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Multiplex Single-Nucleotide Polymorphism Typing by Nanoparticle-Coupled DNA-Templated Reactions

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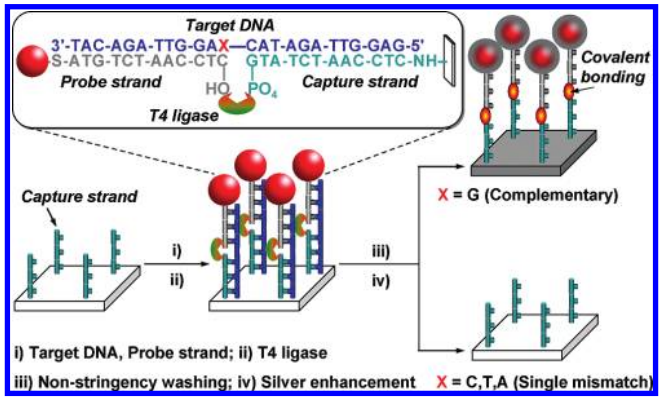
Rapid genotyping of single-nucleotide polymorphisms (SNPs) is essential for early diagnosis, prevention, and treatment of specific human diseases.[1] Conventional methods as well as recently developed techniques, including allele-specific DNA hybridization,[2] ligation or primer extension,[3] molecular-beacon-based fluorescence resonance energy transfer,[4] electrochemical typing,[5] and binary DNA probe-assisted assays,[6] provide accurate validation but have disadvantages either with respect to complex procedures, inadequate sensitivity, lack of multiplex detection capability, or need of large sample volumes. In contrast, oligonucleotide-modified gold nanoparticle (NP) probes[7] coupled with silver enhancement in a chip-based assay format hold great promise for rapid, low-cost, ultrasensitive SNP screening. Despite these attractions, there still exist many of the constraints associated with these detection systems, such as limited sequence specificity and the need for a thermal stringency wash.[7e] Stringent control over melting and washing of probe−target duplexes with single-base-pair mismatches is extremely difficult because of subtle differences in melting temperature between the mismatched and perfectly matched duplexes.[7e] Here we demonstrate a convenient and efficient approach based on NP-coupled DNA-templated reactions that offers both rapid multiplex SNP detection capability and ultrahigh sensitivity without the need for costly instrumentation and stringency washing processes.

Scheme 1



Our system for SNP screening relies on a sandwich assay comprising a capture DNA strand immobilized on a glass substrate, a probe DNA strand attached to a gold NP, and a target DNA (Scheme 1). However, unlike conventional NP-coupled sandwich assays, this system involves the incorporation of DNA-templated reactions[8] (e.g., enzymatic DNA ligation) between the probe and capture strands. Because of the sequence-specific nature of DNA-templated reactions, the probe strand forms a covalent linkage to the capture strand in high yield only in the presence of a perfectly matched target. The covalent linkage results in a firm attachment

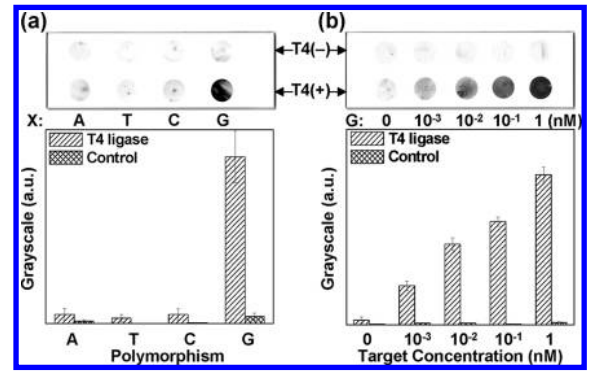


Figure 1. Scanometric images of oligonucleotide-modified glass slides after NP-coupled DNA-templated reactions in the presence of (a) various target DNAs and (b) a perfectly matched target at various concentrations. The corresponding grayscale values of darkened areas are reported below each panel.

of the NP probe to the substrate. After a nonstringency wash and silver amplification, the perfectly matched target can be readily detected with the naked eye or a flatbed scanner. In contrast, targets with a single-base mismatch adjacent to the DNA-templated reaction site might cause instability of the probe−target duplex, particularly at the mismatch position. This should give a low efficiency of coupling between the probe and capture strands, resulting in a background considerably clean of the substrate because of the small number of substrate-tethered particles available for silver enhancement.

As a proof-of-concept experiment, we combined oligonucleotide-functionalized NP probes with an enzymatic DNA-templated ligation reaction for SNP screening. Aqueous solutions containing an NP probe (Au−5′S−ATGTCTAACCTC−OH3′; 100 pM) and different target DNA sequences (5′-GAGGTTAGATAC**X**AGGTTAGACAT-3′, **X** = G, A, T, or C; 1 nM each) were spotted onto a glass microscope slide modified with a capture strand (5′PO_4−GTATCTAACCTC-NH-). After incubation for 30 min in a DNA hybridization chamber, T4 DNA ligase, which catalyzes the formation of a phosphodiester bond between juxtaposed 5′-phosphate and 3′-hydroxyl termini in duplex DNA, was added to the resulting mixture, which was then allowed to stand for 5 min. The glass slide was then directly washed with ultrapure water to remove nonspecifically bound particle probes and target DNA strands. Subsequently, SNP discrimination was enabled with substantially improved sensitivity and reliability by silver amplification of the bound NP probes.

