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# Expression of Human Interferon α-2b and *Escherichia coli* Methionine Aminopeptidase Genes in a Single Host Using Two Incompatible Plasmids

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**Abstract.**- It is a common understanding that incompatible plasmids carrying the same origin of replication cannot co-exist stably in a single *Escherichia coli* cell. However, there are scanty reports indicating that two incompatible plasmids carrying different antibiotic resistance genes can co-exist in *E. coli* for some time under the selection pressure of the two antibiotics. Based on this observation, we used two incompatible plasmids for the co-expression of two different genes. Human interferon α-2b and *E. coli* methionine aminopeptidase were cloned independently into two incompatible vectors, one with ampicillin and the other with kanamycin resistance. Expression of the genes resulted in the production of human interferon α-2b and *E. coli* methionine aminopeptidase in comparable amounts when these genes were expressed independently in *E. coli*. However, human interferon α-2b was produced in a much lesser amount whereas there was no difference in the production of methionine aminopeptidase when the encoding genes were co-expressed in a single cell.

**Keywords:** Human, interferon α-2b, *Escherichia coli*, methionine aminopeptidase, co-expression.

## INTRODUCTION

*Escherichia coli* is the most popular organism for high level production of recombinant proteins. However, not every gene can be expressed efficiently in this organism. This may be attributed to the unique structural features of the gene or major differences in codon usage. Some of the *E. coli* strains have been engineered in order to minimize the codon bias and high levels of recombinant proteins have been produced using these strains. However, some studies, like protein: protein interaction, require co-expression of two genes in a single cell. There are two ways of heterologous co-expression of two genes: the bicistronic (Li *et al*., 1997) and the dual-vector (Johnston *et al*., 2000) systems. The bicistronic system simulates the bacterial operon composition having more than one gene under the control of a single promoter. On the other hand, the dual-vector system consists of two different plasmids which can co-exist in the same host. It is a common understanding that two plasmids carrying the same origin of replication cannot co-exist stably in a single *E. coli* cell (Sambrook and Russle, 2001). However, there is a report on two incompatible plasmids carrying different antibiotic resistance genes, if under the selection pressure of the two different antibiotics, can co-exist in *E. coli* for few hours which are sufficient for the production of recombinant protein (Yang *et al*., 2001).

We evaluated the dual-vector system for the production of human interferon α-2b and *E. coli* methionine amino peptidase in *E. coli*. Interferons are small proteins produced in the human body in response to pathogens and perform functions related to the immune system (Reyes-Vázquez *et al*., 2012). Recombinant proteins are produced with a starting formylmethionine in *E. coli* which is usually cleaved, after deformylation (Flinta *et al.,* 1986), by an enzyme called methionine aminopeptidase that has been characterized from various organisms (Ben-Bassat *et al*., 1987; Kendall and Bradshaw, 1992; Chang *et al*., 1992; Arfin *et al*., 1995). This enzyme has relatively higher affinity towards proteins that contain a physically small residue

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adjacent to the starting methionine (Hirel *et al.*, 1989; Hwang *et al.*, 1999).

In the present study we report on cloning of the genes encoding human interferon α-2b and *E. coli* methionine aminopeptidase in pET-21a and pET-28a expression vectors, respectively, and expression profiles of these genes independently as well as by dual-vector transformation system.

## MATERIALS AND METHODS

### *Materials*

*E. coli* strains used were DH5α and BL21-CodonPlus (DE3)-RIL (Merck KGaA, Darmstadt, Germany). Plasmids pTZ57R/T (Thermo Scintific, MA, USA), pET-28a and pET-21a (Merck) were used for cloning and expression. DNA restriction enzymes, DNA polymerase, DNA extraction kit, InsTA PCR cloning kit and GeneJET Plasmid Miniprep kit were purchased from Thermo Scintific. QIAquick Gel Extraction kit was purchased from QIAGEN (QIAGEN Inc. CA, USA). All other reagents and chemical were analytical grade.

