Potentiometric Detection of Single Nucleotide Polymorphism by Using a Genetic Field-effect transistor

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Potentiometric measurement of allele-specific oligonucleotide hybridization based on the principle of detection of charge-density change at the surface of a gate insulator by using of a genetic field-effect transistor has been demonstrated. Since DNA molecules are negatively charged in aqueous solution, a hybridization event at the gate surface leads to a charge-density change in the channel of the FET and can be directly transduced into an electrical signal without any labeling of target DNA molecules. One of the unique features of our method is to utilize DNA binders such as intercalators as charged species for double-stranded DNA after hybridization, since these are ionized and carry positive charges in aqueous solution. Single-base mismatch of the target DNA could be successfully detected both with the wild-type and with the mutant genetic FETs by controlling the hybridization temperatures and introducing Hoechst 33258 as DNA binder. The genetic FET platform is suitable as a simple, accurate, and inexpensive system for SNP typing in clinical diagnostics.

Introduction

Many types of solid-state biosensors have been developed by combining biotechnology and semiconductor technology. In particular, fabrication and detection technologies in the field of biochips able to detect and monitor specific binding of biomolecules in a parallel way on solid-state substrates have advanced during the past 15 years.[1–4] Several types of DNA chips and DNA microarrays for gene expression and genotyping analyses have been developed, and some of them are used in the field of molecular biology, in the pharmaceutical industry, and in clinical research. While the fluorescent detection method is widely used for DNA chips and DNA microarrays,[5–8] several other methods to detect biorecognition events on the devices—such as chemiluminescence,[9] surface plasmon resonance (SPR),[10] quartz crystal microbalances (QCMs),[11] and electrical current[12,13]—have also been developed. Although fluorescent image analysis is suitable for parallel detection of dense arrays of oligonucleotide spots, it needs expensive optics and instruments with complicated data analysis algorithms. A simpler, more reliable, and inexpensive method and device would be required for the platform used in clinical diagnostics. For genetic analysis in clinical diagnostics, single nucleotide polymorphisms (SNPs) are the most common form of DNA variation in humans, and these are important markers in "tailored medicine". An extensive collection of SNPs would serve as a valuable resource for the discovery of the genetic factors that affect disease susceptibility and resistance. The development of SNP genotyping based on this information has proceeded through the use of diverse methods in recent years[14–21] and should enable clinicians to determine which pharmacological agent would be most effective for treating a given patient's condition.[22]

Recently, field effect devices have been used for electrochemical detection of hybridization events on a solid surface.[23–29] Since DNA molecules in aqueous solution are negatively charged, the amount of negative charge at the gate surface increases as a result of hybridization, and the charge-density change is transduced into an electrical signal by the field effect. On this principle, point mutation analysis using the PCR products amplified with allele specific primers has been carried out.[30] In this case, overall specificity was determined through that of the allele-specific PCR. In this study we report the detection of single nucleotide polymorphism by use of a field-effect transistor in combination with allele-specific oligonucleotide hybridization. The temperatures during the hybridization and washing processes were maintained at their optimum values, depending on the melting temperature of the allele-specific oligonucleotide probe, in order to increase specificity. One of the unique features of our method is that it also utilizes DNA binders such as intercalators as charged species, since they are ionized and positively charged in aqueous solutions, while they are usually used as fluorescent dyes in the field of molecular biology.[31–35] Molecular recognition events such as hybridization and specific binding of DNA binders on the gate of the FET were successfully detected as shifts in the threshold voltage.

Principle of the genetic field-effect transistor

The principle of the genetic FET is based on detection of charge-density change, induced by specific binding of DNA molecules, at the gate surface (Figure 1). Oligonucleotide probes are immobilized on the surface of the gate insulator, and the genetic FET is immersed in a measurement solution

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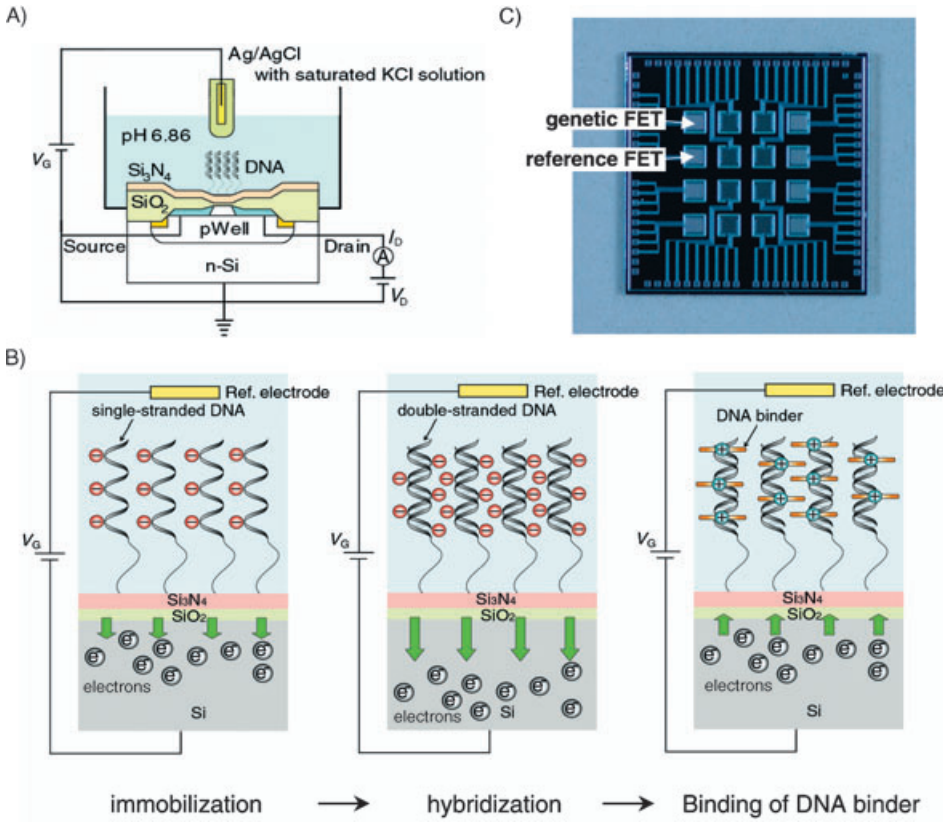

