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实验研究

华支睾吸虫F_0-ATP合酶b亚基基因的克隆表达和重组蛋白的免疫原性分析

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摘要

目的 对华支睾吸虫F_0-ATP合酶b亚基（CsF_0-ATP-synt_B）基因进行克隆、表达及重组蛋白的免疫原性分析。 方法 以华支睾吸虫成虫cDNA文库中含有F_0-ATP合酶b亚基基因的质粒为模板，扩增该基因（去除线粒体靶向序列的成熟肽基因），将其克隆到原核表达质粒pET-28a（+）中，转化大肠埃希菌BL21（DE3），异丙基硫代-β-D-半乳糖苷（IPTG）诱导表达，经亲和层析获得的纯化表达产物免疫SD大鼠，制备抗血清。蛋白质印迹（Western blotting）分析重组蛋白的免疫原性。 结果 华支睾吸虫F_0-ATP合酶b亚基成熟肽基因的编码区含813个碱基，编码271个氨基酸，相对分子质量（*Mr*）为31 171.9。经亲和层析获得的重组蛋白可被其免疫的大鼠血清识别。 结论 华支睾吸虫F_0-ATP合酶b亚基基因可在原核表达系统中获得具有免疫原性的高效表达。

关键词 华支睾吸虫 F_0-ATP合酶b亚基 基因克隆 原核表达 免疫原性

分类号

Cloning and Expression of the F0-ATP Synthase b Chain of *Clonorchis sinensis* and Immunogenicity Identification of the Recombinant Protein

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Abstract

Objective To clone and express the *Clonorchis sinensis* F_0-ATP synthase b chain （CsF_0-ATP-synt_B） gene and analyze immunogenicity of the recombinant protein. Methods The coding region F_0-ATP synthase b chain gene with the mitochondrial targeting sequence （MTS） removed was amplified with PCR using the cloned plasmid as template， and the product was cloned into the prokaryotic expression vector pET-28a（+）， transformed into *E.coli* BL21（DE3） and induced with IPTG. The expressed product was purified by Ni-IDA affinity chromatography，and analyzed by SDS-PAGE for its expression and identified by Western blotting for its immunogenicity. Results The coding sequence of the F_0-ATP synthase b-chain like gene removed off the MTS contains 813 base pairs encoding 271 amino acids with a theoretical molecular weight of 31 171.9. PCR， double enzyme digestion and DNA sequencing confirmed that the recombinant plasmid pET-28a（+）-CsF0-ATP-synt_B was constructed successfully，and the resoluble expression was obtained in *E.coli* BL21. Highly purified recombinant protein was prepared through affinity chromatography. The recombinant protein could be recognized by the immune serum of the SD rat immunized with the recombinant protein. Conclusion The CsF_0-ATP-synt_B like gene has been efficiently expressed in prokaryotic expression system with immunogenicity.

Key words Clonorchis sinensis F_0-ATP synthase b chain Molecular cloning Prokaryotic expression Immunogenicity

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