BLUNT-ENDING DNA FRAGMENTS WITH 5'OVERHANGS
(FILL IN REACTIONS)
WITH DAVID DANKORT

IMPORTANT NOTE

To blunt end 5'overhangs simply fill in the overhang with DNA polymerase (Klenow fragment (NEB M0210S, link). This enzyme works in most restriction enzyme buffers. For 3'overhangs T4 DNA polymerase is more

Reagents Required

DNA polymerase Klenow Fragment	NEB M0210S
dNTPs	10mM stock

1. Cut DNA with restriction enzyme to completion.

2a. If the enzyme is heat sensitive, heat kill the restriction enzyme. For many enzymes a 20minute 65°C incubation will suffice (alternatively 80°C for 20 min). Be sure to check the

2b. (this step is arguable optional). If the enzyme cannot be "heat killed" you can remove the restriction enzyme either through gel purification of through column purification.

3. Set up on ice:

4. To a 20ul digest, add 0.5ul of dNTPs and 0.5 ul Klenow (or 1unit/ug of DNA). Incubate at room temperature for 15 minutes.

5. Stop reaction (Addition of EDTA to 10 mM, purification through column, electrophoresis through gel)

Note: Protocol for blunting ends by 3' overhang removal and 3' recessed end fill-in: DNA should be dissolved in any 1X restriction enzyme NEBuffer or 1X EcoPol Reaction Buffer supplemented with 33µM each dNTP. Add 1 unit Klenow per microgram DNA and incubate 15 minutes at 25°C. Stop reaction by adding EDTA to a final concentration of 10mM and heating at 75°C for 20 minutes. CAUTION: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times may result in recessed ends due to the 3'→ 5' exonuclease activity of the enzyme.