### *Cloning of human interferon α-2b gene*

Human genomic DNA was isolated from the blood of a healthy man using standard protocol (Blin and Stafford, 1976) and used as template for further DNA amplifications by polymerase chain reaction. In the first PCR reaction, a set of forward (5'-ACTTGGATCCTCTGCAACATCTACAATG) and reverse (5'-TAAGAAGCTTCGTGTCATGGTCATAGCA) primers was used to amplify the human interferon α-2b gene. The amplified product was then used as template for the second polymerase chain reaction using a set of forward (5'-CTCTCATATGTGTGATCTGCCTCAAACCCACAGC) and reverse (5'-TGAA AAGCTTTAAATCGTGTCATGGTC) primers. Underlined sequences in the forward and reverse primers are *Nde*I and *Hin*dIII recognition sites, respectively. PCR amplified gene fragment was ligated in pTZ57R/T vector using InsTAclone™ kit (Thermo Scintific) and the resulting plasmid was named pTZ-Ifn. In order to clone human interferon α-2b gene in the expression vector, plasmid pTZ-Ifn was digested with *Nde*I and *Hin*dIII and the liberated gene fragment was ligated in pET-21a. The resulting plasmid was named pET-Ifn.

### *Cloning of* E. coli *methionine aminopeptidase gene*

*E. coli* methionine aminopeptidase gene was amplified by polymerase chain reaction using a set of forward (5'-CATATGGCTATCTCAATCAAGACCCC) and reverse (5'-TTATTCGTCGTGCGAGATTATCGCC) primers as priming strands and genomic DNA of *E. coli* as template. The amplified gene fragment was cloned in pTZ57R/T and the resulting plasmid was named pTZ-Map. For cloning in expression vector, plasmid pTZ-Map was digested with *Nde*I and *Eco*RI restriction enzymes and the liberated gene fragment was ligated in pET-28a. The resulting plasmid was named as pET-Map.

### *Co-transformation of* E. coli *using pET-Map and pET-Ifn*

Host strains *E. coli* DH5α and BL21-CodonPlus (DE3)-RIL were made competent by the standard protocols (Sambrook and Russle, 2001). Equal amounts of pET-Map and pET-Ifn were used for co-transformation. The transformed competent cells were spread on LB agar plates containing 30 μg/ml kanamycin (selection for pET-Map) and 100 μg/ ml ampicillin (selection for pET-Ifn).

### *Expression of interferon α-2b and methionine aminopeptidase genes in* E. coli

In order to express the genes, *E. coli* BL21-CodonPlus (DE3)-RIL cells were transformed using pET-Ifn and pET-Map independently as well as collectively. The cells, containing the respective plasmid, were grown to early log phase and gene expression was induced with 1 mM lactose at 37°C in LB medium containing respective selection marker. The cells were harvested by centrifugation at 5,000×*g* for 10 min at 4°C, resuspended in 50 mM Tris-HCl (pH 8.0), and lyzed by sonication for 10 min at 4°C before analysis by gel electrophoresis.

## RESULTS AND DISCUSSION

*Cloning of interferon α-2b and methionine aminopeptidase genes*

Polymerase chain reaction for the amplification of human interferon α-2b gene, using the set of primers given in the materials and methods section as priming strands and human genomic DNA as a template, resulted in the amplification of 0.5 kb DNA fragment (Fig. 1A) exactly matching the size of human interferon α-2b gene. Similarly, polymerase chain reaction for the amplification of *E. coli* methionine aminopeptidase gene, using the set of primers given in the materials and methods section as priming strands and *E. coli* genomic DNA as a template, resulted in the amplification of 0.8 kb DNA fragment (Fig. 2A) exactly matching the size of *E. coli* methionine aminopeptidase gene.

