3.3.2. Labeling and Termination Reactions for ssDNA

Because the single-stranded DNA needs no prior treatment, the labeling reactions can be started immediately. The same method as described for double-stranded sequencing is followed, except that annealing of the primer to the template is performed at 60°C for 10 min, instead of the quick annealing and slow cooling of the dsDNA method (*see* **Subheading 3.1., steps 5–8**).

3.4. Automatic Sequencing

These notes are based on those supplied with the ABI PRISM (Perkin-Elmer) dye terminator cycle sequencing ready reaction kit. Information is also available from the Perkin-Elmer user bulletins on DNA preparation, and other protocols for template production.

3.4.1. Preparation of DNA for Automatic Reactions

A small amount of DNA is required for automatic sequencing, but it must be extremely clean. The best method for easy and consistent preparation of dsDNA for automatic sequencing is one that involves a column purification step without the use of PEG, or phenol:chloroform. There are many such kits available; one example is Qiagen DNA isolation system, which uses a 5-mL overnight culture. Single-stranded DNA can also be used in these reactions and can be prepared in the same way as previously described. Symmetric PCR templates can be used, and, although these are usually difficult to denature, cycle sequencing can overcome this problem.

3.4.2. Direct Automatic Sequencing of PCR Products

Specifically amplified PCR products can be utilized directly in sequencing reactions if components of the PCR reaction that interfere with sequencing are removed, either by enzymatic degradation or by gel purification. Direct sequencing can be used as a rapid and representative way to sequence PCR products. The following protocol is that recommended by the manufacturers of the Sequenase PCR Product Sequencing Kit.

3.4.2.1. Enzymatic Treatment

1. Examine yield and specificity of PCR by agarose gel electrophoresis. If a single specific product is present, this may be used directly in sequencing reactions.
2. To 5–7 μL of PCR reaction mix, add 1 μL *Exonuclease* I (10 U/μL) and 1 μL shrimp alkaline phosphatase (2 U/μL). Mix and incubate at 37°C for 15 min.
3. Inactivate *Exo*I and SAP by heating to 80°C for 15 min. Both incubation steps can be carried out in a thermal cycler with an appropriate program.
4. To the treated PCR product, add 1 mL of primer solution (10 pm/mL) and SDW to 10 μL.