Figure 1. *Conceptual structure of a genetic field-effect transistor (FET). A) Schematic diagram for measurement of electrical characteristics of genetic FET. The shift of the threshold voltage (V_T) is determined from the gate voltage (V_G)–drain current (I_D) characteristics in a phosphate buffer solution (0.05 M Na_2HPO_4 and 0.05 M KH_2PO_4, pH 6.86). An Ag/AgCl electrode with saturated KCl solution is used as a reference electrode. B) Scheme for potentiometric detection of DNA molecules and DNA binders with a genetic FET. DNA molecules have negative charges in aqueous solution, while DNA binders have positive charges and bind specifically to double-stranded DNA. Since the charged molecules on the gate surface interact electrostatically with electrons in Si through the thin gate insulator, the charge-density change caused by molecular recognition events can be measured as the V_T shift. C) Photograph of the fabricated genetic FET chip. Sixteen FETs and a temperature sensor are integrated in a 5×5 mm chip. The FETs are n-channel depletion type with Si_3N_4/SiO_2 as gate insulator. A pair of FETs were used as a genetic FET and a reference FET.*

together with an Ag/AgCl reference electrode (with saturated KCl solution). The potential of a measurement solution is controlled and fixed by the gate voltage (V_G) through the reference electrode (Figure 1 A). When complementary DNA molecules are contained in a sample solution, hybridization occurs at the surface of the gate area. Since DNA molecules are negatively charged in aqueous solution, hybridization event can in principle be detected by measuring changes in the electrical characteristics of the FET, such as a positive shift of the threshold voltage (V_T; Figure 1 B). When DNA binders are introduced into the double-stranded DNA after hybridization, the threshold voltage of the genetic FET shifts in the negative direction, because DNA binders are positively charged (Figure 1 B). Since some of those DNA binders react specifically with double-stranded DNA, undesirable background noise caused by nonspecific adsorption of single-stranded target DNA can be eliminated, so more precise and reliable detection of hybridization event should be achieved.

We used n-channel depletion mode FET with a double layer of Si_3N_4/SiO_2 as the gate insulator (Figure 1 C). The surface of the Si_3N_4 is not polarized in the aqueous solution but is sensitive to pH in the solution. In order to obtain only the potential change induced by the specific molecular recognition event on the gate, differential measurements were carried out with a pair of FETs: one for the genetic FET with immobilized oligonucleotide probes and the other for the reference FET without oligonucleotide probes. Differential measurement effectively eliminates the common background noise caused by temperature changes, nonspecific adsorption of the charged species, and changes in ion concentrations. For highly sensitive detection of the molecular recognition event on the gate surface, the relationship between the channel width (W) and the channel length (L) was designed to be $W/L = 480$.

Results

Detection of molecular recognition at the gate surface

Specific binding of charged biomolecules at the gate surface can be detected as a shift of the threshold voltage V_T, which can be determined in the gate voltage V_G and the drain current I_D (V_G–I_D) characteristics of the genetic FET (Figure 2). The V_G–I_D characteristics of the genetic FET were shifted along the V_G axis in the positive direction after immobilization of oligonucleotide probes (Figure 2 A). The oligonucleotide probes immobilized were a normal type for the R353Q locus of the factor VII gene (Table 1[21]). In order to evaluate the V_T shift in more detail, the local area shown in Figure 2 A (surrounded area) was magnified (Figure 2 B). The V_T shifts after hybridization and specific binding of DNA binder are also shown in Figure 2 B. When the oligonucleotide probes were immobilized on the gate surface, the V_T was shifted along the V_G axis by the amount of 32 mV. The positive shift is due to negative charges induced at the gate surface after an immobilization process including cleaning, silanization, glutaraldehyde treatment, immobilization of oligonucleotide probes, and blocking with glycine. Immobilization of oligonucleotide probes and glycine blocking is considered to contribute to the V_T shift to a large extent. When the complementary target DNA was introduced onto the gate surface and hybridized with oligonucleotide probes, the V_T was shifted in the positive direction by the amount of

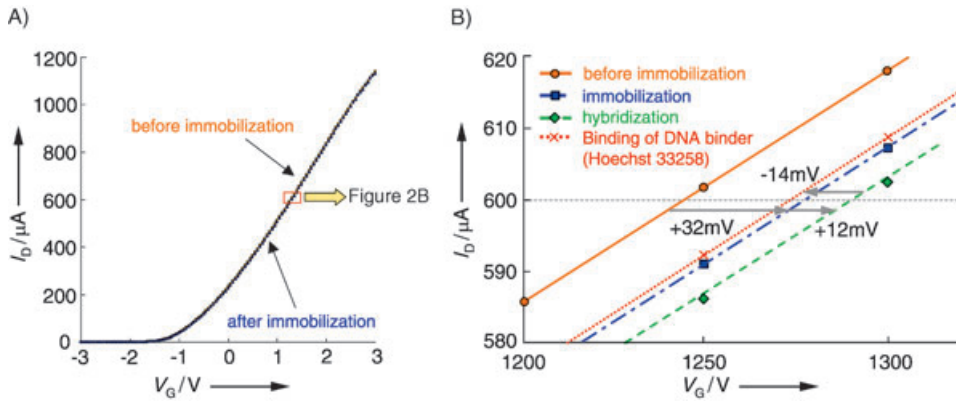

Figure 2. *Electrical signals of molecular recognition events on the genetic field-effect transistor (FET). A) Gate voltage–drain current (V_G–I_D) characteristics of genetic FET before and after immobilization of oligonucleotide probes. B) Threshold voltage V_T shifts after immobilization of oligonucleotide probes, hybridization of target DNA, and specific binding of Hoechst 33258. The size of the V_T shift was determined at a constant drain current of 600 μA.*

Table 1. *Base sequences for oligonucleotide probes at the R353Q locus of factor VII gene.*[21]

Locus	Function	Sequence	T_m [°C]
R353Q	R353Q-normal (N)		
	probe	5′-amino group-CCACTACC**G**GGGCACGT-3′ (17 mer)	60 (T_{m1})
	target	5′-ACGTGCCCCGGTAGTGG-3′ (17 mer)	
	R353Q-mutant (M)		
	probe	5′-amino group-CCACTACC**A**GGGCACGT-3′ (17 mer)	57 (T_{m2})
	target	5′-ACGTGCCCTGGTAGTGG-3′ (17 mer)	

N and M indicate normal (wild-type) and mutant allele-specific oligonucleotides, respectively. T_m shows the melting temperature.

