switch to a fresh batch of seed, or, if this is not possible, extend the sterilization time to 30 min, and reduce the washes to three. Fewer seeds will germinate, but it is worth it if the remaining seedlings are free of any contamination.

4. If there is limited contamination on the plate, then the infected area should be removed as quickly as possible in order to salvage the remaining seedlings.
5. By taking more than one cutting from a shoot-culture, it is very easy to bulk up material rapidly. This may be helpful, for example, if tissue is required for DNA or RNA extractions and a plant is also required for potting out, in order to generate seed.
6. If the shoot-culture medium becomes contaminated during the tissue manipulations, it may be possible to rescue the culture by removing the shoot apex and transferring it to fresh medium. However, if the plant material itself is contaminated, it is usually best to dispose of the whole culture by autoclaving. There are occasions when it is essential to try to rescue contaminated cultures; this can be attempted in three different ways:
   a. Surface-sterilize the entire plant shoot by immersion, for 10 min, in a solution of 10% (v/v) bleach solution, wash five times in sterile distilled H_2O, and transfer to fresh medium. This method is only successful if the plant material is contaminated on the surface, rather than systemically.
   b. Transfer the shoot apex to fresh medium containing 200 mg/L cefotaxime. This will inhibit bacterial growth if the shoot is small (<0.5 cm) and is pushed well into the medium.
   c. Transfer the plantlet (i.e., the shoot plus roots) to compost. Remove all the agar by rinsing under running H_2O, transfer to autoclaved moist compost in a plant pot and cover with plastic film to maintain a high humidity, and allow the plant to harden off gradually over a 2-wk period before transferring to normal greenhouse conditions; allow to flower, then collect seed, from which new shoot cultures can be initiated.
7. It is well worthwhile carrying out a rapid plasmid preparation at this stage to ensure that the right plasmid has been transferred to the *Agrobacterium*, and that no major rearrangements have occurred. Bin19 is a low copy-number plasmid, but we have found that starting with a 50-mL culture and using standard miniprep techniques (e.g., **ref. *7***), enough plasmid DNA is prepared for several restriction enzyme digests.
8. Traditionally, the tobacco leaves were cut into disks with a cork-borer, and, although this eliminates the need to remove the midrib and leaf edges (to expose wounded tissue, which is the tissue infected by the *Agrobacterium*), we find that it is much quicker and simpler to cut the leaves into small pieces with a scalpel.
9. When culturing the leaf explants with the *Agrobacteria*, it is very important that the bacterial suspension is not too concentrated, because this will lead to an overgrowth of the explant by bacteria, which is very difficult to kill off. Therefore the bacterial suspension should be agitated gently (by sucking up and down with a pipet, to break down the clumps of bacteria) and then diluted with fresh LB broth to a final OD_{600} of 1.0.