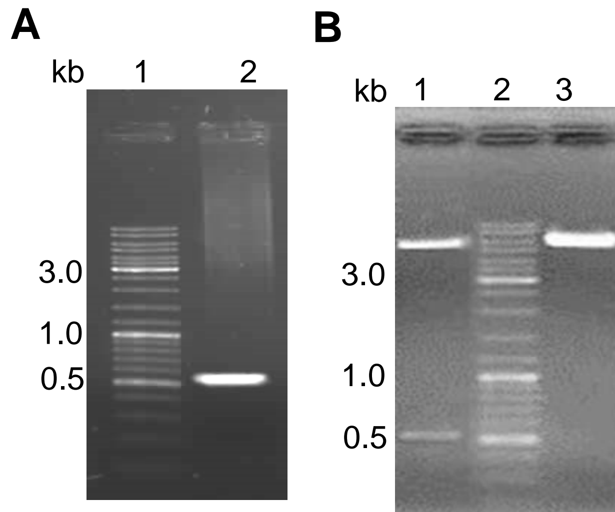


Fig. 1. Ethidium bromide stained agarose gel demonstrating cloning of human interferon α-2b. A) PCR amplification of human interferon α-2b gene. Lane 1, PCR amplified human interferon α-2b gene; lane 2, DNA standard marker. B) Cloning of human interferon α-2b gene in pET-21a expression vector. Lane 1, pET-Ifn plasmid cut with *Nde*I and *Hind*III; lane 2, DNA standard; lane 3, pET-Ifn plasmid cut with *Nde*I.

The amplified human interferon α-2b gene fragment was inserted in pTZ57R/T. When the resulting plasmid pTZ-Ifn was used to transform *E. coli* DH5α competent cells, a total of 26 white and 8 blue colonies appeared on the selection plates. White colonies were further screened for the presence of human interferon α-2b gene by polymerase chain reaction (data not shown). Plasmid DNA was isolated from one of the positive clones and digested with *Nde*I and *Hind*III which resulted in the liberation of a 0.5 kb DNA fragment from the vector (data not shown). Nucleotide sequence of the insert was determined and the results of DNA sequencing confirmed that the cloned fragment encodes human interferon α-2b. In order to clone in the expression vector, plasmid pET-21a was digested with the same two restriction enzymes (*Nde*I and *Hind*III) and resolved on agarose gel. Expression vector pET-21a and human interferon α-2b gene were purified from the gel and ligated. When the resulting plasmid pET-Ifn was used to transform *E. coli* DH5α competent cells, 13 colonies appeared on the selection plate. Positive clones were selected by polymerase chain reaction and one of the positive clones was confirmed by restriction enzyme digestion analysis (Fig. 1B).

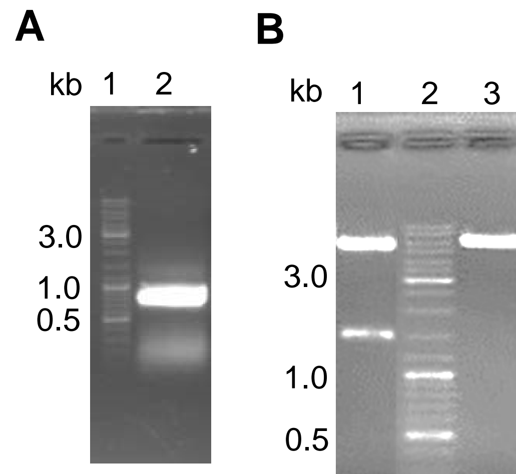


Fig. 2. Ethidium bromide stained agarose gel demonstrating cloning of *E. coli* methionine aminopeptidase gene. A) PCR amplification of *E. coli* methionine aminopeptidase gene. Lane 1, DNA standard marker; lane 2, PCR amplified methionine aminopeptidase gene. B) Cloning of *E. coli* methionine aminopeptidase gene in pET-28a expression vector. Lane 1, pET-Map plasmid cut with *Nde*I and *EcoR*I; lane 2, DNA standard; lane 3, pET-Map plasmid cut with *Nde*I.

