

论著

弓形虫RH株膜表面抗原2全长基因的高效表达与抗原性分析

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摘要

目的 构建原核重组表达质粒pET23aSAG2, 并在大肠埃希菌中实现高效表达, 以及检测表达产物的抗原性。**方法** PCR扩增SAG2编码基因目的片段, 琼脂糖凝胶电泳回收纯化, 克隆至pMD18T载体, 转化大肠埃希菌DH5 α 。测序后亚克隆至表达质粒载体pET23a, 构建重组表达质粒pET23aSAG2, 转化大肠埃希菌DH5 α 。筛选阳性克隆, 经限制性酶切分析鉴定后, 转化大肠埃希菌BL21(DE3), 以异丙基 β D-硫代半乳糖苷诱导表达。十二烷基硫酸钠聚丙烯酰胺凝胶电泳(SDS PAGE)与免疫印迹分析表达产物。**结果** PCR扩增出约500bp的SAG2编码基因目的片段, 与预期片段大小相符, 经测序鉴定无基因突变; 所构建的pET23aSAG2重组表达质粒阳性克隆经PCR与双酶切鉴定, 与预期结果一致; 含有pET23aSAG2重组质粒的大肠埃希菌BL21(DE3)诱导后得到了高效表达, SDS PAGE显示表达产物约Mr19000; 免疫印迹结果表明表达产物具有良好的抗原性。**结论** 成功构建了pET23aSAG2表达质粒, 实现了全长成熟SAG2蛋白在大肠埃希菌中的高效表达; 表达产物具有良好的抗原性。

关键词 [弓形虫属](#) [表面抗原](#) [基因表达](#) [蛋白质印迹法](#)

分类号

High Efficiency Expression and Antigenicity Analysis of the SAG2 Gene From *Toxoplasma gondii* RH Strain

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Abstract

Objective To make high efficiency expression of the SAG2 gene from *Toxoplasma gondii* RH strain in *E. coli* and study the antigenicity of the expressed product. **Methods** The SAG2 gene fragment of *T. gondii* RH strain amplified by PCR method from genome DNA was cloned into the pMD-18T vector and transformed into *E. coli* DH5 α . After nucleotide sequencing, the SAG2 gene fragment was subcloned into the expression vector pET23a with correct orientation and transformed into *E. coli* DH5 α . The plasmid from the correct clone identified by PCR method and endonuclease digestion was transformed into *E. coli* BL21(DE3) and induced for expression. The expressed product was studied by SDS PAGE and Western blot. **Results** 502 bp gene fragment was amplified by PCR as anticipated. Nucleotide sequencing showed a 100% homology with that of the published sequence in GenBank. The molecular weight of the expressed protein was about Mr 19000. Western blotting indicated that the antigenicity of the protein was specific. **Conclusion** The plasmid pET 23a SAG2 was constructed and a high efficiency expression of the SAG2 gene from *T. gondii* RH strain was made. The expressed product shows a specific antigenicity.

Key words [Toxoplasma](#) [Surface antigen](#) [Gene expression](#) [Western blotting](#)

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