# ALKALINE PHOSPHATASE DIGESTION OF DNA

### Introduction

Purpose is to remove the 5' phosphates of DNA fragments and vectors, the former can then be re-phosphorylated with 32P-labelled ATP for Maxam Gilbert DNA sequencing while the latter can be used to improve the efficiency of cloning fragments.

There are two types of alkaline phosphatase used. The first is bacterial alkaline phosphatase (BAP) which is very hardy and reliable but has the disadvantage that preparations are sometimes contaminated with an exonuclease that can ruin a sticky ended vector for cloning purposes. By carrying out the digestion at 68 C and phenol extracting immediately and multiple times, the contaminating exonuclease can be suppressed and then destroyed -- or so you hope. For these reasons, I usually use BAP only for dephosphorylating fragments before labelling for sequencing, and use the cleaner calf intestinal alkaline phosphatase (CIP) for cloning purposes. In both cases it is critically important to remove all traces of phosphatase at the end of the reaction for BAP use multiple (3-4) phenol chloroform extractions since even a trace of residual phosphatase activity will ruin your subsequent experiment. One of the biggest advantages of using CIP is that it is killed by heating to 70 C for 10 min.