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Development of DNA Chip Microarrayer

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Abstract A microarrayer system was developed mainly for manufacturing DNA chips. The 3-axis robot was designed to automatically collect samples from 96- or 384-well microtiter plates using up to 16 simultaneously moving pens and to deposit them on a surface-modified slide glass. This is followed by a wash/dry operation in a clean station. The cycle is repeated with a new set of samples. This system can deposit cDNA or oligonucleotides with spot intervals of 150 μm and the spot size of 80 μm, thus allowing a high density DNA chip containing about 5,000 spots per cm^2. The entire procedure is controlled by the Visual C++ program that was written in our laboratory by using a personal computer with Pentium 100 CPU.

Key words: DNA chip, microarrayer, high density microarray

Biological science is being revolutionized by an availability of much information in regards to a complete genome sequence for many different organisms. Currently, there are 20 completely sequenced genomes including 14 eubacteria, 4 archaea, and 2 eukaryotes [6]. Much effort is being devoted to determine the sequences of many other organisms including humans. Having these enormous amounts of data with the recently developed advanced technology allows us to analyze thousands of genes in parallel [3, 7, 8, 11, 14, 15]. With the advanced technology at hand, the main trend in biological research is rapidly changing from a structural DNA analysis to understanding cellular function of the DNA sequences. Recently developed DNA chips have emerged as prime candidates for the performance of such analyses. Combined with mechanics, computer, bioinformatics and other advanced technologies, DNA chip technology provides numerous applications because of its robustness, accuracy, and automation [2, 10]. With technical development that offers increased sensitivity, the DNA chip is expected to become an indispensable tool in fields of biology, biotechnology, drug discovery, and other application areas [12, 13]. DNA chips can be used for mutation and polymorphism detection, gene expression monitoring, and phenotypic analysis as well. If the DNA chip is used for the development of pharmaceutical products, it can considerably reduce the cost and time for the entire process of drug discovery and development, and can also contribute in developing personal drugs [9].

Unfortunately, the high prices of microarrayers and scanners make it difficult to use the DNA chip as a research tool in normal laboratories [1]. When a decision was made to carry out the gene expression monitoring process using a DNA chip last year, there was no commercially available DNA chip microarrayer. Today, several companies such as Genetic Microsystems (Woburn, Massachusetts, U.S.A.; http://www.geneticmicro.com/) and Cartesian Technologies (Irvine, California, U.S.A.; http://www.cartesiantech.com/) sell microarrayers, but the products are still too expensive to be used in most laboratories. Therefore, Patric Brown Lab. at Stanford Medical School (http://cmgm.stanford.edu/pbrown/index.html) and Albert Einstein College of Medicine (AECOM) (http://sequence.aecom.yu.edu/bioinf/funcgenomic.html) developed their own DNA chip microarrayers [4], which provided initial guidelines for our work.

In this paper, we look into the development of an economical and robust DNA chip microarrayer system. The main purpose for this research is aimed so that the procedures shown in this paper could allow for developing the DNA chip microarrayer at a reasonable cost in any laboratory.

MATERIALS AND METHODS

Microarrayer Parts

The list of the microarrayer parts is shown in Table 1.

Preparation of a DNA Chip

For immobilizing DNA on a solid support, a surface of the slide glass is treated with poly-L-lysine (Sigma, St. Louis,

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Table 1. List of microarrayer parts.

Part	Vendor	Catalog No.	Quantity
Robot axis	Samsung Electronics Co.	RCM4-A (FARAMAN)	2
	(Seoul, Korea)	RCM4-C (FARAMAN)	1
Servo drive	Samsung Electronics Co. (Seoul, Korea)	CSD 02BP (FARACON)	3
Controller	Samsung Electronics Co. (Seoul, Korea)	MMC-PV4 (FARA MMC Series)	1
Vibration isolation table	Daeil System (Seoul, Korea)	DVIO-R-1212M-50 (800H)	1 set
Microspotting tip	Die Tech (San Jose, CA, U.S.A.)	1/16 Printing tips	32 ea
Vacuum pump	Hana Engineering (Taejon, Korea)	TVP90DH250	1
Water pump	Aquarium in Taejon		1
Clean station	Machine shop in KAIST		1
Slide platter	Machine shop in KAIST		1
z-Bracket	Machine shop in KAIST		1
Tip holder	Machine shop in KAIST		1
Adapters	Machine shop in KAIST		6
Hybridization chamber	Machine shop in KAIST		10

