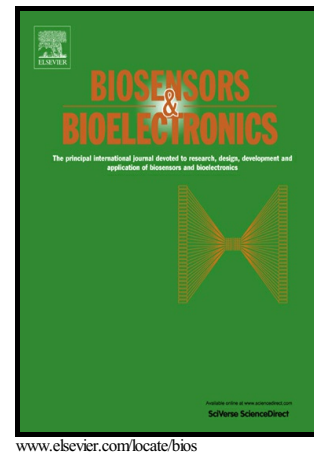


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Enhancing capacitive DNA biosensor performance by target overhang with application on screening test of HLA-B*58:01 and HLA-B*57:01 genes

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Abstract

A highly sensitive label-free DNA biosensor based on PNA probes immobilized on a gold electrode was used to detect a hybridization event. The effect of a target DNA overhang on the hybridization efficiency was shown to enhance the detected signal and allowed detection at a very low concentration. The sensors performances were investigated with a complementary target that had the same length as the probe, and the signal was compared to the target DNAs with different lengths and overhangs. A longer target DNA overhang was found to provide a better response. When the overhang was on the electrode side the signal enhancement was greater than when the overhang was on the solution side due to the increased thickness of the sensing surface, hence produced a larger capacitance change. Using conformationally constrained acpcPNA probes, double stranded DNA was detected sensitively and specifically without any denaturing step. When two acpcPNA probes were applied for the screening test for the double stranded HLA-B*58:01 and HLA-B*57:01 genes that are highly similar, the method differentiated the two genes in all samples. Both purified and unpurified PCR products gave comparable results. This method would be potentially useful as a rapid screening test without the need for purification and denaturation of the PCR products.

Keywords: Label-free capacitive DNA biosensor; acpcPNA probe; Target DNA overhang; HLA-B*58:01; HLA-B*57:01

1. Introduction

Specific-sequence detection of DNA has potential applications in many areas such as clinical diagnostics (O'Connor and Glynn 2010), detection of genetically modified organisms (Manzanares-Palenzuela et al. 2015) and environmental monitoring (Paniel et al. 2013). DNA biosensors are generally composed of single stranded DNA (ssDNA) or peptide nucleic acid (PNA) probes immobilized on a transducer surface that are able to form a duplex with the target DNAs (Odenthal and Gooding 2007). We have recently reported the formation of label-free DNA biosensors based on the sensitive capacitive detection using pyrrolidiny PNA with a conformationally rigid D-prolyl-2-aminocyclopentanecarboxylic acid (ACPC) backbone (acpcPNA) (Sankoh et al. 2013; Thipmanee et al. 2012; Vilaivan 2015). When tested with target DNAs of the same length as the PNA probe, they provided excellent performance. However, real DNA samples are usually much longer than the probe, and exist in double-stranded forms.

It was therefore of interest to investigate the performance of a biosensor with a long and double stranded DNA target, as this was a more realistic situation when testing real samples.

The influence of target length, or more accurately the overhang, on the hybridization response in a label-free biosensor has been reported using cantilevers (Mukhopadhyay et al. 2005) and mostly by detection using electrochemical impedance spectrometry (EIS) (Booth et al. 2011; Corrigan et al. 2014; Riedel et al. 2014; Shamsi and Kraatz 2011). It was expected that the response would increase with the length of the overhang. However, using the EIS response (charge transfer resistance - R_{ct}), the results seemed to vary, from an increased response with the length of the overhang (on the solution side) (Booth et al. 2011) to the existence of an optimal overhang length for the highest response (Corrigan et al. 2014) and to a decrease in the response with the length of overhang despite the increased mass on the sensing surface (Riedel et al. 2014). In all cases the explanation for these behaviors was attributed to the increased negative charges from the overhang target DNA but with different principles on its effect on the electrode/electrolyte charge transfer, hence, the different outcomes. The position of the overhang in the DNA target was also of interest (Riedel et al. 2014; Shamsi and Kraatz 2011), however, the results were inconclusive, possibly due to the different experimental conditions. Since a capacitive system relies on the ability of an electrode to store a charge without any charge transfer, it was important to see how the target DNAs with different overhangs contributed to the capacitive detection. Capacitance determination is generally performed via EIS or by a potentiostatic step method (Berggren et al. 2001). In this case when a potential was given to an electrode, with the insulating sensing layer on the surface, the electrode will possess the same amount of charge as the solution but with an opposite sign. It was expected that the overhang, with its increased mass on the electrode surface changed the thickness of the insulating sensing layer, hence, increased the change in the capacitance.

In this work the effect of the target DNA overhang on the hybridization efficiency of a potentiostatic step capacitive DNA sensor based on an acpcPNA probe was, for the first time, studied with the objective that this effect may help to enhance the detected signal and that the system could be readily applied to a real sample. The detection of the single stranded target DNAs with different lengths and overhangs was first explored. The advantage of using the acpcPNA probe for binding with the double stranded target DNAs was also reported. As a proof of the application we investigated the performance of the developed DNA biosensor on the screening test for the PCR products of two interesting genes HLA-B*58:01 and HLA-B*57:01 in which the targeted sequences have only two nucleotides different (Robinson et al. 2015), and

both had long non-hybridizing segments. HLA-B*58:01 is strongly associated with hypersensitivity to allopurinol that is used to treat hyperuricaemia and recurrent gout, as for HLA-B*57:01, it strongly predicts hypersensitivity to the anti-retroviral drug abacavir (Yun et al. 2012). A detection system that can differentiate and detect the PCR product rapidly with high accuracy is needed, to screen for patients with HLA-B*57:01 or HLA-B*58:01 in the clinic to identify those who should avoid the prescription of potentially harmful drugs. The standard method to detect a specific HLA type in the laboratory is usually conducted by the polymerase chain reaction (PCR) that employs several sets of primers (Hein et al. 1995) or by using nested PCR (Martin et al. 2005) followed by the time consuming detection of the PCR products by gel electrophoresis. The ability of this capacitive DNA biosensor to achieve a rapid, sensitive and selective differentiation between HLA-B*57:01 and HLA-B*58:01 genes was demonstrated.

