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*Full Length Research Paper*

# cDNA, genomic sequence cloning and over-expression of ribosomal protein L15 gene (*RPL15*) from the giant panda

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**RPL15 is a component of the 60S large ribosomal subunit encoded by *RPL15* gene and belongs to the L15e family of ribosomal proteins, which is located in the cytoplasm. The cDNA and genomic sequence of *RPL15* was cloned successfully from the giant panda using RT-PCR and Touchdown-PCR technology, respectively. These two sequences were analyzed preliminarily and the cDNA of the *RPL15* gene was also over expressed in *Escherichia coli* BL21. The length of fragment cloned is 669 bp, containing an open-reading frame (ORF) of 615 bp encoding 204 amino acids. The length of the genomic sequence is 1,835 bp, with three exons and two introns. Primary structure analysis revealed that, the molecular weight of the putative RPL15 protein is 24.142 kDa with a theoretical pI 12.11. Topology prediction shows that there are two cAMP- and cGMP-dependent protein kinase phosphorylation sites, four N-myristoylation site, two Protein kinase C phosphorylation site, two Casein kinase II phosphorylation sites, two Amidation site and one Ribosomal protein L15e signature in the RPL15 protein of the giant panda. Alignment analysis indicates that, the nucleotide sequence of the coding sequence shows a high homology to other known *RPL15* sequences of *Homo sapiens, Bos taurus, Mus musculus*, *Rattus norvegicus*, *Canis familiaris* and *Danio rerio;* as determined by Blast analysis, 94.31, 89.92, 91.54, 91.22, 96.59 and 79.02%, respectively. The homologies for deduced amino acid sequence are all 100% compared with the first five animals and share a high homology with that of *D. rerio* by 95.59%. The cDNA of *RPL15* was cloned successfully from the giant panda in this study. It provides scientific material for enriching and improving the *RPL15* gene database. The *RPL15* gene can be really expressed in *E. coli* and the RPL15 protein fusioned with the N-terminally His-tagged protein gave rise to the accumulation of an expected 30 KDa polypeptide, in good agreement with the predicted molecular weight. The expression product obtained could be used for purification and study of its function further.**

**Key words:** Giant panda, RT-PCR, RPL15, genomic sequences, cloning, over-express.

## INTRODUCTION

Ribosome is responsible for protein synthesis in all organisms, and ribosomal proteins play important roles in the formation of a functional ribosome. Ribosomal proteins are the components of ribosome, which exhibit various secondary functions in DNA repair, apoptosis, drug resistance and proliferation (Wang et al., 2006).

With the continuous advancement of technology, the researchers gradually reveal the physiological functions of ribosomal proteins playing an important role in human disease and development (Yang et al., 2005). *RPL15* gene encodes a ribosomal protein that is a component of the 60S subunit. This protein belongs to the L15E family of ribosomal proteins. It is located in the cytoplasm. This gene shares sequence similarity with the yeast ribosomal protein YL10 gene. Although this gene has been referred to as RPL10, its official symbol is RPL15. As is typical for genes encoding ribosomal proteins, there are a multiple of processed pseudogenes of this gene dispersed through the genome (Kenmochi et al., 1998). Gastric

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cancer is the second most common cancer worldwide and cause of death. The experimental date shows that, the expression of RPL15 is markedly up-regulated in gastric cancer tissues. This gene was also highly expressed in gastric cancer cell lines AGS, MKN45, MKN28, SGC7901 and KATOIII (Wang et al., 2006). In addition, RPL15 protein has been shown to be over-expressed in some esophageal tumors compared to normal matched tissues. The researchers found that *RPL15* gene is expressed efficiently in esophageal cancer and it might play a possible role in carcinogenesis of esophagus (Wang et al., 2001). Therefore, our study of the RPL15 gene of the giant panda is of great value.

At present, the sequence information for *RPL15* gene has been reported in some plants and animals (Lee et al., 1999; Tom et al., 1999; Zhang et al., 2003; Xiang et al., 2006). However, *RPL15* gene from the giant panda (*Ailuropoda melanoleuca*) has not been reported yet. The giant panda (*A. melanoleuca*) is a rare species currently found only in China and belongs to the most endangered animals in the world. In the past years, scientists have won tremendous successes in many respects of giant panda. Recently, functional gene analysis is one of the hot issues in current giant panda research (Du et al., 2007; Hou et al., 2007, 2009; Hou et al., 2008, 2009a, b; Liao et al., 1990; Wu et al., 1990; Zhang et al., 2009).