U.S.A.) [4]. Onto this modified slide glass, DNA samples are arrayed and dried in air for one day. After DNA spotting is completed, the left upside corner of the slide glass is marked by using diamond scriber for verifying the spotting region. DNA samples are immobilized by the process of UV-irradiation. With these procedures, DNA is first covalently attached to the surface, and then it is UV-crosslinked. The rest of the poly-L-lysine surface is blocked by adding 70 mM succinic anhydride dissolved in a solution (315 ml of 1-methyl-2-pyrrolidinone and 35 ml of 0.2 M boric acid (pH 8.0)). Immediately after the blocking reaction, the bound DNA is denatured by incubating for 2 min in distilled water at 95°C. The slides are then transferred into a bath that consists of 95% (v/v) ethanol at room temperature, and the excess liquid is removed from slides by spinning the rack of slides onto the microtitre plate carriers for 3 min at 500 rpm. Slides were stored in a closed box and maintained at room temperature until they were used. Fabricated DNA chips can be stored for as much as 6 month under this condition.

Hybridization and Scanning

E. coli W3110 was used in this study. After cultivation at 37°C and 250 rpm in LB medium, 10 ml of the cell culture at the OD_{600} of 1 were collected by centrifugation and resuspended in 0.3 ml of ice-cold buffer [5]. Thirty µl of 0.2 M Vanadyl Ribonuclease Complex (Gibco BRL, Gaithersburg, U.S.A.), 0.3 ml of hot lysis buffer, and 3 µl of 20 mg/ml Proteinase K were added and this mixture was incubated at 37°C for 30 min. Thereafter, the sample was phenol extracted, ethanol precipitated, and finally dried. The resulting RNA was resuspended in a 200 µl of DEPC-treated water and consecutively treated for 35 min with 12 units RNase-free DNase, followed by 15 min with 40 µg of Proteinase K. An additional phenol extraction was performed before ethanol precipitation. Cy5-dUTP (Amersham, Uppsala, Sweden) was incorporated during reverse transcription of RNA by using a random hexamer (Superscript Preamplification System, Gibco BRL, Gaithersburg, U.S.A.). The reaction mixture was diluted with 470 µl of TE buffer (pH 8.0) and subsequently concentrated to about 20 µl using a Microcentricon-30 (Amicon, Massachusetts, U.S.A.). Purified and labeled cDNA was suspended to 20 µl at 4×SSC buffer. Before hybridization, the solution was boiled for 2 min and then cooled at room temperature. Hybridization was performed for 16 h in a 65°C water bath. To prevent drying of the hybridization solution during the hybridization process, 10 µl of 3×SSC was placed on two corners of the hybridization chamber. After hybridization, DNA chips were first washed in 0.2×SSC with 0.1% (w/v) SDS and subsequently rinsed with 0.2×SSC. Excess liquid on the surface was removed by centrifugation. The DNA chip was scanned by ScanArray 3000 at General Scanning Japan (Tokyo, Japan).

RESULTS AND DISCUSSION

Automatic System

Our DNA chip microarrayer was designed to manufacture high-density, gridded arrays of cDNA or other biological

materials on a solid support. It consisted of combined two axis robots, one axis robot, microspotting tip assembly, clean station, and a computer. The clean station, adapters, hybridization chamber, slide platter, and other mechanical parts were made at the machine shop at KAIST.

