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技术与方法

基于连接检测反应的基因芯片分型技术的建立

肇旭[1，2];秦胜营[1，2];冯国鄞[3];贺林[2]

上海交通大学

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摘 要 目的 建立基于连接检测反应的基因芯片分型技术。方法 将连接检测反应，通用芯片技术及片基异硫氰基化从头活化方法相结合。结果 应用测序技术验证，该方法分型准确性达到100%。另外也不会产生非模板依赖性信号，对未纯化模板的分型效果也较好。结论 连接检测反应能够在高温下进行，显著减少了探针非特异性连接；其通用性使得研究者可基于一张芯片自由选择所研究的位点，无需针对每一组探针进行操作条件的单独优化，并且保证了杂交的特异性；片基从头活化技术更使其摆脱了对商品化片基的依赖。这些特点都极大地提高了该技术的分型准确性，并且降低了费用，使其应用于高通量SNP分型成为可能。

关键词 基因芯片 连接检测反应 分型

分类号

Establishment of DNA chip-based genotyping technology mediated by ligase detection reaction

Zhao Xu[1, 2];Qin Sheng-Ying[1, 2];Feng Guo-Yin[3];He Lin[2]

1. Shanghai Jiao Tong University, Shanghai 200030;
2. Institute for Nutritional Sciences, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031; 3. Shanghai Institute of Mental Health, Shanghai 200030

Abstract Objective DNA chip is a tool for massively parallel analysis of genomes that consists of a solid support containing samples of many DNA probes arranged in a regular pattern. It has now been advocated as one of the most powerful approaches for SNP analysis. Recently, the universal DNA chip method mediated by ligase
detection reaction (LDR) has been demonstrated to be a highly specific and sensitive assay for SNP detection. In the present study, this approach was combined with the slide self-activating method to establish a high-discrimination and cost-effective technology with significant promise for future high-throughput SNP genotyping. Methods Five probes including two discriminating probes, one common probe and two corresponding Zips, which are coupled to the slides at known locations, were designed for each SNP. In addition, the positive and negative marker probes were designed for result evaluation. The standard microscope glass slides were activated by PDITC. The PCR and LDR were all performed using multiplex amplifying method. After hybridization and scanning, the average signals after subtraction of local background were used for calculating the signal ratios of spot pairs corresponding to different alleles. Results Genotyping accuracy of this technology was validated as 100% by comparing with sequencing method. The homozygous and heterozygous could be distinguished easily by this method. The signal ratio of positive and negative dots was higher than 10. No signal was detected in the experiment without DNA template, which demonstrated that the detection result relied on the template directly. When analyzing the same subject one more times, the results could be repeated very well, indicating that the possibility of variation with uncertain conditions is very low. In addition, genotyping of non-purified templates revealed good results. Discussion LDR can be performed at a relatively high temperature, which helps to eliminate non-specific ligations. The characteristic of universal gives researchers freedom to detect spe

cified polymorphic loci based on the same array, with no need to optimize the operation condition of each probe groups and ensures the specificity of hybridization. Self-activating method makes u s avoid the dependence on commercial slides. These improved the genotyping accuracy to a high level and reduced the cost greatly, which make it possible for high-throughput genotyping.

Key words DNA chip Ligase detection reaction Genotyping

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通讯作者 贺林 helin@nhgg.org