In this study according to the related information of ribosomal protein *L15* gene (*RPL15*) of some mammalians designed primer, including *Homo sapiens*, *Mus musculus*, *Rattus norvegicus* and *Bos taurus*. Using RT-PCR technique to amplify the cDNA of *RPL15* gene from the total RNA and Touchdown-PCR technique, to amplify the genomic sequence of the *RPL15* from total DNA from the skeleton muscle of the giant panda, and then analyzed the sequence characteristics of the protein encoded by the cDNA and compared it with those of human and other animals reported. We also over-expressed it in *Escherichia coli* using pET28a plasmids. The availability of the RPL15 sequences from vertebrate and mammals allowed us to examine the relationship among these species, and to test the potential use of this gene in phylogenetic study. The study provides scientific material for inquiring into the hereditary traits and formulating the protective strategy for the giant panda.

## MATERIALS AND METHODS

### Materials

Skeletal muscle was collected from a dead giant panda at the Wolong Conservation Center of the giant panda, Sichuan, China. The collected skeletal muscle was frozen in liquid nitrogen and then used for RNA isolation.

### DNA and RNA isolation

The genomic DNA was isolated from giant panda muscle tissue according to protocol (Gao et al., 2007). The DNA obtained was dissolved in TE buffer and kept at -20°C. Total RNAs were isolated from about 400 mg of muscle tissue using the Total Tissue/Cell RNA Extraction Kits (Waton Inc., Shanghai, China), according to the manufacturer's instructions. The total RNAs extracted were dissolved in DEPC (diethypyrocarbonate) water, and kept at -70°C.

### Primers design, RT-PCR, cloning of RT-PCR products and sequencing

The PCR primers were designed by Primer Premier 5.0, based on the mRNA sequence of *RPL15* from *H. sapiens* (NM_001029), *M. musculus* (NM_013765), *R. norvegicus* (NM_013224) and *B. taurus* (NM_001024568). The specific primers of cDNA sequence are as follows:

Pd-*RPL15*-F: 5′-CGGCAGCCATCAGGTGAGCC-3′;
Pd-*RPL15*-R: 5′-TTAGGTATGAATTTTACAAA-3′.

Total RNAs were synthesized into the first-stranded cDNAs using reverse transcription kit with Oligo dT as the primers, according to the manufacturer's instructions (Promega). The 20 ∝l of first-strand cDNA synthesis reaction system included 1 ∝g of total RNAs, 5 mM of $MgCl_2$, 1 mM of dNTPs, 0.5 ∝g of Oligo $dT_{15}$, 10U/∝l of RNase inhibitor, and 15 U of AMV reverse transcriptase, and was incubated at 42°C for 60 min. The first-strand cDNA synthesized was used as a template. The total reaction volume for DNA amplification was 25 µl. Reaction mixtures contained 1.5 mM of $MgCl_2$, 200 µM of each of dATP, dGTP, dCTP and dTTP (Omega), 0.3 µM of each primer, 5.0 units of Taq plus DNA polymerase (Sangon Co., Shanghai, China). DNA amplification was performed using a MJ Research thermocycler, Model PTC-200 (Watertown, MA) with a program of 4 min at 94.0°C, followed by 30 cycles of 1 min at 94.0°C, 0.5 min at 45°C and 1.5 min at 72.0°C, and then ended with the final extension for 10 min at 72.0°C.

After amplification, PCR products were separated by electrophoresis in 1.5% agarose gel with 1× TAE (Tris-acetate-EDTA) buffer, stained with ethidium bromide and visualized under UV light. The expected fragments of PCR products were harvested and purified from gel, using a DNA harvesting kit (Omega, China), and then ligated into a pET28a vector at 22°C for 12 h. The recombinant molecules were transformed into *E. coli* complete cells (DH5α), and then spread on the LB-plate containing 50 µg/ml ampicillin, 200 mg/ml IPTG (isopropyl-beta-D-thiogalactopyranoside), and 20 mg/ml X-gal. Plasmid DNA was isolated and digested by *Pst*I and *Sca*II to verify the insert size. Plasmid DNA was sequenced by Huada Zhongsheng Scientific Corporation (Beijing, China).

