SOP: SP062

Dephosphorylation of DNA 5'- and 3'- termini

Materials and Reagents:

1. Water, nuclease-free
2. Linear DNA template
3. Shrimp Alkaline Phosphatase (SAP)
4. 10x Reaction Buffer
5. PCR tube
6. Incubator, 37C
7. Water bath, 65C
8. Bench top micro-centrifuge

Protocol:

1. _____ Prepare the following reaction mixture:

Linear DNA (note1)	1ug (~1 pmol termini)
10x reaction buffer (note 2)	2ul
Shrimp Alkaline Phosphatase	1ul (1U)
Water, nuclease-free	to 20ul
Total volume	20ul

2. _____ Mix thoroughly and spin briefly.

3. _____ Incubate at 37C for 30min for 5'-overhangs or blunt ends, 60min for 3'-overhangs (note 3).

4. _____ Stop the reaction by heating for 15min at 65C (note 3).

5. _____ Reaction can be analyzed by gel electrophoresis to confirm non-recircularization of DNA template (note 4).

Notes:

1. For efficient dephosphorylation DNA should be free of RNA and gDNA. SAP may also be used to remove phosphate groups from protein. One unit of SAP enzyme hydrolyzes 1 umol of 4-nitrophenulphosphate in 1min at 37C.
2. SAP is active in virtually all restriction enzyme buffers and may be added directly to digested DNA. Heat inactivation of the restriction enzyme before dephosphorylation reaction is not necessary.
3. May also be done using a Thermocycler set to the appropriate program.
4. Binding of SAP to DNA may result in a band shift in agarose gels. To avoid this, incubate the samples with 6x DNA Loading Dye and SDS Solution at 65C for 10min and chill on ice prior to electrophoresis.

Reference:

Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.

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Khosravi, R., et al., Rapid ATM-dependent phosphorylation of MDM2 precedes p53 accumulation in response to DNA damage, Proc. Natl. Acad. Sci USA, 96, 14973-14977, 1999.

Nilsen, I.W., et al., Thermolabile alkaline phosphatase from Northern shrimp (Pandalus borealis): Protein and cDNA sequence analysis, Comparative Biochemistry and Phusiology, B, 129, 853-861, 2001.