MUNG BEAN NUCLEASE DIGESTION

Used to create blunt ends of either 3' or 5' overhangs by digestion of the single stranded overhang. Advantage over S1 nuclease is its stringent specificity for single stranded DNA -- when double stranded DNA "breathes" S1 may digest it whereas mung bean nuclease does not. Therefore it is the nuclease of choice when exact blunting is needed to achieve correct reading frame. However, mung bean nuclease has some endonuclease actibity associated with it and min my experience most commercial preps have some exonuclease activity. In general, make sure you can check the frame and be prepared to sequence your plasmid when you are done.

Typical protocol:

10 ug of plasmid DNA cut with restriction endonuclease(s) to generate overhangs, phenol/chloroform/ethanol precipitated, spun, washed and respun, air dried.

1. Dissolve in 100 ul of buffer #1
2. Add 10 ul buffer #2
3. Incubate at 37øC for 2 minutes and add 1 ul of mung bean nuclease in buffer #3 at a conc of 4 units/ul.
4. Vortex and incubate at 37øC for 10 minutes
5. Add 1 ul 20% SDS and incubate at 65øC for 5 mins
6. Add 4 ul 2 M Tris pH 8.9
7. Add 6 ul 12 M LiCl2
8. Phenol/chloroform/X 2, chloroform/ethanol precipitate and wash, spin and air dry.

Buffer #1

- 6 mM Tris pH 8
- 6 mM NaCl
- 0.2 mM EDTA

Buffer #2

- 300 mM NaAc pH 4.7
- 500 mM NaCl
- 10 mM ZnCl2

Buffer #3

- #2 in 50% glycerol
- Mung bean nuclease (sold by PL-Pharmacia) 100 units/ul stock. Store at -20øC.