### Cloning the genomic sequence of *RPL15*

The PCR primers were the same as the Pd-*RPL15*-F and Pd-*RPL15*-R presented earlier. The genomic sequence of the *RPL15* gene was amplified using Touchdown-PCR with the following conditions: 94°C for 30 s, 55°C for 45 s, 72°C for 4 min in the first cycle and the anneal temperature decreased 0.5°C per cycle; after 2 cycles conditions changed to 94°C for 30 s, 52°C for 45 s, 72°C for 4 min for another 20 cycles. Finally, the recombinant fragment was sequenced by Sangon (Shanghai, China).

### Construction of the expression vector and over-expression of recombinant RPL15

PCR fragment corresponding to the RPL15 polypeptide was amplified from the *RPL15* cDNA clone with the forward primer: 5'-CAGGATCCATGGGTGCATACAA - 3' (BamHI) and reverse primer,

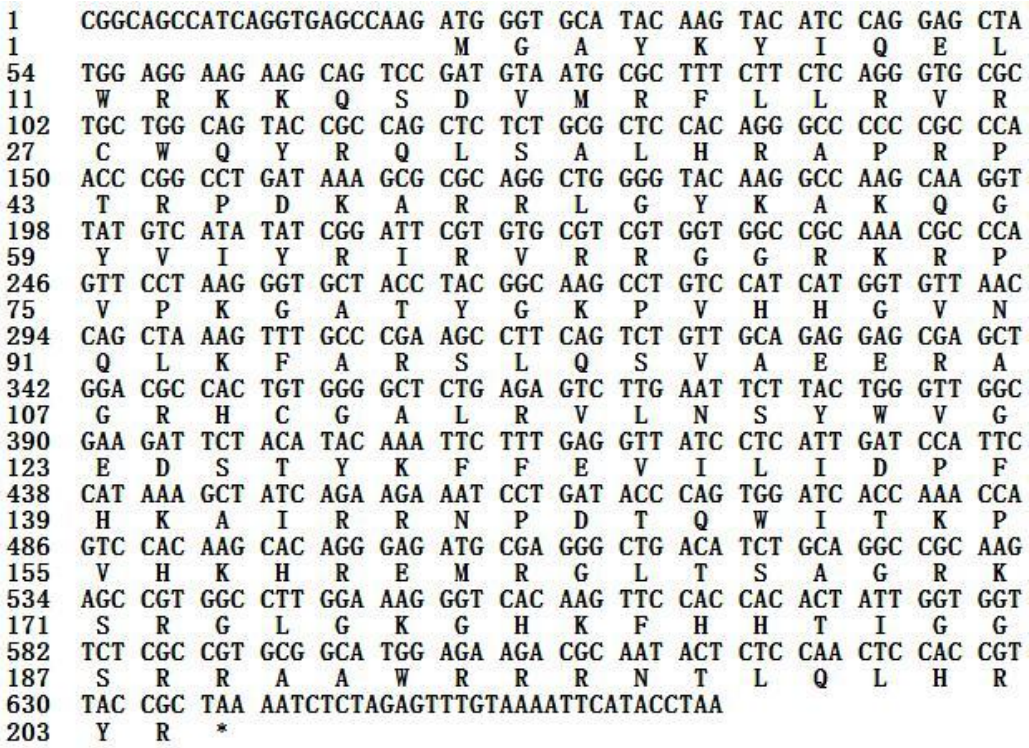


**Figure 1.** Nucleotide and deduced amino acid sequences of cDNA encoding the giant panda *RPL15.* The asterisk (*) represents stop codon.

5'- CGAAGCTTAGCGGTAACGG - 3' (HindIII), respectively. The PCR was performed at 94°C for 3 min; 35 cycles of 30 s at 94°C, 45 s at 55°C and 1 min at 72°C, 10 min at 72°C. The amplified PCR product was cut and ligated into the corresponding site of pET28a vector (Stratagen). The resulting construct was transformed into *E. coli* BL21 (DE3) strain (Novagen) and used for the induction by adding IPTG (isopropyl-b-D-thiogalactopyranoside) at an OD600 of 0.6 and cultured further for 4 h at 37°C, using the empty vector transformed BL21(DE3) as a control. The recombinant protein samples were induced after 0, 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 h and then separated by SDS-PAGE and stained with Commassie blue R 250.

#### Data analysis

The sequence data was analyzed by GenScan software (http://genes. mit.edu/ GENSCAN.html). Homology research of the giant panda *RPL15* compared with the gene sequences of other species was performed using Blast 2.1 (http://www.ncbi. nlm.nih.gov/blast/). ORF of the DNA sequence was searched using ORF finder software (http://www. ncbi.nlm.nih.gov/gorf/gorf.html). Protein structure of the RPL15 sequence cloned was analyzed using PredictProtein software (http://cubic. Bioc. columbia. edu/predictprotein/).

