中国寄生虫学与寄生虫病杂志 2009 27 (3): 210-214 ISSN: 1000-7423 CN: 31-1248/R

论著

东方巴贝虫cDNA文库的构建与免疫学筛选

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收稿日期 修回日期 网络版发布日期 接受日期

摘要
目的 构建东方巴贝虫（*Babesia orientalis*）cDNA文库，从中筛选免疫学阳性的克隆。 方法 用东方巴贝虫感染牛，提纯红细胞内虫体的总RNA，反转录合成cDNA，PCR扩增后将其连接于噬菌体载体（λTriplEx2），通过体外包装，建成东方巴贝虫cDNA文库，并进行扩增。用兔抗东方巴贝虫的血清筛选cDNA文库，阳性克隆经大肠埃希菌BM25.8自身环化酶将噬菌体重组子λTriplEx2转化为相应的质粒重组子pTriplEx2，PCR鉴定插入片段大小，并测序，用Blast对测序结果进行同源性分析，并对序列编码的氨基酸序列进行结构和功能的预测。 结果 未扩增文库的滴度为2.0×10^6 pfu/ml，重组率为98.8%，文库插入片段大小为500~3 000 bp，扩增后的滴度为5.8×10^8 pfu/ml。从东方巴贝虫cDNA文库中筛选得到3个阳性克隆B04、B05和B41，插入片段大小分别约为1 300、1 000和2 400 bp，3个片段均包含开放阅读框，分别与多种原虫的核动蛋白、功能未知的假定蛋白和热激蛋白70具有较高的同源性。B04、B05和B41分别编码310、192和647个氨基酸，相对分子质量(*Mr*)分别约为34 000、21 000和70 700。 结论 构建了较高质量的东方巴贝虫cDNA文库，并发现3个东方巴贝虫阳性克隆。

关键词 东方巴贝虫；cDNA文库；免疫学筛选；序列分析

分类号

Construction and Immunoscreening of cDNA Library of *Babesia orientalis*

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Abstract
Objective To construct a cDNA library for *Babesia orientalis* and screen immunologically positive clones. Methods Total RNA of B. orientalis in red blood cells from an infected calf was isolated. cDNA was synthesized by reverse transcriptase, amplified by PCR and ligated into λTriplEx2 vector. The recombined vectors were packaged and the unamplified cDNA library was constructed. The cDNA library was then amplified and immunologically screened with rabbit anti-B. orientalis serum. The recombinant λTriplEx2 of positive clones were converted to the corresponding recom-binant pTriplEx2. The inserted fragments were identified by PCR amplification. The plasmids were sequenced and compared against GenBank database by Blast. Results The titer of the unamplified library was 2.0×10^6 pfu/ml. The inserted fragment length of the library ranged from 500 to 3 000 bp, and the recombination efficiency accounted for 98.8%. The titer of the amplified library was 5.8×10^8 pfu/ml. Three positive clones were selected by serum immunological screening and named B04, B05, and B41, respectively. The inserted fragments of the B04, B05 and B41 were about 1 300 bp, 1 000 bp, and 2 400 bp, respectively. Sequence analysis revealed that the 3 clones contained open reading frames. Blast results showed that they were highly homologous to the nuclear movement protein gene, the hypothetical protein gene and the heat shock protein 70 (HSP70) gene, respectively. The deduced amino acid sequences of B04, B05 and B41 contained 310, 192 and 647 amino acid residues, with *Mr* of 34 000, 21 000, and 70 700, respectively. Conclusion A qualified cDNA library of *B. orientalis* has been constructed and three positive clones of *B. orientalis* discovered.

Key words *Babesia orientalis*; cDNA library; Immunoscreening; Sequence analysis

DOI:

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