DNA chip replication for a personalized DNA chip

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Abstract

We report the replication technology of DNA chip using sequence specific localization of nucleic acids via hybridization and electric transfer of the nucleic acids onto a new substrate without losing their array information. The denatured DNA fragments are first spotted and UV-cross-linked on a nylon membrane. The membrane is then immersed and hybridized in a DNA mixture solution that contains all complementary sequences of the nucleic acids to be hybridized with the DNA fragments on the membrane. The hybridized DNA fragments are transferred to another membrane at the denatured condition. After separating two membranes, the transferred membrane contains a complementary array of DNA fragments. This method can be used for the replication of the same copy of DNA chip repeatedly and moreover could be applied for a personalized DNA chip fabrication, where specific information of each spot of DNA chip is originated from the genetic information of a personal sample.

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1. Introduction

DNA chip technologies are proving to be powerful tools for advanced diagnostics and genetic analysis (Lemieux et al., 1998; Venkatasubbarao, 2004; Schena et al., 1998). Currently, there are two formats for the fabrication of a DNA chip. The first format involves a method in which oligonucleic acids are directly synthesized on a substrate to make DNA probes (Pease et al., 1994; Singh-Gasson et al., 1999). The second format involves a method in which oligonucleotides (Yershov et al., 1996; Hacia and Collins, 1999; Hacia, 1999), peptide nucleic acid (PNA) (Weiler et al., 1997), or complementary DNA (cDNA) (Shalon et al., 1996; Saghizadeh et al., 2003) are previously synthesized and then arrayed on a chip. Many other efforts to develop the DNA chip have been widely carried out by bead-based arrays (Walt, 2000), microcontact printing of DNA molecules (Lange et al., 2004) and chemical nanoprinting (Kumar and Liang, 2001). Accordingly, some fabrication techniques based on miniaturization and nanotechnology are being substantially put into commercial use (McGlennen, 2001; Henke, 1998; Mazzola, 2003). Very recently, a new fabrication method of DNA chip based on a hybridization–contact–dehybridization cycle of single-stranded DNA molecules was reported (Lin et al., 2005). Yu et al. (2005) have also developed a supramolecular nanostamping method for transferring single-stranded DNA molecules to secondary surface in few tens of nanometer resolution. However, their methods need chemically modified single-stranded DNA molecules and a shearing force generated from contacting two solid surfaces may damage DNA fragments with long sequence.

Conventional DNA chip has only spatial information of known DNA sequences. Thus, NGS-arrayit (http://www.ngs-arrayit.com) has patented a new microarray approach, in which segments of unknown patients DNA are amplified and attached on a chip instead of oligonucleotides or amplicon of known DNA sequence (Schena, 2005). In this approach, fluorescently labeled oligonucleotides whose colors represent normal or disease condition are hybridized with the chip. Then, multiple disease loci from different patients are analyzed with a single chip. However, this method needs cumbersome amplification step for each disease loci and expensive facility for spotting amplified DNA.
In this study, we present simple fabrication method of DNA chip having patients’ DNA sequence by replicating an original DNA chip. Fig. 1 shows the schematic of the DNA chip replication. If a primary DNA chip is put into a solution which contains all complementary sequences of the nucleic acids on the chip and are hybridized with complementary nucleic acids, the hybridized complimentary nucleic acids will be transferred onto a new substrate under specific denatured condition. The fabricated replicated DNA chip has the array information of the primary DNA chip, which is composed of the complementary sequences of nucleic acids. The primary DNA chip can be reused for further replications. In addition, if the fabricated DNA chip is made by a personal DNA sample (that we call a personalized DNA chip), it can be useful for detecting the mutation (Hacia and Collins, 1999; Hacia, 1999) and single nucleotide polymorphism (SNP) (Burmeister et al., 2004) of a sample having multiple genes. In other words, the spatial information of the replicated personalized DNA chip is originated from the sample DNA solution of an individual, unlike a conventional DNA chip. Therefore, the personal DNA in the replicated DNA chip is reversely hybridized with a fluorescence-labeled complementary DNA whose sequences are known. Prior to demonstrate the personalized DNA chip concept, we conducted the replication experiments by use of the DNA chip fabricated on a nylon membrane as a primary DNA chip. Detailed experimental protocol and results of the DNA chip replication experiments are reported herein. We also discussed the potential applications of personalized DNA chip using the replication technology.

2. Experimental protocol

2.1. Primary DNA chip

A primary DNA chip was made with a conventional spotting technique on a nylon membrane. All DNA samples were obtained from LG Life Sciences, Ltd. (Daejeon, Korea). The DNA sequences of HAV (5' UTR of hepatitis A virus; 315 bp, 0.15 \( \mu \)g \( \mu \)L\(^{-1} \)), HBV (surface antigen of hepatitis B virus; 467 bp, 0.14 \( \mu \)g \( \mu \)L\(^{-1} \)) and HCV (5' UTR of hepatitis C virus; 268 bp, 0.16 \( \mu \)g \( \mu \)L\(^{-1} \)) were used in this study. The primary DNA chip has overall nine spots of DNA sequences, where each column of spots is related with HAV, HBV and HCV, respectively. Prior to DNA spotting, the DNA samples containing 1-\( \mu \)L PCR amplified non-labeled DNA solution were denatured at the denatured temperature (90 °C). After the DNA fragments were manually spotted on a nylon membrane (25 mm × 25 mm; Hybond N+; Amersham, IL), the spotted DNA was cross-linked by illuminating an UV light. This ensures the primary DNA chip the repetitive use. The diameter of spot was about 2 mm and distance between two spots was about 5 mm.