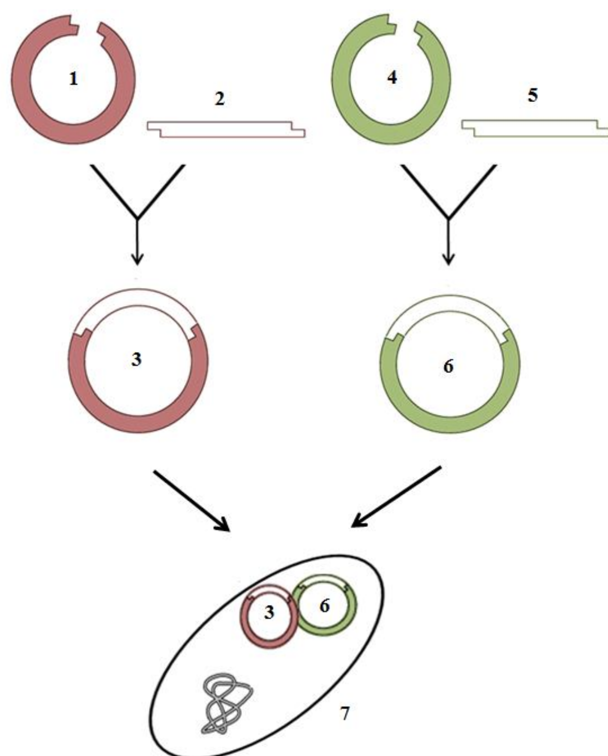


Fig. 3. A schematic representation of cloning of two genes in two incompatible plasmids having different antibiotics resistance and co-transformation of *E. coli*. 1, pET-28a plasmid cut with *Nde*I and *EcoR*I; 2, *E. coli* methionine aminopeptidase gene cut with *Nde*I and *EcoR*I; 3, recombinant pET-Map; 4, pET-21a plasmid cut with *Nde*I and *Hind*III; 5, human interferon α-2b gene cut with *Nde*I and *Hind*III; 6, recombinant pET-Inf; 7, *E. coli* BL21-CodonPlus (DE3)-RIL cell containing pET-Map and pET-Inf.

Similarly when *E. coli* DH5α cells were transformed using pTZ-Map, 12 white and 3 blue colonies appeared on the selection plate. After screening of the white colonies by polymerase chain reaction (data not shown), plasmid DNA was isolated from one of the positive clones and digested with *Nde*I and *EcoR*I which resulted in the liberation of 0.8 kb DNA fragment (data not shown). In order to clone the gene in the expression vector, plasmid pET-28a was digested with *Nde*I and *EcoR*I and ligated with aminopeptidase gene fragment liberated from pTZ-Map. When the resulting plasmid pET-Map was used to transform *E. coli* DH5α cells, a total of 17 colonies appeared on the selection plate. Positive clones were selected by polymerase chain reaction (data not shown) and one of the positive clones was confirmed by restriction enzyme digestion analysis (Fig. 2B).

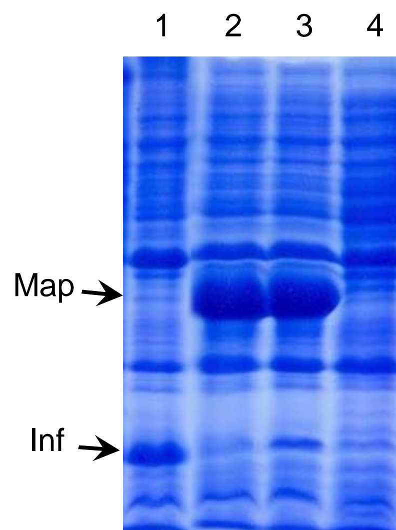


Fig. 4. Coommassie brilliant blue stained SDS-PAGE demonstrating the production of recombinant human interferon α-2b (Ifn) and *E. coli* methionine aminopeptidase (Map). Lane 1, lysate of cells containing pET-Inf; lane 2, lysate of cells containing pET-Map; Lane 3, lysate of cells containing pET-Inf and pET-Map; lane 4, lysate of cells containing pET-21a used as control in comparative analysis.

*Expression of interferon α-2b and methionine aminopeptidase genes in* E. coli

A schematic representation of cloning of the two genes in two incompatible plasmids having different antibiotics resistance and co-transformation of *E. coli* is shown in Figure 3. For expression of the two genes, *E. coli* BL21-CodonPlus (DE3)-RIL cells were transformed using recombinant plasmids pET-Ifn and pET-Map, independently. When the host cells carrying these plasmids were induced overnight with lactose, interferon α-2b and methionine aminopeptidase were produced in comparable amounts (Fig. 4). However, when *E. coli* BL21-CodonPlus (DE3)-RIL cells were transformed using both the recombinant plasmids, pET-Ifn and pET-Map, for co-expression of both the genes, there was no difference in the production level of methionine

aminopeptidase whereas, to our surprise, human interferon α-2b was produced in a much less amount (Fig. 4). We, therefore, propose here that it is not the plasmid that determines the amount of recombinant proteins in *E. coli* strain BL21-CodonPlus (DE3)-RIL, rather it is the preference of native over non-native by the host cells. When there was no competition both the proteins were produced in comparable amounts and when co-expressed the native protein was produced in a much higher amount. This knowledge should be taken into account when two non-identical genes are being co-expressed using dual-vector system.

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