The robot is designed to automatically collect samples from 96- or up to 384-well microtitre plates with as much as 16 simultaneously moving pens and deposit them on a surface-modified slide glass. This is followed by a wash/dry operation where the cycle is repeated with a new set of samples. This system can deposit cDNA with spot intervals of 150 μm and the spot size of 80 μm. A high density DNA chip containing 5,000 DNA spots per cm^2 can be made at this time. With this microarrayer, 100 DNA chips containing 5,000 DNA spots can be made within two days. The programmed orders are converted to electric signal and transferred to a servo drive by using a controller. Then, the servo drive controls the action of the servo motor for the axis robots. The information on the current position of each axis robot is transferred from the encoder to the controller for an accurate position control. Vacuum and water pumps are also connected to the user I/O contact of the controller and the exact operation time is computer-controlled by relay (Fig. 1).

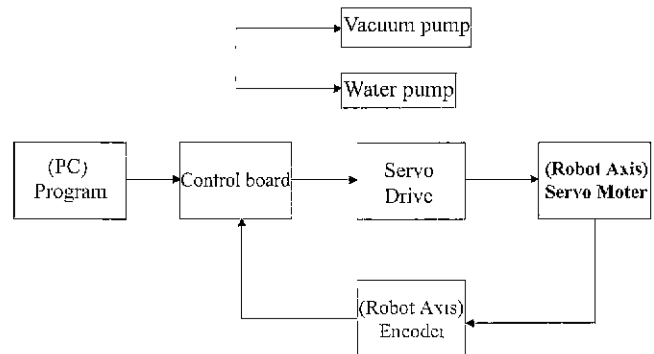


Fig. 1. Microarrayer system overview.

Two RCM4-As were used for x- and y-axis robots and one RCM4-C for the z-axis robot (FARAMAN series, Samsung Electronics Co., Seoul, Korea). The strokes of each axis are 600 mm (x), 600 mm (y), and 200 mm (z). The maximum velocity of each axis is 1,000 mm/sec and the resolution is ± 0.02 mm. The x-axis is mounted directly to the vibration isolation table (1.2 m×1.2 m, height: 80 cm, thickness: 50 mm, Daeil System, Seoul, Korea, Fig. 2A). A choice of the isolation system primarily depends on what the resolution requirement is for the application. It was discovered that the pneumatic isolators

