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# Universal Primers Can Amplify Tumor Necrosis Factor Gene across Species

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**Abstract**

**Universal primers designed in the conserved regions of sequences can be used for cross-species gene amplification and for identification of unsequenced genes from other species. We have designed a universal primer for the tumor necrosis factor gene. This has been tested for the amplification of tumor necrosis factor gene from monkey and guinea pig for which DNA sequence is not yet known. The universal primer thus designed can be used for identification and expression studies for tumor necrosis factor genes in other unknown species. The strategy showed in this paper can be used in designing universal primers for the study of various subjects involving unsequenced DNA.**

Many genes of interest to immunologists have been sequenced in man and mouse, the two most widely studied species. However, information on such genes in other species used as experimental models is often required by the scientific community. Researchers have to resort to re-sequencing of these genes for each new species in order to conduct expression level or other PCR related studies of the gene. Specialized software that could align the sequences from several species and design primers in the consensus region that can amplify across species would facilitate the rapid study of novel genes in new animal models. The design of primers that can successfully amplify the desired gene from multiple species or members of a gene family within the same species is described in this paper. We have selected tumor necrosis factor (TNF) as a model gene and amplified it using universal primers from the consensus region. Universal primers were designed from the human TNF gene sequence, Genbank accession no. M10988 (Wang *et al*., 1985); rat, Genbank accession no. D00475 (Shirai *et al*., 1989); and rabbit, Genbank accession no. M12846 (Ito *et* al., 1986) retrieved using ENTREZ release 24 (NCBI, NIH, USA). The universal primer can be used to prime a PCR or a sequencing reaction for a number of species and for comparative studies of the gene between different species. TNF gene sequences were aligned using tools located at www.ebi.ac.uk and subsequently universal and sequence specific primers were designed using Primer Premier 4.1. (Premier Biosoft International, CA, USA) (Figure 1). Sequence specifications are given in Table 1.

High molecular weight DNA was extracted from 750 µl of fresh blood of human, rat, rabbit, monkey and guinea pig according to Sambrook *et al* (1989). Reactions were carried out in 50µl volume in 0.5ml thin walled PCR tubes (Perkin Elmer, Applied Biosystems, USA) containing 5µl of 10x Taq buffer (Boerhinger Mannheim, USA), 100mM of each deoxynucleoside triphosphates and 20 pM each of the sense primer (Bangalore Genei Pvt. Ltd., India) T1 and anti-sense primer T2. For amplification of TNF gene using sequence specific primers, 40 pM of each human TNF gene specific (TH), 20 pM of rabbit specific (TB) and 20 pM of rat specific (TR) anti-sense primers were used in conjunction with 20 pM of the sense primer T1 in separate reactions. Contents were gently mixed and 1.5 units of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) and 2µl of DNA sample (approx. 50ng) were added to the reaction mixture. After brief centrifugation, two drops of sterile mineral oil were added to each tube to avoid evaporation. These were then subjected to 35 cycles of amplification with a thermal profile of 55°C for 1 min, 72°C for 1 min and 95°C for 1 min with an initial denaturation of 3 min at 96°C and a final extension at 72°C for 5 min in a thermal cycler (Perkin Elmer 2400, Applied Biosystems, USA). After amplification, PCR products were electrophoresed on 2.5% Nusieve agarose (3 parts Seakam® and 1 part of agarose gel) at 80V for 45 min and stained with ethidium bromide, and subsequently visualized using a UV Transilluminator.

The amplifications of TNF genes from different species are shown in Figure 2. The TNF gene was successfully amplified from rat and human DNA using sequence specific primers whereas it was amplified from DNA of human, rat, rabbit, monkey and guinea pig using the universal primers. Despite the fact that the DNA sequence for monkey and guinea pig TNF gene was not considered for primer design, results of this set of experiment revealed that universal primers designed in the conserved region of the sequences can be used for cross species gene amplification and for the identification of the same un-sequenced genes from

