 μg/mL kanamycin and 100 μg/mL rifampicin. The *Agrobacterium* culture is incubated in a bacteria shaker at 30°C.
4. The cotyledons of the sterile tomato seedlings are cut off and the tips of the leaves are removed. Then, the cotyledons are cut in half and placed upside up on plates with MSOZR medium containing 0.6% agar. The Petri dishes are sealed with parafilm and incubated in a plant growth chamber (25°C, photoperiod of 16 h light/8 h dark).
5. Forty-eight hours after starting the *Agrobacterium* culture, the bacteria are harvested by centrifugation at 4300*g* and resuspended in 3 mL of sterile *Agrobacterium* induction medium (*see* **Note 2**). The *Agrobacteria* are now incubated for another 12 h at 30°C in a bacteria shaker.
6. After 12 h of incubation in the induction medium, the *Agrobacteria* are pelleted at 4300*g*, resuspended in 3 mL of MSO medium, and diluted by adding 15 mL MSO medium (*see* **Note 3**). The cut pieces of the tomato cotyledons are removed from the MSOZR plates, dipped into the *Agrobacteria* solution, blotted dry on sterile filter paper, and placed back on the same MSOZR plates.
7. The MSOZR plates are sealed again and incubated for 2 d in a plant growth chamber under the same conditions.