4. Tris-buffered phenol, prepared as described in **Subheading 2.1.2.** (**Caution:** Handle solutions containing phenol with caution, and in a fume hood: Phenol is toxic and highly corrosive.)
5. Chloroform (**Caution:** to be handled in a fume hood, since chloroform is carcinogenic).
6. Isopropanol (propan-2-ol).
7. Absolute ethanol and 70% ethanol.
8. TE buffer: 10 m*M* Tris-HCl, 1m*M* EDTA, pH 8.0, sterilized by autoclaving.

2.3. Isolation of RF-DNA by Alkaline Denaturation and Anion-Exchange Chromatography

1. Commercially available plasmid isolation kit, for example the Qiagen-tip 20 kit (Qiagen GmbH and Qiagen, Hilden, Germany), with Qiagen-tip 20 columns and solutions:
   P1: 50 m*M* Tris-HCl, 10m*M* EDTA, pH 8.0, 100 μg Rnase A.
   P2: 200 m*M* NaOH, 1% SDS.
   P3: 3.0*M* KOAc, pH 5.5.
   QBT: 750 m*M* NaCl, 50 m*M* MOPS, 15% ethanol, pH 7.0, 0.15% Triton X-100.
   QC: 1.0*M* NaCl, 50 m*M* MOPS, 15% ethanol, pH 7.0.
   QF: 1.25*M* NaCl, 50 m*M* Tris-HCl, 15% ethanol, pH 8.5.
2. Sterile 1.5-mL microcentrifuge tubes.
3. Sterile TE buffer: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0.

3. Methods

3.1. Isolation of Maize Streak Virus Virions and ssDNA

1. Homogenize freshly harvested leaf material (*see* **Note 2**) from infected plants with an equal weight/volume of 0.1*M* acetate buffer, pH 4.8, at room temperature.
2. Squeeze the homogenate through a layer of cheesecloth.
3. Immediately adjust the pH of the homogenate back to pH 4.8 with 10% glacial acetic acid.
4. Remove the precipitated plant components by low speed centrifugation (12,000*g* for 10 min in a Sorval GSA rotor).
5. Pellet the virions by ultracentrifugation (130,000*g* for 150 min in a Beckman Type 35 rotor).
6. Resuspend the high-speed pellet in 0.05*M* sodium phosphate buffer, pH 7.5, and repeat **steps 3** and **4**.
7. Resuspend the virus pellet in 0.05*M* sodium phosphate buffer, pH 7.5. Alternatively, if the virus is to be used for extraction of ssDNA, resuspend in DNA extraction buffer.
8. Isolate ssDNA from virions by extraction with an equal volume of phenol:chloroform (1:1). Remove the aqueous phase containing ssDNA to a fresh tube and re-extract with phenol:chloroform, if necessary. Remove residual phenol by extraction with chloroform.