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实验研究

PCR检测大瓶螺体内广州管圆线虫幼虫方法的建立

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摘要

目的 建立一种基于PCR方法检测大瓶螺体内广州管圆线虫幼虫的方法。 方法 从美国生物信息中心GenBank中获得广州管圆线虫感染性III期幼虫（L_3）cDNA特异性片断，应用美国 DNASTAR公司Lasergene软件，设计特异性引物。TRIzol 一步法抽提广州管圆线虫感染性L_3和大瓶螺总RNA，按RT-PCR试剂盒提供方法进行PCR 扩增。 结果 用RT?鄄PCR方法能检测出阴性与感染性螺，其最低检出的总RNA量相当于1条广州管圆线虫L_3；将阴性大瓶螺总RNA与感染期幼虫总RNA不同浓度混合，PCR法可检测出肉眼能分辨的电泳条带相当于总RNA浓度为128 pg。此方法可以检测出广州管圆线虫III期幼虫RNA的最低值为105 pg。 结论 建立了PCR检测大瓶螺体内广州管圆线虫幼虫的方法。

关键词 大瓶螺 PCR cDNA 广州管圆线虫 幼虫

分类号

Development of PCR Assay for Detection of *Angiostrongylus cantonensis* in *Pomacea canaliculata*

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Abstract

Objective To establish a PCR assay for detecting the third-stage larvae of *Angiostrongylus cantonensis* in *Pomacea canaliculata*. Methods Polymerase chain reaction primers were designed by the software Lasergene， based on the specific cDNA of the third-stage larvae of *A.cantonensis* in Genbank. The total RNA was prepared from the third-stage larvae of *A.cantonensis* and of the snails by TRIzol one-step protocol. Amplification by RT-PCR was carried out following the kit protocol. Results RT-PCR assay revealed a clear differentiation between infected and negative snails. When a mixture of the total RNA from the negative snails and the third-stage larvae of *A.cantonensis* was tested by the PCR assay， the detectable level was 128 pg RNA， a concentration close to one third-stage larva of *A.cantonensis*， mini-mum concentration that could be found by naked eyes. The minimum detected total RNA concentration of the third-stage larvae of *A.cantonensis* was 105 pg by PCR assay. Conclusion A PCR assay has been developed for detecting *A.cantonensis* larva in *Pomacea canaliculata*.

Key words *Pomacea canaliculata* PCR cDNA *Angiostrongylus cantonensis* Larva

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