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# Molecular Cloning of Gene Encoding Human Bone Morphogenetic Protein-2

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Bone morphogenetic proteins-2 (BMP-2) is a potent growth and differentiation factor for cartilage and bone induction and regeneration. BMP-2 regulates the chondrogenic and osteogenic differentiation and stimulates the synthesis of chondrocyte matrix components by human articular chondrocytes. To investigate the mechanism of chondrogenesis and osteogenesis underlying the role of BMP-2, the sufficient amount of recombinant human BMP-2 with functionally active is needed. Here, a gene encoding mature human BMP-2 was isolated and sequenced. Human BMP-2 expressed cell was cultured and extracted the total RNA. The complementary DNA was prepared by using reverse transciptase and oligo $dT_{18}$ primer. A single band at 342 bp in length (114 amino acids) of gene encoding mature human BMP-2 was amplified by polymerase chain reaction (PCR). The secondary PCR was performed to add nucleotide sequences coding for the restriction sites of *Eco*R I, 6xHistidine tag and factor Xa at 5′ end, whereas *Bam*H I was incorporated at its 3′ end. A 406 bp of amplified product obtained was ligated into the cloning vector using blunt-end ligation and transformed into *E.coli* host cells. Recombinant plasmids were randomly selected, characterized and verified the nucleotide sequences by using automated DNA sequencing. Analysis of insert nucleotide sequences showed the perfect similarity with human BMP-2 gene. In conclusion, we successfully cloned the gene encoding human BMP-2. This construct will be utilized for production recombinant human BMP-2, which will provide an important material for cartilage tissue engineering and might be applied for clinical therapy.

## 1. Introduction

Bone Morphogenetic Proteins (BMPs) are the key regulatory factors in chondrogenic and osteogenic differentiation, and also function in repair and remodeling of the adult skeletal system [1]. BMPs have been involved in the regulation of cell proliferation, survival, differentiation, apoptosis and stem cell properties. BMPs are the multi-functional growth factors belonging to the TGF-β superfamily, their binding lead to dimerization of receptors prior to phosphorylation and signaling through the Smad pathway [2]. Among others, BMP-2 is an attractive growth factor which regulates chondrogenic development of mesenchymal progenitors and stimulates the synthesis of chondrocyte matrix components by human articular chondrocytes *in vitro*. Therefore, the more information about the action of human BMP-2 on development and its role on chondrocyte is needed to be fulfilled; however, the limitation of studies involves the lack of sufficient amount of human BMP-2. The aim of the present study was to clone the gene encoding the human BMP-2, as an important material for production of the recombinant human BMP-2 for studying its function on chondrocyte development and proliferation and potential clinical applications.

## 2. Methodology

Human fetal osteoblast cell line (hFOB) was cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 50 units/ml penicillin G and 50 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% $CO_2$. Total RNA extraction was prepared and reverse transcribed into the complementary DNA (cDNA) by using the oligo $(dT)_{18}$ primer using the first strand cDNA Synthesis kit. The primers mBMP-2_1: 5′ CAAGCCAAACACAAACAGCG 3′ (forward primer), and mBMP-2_2: 5′ GCGACACCCA CAACCCTC 3′ (reverse primer), corresponding to sequences in the coding region of the mature human BMP-2 gene, were

selected for PCR amplification. The condition that used for PCR is 35 cycles of 94°C for 45 s, 55°C for 1 min and 72°C for 1 min. The reaction (25µl) consisted of cDNA template, 10 pmol each of primers, 200µM of each dNTPs, 1.5mM $MgCl_2$ in reaction buffer, pH8.5. The amplified gene was gel-purified and used as template for re-amplification. The *Eco*RI and *Bam*HI recognition sites were introduced at its 5′ and 3′ends respectively, and the recognition sequences for histidine 6 residues and factor Xa are added at its 5′end. The amplified DNA was subsequently ligated into pJET1.2/blut cloning vector and transformed into *E.coli* XL1 Blue. The recombinant clones which appeared on the culture plate were randomly screened by PCR, the desired recombinant clones were extracted the plasmid DNA and then analyzed with *Bgl*II restriction enzyme. The desired recombinant plasmid was subsequently characterized by *Eco*RI/*Bam*HI double-digestion and verified the insert DNA sequence by using automated DNA sequencing. The resulting nucleotide sequence was analyzed using Blast Similarity search tool on NCBI database. The recombinant plasmid harboring the correct nucleotide sequence was designated as pJET1.2mBMP-2(His).

