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Single-Base Extension and ELISA-Based Approach for Single-Nucleotide Polymorphisms Genotyping

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Abstract Single-nucleotide polymorphisms (SNPs) emerge as a fundamental tool in personalized medicine due to their association with drug responses or disease predisposition. Single-base extension (SBE) is a common method for characterizing known SNPs, but involves complicated procedures or requires costly analytical instruments. Here, we describe a novel SNP genotyping based on SBE and enzyme-linked immunosorbent assay (ELISA). During the SBE, the 5′ end fluorescein isothiocyanate-labeled allele-specific primer will extend with biotinylated dideoxynucleotides which are complementary to the SNP sites. The extension product will then be captured by streptavidin-coated nanoparticle and develop blue color in the ELISA assay. We validated this method by detecting SNPs for TP53 gene codon 273 from 68 individuals and the data were 100% in concordant with DNA sequencing. Thus, SBE and ELISA-based SNPs assay is a simple and accurate method for SNP genotyping.

Keywords Single-nucleotide polymorphisms · Genotyping · ELISA · Single-base extension

Introduction

Single-nucleotide polymorphisms (SNPs) are the most abundant source of genetic variation, and there are more than 3 million SNPs present in human genome [1]. SNPs emerge as a fundamental tool utilized in furthering personalized medicine due to their association with drug responses or disease predisposition [2]. Therefore, up to now, numerous methods have been developed for SNPs detection, such as DNA sequencing [3], gene microarray [4], denaturing capillary electrophoresis [5], electrochemical detection [6], mass-based spectrometry [7], oligonucleotide ligation reaction [8], and molecular beacon assay [9].

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Single-base extension (SBE), in which a specific primer is annealed to the template and extended by DNA polymerase with specific dideoxynucleotide complementary to the SNPs site, is one of main techniques for accurate genotyping of the known SNPs [10]. SBE-based method adapts to various detection formats such as solid-phase microtiter plates [11, 12], mass-based spectrometry [7], fluorescence resonance energy transfer [13, 14]. However, most SBE-based methods need costly analytical instruments. Here, we describe a novel and simple SBE-based approach for the SNP genotyping by enzyme-linked immunosorbent assay (ELISA). The idea is that PCR is used to amplify the region containing SNP site. Next, the 5′ end fluorescein isothiocyanate (FITC)-labeled specific primer extends with specific biotin-dideoxynucleotides (ddNTPs) complementary to the SNP sites in the SBE. The SBE product will conjugate with streptavidin-coated nanoparticles and produce blue color in ELISA assay. The use of ELISA and the specificity of SBE provide a simple and accurate approach for the genotyping of SNPs.

Materials and Methods

Materials

All the oligonucleotide and Taq DNA polymerase were purchased from Sagon Biotechnology Co., Ltd (China). Biotin-ddNTPs were from Perkin Elmer (Germany) and goat anti-fluorescein isothiocyanate-horseradish peroxidase (anti-FITC-HRP) from Roche (Switzerland), ThermoSequenase DNA polymerase from Amersham Biosciences (USA), shrimp alkaline phosphatase (SAP) from New England Biolabs (USA), streptavidin-coated nanoparticles from Ustar Biotechnology Ltd (China), 3, 3′, 5, 5′-tetramethylbenzidine and hydrogen peroxide (H_2O_2) from PIERCE (USA).

PCR Amplification

Genomic DNA was extracted from 200 μl blood using QIAGEN kit (Qiagen, Germany) according to the manufacture's manual. PCR amplification was performed in 20 μl reactions with following components: 200 nM forward primer: 5′-GTCCTGCTTGCTTACCTCGCT-TAGT-3′ and reverse primer: 5′-ACCTGATTTCCTTACTGCCTCTTGC-3′ [14]; 1×PCR buffer (50 mM KCl, 1.5 mM MgCl2, 10 mM Tris–HCl), 0.2 mM dNTPs, 20 ng genomic DNA and 1 U Taq DNA polymerase. The mixture was held at 95 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, and then kept at 72 °C for 5 min.

The Treatment of PCR Product and SBE Reaction

After PCR reaction, 5 U SAP and 2 μl of 10×SAP buffer were added to the PCR reaction and incubated at 37 °C for 30 min to degrade dNTP followed by incubation at 95 °C for 10 min to inactivate enzymes. Then, 5 μl of treated PCR product was used as the template for SBE. Since TP53 gene contains a polymorphic site (C/C, C/T, T/T) at codon 273, the SBE reaction was performed at 94 °C for 2 min and 72 °C for 60 s with the following components: 50 nM 5′ end FITC labeled specific primer 5′-FITC-GGACGGAACAGCTTTGAGGTG-3′, 0.2 μM biotin-ddCTP or biotin-ddUTP (Perkin Elmer, Germany) separately, 1.5 U ThermoSequenase DNA polymerase (Amersham Biosciences, USA), in a total volume of 20 μl (1×ThermoSequenase buffer).

