Application of partially double-stranded DNA probes to high-throughput SNPs genotyping

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\textbf{ABSTRACT}

We have focused on DNA strand exchange reaction (SER) with partially double-stranded (PDS) probes as a novel platform of SNPs genotyping. In this report, we elaborated the method for reliable and high-throughput SNPs typing. Competitive reactions using a couple of PDS probes designed for wild and mutant sequences significantly increase resolution efficiency to SNP types that is hardly discriminated by a non-competitive reaction with a single probe. Integration of the PDS probes with DNA microarray technology enable us to increase throughput efficacy. Simple and quick typing of fifteen SNPs with reliability as high as a ligase-based assay was demonstrated.

\textbf{INTRODUCTION}

Single nucleotide polymorphisms, SNPs, may be linked to genetic predispositions, frank disorders or adverse drug response. There are variety of SNPs genotyping methods including the Taq-Man\textsuperscript{3}, the primer extension\textsuperscript{2} and the invader assays\textsuperscript{3}. However, in these assays, strict optimizations of probe/primer design and/or reaction conditions are required. These methods are also lack in resource effectiveness and throughput efficacy.

As a resource effective and simple method for SNPs typing, we have focused on DNA strand exchange reaction (SER) of partially double-stranded (PDS) probes with target DNAs. The PDS probe consists of double-strand and single-strand (ss) regions (Fig.1)\textsuperscript{6}. When the PDS probe was added to fully matched target DNA, rapid SER was initiated by nucleation between the target DNA and the ss region of the probe\textsuperscript{7}. On the other hand, a single-base mismatch at the nucleation site of the target DNA significantly impeded with the nucleation, resulting in considerably slow SER. We previously reported that the PDS probes can effectively discriminate between fully matched and single-base mismatched target DNAs. However, the PDS probe method showed insufficient resolution for some of single base mutations such as A:T vs. A:G base pairs. In this study, we demonstrated that resolution efficacy of the PDS probe method can be improved by a competitive reaction assay where a couple of PDS probes designed for both wild and mutated sequences are simultaneously mixed with target DNAs in a reaction. We further elaborated the PDS probe method to high-throughput SNP genotyping assay by employing DNA microarray technology.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Schematic representation of mismatch detection by SER using the PDS probe}
\end{figure}

\textbf{RESULTS AND DISCUSSION}

The PDS probes were obtained by mixing FITC-oligodeoxynucleotides containing complementary sequence to SNP site at single-stranded portion (~6 mer) and its complementary DABCYL-labeled oligodeoxynucleotides in the equimolar amount. When a target ss DNA was added to fully matched PDS probes, fluorescence intensities drastically increased within 10 min due to SER. In contrast, the addition of a single-base mismatched target DNA to each PDS probe resulted in slow increase in the intensity. Thus, we can kinetically discriminate between target DNAs with and without a single-base mismatch. However, when the PDS probe with an adenosine base (A) at single-base mismatch site (Probe A) was added to target DNAs with T (Sample T) or G (Sample G), no significant change in time course profiles of SER was shown, indicating insufficient discrimination efficacy of the PDS probes for A:T vs. A:G base pairs.
single-stranded probes were added to the target DNAs, as shown in Fig.3a. The cluster separation was further improved when the PDS probes were reacted in the presence of poly(L-lysine)-graft-dextran copolymer, which accelerates DNA hybridization \(^{9,11}\) (Fig 3c). Similar allele discriminations at other allele sites were achieved. The typing results were consistent with those evaluated using a ligase-based method, indicating reliability of the PDS probe method. These results suggested that the integration of the PDS probes with PLL-g-Dex would offer a novel high-throughput genotyping with simple and resource effective format.

**CONCLUSION**

We demonstrated that mismatch discrimination with PDS probe was improved by a competitive reaction protocol, allowing reliable discrimination of most of SNP alleles. The integration of the PDS probe method with DNA microarray technology was shown to be promising for a high-throughput and resource-effective genotyping method.

**REFERENCES**


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