4. A 6% acrylamide gel should produce 300–400 nucleotides of clear sequence. To visualize sequences close to the primer (within 50 nucleotides), use a higher-percentage gel (12–20%); to read more distal sequences, use a lower percentage gel (4%). Take care when running these gels, because high-percentage ones may have a tendency to overheat as a result of their increased resistance, and, conversely, lower-percentage gels may melt while running, and are less robust on removal of the top plate and subsequent transfer to paper. For a more uniform spacing of gel bands, use wedge-shaped spacers or a gradient buffered gel (*see* **Subheading 3.2.2.**).
5. 40% 19:1 acrylamide:*bis*-acrylamide. This should be prepared in advance and can be stored indefinitely at 4°C, if protected from the light. **Note:** Take great care when handling acrylamide: It is a neurotoxin. Always wear gloves, and weigh out and dissolve in a fume hood. Heat will be needed to dissolve the urea. Deionize for 1 h using amberlite, filter through Whatman paper to remove any metal ion impurities that may be present.
6. When loading gels, it is important to wash the wells with buffer before every loading, especially when loading onto a gel that has already been running for several hours. This removes any crystals of urea that form as the gel heats up and cause the bands to look very jagged, which can make them difficult to resolve. The wells can be rinsed easily with a syringe and narrow gage needle, using the buffer in the backplate reservoir.
7. The migration of loading dyes in TBE denaturing polyacrylamide gels:

Gel %	Comigrating DNA fragment size	
	Bromophenol blue	Xylene cyanol
6%	26 bp	106 bp
8%	19 bp	75 bp
10	12 bp	55 bp

8. To read sequences close to the primer use a manganese buffer supplied with the Sequenase kit.
9. Band compressions often occur in G–C-rich regions of sequence and they can mask the correct sequence in a particular region of the gel. Compressions are fragments that have comigrated because they are not properly denatured as a result of their formation of stable intrastrand secondary structures. This problem can sometimes be overcome by repeating the sequencing reactions using different termination mixes that contain 7-deza dGTP, 7-deza dATP, or ITP, which are purine analogs.
10. Parallel bands in all four lanes at sequences 200 bp or more from the primer can be a common problem. It may be caused by a decrease in enzyme activity, if the termination or labeling reactions were carried out at temperatures above 37°C. Take care to use a properly equilibrated water bath or incubator for these stages. Another possibility is that the enzyme may have lost its activity by repeated freeze–thaw cycles. Increase twofold the amount of enzyme used in each reaction. The template may also have strong secondary structures that cause the polymerase to pause. Following the annealing step, incubate the primer–template