## RESULTS

### Analysis of the cDNA of *RPL15* from the giant panda

About 670 bp of cDNA fragment was amplified from the giant panda. The length of the cDNA cloned is 669 bp. Blast search showed that the cDNA sequence cloned is highly homologous with the *RPL15* from *H. sapiens* and some other animals reported. On the basis of the high identity, we concluded that the cDNA isolated is the cDNA encoding the giant panda RPL15 protein. The *RPL15* sequence has been submitted to Genbank (accession number: HM047806). An ORF of 615bp encoding 204 amino acids was found in the cDNA sequence (Figure 1).

### Analysis of the genomic sequence of *RPL15* from the giant panda

A DNA fragment of about 2000 bp was amplified with primers *RPL15* -F and *RP L15* -R. The length of the DNA fragment cloned is 1835 bp, containing the 5′-untranslated sequence of 23 bp and the 3′-untranslated region of 31 bp size length. Comparison between the cDNA sequence and the DNA fragment sequence of the *RPL15* amplified from giant panda was performed by software lasergene. the result indicated that, the cdna sequence is in full accord with three fragments in the DNA fragment, which manifests that the DNA fragment amplified is the genomic sequence of the *RPL15* from giant panda. The genomic sequence of the *RPL15* has been submitted to Genbank (accession number: HM047807).


**Figure 2.** Comparison of the RPL15 amino acid sequences among the different species. __: cAMP- and cGMP-dependent protein kinase phosphorylation site; ▮: Protein kinase C phosphorylation site; : Ribosomal protein L15e signature; I: Amidation site; __ : N-myristoylation site; I: Casein kinase II phosphorylation site : polymorphism site. (Pa: *Ailuropoda melanoleuca*; Ho: *Homo sapiens*; Mu: *Mus musculus*; Ra: *Rattus norvegicus*; Bo: *Bos taurus*; Ca: *Canis familims*; Da: *Danio rerio*).

**Prediction and analysis of protein functional sites in RPL15 protein of the giant panda**

Primary structure analysis revealed that, the molecular weight of the putative RPL15 protein of the giant panda is 24.142 kDa with a theoretical pI 12.11. Topology prediction which shows that there are two cAMP- and cGMP- dependent protein kinase phosphorylation sites, four N-myristoylation sites, two Protein kinase C phosphorylation sites, two Casein kinaseII phosphorylation site, one Amidation site and one ribosomal protein L15e signature in the RPL15 protein of the giant panda (*A. melanoleuca*) (Figure 2).

**Over-expression of the *RPL15* gene in *E. coli***

The *RPL15* gene was over-expressed in *E. coli*, using pET28a plasmids carrying strong promoter and terminator sequences derived from phage T7. For this purpose, the *RPL15* gene was amplified individually by PCR and cloned in a pET28a plasmid, resulting in a gene fusion coding for a protein bearing a His-tag extension at the N-terminus. Expression was tested by SDS-PAGE analysis of protein extracts from recombinant in *E. coli* BL21 strains (Figure 3).

The results indicated that the protein *RPL15* fusion with the N-terminally His-tagged form, gave rise to the accumulation of an expected 30 kDa polypeptide that formed inclusion bodies. Apparently, the recombinant protein was expressed after half an hour (½ h) of induction and after 2 h, it reached the highest level. These results suggested that the protein is active and just the protein encoded by the RPL15 from the giant panda. The expression product obtained could be used to purify the protein and further study its function.

**DISCUSSION**

Here, we report the identification and characterization of genomic sequence and cDNA clone encoding ribosomal

