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刚地弓形虫尿苷磷酸化酶的克隆、表达与免疫反应性检测

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Gene-cloning, Expression and Immunoreactivity Detection of Toxoplasma gondii Uridine Phosphorylase

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摘要 【摘要】 目的 预测刚地弓形虫尿苷磷酸化酶（uridine phosphorylase，TgUPase）基因编码蛋白的主要特性和抗原表位，克隆、表达TgUPase基因，并检测其免疫反应性。 方法 采用生物信息学在线分析程序和软件预测TgUPase蛋白的理化性质和抗原表位。提取RH株弓形虫速殖子总RNA。根据TgUPase基因全长编码序列（GenBank登录号为DQ385446.1）的开放阅读框设计引物，并用RT-PCR扩增，扩增产物经双酶切后连接入pET-30a（+）载体。用重组质粒转化大肠埃希菌（E. coli）DH5α，阳性菌落经PCR、双酶切和测序鉴定。将重组质粒pET-30a（+）-TgUPase转化至E. coli BL21（DE3），并加入异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达。十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）结合考马斯亮蓝染色检测表达产物，分别以抗His标签抗体和人抗弓形虫血清为一抗，蛋白质印迹（Western blotting）分析重组蛋白及其免疫反应性。 结果 生物信息学预测结果显示，TgUPase蛋白由303个氨基酸组成，相对分子质量（Mr）为33 042.9，为可溶性蛋白，有3个潜在的T/B细胞联合抗原表位。RT-PCR扩增产物约为921 bp。菌落PCR、双酶切以及测序结果表明，重组质粒pET-30a（+）-TgUPase构建成功。SDS-PAGE结果表明，经IPTG诱导获得约Mr 38 000的可溶性重组蛋白（带His标签）。Western blotting结果显示，重组蛋白能被His标签抗体和人抗弓形虫血清识别。 结论 成功构建了重组质粒pET-30a（+）-TgUPase，获得刚地弓形虫重组尿苷磷酸化酶，且重组蛋白具有免疫反应性。

关键词： 刚地弓形虫 尿苷磷酸化酶 生物信息学分析 基因克隆 原核表达 免疫反应性

Abstract： 【Abstract】 Objective To predict the physicochemical properties and antigenic epitopes of Toxoplasma gondii uridine phosphorylase（TgUPase）, clone, and express TgUPase gene, and analyze its immunoreactivity. Methods The physical and chemical characters and specific epitopes of TgUPase protein were predicted by bioinformatics software tools. Total RNA was extracted from RH strain T. gondii tachyzoites. A pair of specific primers was designed according to the open reading frame of TgUPase gene （GenBank Accession No. DQ385446.1）. RT-PCR product was digested with restric-tion enzyme and ligated into a pET-30a（+） vector. The recombinant plasmid pET-30a（+）-TgUPase was transformed into E. coli DH5α and the positive clones were selected by colony PCR and confirmed by double restriction enzyme digestion and sequencing. The constructed pET-30a（+）-TgUPase was then transformed into E. coli BL21（DE3） and induced with IPTG for expression. The expression product was analyzed through SDS-PAGE followed by Coomassie blue staining. Western blotting assay with His primary antibody and human anti-T. gondii serum was used to confirm the expression of rTgUPase and detect its immunoreactivity. Results Bioinformatics prediction results showed that rTgUPase protein was 303 amino acids in length with a predicted molecular mass of Mr 33 042.9, and this soluble protein had three potential T/B cell epitopes. The product of RT-PCR was 921 bp. Colony PCR, double restriction enzyme digestion and DNA sequencing confirmed that the recombinant plasmid pET-30a（+）-TgUPase was constructed. SDS-PAGE showed that bacteria containing recombinant plasmid pET-30a（+）-TgUPase expressed a soluble protein of His-TgUPase （about Mr 38 000） after being induced with IPTG. The recombinant protein reacted positively with His primary antibody and human anti-T. gondii serum by Western blotting analysis. Conclusion The recombinant plasmid pET-30a（+）-TgUPase is constructed and the soluble rTgUPase shows immunoreactivity.

Keywords： Toxoplasma gondii Uridine phosphorylase Bioinformatics analysis Gene cloning Prokaryotic expression Immunoreactivity

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