Table 1. Primer sequences used in the study

| Name | Sequence |
|---|---|
| Universal primer T1 (sense) | 5'-gAg CAC TgA AAg CAT gAT CCg |
| T2 (anti-sense) | 5'-CAg CAg gCA gAA gAg CgT ggT |
| Human specific TH (anti-sense) | 5'-ggA gAT ggg TAC ACg Agg Ag |
| Rabbit specific TB (anti-sense) | 5'-gCT gAT CTg Agg gTg ACC AT |
| Rat specific TR (anti-sense) | 5'-CTC CCA TCT CCg TCT CgA AT |
| Control primer β-actin (sense) | 5'-CAC TCT TCC AgC CTT CCT TCC |
| β-actin (anti-sense) | 5'-Cgg ACT CgT CAT ACT CCT gCT T |

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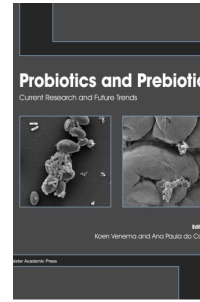


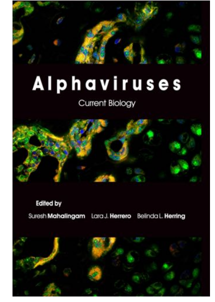

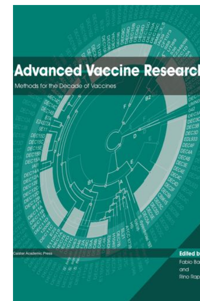

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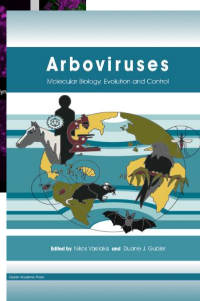


