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Simplified preparation of unidirectional deletion clones

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The unidirectional digestion of DNA, first cut with two different restriction enzymes generating one 3'-protrusion and than with exonuclease III and S1-nuclease allows a very defined removal of DNA for sequencing (1,2) or mapping of biological functions. The DNA is cloned into the polylinker of e.g. pUC18 or mp18 (2), double digested with SstI/SmaI or PstI/XbaI, phenol extracted, ethanol precipitated and taken up in 10 mM Tris-HCl, 0.1 mM EDTA pH 8.0 at 0.2 mg/ml. For avoiding further phenol and ethanol steps (1) the following protocol is very helpful:

12.5 µl linearized DNA, 5 µl 5x-Exo-buffer (75 mM Tris-HCl pH 8.0, 3.3 mM $MgCl_2$) and water to final 25 µl are mixed and incubated for 2 min at the desired temperature (200 base pairs/min: 37°C, 30/min: 22°C), than 135 units exonucleaseIII/pmole susceptible 3' ends are added at time zero (about 190 units for 2.5 µg pUC18). Every minute a 3 µl aliquot is mixed quickly with 3 µl water, which is heated to 100°C, and incubated for 5-7 minutes at 70°C to stop and inactivate exonuclease III.

On ice 15 µl S1-buffer (16 mM Na-acetate pH 4.6, 400 mM NaCl, 1.6 mM ZnSO4, 8% glycerol) and 4 µl S1-nuclease (4.5 U/µl) are added and incubated at room temperature for 10-20 min to digest single stranded DNA and stopped by a pH-shift upon adding 5 µl of 800 mM Tris-HCl pH 8.0, 20 mM ETDA pH 8.0, 80 mM $MgCl_2$. (8 µl are run on a 1% agarose gel to check the extend of deletion).

The remaining 22 µl are incubated for 2 min at 37°C with 2 µl of Klenow-fragment; 2 µl of a mixture of the four dXTP's (each at 0.25 mM) are added for 10 min at 37°C to create blunt ends. 13 µl ligation-buffer (80 mM Tris-HCl pH 7.5, 30mM DTT, 20 mM MgC12, 3mM spermidine), 2 µl of 10 mM ATP and 4 µl T4-DNA ligase are added and the ligation run for 6 hrs at 25°C or overnight at 18°C. Calcium treated competent JM83 cells are transformed with 10 µl of the ligation mix and spread on LB-plates with ampillicin (100 µg/ml). About thousand colonies (1-2% of that for ccc-DNA) are obtained.

1. Henikoff, S. (1984) Gene 28,351-359.
2. Yanisch-Perron,C., Vieira,J. and Messing,J. (1985) Gene 33,103-119.

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