10. Another important step to ensure that the bacteria do not overgrow the leaf explants is to remove excess bacteria by thoroughly blotting the leafs on sterile filter paper prior to transferring them to the shooting medium.
11. By sealing the plates with laboratory sealing film, a humid environment is created for the 2 d during which the *Agrobacterium* infection takes place.
12. After the infection period, the *Agrobacterium* should be killed off as quickly as possible. This can be achieved by embedding the leaf explants well into the medium, and by not sealing the lids on the Petri dishes for a few days. After this time, the lids should be sealed with laboratory sealing film again; otherwise, the plates will dry out too rapidly; but this time, cut small slits in the laboratory sealing film to allow gas exchange to occur freely.
13. If, while waiting for the transformed shoots to appear, the plates do become overgrown with bacteria, then transfer the leaves to fresh plates. The leaves will have expanded considerably and will have become wrinkled; they will therefore have come out of contact with the medium, so, when transferring the tissue, try to embed the edges into the medium.
14. Ideally only remove shoots that have regenerated from the edges of the explants that are in good contact with the medium, and, therefore, are kanamycin-resistant. It is usual for the edges of the explant to curl away from the medium, thus increasing the opportunity for escapes, but only shoots that are kanamycin-resistant will produce roots in the rooting medium (which contains kanamycin).

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