

cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca)

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ABSTRACT. The ribosomal protein L9 (RPL9), a component of the large subunit of the ribosome, has an unusual structure, comprising two compact globular domains connected by an α-helix; it interacts with 23 S rRNA. To obtain information about rpL9 of Ailuropoda melanoleuca (the giant panda), we designed primers based on the known mammalian nucleotide sequence. RT-PCR and PCR strategies were employed to isolate cDNA and the rpL9 gene from A. melanoleuca; these were sequenced and analyzed. We overexpressed cDNA of the rpL9 gene in Escherichia coli BL21. The cloned cDNA fragment was 627 bp in length, containing an open reading frame of 579 bp. The deduced protein is composed of 192 amino acids, with an estimated molecular mass of 21.86 kDa and an isoelectric point of 10.36. The length of the genomic sequence is 3807 bp, including six exons and five introns. Based on alignment analysis, rpL9 has high similarity among species; we found 85% agreement of DNA and amino acid sequences with the other species that have been analyzed. Based on topology predictions, there are two N-glycosylation sites, five protein kinase C phosphorylation sites, one casein kinase II phosphorylation site, two tyrosine kinase phosphorylation sites, three N-myristoylation sites, one amidation site, and one ribosomal protein L6 signature 2 in the L9 protein of *A. melanoleuca*. The *rpL9* gene can be readily expressed in *E. coli*; it fuses with the N-terminal GST-tagged protein, giving rise to the accumulation of an expected 26.51-kDa polypeptide, which is in good agreement with the predicted molecular weight. This expression product could be used for purification and further study of its function.

Key words: Giant panda (*Ailuropoda melanoleuca*); Genomic sequence; Ribosomal protein gene L9 (rpL9); cDNA; Cloning; Overexpression