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## **Amplification and Cloning of human interleukin-18 gene in mammalian expression vector and its expression study**

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### **Abstract**

The human interleukin -18 gene was amplified by reverse transcriptase polymerase chain reaction with M-MuLV reverse transcriptase enzyme from the total RNA of human peripheral blood lymphocytes. Taq DNA polymerase enzyme was used to amplify the gene as it adds 'A' overhang in the PCR product. This 'A' tailed product was cloned in TA cloning vector pTarget and transformed in *E. coli* DH5 $\alpha$  cells. The recombinant plasmid containing the human interleukin -18 gene insert in right orientation was selected after characterization using restriction enzyme analysis, directional PCR and nucleotide sequencing. The recombinant plasmid pTarget.hu IL-18 was observed to express IL-18 protein in vero cell as detected by fluorescent antibody and immunoperoxidase tests.

**Keywords:** Interleukin -18, cloning, human, PCR,

**Introduction**

Interleukin-18 is also known as interferon-gamma-inducing factor (Sarvetnick, 1997). Interleukin-18 (IL-18) is a novel proinflammatory cytokine with potent interferon (IFN)- $\gamma$  inducing activity that plays an important biological role in the enhancement of the activity of natural killer cells and cytotoxic T lymphocytes. The gene encodes a precursor protein of 192 amino acids and a mature protein of 157 amino acids. By analysis of a human/rodent somatic cell hybrid panel and radiation hybrid analysis, (Nolan *et. al.*, 1998) mapped the IL18 gene to 11q22.2-q22.3, close to the DRD2 gene. Interleukin-18 (IL-18), originally identified as an interferon (IFN)-  $\gamma$  inducing factor, was first isolated from livers of mice stimulated with *Propionibacterium acnes* and lipopolysaccharide (LPS) Okamura *et. al.*, (1995). IL-18 is a potent pro-inflammatory cytokine that induces IFN- $\gamma$  production in T cells and natural killer (NK) cells (Ushio *et. al.*, 1996) enhances the Fas ligand and perforin-mediated T cell and NK cell cytotoxicity (Dao *et. al.*, 1996, Takeda *et. al.*, 1998), and plays a critical role in the T-lymphocyte helper type 1 response (Takeda *et. al.*, 1998). IL-18 also induces expression of GM-CSF, inflammatory cytokines (e.g., tumor necrosis factor- $\alpha$ , IL-1 and IL-13), and chemokines, such as IL-8, macrophage inflammatory protein (MIP)-1 and MIP-1 (Ushio *et. al.*, 1996; Netea *et. al.*, 2000, Fehniger *et. al.*, 1999). Okamura *et. al.*, (1995) speculated that IGIF may be involved in the development of Th1 cells and also in mechanisms of tissue injury in inflammatory reactions. Administration of anti-IGIF antibodies prevented liver damage in mice inoculated with *Propionibacterium acnes* and challenged with lipopolysaccharide that induces toxic shock. Shida *et. al.*, (2001) found that 30% of normal subjects had a detectable, functionally inactive IL18 fragment, which they termed IL18 type 2, bound to IgM in plasma. The level of IL18 type 2 was 10- to 100-fold higher than that of conventional, active IL18 type 1 in these subjects. The IL18 cytokine increased expression of vascular cell adhesion molecule-1 (VCAM1) and the adherence of melanoma cells. Pizarro *et. al.*, (1999) detected increased IL18 mRNA and protein expression in intestinal epithelial cells and lamina propria mononuclear cells in Crohn disease tissue compared with ulcerative colitis and normal tissue. By immunohistochemical analysis, Corbaz *et. al.*, (2002) showed that IL18-binding protein (IL18BP) expression in intestinal tissue is increased in endothelial

cells as well as cells of the submucosa and overlying lymphoid aggregates in Crohn disease patients compared with controls. Okamura *et. al.*, (2000) showed that I118 has a protective effect against the development of chronic graft-versus-host disease (GVHD) in mouse. Using a murine bone marrow transplant (BMT) model, Reddy *et. al.*, (2001) showed that blockade of I118 accelerated acute GVHD mortality

## **Materials and Methods**

### **Isolation of RNA**

The peripheral blood mononuclear cells were isolated from the blood of healthy human using histopaque (Sigma, USA) as per manufacturer's instructions (Boyum, 1976). The total RNA was isolated from lymphocytes using TRIZOL reagent.

