3.2. Preparation Sequencing Gels

Gel kits will vary in size and shape, but this general method can be adapted. A TBE gel is suitable for short runs (up to 400 bp from the primer site) and long runs, though it may be necessary to change the running buffer on runs of over 4 h, because it may become exhausted. To increase the number of bands that can be read from one gel, particularly on a long run, a buffer gradient gel can be used. For very long runs, it may be necessary to use a different percentage of acrylamide (*see* **Note 4**) to improve resolution. Many ready-prepared commercial gel mixes are now available and reduce the risks involved with handling powdered acrylamide. The mixes of 40% 19:1 acrylamide not only have a longer shelf life than premade mixes, but are also more versatile, allowing you to customize your gels, as needed, to maximize the amount of sequence read from one gel (*see* **Note 5** for making 40% acrylamide).

3.2.1. TBE Gel Preparation

This method applies to the BioRad Sequi-Gen kit 21 × 50 cm gel, but is similar to that required for other kits and can be easily adapted.

1. Prepare the gel kit. Ensure that clips fit well and make a tight seal. Clean the surfaces of the glass plates that will be in contact with the gel, first with water and detergent; then rinse and wash with ethanol or industrial methylated spirits. Dry and coat the top plate with siliconate to facilitate its removal once the gel has run. Allow to dry in a fume hood.
2. Prepare the gel mix. This will vary depending on the percentage of gel required (*see* **Note 4**); for most short runs, a 6% acrylamide gel is used. For 100 mL (enough for one gel), mix 15 mL of the ready-prepared 40% acrylamide mix with 50 g of urea (*see* **Note 5**). The large amount of urea will be difficult to dissolve, especially because this is an endothermic reaction. To facilitate this process, warm the mixture on a heated stirrer. Finally, add 10 mL of 10X TBE and 35 mL of distilled water to make the volume up to 100 mL.
3. Assemble the kit, clipping both plates together, with spacers inserted, ensuring that the bottom of the spacers and the two glass plates are flush with the bottom of the clips.
4. Line the casting tray with a strip of Whatman paper. To 40 mL of gel mix, add 480 μL of 10% AMPS and 64 μL TEMED, mix well, and pour into casting tray. Push the bottom of the gel plates into the tray so that the acrylamide is forced up between the plates. Secure the position of the plates by tightening the screws on the tray. Leave to stand in a vertical position for 10 min to allow the plug to set.
5. Pour enough water down the back plate into the buffer reservoir to cover the electrode. This will prevent any acrylamide setting round the electrode, if it overflows into the reservoir tank by mistake.
6. To 60 mL of gel mix, add 480 μL of 10% AMPS and 64 μL TEMED; mix well. Using a 100-mL clean syringe, suck up the acrylamide mixture and pour