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High-throughput SNP genotyping based on solid-phase PCR on magnetic nanoparticles with dual-color hybridization

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Abstract

Single-nucleotide polymorphisms (SNPs) are one-base variations in DNA sequence that can often be helpful when trying to find genes responsible for inherited diseases. In this paper, a microarray-based method for typing single nucleotide polymorphisms (SNPs) using solid-phase polymerase chain reaction (PCR) on magnetic nanoparticles (MNPs) was developed. One primer with biotin-label was captured by streptavidin coated magnetic nanoparticles (SA-MNPs), and PCR products were directly amplified on the surface of SA-MNPs in a 96-well plate. The samples were interrogated by hybridization with a pair of dual-color probes to determine SNP, and then genotype of each sample can be simultaneously identified by scanning the microarray printed with the denatured fluorescent probes. The C677T polymorphisms of methylenetetrahydrofolate reductase (MTHFR) gene from 126 samples were interrogated using this method. The results showed that three different genotypes were discriminated by three fluorescence patterns on the microarray. Without any purification and reduction procedure, and all reactions can be performed in the same vessel, this approach will be a simple and labor-saving method for SNP genotyping and can be applicable towards the automation system to achieve high-throughput SNP detection.

Keywords: Magnetic nanoparticles; Single nucleotide polymorphisms; Solid-phase PCR; Dual-color hybridization

1. Introduction

Single nucleotide polymorphisms (SNPs) are the most abundant forms of sequence variations between individuals and occur at about one per 500–1000 bp in the human genome (Marshall, 1997). SNPs, because they are usually biallelic, are more amenable to automated detection; they are also regarded as ideal genetic markers in linkage disequilibrium analysis for identifying genetic factors associated with common diseases or adverse drug responses due to their accessible class of polymorphisms present and genetic stability (Pennisi, 1998). So methods for SNP analysis are needed as a technology basis for a better understanding of the genetic basis for complex diseases, and to realize the potential of pharmacogenetics (Ranade et al., 2001). Microarray platform has been widely used for highly parallel genomic analyses, due to their highly multiplex capabilities, low cost and can be highly parallel readout for large-scale samples. Over the past several years, multiplexing high-throughput methods based on microarrays to discover and measure SNPs have been developed and commercialized (Flavel et al., 2003; Erdogan et al., 2001; Hultin et al., 2005), while, with the procedures for purification and concentration of targets in sample preparation these technologies are usually time-consuming and not suitable for automatic operation. So these methods have limited their utility in a high-throughput polymorphism detection to meet the challenges of the new genomics era. Furthermore, a rapid, simple, high-throughput parallel screening protocol for SNPs detection over thousands of samples is still required.

Magnetic nanoparticles (MNPs) have already been successfully used in various fields of biology and medicine such as magnetic targeting (of drugs, genes), magnetic resonance imag-

Abbreviations: SNP, single nucleotide polymorphism; MNP, magnetic nanoparticle; MTHFR, methylenetetrahydrofolate reductase; SA, streptavidin; SSC, standard saline citrate; PBS, phosphate-buffered saline.

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Table 1
Oligonucleotides used in this study

Name	Type	Sequence 5′-3′
C677T FP	Forward primer	TGAAGGAGAAGGTGTCTGCGGGA
C677T RP	Biotin-labeled reverse primer	Biotin-$(T)_{15}$-AGGACGGTGCGGTGAGAGTG
677CC probe	Wild type probes	Cy3-CGGGAG*C*CGATTT
677TT probe	Mutant type probes	Cy5-CGGGAG*T*CGATTT
C677T-WT	Wild simulated target	Biotin-$(T)_{15}$-GCCCTC*G*GCTAAA
C677T-MT	Mutant simulated target	Biotin-$(T)_{15}$-GCCCTC*A*GCTAAA

The italicized base representing the recognition position.