### **RT-PCR**

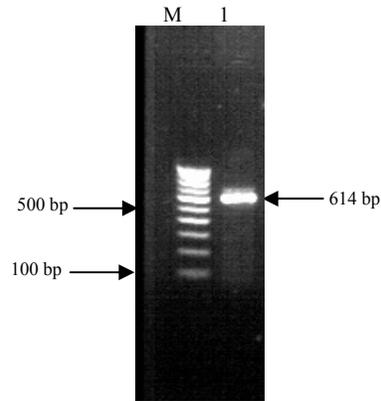
The oligonucleotide primers were designed as forward primer 5' CAG GAA TAA AGA TGG CTG CTG A -3' and reverse primer 5'- CCC GGC ATG AAA TTT TAA TAG C -3' using the published sequence (Genbank accession no. BC007461). RT-PCR reaction was performed to obtain cDNA using 10 µl of total RNA with 25 pmol random hexamer primer, 200mM dNTPs, and 40U of M-MuLV reverse transcriptase enzyme (Fermentas, USA) in 1X reaction buffer in total volume of 20 µl. The amplification step was performed at 37°C for 1 hour and 72°C for 10 min. The PCR was performed using 5 µl of cDNA along with forward and reverse primers (25 pmol each), 200 µM dNTPs, 1.5 mM MgCl<sub>2</sub> and 3U of Taq DNA polymerase (Fermentas, USA) in 1X reaction buffer in total volume of 50 µl. The cyclic conditions were initial denaturation at 94°C for 5 min followed by 32 cycles of denaturation at 94°C for 30 sec, primer annealing at 55°C for 50 sec and primer extension at 72°C for 50 sec (Sambrook and Russell, 2001) with final extension time of 8 min to facilitate the addition of 'A' nucleotide at 3' ends of amplified products.

### **Gene cloning**

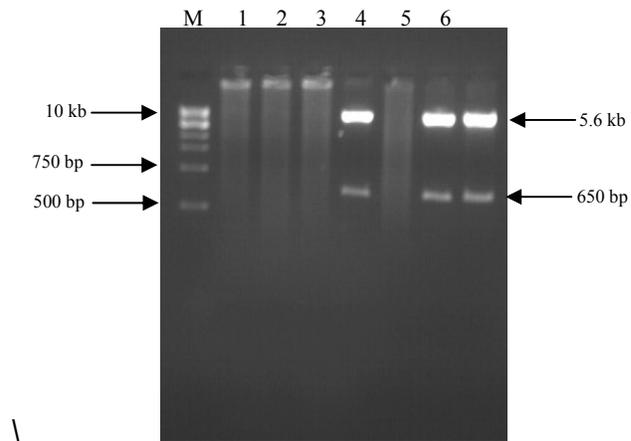
The PCR product was purified using QIAEX II gel extraction kit (Qiagen, Germany) and then ligated into pTarget vector (Promega, USA) using T4 DNA ligase (Promega, USA) at 16°C overnight. The description of pTarget mammalian expression vector has been given by (Rai *et al.* 2006). The ligated plasmid after transformation into *E. coli* DH5 $\alpha$  competent cells produced blue and white colonies. The white colonies were selected, subcultured and recombinant plasmids were isolated using QIAGEN Miniprep plasmid isolation kit (Qiagen, Germany). The presence of Il-18 gene in the recombinant plasmid was checked by restriction digestion with EcoR1 (Fermentas, USA) and the orientation of the gene insert was checked by colony PCR using T7 promoter primer present flanking 5' end of gene in the vector and reverse primer of the gene. The digested products and PCR products were analyzed on 1.0% agarose gel along with DNA molecular weight marker.

### **Results and Discussion**

The 614 bp human interleukin-18 gene was successfully amplified from the total RNA isolated from PBMC's (**fig 1**). The basic property of Taq DNA polymerase was used for TA cloning by addition of 'A' nucleotide to PCR product, since pTarget mammalian expression vector has T overhang at 5' end assisting the ligation process. The presence of interleukin-18 gene in the recombinant plasmid designated as pTarget.IL-18 was successfully detected by release of 650 bp gene insert on digestion with EcoRI enzyme (**fig. 2**). The orientation of gene was confirmed by PCR using T7 promoter primer and reverse primer of gene using recombinant plasmid DNA as template which yielded full length 634 bp PCR product in correct orientation while no product was obtained in wrong orientation (**fig.3**).

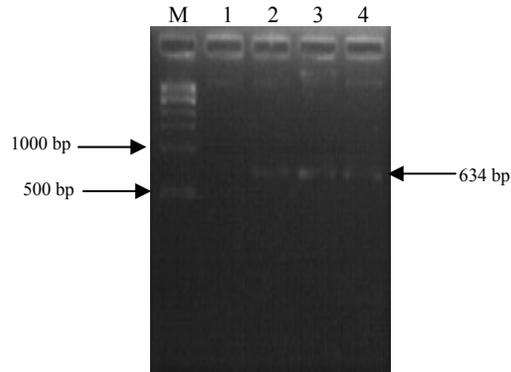


**Fig 1:** PCR amplification of human IL-18 gene 614bp. Lane M: 100 base pair DNA ladder, 1: PCR product of human IL-18 - 614bp.



**Fig 2:** Digestion of pTargetT.IL-18hu with EcoRI enzyme.

Lane M: 1 kb DNA ladder, 1, 2, 3&5: r-plasmid without insert; 4-7: 650 bp insert release of human IL-18 gene from pTargetT.IL-18hu,



**Fig 3** : PCR amplification of pTarget.IL-18hu with T-7 forward and gene specific reverse primer. Lane M : 1 kbDNA ladder, 1: No amplification, 2-4: 634 bp amplified IL-18hu gene.

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