

实验研究

伯氏疟原虫 *pbmag-1* 基因片段克隆及原核表达优化

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摘要

【摘要】 目的 克隆并表达伯氏疟原虫 *pbmag-1* 基因cDNA片段。方法 在GenBank中检索伯氏疟原虫编码基因 *pbmag-1* 部分cDNA序列, 设计特异引物, 经RT-PCR从伯氏疟原虫ANKA株扩增出该基因的部分cDNA片段。以锚定Oligo dT引物反转录mRNA获得的cDNA为模板, 利用已知序列设计特异引物, 通过cDNA末端快速扩增(RACE)技术延伸 *pbmag-1* 3'端未知的编码序列, 并将其克隆于原核表达载体后转入大肠埃希菌 (*E. coli*) BL21-(DE3)-RIL 株, 经优化诱导条件, 表达了重组蛋白PbMAG-1并用其免疫小鼠。结果 获得1 341 bp具有完整3'末端序列的 *pbmag-1* 基因片段, 其A/T含量为73%。以包涵体形式表达的重组蛋白免疫小鼠, 其血清抗体经蛋白质印迹 (Western blotting) 分析, 能特异性地识别伯氏疟原虫感染红细胞相对分子质量约为 *Mr* 64 000 的蛋白。结论 获得重组蛋白PbMAG-1的3'端完整的 *pbmag-1* 基因cDNA片段, 为研究伯氏疟原虫PbMAG-1蛋白在鼠疟免疫反应中的作用奠定了实验基础。

关键词 [pbmag-1](#) [伯氏疟原虫](#) [基因克隆](#) [原核表达](#)

分类号

Cloning and Optimized Prokaryotic Expression of a *pbmag-1* cDNA Fragment from *Plasmodium berghei* ANKA

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Abstract

【Abstract】 Objective To clone and express a novel gene cDNA fragment, *pbmag-1*, from *Plasmodium berghei* ANKA strain. Methods The cDNA sequence of *pbmag-1* was obtained from the GenBank of *P. berghei* ANKA genomic databases, with which a pair of primers was designed and RT-PCR was used to get a cDNA fragment of the gene from the parasite. The expanded cDNA 3' fragment of the gene was obtained by 3'-RACE using the oligo dT primer and a set of specific primers. The intact cDNA 3' fragment was cloned into a prokaryotic expression vector and transformed into the BL21-(DE3)-RIL strain of *Escherichia coli*. The recombinant protein of PbMAG-1 was expressed with an optimized strategy and used to immunize mice. Results The *pbmag-1* cDNA fragment obtained was 1 341 bp in length, A/T rich (73%) and with a correct 3' end sequence. By Western blot, the anti-serum of mice immunized with the recombinant protein of PbMAG-1/GST, which was expressed as inclusion bodies, specifically recognized a band with *Mr* 64 000 molecule from the protein extracts of *P. berghei*-infected mouse erythrocytes. Conclusion The *pbmag-1* cDNA sequence with intact 3' has been obtained, which will be used for further study on its role in the immune response of *P. berghei* infection.

Key words [pbmag-1](#) [Plasmodium berghei](#) [Gene cloning](#) [Prokaryotic expression](#)

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