ing, immunoassays, cell separation, RNA and DNA purification, for their unique higher dispersion capability in aqueous solution, higher separation efficiency in magnetic field and easy operation in autoworkstations (Niemeyer, 2001; Zhao et al., 2003; Ingram et al., 2005). Furthermore, some methods using magnetic nanoparticles as platforms for SNPs genotyping were developed soon. Typically, Yoshino et al. developed a SNP detection using bacterial magnetic particles (BMP), the results were observed at a single particle level by fluorescence microscopy (Yoshino et al., 2003). They have succeeded in detecting a single particle as the minimal detectable unit in BMP assays, while the detection of this approach could not avoid the background of bacterial magnetic particles, and not suitable for high-throughput analysis.

With the technique described above, the combination of elements referred both array-based "readout" technology and magnetic nanoparticles will facilitate the development of high-throughput genotyping methods (Fan et al., 2006). Herein, we present a methodology with PCR amplification directly on MNPs, hybridization with allele-specific probes labeled with dual-color fluorescence (Cy3, Cy5) for multiplex SNP profiling in conjunction with microarrays. In our method, all steps of the preparation can be performed in the same vessel by simple additions of solution and incubation, and then genotypes are discriminated by scanning the microarray printed with the denatured fluorescent probes onto an unmodified glass slide. Therefore, this method is particularly suitable for automation. Without the necessary procedures for purification and complex reduction of PCR products, the application of this strategy to large-scale SNP studies will be simple, labor-saving, high sensitive and potential for automation.

2. Materials and methods

2.1. DNA Samples

Peripheral bloods of 126 different patients with gastric carcinoma were obtained from Changsha Central Hospital (Changsha, China). A written informed consent was signed by all participants. Procedures were in accordance with the Helsinki Declaration for the Ethical Treatment of Human Subjects. Genomic DNA sample was extracted from 100 μl blood for each patient using a QIAamp DNA Blood Mini Kit (Qiagen).

2.2. Preparation of oligonucleotides

The 677 methylenetetrahydrofolate reductase (MTHFR) polymorphisms were selected as targets. One set of PCR primers was designed to amplify a section (213 bp) of the methylenetetrahydrofolate reductase (MTHFR) gene containing the C677T SNP locus. The sequences of all oligonucleotides including PCR primers, allele-specific dual-color probes and simulated targets used in this study are shown in Table 1. All oligonucleotides were synthesized and purified by Shanghai Sangon Biologic Engineering Technology and Service Co. Ltd. (Shanghai, PR China).

2.3. Immobilization of streptavidin onto MNPs

MNPs about 100 nm in diameter were prepared according to the previously published method (Wang et al., 2006). MNPs were modified firstly with 3-aminopropyl triethoxysilane (APTS) and then with glutaraldehyde as a linkage between the amine group of APTS on MNPs surface and streptavidin. The aldehyde-MNPs (10 mg) were washed thrice with 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 20 min at room temperature with pulsed sonication. Then MNPs were magnetically separated from the mixture using a neodymium-boron (Nd-B) magnet and the supernatant was discarded. They were then incubated with streptavidin (100 mg/mL) in PBS buffer for 30 min at room temperature with pulsed sonication, and streptavidin were immobilized onto the MNPs surface. The streptavidin coated MNPs (SA-MNPs) were magnetically washed thrice with PBS buffer to remove excess unconjugated streptavidin and finally dispersed in PBS buffer with the concentration of 4 mg/mL and stored at 4 °C.

2.4. Solid-phase PCR

MNPs-bound primers were prepared using every 15 pmol biotin-labeled reverse primers covalently immobilized onto 50 μg SA-MNPs. The PCR reaction was performed in 30 μl mixture contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM each of the deoxynucleotide triphosphates, 100 ng template DNA, 1.25 U of Taq DNA polymerase (TaKaRa), 0.5 μM forward primer and 50 μg MNPs-bound reverse primer. As negative controls, SA-MNPs without biotin-labeled reverse primers were used to replace the SA-MNPs with bounded primers in amplification and genotyping procedure,