The unidirectional digestion of DNA, first cut with two different restriction enzymes generating one 3'-protrusion and then 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:

1. 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 ZnSO$_4$, 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 EDTA 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 MgCl$_2$, 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 ampicillin (100 μg/ml). About thousand colonies (1-2% of that for ccc-DNA) are obtained.