As anticipated, in the presence of a single-base-mismatched target sequence (**X** = A, T, or C), no marked darkening of the spotted surface was observed or measured by the relative grayscale values taken from flatbed scanner images of the oligonucleotide-modified glass surface (Figure 1a). In a stark contrast, significant darkening of the spotted surface was observed when the perfectly matched target (**X** = G) was used. Importantly, our control experiments showed that without added T4 DNA ligase, the spotted areas did

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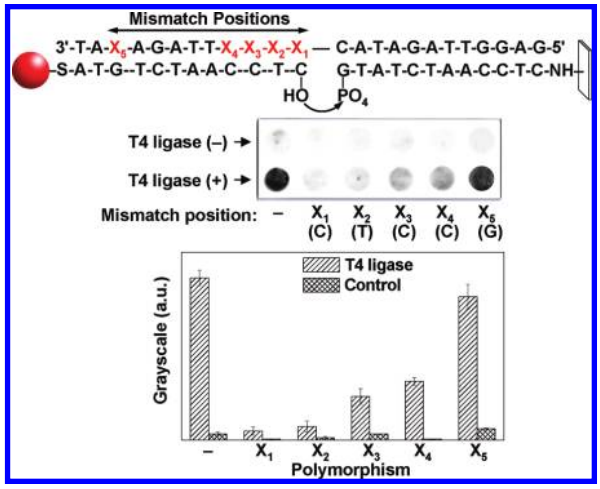

Figure 2. Effect of mismatch position on SNP screening. A representative scanometric image of an oligonucleotide-modified glass slide after NP-coupled DNA-templated reactions in the presence of a perfectly matched target and singly mismatched targets with the mismatches at different positions (denoted as $\mathbf{X_1}$ to $\mathbf{X_5}$) is shown. The corresponding grayscale values of darkened areas are reported below the scanometric image panel.

not show marked darkening effects in all cases, demonstrating that the nonstringency wash is an effective way to remove nonspecifically bound particle probes from the surface. We also found that the NP-coupled DNA-templated reaction allowed the facile semiquantification of target hybridization without stringent washing steps, as indicated by the imaged grayscale intensity value of the darkened spot (Figure 1b).

Another intriguing feature of the present approach is its multiplex ability to recognize SNPs at different positions adjacent to the DNA-templated reaction site. A series of target DNA sequences with a single-base mismatch at different positions (labeled as $\mathbf{X_1}$ to $\mathbf{X_5}$) were selected and annealed to the probe and capture strands in the presence of T4 DNA ligase. After nonstringent washing and silver amplification under standard conditions, our results showed strong position-dependent effects of sequence mismatches on the DNA-templated ligation reactions (Figure 2). The efficiency of the ligation reactions increased with increasing distance ($\mathbf{X_1}$ to $\mathbf{X_4}$) between the mismatch and the ligation site, as measured by the grayscale intensities of the darkened areas (Figure 2). In excess of a distance of nine nucleotide bases (e.g., $\mathbf{X_5}$), the effect of the mismatched site on the DNA-templated reaction was almost unnoticeable.[9] In contrast, solution-based DNA-templated ligation reactions can also be used to detect a perfectly matched target, but mismatched targets

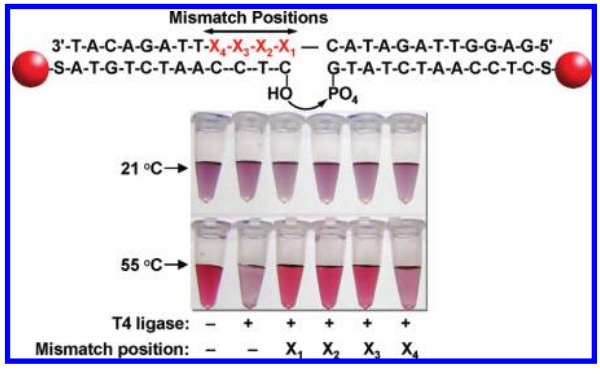

Figure 3. Color responses of the probe solutions for targets with various mismatch positions ($\mathbf{X_1}$ to $\mathbf{X_4}$) at 21 and 55 °C in the presence (+) or absence (−) of T4 DNA ligase. Notably, the irreversible color responses for lanes 2 and 6 indicate the high-yield formation of covalent bonds that hold the particles together at elevated temperatures.

with SNP positions (e.g., $\mathbf{X_1}$−$\mathbf{X_3}$) adjacent to the ligation site cannot be colorimetrically differentiated (Figure 3). This can be attributed to colorimetric assay interference resulting from the presence of large amounts of noncovalently linked particles in solution.

In conclusion, we have developed a straightforward approach for rapid and ultrahigh-sensitivity detection of SNPs without complex stringency washing steps. Although this approach requires reactions and conditions that are compatible with particle probes, we expect its ability to quickly identify the precise location of the single-base mismatch in a target DNA sequence via an array assay format to provide a time-efficient approach for high-throughput multiplex SNP genotyping. Ongoing efforts in our group seek to further develop this new approach by expanding the reaction scope and coupling it with lanthanide-doped multicolor luminescent nanocrystals[10] for improved sensitivity and multiplex capability.

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Supporting Information Available: Details of the experimental procedure as well as TEM and spectroscopic characterization of solution-based SNP detection. This material is available free of charge via the Internet at http://pubs.acs.org.

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