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Improved double-stranded DNA sequencing using the linear polymerase chain reaction

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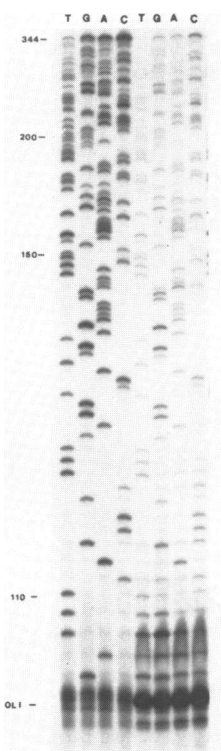
One of the problems associated with double-stranded DNA sequencing is that most of the [5'-^{32}P]-labelled oligonucleotide primer is not extended by the DNA polymerase. Hence, most of the labelled primer is wasted and gels have to be autoradiographed for several days. In the method described in this 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'GTGGAATTTGCAAGTGGA3' 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 $MgCl_2$, 10mM DTT, 2 units polynucleotide kinase (Pharmacia), and 5µCi ɣ-ATP (Bresa, Australia-4000 Ci/mmole) for 20 min at 37°C. One µl of this reaction was added to 16.6 mM $(NH_4)_2$ SO_4, 67mM Tris .HCl, pH8.8, 6.7mM $MgCl_2$, 10mM DTT, 2 mg/ml BSA, 2.5µM each dATP, dGTP, dCTP, dCTP, 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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