Focal Extraction of Surface-Bound DNA from a Microchip Using Photo-Thermal Denaturation

BioTechniques 28:1006-1011 (May 2000)

Kenji Yasuda, Kazunori Okano¹ and Shin'ichi Ishiwata²

The University of Tokyo,
¹Hitachi, Ltd. and ²Waseda
University, Tokyo, Japan

ABSTRACT

High-throughput, selective extraction of a particular DNA fragment from a mixture of DNA before PCR amplification is becoming increasingly important in the DNA analysis field. Although the latest microchip technology has enabled real-time DNA expression analysis using hybridization between surface-bound probe DNA and sample DNA, the potential of this technology in purification of a small amount of DNA has not been demonstrated. We report here a method for area-selective release and collection of specific DNA, in which an IR laser beam is focused onto surface-bound sample DNA at the target-spotted area to denature hybridized DNA. First, sample DNA labeled with a fluorescent dye was hybridized to a probe DNA immobilized on a chromium-coated chip. A 1053-nm IR laser beam with an intensity of 10–100 mW was then focused on the target area with a spatial resolution of 10 μm , causing the release of the fluorophore-labeled sample DNA as a result of photo-thermal denaturation. Confirmation of the amount of eluted DNA by PCR amplification after collection indicated that more than 10^{-20} mol DNA/ μm^2 area was eluted from the microchip, representing more than 70% of the chip-bound sample DNA. These results indicate that this method can be applied to the highly sensitive purification of DNA in microchip technology.

INTRODUCTION

Purification and analysis of DNA from a mixture of cell extract are fundamental in molecular and cellular biology and molecular diagnosis. In purification, a DNA library constructed from cloned DNA is usually used. Although the cloning method is widely used and is suitable for the preparation of a large number of DNA, the cultivation step of this method is laborious and time consuming. Recently, molecular biology has moved rapidly towards the study of functional genomics (10) and proteomics. These studies require rapid selection and collection of the target parts of a gene and mRNA from crude samples (3,6). Although the latest chip technology is capable of simple, high-throughput analysis in distinguishing different sequences of DNA by hybridization detection between a chip-bound probe DNA and sample DNA (1,2,8,9), it has not yet been applied to high-throughput purification of DNA, in which a portion of the sample DNA having particular DNA segments is picked up for further analysis.

Here, we propose a new type of DNA purification method using a photo-thermal approach to extract specific DNA bound on a microchip surface. This method is based on the principle that a focused IR beam heats a spotted area on the metal-coated chip surface (4) and that the resulting increase in temperature leads to denaturation of the sample probe DNA. As shown in Figure 1, the method consists of the following four steps: (i) sample DNA are loaded onto a chip surface coated with a metal thin layer, on which a group of surface-bound probe DNA are immobilized; (ii) sample DNA is hybridized with probe DNA; (iii) the chip surface is washed, the hybridized sample DNA are observed and the plate is

spot-heated to extract DNA from the spotted area; and (iv) the released sample DNA is collected. The advantages of this spot-heating method are that it provides non-contact, spatially distributed denaturation and faster relaxation time in the increase in temperature. It should thus prove highly suitable for extracting target DNA individually from a chip surface on which a crude DNA mixture has been loaded. Steps *i* and *ii* have already been realized in DNA chip technology (1,2,8,9), but steps *iii* and *iv* are not yet tested. Here, we focused on steps *iii* and *iv*, namely the technique for area-specific denaturation and release of surface-bound DNA and subsequent collection of the released DNA.

MATERIALS AND METHODS

Photo-Thermal Denaturation Microscopy System

Figure 2 shows a diagram of the photo-thermal denaturation microscopy system for area-specific extraction of surface-bound DNA. It consists of three different optical units for phase-contrast, fluorescence and 1053-nm IR laser irradiation, respectively (Nd:YLF laser, 1053-100p; Amoco Laser, Naperville, IL, USA; maximum power 100 mW at the focal point when a $\times 10$ objective lens is used). Different probe DNA areas in the matrix are distinguished by phase-contrast microscopy. The amount of surface-bound sample DNA on the microchip was measured by fluorescence microscopy. Phase-contrast and fluorescence images were simultaneously acquired with a dual-view microscopy system (4,5,7): the former through a charge-couple device (CCD) camera and the latter through a CCD camera equipped with an image intensifier. The area of DNA denaturation was brought into focus using the

fluorescence image, and the IR laser beam irradiated the spotted area. The intensity of the IR laser beam can be controlled up to 100 mW at the focal position on the microchip.

