REAGENTS AND SOLUTIONS

5× exonuclease III buffer

75 mM Tris·Cl, pH 8.0
3.3 mM $MgCl_2$

Exo III ligation buffer

80 mM Tris·Cl, pH 7.5
30 mM dithiothreitol
20 mM $MgCl_2$

Phage loading buffer

0.25% Bromphenol Blue
15.0% Ficoll 400
2.0% sodium dodecyl sulfate
10 mM EDTA

S1 nuclease buffer

16 mM sodium acetate, pH 4.6
400 mM NaCl
1.6 mM $ZnSO_4$
8% glycerol

S1 nuclease stop buffer

0.8 M Tris·Cl, pH 8.0
10 mM EDTA
80 mM $MgCl_2$

COMMENTARY

Background Information

Exonuclease III. The experimental strategy of the exo III method is based on the unique properties of *E. coli* exo III, which is a 3′→5′ double-stranded specific exonuclease that catalyzes release of 5′ nucleotides from the 3′ hydroxy end of double-stranded DNA (see *UNIT 3.11* and Fig. 3.11.3). Blunt DNA ends (e.g., created by *Sma*I) or overhanging 5′ ends (e.g., created by *Hin*dIII, *Xba*I, *Bam*HI) are substrates for an exo III attack. However, exo III will not initiate digestion at an overhanging 3′ end of four bases created by restriction enzymes such as *Pst*I, *Apa*I, *Sac*I, *Kpn*I, and *Bst*XI. Exo III also will not digest ends of DNA that contain an [αS]dNTP (see second support protocol). A cloned fragment of DNA can be specifically digested with exo III if it is adjacent to a 5′ overhanging end or blunt end, whereas a primer sequencing site and vector DNA can be protected from digestion if they are adjacent to a 3′ overhanging end or an end "capped" with a thio nucleotide analog. Exo III is particularly suited for generating nested sets of deletions because of the synchrony and uniformity of its exonucleolytic activity (see Fig. 7.3.2). The number of nucleotides to be deleted can be varied by adjusting amount of exo III or incubation time.

The exo III method has one major advantage over the *Bal* 31 nuclease method (Poncz et al., 1982; Guo and Wu, 1982) for constructing nested sets of deletions. The *Bal* 31 procedure requires gel purification of the set of digested fragments and subsequent recloning; in contrast, the exo III procedure is carried out on a single linearized plasmid and is followed by religation. Double digestion of the DNA, exo III treatment, and religation can be accomplished in 3 hr (Hoheisel and Pohl, 1986). Another advantage of the exo III procedure over the *Bal* 31 procedure is that larger DNA fragments (up to 14 kb; Henikoff, 1984) can be easily subjected to construction of progressive deletions. On the other hand, the exo III procedure depends on the availability of appropriate restriction sites between the insert and primer site. If the required sites are not available for the exo III procedure, the *Bal* 31 procedure can be used.

Bal 31 exonuclease. The basis of this method is the ability of *Bal* 31 exonuclease to progressively shorten a DNA restriction