

# A gel-based solid-phase amplification and its application for SNP typing and sequencing on-chip

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As conventional solid-phase amplification (SPA) on a two-dimensional slide has a low amplification capacity due to a limited amount of immobilized primers, we propose a three-dimensional SPA by immobilizing primers in hydrogel attached to a slide. One of the PCR primers, modified with an acrylamide group at the 5'-terminal, was copolymerized with both polyacrylamide gel and an acryl-modified glass slide, resulting in a high amplification capacity. The immobilization process was carried out by adding the catalysis reagent *N,N,N',N'*-tetramethylethylenediamine (TEMED) volatilized in vacuum, with uniform sample-concentration and gel-viscosity in the course of one-step nucleic acid immobilization. The porous structure of polyacrylamide gel, which allows PCR reagents such as *Taq* DNA polymerase, primers, dNTPs and DNA templates to freely enter the gel matrix, provides a homogeneous solution-mimicking environment for SPA on the interface or the inside of gel pads. Based on gel-based SPA, genotypes of different samples were accurately discriminated by either dual-color fluorescence hybridization or BAMPER (Bioluminometric Assay coupled with Modified Primer Extension Reactions). Pyrosequencing was also successfully carried out on SPA products. As the linkage between DNA molecules and gel is very strong, SPA products immobilized on gel pads could be reused several times if extended strands were removed by electrophoresis. Thus, the gel-based SPA provides a powerful tool for directly using on-chip amplicons for parallel detection.

## Introduction

SNP typing and sequencing are widely used in molecular diagnosis and large-scale genetic association studies. If there is a limited amount of target, most approaches for SNP typing and DNA sequencing need polymerase chain reaction (PCR), which can generate millions of DNA copies from the amplification of a single or several copies of DNA.<sup>1–4</sup> For a high throughput detection format such as a DNA chip, PCR in parallel is required. Solid-phase amplification (SPA), which is an improved PCR technique for an efficient high-throughput assay on a chip, has been proposed for molecular diagnosis.<sup>5–8</sup> As SPA has the advantage of easy purification of amplicons on slides, it has been widely used for various applications.<sup>9–11</sup> Immobilized primers in SPA should meet the requirements of a high surface density and a strong linkage between primers and substrates,<sup>9</sup> thus the selection of supporting matrix and an immobilization method is very important for SPA on a chip. In the case of rigid supports on a flat surface, such as glass and microwell used in conventional SPA,<sup>9,12,13</sup> the binding capacity is very limited due to a small surface area. Therefore three-dimensional (3-D) hydrophilic-gel (e.g., polyacrylamide) has been employed as a substrate to improve the surface area in SPA,<sup>14</sup>

but DNA attachment still requires the following steps: preparation of polyacrylamide gel-pads, activation of the gel to produce aldehyde groups and immobilization of 5'-terminal amino-modified primers. These steps are tedious and labile. Although the copolymerization step and the polymerization-mediated immobilization step were further improved,<sup>15</sup> the whole process is still complex and time consuming. Thus, a simple (ideally one-step) and rapid method for immobilization is preferable for controlling the quality of immobilization.

A simple process was developed for immobilizing oligonucleotides bearing an acrylamide group at the 5'-terminal onto a solid support by using acrylamide monomers for copolymerization, and the gel activation step was skipped.<sup>16</sup> However, the direct addition of the ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine (TEMED) into prepolymers would cause the polymerization to be uncontrollable, so that it becomes very difficult to spot the mixed prepolymers homogeneously onto a large scale microarray. To get a uniform sample-concentration and gel-viscosity in the course of nucleic acid immobilization, we proposed a novel method to control the polymerization process by adding the catalysis reagent TEMED separately, the copolymerization was catalyzed after volatilized TEMED molecules in vacuum reached onto the spotted prepolymers.<sup>16</sup> This method enables the spotting time to be controlled, and the attachment of gel to slides is extremely stable. In contrast to flat surfaces, polyacrylamide gel can provide a 3-D surface with a very high probe-density (200 fmol/mm<sup>2</sup>).<sup>16</sup> We have successfully used this method to bind PCR products for SNP typing and DNA sequencing.<sup>16</sup> However the whole process is tedious as a regular amplification reaction is still needed to supply amplicons for gel

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