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论著

# 荧光定量PCR检测异尖线虫类病原体

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摘要
【摘要】 目的 运用荧光定量PCR法检测异尖线虫类病原体。 方法 于鱼类内脏中检获6种异尖线虫类幼虫：抹香鲸异尖线虫、简单异尖线虫、内弯对盲囊线虫、带鱼针蛔线虫、灰海鳗对盲囊线虫和台湾海峡鱼类中一优势种对盲囊线虫。提取各虫体DNA，PCR扩增ITS?鄄2序列，测序并进行数据库比对。依据测序结果设计特异引物，常规PCR检验引物特异性。将ITS?鄄2序列扩增产物回收、纯化后经T克隆转入大肠埃希菌DH5α，提取重组质粒，鉴定后作为标准品模板建立荧光定量PCR标准曲线，并做敏感性和重复性试验。 结果 构建的荧光定量PCR标准曲线循环阈值与模板浓度呈良好的线性关系，相关系数均在0.998以上。重复性实验中，6种虫体对应的变异系数（cv）最小值为0.18%，最大值为2.80%，试验间平均cv最小值为0.55%，最大值为1.94%，无非特异性扩增，溶解曲线的特异性和重复性良好。灵敏度实验中，可检出的最低模板浓度为1×102拷贝/ μl，比常规PCR灵敏度高100倍。 结论 初步建立了SYBR Green Ⅰ荧光定量PCR检测异尖线虫类病原体的方法。

关键词 异尖线虫病；异尖线虫；ITS?鄄2序列；荧光定量PCR；SYBR Green Ⅰ
分类号

# Detection of Anisakid Nematodes by an SYBR Green Ⅰ Real-time PCR

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Abstract
【Abstract】 Objective To establish an SYBR Green Ⅰ real-time quantitative PCR method for the detection of anisakid nematodes with zoonotic potential from Taiwan Strait. Methods Anisakid larvae of six species （Anisakis sim-plex, A. physeteris, Raphidascaris trichiuri, Contracaecum aduncum, C. muraenesoxi, and Contracaecum sp., a predominant species in fishes in the strait） were obtained from the guts of marine fishes and identified chiefly based on the morphol-ogical features. The ITS-2 rDNA sequences from the larvae were amplified by PCR using universal primers, then cloned and bidirectionally sequenced. According to these sequences, six specific forward primers were designed and synthesized. Specificity was determined by a series of conventional PCR respectively, the ITS-2 sequences amplified above were cloned into T vector which was subsequently transformed into E. coli DH5α. Following extraction and identification, the positive recombinant plasmid was used as quantitative template to generate standard curve and melt curve. Sensitivity and reprod-ucibility were determined. Results All the 6 standard curves established by the recombinant plasmids showed adequate linear relationship between threshold cycle（Ct） and template concentration. Melt curves were specific and all the 6 corr-elation coefficients were above 0.998. In the reproducibility test, the coefficients of variation （cv） of Ct values for detection of the 6 nematodes ranged between 0.18% and 2.80%, and the cv of the inter-assay ranged between 0.55% and 1.94%. The sensitivity of the real-time PCR was 1×102 copies/μl, about 100 times higher than the conventional PCR assays. The real-time quantitative PCR detection needed only 3.5 hours from the sample treatment to result report. Conclusion An SYBR Green Ⅰ fluorescent quantitative PCR has been developed for detecting anisakid nematodes with adequate sensitivity and specificity.
Key words Anisakiasis；Anisakid nematode；ITS-2 sequence；Real-time PCR；SYBR Green Ⅰ
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通讯作者

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