2. Experimental

2.1 Materials

The 11-mer lysine-modified acpcPNA probes were synthesized by Mrs Chotima Vilaivan at Chulalongkorn University, Thailand according to the published protocol (Vilaivan and Srisuwannaket 2006). The acpcPNA probes were purified by reverse phase HPLC (to 90% purity) and their identities were verified by MALDI-TOF mass spectrometry. The sequence of the lysine-modified acpcPNA probes for both the HLA-B*58:01 (P1 and P2) and the HLA-B*57:01 (P3) genes are shown in Table 1.

The synthetic target DNAs used in this work were synthesized and purified by the Bioservice Unit, National Science and Technology Development Agency and BioDesign Co., Ltd., Thailand, and their sequences are shown in Table 1. The HLA-B*58:01 and HLA-B*57:01 PCR-amplified DNA samples were 200 bases in length, with only a 2-nucleotide difference (Table 1). These genes were amplified from the genomic DNA, extracted from white blood cells (see Supplementary data for the PCR protocol) and their concentrations were determined from the optical density at 260 nm using a Nanodrop spectrophotometer (Wilmington, USA).

The blocking thiol, 11-mercapto-1-undecanol (11-MUL) was from Aldrich (Steinheim, Germany). para-phenylenediamine (*p*-PD) was from UNILAB reagent (Sydney-Melbourne, Australia). Glutaraldehyde was from Sigma-Aldrich (Steinheim, Germany). All buffers were prepared with deionized water treated with a reverse osmosis-deionizing system (Pentair, Inc., USA). Before use, buffers were filtered through a nylon membrane (0.2 µm pore size, 47 mm

diameter, Vertical®, Spain) before being degassed. Other chemicals were analytical reagent grade and were used as received.

2.2 Immobilization of acpcPNA probe

Gold rod electrodes (99.99% purity) with a diameter of 3.0 mm were cleaned by dipping in piranha solution (3:1 %v/v of conc. H₂SO₄: 30% H₂O₂) for 20 min followed by rinsing with distilled water. Then, they were polished using an alumina slurry (5, 1, 0.3 µm), on a polishing cloth until a mirror-like surface was obtained and subsequently washed with distilled water. The electrodes were placed inside a plasma cleaner (Model PDC-32G, Harrick, New York, USA) to remove organic and inorganic molecules adsorbed on the surface of the electrodes.

The P1, P2 and P3 PNA probes were immobilized on separate electrodes via a polymer layer of *p*PD (*Pp*PD) following an earlier reported protocol (Sankoh et al. 2013) (Supplementary data Fig.S1). The *p*PD monomer of 5.0 mM was first prepared in 1.0 mM sodium acetate buffer pH 5.18. Electropolymerization was carried out by cyclic voltammetry at 15 scans using the potential range from 0.0 to 0.8 V vs. Ag/AgCl with a scan rate of 50 mVs⁻¹. The *Pp*PD coated electrode was cleaned by rinsing with distilled water and treated with 5.0 % (v/v) glutaraldehyde in 10 mM phosphate buffer pH 7.00 at room temperature for 20 min to activate the aldehyde group. Then 20 µL of 5.0 µM of acpcPNA probe was placed on the *Pp*PD coated electrode and left for 24 h at 4 °C. The electrode was then treated with 1.0 mM ethanolamine pH 8.00 for 7 min to deactivate all the remaining aldehyde groups not coupled to the immobilized acpcPNA probe. Finally, 1.0 mM 11-MUL solution was applied for 1 h to block the remaining pinholes, hence, preventing any non-specific binding on the electrode surface.

2.3 Capacitance measurement

The measurements were performed in a flow-injection system. The modified gold electrode was used as the working electrode together with an Ag/AgCl reference electrode and a Pt wire counter electrode connected to a potentiostat (Model EA161, EDAQ, New South Wales, Australia). The conditions were, carrier buffer: 10 mM phosphate buffer pH 7.00, a flow rate: 50 µL min⁻¹, sample volume: 300 µL and the regeneration solution: 50 mM NaOH, 300 µL.

