4. On the day before the inoculation, set up a 75-mL LB broth culture of the LBA4404 strain harboring the DNA construct from a fresh overnight culture. Add 100 mg/L of both kanamycin sulfate and rifampicin to the LB broth and incubate overnight at 28–30°C, with shaking (200 rpm).
5. Measure the optical density of the culture at 600 nm and dilute it, if necessary, with fresh LB broth to an OD_{600} of 1.0 (*see* **Note 9**). Pour the culture into a sterile beaker or polypot.
6. With sterile forceps, remove the leaf explants from the plates and immerse them in the bacterial suspension. Mix by swirling and leave for 1 min.
7. Remove the explants and blot them dry on sterile filter paper (*see* **Note 10**).
8. Replace the explants on the same shooting medium 1 plates, again adaxial-side up, seal the plates with laboratory sealing film, and incubate as before for 2 d (*see* **Note 11**).
9. Transfer the explants to shooting medium 2. Leave the plates unsealed and return them to the growth room for a further 2 d (*see* **Note 12**).
10. Reseal the plates with Micropore tape or laboratory sealing film. The first regenerating shoots should be visible after 3 wk. If the plates become contaminated, *see* **Note 13**.

3.4. Growth of Transformed Material

1. When the shoots are big enough to handle (approx 1 cm in length), they should be removed from the leaf explant and transferred to rooting medium in 60-mL polypots. The shoots usually fall away for the leaf explant very easily, because, by this stage, the explant tissue is brown and dying. In contrast, the shoots should be bright green and healthy. Only single and well-separated shoots should be removed from each explant, to avoid propagating genetic clones (*see* **Note 14**).
2. Return the shoots to the growth room, making sure that the lids on the polypots are attached loosely, to allow gas exchange. Roots should develop within 2–3 wk.
3. When the plantlets have three to four pairs of leaves, they should be transferred to either Kilner jars containing MS30 or directly into compost. If the plantlet is to be grown in compost, it is important to carefully rinse all the agar from the roots and to autoclave the compost before potting out, to avoid bacterial and fungal growth. The plants should be hardened off gradually over a period of 1–2 wk.

4. Notes

1. Treatment of the seeds with 70% (v/v) ethanol removes waxy substances from the surface of the seeds and kills some contaminating organisms present. Do not exceed the immersion time of approx 20 s, because this will result in a loss of viability of the seed.
2. Tween-20 acts as a surfactant, to allow access of the sterilizing bleach solution.
3. Washing the sterilized seeds five times in H_2O is necessary to remove all of the bleach solution. Failure to do this will result in a loss of viability in a portion of the seed. However, if the germination plates are contaminated with fungi or bacteria and this contamination appears to be associated with the seeds, then either