

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 μ g of DNA in 10 μ l)
2 mM solution of all four dNTPs	1 μ l
10X nick-translation buffer	2.5 μ l
H ₂ O	to 25 μ l

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

Reference: Wartell and Reznikoff. 1980. *Gene* 9, 307.