中国寄生虫学与寄生虫病杂志
CHINESE JOURNAL OF PARASITOLOGY AND PARASITIC DISEASES
ISSN 1000-7423
CN 31-1248/R
主管：中华人民共和国卫生部
主办：中华预防医学会
中国疾病预防控制中心寄生虫病预防控制所

中国寄生虫学与寄生虫病杂志 » 2011, Vol. 29 » Issue (1) :29-32 DOI:

微小隐孢子虫病毒衣壳蛋白的原核表达及鉴定

刁玉梅, 宫鹏涛, 李巍, 苏利波, 黄祥盛, 李建华, 张西臣*

吉林大学畜牧兽医学院，长春 130062

Prokaryotic Expression and Identification of S-dsRNA Gene from *Cryptosporidium parvum* Virus

DIAO Yu-Mei, GONG Feng-Chao, LI Wei, SU Li-Bei, HUANG Xiang-Cheng, LI Jian-Hua, ZHANG Xi-Chen-*

College of Animal Science and Veterinary Medicine，Jilin University，Changchun 130062，China

摘要 | 参考文献 | 相关文章

Download: PDF (230KB) HTML 1KB Export: BibTeX or EndNote (RIS) Supporting Info

摘要 目的 对微小隐孢子虫病毒衣壳蛋白S-dsRNA 基因进行克隆、表达和反应原性分析。 方法 以微小隐孢子虫总RNA逆转录的cDNA为模板，克隆S-dsRNA基因，并转化至原核表达载体pET-28a（+）中，构建重组原核表达载体pET-28a（+）-S， 转入大肠埃希菌BL21（DE3）中，用异丙基-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）观察重组蛋白的表达情况，蛋白质印迹（Western blotting）分析重组蛋白与鼠抗微小隐孢子虫阳性血清的反应原性。 结果 PCR和双酶切鉴定表明，重组质粒pET-28a（+）-S构建成功。SDS-PAGE 结果显示，37 ℃下经1 mmol/L IPTG 诱导4 h，重组蛋白表达量最大。重组蛋白主要以包涵体形式表达，相对分子质量（Mr）约为37 000，与预期大小一致，重组蛋白约占蛋白总量的72.6%。Western blotting分析结果表明，重组蛋白能识别抗微小隐孢子虫阳性鼠血清。 结论 微小隐孢子虫病毒衣壳蛋白S-dsRNA 基因表达成功，重组蛋白具有反应原性。

关键词： 微小隐孢子虫 dsRNA 病毒 S-dsRNA 原核表达

Abstract: Objective To clone and express S-dsRNA gene of *Cryptosporidium parvum* virus, and investigate the reactionogenicity of the recombinant. Methods Total RNA was extracted from *Cryptosporidium parvum* and S-dsRNA gene was amplified by RT-PCR. The PCR product was cloned into pET-28a（+） expression vector. The recombinant plasmid pET-28a（+）-S was transformed into *E. coli* BL21 （DE3） and induced with IPTG. The expression situation of recombinant protein was analyzed by SDS-PAGE. Its reactionogenicity was examined by Western blotting analysis. Results pET-28a（+）-S was identified by PCR and double endonuclease digestion. SDS-PAGE result showed that the recombinant protein （M_r 37 000） was expressed in the form of inclusion body. High level expression of recombinant protein was found at 1 mmol/L IPTG condition after incubation at 37 ℃ for 4 h and reached up to 72.6 % of the total protein. The protein was recognized by the antisera from mice immunized with antigens from Cryptosporidium parvum oocysts. Conclusion The S-dsRNA gene of *Cryptosporidium parvum* virus has been expressed with adequate reactionogenicity.

Keywords: *Cryptosporidium parvum* dsRNA virus S-dsRNA Prokaryotic expression

Service
把本文推荐给朋友
加入我的书架
加入引用管理器
Email Alert
RSS
作者相关文章
刁玉梅
宫鹏涛
李巍
苏利波
黄祥盛
李建华
张西臣*

引用本文:
刁玉梅, 宫鹏涛, 李巍, 苏利波, 黄祥盛, 李建华, 张西臣*.微小隐孢子虫病毒衣壳蛋白的原核表达及鉴定[J] 中国寄生虫学与寄生虫病杂志, 2011,V29(1): 29-32

DIAO Yu-Mei, GONG Feng-Chao, LI Wei, SU Li-Bei, HUANG Xiang-Cheng, LI Jian-Hua, ZHANG Xi-Chen-*.Prokaryotic Expression and Identification of S-dsRNA Gene from *Cryptosporidium parvum* Virus[J] , 2011,V29(1):29-32