

Plasmid DNA sequencing using highly degenerate oligonucleotides as primers

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Sequencing double-stranded DNA templates has become an efficient procedure (1) being much less time-consuming than the preparation and subsequent sequencing of inserts in phage single-stranded DNA. The ability to use highly degenerate oligonucleotides instead of sequence-specific primers would make this method even more powerful for the rapid sequencing of DNA that codes for a protein for which amino acid sequence information is already available. Here I report the applicability of this method, using oligonucleotides of almost 1000-fold degeneracy (i.e. only one out of a mixture of 1000 oligomers has the correct base sequence) at appropriate primer:template ratios, to directly start sequencing within the gene coding for *S. cerevisiae* guanylate kinase, designated GUK1, whose amino acid sequence has recently been published (2).

Two mixed oligonucleotide probes were prepared which corresponded to the amino acid sequence from position 61 to 70 (Oligo I: 5′-CCA (T, C) TC (A, G, T) AT (A, G) AA (T, C) TC (A, G) TT (A, G) TT (T, C) TT (A, G, T) AT CAT-3′), and from 179 to 186 (Oligo II: 5′-(T, C) TT (T, C) TC (A, C, G, T) GC (A, G) AA (A, G, T) AT (A, G) AA (A, G) TC (T, C) TT-3′). They were synthesized with a Biosearch 8700 DNA synthesizer and used without further purification by HPLC or polyacrylamide gel electrophoresis. A 1.7 kb genomic DNA fragment carying the GUK1 gene was cloned in pUC-8 in a way similar to that reported for the adenylate kinase gene (3). CsCl purified plasmid DNA was further treated as described (4) and sequenced using the T7 Sequencing™ (Pharmacia LKB) kit with ^{35}S-dATP as the label nucleotide. 3 μg plasmid DNA (about 1 pmole) were incubated with varying amounts (from 5 to 200 pmoles) of Oligo I and Oligo II, respectively. In either case, excellent results were obtained when applying primer:template ratios from 10:1 to 50:1, producing only a low unspecific background in all four sequencing lanes (Fig. 1). Cumbersome subcloning could thus be avoided and sequence information of the protein coding region became directly available allowing for the immediate synthesis of specific primers.

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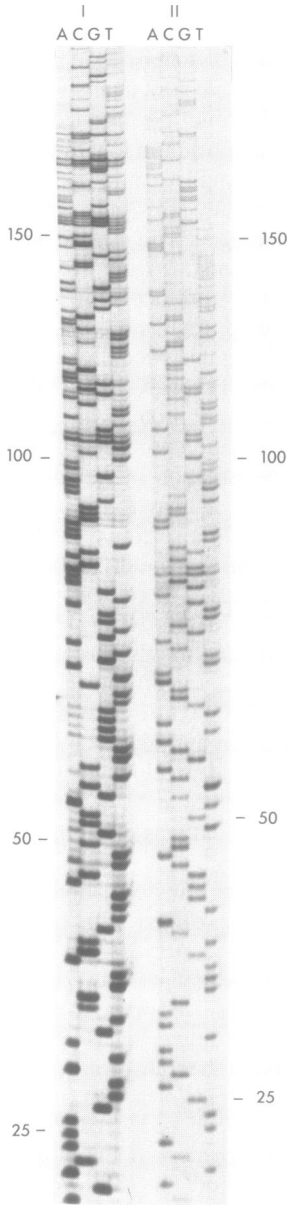

Figure 1. Autoradiogram of 6% denaturing polyacrylamide sequencing gel. Sequencing reactions were primed with 50 pmoles of either the 576-fold degenerate 30-mer (I) or the 768-fold degenerate 24-mer (II). Numbers indicate the distance of bases from the primer. Exposure time was 12 hours.