CIP

To 10 ug DNA linearized and dissolved in 40 ul H2O, add 10 ul 5 X CB CIP and 1 ul of 1/10 diluted CIP 1 units/ul Boehringer (ammonium sulfate suspension pelleted in microfuge at 4C for 5 mins, sup aspirated and pellet dissolved in H2O at this concentration. Usable for many months stored at 4C in this form. You need .01 units CIP to dephosphorylate 1 pmole of 5' ends (1 pmole of 5' ends of a 4 kb DNA fragment is 1.6 ug). Thus, I use a slight excess of CIP.

Unlike BAP, incubations with CIP are done at 37C for 5' overhangs for 1/2 hr with two additions, or at 37C for 1/2 hr then at 56C for 1/2 hr after another addition, for 3' overhangs or blunt DNA. In all cases the reaction is terminated by the addition of 2.5 ul 20% SDS, 40 ul H2O, 10 ul 10 X STE (TNE) vortexed and heated at 65C for 15 mins followed by multiple phenol chloroform extractions and ethanol precipitation.

This procedure only works well for sticky end ligations and cannot be used with one or two blunt ends. Always do control transformations of vector before phosphatase religated, vector after phosphatase religated and not religated to determine the efficiency of linearization in the first place, as well as the efficiency of dephosphorylation. The dephosphorylated vector should not be able to religate to itself and thus needs a phosphorylated fragment to contribute the 5' phosphate at each end to create a nicked but circular molecule (catalyzed by DNA ligase) that can transform with moderate efficiency.

If you get no colonies with addition of insert to ligation reaction with phosphatased vector, you need to worry about the possibility that a nucleolytic contaminant of the phosphatase has destroyed your sticky ends, or that residual phosphatase activity is dephoshorylating your insert in the ligation reaction, versus a problem with the insert, e.g. inhibition of ligation due to some poison from agarose, acrylamide gels or centrifuge desalting or elution columns, etc. The control in this case is to ligate your phosphatased vector to the digest of a plasmid with a different antibiotic resistance marker which also contains a reasonable sized (100 to 1000 bp) similarly sticky ended insert. Thus, without purifying the DNA you can mix the digest with the phosphatased vector, ligate, select for the phosphatased vectors antibiotic resistance and see if sticky fragments were able to "rescue" the phosphatased vector. If yes, then the problem is with your insert, if no, then the problem is with your phosphatased vector.

5 X CB CIP

0.25 mM Tris pH 8.9	125 uL 2M Tris pH 8.9
5 mM MgCl2	50 uL 0.1 M MgCl2
0.5 mM ZnCl2	0.5 uL 1.0 M ZnCl2
5 mM spermidine	500 uL 10 mM spermidine
	320 uL H2O

Total = 10mL

10 X STE (TNE)

100 mM Tris pH 8.0	0.5 mL 2M Tris pH 8.0
1.0 M NaCl	2.5 mL 4M NaCl
10 mM EDTA	0.5 mL 0.2M EDTA
	6.5 mL H2O
Total = 10 mL	