CONVERSION OF FRAGMENTS WITH PROTRUDING 5' ENDS TO BLUNT ENDS

Protruding 5' ends are filled using the DNA polymerizing activity of the Klenow fragment of E. coli DNA polymerase I.

1. Set up the following reaction:

   restriction fragment (up to 1 µg of DNA in 10 µl)
   2 mM solution of all four dNTPs 1 µl
   10X nick-translation buffer 2.5 µl
   H₂O to 25 µl

2. Add 2 units of the Klenow fragment of DNA polymerase I. Mix and incubate for 15-30 minutes at 22°C. Heat to 70°C for 5 minutes to inactivate the enzyme.

3. Ligate the blunt-ended DNA fragment to synthetic, phosphorylated linkers or vector as desired.

   There is no need to purify the blunt-ended DNA since the end-filling and ligation reactions can be carried out sequentially in the same reaction mixture.

Procedure from Maniatis cloning manual