12 mV, due to the increasing of the negative charges of the target DNA introduced by hybridization. After hybridization, a DNA binder—Hoechst 33258—was introduced onto the gate surface, and the V_T was shifted in the negative direction by the amount of 14 mV. The negative shift of the V_T indicates an increase in positive charge at the gate surface and is due to specific binding of Hoechst 33258 to the double-stranded DNA. This is in contrast to the positive change of the V_T due to negatively charged DNA molecules. The charge-density change at the gate surface after each molecular recognition event could thus be successfully detected by use of genetic FETs.

The V_T shifts for various DNA binders were investigated with genetic FETs (Figure 3). All the DNA binders tested are ionized and carry positive charges in aqueous solution (Figure 3A). When each DNA binder was introduced onto the gate surfaces of the genetic FETs after hybridization, the V_T was shifted in the negative direction due to the positive charge of the DNA binder (Figure 3B). The magnitudes of the V_T shifts were different for the different DNA binders, since the densities of DNA binder attachment along the double-stranded DNA are dependent on the DNA binders. The use of some of the DNA binders after hybridization is effective for discriminating between the signals of double-stranded DNA and those of non-specifically adsorbed single-stranded DNA at the gate surfaces, as some of the DNA binders used in this study, such as EB and PI, react specifically with double-stranded DNA.

Discrimination of single nucleotide polymorphism

We prepared two types of genetic FETs to detect single base changes in the target DNA, using the R353Q locus of factor-VII gene as a model sample (Table 1[21]). Normal (wild-type) oligonucleotide probes were immobilized on the gate surface of one of the genetic FETs (N-type genetic FET), while mutant oligonucleotide probes were immobilized on the gate surface of the other genetic FET (M-type genetic FET). The N-type and M-type genetic FETs were placed in a buffer solution and the temperature of the buffer solution was maintained at 60 °C for hybridization with target normal DNA in a normal sample and at 57 °C for hybridization with target mutant DNA in a mutant sample (Figure 4A). When normal DNA was hybridized at 60 °C with N-type and M-type genetic FETs, the V_T shift produced by the fully matched N-type genetic FET was bigger than that due to the one-base mismatched M-type genetic FET (Figure 4B (**1**)). On the other hand, when mutant DNA was hybridized at 57 °C with both FETs, the V_T shift obtained for the fully matched M-type genetic FET was bigger than that for the one-base mismatched N-type genetic FET (Figure 4B (**2**)). This means that it is possible to detect a one-base change of a target DNA with the genetic FETs. However, the difference between the signals of the N-type genetic FET and the M-type genetic FET in response to the mutant sample (Figure 4B (**2**)) was small in relation to that observed in response to the normal sample (Figure 4B (**1**)). The ratio of the signals between the N-type genetic FET and the M-type genetic FET was 4.9:1 in the case of the normal sample, while the signal ratio between the N-type and M-type FETs was 0.6:1 in the case of the mutant sample. Precise control and optimization of the temperatures during the hybridization and washing processes are important for a robust detection system. After hybridization, Hoechst 33258 was introduced onto the gate surfaces of the N-type genetic FET and the M-type genetic FET (Figure 4C). The ratio of the signals between the N-type genetic FET and the M-type genetic FET was 10.1:1 in the case of the normal sample, while the signal ratio between the N-type and M-type FETs was 0.1:1 in the case of the mutant sample. The ability to discriminate a single base change could thus be increased by the use of the DNA binder in combination with the genetic FETs. It is notable that the directions of the V_T shifts were positive for hybridization with

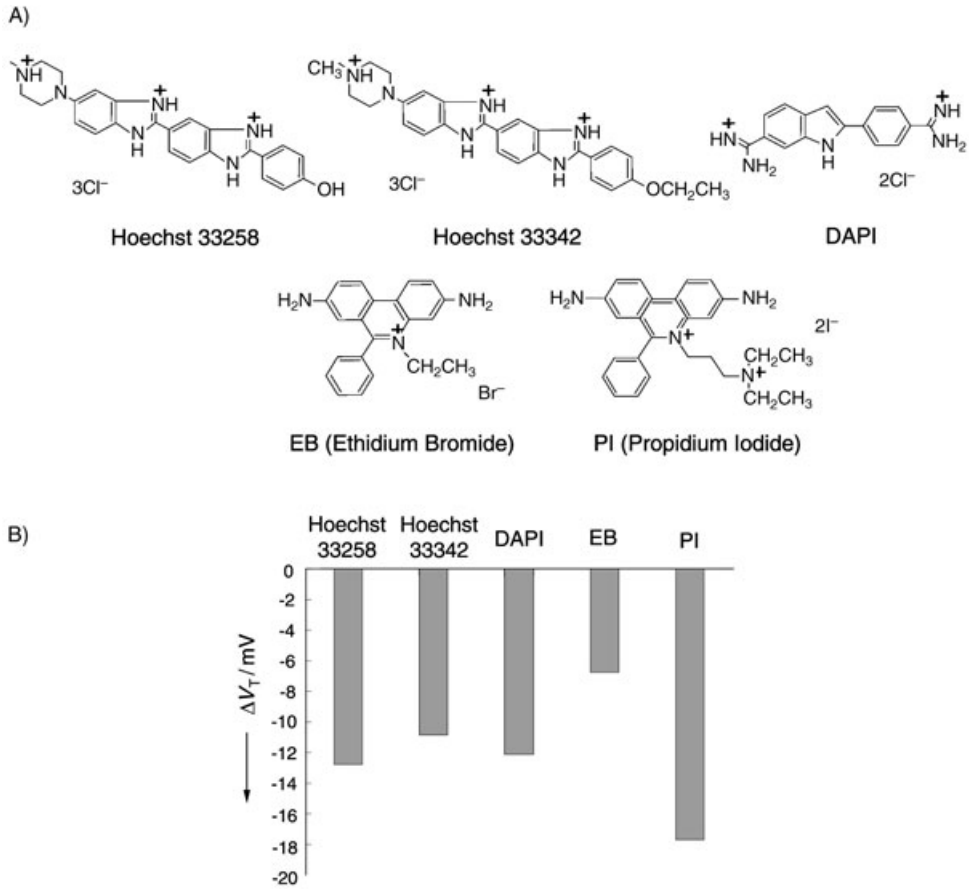

Figure 3. *Potentiometric detection of DNA binders. A) Chemical structures of various DNA binders. All the DNA binders shown here have positive charges in aqueous solution and react with double-stranded DNA. B) Shifts of the threshold voltage (V_T) for various DNA binders. Each DNA binder was dissolved in deionized water at a concentration of 100 μM. The number of charges in aqueous solution is different for the different DNA binders shown in (A).*

target DNA and negative for the DNA binder because of the intrinsic charges of DNA and DNA binder.