ELISA Detection of SBE Product

Ten microliter SBE product was combined with 20 μl of streptavidin coated nanoparticles (Ustar Biotechnology Ltd, China; 400 nm, 1 mg/ml) for 10 min. After centrifuging and discarding the supernatant, the nanoparticles were incubated with 20 μl goat anti FITC-HRP (Roche, Switzerland) at 1:2,000 for 5 min, and then washed with 50 μl 10 mM Tris-HCI (pH 7.5) twice. Finally, the nanoparticles were incubated with 20 μl H_2O_2 (0.02%) and 20 μl 3, 3′, 5, 5′-tetramethylbenzidine (0.4 g/L) (PIERCE, USA) at 37 °C for 5 min to observe the color change. The blue color indicated that the added biotin-ddNTPs were complementary to the SNP sites.

DNA Sequencing

The PCR products were purified from agarose gel by the JETsorb DNA extraction kit (Genomed, GmbH, Bad Oeynhausen, Germany) and sequenced and analyzed (Dalian Bao biotechnology Co., Ltd, China).

Results and Discussion

We analyzed DNA samples from 68 individuals by SBE-ELISA method and compared the results with independent DNA sequencing. Our results demonstrate that SBE-ELISA was 100% in accordance with DNA sequencing (an example was shown in Fig. 1).

The SBE and ELISA-based SNPs assay described here has four steps: PCR amplify the regions containing SNP sites, treat PCR product with phosphatase to remove the remaining dNTPs, perform SBE reaction with the treated product, and finally detect SBE product by ELISA. In SBE, a specific primer with 5′ end FITC labeled and 3′ end immediately adjacent to the SNP

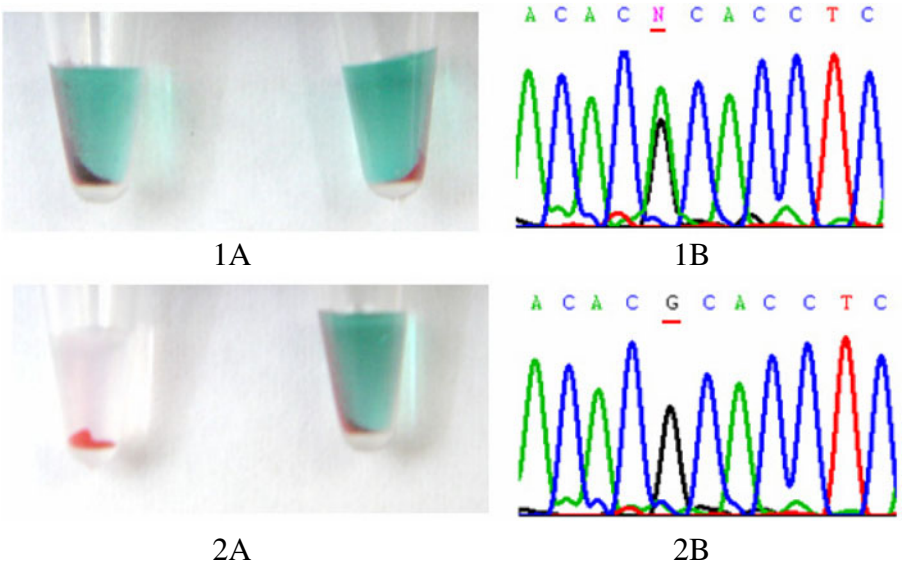

Fig. 1 Genotyping for TP53 codon 273 by SBE-ELISA. Biotin-ddCTP was added to the first tube, while biotin-ddUTP added to the other tube in SBE reaction. The *blue color* indicates that the added dd-NTP is complementary to the SNP polymorphic site. *1A/1B* SNP polymorphic site was G/A, while the *2A/2B* was G

polymorphic site will only extend with the biotin-ddNTP that complementary to the SNP site. The SBE products will then conjugate with streptavidin-coated nanoparticles and develop blue color by anti-FITC-HRP in ELISA. The accuracy of our SNP assay for TP53 codon 273 in 68 individuals is 100% as confirmed by independent DNA sequencing.

Like other SBE-based SNP assay, SBE-ELISA has many advantages: it is simple, accurate, and robust [11–14]. Most notably, SBE-ELISA is an economical approach because the result can be visualized by the naked eye without use of any equipment. Although SBE-ELISA has disadvantage in that it is hard to discriminate polymorphic sites simultaneously in a single SBE, it is still a very useful method for SNP genotyping especially when the polymorphic sites are known.

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Competing Interests The authors declare no competing interests.

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