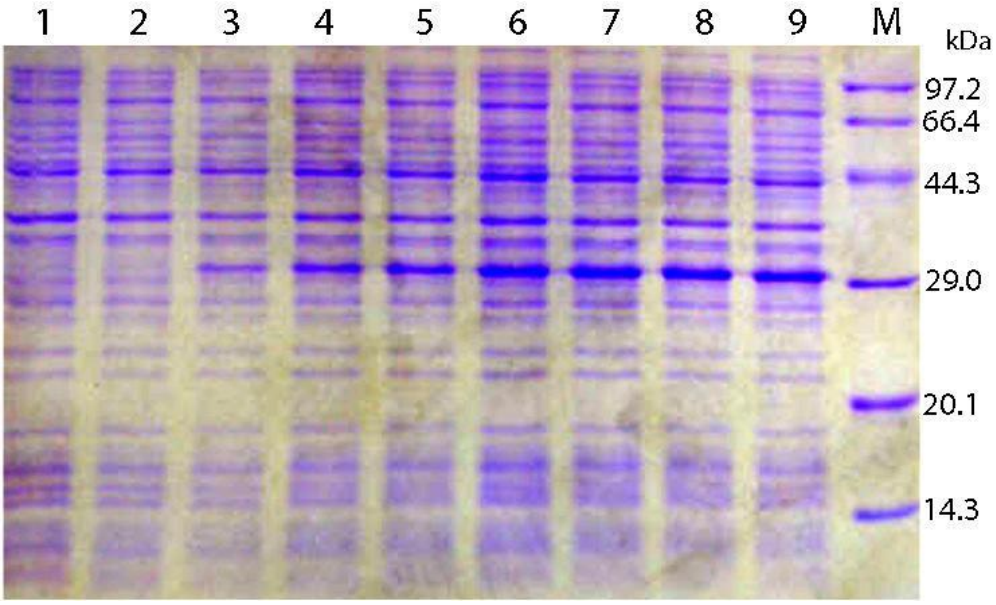


**Figure 3.** Protein extracted from recombinant *E. coli* strains were analyzed by SDS-PAGE gel stained with Commassie blue R 250. Numbers on the right shows the molecular weight, and the site of 30 indicates the recombinant protein bands induced by IPTG with 0, 0.5, 1,1.5, 2 , 2.5 **,** 3 and 3.5 h (lane 2 to 9), respectively. Lane 1 represents the products of the *E. coli* strains with the empty vectors.

protein L15 from giant panda. The genomic sequence of *RPL15* is 1835 bp in size. A comparison of the nucleotide sequences of the genomic and cDNA sequences indicated that, the genomic sequence of *RPL15* possesses three exons and two introns, which is also supported by restriction mapping of the genomic and cDNA sequences. Compared with some mammals and vertebrates including *H. sapiens, B. taurus, M. musculus*, *R. norvegicus*, *Canis familims* and *Danio rerio*, the three exons, which comprise the cDNA sequence of *RPL15* gene after RNA splicing, is highly conserved and remain essentially the same. The restriction sites in the exons are the same in both the cDNA and the genomic sequences. On the contrary, the two introns are different in length (Table 1). From the table, we can see that the length of the first intron of the *RPL15* gene from giant panda is 311 bp, while the length ranges from124 bp (*B. taurus*) to 2094 bp (*D. rerio*) in the aforestated six species. The length of the second intron of the *RPL15* gene from giant panda is 856 bp, while the length ranges from 590 bp (*R. norvegicus*) to 3131 bp (*D. rerio*) in the earlier stated six species. The variations in lengths of the introns determine the lengths of the *RPL15* genes. The length of cDNA fragment cloned is 669 bp with a 615 bp open reading frame with ATG as initiation codon and TAA as stop codon, which contains 24.1% A; 27.2% C; 27.0% G and 21.8% T. Alignment analysis of *RPL15* among the giant panda and other animals, indicated that both the nucleotide sequence and the deduced amino acid sequence are highly conserved. There is not any deletion and insertion of nucleotide and amino acid residue.

The deduced amino acid sequence encodes a protein of 204 residues with a molecular mass of 24.142 kDa and isoelectric point of 12.11. As with most ribosomal proteins, L15 is highly basic, containing a combined 61 Arg, Lys, and His residues and only 11 Asp and Glu residues, in which the highest content of Asp residues (16.18%), is far higher than other amino acids and Cys content of the lowest, only 0.98%. Physical and chemical analysis showed that, the molecular weight and theoretical pI of the putative protein among those animals are very close (Table 2). We analyzed the functional sites of the amino acid sequences encoded by RP*L15* genes and found that there are two cAMP- and cGMP-dependent protein kinase phosphorylation sites, four N-myristoylation site, two protein kinase C phosphorylation sites, two casein kinaseII phosphorylation sites, two amidation site and one ribosomal protein L15e signature in the RPL15 protein of the giant panda, *H. sapiens*, *B. taurus*, *M. musculus*, *R. norvegicus*, *C. familiaris* and *D. rerio* (Figure 2). That is to say, the functional sites are entirely identical in RPL15 proteins of these animals. However, the identity among the giant panda, *H. sapiens*, *B. taurus*, *M. musculus*, *R. norvegicus* and *C. familiaris* L15 sequence and their slight difference from the *D. rerio* L15 sequence were somewhat more enigmatic.

