

### 2.3. Isolation of DNA for Genomic Blot Analysis

1. Extraction buffer (Stable for months at room temperature): 2% Hexadecyltrimethylammonium bromide (CTAB, no. 5882, Sigma), 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4M NaCl, 2%  $\beta$ -mercaptoethanol added just prior to use.
2. Precipitation buffer (stable for months at room temperature): 1% CTAB, 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 1%  $\beta$ -mercaptoethanol added just prior to use.
3. Mortar and pestle.
4. Liquid nitrogen ( $N_2$ ).
5. Shaking water bath or hybridization oven (optional).
6. Chloroform:isoamyl alcohol 24:1.
7. Polypropylene oak ridge (or similar) centrifuge tubes.
8. Polypropylene tubes (17  $\times$  100 mm) with caps.
9. 1M Ammonium acetate.
10. 7.5M Ammonium acetate.
11. Isopropanol.
12. TE + RNase: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 20  $\mu$ g/mL RNase A.
13. Ethanol (EtOH, 70%).

### 2.4. Supplies for Genomic Blot Analysis

#### 2.4.1. Agarose Gel Electrophoresis

1. 40X Tris-acetate buffer (stable for months at room temperature): 193.5 g Trizma, 65.7 g sodium acetate (anhydrous), 29.8 g EDTA, 650 mL ddH<sub>2</sub>O, adjust pH to 8.0 with glacial acetic acid, adjust volume to 1 L.
2. Loading buffer (100 mL) (filter-sterilize, stable for months at room temperature): 0.1 g bromophenol blue, 15 g Ficoll 400, to 100 mL with ddH<sub>2</sub>O.
3. Peristaltic pump to recirculate buffer in gel box.
4. Orbital shaker (e.g., Model G2, New Brunswick Scientific, Edison, NJ).
5. Ethidium bromide solution (0.5  $\mu$ g/mL in ddH<sub>2</sub>O).
6. Pyrex dish.
7. UV light box (preferably 302 nm).
8. UV germicidal lamp (254 nm) or UV crosslinker (e.g., Stratalinker, Stratagene, San Diego, CA).
9. Camera equipped with filters (i.e., UV haze and Wratten 23a filters) for documentation.

#### 2.4.2. Capillary Blotting

1. Nitrocellulose (e.g., no. BA 85, Schleicher and Schuell, Keene, NH) or nylon membrane (e.g., Hybond N, Amersham, Arlington Heights, IL).
2. Chromatography paper (e.g., no. 3030917, Whatman, Maidstone, UK).
3. Blotting table (*see Note 2*).
4. Paper towels cut to the size of the gel to be blotted.
5. Heat sealable plastic bags (e.g., Seal-A-Meal bags, Dazey, Industrial Airport, KS).
6. 50X Denhardt's solution (100 mL, can store for months at  $-20^\circ\text{C}$ ): 1 g BSA (fraction V), 1 g Ficoll 400, 1 g polyvinylpyrrolidone (mol wt  $\sim$ 40,000), to 100 mL with ddH<sub>2</sub>O; mix extensively to dissolve.