For SNP typing analysis it is preferable to use the genetic FETs at a constant temperature. The genetic FETs were evaluated for SNP typing at 57 °C with the artificial samples of three different genotypes (Figure 5). When the N-type genetic FET and M-type genetic FET were hybridized with a normal/normal (n/n) homozygote sample, a normal/mutant (n/m) heterozygote sample, and a mutant/mutant (m/m) homozygote sample (Figure 5 A), the ratios of the V_T shifts of the N-type genetic FET and the M-type genetic FET were 2.4:1, 1.2:1, and 0.6:1 for a n/n homozygote sample, a n/m heterozygote sample, and a m/m homozygote sample, respectively (Figure 5 B). It was therefore possible to distinguish three genotypes of the target DNA by allele-specific oligonucleotide hybridization through the use of the genetic FETs. When a DNA binder (Hoechst 33258) was introduced and allowed to react with double-stranded DNA at the gate surface after hybridization, the ratios of the V_T shifts of the N-type genetic FET and the M-type genetic FET were 9.6:1, 1.2:1, and 0.1:1 for a n/n homozygote sample, a n/m heterozygote sample, and a m/m homozygote sample, respectively (Figure 5 C). We found that the use of DNA binder after hybridization was effective for distinguishing genotypes of the target DNA more clearly than hybridization only. The results of target hybridization and specific binding of DNA binder demonstrate the ability of the genetic FETs to distinguish the three different genotypes.

Discussion

Molecular recognition events such as hybridization and specific binding of DNA binder could be directly transduced into electrical signals by use of the genetic FETs (Figure 2). A change in the surface charge density could be detected as a shift of the threshold voltage (V_T) of a genetic FET. The V_T shift after hybridization (ΔV_T) is expressed in Equation (1), where $Q_{ds\text{-}DNA}$ is the charge per unit area of the double-stranded DNA after hybridization, $Q_{ss\text{-}DNA}$ is the charge per unit area of the single-stranded oligonucleotide probes, ΔQ_{DNA} is the charge difference per unit area after hybridization, and Ci is the gate capacitance per unit area.

$$\Delta V_T = (Q_{ds\text{-}DNA} - Q_{ss\text{-}DNA}) \times Ci^{-1} = \Delta Q_{DNA} \times Ci^{-1} \quad (1)$$

Since $\Delta V_T = 12$ mV and $Ci = 4.3 \times 10^{-4}$ F m^{-2} for the genetic FET, the charge increase after hybridization is calculated to be 5.1×10^{-6} C m^{-2}. The base lengths of the oligonucleotide probe and the target DNA used in this study are both 17 bases, which corresponds to 5.78 nm in length. Negative charges derived from phosphate groups are distributed along the double-stranded DNA from the gate surface to the bulk of the sample solution. We assume that these negative charges along the DNA molecules contributed to the V_T shift equally and that all the oligonucleotide probes were hybridized with the target DNA. Given these assumptions, the number of oligonucleotide probes on the channel region can be calculated as 2.3×10^4, which corresponds to 1.9×10^8 cm^{-2}. The surface densities of oligonucleotide probes immobilized on glass, silicon dioxide, and gold have been reported to be in the order of 10^9 to 10^{13} cm^{-2}, determined by different methods.[36–41] Since the densities of the oligonucleotide probes are strongly dependent on the method and materials used for a substrate and immobilization, the number of oligonucleotide probes immobilized on silicon nitride could be increased by optimizing the immobilization method. It is noted that hybridization with 2.3×10^4

