solution at 60–70°C for 4 min, return the solution to room temperature for 2 min and then immediately proceed to the labeling reaction.

11. Single-stranded DNA quality can be assessed by the A_{260}/A_{280} ratio, which should not be greater than 1.8 for successful sequencing. If the absorbance is greater than 1.8, then repeat phenol:chloroform extractions. If the preparation is clean, then the A_{260} can be a good estimate of the concentration of the ssDNA. A 1 mg/mL solution of ssDNA has an A_{260} of 33. Quality and quantity of ssDNA can also be determined by agarose gel electrophoresis, using a 1% EtBr stained gel. Two major bands are usually seen: the helper phage DNA and the single-stranded plasmid. There may be genomic chromosomal contamination, which will appear as a large product, or RNA contamination, which will appear on the gel as a smear of small fragments.
12. With the Sequenase PCR product kit, the polymerase is supplied prediluted in a modified buffer. This contains inorganic pyrophosphatase to prevent pyrophosphate accumulation and also a higher than normal glycerol concentration to aid enzyme stability. For this latter reason, direct sequencing reactions must be run on glycerol-tolerant sequencing gels (i.e., not TBE gels). This preparation is compatible with the use of 7-deaza-dGTP to resolve compressions and manganese buffer to read close to the primer.

Reference

1. Shukla, D. D. and Ward, C. W. (1988) Amino acid sequence homology of coat protein as a basis for identification and classification of the potyvirus group. *J. Gen. Virol.* **69,** 2703–2710.