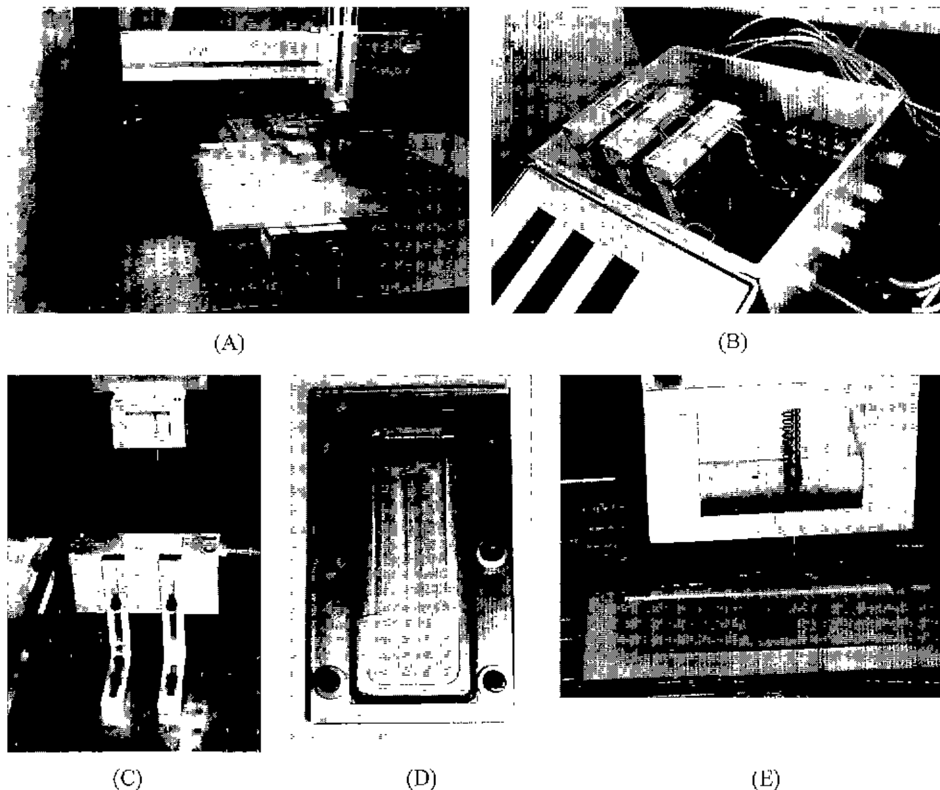
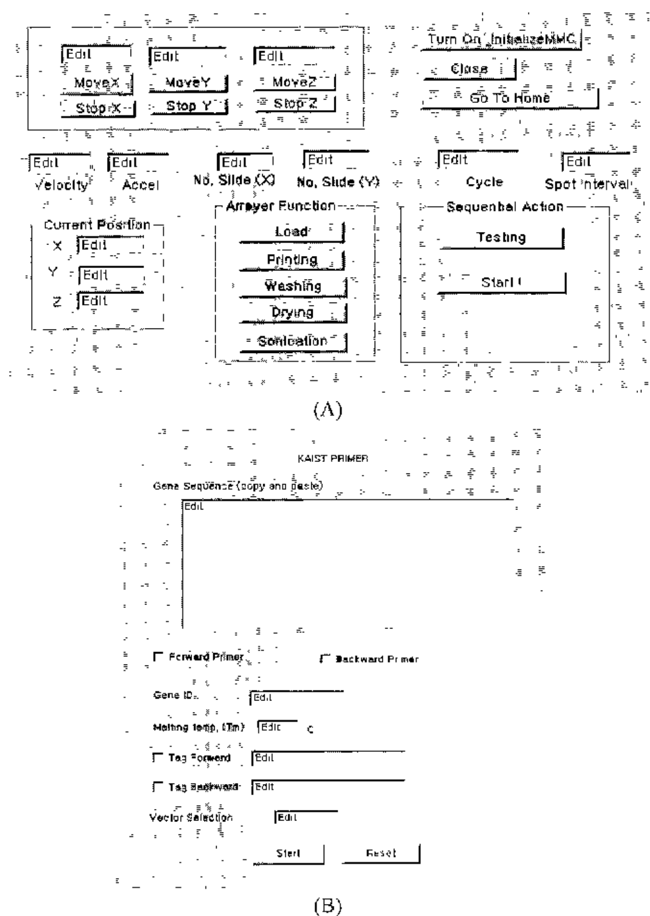


Fig. 2. Microarrayer (A) system, (B) control box, (C) clean station, (D) hybridization chamber, and (E) printing test with ink spotting (4,900 ea/3.24 cm^2, cover glass size)

are not required for the DNA microarrayer system. The slide platter, on which slide glasses are moved to the spotting position, is loaded on the x-axis. For carrying the microspotting tip for spotting DNA on a slide glass, the z-axis is perpendicularly attached to the y-axis. The y-axis robot is located 300 mm above the table and perpendicular to the x-axis robot. Adapters are used for fixing each robot slide to the table. Three servo drives (FARACON CSD series, 200 W, 220 V, Samsung Electronics Co., Seoul, Korea) are connected to each robot slide to supply power and to amplify signals from the controller. Two terminal block modules were used for wiring the microarrayer control system in which one was for axis-robots and the other was for vacuum and water pumps. The microarrayer system could be simplified by putting servo drives, terminal blocks, and relays in a control box (Fig. 2B).

The controller (Multi-Motion Controller, Samsung Electronics Co., Seoul, Korea) was installed on a personal computer with Pentium 100 CPU, and the robots were operated by following the user-defined program. The system was controlled by a Visual C++ program that was written in our laboratory and runs in a Microsoft Windows 95 environment (Fig. 3A). The control program provides the user with a friendly interface where users can select the appropriate spot position, spot intervals, and the number of DNA chips intended to be fabricated in a batch. The results from spotting test with a printing ink are shown in Fig. 2E.



(A)

(B)

Fig. 3. Programs for microarrayer control (A) and primer design (B).

