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Description	

Sequence Specific Interstrand Photocrosslinking for Effective SNP Typing

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We describe a simple and inexpensive SNP typing method by using sequence specific interstrand photocrosslinking via *p*-carbamoylvinyl phenol nucleoside. Interstrand photocrosslinking showed a high degree of single nucleotide specificity up to 10^3-fold and more, and can be used in the
10 diagnostic detection of DNA sequences.

Introduction

Single nucleotide polymorphisms (SNPs) are the most common form of variation in the human genome and can be diagnostic of particular genetic predispositions toward
15 disease.[1] Most methods of DNA detection involve hybridization by an oligodeoxynucleotide (ODN) probe to its complementary single-strand nucleic acid target leading to signal generation.[2] In as far as the detection relies on hybridization events,[3] however, such ODN probes have
20 inherent limitations in their selectivity. Differences in hybridization efficiency vary with sequence context and are often very small for the detection of a single base mismatch in a target strand of DNA. To attain the high sequence selectivity, the hybridization and washing conditions need to be carefully
25 selected to minimize any undesirable responses from mismatched hybridization probes. From this perspective, alternative methods that do not rely on hybridization events are urgently required. The enzymatic ligation method[4] and chemical ligation method[5] have been developed in use for
30 engendering high specificity in sequence detection. A single primer extension with DNA polymerase is the representative key reaction in enzyme-coupled SNP typing.[6] We have been studying artificial DNA bases as a tool for photochemical DNA ligation method.[7] We recently reported that a modified
35 ODN containing *p*-carbamoylvinyl phenol nucleoside (^{CV}P) could be photocrosslinked by irradiating at 366 nm with adjacent adenine residue in a [2 + 2] manner as shown in Fig. 1a.[8] Photocrosslinking-coupled SNP typing is expected to demonstrate the high performance for allele discrimination.
40 Here, we describe the coupling of DNA chip technology in a interstrand photocrosslinking via ^{CV}P as a method for SNP-typing. This photocrosslink reaction is shown to proceed with very high sequence specificity, and can be used in the diagnostic detection of DNA sequences.

45 ## Results and discussion

To demonstrate that interstrand photocrosslinking could be incorporated into platforms suitable for DNA chip

technologies, we constructed the DNA chip by attaching amino-labeled ODN containing ^{CV}P (S corresponds to a
50 hexa(ethylene glycol) linker fragment) as the capture strand onto the aldehyde-modified glass surface (Fig. 1b).[7] The sequences were taken from the H-*ras* protooncogene (wild-type) and the activated H-*ras* oncogene (mutation), which has a C → A point mutation in codon 12.[9]
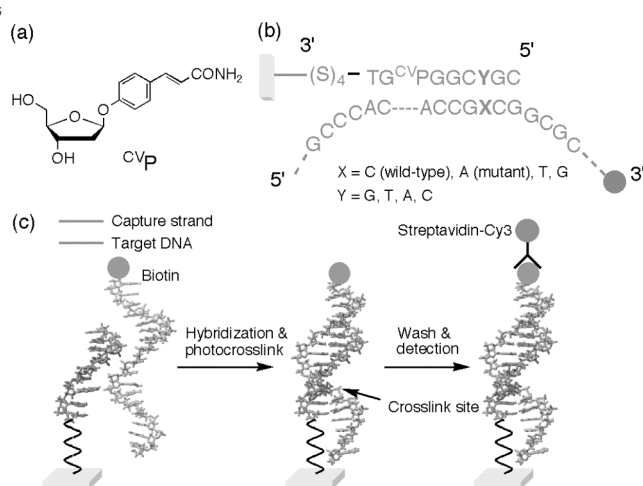55

Fig. 1 Structure, sequences, and progress of interstrand photocrosslinking. (a) Structure of ^{CV}P. (b) Capture and target sequences used in this study. (c) Strategy for the detection of single
60 nucleotide differences on a DNA chip.

