3. Stand the stem sections vertically in MS30 medium, poured in Petri dishes, and smear the apical end with *Agrobacterium* taken from the LB plate, using a sterile inoculating loop (*see* **Note 3**).
4. Seal the Petri dishes with sealing film (*see* **Note 4**) and allow to cocultivate for 48 h (for incubation conditions, *see* **Subheading 2.3.**).
5. Following cocultivation, transfer the explants to callus-inducing medium; the sections can now be laid horizontally (*see* **Note 5**).
6. Seal the Petri dishes with Micropore tape (*see* **Note 6**) and incubate as before.
7. Green callus will develop at the inoculated, apical end of the stem section within 2–4 wk. Transfer the callus, or the whole explant, to shoot-inducing medium and seal the Petri dishes with Micropore tape.
8. When regenerated shoots have reached a height of 0.5–1.0 cm, transfer them onto MS30 medium to induce rooting. The medium should be supplemented with both cefotaxime and kanamycin (*see* **Note 7**).
9. Rooted shoots are maintained on MS30 medium supplemented with kanamycin in the same way as the potato stocks (*see* **Subheading 2.3.**) and tested by PCR and Southern analysis to confirm transformation (*see* Chapters 41–43).

Regeneration of shoots from the callus should occur within 2–6 wk, and the entire process of transformation and regeneration of rooted transgenic plantlets can be accomplished in 2–3 mo.

It is advisable to include a full range of controls with each transformation experiment carried out (*see* **Note 8**).

4. Notes

1. A prerequisite of plant transformation is a reliable, efficient regeneration protocol, because the transformation procedure reduces the regeneration efficiency significantly. Different cultivars have their own individual preferences of hormone combinations and concentrations for optimal regeneration ***(6,13)***. An appropriate ratio of cytokinin (BAP) to auxin (NAA) in the medium induces plant cells to undergo proliferation into undifferentiated callus, and, by decreasing or removing auxin, the cells are induced to differentiate into shoots. Inclusion of gibberellic acid (GA_3) can promote stem elongation. The callus phase needs to be kept to a minimum to reduce the risk of somaclonal variation (phenotypic variation, either genetic or epigenetic in origin).
2. Different cultivars vary in their amenability to transformation and regeneration. As well as variation in hormone levels and combinations (*see* **Note 1**), the transformation protocol can include preconditioning the explants before cocultivation with the bacterial cells ***(7,13)***, addition of silver nitrate in the growth medium to obtain efficient shoot regeneration ***(4)***, and the use of a nurse or feeder layer ***(5,9)***. During cocultivation, the explants are incubated on filter paper above a feeder layer of plant cell suspension, which is in contact with the culture medium. This requires the availability of an exponentially growing cell suspension (normally tobacco, petunia, or potato). Acetosyringone is the prominent inducer of