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PCR mediated gene synthesis

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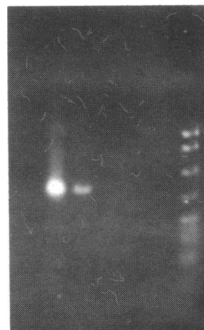
Mullis *et al.* have described a procedure[1] for synthesizing double stranded DNA fragments from oligonucleotides. This procedure involves sequential insertion of oligonucleotides by Polymerase Chain Reaction (PCR) without the use of DNA ligase. We have developed an alternate method for gene synthesis which involves a combination of a single-step ligation of oligonucleotides and PCR amplification of this crude ligation mixture. In our approach, all the overlapping oligonucleotides comprising the entire gene are synthesized, phosphorylated (except the two 5' end oligonucleotides of the top and bottom strands) and ligated in a single reaction and then an aliquot of this crude ligation mixture is used as a target for PCR amplification. The two 5' end oligonucleotides of the gene are used as primers for PCR amplification. The PCR reaction mixture is run on a low-melting agarose gel and the product band corresponding to the size of the gene is directly used for cloning. We describe here a successful application of this approach to the synthesis of tuna and horse cytochrome *c* (104 and 103 amino acids respectively) genes. The method is simple and potentially very useful in the synthesis of large genes.

Sixteen oligonucleotides ranging from 31-62 bases long, were designed for each gene, synthesized (ABI Model 380B or Biosearch Model 8750) phosphorylated (except the two 5' end oligonucleotides) and ligated in a single reaction (50-100 µl volume). A small aliquot (5-10 µl) of this crude reaction mixture was used as a target for PCR amplification. The two 5' end oligonucleotides (#1 and #16) were used as primers. The PCR reaction mixture was ethanol precipitated, digested with *Eco*R1 and *Bam*H1 and electrophoresed on a 3% nusieve gel. A clear band for both the tuna and horse cytochrome *c* genes were obtained as shown in Figure 1 (Lanes 1 and 2, respectively). Exchange of primers and targets between these two PCR reactions did not produce the product bands confirming specificity (Lanes 3 and 4). The products were cloned into M13mp19 and verified by dideoxy sequencing. Synthesis of larger genes (1 kb and above) are in progress.



REFERENCES

1. Mullis,K.B., Erlich,H.A., Arnheim,N., Horn,G.T., Saiki,R.K. and Scharf,S.J. (1987) U.S. Patent No. 4,683,195.