We determined the feasibility of the interstrand photocrosslinking through ODN containing ^{CV}P on a DNA chip (Fig. 1c). A glass chip spotted with 1 μM target DNA
65 was irradiated at 366 nm for 45 min in 50 mM sodium cacodylate buffer (pH 7.0) and 50 mM sodium chloride. After the chip had been washed with deionized water at 98 °C for 5 min, a phosphate-buffered saline (PBS) solution of streptavidin-Cy3 conjugate was added to the surface, and the
70 chip was washed twice in PBS. Fluorescence signals were detected on a microarray scanner. As shown in Fig. 2, we measured the strong fluorescence signal of photocrosslinked product with the completely complementary case. To investigate the generality of sequence discrimination, we
75 constructed five target ODNs with mismatch at various position. Results show that a single mismatch at the seventh

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position in ODN context yielded very little photocrosslinked product, with a measured fluorescence signal that is 10^3-fold lower that the completely complementary case (Table 1).
80 Then we constructed a set of four closely related target ODNs with a single variable base (A, T, G, or C) in the seventh position. Most mismatches give relative fluorescence signals of 0.1% or less, compared to the correctly matched case.
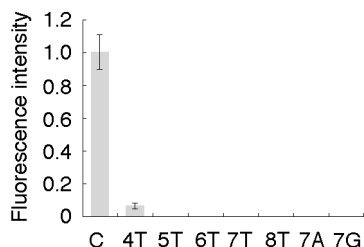

85 **Fig. 2** Fluorescence intensity acquired on a microarray scanner for the product of photocrosslinking on matched and singly mismatched target ODNs.

90 **Table 1** Normalized fluorescence intensity for the photocrosslinked product of capture strand and target ODN, correctly base paired to eight ODNs that differed in a single nucleotide position.

	Target ODN[a]	Fluorescenceintensity[b]
ODN(C)	5´-d(ACACCGCCG)-biotin-3´	1.0 ± 0.11
ODN(4T)	5´-d(ACAT̲CGCCG)-biotin-3´	0.064 ± 0.018
ODN(5T)	5´-d(ACACT̲GCCG)-biotin-3´	0[c]
ODN(6T)	5´-d(ACACCT̲CCG)-biotin-3´	0[c]
ODN(7T)	5´-d(ACACCGT̲CG)-biotin-3´	0[c]
ODN(8T)	5´-d(ACACCGCT̲G)-biotin-3´	0[c]
ODN(7A)	5´-d(ACACCGA̲CG)-biotin-3´	0[c]
ODN(7G)	5´-d(ACACCGG̲CG)-biotin-3´	0[c]

[a]Underlined character indicates the mismatched base. [b]Each experiment has been repeated at least three times. [c]The average intensity as measured
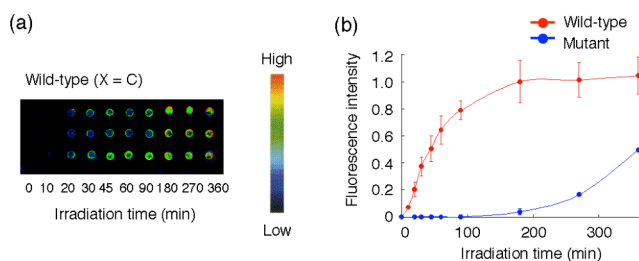95 is approximately equal to zero.

Fig. 3 (a) Fluorescence image for a time course of wild-type detection. The capture strands (Y = G) were attached to the surface.
100 (b) Differing rates of photocrosslinking of matched and singly mismatched target DNA.

As shown in Fig. 3a and 3b, results show that the interstrand photocrosslinking reached the 50% stage in 45 min.
105 Significantly, a single mismatch in the target DNA yielded very little photocrosslinked product, with a measured rate that was 10^3-fold lower than that of the completely complementary case.