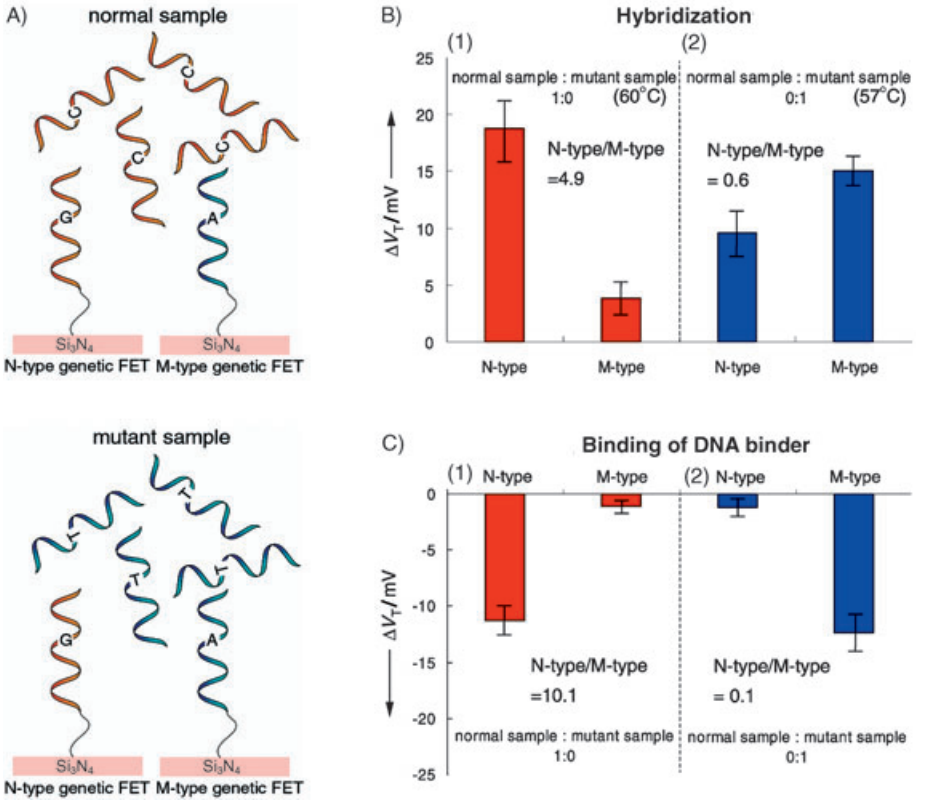

Figure 4. *Discrimination of single nucleotide polymorphism (SNP) with genetic field-effect transistors (FETs). The V_T shifts obtained with three different genetic FETs are averaged for the same conditions and the standard deviation is expressed as a bar. A) Schematic representation showing the method for SNP analysis with genetic FETs. The N-type and M-type genetic FETs were prepared by immobilizing oligonucleotide probes for normal (wild-type) and mutant-type genes, respectively, at the locus of R353Q. A pair of N-type and M-type genetic FETs were hybridized with normal target or mutant target at optimum temperatures. B) SNP detection by allele-specific oligonucleotide hybridization at controlled temperatures. The change in the threshold voltage V_T after hybridization was measured for N-type and M-type genetic FETs hybridized with normal target DNA (1) and mutant target DNA (2). Hybridization was carried out at 60°C for the normal sample, and at 57°C for the mutant sample. C) SNP detection with genetic FETs in combination with DNA binders. Signal-to-noise ratios to distinguish one-base change drastically improved when Hoechst 33258 was used after hybridization.*

target DNA molecules resulted in a V_T shift of 12 mV. Therefore, detection of DNA molecules by use of a genetic FET can be very sensitive, if hybridization is carried out suitably. In this study, 100 μL of synthesized oligonucleotide at a concentration of 100 μM was used as target DNA. On comparison of the numbers of oligonucleotide probes and the target DNA molecules in the sample solution, almost all the oligonucleotide probes on the gate surface were considered to be hybridized. This would be the reason why the size of the V_T shift of the N-type genetic FET for the n/m heterozygote sample was similar to that of the n/n homozygote sample, although the concentration of the normal DNA in a n/m heterozygote sample was half that in the n/n homozygote sample. The lower detection limit and the linear concentration range of the genetic FET are under investigation.

A single molecule of Hoechst 33258 has three positive charges in aqueous solution, as shown in Figure 3A. We therefore expected that a larger V_T shift would be obtained by use of Hoechst 33258 (based on larger charge-density changes) than would be found with the use of DNA binders other than Hoechst 33342. However, the V_T shifts due to DNA binders are more complicated. The binding between double-stranded DNA and a DNA binder is dependent on charges, chemical structure of the DNA binder, base sequence of the DNA, and so on. DNA binders such as Hoechst 33258, Hoechst 33342, and DAPI have been reported to bind selectively in the minor groove to AT-rich sites in DNA molecules,[32,35] and to show weak binding to GC sites.[31] On the other hand, EB and PI are known as intercalators and show high affinity toward double-stranded DNA and generally exhibit modest base pair selectivity.[33,34] The V_T shift caused by PI was approximately twice that produced by EB (Figure 3). This can be explained by the difference in the number of charges in PI and in EB (Figure 3A). The ability to distinguish genotypes drastically improved when Hoechst 33258 was used after hybridization (Figures 4 and 5). Although undesirable signals of mishybridization between the mutant sample and the N-type genetic FET were observed to large extents after hybridization (Figures 4B and 5B), they were eliminated after introduction of Hoechst 33258 (Figures 4C and 5C). A significant improvement in the signal-to-noise ratio after introduction of DNA binder could thus be obtained in this study. From these results, DNA binders are considered to show weak affinity to mismatched double-stranded DNA. This feature of the DNA binder is important for achievement of precise genotyping through the use of the genetic FETs.

For the simultaneous analysis of many different SNPs with high precision, it is important to optimize the temperature of each oligonucleotide probe during the hybridization and washing process. A thermal gradient DNA chip intended for this purpose has already been proposed[21] and evaluated for genotyping, in which temperatures of oligonucleotide probes could be controlled independently. Unlike other types of DNA microarray, the thermal gradient DNA chip should allow multiplex loci typing with use of different optimal temperatures for each locus on the same chip. Since the fabrication process for the genetic FETs is compatible with that for the thermal gradient DNA chip, the genetic FETs could be integrated in Si islands in

