

DEPHOSORYLATION OF DNA WITH CALF INTESTINAL PHOSPHATASE

- BUFFER: 10 x CIP buffer: 0.5 M Tris pH 9.0
 10 mM MgCl₂
 1 mM ZnCl₂
 10 mM Spermidine
1. Resuspend DNA in: 10 µl 10 mM Tris pH 8.0
 30 µl dH₂O
 4.5 µl 10 x CIP buffer
 1/2 µl CIP, 37°C 30 min
 2. Add: 10 µl 10x STE (100 mM Tris 8.0, 1 M NaCl
 10 mM EDTA)
 1 µl 20% SDS
 3. Heat at 68°C 15'-30'
 4. Add 50 µl TE
 5. Phenol Extract
 6. Pass aqueous over G-50 column
 7. EtOH ppt. with NaOAc

Alternative method:

Add CIP to restriction enzyme reaction for 60' at 37°C using 1U/µg DNA

Reference: Maniatis Cloning Manual