In such an interstrand photocrosslinking, there are two
110 possible sources of specificity: the interstrand

photocrosslinking itself and the hybridization selectivity between the target DNA and capture strand. We also conducted wild-type detection in the conventional method for the same target to verify the potential selectivity of our
115 interstrand photocrosslinking method for SNP detection. A conventional method of increasing target selectivity is to wash the DNA chip with a buffer solution at an adequate temperature, which results in the dehybridization of DNA duplexes formed from non-complementary strands. In the
120 wild-type detection by the traditional method, which shows only hybridization specificity, the observed selectivity for the matched (wild-type) sequence was about 3, even after washing with a stringent solution at the appropriate temperature (Fig. 4a). One possible reason for the decrease in
125 the selectivity is the loss of DNA duplexes of matched sequences, particularly during the washing step employed to remove non-complementary strands from the DNA chip. A second possible source of low selectivity is incomplete washing of the mismatched sequences (mutant). By contrast,
130 the fluorescence image after use of the interstrand photocrosslinking method showed no loss of the target DNA, due to covalent bonding between the capture strand and target DNA (Fig. 4b). Moreover, mismatched duplexes with the potential to harm the fluorescence imaging were eliminated
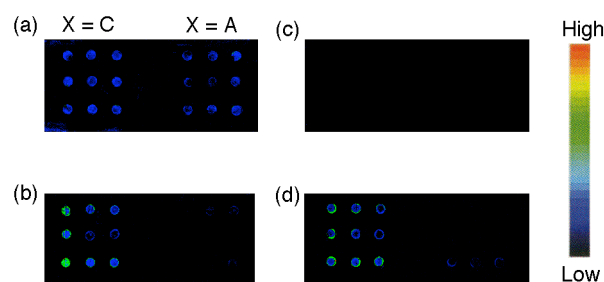135 completely by high temperatures (Fig. 4c, 4d).

Fig. 4 Influence of washing and interstrand photocrosslinking on specificity. The capture strands (Y = G) or traditional probe (5´-
140 d(CGGCGGTGT)-SSSS-NH$_2$-3´) were attached to the surface, and targets (left: X = C, 3 × 3 spots; right: X = A, 3 × 3 spots) were applied. (a) Use of only hybridization specificity and washing with buffer under stringent conditions (25 °C). The observed specificity was 3. (b) Use of both hybridization specificity and interstrand
145 photocrosslinking specificity and washing with buffer under stringent conditions. The observed specificity was 13. (c) Use of only hybridization specificity and washing with the deionized water at 98 °C for 5 min. No signal was observed. (d) Use of both hybridization specificity and interstrand photocrosslinking specificity
150 and washing with the deionized water at 98 °C for 5 min. The observed specificity was 52.

Finally, to demonstrate the generality of sequence discrimination, we constructed a set of four closely related
155 targets (all in the H-*ras* context) with a single variable base (A, G, C, or T), along with 9-mer immobilized capture strands with the same four bases. We then performed kinetic measurements for all 16 possible combinations of capture strands and target DNAs (Table 2). As shown in Fig. 5, The
160 photocrosslinking of each of the 12 possible mismatched sequences is presented as a ratio between the

photocrosslinking efficiency of each mismatched reaction and that of the corresponding matched one. Most mismatches are discriminated by a factor greater than 10^3-fold, as compared to the corresponding matched sequence (G-T, G-A, G-G, A-C, A-A, C-C, T-C, T-T), and almost all of the remaining sequences are discriminated by greater than 10^2-fold. Although the discrimination of G-T mismatch is more difficult than other kinds of mismatches,[10] use of the interstrand photocrosslinking method exhibits the adequate sequence specificity.

Table 2 Survey of photocrosslinking rates for all base-pairing combinations in the context of the H-*ras* system.

