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技术与方法

一种基于适配器连接介导的等位基因特异性扩增法测定多重SNP

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摘要

建立了一种基于DNA适配器连接介导的等位基因特异性扩增法测定多重SNP。以*CYP2D6*基因中的5个SNP位点（100C>T，1661G>C，1758G>T，2470T>C和2850C>T）为例，用PCR法预扩增得一段含所有待测SNP位点的长片段，然后用限制性内切酶将其消化成短片段，在连接酶的作用下与设计的DNA适配器（adapter）相连；该适配器的一端与限制性内切酶降解后留下的粘性末端相同，另一端带有一段公共序列。在两管中加入与适配器连接的片段作为PCR扩增模板，并分别加入SNP特异性引物和一种适配器特异性的通用引物进行PCR扩增，最后用凝胶电泳法分离PCR扩增产物。由于每管与SNP的两种特异性引物中的一种对应，可以根据每管中扩增片段的大小判断SNP的类型。通过凝胶电泳法可以一次分离与5种SNP类型相对应的引物特异性延伸反应产物；采用该法成功测定了20名健康中国人的*CYP2D6*基因中5个SNP位点的基因多态性，与限制性片段长度多态性法（RFLP）测定结果完全一致。该方法采用n+1种引物（n种SNP特异性引物和一种通用引物）进行n重PCR反应，极大提高了PCR反应的特异性，结果准确，可用于同时测定多个SNP位点。

关键词 DNA适配器；SNP；多重PCR；*CYP2D6*

分类号 Q75

Adapter-ligation Mediated Allele-specific Amplification (ALM-ASA) for Multiplex SNP Genotyping

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Abstract

<DIV align=left>To establish adapter-ligation mediated allele-specific amplification (“ALM-ASA” for short) for multiplex SNP genotyping, five SNPs, 100C>T, 1661G>C, 1758G>T, 2470T>C and 2850C>T in <I>CYP2D6</I> gene were used as an example for evaluating the method. Firstly, a preamplification was carried out for producing a long target containing all SNPs of interest. Secondly, the preamplified DNA fragments were digested with a restriction endonuclease to form sticky ends. Thirdly, an adapter was ligated to either end of the digested fragment. One end of the adapter was designed as a sequence sticky to the ends of the enzymatically digested fragments, and the other end had a common sequence. Fourthly, an allele-specific amplification was performed by allele-specific primers and a universal primer in one tube by using the adapter-ligated fragments as templates. Finally, the allele-specific amplification products were separated by agarose gel electrophoresis. Because each tube corresponds to one kind of allele-specific primers, the genotype of an SNP can be easily discriminated by the length of the amplified products in each tube. The products of 5-plex allele-specific amplification can be separated by agarose gel electrophoresis. Five SNPs in the <I>CYP2D6</I> gene were successfully typed for 20 healthy Mainland Chinese and the results were in agreement with those by RFLP. By ALM-ASA, n+1 primers (n SNP allele-specific primers and a universal primer)

can be used for an n-plex PCR amplification; the specificity of PCR is thus enhanced significantly. It is concluded that ALM-ASA can be used for typing multiple SNPs simultaneously.</DIV>

Key words DNA adapter SNP Multiplex PCR *CYP2D6*

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