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PCR amplification of long DNA fragments

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The polymerase chain reaction (PCR) has recently evolved as a standard laboratory technique, popular in all areas of molecular biology research. However, the technique still has two limitations: the relatively low fidelity of Taq polymerase when compared with other polymerases (1), and its inability to efficiently amplify fragments higher than 3 kbp (2, 3). Although these two issues are irrelevant in most PCR applications, they are limiting factors in some cases such as the amplification of large constructs for in vitro mutagenesis and the amplification of eukaryotic genomic DNA segments containing introns of unknown length.

We have developed PCR conditions allowing the efficient amplification of DNA segments with 6 kbp (see fig. 1 legend). We were interested in the amplification of several segments (ranging 1 to 6 kbp) of the CyIIIa·CAT construct (4), in order to develop a PCR based method for the in vitro mutagenesis of the upstream regulatory domain of the CyIIIa cytoskeletal sea urchin gene. We found the absence of KCl to be optimal for the amplification of DNA molecules in the range of 3–6 kbp: bands of these sizes were faint or undetectable in ethidium bromide stained agarose gels after 30 PCR cycles (using 1–100 ng of template and buffers PEC and I in Table 1), yet several micrograms of 3–6 kbp molecules were obtained after 30 cycles (using 10 ng of template and buffer II). Given the large temperature dependence of pH in buffers made with Tris (5), we tested a Tricine buffer (III in Table 1) without KCl. As shown in Figure 1, using buffer III it is possible to obtain several micrograms of a 6.2 kbp PCR product even after only 10 PCR cycles. Template amounts used in these 10 cycle experiments were 100 ng to 1 μg, with only small differences in product yield.

In a PCR based mutagenesis experiment, it is crucial to obtain molecules carrying exclusively the desired mutation. It is necessary to try to reduce the probability of occurrence of mutations randomly introduced by the polymerase due to its known low fidelity (1). The presence of undesired mutations could be minimized by using large amounts of template and a low number of cycles. Reaction conditions described above make it possible to reduce the probability of finding mutations introduced by Taq polymerase in the PCR amplified molecules. Genomic DNA could also be amplified using 30 or more PCR cycles. Buffer III (Table 1) could be useful to efficiently amplify DNA molecules with more than 6 kbp, by increasing the length of the polymerization step in the thermo-cycle program.

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REFERENCES

1. Kunkel,T.A. and Eckert,K.A. (1989) In Erlich,H.A., Gibbs,R. and Kazazian,H.H.Jr. (eds.). Polymerase Chain Reaction. Current Communications in Molecular Biology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 5–11.
2. Innis,M.A., Myambo.K.B., Gelfand,D.H. and Brow,M.A.D (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9436–9440.
3. Schwarz,K., Hansen-Hagge,T. and Bartram,C. (1990) *Nucl. Acids Res.* **18**, 1079.
4. Flytzanis,C.N., Britten,R.J. and Davidson,E.H. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 151–155.
5. Good,N.E. (1986) *Biochemistry* **5**, 467–476.

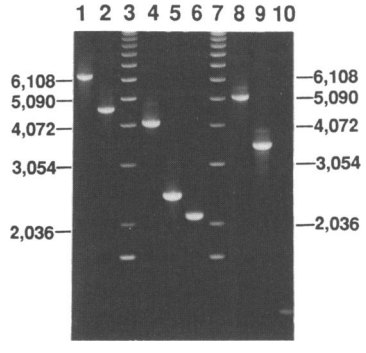

Figure 1. 5 μl of each 100 μl reaction were electrophoresed per track on a 1% agarose gel stained with ethidium bromide. Lanes 3 and 7: size markers (1 kb ladder, BRL). The remaining lanes show PCR products ranging from 6.2 kbp (lane 1) to 1.2 kbp (lane 10), obtained using different sets of 2 primers from a group of 7 oligonucleotides (18-mer to 25-mer). PCR reactions were performed in 100 μl mixtures covered with 100 μl mineral oil. Reaction mix included 10μl buffer III (Table 1), 2.5 units of Cetus Taq polymerase, 200 μM of each dNTP, 0.1 μM of each primer and 100 ng of linearized CyIIIa·CAT as template. Initial denaturation was for 5 min at 94°C. Amplification was for 10 cycles on a Perkin Elmer Cetus thermal cycler (40 sec of transition to 94°C, 1 min at 94°C, 35 sec of transition to 55°C, 1 min at 55°C, 1 min of transition to 72°C and 5 min at 72°C) followed by a final incubation for 10 min at 72°C.

Table 1. PCR 10× buffers used in this work.

	PEC	I	II	III
Tris-HCl pH 8.5		300 mM	300 mM	
Tris-HCl pH 8.3	100 mM			
Tricine pH 8.4				300 mM
KCl	500 mM	500 mM		
$MgCl_2$	15 mM	20 mM	20 mM	20 mM
β-Mercaptoethanol	50 mM	50 mM	50 mM	50 mM
Gelatin	0.01%	0.1%	0.1%	0.1%
Thesit	1%	1%	1%	1%

All pH measurementss were done at 20°C. PEC: Perkin Elmer Cetus buffer.

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