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Short Communication

# Analysis of unknown DNA sequences by polymerase chain reaction (PCR) using a single specific primer and a standardized adaptor

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## Summary

A new procedure for the PCR amplification of unknown DNA sequences adjacent to a known sequence is described. The required but not readily available second primer sequence in the unknown DNA sequence is obtained by creating an overhanging restriction site in the unknown sequence to which a double-stranded oligonucleotide adaptor of known sequence is ligated.

Polymerase chain reaction; Amplification

Minute amounts of specific DNA sequences can be amplified in a few hours by the polymerase chain reaction using two primers complementary to known sequences at both sides of the sequence of interest (Saiki et al., 1985; Puchta et al., 1989). The inverse PCR permits the amplification of unknown DNA sequences flanking a region of known DNA sequence in circularized DNA (Ochman et al., 1988; Silver et al., 1989). Recently, the anchored PCR (Berchtold, 1989; Loh et al., 1989) was developed for the analysis of unknown sequences adjacent to a known RNA sequence. We describe an anchored PCR for the analysis of unknown DNA sequences in linear DNA molecules. The principle of the assay is to create a known primer sequence in the unknown DNA sequence by cutting the DNA with a restriction enzyme. A double-stranded oligonucleotide adaptor is then ligated to the restriction site and an adaptor specific oligonucleotide is used as second primer for the PCR. The new assay (Fig. 1) was tested in a model system by amplification of a small stretch of known phage λ-

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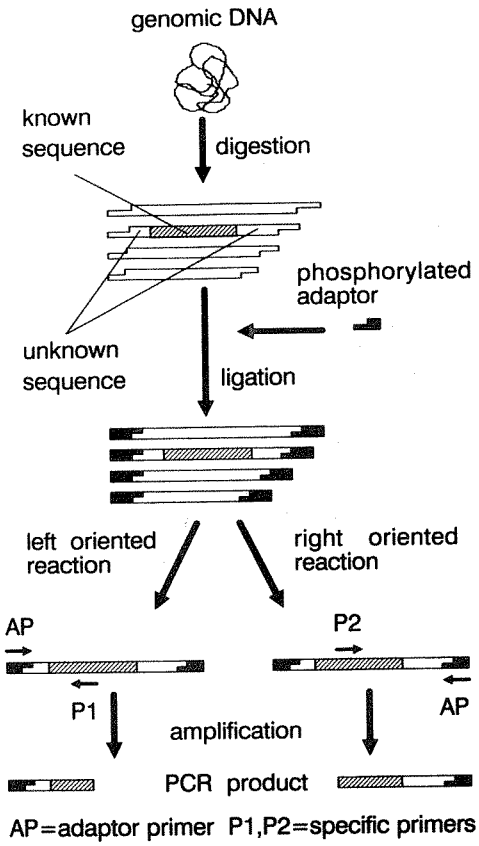


Fig. 1. Schematic representation of the single sequence specific primer DNA amplification system.

DNA sequence. This permitted the verification of the amplification product. A large excess of cellular human genomic DNA (100 ng) was digested with *Pst*I restriction enzyme in the presence of a small amount of double stranded phage λ-DNA (1–100 pg) to generate fragments with defined sticky ends in both the phage and the excess genomic DNA. The generation of sticky ends is important for a high ligation efficacy of the double stranded adaptor because this influences the sensitivity of the assay directly. The double-stranded adaptor 5′-CGACGGATCGTAGCAACAA′TGCA-3′ with an overhanging 5′-TGCA-3′ end, which is complementary to the overhanging end generated by the *Pst*I enzyme, was synthesized. The adaptor was phosphorylated and added to the digested DNA (100 ng) in a molar ratio of 10:1 to the approximately $3.7 \times 10^9$ restriction sites per 100 ng DNA. This number of restriction sites was estimated as number of *Pst*I cuts in a random DNA sequence. The DNA fragments were ligated overnight at room temperature using the linker-kinase reaction system according to Maniatis et al. (1982). The PCR was performed in a standard buffer (Cetus, 1988) on the crude ligation assay. The adaptor specific primer was added in 4 μM concentration, because of the excess of ligated restriction sites in the digested DNA as compared to the number of specific primer sites. The sequence specific primer 5′-GCACCATGCAACATGAATAA-3′ is complementary to a stretch of phage lambda

