REPAIR OF STICKY ENDS WITH KLENOW (LARGE) FRAGMENT OF DNA POLYMERASE I

Klenow fragment of DNA pol I contains the 5' to 3' polymerase activity and the 3' to 5' exonuclease activity but lacks the 5' to 3' exonuclease activity of intact DNA pol I. Therefore, in the presence of the four deoxynucleoside triphosphates it will fill in 5' overhangs and blunt back 3' overhangs with fidelity.

It can be used not only to generate blunt ends from sticky ones, but also to correct the reading frame of a piece of DNA to be cloned into another reading frame.

Typically, 5-10 ug of a plasmid are digested with the enzymes of choice and the equivalent molar ratio of the supercoiled plasmid taken in a volume of 20 ul sterile distilled water (after phenol/chloroform, chloroform, ethanol and wash spins, and air drying). For example, if the 10 ug of plasmid were converted into two fragments upon digestion, the dried digest would be dissolved in 40 ul of water and 20 saved; if the plasmid is just being linearized, dissolve it all in 20 ul and use the whole thing; if 10 fragments were generated, use only 2 ul of the 20 ul its dissolved in.

CB 10 X Klenow contains BSA so store it at -20C not 4 C. 4 dNTPs are 2 mM each adjusted to pH 7 and should also be stored at -20øC.

A typical reaction:

- DNA (2-3 pmoles of fragments) ..... 20 ul (reaction of restriction digestion of 5 ug DNA)
- CB 10 X Klenow ..... 3 ul
- EDTA 2 mM ..... 3 ul
- 4 dNTPs..... 3 ul

Now check pH of a small droplet as for the (pH 7.6) ligation protocol, then add 1 ul Klenow 1 ul

Incubate at room temperature for « hr then phenol/chloroform/ethanol precipitate/wash (or gel purify by glassmilk method), dissolve in X volume for the next reactions.

CB 10 X Klenow

0.5 M Tris Cl pH 7.2	250 uL Tris Cl pH 7.2
0.1 M MgCl2	500 uL 0.2M MgSO4
1.0 mM DTT	1 uL 1M DTT
500 ug/mL BSA	25 uL 20mg/mL BSA
	224 uL H2O

Total = 1 mL