Preparation of DNA Chip

Probe DNA was immobilized on a 6-nm-thick chromium-coated glass plate. The chromium surface was modified with an active residue having a glysidoxo group for coupling dsDNA (619 bp, 0.5 pmol, 0.5 μ L) onto the surface by the following two reaction steps. First, the glass plate with a chromium surface ($45 \times 25 \times 0.4$ mm) was dipped into a solution of 3-glysidoxypropytrimethoxysilane for 1 h at room temperature (25°C) and dried at 110°C for 30 min. Next, dsDNA was prepared with an amino residue at the 5'-terminus of one strand and sulforhodamine-101 fluorophore at the 5'-terminus of the other strand so as to immobilize the DNA through its amino residue with glysidoxo groups on the chip surface. The dsDNA was a product amplified from a cloned human DNA. The clone has M13 priming sites 5'-CATGACTG-CCGTCGTT-3' at both ends. It was amplified by PCR with *Taq* DNA polymerase (Amersham Pharmacia Biotech, Little Chalfont, UK) and two primers, 5'-fluorophore-AACGACGGCCAGT-CATGCG-3' and 5'-NH₂-AACGAC-

GGCCAGTCATGTG-3'. Thermal cycling for PCR was carried out 35 $\times$ at 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. A solution of the product (10 μ M dissolved in 0.2 M sodium carbonate buffer, pH 9.5) was dropped onto the activated chip, incubated at 50°C for 45 min and kept at room temperature so that the 5'-terminus of the probe DNA was attached to the chip. The quantity of immobilized DNA was checked by fluorescence microscopy with a confocal scanning fluorescence microscope (LSM-200; Olympus, Tokyo, Japan).

Recovery of DNA Using Photo-Thermal Denaturation

The DNA chip was rinsed and overlaid with 5 μ L 20 mM Tris-HCl (pH 7.4) containing 2 mM EDTA (2 $\times$ TE buffer). A small area on the chip was heated with IR laser (10, 25, 50 and 100 mW on the surface of the chip) using the microscopy system. After IR irradiation, a 4- μ L drop of solution from the heated spot was collected into a vessel.

The recovered DNA in the drop was amplified to check quantity. PCR amplification was carried out under the same conditions as described above. The products of this amplification were checked by electrophoresis using a 2% agarose gel followed by staining with 0.5 μ g/mL ethidium bromide and analyzed with a fluorescence-image ana-

lyzer (FM-Bio® 100; Hitachi Software Engineering, Tokyo, Japan).

Rehybridization of Fluorophore-Labeled DNA with an ssDNA on the Microchip

A 60-nucleotide fluorophore-labeled oligomer ssDNA (60-nucleotide sample DNA) complementary to the 60 nucleotides from the 3'-end of the immobilized strand on the DNA chip was diluted to 2 μ M. For rehybridization, the solution was dropped on the chip surface and incubated for 3 min at 60°C. After rehybridization, the chip was rinsed with 2 $\times$ TE buffer at room temperature and analyzed using the microscopy system.

RESULTS AND DISCUSSION

The purpose of this study was to demonstrate the feasibility of denaturing and extracting sample DNA from a small area by a photo-thermal denaturation procedure. First, we examined the efficiency of denaturation of sample DNA from the chip surface with a conventional heating procedure using a heat block. When the chip was maintained at 95°C for 1 min, all of the sample DNA, which had been hybridized to the surface-bound probe DNA, was released from the chip surface (Figure 3, a and b). As shown in Figure 3b, more

Figure 1. Schematic drawing of the photo-thermal denaturation method. First, the target DNA sample is captured by the immobilized probe DNA on the microchip surface. After the area for spot heating is selected by fluorescence microscopy, the area is heated by irradiation with an IR laser. The sample DNA released into the solution is collected and used for further analysis. The chosen DNA samples attached to the particular area on the chip surface are released by spot heating, which is controlled by the IR focused beam.

Figure 2. Schematic drawing of the photo-thermal denaturation microscopy system for area-specific extraction of surface-bound DNA. The microscope has three different optical units for phase-contrast, fluorescence and IR laser irradiation (5,7). Dichroic mirrors and band-pass filters direct the beams of the three different wavelengths of light.

Research Report

than 95% of the fluorescent sample DNA was released from the chip surface. Next, a 2- μ M 60-nucleotide sample DNA in 50 mM Tris-HCl (pH 7.5) was loaded onto the chip and successfully rehybridized with the surface-bound probe DNA (Figure 3c). The results indicate the following two facts. First, the integrity of the probe DNA remained intact, with the whole strands undamaged even after denaturation, as indicated by the 60-nucleotide sample DNA successfully attaching to the 3'-end (free end) of probe DNA. Second, the chromium-coated microchip served

as a good substrate for the immobilized probe DNA and could release almost 100% of sample DNA, and 70% could be rehybridized on average through the thermal denaturation and renaturation steps. Note that the proportion of irreversibly attached sample DNA on the chromium-coated chip is negligibly small in contrast to the glass surface (Figure 3d). As shown in Figure 3d, the amount of undetached sample DNA on the glass surface was 90% after the denaturation process, in strong contrast to the case with the chromium-coated surface, which was less than 5%. This may

Figure 3. Fluorescence micrographs of the chromium-coated microchip surface and glass surface. (a) Fluorescence image of the chromium-coated microchip with attached fluorescent DNA; (b) the chip after the photo-thermal denaturation process; (c) the chip after rehybridization with the sample; and (d) the glass surface after the denaturation process. Bar, 200 μ m.