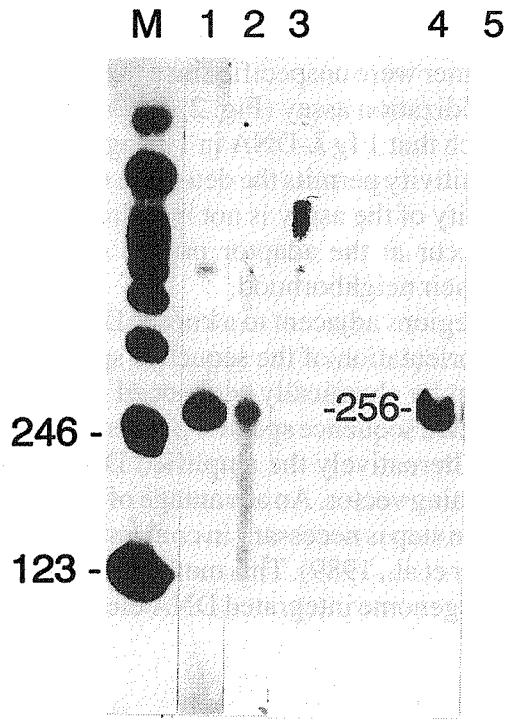


Fig. 2. Analysis of the amplification product (lanes 1–3). Direct detection of the amplified 256 bp fragment using a radioactive labeled sequence specific primer for the PCR; (M) $^{32}$P-5′labeled 123 bp-molecular weight standard (Gibco-BRL); (1) 0.1 pg; (2) 1.0 fg *PstI* cut lambda DNA in 100 pg genomic DNA (3) negative control, (lanes 4,5) amplified fragment detected by Southern hybridization; (4) 256 bp fragment; (5) negative control.

sequence and was provided in a concentration of 0.4 μM (Katz et al., 1989). For radioactive detection of the PCR amplification product, 1 pmol of a $^{32}$P-end-labeled sequence specific primer was added. For Southern blot detection of the amplified DNA, both primers remained unlabeled. The desired DNA sequence was amplified through 30 PCR cycles at 92°C 1.0 min; 60°C 1.0 min and 72°C 1.0 min with the Cyclotherm instrument (Collasius et al., 1989) using *Taq* polymerase (Amersham, U.S.A.) as DNA synthesizing enzyme. In case of radioactive assays an aliquot of 10 μl of the 100 μl of amplified assay was separated by PAGE on a 8% gel and autoradiographed (Fig. 2, lanes 1–3). The non-radioactive amplification assay was ethanol precipitated, glyoxalated (McMaster et al., 1977), separated by PAGE on a 5% gel in 10 mM sodium-phosphate buffer, pH 6.0, and electroblotted (Stellwag et al., 1980) onto a nylon membrane (Genescreen, NEN, Boston, MA, U.S.A.). A $^{32}$P-end-labeled oligonucleotide (Maniatis et al., 1982) 5′-CGGCAACACAGGATCTCTCTTTTA-AGTTACTCTCTA-3′, complementary to the inner part of the PCR amplified sequence, hybridized (Albretsen et al., 1988) well at 42°C in the Southern blot (Fig. 2, lanes 4,5) proving the specificity of the DNA amplification. The amplified DNA sequence corresponded in size (Fig. 2) to the computer determined length of the expected phage lambda fragment (256 bp).

The control assay without λ-DNA was, as expected, negative for the major amplifi-

cation band (Fig. 2, lane 3). Some faint bands present on the gel with the radioactively labeled sequence specific primer were unspecific since they were not detectable in the more specific Southern hybridization assay (Fig. 2, lanes 4,5). The sensitivity of the amplification system was such that 1 fg λ-DNA in 100 pg genomic DNA was detectable (Fig. 2, lane 2). This sensitivity permits the detection of single copy gene in eukaryotic genomes. The sensitivity of the assay is not impaired by the nonspecific DNA amplification which may occur at the adaptor primer sites which do not have a sequence specific primer in their neighborhood.

The left and right handed regions adjacent to a known DNA sequence can be analyzed by simply inverting the orientation of the sequence specific primer (Fig. 1). The amplified DNA fragment can be chemically sequenced following elution from the gel, when a radioactively labeled sequence specific primer is used (Puchta et al., 1988; 1989; Tabler et al., 1989). Alternatively the amplified DNA sequence can be introduced into an appropriate cloning vector. An advantage of the system described is that only one enzymatic restriction step is necessary in contrast to two steps for the inverse PCR reaction for DNA (Silver et al., 1989). This method is rapid and seems of particular interest for the analysis of genome integrated DNA elements.

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