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论著

长角血蜱饥饿雌蜱cDNA表达文库的构建及免疫学筛选

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摘要

目的 构建长角血蜱饥饿雌蜱cDNA表达文库，筛选长角血蜱功能性抗原基因。 方法 在无RNA酶污染的条件下提取长角血蜱总RNA，进而纯化mRNA，以寡脱氧胸腺核苷酸［oligo（dT）］为引物合成双链cDNA，并在其两端加*Eco*RⅠ/*Hin*dⅢ定向接头。将cDNA分子定向克隆至具有*Eco*RⅠ/*Hin*dⅢ黏性末端的λSCREEN载体。用噬菌体包装蛋白对以上连接产物进行体外包装以形成完整的噬菌体，转化大肠埃希菌（*Escherichia coli*）ER1647，从而构建长角血蜱饥饿雌蜱cDNA表达文库。使用兔抗长角血蜱全蜱血清对该文库进行免疫学筛选，经过2次筛选得到的阳性噬菌体转化*E. coli* BM25.8亚克隆为重组质粒，转化*E. coli* JM109，提取重组质粒进行PCR和测序分析。 结果 长角血蜱饥饿雌蜱cDNA表达文库的基础库容量为1.8×106 pfu，重组率为100%，扩增后的滴度为2.4×109 pfu/ml。筛选获得42个阳性克隆，序列分析表明有12个新cDNA序列，其编码蛋白与长角血蜱原肌凝蛋白、环状扇头蜱幼蜱未知蛋白、黑腹果蝇染色体2R、褐黄血蜱线粒体DNA、青海血蜱HqL09、Hq05和肌球蛋白轻链mRNA等具有同源性。 结论 构建了长角血蜱饥饿雌蜱cDNA表达文库，获得的阳性克隆为长角血蜱功能性抗原的研究奠定基础。

关键词 长角血蜱 雌蜱 cDNA表达文库 免疫学筛选

分类号

Construction of cDNA Expression Library of Unfed Female Haemaphysalis longicornis and Immuno-Screening

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Abstract

Objective To construct a cDNA expression library from unfed female tick *Haemaphysalis longicornis* for screening and cloning potential antigenic genes. Methods Total RNA was isolated from unfed female ticks，mRNA was purified and a library of oligo（dT）-primed cDNA with added directional *Eco*RⅠ/*Hin*dⅢ linkers was constructed from the purified mRNA. The constructed cDNA was ligated to the *Eco*RⅠ/*Hin*dⅢ arms of the λSCREEN vector. Pure phage stocks were harvested by plaque purification and converted to plasmid subclones by plating phage on host strain BM25.8. Recombinant plasmids that were subcloned to E. coli BM25.8 were isolated and transformed into *E. coli* JM109. Recombinant plasmids abstracted from JM109 were identified by PCR and sequencing. Rusults The recombinant phage DNA was packaged by using phage-marker packaging extracts，resulting in a primary cDNA library with a size of 1.8×106 pfu. Data showed 100% of the library were recombinant and the titer of the amplified library was 2.4×109 pfu/ml. Forty-two clones of encoding immunodominant antigens were obtained from the cDNA library. Sequence analysis revealed 12 unique cDNA sequences and the encoded putative proteins showed similarities to *H. longicornis* tropomyosin mRNA，Rhipicephalus annulatus unknown larval protein mRNA, chromosome 2R of Drosophila melanogaster, mitochondrial DNA of *H. flava*, clones HqL09 unkown mRNA and Hq05 mRNA of *H. qinghaiensis*, and myosin alkali light chain protein mRNA. Conclusion The cDNA expression library from unfed female *H. longicornis* was successfully constructed and screening of protective genes may provide candidate antigens of the tick.

Key words *Haemaphysalis longicornis* Female tick cDNA expression library Immunoscreening

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