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实验研究

# 实时荧光定量PCR法检测日本血吸虫

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摘要

目的 运用实时荧光定量PCR法检测日本血吸虫。 方法 根据日本血吸虫18 S小亚基单位核糖体核酸（18S rRNA）基因设计特异性引物，PCR扩增出1 450 bp序列，经TA克隆后转入大肠埃希菌DH5α，提取重组质粒，鉴定后作为模板制作荧光定量PCR 标准曲线。方法重现性评价，用初始循环数（Ct，拷贝/反应）进行标准差分析，并计算变异系数（CV）。 结果 制作的标准曲线循环阈值与模板浓度具有良好的线性关系，相关系数为0.998 7。方法重现性评价，在1.05×107～1.05×103个拷贝范围内，对应的Ct平均值分别为17.55、20.93、24.32、27.59和30.95；CV值分别为1.31%、1.53%、0.90%、1.85%及0.90%，在重复性试验中试验间数据平均变异系数为1.27%，无非特异性扩增。 在试验检测范围内（Ct≤30.95），可检测的日本血吸虫基因组浓度为6.15 pg，3 h内完成。 结论 运用荧光定量PCR方法检测日本血吸虫DNA，快速、灵敏、特异性高。

关键词 日本血吸虫 荧光定量PCR TaqMan探针 18S小亚基单位核糖体核酸

分类号



# Fluorescent Quantitative Real-time PCR for Detection of Shistosoma japonicum

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Abstract

Objective To establish a sensitive and specific fluorescent quantitative real-time PCR method for the detection of Schistosoma japonicum. Methods Based on 18SrRNA sequence of S. japonicum, a PCR assay was established. The 1 450 bp fragment was amplified and 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. Reproducibility and specificity of the assay was determined as well. Results The standard curve established by recombinant plasmid showed a fine linear relationship between threshold cycle （Ct） and template concentration, and the correlation coefficient was 0.998 7. Using the coefficient of variation （CV） value to evaluate the reproducibility, at the template concentration of 1.05×107-1.05×103 copies per reaction， the average Ct values were 17.55，20.93，24.32，27.59，30.95，and the CV values were 1.31%，1.53%，0.90%，1.85% and 0.90% respectively. In the evaluation of the reproducibility， the mean interassay CV was 1.27% and no unspecific amplification was observed. The real-time PCR assay could quantitatively detect as low as 6.15 pg S. japonicum genome in the study（Ct≤30.95）， and the detection should be done in 3 hours. Conclusion A fluorescent quantitative real-time PCR for the detection of S. japonicum is developed, which is rapid， sensitive and specific for pathogen detection.

Key words Schistosoma japonicum Real-time PCR TaqMan probe 18SrRNA

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