Base pair (Y-X)	Fluorescence intensity[a]	Base pair (Y-X)	Fluorescence intensity[a]
G-C	1.0 ± 0.145	C-C	0[b]
G-T	0[b]	C-T	0.017 ± 0.029
G-A	0[b]	C-A	0.019 ± 0.023
G-G	0[b]	C-G	1.0 ± 0.025
A-C	0[b]	T-C	0[b]
A-T	1.0 ± 0.042	T-T	0[b]
A-A	0[b]	T-A	1.0 ± 0.027
A-G	0.025 ± 0.024	T-G	0.041 ± 0.074

[a] Each experiment has been repeated at least three times. [b] The average intensity as measured is approximately equal to zero.
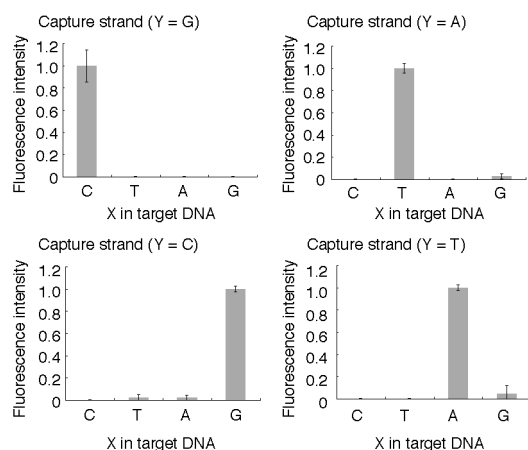

Fig. 5 Fluorescence intensity for all 16 possible combinations of capture strands and target DNAs. Each experiment has been repeated at least three times.

Conclusions

In conclusion, we demonstrated a simple and inexpensive SNP typing method by using sequence specific interstrand photocrosslinking via ^{CV}P. Interstrand photocrosslinking showed a high degree of single nucleotide specificity up to 10^3-fold and more. Therefore, this system can be widely used for sensitive assays for clinical diagnostics of genetic and infectious disease.

Experimental

General

Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems. Irradiation was performed by 25 W transilluminator (FUNAKOSHI, TFL-40, 366 nm, 5,700 $\mu W/cm^2$). HPLC was performed on a Chemcobond 5-ODS-H column (4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. The reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from Glen Research. Calf intestine alkaline phosphatase (AP) was purchased from Promega. Nuclease P1 was purchased from Yamasa.

Preparation of ODN

ODN sequences were synthesized by the conventional phosphoramidite method by using an Applied Biosystems 3400 DNA synthesizer. The coupling efficiency was monitored with a trityl monitor. The coupling efficiency of crude mixture of ^{CV}P was 97% yield. The coupling time of crude mixture of ^{CV}P was 999 sec. They were deprotected by incubation with 28% ammonia for 4 h at 65 °C and were purified on a Chemcobond 5-ODS-H column (10 × 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% CH_3CN, linear gradient (30 min) at a flow rate of 3.0 mL/min. Preparation of ODNs was confirmed by MALDI-TOF-MS analysis.[11] The target DNAs were 5′-TCTGGATCAGCTGGATGGTCAGCGCACTCTTG CCCACACCGXCGGCGCCCACCACCACCAGCTTATATT CCGTCATCGCTCCTCAGGGGCCTGCGGCCCG-biotin-3′, where X was either A, G, C, or T.

DNA probe immobilization

The amino-labeled ODN probe containing ^{CV}P was diluted to a concentration of 10 μM in 100 mM sodium cacodylate buffer (pH 7.0). Spotting was accomplished by using 4 μL aliquots from a standard micropipette. Binding of amino-labeled ODN probe containing ^{CV}P to the surface was performed over a period of 12 h at room temperature in a humid chamber. After probe immobilization, the glass surface was rinsed with 0.1% SDS and deionized water. The surface was deactivated with a solution made of $NaBH_4$ (3.75 mg), PBS (1.5 mL), and ethanol (375 μL) over a period of 5 min. The surface was subsequently washed with deionized water, and dried.

Interstrand photocrosslinking on a DNA chip

A glass chip spotted with a solution (4 μL) made of 1 μM target DNA in 50 mM sodium cacodylate buffer (pH 7.0) and 50 mM sodium chloride was irradiated at 366 nm for 45 min. After the chip had been washed with deionized water at 98 °C for 5 min, a PBS solution of streptavidin-Cy3 conjugate (20 μg/mL) was added to the surface, and the chip was washed twice in PBS. Fluorescence measurements were performed on a microarray scanner CRBIO II e (Hitachi), which was equipped with a laser with excitation wavelength of 532 nm.

