suitable culture conditions, the leaf explants will readily regenerate transgenic plantlets which can then be potted out and grown to maturity.

This chapter will describe and discuss the techniques involved in the establishment and maintainance of tobacco shoot cultures, which are source of leaf material for *Agrobacterium*-mediated transformation, the introduction of plasmids into *Agrobacterium* by triparental mating, the inoculation of leaf explants with *Agrobacterium*, and the subsequent selection and regeneration of the transformed material.

Our laboratory has found it very convenient to use aseptic leaves from shoot cultures as the source material for transformations. However, it is possible to use greenhouse grown plants as a source of leaf material, provided that the leaves are free from pests and diseases, and that they are surface-sterilized prior to transformation.

2. Materials

2.1. Plant Growth Regulators and Antibiotics

1. Benzylaminopurine (BAP, Sigma, Poole, UK): Make a stock solution of 1 mg/mL by dissolving the BAP in a minimum volume of dilute HCl (0.01*M*), and make up to the final volume with ddH_2O. The BAP solution can either be filter-sterilized through a 0.2-μm acrodisk and added to the autoclaved medium or it can be co-autoclaved with the medium. The solution is stable for several months at –20°C.
2. Kanamycin sulfate (Sigma): Make a stock solution of 100 mg/mL in ddH_2O and filter-sterilize through a 0.2-μm acrodisk. The solution is stable for several months at –20°C.
3. Cefotaxime: Cefotaxime can be purchased from Sigma, but it is cheaper if purchased from Roussel Laboratories (Uxbridge, UK) under the trade name Claforan. Make a stock solution of 100 mg/mL in ddH_2O, filter-sterilize, and store in the dark at –20°C.
4. Rifampicin (Sigma): Make up a stock of 20 mg/mL in methanol and store at –20°C.

2.2. Plant Culture Medium

1. 1/2 MS10 (Germination medium): 2.2 g/L Murashige and Skoog basal medium (Sigma), 10 g/L sucrose, 8 g/L agar (Becton and Dickinson, Plymouth, UK). Adjust pH to 5.8 with 1*M* KOH, and autoclave (121°C, 20 min). The media can be stored for several months at room temperature.
2. MS30: 4.4 g/L Murashige and Skoog basal medium (Sigma), 30 g/L sucrose, 8 g/L agar (Becton and Dickinson). Adjust pH to 5.8 with 1*M* KOH, and autoclave (121°C, 20 min). The media can be stored for several months at room temperature.
3. Shooting medium 1: MS30, 1 mg/L BAP. Add the appropriate volume of the stock BAP solution to the molten MS30 when it has cooled to 50–60°C.