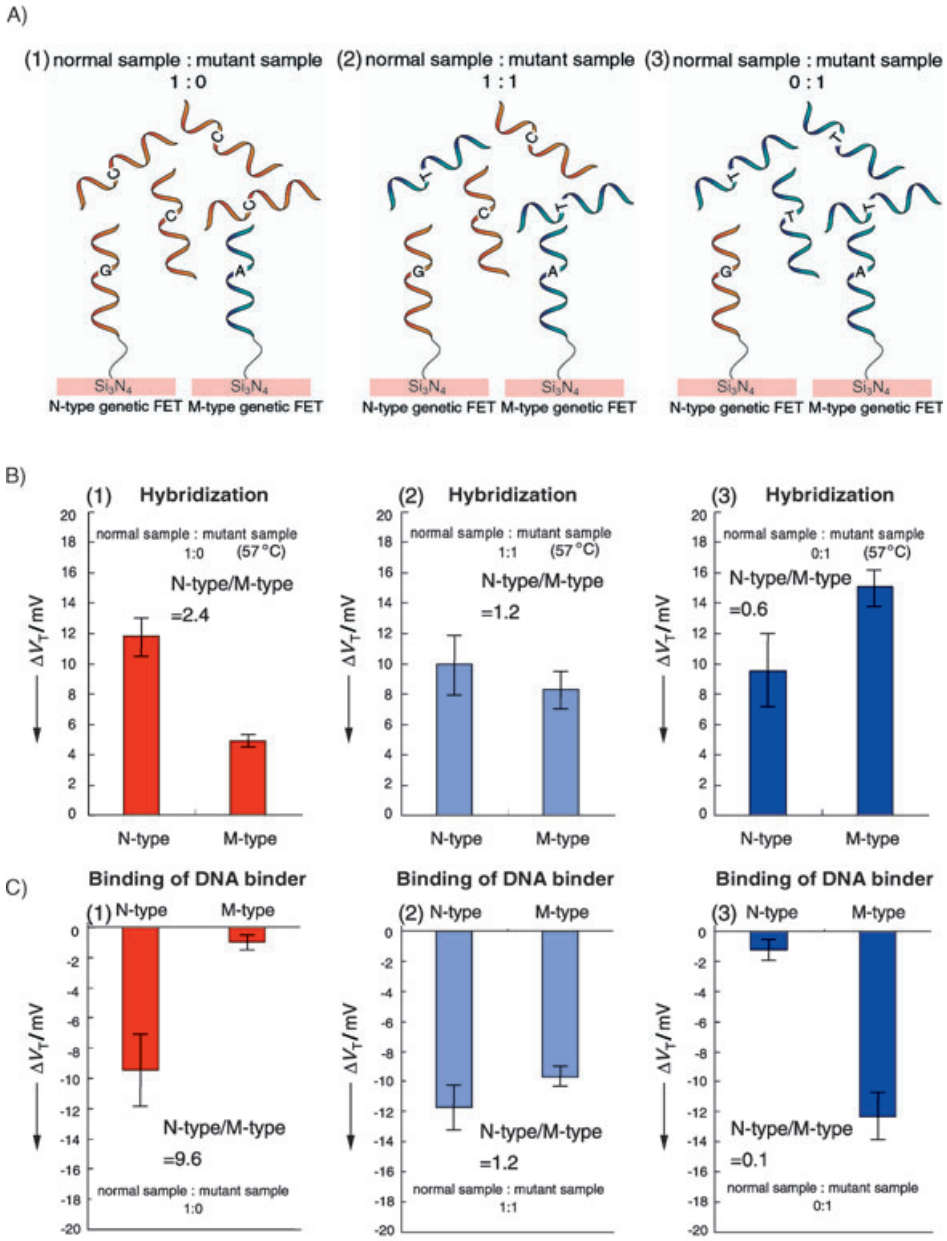

Figure 5. *Genotyping analysis with genetic field-effect transistors (FETs). The V_T shifts obtained with three different genetic FETs are averaged for the same conditions and the standard deviation is expressed as a bar. A) Schematic representation showing the method for genotyping analysis with genetic FETs. The N-type and M-type genetic FETs were prepared by immobilizing oligonucleotide probes for normal (wild-type) and mutant-type genes, respectively, at the locus of R353Q. A pair of N-type and M-type genetic FETs were hybridized with a n/n homozygote sample, a n/m heterozygote sample, and a m/m homozygote sample. B) Genotyping of R353Q locus by hybridization at 57°C. The N-type genetic FET and M-type genetic FET were hybridized with a n/n homozygote sample, a n/m heterozygote sample, and a m/m homozygote sample. The relationships of the concentration of normal sample to mutant sample were 1:0, 1:1, and 0:1, respectively, for the three different genotypes. The ratios of the V_T shifts of the N-type genetic FET and M-type genetic FET—the N-type/M-type ratios—were 2.4:1, 1.2:1, and 0.6:1, respectively, for the three different genotypes. C) Genotyping with genetic FETs in combination with Hoechst 33258 as DNA binder. The N-type/M-type ratios were 9.6:1, 1.2:1, and 0.1:1, respectively, for the three different genotypes. The ability to distinguish genotypes was drastically improved by the use of Hoechst 33258.*

the thermal gradient DNA chip. By combining the genetic FETs with the thermal gradient DNA chip, more precise and reliable SNPs analysis should be achievable.

Conclusion

Insulated gate field-effect transistors have been studied in various biosensors, such as enzyme FETs[42–44] and immunological FETs,[45] since the first ion-sensitive field-effect transistor (ISFET) was reported.[46] There are several advantages to these solid-state biochemical sensors, such as integration of multiple sensing spots and miniaturization including signal transduction, although they are subject to drift of the output signal.[47–49] These characteristics are more suitable for genetic analysis, in which parallel detection is often required with qualitative output, than for quantitative analyses of ions and biochemical components with high precision. In this study, a new method to detect single nucleotide polymorphism by use of a field-effect transistor in combination with allele-specific oligonucleotide hybridization has been developed. The charge-density change due to temperature-controlled hybridization at the surface of the gate insulator could be successfully detected by using a genetic FET. Specific binding of a DNA binder could be utilized in the form of a negative shift of the threshold voltage, because DNA binders have positive charges in aqueous solution. One of the potential advantages of the genetic FET together with a DNA binder is to eliminate undesirable signals of mishybridization. By use of this feature, single nucleotide polymorphism could be successfully detected by using a genetic FET and a DNA binder. In particular, the introduction of DNA binder after hybridization resulted in a better signal-to-noise ratio to discriminate genotypes of the target DNA than could be achieved through the hybridization event alone.

As described in the Introduction, a simpler, more reliable, and inexpensive method and device would be required for platforms used in clinical diagnostics. In addition to these re-

quirements, standardization of data is important for the devices to be used in clinical diagnostics. The data obtained with DNA microarray, SPR, or QCM techniques can be used consistently in the laboratory, but are in general difficult to compare between instruments, or laboratories, because the data-analysis algorithms are completely different. In the case of genetic FETs, the electrical output—the threshold voltage—shifts as a function of the number of intrinsic charges of biomolecules induced on the gate area. It is therefore easier to control and standardize the threshold-voltage shift than it is for fluorescent emission, refractive index change, or frequency change. This characteristic of the genetic FET is more suitable than fluorescent detection, SPR, QCM, etc. for standardization of the data.

One of the ways to achieve cost-effective analysis through the use of micro-fabricated devices with additional value is to use the device repeatedly in a flow-through cell. Since the principle of the genetic FET is based on potentiometric detection of charged biomolecules, it is easier for the genetic FET to be used repeatedly than it is for the electrical current (amperometric) detection method for DNA chips. In the case of the amperometric detection method, irreversible reactions take place at the electrode surfaces on which DNA probes are immobilized and it is in general difficult to obtain a reproducible signal for an amperometric detection device in repeated use. Therefore, the genetic FET is suitable for producing a simpler, more reliable, and less expensive method and device for platforms used in clinical diagnostics.

Experimental Section

Measurement of the electrical characteristics of genetic FETs: Insulated gate field-effect transistors were fabricated by standard integrated circuit technology except for deposition of the gate electrode. Sixteen FETs and a temperature sensor were integrated in a 5×5 mm chip (Figure 1C). The thicknesses of the Si_3N_4 layer and the SiO_2 layers were 140 and 35 nm, respectively. The fabricated FET chip was mounted on a flexible polyimide film with patterned metal electrodes and wire-bonded. The FET chip was encapsulated with an epoxy resin (ZC-203, Nippon Pelnox) except for the gate areas. Both the fabricated FETs and the commercial ISFETs (BAS Inc.) were used for the experiments in this study. The FETs were immersed in a phosphate buffer solution (0.05 M Na_2HPO_4 and 0.05 M KH_2PO_4, pH 6.86, Wako) with a reference electrode. The electrical characteristics of the FETs—such as the gate voltage (V_G)–drain current (I_D) characteristics—were measured at 25 °C with the aid of a semiconductor parameter analyzer (4155C, Agilent). The threshold voltage (V_T) shift was calculated after immobilization, hybridization, and specific binding of DNA binder. The V_T shift was defined as the difference in the V_G–I_D characteristics at a constant drain current of 600 μA.

