by Rybicki and Hughes ***(25)***; similarly, Rojas et al. ***(26)*** reported sets of primers useful for amplification of parts of the genome of all Curto- and Begomoviruses. If the PCR products are cloned and sequenced, one can then design abutting or partially overlapping PCR primers that will facilitate amplification and cloning of an entire genomic component of the virus of interest (*see* **refs. *27–29***).

2. Materials

2.1. Isolation of Maize Streak Virus Virions and ssDNA

2.1.1. Isolation of MSV Virions

1. Infected leaf material (about 100 g) (*see* **Note 1**).
2. 0.1*M* Sodium acetate buffer pH 4.8 ***(30)***.
3. 0.05*M* Sodium phosphate buffer, pH 7.5.
4. Waring-type blender.
5. Cheesecloth.
6. Polypropylene centrifuge tubes (Sorvall GSA, or equivalent; Newtown, CT), polycarbonate centrifuge tubes (for Beckman type 35 rotor, or equivalent; Palo Alto, CA).

2.1.2. Isolation of ssDNA from MSV virions

1. DNA extraction buffer: 0.1*M* Tris-HCl, 0.1*M* NaCl, 0.1*M* EDTA, pH 7.0, autoclaved. After autoclaving, add SDS to 1% (w/v).
2. Tris-buffered phenol, prepared as described in Sambrook et al. ***(31)***: Melt solid phenol at 68°C. Add 8-hydroxyquinoline to a final concentration of 0.1%. Add an equal volume of 0.5*M* Tris-HCl, pH 8.0, and stir the mixture on a magnetic stirrer for 15 min. Allow the two phases to separate, then aspirate as much of the upper aqueous phase as possible. Add an equal volume of 0.1*M* Tris-HCl to the phenol, stir again, and remove the aqueous phase as before. Repeat these extractions until the pH of the phenolic phase is >7.8. Store equilibrated phenol at 4°C for short term storage, or at –20°C. **Caution:** Handle solutions containing phenol with caution, and in a fume hood: phenol is toxic and highly corrosive.
3. Chloroform (**Caution:** to be handled in a fume hood, since chloroform is carcinogenic).
4. Sterile 1.5-mL microcentrifuge tubes.
5. Sterile TE buffer: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0.

2.2. Isolation of Total DNA from Infected Plant Material

1. Liquid nitrogen, mortar, and pestle.
2. Polypropylene centrifuge tubes (Sorvall SS34, or equivalent), 1.5- and 2.0-mL microcentrifuge tubes (sterilized by autoclaving).
3. DNA extraction buffer: 0.1*M* Tris-HCl, 0.1*M* NaCl, 0.1*M* EDTA, pH 7.0, autoclaved. After autoclaving, add SDS to 1% (w/v).