2. Materials

2.1. Double-Stranded Sequencing

1. Sterile dH_2O (SDW).
2. Freshly made 2*M* NaOH.
3. 3*M* NaOAc.
4. 100 and 70% ethanol.
5. ^{35}S αdATP.
6. Either ^{T7}Sequencing kit (Pharmacia, Uppsala, Sweden) or Sequenase kit (USB, Cleveland, OH).
7. X-ray film.

2.2. Preparation of Sequencing Gels

2.2.1. TBE Gel

1. A siliconate such as Replicote (BDH, Poole, UK).
2. Industrial methylated spirits or ethanol for cleaning plates.
3. Acrylamide mix: 40% 19:1 acrylamide:*bis*-acrylamide (*see* **Note 5**).
4. Amberlite (BDH).
5. 10X TBE for 1 L: 108 g Tris, pH 8.5, with HCl, 55 g boric acid, 9.3 g EDTA.
6. Urea.
7. 10% Ampersulfate.
8. TEMED.
9. Gel fixer: 10% methanol, 10% acetic acid.
10. Whatman paper.
11. Gel kit (e.g., Bio-Rad Sequi-Gen 21 × 50 cm).

2.2.2. Buffer Gradient Gel

1. 10X Bromophenol blue: 0.5 mg/mL.
2. Sucrose.

2.3. Single-Stranded Sequencing

2.3.1. Preparation of ssDNA

1. 2X YT broth—per liter: to 900 mL deionized water, add 16 g bacto-tryptone, 10 g bacto-yeast extract, and 5 g NaCl, shake well, until all solutes have dissolved, and adjust to pH 7.0 with NaOH; make up to 1 L and autoclave.
2. Helper phage: as appropriate for the vector used, e.g., M13K07 for pBSK+.
3. Ampicillin: Made at 25 mg/mL; store for 1 mo at –20°C.
4. 2.5*M* NaCl, 20% PEG 6000.
5. Phenol:chloroform.
6. 10*M* Ammonium acetate.
7. Ethanol: 100 and 70%.