Oligonucleotide probes and target DNA: Oligonucleotides were synthesized by the phosphoramidite method and purified by HPLC (Espec). The 5′-end of the synthesized oligonucleotide was modified with an amino group for attachment to the Si_3N_4 surface. The factor VII gene, related to blood coagulation factor, was used for the genotyping experiment. The base sequences of the oligonucleotide probes and targets for the normal-type (wild-type) and the mutant-type genes at the locus of R353Q were designed as shown in Table 1 according to ref. [21].

Immobilization of oligonucleotide probes: The surface of the Si_3N_4 layer was cleaned with NaOH (1 M) for 1 h at room temperature and silanized in toluene (Sigma–Aldrich) containing 3-aminopropyltriethoxysilane (Sigma–Aldrich, 2 wt.%). The amino-silanized Si_3N_4 surface was rinsed in toluene and dried in vacuo at 110 °C for 1 h. Reactive amino groups were then introduced at the Si_3N_4 surface.

Oligonucleotide probes were immobilized on the modified Si_3N_4 surface by using glutaraldehyde as a bifunctional cross-linking agent. The amino-silanized Si_3N_4 surface was soaked in a glutaric dialdehyde solution (Sigma–Aldrich, 25 wt.%) with 0.5 g of sodium cyanoborohydride (Sigma–Aldrich) per 50 mL for 4 h at room temperature, followed by rinsing in deionized water and drying in vacuo at room temperature for 1 h. Oligonucleotide probes were dissolved in a TE buffer (pH 8.0, Nippon Gene) at a concentration of 100 μM. To couple amino-modified oligonucleotides with the glutaraldehyde-treated Si_3N_4 surface, the FET chip was kept overnight at 50 °C in the oligonucleotide solution with 0.5 g of sodium cyanoborohydride per 50 mL to complete the coupling reaction. The FET chip was then soaked for 1 h in a phosphate buffer solution (0.04 M Na_2HPO_4 and 0.03 M KH_2PO_4, pH 7.0, Wako) with glycine (Wako, 1 M) at 50 °C to block any remaining glutaraldehyde groups. The FET chip was washed with the phosphate buffer solution (pH 7.0) and with deionized water and dried in vacuo at room temperature for 1 h. The surface-modified FET chip was ready for use in hybridization and specific binding studies.

Hybridization with target DNA: Target DNA used for hybridization was prepared by dissolving target oligonucleotides in a hybridization buffer solution, which was composed of 4xSSC + 0.1 %SDS (Invitrogen), at a concentration of 100 μM. The genetic FET with immobilized oligonucleotide probes was kept in the hybridization buffer solution containing target oligonucleotides for 15 h at controlled temperatures: 60 °C for hybridization with normal targets, and 57 °C for hybridization with mutant targets (Table 1[21]). After hybridization, the genetic FET was washed with 1xSSC + 0.03 %SDS, 0.2xSSC, 0.05xSSC, and deionized water at each temperature in order to remove nonhybridized oligonucleotides.

Introduction of DNA binders onto genetic FETs: The DNA binders used in this work are as follows: 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-*1H*-benzimidazole trihydrochloride, Hoechst 33258 (Dojindo); 2′-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-*1H*-benzimidazoletrihydrochloride, Hoechst 33342 (Dojindo); 4′,6-diamidino-2-phenylindole dihydrochloride, DAPI (Dojindo); 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide, ethidium bromide, EB (Dojindo); 3,8-diamino-5-[3-(diethylmethylammonio)-propyl]-6-phenylphenanthridinium diiodide, propidium iodide, PI (Dojindo). All the DNA binders were dissolved in deionized water at a concentration of 100 μM. Hoechst 33258 was mainly used for the V_T shift experiments in this study. After hybridization, the genetic FET was soaked in each DNA binder solution at room temperature for 12 h and washed with deionized water.