The capacitance was obtained based on a potential step method (Berggren et al. 2001) as previously described (Sankoh et al. 2013) (Supplementary data Fig.S1). In brief, the transient current response of an applied potential step (50 mV, 1 pulse/min) was used to obtain the linear least-square fitting of $\ln i(t)$ versus t . The value of the capacitance of the electrode surface, C_{total} ,

was then obtained from the slope of the linear regression equation. The measuring flow cell had a dead volume of approximately 10 μL . The flow of the carrier buffer through the measuring cell was maintained by a peristaltic pump (Miniplus3, Gilson, France) that provided a baseline capacitance. Then, the synthetic target DNA or the gene sample with a volume of 300 μL was injected into the flow system and the hybridization between the PNA probes on the electrode surface and the target DNAs in the flow through sample was performed at room temperature. The decrease in the total capacitance upon hybridization due to the increase of the dielectric layer thickness can be described by Equation 1

$$\frac{1}{C_{total}} = \frac{1}{C_{PpPD}} + \frac{1}{C_{acpcPNA}} + \frac{1}{C_{DNA}} \quad (1)$$

where C_{total} is the total double layer capacitance measured at the electrode-solution interface, C_{PpPD} is the capacitance of the PpPD modified layer, $C_{acpcPNA}$ is the capacitance of the acpcPNA layer, and C_{DNA} is the capacitance of the DNA layer. The measure C_{total} was plotted as a function of time. The capacitance change (ΔC) is the difference between the capacitance obtained before and after the binding of the PNA probe and the target DNA (Supplementary data Fig. S1). The regeneration solution (50 mM NaOH, 300 μL) was then injected and flowed through the electrode surface to break the binding between the probe and the target and the capacitance returned to its baseline, ready for a new analytical cycle.

3. Results and discussion

3.1 Effect of target DNA overhang

The hybridization between the PNA probe and target DNA of unequal lengths results in non-hybridized segments. The target DNA overhang was first investigated, with an immobilized P1 probe, using different lengths of synthetic ssDNAs with overhangs on either side between 1.0×10^{-14} and 1.0×10^{-6} M. Linear relationship between ΔC and the logarithm of the concentration of all targets were between 1.0×10^{-12} and 1.0×10^{-8} M. The limit of detection (LOD) of the capacitive biosensor for the complementary target DNA (T1) was 1.0 pM (Buck and Lindner 1994). For the 41 and 61 bases target DNAs (T2 and T3) with a 15 and 25 nt overhang on each side, the limit of detection improved slightly with the length of the overhang, i.e., 0.8 pM and 0.5 pM for the target with a 15 and 25 nt overhang, respectively. For the the sensitivity (expressed by the slope of the calibration plot), the results in Fig. 1a indicated that the targets T2 and T3 had higher sensitivities (18.0 ± 1.0 and 23.2 ± 1.1 $-\text{nF cm}^{-2} (\log \text{M})^{-1}$, respectively) than the 11 bases

DNA without the overhang ($11.1 \pm 0.2 \text{ nF cm}^{-2} (\log M)^{-1}$). As anticipated a longer overhang (25 bases of the 61 bases T3), which added more mass to the surface, gave a higher sensitivity (and a better LOD). This was in contrast to some earlier reports based on EIS in which the highest R_{ct} was obtained from the hybridization between the DNA probe and the complementary target DNA that has no overhang. The lower response was said to be due to the lower charge transfer resistance caused by the lower hybridization efficiency of a target with an overhang on the electrode side (Shamsi and Kraatz 2011) or to the direct increase of the negative charges (of the target DNA) near the electrode, which disturbed the permeation of ferri-/ferrocyanide on the electrode (Riedel et al. 2014). These effects were probably overcome by the use of the PNA probe, which has proven to be very efficient for hybridization with target DNA (Vilaivan 2015; Vilaivan and Srisuwannaket 2006) and for the detection of the change in capacitance as it does not depend on the charge transfer.

Further experiments were carried out to see the effect of the position of the overhanging part. This was investigated using two 65 bases as target DNAs with a 54 base overhang on either side (T4 and T5). As shown in Fig. 1b, a much higher sensitivity was obtained from the target DNA with the overhanging part on the electrode surface ($27.1 \pm 1.5 \text{ nF cm}^{-2} (\log M)^{-1}$) compared to the one with the overhang in the solution ($11.1 \pm 0.2 \text{ nF cm}^{-2} (\log M)^{-1}$). This is most likely because the overhang on the electrode surface pushed the electrolyte further away from the electrode surface, i.e., making the interface thicker resulted in a decreased capacitance (Berggren et al. 2001; Gebbert et al. 1992), and thus increased the ΔC . On the other hand the target DNA with the overhang in the solution gave the same sensitivity as the fully complementary DNA (11 bases) but with a slightly higher signal. It was therefore likely that an overhang of the target DNA on the electrode surface was more effective for displacing water and electrolyte molecules away from the surface, and provided for a more enhanced sensitivity. This is in contrast to the results of Shamsi and Kraatz (Shamsi and Kraatz 2011). Using DNA probes they found that the same length of an overhang on the electrode side, compared to the solution side, decreased the response (ΔR_{ct}). They reasoned that this was most likely due to the decrease in hybridization efficiency caused by steric congestions at the electrode surface. This again confirmed the advantage of this newly reporting system, i.e., the use of a neutral PNA probe can reduce such a steric effect from the DNA-DNA hybridization.

3.2 Detection of double stranded target DNA

As most DNA targets are in duplex forms (dsDNA), almost all previous DNA sensors require denaturation of the target dsDNA prior to detection. One advantage of using PNA is that it can

also bind directly to dsDNA or other structured DNA targets in various configurations depending on the base sequence (Armitage 2003; Wang and Xu 2004). For the first time, a direct capacitive detection with immobilized PNA probes was investigated for its binding capability to dsDNA targets, with and without denaturation. Two dsDNAs were tested with the P1 probe, i.e., the PCR products of the target HLA-B*58:01 gene and the HLA-B*57:01 gene that had only two nucleotides different. The denaturation was carried out by heating at 90°C in a water bath for 15 min and rapidly cooled down on ice for 10 min. The responses from the DNAs with and without denaturation are shown in Fig. 2. The slightly higher response of the denatured DNA was most likely due to the better accessibility of the PNA probe to the hybridization segment. However, the responses of the DNA without denaturation were only about 10% lower than those from non-denatured DNA. As expected the responses to the HLA-B*57:01 gene were much lower than those to the HLA-B*58:01, but a similar behavior was obtained, i.e., the denatured DNA gave a higher response than the dsDNA. From the results, this system could be applied to detect target dsDNA without the need for denaturation, and therefore reduced the time taken and simplified the method.

