FILLING IN STAGGERED DNA ENDS

5' protruding ends

1. Set up:

   20 μl DNA sample (up to 1 μg)
   3 μl 10 x polymerase buffer (manufacturer's)
   3 μl 0.5mM dNTP mix
   1 μl Klenow polymerase

   Incubate at 25°C for 15'.

2. Add 1 μl 0.5 M EDTA, heat at 70°C for 10'.

3. Extract 2 x with phenol/chloroform.

4. Ethanol precipitate.

3' protruding ends

1. Set up:

   23 μl DNA sample (up to 1 μg)
   3 μl 10 x T4 polymerase buffer (manufacturer's)
   3 μl 0.5mM dNTP mix
   1 μl T4 DNA polymerase

   Incubate at 37°C for 15'.

2. Add 1 μl 0.5 M EDTA, heat at 65°C for 5'.

3. Extract 2 x with phenol/chloroform.

4. Ethanol precipitate.