论著

日本血吸虫组织蛋白酶L1基因的编码区全序列分析及克隆

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目的 分析日本血吸虫组织蛋白酶L1(SjCL1)基因编码区的完整序列,并定向克隆到真核表达质粒pcD-NA3中。 方法 从日本血吸虫成虫提取总RNA,进行反向巢式RT-PCR,T载体克隆后测序。PCR扩增SjCL1 基因的编码区序列,并将扩增产物克隆到pcDNA3质粒的BamHI和Xhol位点上。结果 通过反向巢式RT-PCR扩增出332 bp SjCL1基因5′端序列,测序后与报道的SiCL1基因部分序列拼接,可得到一个编码317 个氨基酸的完整编码区序列。PCR特异性扩增出SjCL1编码区基因序列,其大小约为1 kb。经酶切、PCR 鉴定和测序表明所构建的质粒pcDNA-SjCL1中含有所扩增的基因序列。 结论 构建了含SjCL1基因的编 码区序列的真核表达质粒pcDNA-SiCL1。

关键词 RT-PCR 日本血吸虫 组织蛋白酶L 基因克隆 分类号

Analysis and Cloning of Proteinase Cathepsin L1 Gene Coding Sequence of Schistosoma japonicum

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Abstract

Objective To analyze the full coding sequence of proteinase cathepsin L1 (SjCL1) of Schistosoma japoni-cum, and clone it into the eukaryotic expression vector pcDNA3. Methods Total RNA was isolated from adult worms of S. japanicum, the sequence of SjCL1 gene 5'-end was attained by performing averse nested PCR, and the sequence of SjCL1 gene 5'-end was determined by sequencing after being cloned into T vector. The coding region gene of SjCL1 was amplified by PCR, and the fragment from PCR was cloned into eukaryotic expression vector pcDNA3 via Bam HI and Xho I sites. The resulting construct was determined by PCR, restriction analysis and sequencing. Results A 320 bp sequence of SjCL1 gene 5'-end of Schistosoma japonicum was obtained by using averse nested PCR. After combined with the reported segment of SjCL1 gene, an integral coding sequence was obtained. The coding region of SjCL1 gene was specifically amplified by PCR, with a size of about 1 kb. The expression plasmid pcDNA-SjCL1 contained the amplified fragment, which is validated by PCR, restriction analysis and sequencing. Conclusion The eukaryotic expression plasmid containing the coding sequence of SjCL1 gene was constructed.

Key words RT-PCR Schistoscma japonicum cathepsin L gene cloning

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