## 3. Results and Discussion

A gene encoding for mature human BMP-2 was isolated from human fetal osteoblast cell line (hFOB). The total RNA was extracted and converted to cDNA by using oligo $dT_{18}$ primer. A single band at 342 bp was obtained after PCR amplification (Fig.1). In order to facilitate the cloning step, the *Eco*RI and *Bam*HI restriction sites were added at its 5′ and 3′ends respectively, whereas the histidine 6 amino acids and factor Xa were fused at N-terminus to facilitate the purification step. The amplified product at 406 bp in length was observed after analysis on 1.5% agarose gel electrophoresis. The ligated product into pJET1.2 plasmid vector was transformed into the *E.coli* XL1-Blue. Screening of recombinant clones showed that the recombinant clones containing the insert DNA fragment at approximately 406 bp, presumably to harbor human BMP-2 gene. After restriction analysis with *Bgl*II and the restriction analysis with *Eco*RI/*Bam*HI, the results revealed that the recombinant plasmid harboring the insert DNA sequence (Fig.2). Nucleotide sequence analysis of the recombinant plasmid was the perfect match (100%) for the mature human BMP-2 gene, confirming that the recombinant clone contained the gene encoding the human BMP-2.

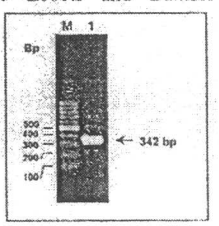


Fig. 1. Agarose gel electrophoresis of human BMP-2 gene from PCR amplification.

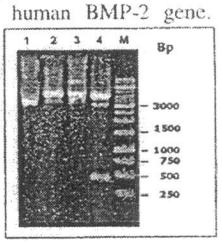


Fig.2. Agarose gel pattern of pJET1.2mBMP-2(His) characterization with restriction digestion.

## 4. Conclusions and Recommendations

We succeeded to isolate and construct the recombinant plasmid harboring the human BMP-2 gene. This human BMP-2 gene will be as the crucial source for construction of the recombinant human BMP-2, which can be applied for better understanding of its action and therapeutics based on cartilage tissue engineering, adult stem cell therapy, and bone or cartilage diseases in Thailand.

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# Production of Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2) in *Escherichia coli*

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**Abstract**

Bone morphogenetic proteins-2 (BMP-2) is a crucial growth factor for chondrogenic and osteogenic differentiation and regeneration. The aim of the present study is the cloning, expression, and characterization of the recombinant human bone morphogenetic protein-2 (rhBMP-2). A hBMP-2 gene was isolated from osteoblast by extraction of the total RNA and reverse-transcribed into the complementary DNA. A single band at 342 bp (114 amino acids) was amplified by polymerase chain reaction (PCR). To facilitate the cloning and purification step, the restriction sites of *Eco*R I, 6xHistidine tag and factor Xa were introduced at 5′ end, whereas *Bam*H I was incorporated at its 3′ end. The amplified product was ligated into the plasmid vector and sub-cloning into the expression vector using blunt-end ligation and transformed into *E.coli* XL1-Blue. Nucleotide sequence analysis of insert fragment was the perfect similarity with human BMP-2 gene. Recombinant hBMP-2 protein was successfully expressed as the soluble protein under the control of the *lac*UV5 and protein A promoters by IPTG induction. Analysis of purified protein by immunodetection and N-terminal amino acid sequencing confirmed that it was the recombinant human BMP-2. The availability of purified rhBMP-2 could be used as therapeutic agent for bone, dental and cartilage tissue repair.

**Keywords:** bone morphogenetic protein-2, growth factor, transforming growth factor-beta, recombinant protein, stem cells, cartilage tissue engineering

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