Figure 4. Fluorescence and phase-contrast micrographs of the microchip surface. Fluorescence image of the microchip surface before (a) and after (b) IR laser beam irradiation; (c) the chip after rehybridization with the sample DNA on the IR-irradiated microchip; and (d) phase-contrast micrograph of (c). The numbers traced on the surface of the chromium-coated microchip (100, 50, 25 and 10, respectively) indicate the power of IR laser irradiation for tracing, 100, 50, 25 and 10 mW. The word "HARL" was traced with a 10-mW IR laser beam. Bars, 100 μ m.

Research Report

be due to the charge of chromium (zeta potential) at the boundary of the chip surface, which prevents the nonspecific adsorption of sample DNA directly onto the chip surface.

The chromium-coated microchip has three advantages in the photo-thermal denaturation method. The first is its improvement of the absorbance of the IR laser on the surface of the chip during heating. While absorbance of a glass slide and water is negligibly small at 1053 nm, the 6-nm-thick chromium-coated surface achieved 40% absorption of the IR laser. The second advantage is the high efficiency of silane coupling on the oxydized chromium at the surface of the plate compared with that of the glass surface. In general, silane coupling works far more efficiently on a metal oxide than a glass surface. The third advantage is, as mentioned above, the prevention of nonspecific DNA adsorption.

Before photo-thermal spot heating, the fluorophore-labeled 619-nucleotide ssDNA (standard sample DNA), the complementary pair of whole-length probe DNA was hybridized onto the chromium-coated chip (Figure 4a). Importantly, although the whole area of the microchip was exposed to the standard sample DNA solution, the DNA attached only to the area coated with probe DNA. Next, the 1053-nm

focused IR beam irradiated the surface of the chip and scanned it with varying laser power (10–100 mW) (Figure 4b). Bubbles generated by boiling water were sometimes observed at the irradiated area, indicating that the temperature of solution in the focused area exceeds boiling point. Figure 5a shows a magnified image of the area irradiated at 25 mW in Figure 4b. Figure 6a is the trace of fluorescence intensity along the line A-A in Figure 5a. As shown in Figures 5a and 6a, fluorescence intensity decreased in two steps, first to less than 30% in region P with a width of 30 μm and then to less than 5% in region

Q with a width of 10 μm .

After photo-thermal extraction, we examined whether the probe DNA is maintained intact after IR irradiation by rehybridizing the 60-nucleotide sample DNA. Figure 4c shows the result of rehybridization between the 60-nucleotide sample DNA and the probe DNA after 10–100 mW IR laser irradiation. As analyzed in the 25-mW case (Figure 5b and Figure 6b), fluorescence intensity in region P recovered to the original level after the rehybridization procedure (width R), but the intensity in region Q did not. Thus, although all non-fluorescent lines traced by the IR beam

Figure 6. Fluorescence intensities of the IR-irradiated microchip surface. (a) and (b) fluorescence intensity profile along the A-A line in Figure 5, a and b, respectively.

Figure 5. Fluorescence micrographs and intensity profiles of the 25-mW IR-irradiated microchip surface. (a) Fluorescence image of the microchip surface after 25 mW IR laser irradiation (magnified from Figure 4b); and (b) the chip after rehybridization with the sample DNA on the IR-irradiated microchip (magnified from Figure 4c). Bar, 100 μm .

Figure 7. Laser power dependence of DNA denaturation on the microchip surface. Open box, width of the denatured area of the fluorophore-labeled sample DNA after IR irradiation; closed box, width of the damaged area on which no rehybridization occurred after rehybridization; hatched box, width of the rehybridized area after rehybridization.

at greater than 10 mW remained even after the rehybridization process, those traced at 10 mW were completely re-decorated with fluorophores (Figure 4c). The width of the traced lines increased as laser power increased (Figure 7). A phase-contrast image (Figure 4d) shows that the chromium film was damaged by IR irradiation stronger than 10 mW. These results demonstrate that the probe DNA remains undamaged after IR irradiation unless the chromium coating has been damaged.

