USING BACTERIAL ALKALINE PHOSPHATASE TO DEPHOSPHORYLATE DNA

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Procedure:

1. BAP can be used in virtually any restriction enzyme buffer since the enzyme requires only Mg^{+2} and a pH in the 7.0-8.0 range.
2. After restriction digestion of DNA, add BAP directly to reaction and place at 65°C. 50-100 units (BRL units) is sufficient to dephosphorylate 10µg of linearized vector DNA. Incubate at 65°C for 1 hour.
3. Add 6 X proteinaseK buffer (1.2 M LiCl, 60 mM Tris pH 7.6, 60 mM EDTA, 1.2% SDS) to 1 X and proteinase K to 250-500 µg/ml; incubate at 37°-50°C for 30-60 minutes.
4. Phenol: chloroform extract 2 X.
5. Add 3 M Na Acetate pH 5.5 to 0.3 M and EtOH precipitate.

Reference: Chaconas, G. and J.H. Van de Sande. 1980. Methods Enzymol.65:75