3.3 Analysis of the purified HLA-B*58:01 and HLA-B*57:01 genes

Using the electrode with the immobilized P1 probes, the purified HLA-B*58:01 and HLA-B*57:01 genes were first tested in the concentration range from between 1.0×10^{-14} and 1.0×10^{-7} M. The linear range of both targets were from 1.0×10^{-12} to 1.0×10^{-8} M, with the same LOD of 0.4 pM. However, the %signal suppression values ($\%SS = 100 \times (\text{complementary response} - \text{mismatched response}) / \text{complementary response}$) within the linear range were only 15 – 30 %. This led to the investigation of using a hybridization suppressor (additive) that helped to control the hybridization behavior of the perfect matched and mismatched target DNA (Lao et al. 2009). Two additives were tested, using the carrier buffer of 100 mM phosphate buffer, pH 7.00. Acetamide (0.10, 0.25, 0.50, 0.80, 0.90 and 1.0 M) and formamide (0.25 M) were investigated with the P1 probe to detect a 41 base target DNA (T2) at 1.0×10^{-10} M.

The additives did lower the responses for both the complementary (T2) and mismatched DNAs (T2mis1 and T2mis2). However, the presence of the additive considerably improved the %SS values (Supplementary data Table S1). Comparing the presence of formamide and acetamide at the same concentration (0.25 M) the latter provided a better %SS. This is because acetamide has a lower dielectric constant (D) than formamide (D = 4.7 and 109.5 at 25 °C, respectively) (Covington and Dickinson 1973), so gave a lower baseline that resulted in a larger ΔC response. The optimum concentration for acetamide was studied and found to be 0.25 M. This provided

the highest %SS for both a single (75%) and double (81%) mismatched DNAs. The HLA-B*58:01 and HLA-B*57:01 genes within the same concentration range were then tested again by adding 0.25 M acetamide in the buffer. The %SS improved by 50 – 70%, compared to when there was no additive, and therefore the acetamide additive was included for the remaining experiments.

The use of the P2 probe with a slightly different sequence to P1 (one base shift towards the C-terminus: Table 1) for the detection of HLA-B*58:01 was also tested. A calibration curve was established from a purified PCR product of HLA-B*58:01. The linearity between the capacitance change ($-nF\text{ cm}^{-2}$) and the logarithm of the HLA-B*58:01 concentration ($\log M$) was observed between 1.0×10^{-12} and 1.0×10^{-8} M (Fig. 3a). The very low concentration of the linear range would allow for detecting a sample with a very small amount of DNA. For samples with high concentration, dilution can be easily performed, hence only a small sample volume is required. The dilution will also reduce any interfering effect from the sample matrix. The performance of the P2 probe was comparable to P1 (data not shown), P2 was then used for further experiments. It should also be noted that the dsDNA of the HLA-B*58:01 gene with a 107-base non-hybridizing segment on the electrode side provided a much higher sensitivity ($36.0 \pm 1.3 -nF\text{ cm}^{-2} (\log M)^{-1}$) (Fig. 3a) than the ssDNA target with a 54 base overhang ($27.1 \pm 1.5 -nF\text{ cm}^{-2} (\log M)^{-1}$) (Fig. 1b). This also confirmed that a longer non-hybridizing segment provided a better sensitivity.

The detection for the HLA-B*57:01 genes yielded a sensitivity of $8.1 \pm 0.2 -nF\text{ cm}^{-2} (\log M)^{-1}$ (Fig. 3a). Although the sensitivity from the purified HLA-B* 58:01 was 4.4 times more than the one from the HLA-B*57:01 genes, an incorrect quantitative result may still be obtained. For example, a sample with a large amount of the HLA-B*57:01 genes might give the same response as a sample with a lower amount of the HLA-B*58:01 genes. Therefore, the same samples were detected twice using two probes, and the signals were compared to eliminate the effect of concentration. In this set up, the same sample was tested with two modified electrodes, one with the P2 probes and the other with the P3 probes. Using the P3 probes, the sensitivity to the complementary HLA-B*57:01 was 4.6 times higher than that for the highly similar HLA-B*58:01 (Fig. 3b).

By following this protocol of using two PNA probes, a total of 21 blind purified PCR gene samples (SP1-SP21) from human white blood cells were analyzed. Samples were sequentially diluted from the initial concentration to be approximately 10^{-10} M. This was done to eliminate any interferences from the PCR matrix as well as to adjust the concentration of the sample to be

within the linear range of the sensor. The results in Table 2 clearly showed that the responses of each sample based on the two probes differed by at least 3 fold. A higher response from the P2 probe indicated a HLA-B*58:01 gene and vice versa. That is, this method had a distinct ability to distinguish between the HLA-B*58:01 and the HLA-B*57:01 genes.

