between the plates. Pour smoothly and continuously down the far side of the gel, holding the plates at an angle away from your body. Then tilt the gel, still pouring continuously, toward your body to allow the acrylamide to cover the bottom of the gel. Continue to pour until the gel is almost complete, lower the top end of the gel, when the acrylamide is a few centimeters from the top of the plates, onto an empty tip box, or some other stand. This will allow the acrylamide to settle into position. If any bubbles form in the gel, raise the plates to a more vertical position to force them to rise. They can then be reached by a bubble getter (these are available commercially, but a piece of developed X-ray film cut into an extended hook will work just as well) and hooked out of the gel. Push in the shark-tooth comb, with the flat edge toward the bottom of the gel, to make an insert for the teeth of the comb when it is set.

7. Clamp the comb to ensure a close fit and leave the gel to set in this propped-up position for 1 h. The gel can be left overnight and run within 24 h, if the top of the gel is covered with cling film or a layer of 1X TBE running buffer, as oxygen will inhibit polymerization of the gel.
8. Once set, remove the plug and casting tray and the clamps, leaving the comb in place. Make up 1 L of 1X TBE running buffer. Place the gel in the running tank and secure in place. Pour the water out of the buffer reservoir at the back of the plates and fill it with running buffer. Pour the rest of the buffer into the bottom reservoir to cover the electrodes.
9. Remove the comb carefully, using forceps, pulling it out evenly, to prevent it from slipping on an angle and denting the top of the gel.
10. Using a syringe with a fine needle, wash out the space left by the comb to remove any crystals of urea or pieces of loose acrylamide. Wash the comb and replace it, teeth down, into the space at the top of gel, taking care not to push it into the gel, but leave it just touching on the top edge. Using the syringe, wash out each well carefully, and check that the seal on each is tight by looking for buffer leaking between wells as you wash.
11. Attach the electrodes and prerun the gel at 55 W or 2500 V until the temperature indicator on the gel registers 50–55°C. Alternatively, prewarm the running buffer to 50°C in a microwave before pouring into the gel apparatus; this will cut down the time needed for the gel to reach the set temperature.

3.2.2. Preparation of a Buffer Gradient TBE Gel

This gel has a graded concentration of salt, which increases toward the bottom of the gel. This has the effect of creating a voltage gradient down the length of the gel, which slows the migration of low mol-wt fragments near the bottom of the gel. The bands are more evenly spaced on the final autoradiogram, and thus more sequence can be read from one run (up to 600 nucleotides), as compression at the top of the gel is reduced, and resolution is improved.