SHORT PROTOCOL

USING EXONUCLEASE III TO CONSTRUCT NESTED DELETIONS

1. Completely linearize plasmid::insert DNA or M13mp::insert RF DNA, creating a unique 3′ overhang restriction site and a unique 5′ or blunt restriction site situated between the insert and the 3′ site.
2. Phenol extract, phenol/chloroform extract, and precipitate with ethanol. Dissolve to 0.2 μg/μl in TE buffer.
3. Mix 12.5 μl linearized DNA with 5 μl 5× exonuclease III buffer and 7.5 μl water. Add 150 U exo III per pmol susceptible 3′ ends. Remove 3-μl aliquots at 1 min intervals and inactivate the enzyme activity by incubating 10 min at 70°C.
4. Add 3 μl water, 15 μl S1 nuclease buffer, and 4 μl (4 U) S1 nuclease. Mix and incubate 20 min at room temperature.
5. Stop S1 nuclease activity by adding 5 μl S1 nuclease stop buffer.
6. Run 8-μl aliquots on an agarose gel to determine the rate of exo III degradation.
7. Repair the ends by adding 1 μl dNTP mix and 1 μl (2 U) Klenow polymerase. Incubate 10 min at 37°C.
8. Recircularize the deleted molecules by adding 13 μl ligation buffer, 2 μl of 10 mM ATP, and 1 μl (1 U) T4 DNA ligase. Incubate 5 hr at room temperature or overnight at 15°C.
9. Transform 10 μl of each of the ligation reactions into appropriate competent cells and spread on ampicillin-containing agar plates.
10. Characterize the deleted clones by electrophoresis and prepare selected templates for sequencing (see support protocol, *UNIT 7.4*).

BASIC PROTOCOL

USING *BAL* 31 EXONUCLEASE TO CONSTRUCT NESTED DELETIONS

This protocol is written for use with M13mp (Yanisch-Perron et al., 1985) vectors but can be readily adapted for use with a variety of plasmid vectors that contain suitable restriction sites in a polylinker. As illustrated in Figure 7.3.3, the *Bal* 31 procedure involves linearizing plasmid or M13 RF DNA at one end of the region to be sequenced. In a second step, a series of deletions are created which extend various amounts into the desired region using *Bal* 31 exonuclease. The linearization and *Bal* 31 digestion procedures are then repeated by linearizing the plasmid at the other end of the region to be sequenced. Finally, the deleted fragments are cloned into an appropriate M13mp vector such that the deleted *Bal* 31 ends are adjacent to the DNA sequencing priming site. By generating the nested set of deletions from both sides of the insert, both strands of the insert DNA can be sequenced.

A nested set of deletions spanning a 1.5- to 2.0-kb region can reliably be generated using this procedure. If a larger region is to be sequenced, it is best to choose two large deletions (one from either end of the fragment) from the first set of *Bal* 31 digestions and then to repeat the procedure with these deleted fragments. Alternatively, a series of ~2.0-kb fragments spanning the region to be sequenced can be cloned individually. If this latter procedure is adopted, choose a set of overlapping rather than adjacent fragments to avoid the problem of overlooking very small restriction fragments.