To verify the results, the blind samples were independently tested by the Sanger sequencing technique (Ward Medic Ltd., PART, Malaysia) using the forward primer (5'-TTT CTA CAC CGC CAT GTC C-3') and the reverse primer (5'-AAG TCT GCG CGG AGG C-3'). The results of the capacitive system and the Sanger sequencing technique were in complete agreement (Table 2). Therefore, a direct capacitive DNA biosensor can be used to differentiate between the purified PCR products of HLA-B*58:01 and HLA-B*57:01 with a high specificity. In addition, it required a very small sample volume, of only 1 – 2 μ L of the original PCR product for each measurement.

3.4 Analysis of unpurified PCR samples

Although there was an exceptionally good discrimination between the two genes, the experiments above were carried out with purified PCR products. If a similar discrimination could be achieved with an unpurified product much process time and cost could be reduced. Eight unpurified DNA samples (SU1-SU8, unpurified versions of blind samples SP1-SP8: diluted to be approximately 10^{-10} M) were then tested. A good differentiation between the two genes was obtained (Fig. 4). The results provided a high %SS and the type of genes were in complete agreement with the purified samples SP1-SP8 (Supplementary data Table S2). It is clear that the two genes can be easily distinguished without the need for purification of the PCR sample.

4. Conclusions

This research has shown that enhancement of the capacitive signal can be achieved by using the overhanging part of the target DNA. The longer the overhanging part on the electrode surface the better the signal, i.e., more capacitance change. This would be useful for the detection of samples that have a small amount of DNA. Another advantage is that dsDNA targets can be detected by the immobilized PNA probe without an additional denaturation step so the method for detection becomes much simpler. This work also demonstrated the successful use of a direct capacitive system based on two acpcPNA probes that could discriminate between the HLA-B*58:01 and HLA-B*57:01 genes from both purified and unpurified PCR samples. Adding acetamide to the carrier buffer improved the discrimination. This method provided for a rapid

screening test, with a high sensitivity and specificity, and required only a small sample volume (1 – 2 μ L from the original PCR product). In addition, the unpurified PCR products can be tested directly, without the need for a denaturing step.

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Figures captions

- Fig. 1.** Capacitance change from the hybridization of probe P1 with the same length target DNA (T1) and (a) target DNAs with different lengths of overhang on both sides, T2 and T3 (b) target DNAs with the same length of overhang (54 bases) but on opposite side (T4 and T5) ($n = 3$).
- Fig. 2.** Capacitance change from the hybridization of probe P1 with 1.0×10^{-11} M PCR product of the HLA-B*58:01 and HLA-B*57:01 genes, with and without denaturation ($n = 3$).
- Fig. 3.** Calibration plots of purified HLA-B*58:01 and HLA-B*57:01 using modified electrodes with (a) P2 probe (for HLA-B*58:01) and (b) P3 probe (for HLA-B*57:01) with 0.25 M acetamide in phosphate buffer ($n = 3$).
- Fig. 4.** Capacitance change of the blind unpurified HLA-B*58:01 and HLA-B*57:01 gene samples with 0.25 M acetamide in phosphate buffer ($n = 3$).

