1. Prepare the plates as for **Subheading 3.2.1.**
2. Mix two gel solutions:
   a. 75 mL Light solution (0.5X TBE, 6% acrylamide, 7.67*M* urea).
   b. 40 mL Heavy solution (2.5X TBE, 6% acrylamide, 7.67*M* urea, 10% sucrose, 1X bromophenol blue).
3. Pour the light and heavy solutions into separate beakers.
4. Add 10% AMPS and TEMED to achieve a final concentration of 0.25% AMPS and 0.1% TEMED to 20 mL of the heavy solution to use for a casting plug, as described in **Subheading 3.2.1.** Once set, add AMPS and TEMED to both remaining solutions.
5. Take up 50 mL of the light solution into a 50-mL syringe and set aside.
6. Take up 12 mL of light solution into a glass 25-mL pipet. Then take up 12 mL of the heavy solution into the same pipet. This should mix slightly on the interface to form a gradient; if it does not, allow a couple of air bubbles to be drawn into the pipet.
7. Pour this gradient down the center of the gel.
8. Pour the rest of the light solution into the gel, making sure that the flow is kept in the center of the gel, so that the gradient forms evenly across the gel, as well as vertically along its length. The bromophenol blue dye will show how well the gradient has formed.
9. The gel can be run in the usual way. The bromophenol blue dye will run out of the gel as electrophoresis proceeds, so the progress of the fragment dye front can be seen. The dark-blue dye front should take at least 4 h to run off the gel.

3.2.3. Loading, Running, and Drying Sequence Gel

1. It is important to clean the wells created by the shark-tooth comb before every loading of the samples, particularly if the gel is reloaded after a period of time (*see* **Note 6**).
2. Place the sequencing reactions in a 80°C water bath for 2 min. Place immediately on ice and load 2–3 µL of each reaction on the gel. The loading order is a matter of personal choice, though for a long run a special method of loading, which places every base next to every other base, is recommended to enable more sequence to be read (*see* **Note 6**).
3. Run the gel at 2500 V or 50 W until the dark blue dye has reached the bottom of the gel for a short run (approx 2 h). Running a gel on constant power (watts = volts × amps) prevents large surges of voltage or overheating occurring; the amount of heat generated will affect the resistance of the gel and, therefore, the current through it.
4. Short runs to cover distances up to 250 bp from the primer usually take 2–3 h, depending on the type of gel used. A good indicator of how far the gel has run can be gained from the position of the two dye fronts (*see* **Note 7**). Once the gel had finished running, disconnect it from the running apparatus and lay it down, resting on the back plate. Remove the clips carefully and prize off the top plate using first finger and thumb at the top corner of the plates. Carefully lift off the upper plate, leaving the gel on the back plate.