

2. MS30 medium: Composition as for MS liquid; solidify with 8 g/L bacto-agar (Difco).
3. Callus-inducing medium: MS30 supplemented with 0.186 mg/L NAA, 2.25 mg/L BAP, 10 mg/L GA<sub>3</sub>, 50 mg/L kanamycin, and 200 mg/L cefotaxime.
4. Shoot-inducing medium: Composition as for callus-inducing medium, but omitting the NAA.

Sterilize all media by autoclaving (121°C, 15 min). Because many antibiotics and hormones are heat-labile, add to the medium after autoclaving, when it has cooled to approx 45–55°C.

### 2.5. Antibiotic Stocks

1. Cefotaxime: bought commercially as Claforan (Roussel); dissolve in deionized water to a concentration of 100 mg/mL, filter-sterilize (0.2- $\mu$ m filter), and store at –20°C. Cefotaxime is not stable in the light and breaks down rapidly; therefore medium containing cefotaxime should be prepared immediately before use, and not stored.
2. Kanamycin sulfate (Sigma): Dissolve in deionized water to a concentration of 50 mg/mL, filter-sterilize, and store at –20°C.
3. Rifampicin (Sigma): Dissolve in methanol to a concentration of 50 mg/mL and store at –20°C.

**Caution:** Since these chemicals can be harmful, wear suitable protective clothing when preparing stocks.

### 2.6. Hormone Stocks

1. BAP, 6-benzylaminopurine (Sigma): Dissolve in 1N NaOH to a concentration of 4.5 mg/mL, filter-sterilize (0.2- $\mu$ m filter), and store at 0–5°C.
2. GA<sub>3</sub>, gibberellic acid (Sigma): Dissolve in deionized water to a concentration of 20 mg/mL, filter-sterilize, and store at 0–5°C.
3. NAA, 1-naphthalene acetic acid (Sigma): Dissolve in 1N NaOH to a concentration of 1.86 mg/mL, filter-sterilize, and store at 0–5°C.

### 2.7. Miscellaneous

1. Laboratory sealing film and Micropore tape (3M, Loughborough, UK).
2. Presterilized 9-cm plastic Petri dishes (Sterilin, Stone, UK).
3. Inoculating loop and tissue culture implements.

## 3. Methods

This method is based on that described by Twell and Ooms (8). All tissue culture manipulations must be carried out under sterile conditions in a laminar flow hood.

1. Streak the *Agrobacterium* onto LB agar plates containing rifampicin and kanamycin, so that a confluent bacterial lawn will be produced, and incubate at 29°C for 48 h.
2. Prepare the potato material by cutting stems from the in vitro-grown plants into internodal sections approx 0.5–1.0 cm in length. Ensure the sections are devoid of leaves and axillary buds.