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[1] B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, R. J. Pace, *Nature* **1997**, *387*, 580 – 583.
[2] K. K. Jain, *Science* **2001**, *294*, 621 – 623.
[3] P. P. Zarrinkar, J. K. Mainquist, M. Zamora, D. Stern, J. B. Welsh, L. M. Sapinoso, G. M. Hampton, D. J. Lockhart, *Genome Res.* **2001**, *11*, 1256 – 1261.
[4] T. G. Drummond, M. G. Hill, J. K. Barton, *Nat. Biotechnol.* **2003**, *21*, 1192 – 1199.
[5] S. P. Fodor, R. P. Rava, X. C. Huang, A. C. Pease, C. P. Holmes, C. L. Adams, *Nature* **1993**, *364*, 555 – 556.
[6] D. J. Lockhart, E. A. Winzeler, *Nature* **2000**, *405*, 827 – 836.
[7] C. C. Xiang, O. A. Kozhich, M. Chen, J. M. Inman, Q. N. Phan, Y. Chen, M. J. Brownstein, *Nat. Biotechnol.* **2002**, *20*, 738 – 742.
[8] X. Zhao, R. Tapec-Dytioco, W. Tan, *J. Am. Chem. Soc.* **2003**, *125*, 11474 – 11475.
[9] D. J. Lockhart, H. L. Dong, M. C. Byrne, M. T. Follettie, M. V. Gallo, M. S. Chee, M. Mittmann, C. W. Wang, M. Kobayashi, H. Horton, E. L. Brown, *Nat. Biotechnol.* **1996**, *14*, 1675 – 1680.
[10] K. Nakatani, S. Sando, I. Saito, *Nat. Biotechnol.* **2001**, *19*, 51 – 55.
[11] M. A. Cooper, F. N. Dultsev, T. Minson, V. P. Ostanin, C. Abell, D. Klenerman, *Nat. Biotechnol.* **2001**, *19*, 833 – 837.
[12] D. J. Caruana, A. Heller, *J. Am. Chem. Soc.* **1999**, *121*, 769 – 774.
[13] S. J. Park, T. A. Taton, C. A. Mirkin, *Science* **2002**, *295*, 1503 – 1506.
[14] M. Chee, R. Yang, E. Hubbell, A. Berno, X. C. Huang, D. Stern, J. Winkler, D. J. Lockhart, M. S. Morris, S. P. A. Fodor, *Science* **1996**, *274*, 610 – 614.
[15] U. Landegren, M. Nilsson, P.-Y. Kwok, *Genome Res.* **1998**, *8*, 769 – 776.
[16] W. M. Howell, M. Jobs, U. Gyllensten, A. J. Brookes, *Nat. Biotechnol.* **1999**, *17*, 87 – 88.
[17] S. Tyagi, S. A. E. Marras, F. R. Kramer, *Nat. Biotechnol.* **2000**, *18*, 1191 – 1196.
[18] S. Sauer, D. Lechner, K. Berlin, H. Lehrach, J.-L. Escary, N. Fox, I. G. Gut, *Nucleic Acids Res.* **2000**, *28*, e13.
[19] A.-C. Syvanen, *Nat. Rev. Genet.* **2001**, *2*, 930 – 942.
[20] M. Jobs, W. M. Howell, L. Stromqvist, T. Mayr, A. J. Brookes, *Genome Res.* **2003**, *13*, 916 – 924.
[21] T. Kajiyama, Y. Miyahara, L. J. Kricka, P. Wilding, D. J. Graves, S. Surrey, P. Fortina, *Genome Res.* **2003**, *13*, 467 – 475.
[22] M. Schene, D. Shalon, R. W. Davis, P. O. Brown, *Science* **1995**, *270*, 467 – 470.
[23] E. Souteyrand, J. P. Cloarec, J. R. Martin, C. Wilson, I. Lawrence, S. Mikkelsen, M. F. Lawrence, *J. Phys. Chem. B* **1997**, *101*, 2980 – 2985.
[24] J. Fritz, E. B. Cooper, S. Gaudet, P. K. Sorger, S. R. Manails, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14142 – 14146.
[25] T. Sakata, H. Otsuka, Y. Miyahara, *Solid State Devices and Materials (SSDM), Tokyo*, **2003**, 356 – 357.
[26] T. Sakata, H. Otsuka, Y. Miyahara, *Material Research Society (MRS), Boston*, **2003**, *782*, 393 – 398.
[27] F. Uslu, S. Ingebrandt, D. Mayer, S. Böcker-Meffert, M. Odenthal, A. Offenhäusser, *Biosens. Bioelectron.* **2004**, *19*, 1723 – 1731.
[28] D.-S. Kim, Y.-T. Jeong, H.-J. Park, J.-K. Shin, P. Choi, J.-H. Lee, G. Lim, *Biosens. Bioelectron.* **2004**, *20*, 69 – 74.
[29] F. Pouthas, C. Gentil, D. Cote, U. Bockelmann, *Appl. Phys. Lett.* **2004**, *84*, 1594 – 1596.
[30] D.-S. Kim, Y.-T. Jeong, H.-K. Lyu, H.-J. Park, H. S. Kim, J.-K. Shin, P. Choi, J.-H. Lee, G. Lim, M. Ishida, *Jpn. J. Appl. Phys. Part 1* **2003**, *42*, 4111 – 4115.
[31] W. D. Wilson, F. A. Tanious, H. J. Barton, L. Strekowski, D. W. Boykin, *J. Am. Chem. Soc.* **1989**, *111*, 5008 – 5010.
[32] M. Sriram, G. A. van der Marel, H. L. P. F. Roelen, J. H. van Boom, A. H.-J. Wang, *Biochemistry* **1992**, *31*, 11823 – 11834.
[33] H. S. Rye, S. Yue, M. A. Quesada, R. P. Haugland, R. A. Mathies, A. N. Glazer, *Methods Enzymol.* **1993**, 217, 414 – 431.
[34] W. A. Dengler, J. Schulte, D. P. Berger, R. Mertelsmann, H. H. Fiebig, *Anticancer Drugs* **1995**, *6*, 522 – 532.
[35] D. L. Boger, B. E. Fink, S. R. Brunette, W. C. Tse, M. P. Hedrick, *J. Am. Chem. Soc.* **2001**, *123*, 5878 – 5891.
[36] L. A. Chrisey, G. U. Lee, C. E. O'Ferrall, *Nucleic Acids Res.* **1996**, 24, 3031 – 3039.
[37] A. B. Steel, T. M. Herne, M. J. Tarlov, *Anal. Chem.* **1998**, *70*, 4670 – 4677.
[38] A. Kumar, O. Larsson, D. Parodi, Z. Liang, *Nucleic Acids Res.* **2000**, 28, e71.
[39] A. W. Peterson, R. J. Heaton, R. M. Georgiadis, *Nucleic Acids Res.* **2001**, 29, 5163 – 5168.
[40] E. Huang, M. Satjapipat, S. Han, F. Zhou, *Langmuir* **2001**, *17*, 1215 – 1224.
[41] Z. Guo, M. S. Gatterman, L. Hood, J. A. Hansen, E. W. Petersdorf, *Genome Res.* **2001**, 12, 447 – 457.
[42] S. Caras, J. Janata, *Anal. Chem.* **1980**, *52*, 1935 – 1937.
[43] Y. Miyahara, T. Moriizumi, S. Shiokawa, H. Matsuoka, I. Karube, S. Suzuki, *Nippon Kagaku Kaishi* **1983**, *6*, 823 – 830.
[44] Y. Miyahara, T. Moriizumi, K. Ichimura, *Sens. Actuators* **1985**, *7*, 1 – 10.
[45] S. Collins, J. Janata, *Anal. Chim. Acta* **1982**, *136*, 93- 99.
[46] P. Bergveld, *IEEE Trans. Biomed. Eng.* **1970**, *17*, 70 – 71.
[47] S. D. Moss, J. Janata, C. C. Johnson, *Anal. Chem.* **1975**, *47*, 2238 – 2243.
[48] R. P. Buck, *IEEE Trans. Electron Devices* **1982**, *29*, 108 – 115.
[49] Y. Miyahara, K. Yamashita, S. Ozawa, Y. Watanabe, *Anal. Chim. Acta* **1996**, *331*, 85 – 95.

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