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研究报告

多重实时荧光PCR相对定量法快速诊断唐氏综合征

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摘要

为了建立一种基于多重实时荧光相对定量PCR技术并应用之于唐氏综合征分子诊断，选择21号染色体上唐氏综合征特异区域基因片段(*DSCR3*)为目的基因，以12号染色体上的磷酸甘油醛脱氢酶基因(*GAPDH*)为参照基因，设计合成两对引物以及分别以不同荧光标记的*Taq*Man探针，在同一个反应管中进行扩增。以相对定量指标△CT值区分唐氏综合征患者与正常人。采用EB 病毒转化技术，把唐氏综合征患者外周血B 淋巴细胞转化成永生淋巴母细胞系作为标准品。通过优化反应条件，使得目的基因和参照基因的扩增效率基本一致，接近100%，模板浓度在3～300 ng/μL范围内，△CT值的变异系数小于15%，浓度在30 ng/μL时，变异系数最小(＜10%)，以该浓度的DNA作为模板进行批内和批间实验的△CT值重复性好，变异系数分别为9.8%和13.3%。运用建立的方法检测20例唐氏综合征患者的血标本和30例正常人的血标本，正常人△CT值范围是-1.90～-1.30，患者的△CT值范围是-2.95～-2.15，两组之间无交叉重叠，有明显差异(P＜0.001)。唐氏综合征患者永生细胞系建系成功，染色体核型和DNA 分析表明建系前后遗传是稳定的。因此，实时荧光定量PCR比较△CT值的相对定量法快速诊断唐氏综合征是可行的。

关键词 唐氏综合征 荧光定量PCR 相对定量 △CT值 永生细胞系
分类号

Rapid diagnosis of Down’s syndrome by multiplex real-time fluores-cence relative quantitative PCR

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Abstract

<P>To establish a multiplex real-time fluorescence relative quantitative PCR method for diagnosis of Down’s syndrome. The fragment from Down’s syndrome critical region gene 3 (<EM>DSCR3</EM>) on chromosome 21 was used as the target gene, and the glyceraldehyde-3-phosphate dehydrogenase (<EM>GAPDH</EM>) gene on chromosome 12 was used as the control gene. The two genes were amplified in the same tube. The relative quantitative index-<I>&#</I>8710;CT value was used to differentiate trisomy 21 patient from normal person. The peripheral blood sample from a Down’s syndrome patient was collected and the B-lymphocytes were transformed by Epstein-Barr virus to establish the immortalized cell lines as standard material. The reaction conditions were optimized to obtain an equal amplification efficiency from both the target and the control genes. The slopes of both genes were almost -3.32, indicating that the efficiencies of the two amplifications were approximately equal. Among a certain range from 3-300

ng/PCR, the variation of detected <I>&#</I>8710;CT value were less than 15%, and amplifica-tion showed the highest reproducibility when the concentration of DNA template was 30 ng/μL. Then, the variation of <I>&#</I>8710;CT value with inter- and intra-assay were 9.8% and 13.3% at this DNA concentration of the templates. Clinical samples, in-cluding 20 blood samples from patients and 30 blood samples from normal persons, were detected using the established method. The <I>&#</I>8710;CT value from Down's syndrome group were dramatically different from normal group (<EM>P</EM><0.001). The trisomy 21 immortalized cell lines were established and the genetic integrity of the cell lines was stable as evaluated by karyotype and DNA analysis. The relative quantitative PCR with <I>&#</I>8710;CT value could be used to rapidly diagnose Down's syn-drome.</P>

Key words Down's syndrome fluorescence quantitative PCR Δ CT value immortalized cell line

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