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研究论文

一种基于磁性纳米粒子PCR的高通量SNP分型方法

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摘要 利用磁性纳米粒子PCR扩增(MNPs-PCR)和等位基因特异性双色荧光探针(Cy3, Cy5)杂交, 建立了一种单核苷酸多态性(SNP)分型的新方法. 应用该方法对9个样本MTHFR基因的C677T多态进行检测, 野生和突变型样本正错配信号比大于9.0, 杂合型正错配信号比接近1.0, 分型结果经测序验证. 此方法无须产物纯化、浓缩, 扫描分型结果快速、直观, 是一种操作简单、快速、高通量、高灵敏度的分型方法.

关键词 磁性纳米粒子 PCR 双色荧光杂交 SNP分型

分类号 0657

High-throughput SNP Genotyping Method with PCR on Magnetic Nanoparticles

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Abstract Single nucleotide polymorphisms(SNPs) assay is important for elucidating the genetics of indivi-dual differences in drug response and disease susceptibility. This report described a novel high-throughput SNP genotyping method using magnetic nanoparticles as PCR primers carriers. PCR products were directly amplified on MNPs and interrogated by hybridization with a pair of dual-color fluorescence(Cy3, Cy5) probes to determine SNP, and then genotype of each sample can be simultaneously identified by scanning the microarray printed with the denatured fluorescent probes on an unmodified glass slide. As the results, the homozygous wild type, homozygous mutant type and heterozygote type yield strongly green, red and yellow fluorescent signals, respectively. The methylenetetrahydrofolate reductase(MTHFR) gene C677T polymorphism of nine di-fferent samples was detected and their fluorescent signals of the nine samples were quantified. The fluorescent ratios(match probe signal to mismatch probe signal) of homozygous samples were over 9.0, whereas heterozygous samples had ratios near 1.0. The genotyping results were additionally validated by sequencing. Without any purification and concentration of PCR products, the approach reported here should be a simple, sensitive, high-throughput and high accurate genotyping method.

Key words Magnetic nanoparticles PCR Dual-color fluorescence hybridization SNP genotyping

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