研究简报

马来丝虫肌球蛋白部分编码基因Bm-M55的克隆与真核表达

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摘要

根据马来丝虫肌球蛋白部分编码基因(*Bm*-M55)序列设计引物,以其微丝蚴总RNA为模板,反转录PCR扩增目的基因。用TA克隆方法将目的基因克隆至载体pGEM?鄄T Easy中,经PCR和双酶切鉴定并测序后,亚克隆至真核表达质粒pcDNA3.1(+),构建真核表达载体pcDNA3.1(+)/Bm-M55,转染COS-7细胞后进行RT-PCR验证。用十二烷基硫酸钠-聚丙烯酰胺电泳(SDS-PAGE)对获得重组蛋白Bm?鄄M55进行分析和鉴定。RT-PCR鉴定结果显示,转染的COS-7细胞表达了Bm-M55基因,根据克隆的目的基因序列推导的氨基酸序列与GenBank(登录号为AAA27858)中的一致,重组蛋白Bm-M55相对分子质量(*M*r)约为 55 000。

Cloning and Eukaryotic Expression of the Gene Encoding Myosin from *Brugia malayi*

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Abstract

Total RNA was extracted from periodic microfilariae of Brugia malayi and its myosin partial gene (*Bm*-M55) was amplified by RT-PCR. The PCR product was cloned and then subcloned into pcDNA3.1 (+) vector. The recombinant eukaryotic plasmids were screened and identified by digestion with restriction enzyme and PCR amplification, and was transfected into COS-7 cells subsequently. The expressed protein was identified by SDS-PAGE. Bm-M55 mRNA was highly expressed in transfected COS-7 cells. The deduced amino acid sequence showed to be identical with that of Bm-M55, and the recombinant protein was about *M*r 55 000.

Key words <u>Brugia malayi</u> <u>Myosin</u> <u>Eukaryotic expressing vector</u> <u>COS-7 cell</u>

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