2.2. Replication apparatus of DNA chip

Fig. 2A shows the schematic of DNA transfer apparatus used. A primary DNA chip (the lower membrane in Fig. 2B) was put on the stainless steel block and the poly(dimethylsiloxane) (PDMS) plate was placed on the membrane. After filling the thru-holes of the PDMS plate with a degassed phosphate buffered saline (PBS) buffer, a new substrate (the upper membrane in Fig. 2B) for replicated DNA chip and stainless steel plate was place on the PDMS plate, and loosely fastened with four bolts. Multifunction board (PCI-6035E; National Instrument, Austin, TX) was used to apply a voltage and monitor a current between stainless steel plate and block. A temperature control system including heating rods, thermocouple and temperature controller was purchased from Watlow (St. Louis, MO). The PDMS plate (thickness: 1 mm) was made by conventional molding process. The mixture of PDMS prepolymer and curing agent (Sylgard 184; Dow Corning, MI) was poured onto the plate mold and cured at 100 °C for 30 min. An aluminium block consists of two blocks and the lower one has a channel to allow water flowing.

2.3. DNA hybridization and electrical transfer

The primary DNA chip (Fig. 3A) was hybridized in the solution containing 20 \( \mu \)L of the Cy3-labeled DNA mixture of 0.033 \( \mu \)g \( \mu \)L\(^{-1} \) HAV, 0.025 \( \mu \)g \( \mu \)L\(^{-1} \) HBV and 0.033 \( \mu \)g \( \mu \)L\(^{-1} \) HCV (Fig. 3B). Hybridization conditions are as follows: pre-hybridization at 60 °C for 1 h in ExpressHyb Hybridization buffer (Clontech, Palo Alto, CA) and hybridization at 60 °C.

Fig. 1. Concept of DNA chip replication.
for 1 h in a hybridization oven (Hybaid, Middlesex, UK). Then, the hybridized DNA fragments were transferred to another nylon membrane at the denatured temperature (90 °C) using the DNA transfer apparatus as shown in Fig. 2 (Fig. 3C). To facilitate a DNA transfer (Toneguzzo et al., 1986), we also applied an electrical voltage of 0.1–3.0 V between the membranes for 10 min. In the transfer stage, the poly(dimethylsiloxane) (PDMS) spacer with thru-hole array was placed between two membranes to prevent contamination of the DNA fragments on each spot. After separating two membranes, the transferred membrane contains a complementary array of DNA fragments and can be used for a personalized DNA chip application. The primary DNA chip can also be reused for building another replicated DNA chip. The transferred membrane, the replicated DNA chip, was UV-illuminated and hybridized with one of Cy5-labeled probe DNA (HAV, HBV, or HCV) solution (Fig. 3D). The concentrations of Cy5-labeled DNA were 0.027 μg μL⁻¹ HAV, 0.030 μg μL⁻¹ HBV and 0.027 μg μL⁻¹ HCV, respectively. The other hybridization conditions are the same as those of Cy3-labeled DNA.

2.4. Measurement set-up

A commercial microarray scanner (ScanArray LITE; Parkard Bioscience Inc., Meriden, CT) was used to capture the two channel fluorescence images of the replicated DNA chip after being dried and attached on a slide glass using double stick tape. However, as the primary DNA chip was used repetitively, it cannot be imaged with the commercial scanner. We constructed a fluorescence imaging system to visualize the membrane images of Cy3-labeled DNA chip. A laser light emitted from a green laser (532 nm; DTL-312-100; Power Technology Inc., Little Rock, AR) was expanded with two lenses and reflected with a mirror to illuminate on a membrane strip with about 40° angle. A charge coupled device (CCD; MicroMax-512BFT; Roper Scientific; Trenton, NJ) camera placed right above and equipped with a macro lens was used to capture the fluorescence images of the membrane after cutting off the scattered laser light with a long wave pass filter (LP-560-2 × 2; CV I Laser, Albuquerque, NM).

3. Results and discussion

The objective of the work was to develop the replication technology of a primary DNA chip and its application to a personalized DNA chip. First, we conducted the experiments with the fabrication of model primary DNA chip. To prove the concept of experiments, we have exploited the low-density DNA probes with a large size of spot to investigate the DNA
transfer condition easily. The primary DNA chip with nine spots of DNA sequences was then immersed and hybridized in a DNA mixture solution that contained all complementary sequences of the nucleic acids to be hybridized with the DNA fragments on the primary DNA chip.

