the bacterial virulence genes, involved in T-DNA transfer, released in solanceous plant tissue following wounding ***(2)***, and can be included in bacterial growth medium to increase the frequency of T-DNA transfer ***(6)***. With certain cultivars the choice of explant, vector, and *Agrobacterium* strain may prove important in developing an efficient transformation protocol.

3. Both ends of the stem section can be smeared with bacteria and the explant laid horizontally on the medium. Incubate 15–20 explants per Petri dish.

   An effective alternative to this method is to immerse the stem sections, and leaves (cut into halves) in a liquid culture of *Agrobacterium*. Grow an overnight culture of *Agrobacterium* in LB broth (remember to include antibiotics) at 29°C, with shaking at 200 rpm, and pellet by centrifugation at 3000*g* for 5 min. Resuspend the cells in twice the volume of MS liquid (this dilution varies, from 2- to 100-fold, with different protocols; *see* **refs. *5***, ***6***, and ***13***) and immerse the explants for 10 min. Briefly blot the explants on sterile filter paper, being careful not to let them dry out, and transfer them to MS30, making sure they are in close contact with the culture medium. The remainder of the protocol is exactly the same as that described, and regeneration, from callus at the cut explant surfaces, occurs within the same time-scale. Because in vitro-grown leaves are small, leaves taken from soil-grown plants can be used, but need to be surface-sterilized. A sterile cork-borer is a quick way of cutting uniformly sized leaf disks.
4. Sealing the plates maintains humidity, which is thought to enhance infection.
5. Decrease the number of explants to 5–7 per Petri dish. Callus-inducing medium is supplemented with kanamycin to select for transformed plant cells, and with cefotaxime to kill the *Agrobacterium*. The level of kanamycin (50 mg/L) is above the concentration on which nontransformed tissue is able to grow.
6. Micropore tape allows gas exchange and prevents the plates from becoming too humid. If the plant tissues become overrun with *Agrobacterium*, then transfer them to fresh plates (cefotaxime is degraded rapidly in light) and leave the plates unsealed. If this becomes a serious problem, the explants can be washed in a cefotaxime solution.
7. Either take one shoot per callus or identify shoots obtained from the same callus, because they may be clonal in origin. It is important to include cefotaxime and kanamycin in the medium during rooting, to help prevent bacterial regrowth that can interfere with subsequent analyses, and to ensure selection against escapes. Occasionally, shoots that are not transformed or do not express the foreign DNA can survive on medium containing selection. Roots are relatively sensitive to antibiotics, and inclusion of selection in the rooting medium should prevent root formation on nontransformed shoots.
8. Controls should be included with each experiment. A full set of controls would consist of inoculated explants incubated on culture medium lacking antibiotic selection, explants inoculated with the *Agrobacterium* strain (no binary vector) incubated on medium with and without antibiotic selection, and uninoculated explants incubated on medium with and without antibiotic selection and cefotaxime.