Finally, we measured the amount of sample DNA extracted from the chip after spot-heating a $100 \times 10 \mu\text{m}$ area with a 10-mW IR focused beam. From fluorescence intensity analysis of the beam, more than 70% of the sample DNA was released from the chip surface after irradiation. As shown in Figure 8, the total amount of extract was in the order of 10^{-17} mol, on the basis that the fluorescence intensity of the extracted sample DNA was between that of the 1×10^{-16} mol and 1×10^{-17} mol reference samples after 15 cycles of PCR amplification. This result indicates that this photo-thermal denaturation extracted at least one DNA molecule from a $13 \times 13 \text{ nm}^2$ area (10^{-17} mol/ $1000 \mu\text{m}^2$) and further indicates that this method allows the area-specific extraction of a

specified sample DNA.

The present study shows the potential of this DNA chip as a DNA purification apparatus. Spatial resolution of the denaturation spot size can be improved by increasing the objective lens magnification. We feel that this photo-thermal method will greatly enhance separation efficiency of different DNA from crude samples.

ACKNOWLEDGMENTS

We thank Drs. K. Miyauchi, T. Iwayanagi and T. Sakamoto of Hitachi, Ltd. for their support, and Dr. G. Harris for English editing. We also thank Prof. K. Kinoshita, Jr. of Keio University and Dr. T. Nishizaka of Core Research for Evolutional Science and Technology (CREST) for their cooperation in consulting on the microscopy system. This research was partly supported by Grants-in-Aid for Scientific Research, for Scientific Research on Priority Areas and for the High-tech Research Center Project from the Ministry of Education, Science, Sports and Culture of Japan [grant no. 11559016 to S.I., no. 11878129 to S.I. and no. 11CE2006 (Komaba Complex Systems Life Science Project) to K.K.] and Grants-in-Aid from the Japan Science and Technology Corporation (CREST).

REFERENCES

1. Burns, M.A., B.N. Johnson, S.N. Brahma-sandra, K. Handique, J.R. Webster, M. Krishnan, T.S. Sammarco, P.M. Man et al. 1998. An integrated nanoliter DNA analysis device. *Science* 282:484-487.
2. Cheng, J., E.L. Sheldon, L. Wu, A. Uribe, L.O. Gerrue, J. Carrino, M.J. Heller, and J.P. O'Connell. 1998. Preparation and hybridization analysis of DNA/RNA from *E. coli* on microfabricated bioelectronic chips. *Nat. Biotechnol.* 16:541-546.
3. Kambara, H. 1998. Recent progress in fluorescent DNA analyzers and methods. *Curr. Topics Anal. Chem.* 1:21-36.
4. Kato, H., T. Nishizaka, T. Iga, K. Kinoshita, Jr. and S. Ishiwata. 1999. Imaging of thermal activation of actomyosin motors. *Proc. Natl. Acad. Sci. USA* 96:9602-9606.
5. Kinoshita, K., Jr., H. Itoh, S. Ishiwata, K. Hirano, T. Nishizaka and T. Hayakawa. 1991. Double-view microscopy with a single camera: real-time imaging of molecular orientations and calcium. *J. Cell Biol.* 115:67-73.
6. Lockhart, D.J., H. Dong, M.C. Byrne, M.T. Follettie, M.V. Gallo, M.S. Chee, M. Mittmann, C. Wang et al. 1996. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotechnol.* 14:1675-1680.
7. Nishizaka, T., H. Miyata, H. Yoshikawa, S. Ishiwata and K. Kinoshita, Jr. 1995. Unbinding force of a single motor molecule of muscle measured using optical tweezers. *Nature* 377:251-254.
8. O'Donnell, M.J., K. Tang, H. Koster, C.L. Smith and C.R. Cantor. 1997. High-density, covalent attachment of DNA to silicon wafers for analysis by MALDI-TOF mass spectrometry. *Anal. Chem.* 69:2438-2443.
9. Service, R.F. 1998. Microchip arrays put DNA on the spot. *Science* 282:396-398.
10. Wang, D.G., J.-B. Fan, C.-J. Siao, A. Berno, P. Young, R. Sapolsky, G. Ghandour, N. Perkins et al. 1998. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 280:1077-1082.

Received 5 March 1999; accepted 1 December 1999.

Address correspondence to:

Dr. Kenji Yasuda
Department of Life Sciences
Graduate School of Arts and Sciences
The University of Tokyo
3-8-1 Komaba, Meguro
Tokyo 153-8902, Japan
Internet: cyasuda@mail.ecc.u-tokyo.ac.jp

Figure 8. Agarose gel electrophoresis of PCR products amplified from the microchip extract. Five microliters of the DNA products were applied to a 2% agarose gel. Lane A, DNA molecular markers; lane B, sample DNA extracted from $10^3 \mu\text{m}^2$ microchip surface; lanes C-E, concentration references, 1×10^{-15} , 10^{-16} and 10^{-17} mol of the same DNA, respectively.