High speed chemical DNA sequence determination

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The time consuming disadvantages of the chemical degradation method for DNA sequencing (1)(e.g. large scale plasmid DNA preparation, secondary digests, gel purification) have been overcome partially by labeling selectively one of the four ends created by certain restriction enzymes in special plasmids (2) and thereby saving several experimental steps. By using the method described here, the advantages of chemical DNA sequencing (1,3) can be utilized with a minimum of expense being well suitable for the rapid DNA sequence determination of a large number of recombinants.

The procedure consists of four steps: 1) Plasmid preparation from a 1 ml saturated culture: Cells are spun down, the pellet is suspended in 80 µl lysis-buffer (50 mM Tris/HCl pH 8.0; 50 mM EDTA; 8% sucrose; 5% Triton-X), and 5 µl lysozyme (10 mg/ml in H2O) are added. The mixture is heated to 95°C for 1 min. After centrifugation and removing of the pellet an iso-propanol precipitation (same volume, incubation for 2 min at room-temperature, centrifugation for 2 min) is carried out. The pellet is dissolved in 100 µl TE-buffer (10 mM Tris/HCl, pH 8.0; 1 mM EDTA) followed by 2x extraction with the same volume of phenol, 2x ether-extraction and EtOH-precipitation. 2) DNA cleavage with an appropriate restriction enzyme (>40; 20 min; other conditions according to (4)). 3) Labeling reaction with [α-32P]dNTP (20 µCi; 3 Ci/µMol) and Klenow-fragment (5 U) at room temperature for 10 min followed by EtOH-precipitation. This reaction can be carried out in the medium restriction buffer recommended by Maniatis et al. (4). The total incorporation of radioactivity is approximately 2.5 x 10^6 cpm. Finally DNA is dissolved in TE-buffer (2.5 x 10^6 cpm/µl). 4) DNA sequencing using the solid phase method described by Rosenthal et al. (3).

All four steps can be done for a large number of fragments parallely within a total time of 4 hrs, which strongly recommends this method for screening recombinant DNA (e.g. from mutants) in the case of appropriate restriction sites. The resulting reaction mixtures then have to be lyophilized and are sufficient for at least two sequencing gels with an exposure time of 24 hrs.

In certain restriction sites the base distributions allow selective labeling of one of the two recessive 3'-ends with Klenow-fragment and deoxynucleotidase (e.g. AccI: GT'AGAC, GT'CTAC; AvaI: C'TCGGG, C'CCGAG; BanI: G'GTGCC, G'GCACC; BstEII: G'GTMACC; Tth111I: GACN'NNGTC; for other useful restriction sites see (5)). Consequently sequencing reactions can be done without any further steps.
Autoradiography of DNA fragments after application of the described method and separation on a 7M-urea-8% polyacrylamide gel. The AccI-site GTCTAC of plasmid pfdWBC91 (Maeda et al., in preparation) was labelled using [α-32P]dATP. The two remaining AccI-sites have G in the fourth position and therefore are not labelled. The autoradiography does not show any radioactive contamination caused by unspecific labeling of plasmid or chromosomal DNA. The T-reaction seems to be less specific (bands sometimes occur in G-positions), which has been observed earlier (3). In the case of T-positions, even weak signals in the T-lane are unequivocal, because no unspecific bands can be seen in the other lanes. The shown sequence from bottom to top is: AGTCCGAGGT TCTGGATCAC CTATTTGACG T.

References