One of the problems associated with double-stranded DNA sequencing is that most of the [5'-32p]-labelled oligonucleotide primer is not extended by the DNA polymerase. Hence, most of the labelled primer is wasted and gels have to be autoradiographed for several days. In the method described in this paper, almost all of the labelled primer is extended by the DNA polymerase and thus a shorter autoradiograph exposure is needed. This method is a modification of the linear polymerase chain reaction (LPCR) where only one oligonucleotide is present (1). It uses Taq DNA polymerase and, since polymerisation is at 72°C, results in a low level of sequencing artifacts. Multiple LPCR cycles also removes sequencing artifacts. The use of lower than normal dNTP concentrations is necessary to achieve sufficient chain termination by the ddNTPs.

Methods

One pmole of an 18 mer (5'GTGGAATTGCAAGTGGA3' which is homologous to bps 89-106 of the human alpha RI DNA clone alpha B3 (2)) was 5'-end labelled in reaction volume of 20μl containing 60mM tris .HCl, pH 7.5, 9mM MgCl2, 10mM DTT, 2 units polynucleotide kinase (Pharmacia), and 5μCi γ-ATP (Bresa, Australia-4000 Ci/m mole) for 20 min at 37°C. One μl of this reaction was added to 16.6 mM (NH4)2 SO4, 67mM Tris .HCl, pH 8.8, 6.7mM MgCl2, 10mM DTT, 2 mg/ml BSA, 2.5μM each dATP, dGTP, dCTP, dTTP, 50 ng 340 bp alpha B3 (2), 0.6 unit Taq DNA polymerase (Pharmacia), either 1mM ddT or 50μM ddG or 500μM ddA or 500μM ddC (final volume 20μl) and overlaid with 50μl mineral oil. Either one (right 4 lanes) or ten (left 4 lanes) LPCR cycles were performed at 95°C (1 min), 45°C (2 min) and 72°C (2 min). Two μl of each reaction was loaded on a 16% polyacrylamide-urea sequencing gel.

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References

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