Acknowledgements

Notes and references

1   (a) A. Brookes, *Gene* 1999, **234**, 177. (b) J. J. McCarthy and R. Hilfiker, *Nat. Biotechnol.* 2000, **189**, 505.

2   (a) T. G. Drummond, M. G. Hill, and J. K. Barton, *Nat. Biotechnol.* 2003, **21**, 1192. (b) K. Nakatani, *ChemBioChem* 2004, **5**, 1623.

3   (a) Z. Guo, Q. Liu, and L. M. Smith, *Nat. Biotechnol.* 1997, **15**, 331. (b) A. G. Frutos, S. Pal, M. Quesada, and J. Lahiri, *J. Am. Chem. Soc.* 2002, **124**, 2396. (c) M. F. Ali, R. Kirby, A. P. Goodey, M. D. Rodriguez, A. D. Ellington, D. P. Neikirk, and J. T. McDevitt, *Anal. Chem.* 2003, **75**, 4732. (d) K. Ketomäki, H. Hakala, O. Kuronen, and H. Lönnberg, *Bioconjugate Chem.* 2003, **14**, 811.

4   (a) M. Nilsson, G. Barbany, D. Antson, K. Gertow, and U. Landegren, *Nat. Biotechnol.* 2000, **18**, 791. (b) X. B. Zhong, R. Reynolds, J. R. Kidd, K. K. Kidd, R. Jenison, R. A. Marlar, and D. C. Ward, *Proc. Natl. Acad. Sci. USA* 2003, **100**, 11559. (c) J.-Y. Deng, X.-E. Zhang, Y. Mang, Z.-P. Zhang, Y.-F. Zhou, Q. Liu, H.-B. Lu, and Z.-J. Fu, *Biosen. Bioelectron.* 2004, **19**, 1277.

5   (a) S. M. Gryaznov, R. Schultz, S. K. Chaturvedi, and R. L. Letsinger, *Nucleic Acids Res.* 1994, **22**, 2366. (b) Y. Xu, N. B. Karalkar, and E. T. Kool, *Nat. Biotechnol.* 2001, **19**, 148. (c) C. Dose, S. Ficht, and O. Seitz, *Angew. Chem., Int. Ed.* 2006, **45**, 5369.

6   Syvänen, A.-C., *Hum. Mutat.* 1999, **13**, 1.

7   (a) K. Fujimoto, S. Matsuda, N. Takahashi, and I. Saito, *J. Am. Chem. Soc.* 2000, **122**, 5646. (b) S. Ogasawara and K. Fujimoto, *ChemBioChem* 2005, **6**, 1756. (c) Y. Yoshimura, Y. Noguchi, H. Sato, and K. Fujimoto, *ChemBioChem* 2006, **7**, 598. (d) Y. Yoshimura, D. Okamura, M. Ogino, and K. Fujimoto, *Org. Lett.* 2006, **8**, 5049. (e) Y. Yoshimura, Y. Noguchi, and K. Fujimoto, *Org. Biomol. Chem.* 2007, **5**, 139.

8   Y. Yoshimura, Y. Ito, and K. Fujimoto, *Bioorg. Med. Chem. Lett.* 2005, **15**, 1299.

9   E. P. Reddy, R. K. Reynolds, E. Santo, and M. Barbacid, *Nature* 1982, **300**, 149.

10 D. Wu and R. B. Wallace, *Gene* 1989, **76**, 245.

11 MALDI-TOF-MS: calcd 4365.25 for capture strand (Y = G) $[(M + H)^+]$, found 4365.21; calcd 4340.24 for capture strand (Y = T) $[(M + H)^+]$, found 4340.18; calcd 4349.25 for capture strand (Y = A) $[(M + H)^+]$, found 4349.87; calcd 4325.22 for capture strand (Y = C) $[(M + H)^+]$, found 4325.27; calcd 4328.19 for traditional probe $[(M + H)^+]$, found 4328.15.

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