Further analysis detected nine polymorphic sites in the amino acid sequences of the six species compared. In particular, the nonconservative substitution of Ile for Met at position 7, Ala for Ser at position 35, Leu for Ile at

**Table 1.** Comparison of *RPL15* genomic among 7 species.

| Species | Genomic length | Number of exons | Number of introns | 5'UTR (bp) | 3'UTR (bp) | Intron 1 | Intron 2 | Joint sites in the CDS | GenBank accession Nos. |
|---|---|---|---|---|---|---|---|---|---|
| *Ailuropoda melanoleuca* | 1835 | 3 | 2 | 23 | 31 | 311 | 856 | 24-195,507-643, 1499-1801 | HM_047807 |
| *Homo sapiens* | 3696 | 3 | 2 | 712 | 1342 | 408 | 619 | 713-884,1293-1429, 2049-2354 | NC_000003 |
| *Mus musculus* | 3223 | 3 | 2 | 487 | 1239 | 241 | 641 | 488-659,901-1037, 1679-1984 | NC_005114 |
| *Rattus norvegicus* | 3164 | 3 | 2 | 536 | 1188 | 235 | 590 | 537-708,944-1080, 1671-1976 | NC_000080 |
| *Bos taurus* | 3949 | 3 | 2 | 451 | 1543 | 124 | 1216 | 452-623,748-884, 2101-2406 | NC_007328 |
| *Canis familims* | 12316 | 3 | 2 | 1118 | 9340 | 386 | 842 | 1119-1290,1677-1813, 2656-2961 | NC_006605 |
| *Danio rerio* | 7595 | 3 | 2 | 802 | 52 | 2094 | 3131 | 803-974,3970-4106, 7238-7543 | NC_007130 |

**Table 2.** The comparison of *RPL15* gene's and encoding sequence among *Ailuropoda melanoleuca* and six other species.

| | ***Ailuropoda melanoleuca*** | ***Homo sapiens*** | ***Mus musculus*** | ***Rattus norvegicus*** | ***Bos taurus*** | ***Canis familims*** | ***Danio rerio*** |
|---|---|---|---|---|---|---|---|
| Molecular weight (kDa) | 24.1420 | 24.1420 | 24.1420 | 24.1420 | 24.1420 | 24.1420 | 24.0599 |
| Theoretical (pI) | 12.11 | 12.11 | 12.11 | 12.11 | 12.11 | 12.11 | 12.04 |

position 92, Ala for Gly at position 112, Pro for Thr at position 137, Arg for Lys at position 169 and 193, Pro for Ala at position 154 and Hist for Leu at position 182, respectively, of the giant panda, *H. sapiens*, *B. taurus*, *M. musculus*, *R. norvegicus* and *C. familiaris* sequence in the *D. rerio* suggest that these 9 residues are not critical for the function of this molecule (Figure 2). These polymorphic sites are located irregularly in the amino acid sequences all of which result from the transversion or transition of the corresponding codons without any deletion and insertion of base. Among these polymorphic sites, site 46 and 69 are located in ribosomal protein L15e signature, but it does not result in any differences from giant panda and other six species in the functional site. Alternatively, any one of the sequence differences at these nine positions in the *D. rerio* may serve to compensate for the others in preserving the structural and functional integrity of the L15 protein. The fact shows that, the variation of sites has no affect on the structure and function of RPL15 protein and it may be the result during the evolution of these species.

However, what changes caused by other mutations outside the functional sites in the structure and the function of RPL15 need further studies. The *RPL15* gene obtained is expressed efficiently in prokaryotic organism such as the *E. coli* using Pet28a plasmids, and the gained fusion protein is in accordance with the expected 30 kDa polypeptide (Figure 3). These results suggest that, the protein is active and it is just the protein encoded by the RPL15 from the giant panda. The expression product obtained could be used for purification and further study of its function.

The characterization of genomic sequence and cDNA clones encoding ribosomal proteins would be beneficial in the study of ribosomal biogenesis and would allow the elucidation of structure and regulation of genes encoding ribosomal proteins in eukaryote. These data will enrich and supplement the information about *RPL15*. This paper provides theoretical references for the construction of molecular phylogenetic tree. In addition, it will contribute to the protection of gene resources and the discussion of the genetic polymorphism.


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