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THE USE OF CONSENSUS SEQUENCE FOR AMPLIFICATION AND ORIENTED CLONING OF HUMAN ALPHA INTERFERON GENES

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Summary. – A clone from human cDNA library was amplified in polymerase chain reaction (PCR) by the synthetic oligonucleotides. The final construct after linker ligation and digestion with restriction endonucleases was suitable for oriented cloning into *E. coli* expression vector. The interferon (IFN) expression could be detected by SDS-PAGE electrophoresis, Western blotting and biological antiviral assay. This set of oligonucleotides can be used also for amplification of genomic DNA or cDNA libraries.

Key words: polymerase chain reaction; interferon; oligonucleotides; amplification; oriented cloning; expression

PCR is a powerful tool for gene technology. Previously a possibility of cDNA amplification by PCR has been demonstrated (Saiki *et al.*, 1988; Liang and Johnson, 1988). The modified PCR was successfully applied for detection of specific IFN mRNAs (Dieffenbach, 1988). We have used this technique and synthetic oligonucleotides to obtain the human alpha IFN gene for the direct cloning and expression of the biologically active protein in *E. coli*.

Plasmid pUC 9 carrying the human alpha IFN cDNA, confirmed by hybridization was amplified using primers OK2 and OK4 (Fig. 1). OK2 (20-mer) is sense consensus sequence from the beginning of the mature sequence (Henco *et al.*, 1985) and OK4 (23-mer) is modified consensus sequence from the 3'end including the stop codon and the *Hind*III site.

PCR mixture (100 μl total volume) contained 0.5 ng of the recombinant linearized plasmid, 1 μmol/l each of the OK2 and OK4 primers (HPLC purified), 50 mmol/l KCl, 10 mmol/l Tris-HCl pH 8.3, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 0.01 % gelatine and 2.5 U Taq polymerase (Cetus). 30 cycles were performed on Hybaid Thermal Reactor with following parameters: 2 min at 94 °C, 3 min at 37 °C, 4 min at 72 °C. The final extension was 7 min. After amplification and purification procedure the phosphorylated synthetic linker OK1 (Fig. 1) containing ATG codon was ligated to the product of the PCR. The

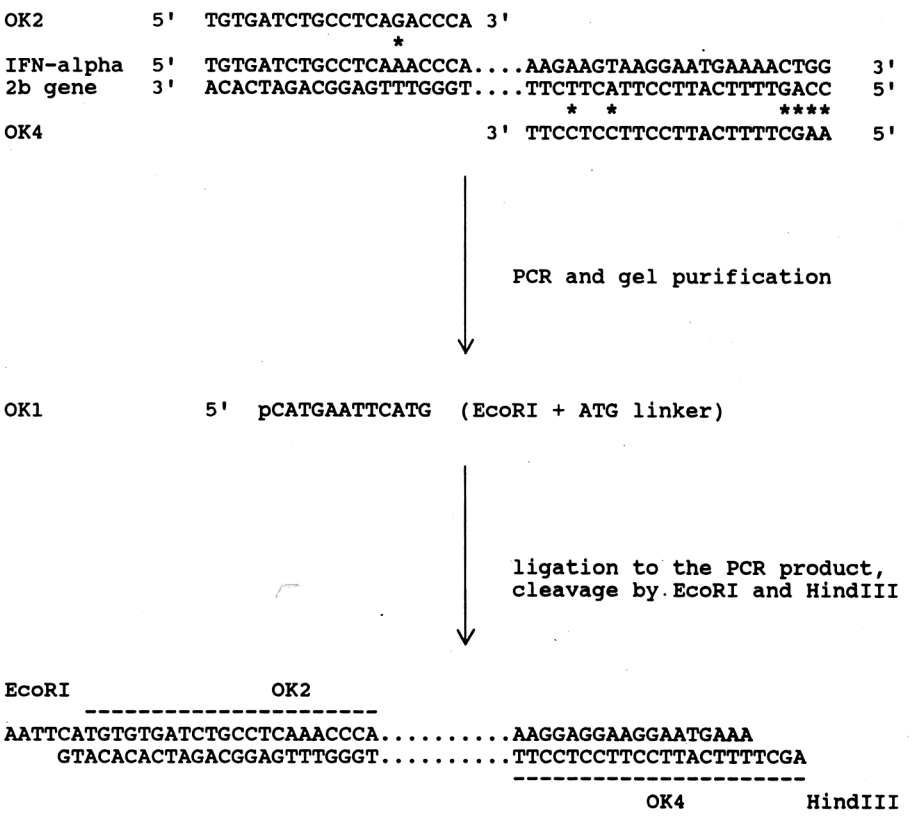

Fig. 1
Schematic diagram of human alpha IFN gene amplification and its arrangement for the direct insertion

* - denotes mismatched bases

OK1-PCR construct was cleaved by *Eco*RI and *Hind*III endonucleases (Fig. 1).

After insertion of *Eco*RI-*Hind*III fragment into M13mp9 plasmid the sequence of OK1, OK2 and OK4 was verified by standard dideoxysequencing protocol. The internal sequence was homologous to the human alpha 2b IFN gene (Gabain *et al.*, 1990). By the direct cloning of this gene into *E. coli* expression vector containing P_L promotor IFN synthesis was demonstrated by the SDS-PAGE electrophoresis, Western blotting and biological antiviral activity - 10^9 i. u./ml (Kontsek *et al.*, 1991). The amplification with OK2 and OK4 primers has no effect on the amino acid composition of the majority proteins encoded by alpha IFN genes. These primers were also used for the searching of IFN sequences in human genomic DNA (data not shown).

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