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人类血小板抗原1～6系统同步基因分型的研究Study on the Simultaneous Genotyping of Human Platelet Antigens of 1, 2, 3, 4, 5, 6 System by PCR-SSP and Its Applications

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摘要

为研究采用PCR—SSP技术，建立可靠的人类血小板抗原HPA-1,2,3,4,5,6系统的同步基因分型方法，并以所建立的方法研究血小板抗原。设计合成18条序列特异性引物，探索最佳退火温度，通过调整引物浓度、Mg2＋离子浓度，使HPA-1~6系统等位基因在同一条件下进行同步扩增和扩增产物在同一凝胶中进行同步电泳。引物的特异性和灵敏度采用基因型已知的质控DNA进行验证。应用此方法，对2000年度国际输血协会（ISBT）第十届血小板基因定型与血清学工作组送检的15份考核样本（其中血样2份，DNA样本13份）进行了基因分型。用此方法检测质控DNA，结果与已知的HPA基因型完全相符；15份第十届血小板基因定型与血清学工作组的考核样本的检测结果，与ISBT公布的结果完全相同，准确率达100%。Abstract: To set up the simultaneous genotyping of human platelet antigens of 1,2,3,4,5,6 system by PCR—SSP assay and use the genotyping method for the study of platelet antigens. In this study, 18 sequence-specific primers were designed and synthesized. The annealing temperature for all sequence-specific primer pair, the concentration of each primer pair and the concentration of Mg2+ were adjusted to the optimum so that HPA-1 to 6 systems could be amplified simultaneously under the same PCR cycling parameters. The electrophoresis of PCR products was conducted simultaneously on the same agarose gel. Control DNA samples that genotypes known were used to confirm the sensitivity and specificity of each sequence-specific primer. 15 coded samples (including 2 blood samples and 13 DNA samples) distributed by 10TH Platelet Genotyping and Serology Workshop of the International Society of Blood Transfusion (ISBT) were typed for HPA-1 to 6 systems by this method. A concordance rate of 100 percent was observed between the results of control DNA samples typed by our PCR—SSP assay and the data of known specificity of control DNA. The results of 15 coded samples tested by our method agreed well with the results provided by ISBT report.

关键词 序列特异性引物—PCR 人类血小板抗原 基因分型 Key words PCR—SSP Human Platelet Antigens (HPA) Genotyping

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Abstract

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