Fig. 4 shows the CCD camera images of five successive replication of a primary DNA chip. For each replication experiment, the same conditions (block temperature, 90 °C; transfer time, 10 min) were applied to the DNA transfer apparatus loaded with two membranes. Optimum transfer voltage was selected at 2.0 V in the range of 0.1–3.0 V operating conditions. During the experiments, gas bubbles, which can be caused by degassing of buffer solution and electrochemical reaction on the stainless steel electrodes, were prevented from the extension of the replication time and elevation of transfer voltage. Fig. 4A shows CCD images of a primary DNA chip hybridized with Cy3-labeled DNA before each replication step. Interference patterns on CCD images were caused by the lens to expand excitation laser beam and those were not shown in the scanner images. Although we use the membrane allowing fluorescence detection, the membrane used for DNA chip fabrication had some substances generating background fluorescence and those were reduced after first replication. In addition, some hybridized Cy3-labeled DNA was
still remaining in the primary DNA chip after replication process, and it results in accumulation of the spot signal after repetitive replication (Fig. 4B). Because the sequence of Cy3-labeled DNA is similar to that of originally spotted on the primary DNA chip, remaining DNA fragments will not give significant influence on the replicated DNA chip (Fig. 4C). However, the spot to spot resolution of the DNA chip was poor because a PDMS plate with thru-holes was interposed to prevent cross-contamination. If more rigid substrate (such as plastic and glass) with thru-hole structures and sophisticated positioning tool to align the thru-holes with the spots were used as a surface of DNA immobilization, this could be improved to the point where it would be comparable to existing DNA chips.

From the CCD images for Cy3 dye, we can find nine spots for three different genetic loci, all of which were labeled with the same Cy3 dye. To verify the fact that the replicated DNA chip preserves spatial information of the primary DNA chip, we employed the DNA hybridization experiments with the replicated DNA chips. Fig. 5 shows the fluorescence images of DNA chip. The Cy5-labeled HAV, HCV and HBV DNA probes were used for Experiments 1–3, respectively. From the results, it was confirmed that the DNA location on the replicated DNA chip was mirrored with the primary DNA chip. In this case, we used single probe for each genetic loci to confirm DNA sequence information which was preserved. However, probes for a locus can be multiplexed and differently labeled to analyze genetic information of a person.

This replication technology of the DNA chip has difficulty to provide a reproducible and high-throughput fabrication method although a number of DNA chip can be replicated from a primary DNA chip. In addition, every time a new DNA chip is made it is necessary to hybridize DNA to the master. This takes
an hour during which time one could spot a large number of DNA chips with a conventional robot. However, the replication technology could be useful to construct a customized and personalized DNA chip without using expensive spotting facility. For example, the primary DNA chip designed for capturing nucleic acids of interest is put into the solution which contains nucleic acids of a personal sample and the nucleic acids are hybridized according to their sequences. Thus, the hybridized nucleic acids originated from the individual sample are transferred onto a new substrate. Compared with the NGS-array format technology (Schena, 2005), multiplex PCR for multiple disease loci can also reduce the cost of the DNA amplification.

Fig. 6 shows comparison of three different types of DNA chip. Generally, cDNA chip analyzes multiple genes but ignores minor sequence differences (Fig. 6A). Oligonucleotide chip analyzes minor sequence differences but it is hard to analyze multiple genes at a time (Fig. 6B). In a personalized DNA chip, however, each disease loci could be discriminated with the primary DNA chip like cDNA chip and the loci information is transferred to the replicated DNA chip. The minor sequence differences could be determined via hybridization of short probe labeled with different fluorescence tag (Fig. 6C). In addition, probes for different loci, which can have completely different sequence between each other, can be labeled with the same color as they can be discriminated with their location on the chip. Consequently, a personalized DNA chip replicated has the spatial information (gene location) of DNA chip, and the sequence information of each spot is originated from the personal DNA sample. A personalized DNA chip could be analyzed with fluorescence tagged probes, if fluorescently labeled DNA probes are prepared for each gene, such as normal for green dye and mutated for red dye. From the fluorescence images of DNA microarray, genetic mutation of a sample could be explored simultaneously for multiple genes.

In summary, the same copy of DNA chip was fabricated repeatedly and a number of DNA chips can be replicated from the primary DNA chip. The DNA information on a nylon membrane was transferred onto the other nylon membranes. With the experimental condition we used, however, the hybridized DNA fragments were not completely transferred. The suitable structure of primary DNA chip and DNA immobilization strategy for electric transfer of DNA should be explored to achieve the reproducible replicated DNA chip. The replication technology could be applied to produce a personalized DNA chip, which contains genetic information of a real human DNA sample. For personalized DNA chip technology, DNA transfer efficiency is not important because genetic information can be analyzed by not intensity of each spot but fluorescence color differences of each spot. With these backgrounds, it was thought that the membrane-based personalized DNA chip technology can be a cost effective method for clinical genetic analysis.

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References