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论著

一种新的高效快速检测遗传性耳聋的方法

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摘要:

目的: 探寻在不同人群中应用快速、准确的检测非综合征性耳聋的方法。方法: 将引物延伸及变性高效液相色谱法相结合,检测180例中国人群的6种常见耳聋突变点(*GJB2-235delC, GJB2-299delAT, PDS-A2168G, PDS IVS7-2A>G*, *mtDNA-A1555G*, 以及 *mtDNA-C1494T*) 。PCR扩增的靶序列, 经引物延伸, 得到中国人遗传性非综合征性耳聋6个常见突变的特异性延伸片段, 用全变性高效液相色谱分析延伸片段混合物, 分离图谱可鉴定被检样本的基因型。结果: 盲法分析显示180例样品的引物延伸变性高效液相色谱法与直接测序法检测结果完全符合。结论: 该法是一种准确、高效的检测非综合征性遗传性耳聋突变的分析方法, 并可以探讨将其应用到其他遗传病的检测中。

关键词: 遗传性耳聋 变性高效液相色谱 基因突变 引物延伸

Novel multiplex primer extension and denaturing high-performance liquid chromatography for genotyping of the deafness gene mutations

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Abstract:

Objective To find a rapid and accurate genotyping method for specific non-syndromic hearing loss (NSHL)-causing gene mutations for disease diagnosis in different ethnic populations. Methods We performed a novel multiplex primer extension (PE) reaction in combination with denaturing high-performance liquid chromatography (DHPLC) to simultaneously detect and genotype the 6 most common mutations in 180 patients with NSHL (*GJB2-235delC, GJB2-299delAT, PDS-A2168G, PDS IVS7-2A > G*, *mtDNA-A1555G*, and *mtDNA-C1494T*) in Chinese population. This method involved the amplification of the target sequence, followed by a purification step, a multiplex PE reaction, and DHPLC analysis performed on the Transgenomic Wave DNA fragment analysis system under fully-denaturing conditions. Results In a blind analysis, this technique successfully and accurately genotyped 100% of the samples simultaneously characterized by direct sequencing. Conclusion Combination of PE and DHPLC is simple, rapid, accurate, and cost-effective for genotyping common disease-causing mutations, including substitutions, insertions, and deletions in NSHL, and may be successfully used in other genetic diseases.

Keywords: genetic deafness denaturing high-performance liquid chromatography mutation primer extension

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