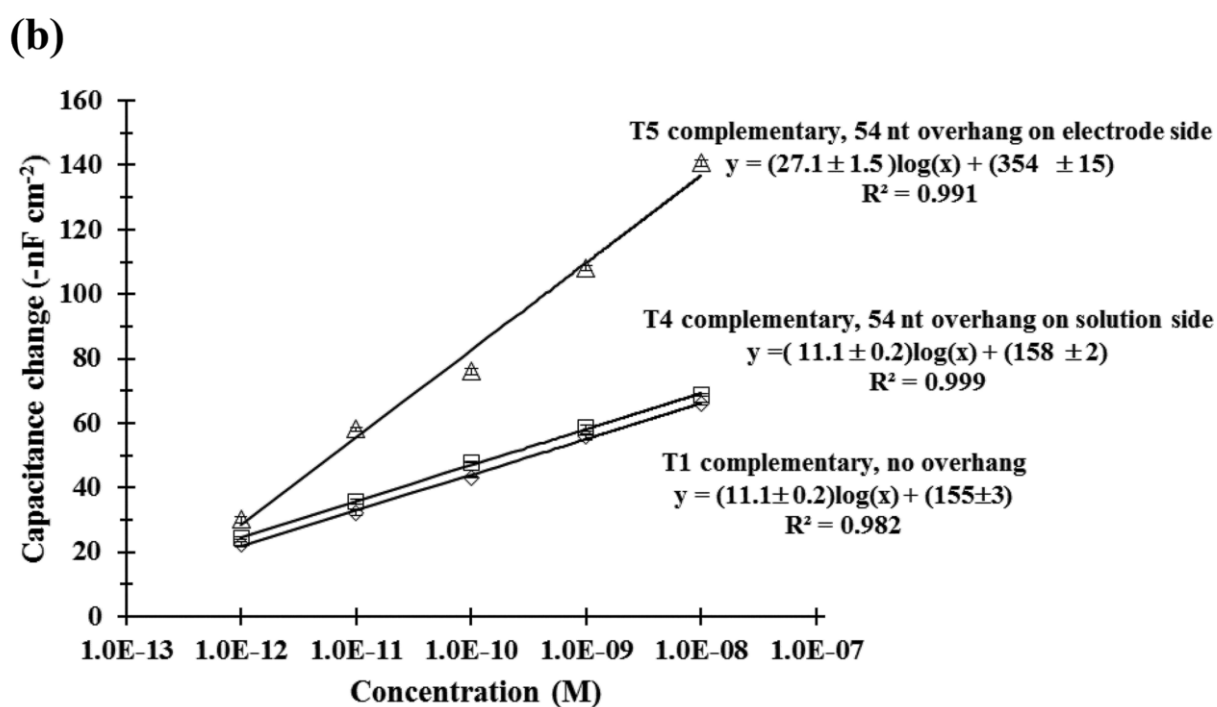
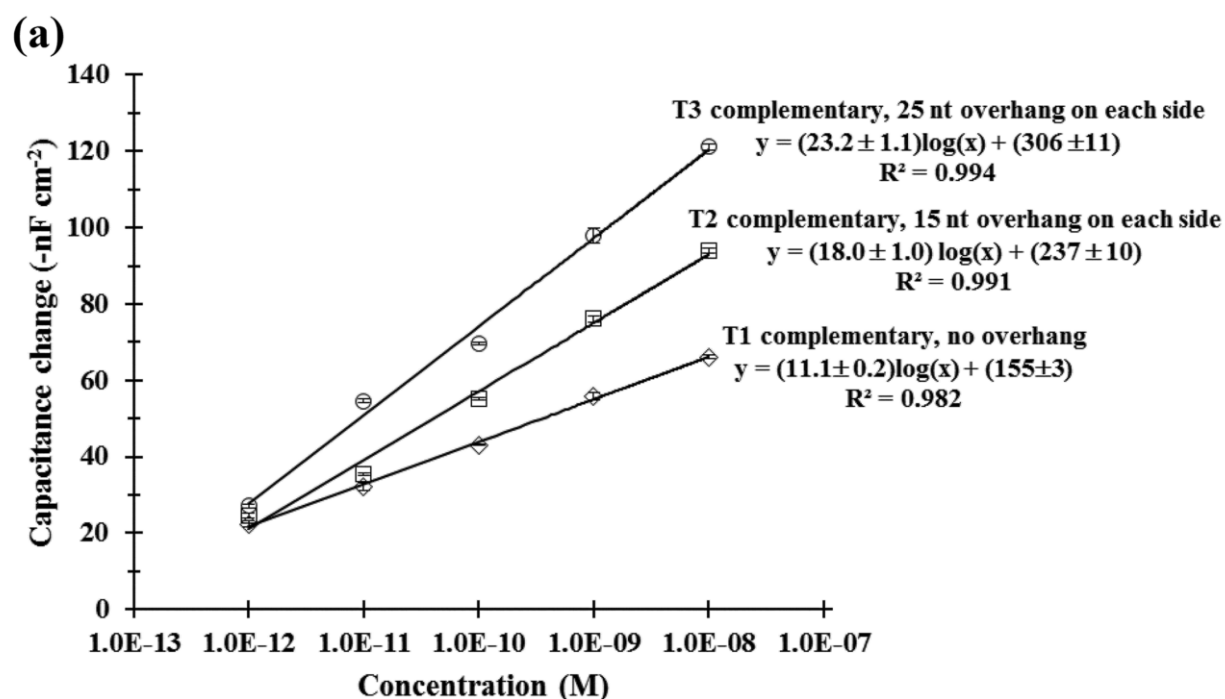
Table 1 Sequence of PNA probes and target DNAs. The italic and underlining alphabets indicate the mismatched and non-hybridizing segment, respectively.

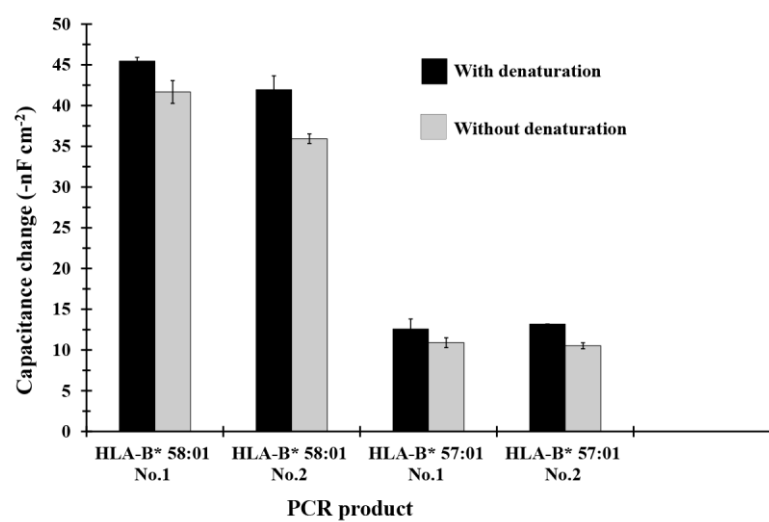
Name	Type	Sequence	Number of base
P1	acpcPNA probe for HLA-B*58:01 gene	Ac-GCTCCGTCCTC-LysNH ₂ (N--C)	11
P2	acpcPNA probe for HLA-B*58:01 gene (one base shift from P1)	Ac-CTCCGTCCTCG-LysNH ₂ (N--C)	11
P3	acpcPNA probe for HLA-B*57:01 gene	Ac-CGCCATCCTCG-LysNH ₂ (N--C)	11