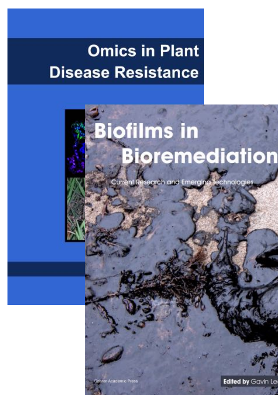


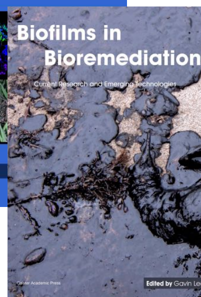


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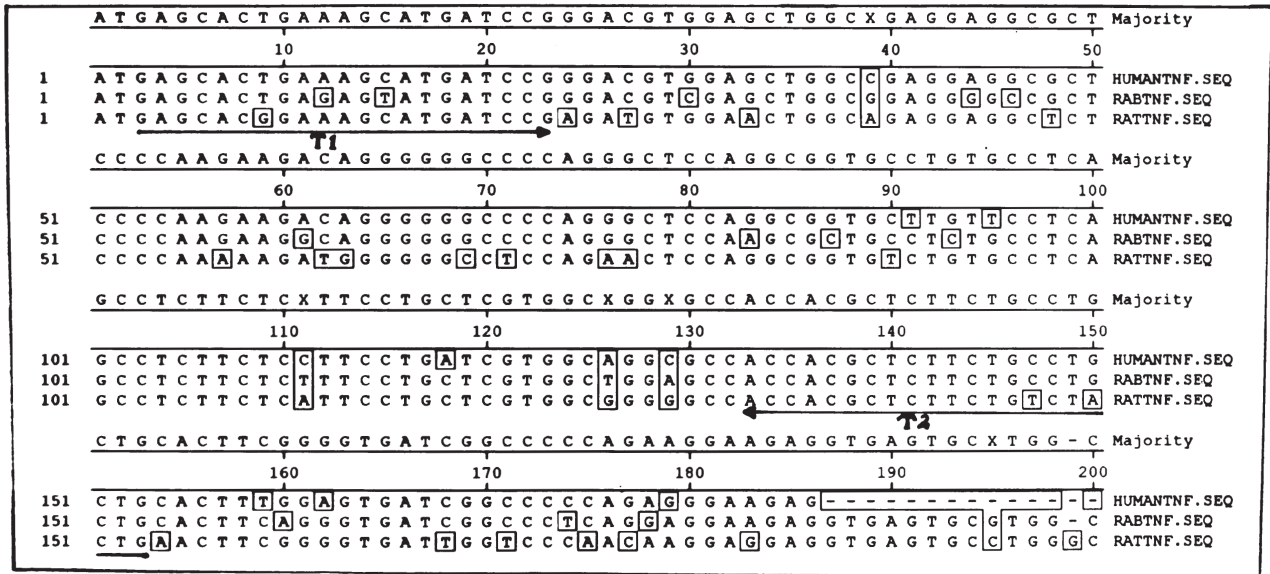
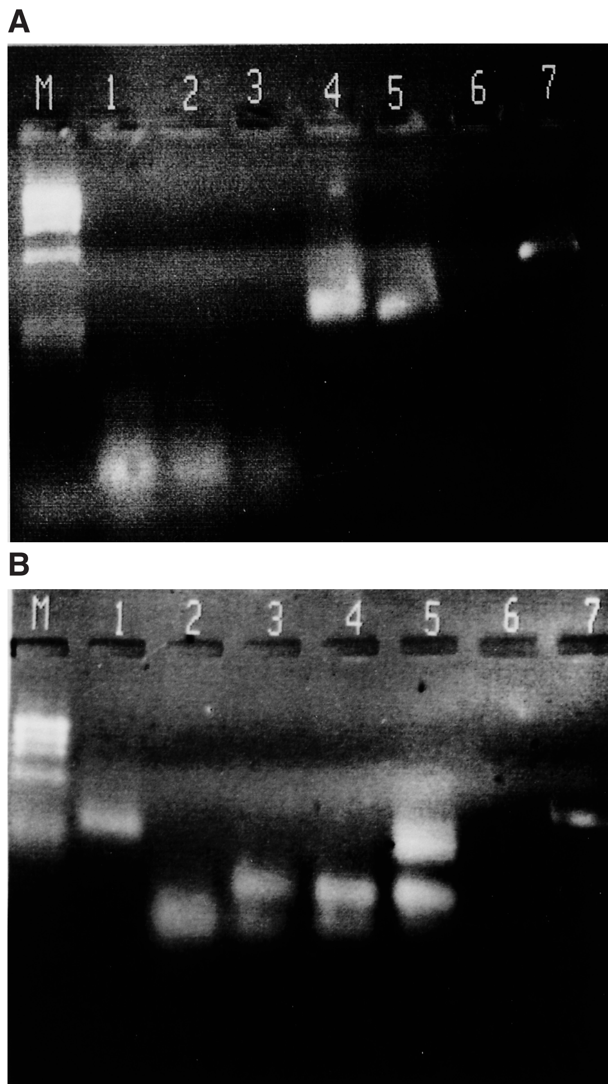



Figure 1. Position of universal primers on the conserved region produced after multiple sequence alignment of human, rat and rabbit TNF gene sequence.




other species. Universal primers thus designed can also be used to differentiate and amplify genes from the members of other species as well as for evolutionary and phylogenic related studies.

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**References**

Ito, H., Shirai, T., Yamamoto, S., Akira, M., Kawahara, S., Todd, C. and Wallace, R.B. 1986. Molecular cloning of the gene encoding rabbit tumor necrosis factor. DNA. 5: 157-165.

Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular Cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Shirai, T., Shimizu, N., Horiguchi, S. and Ito, H.1989. Cloning and expression in Escherichia coli of the gene for rat tumor necrosis factor. Agric. Biol. Chem. 53: 1733-1736.

Wang, A.M., Creasey, A.A., Ladner, M.B., Lin, L.S., Strickler, J., Van Arsdell, J.N., Yamamoto, R. and Mark, D.F. 1985. Molecular cloning of the complementary DNA for human tumor necrosis factor. Science. 228: 149-154.

Figure 2. (A) Band profile of PCR products on 2.5% Nusieve agarose gel; Lane M: marker lane (ϕX174-HaeIII digest); Lanes 1, 2 and 3: TNF gene from human, rat and rabbit, respectively using universal primers; Lanes 4 and 5: TNF gene from human and rat, respectively amplified using species specific primers; Lane 6: negative control and Lane 7: β-actin run as positive control
(B) Band profile on 2.5% Nusieve gel; Lane M: marker (ϕX174 -HaeIII digest); Lane 1: TNF gene from human using species specific primer; Lane 2: TNF gene using universal primers from human; Lanes 3 and 4: TNF gene from guinea pig using universal primers, Lane 5: TNF gene from monkey using universal primers; Lane 6: negative control and Lane 7: β-actin run as positive control.