Name	Type	Sequence	Number of base
T1	Single stranded complementary to P1	5'- GAGGACGGAGC -3'	11
T2	Single stranded complementary to P1 Overhang, 15 bases on each side	5'-CGACGCCGCGAGTCC GAGGACGGAGC CCCGGGCGCC ATGGA-3'	41
T2mis1	Single stranded, single mismatched to P1 Overhang, 15 bases on each side	5'-CGACGCCGCGAGTCC GAGGATGGAG CCCCGGGCGCC ATGGA-3'	41
T2mis2	Single stranded, double mismatched to P1 Overhang, 15 bases on each side	5'-CGACGCCGCGAGTCC GAGGTCTGAG CCCCGGGCGCC ATGGA-3'	41
T3	Single stranded complementary to P1 Overhang, 25 bases on each side	5'-GGTTCGAGAGCGACGCCGCGAGTCC 61 GAGGACGGAGC CCCCGGGCGCCATGG ATAGAGCAGGA-3'	61
T4	Single stranded complementary to P1 Overhang, 54 bases on solution side	5'- GAGGACGGAG CCCCGGGCGCC ATGGATAGAGCAGGAGGGGCCGGA GTATTGGGACGGGGAGACAC-3'	65
T5	Single stranded complementary to P1 Overhang, 54 bases on electrode side	5'-GGGCTACGTGGACGACACCCAGTT 65 CGTGAGGTTTCGACAGCGACG CCGCGAGTCC GAGGACGGAGC -3'	65
HLA-B*58:01	Double stranded PCR product Overhang, 107 bases on electrode side, 82 bases on solution side Bold alphabets show the sequence complementary to P2, the sequence complementary to P1 is one base shift to the left	52-TTTCTACACCGCCATGTC CCGGCCCGGCCGCGGGGAGC CCCGCTTCATCGCAGTGGGC TACGTGGACGACACCCAGTT CGTGAGGTTTCGACAGCGACG CCGCGAGTCC GAGGACGGAG CCCCGGGCGCCATGGATAGA GCAGGAGGGGCCGAGTATT GGGACGGGGAGACACGGAAC ATGAAGGCCTCCGCGCAGACTT-32	200
HLA-B*57:01	Double stranded PCR product, 2- nucleotide difference from the HLA-B* 5801 Overhang, 107 bases on electrode side, 82 bases on solution side Bold alphabets show the sequence complementary to P3	52-TTTCTACACCGCCATGTC CCGGCCCGGCCGCGGGGAGC CCCGCTTCATCGCAGTGGGC TACGTGGACGACACCCAGTT CGTGAGGTTTCGACAGCGACG CCGCGAGTCC GAGGATGGCG CCCCGGGCGCCATGGATAGA GCAGGAGGGGCCGAGTATT GGGACGGGGAGACACGGAAC ATGAAGGCCTCCGCGCAGACTT-32	200

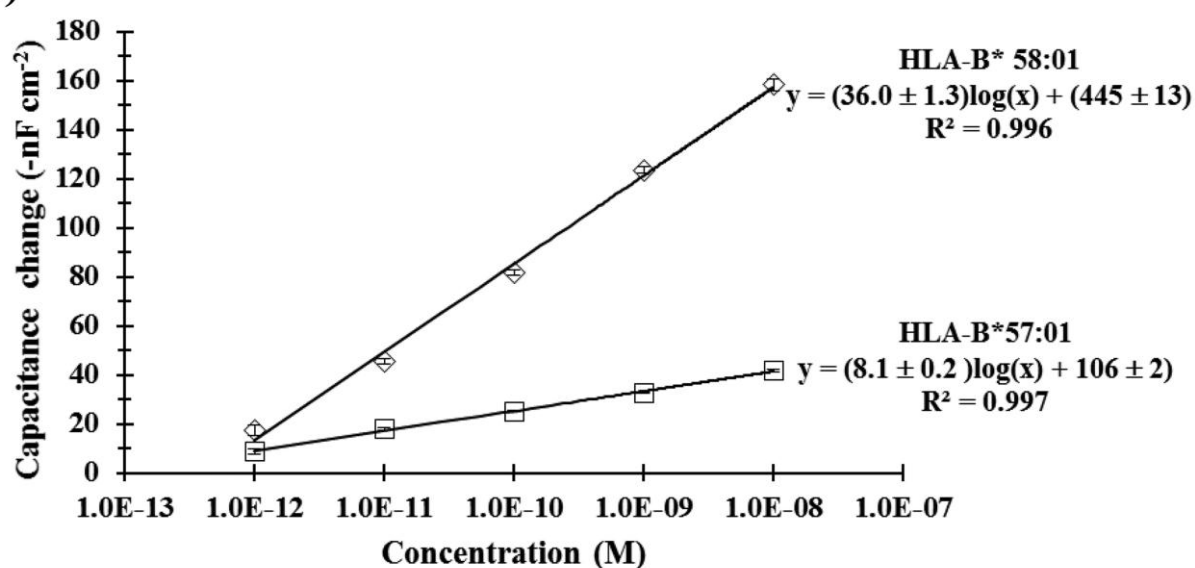
Table 2 The results of the blind purified PCR gene samples obtained from the white blood cell using a direct capacitive system (this work, $n = 3$) and the Sanger sequencing technique.

Sample	Concentration (M)	Capacitive DNA biosensor			Sanger sequencing
		Capacitance change		Gene	
		(-nF cm ⁻²)			
		Probe P2	Probe P3		Gene
SP1	2.3 × 10 ⁻¹⁰	34 ± 1	105 ± 2	5701	5701
SP2	2.2 × 10 ⁻¹⁰	95 ± 2	23 ± 1	5801	5801
SP3	1.7 × 10 ⁻¹⁰	29 ± 1	99 ± 2	5701	5701
SP4	2.3 × 10 ⁻¹⁰	99 ± 3	25 ± 1	5801	5801
SP5	1.3 × 10 ⁻¹⁰	88 ± 2	23 ± 2	5801	5801
SP6	2.1 × 10 ⁻¹⁰	97 ± 3	28.0 ± 0.2	5801	5801
SP7	2.9 × 10 ⁻¹⁰	35 ± 1	103 ± 3	5701	5701
SP8	2.1 × 10 ⁻¹⁰	98 ± 1	27 ± 2	5801	5801
SP9	3.3 × 10 ⁻¹⁰	102 ± 3	25.0 ± 0.2	5801	5801
SP10	4.1 × 10 ⁻¹⁰	34 ± 2	112 ± 1	5701	5701
SP11	4.1 × 10 ⁻¹⁰	33 ± 1	113 ± 1	5701	5701
SP12	4.3 × 10 ⁻¹⁰	103 ± 2	28 ± 1	5801	5801
SP13	4.1 × 10 ⁻¹⁰	33 ± 1	111.0 ± 0.3	5701	5701
SP14	4.2 × 10 ⁻¹⁰	106 ± 1	27 ± 1	5801	5801
SP15	4.1 × 10 ⁻¹⁰	108 ± 4	27 ± 1	5801	5801
SP16	4.1 × 10 ⁻¹⁰	34 ± 2	112 ± 1	5701	5701
SP17	4.1 × 10 ⁻¹⁰	34 ± 2	110.0 ± 0.2	5701	5701
SP18	1.8 × 10 ⁻¹⁰	32 ± 1	100 ± 1	5701	5701
SP19	1.6 × 10 ⁻¹⁰	93 ± 1	25 ± 1	5801	5801
SP20	1.7 × 10 ⁻¹⁰	31 ± 1	99 ± 1	5701	5701
SP21	1.8 × 10 ⁻¹⁰	97 ± 1	28 ± 1	5801	5801

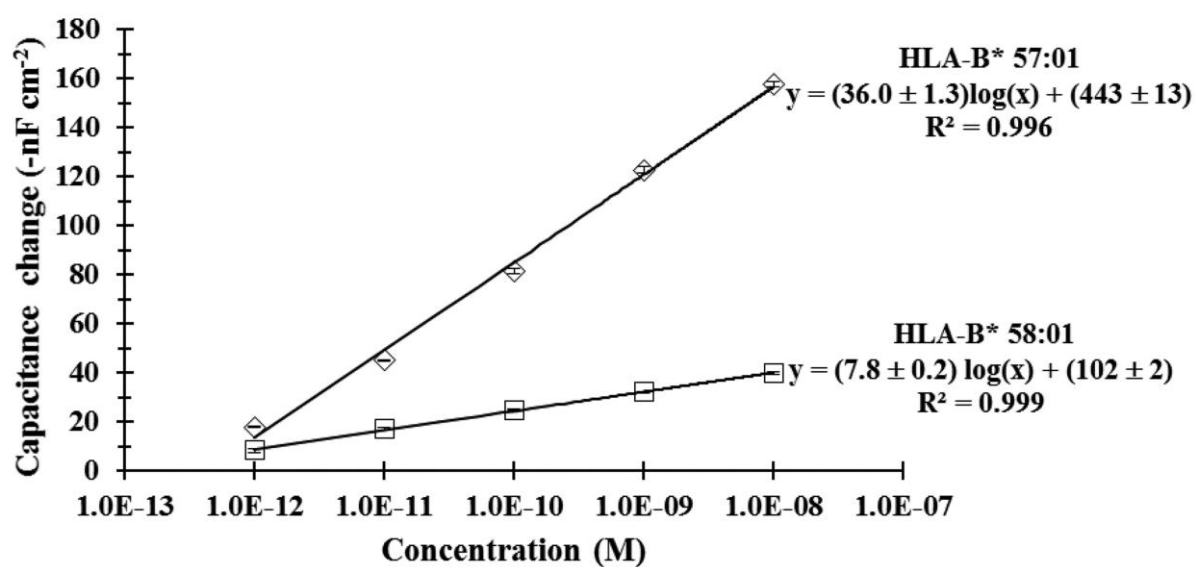


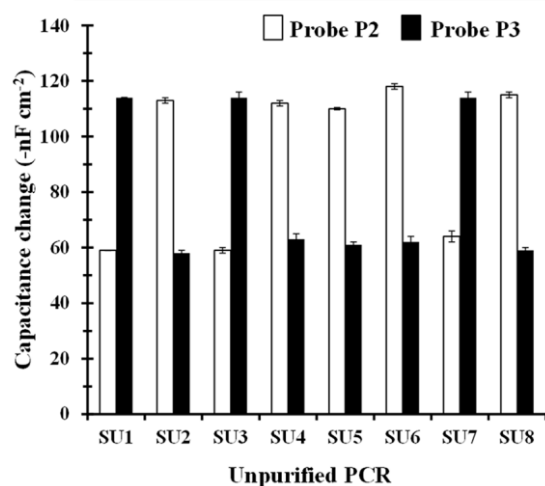


(a)



(b)





Highlights

- The longer the overhanging part on the electrode surface, the better the capacitive response.
- Double stranded DNA targets were detected using a PNA probe without denaturation.
- A direct capacitive system based on two acpcPNA probes differentiated between the highly similar HLA-B*58:01 and HLA-B*57:01 genes
- Both purified and unpurified PCR samples can be used.
- Only 1-2 μ L from the original PCR product was needed.