Mechanical System

In order to carry the microspotting tips, the z-stage bracket and a tip holder are assembled and attached to the z-axis (Fig. 2A). The tip contained in the tip holder is designed to freely move up and down during a process of spotting DNA onto the slide glass. The microspotting tip (Die Tech, San Jose, U.S.A.) has a diameter of 1/16 inch and it was wire-cut to 0.001 inch at the end of the tip. This tip can load DNA from a 96- or 384-well plate by capillary action and can spot DNA onto the surface-modified slide glass by mechanical contact. In the middle part of the tip, there is a round groove for fitting the E-ring, which prevents the tip from slipping out of the tip holder. The tip holder contains four holes at each side of both tops and bottom sections. For reducing abrasion of the tip round during the DNA spotting procedure, linear bearing can be fixed in a hole of the tip holder. However, if the spotting tip is considered as articles of consumption and if the tip end should be worn out before the abrasion of the tip round, the use of linear bearing is not necessary. It should be noted that since tips must be aligned with a hole, much attention and caution are required for making holes in the tip holder. This first generation microarrayer system used spring for DNA spotting. Unfortunately, this resulted in a rapid abrasion of the tip end. As a result, spring is not in use anymore and DNA spotting is done mostly by gravity.

A clean station consists of wash and drying parts and there are 4 holes on top of each part. (Fig. 2C). The hole intervals are exactly the same as those of the tip holder, and the size of the hole is slightly larger in order for the tip to go into the hole without touching anything. To clean the tip end, distilled water flows into the wash station by using the water pump which was purchased from an aquarium shop in Taejon. The water pump is submerged in distilled water in a carboy, and is connected to the inlet of the washing part by Tygon tubing. For controlling the water flow rate, a tube connector at the inlet tube is used. After washing, the wet tip end is dried in the drying station, which is connected to a vacuum pump and it is computer-controlled by relay. A moisture trap is used to protect the vacuum pump from water damage or dirt. The hybridization chamber is made of a metal body with a transparent acrylic polymer cover that can handle any high reaction temperature, and it is designed for handling hybridization reactions which involve DNA chips (Fig. 2D).

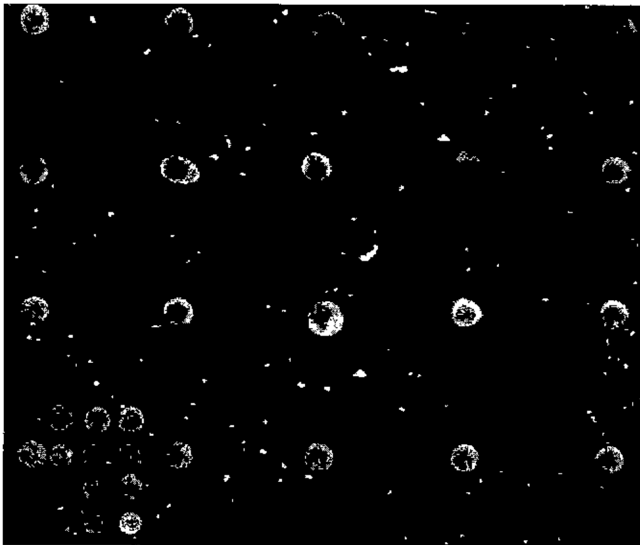


Fig. 4. A hybridized DNA chip printed by our microarrayer. *E. coli* genes were immobilized on a poly-L-lysine treated slide glass, hybridized with Cy5-labeled cDNA, and scanned. Spot intervals are 1,000 μm and 200 μm (left bottom corner).

Sample Test with *E. coli* Genes

DNA chips containing 20 genes of *E. coli* were made by the microarrayer described above, and hybridized with a labeled cDNA (Fig. 4). For acquiring the whole round DNA spot, it was found that the upward speed of the z-axis robot was about twice as high than the downward speed in the spotting procedure. To minimize any abrasion of the tip end, it was operated to touch glass surface gently. Genes were spotted with intervals of 1,000 μm and 200 μm (left bottom corner). It can be recognized that the DNA samples were regularly spotted on the slide at desired spot intervals. The spotted *E. coli* genes are as follows: *talE*, *pgm*, *dnaK*, *groEL*, and *groES* in the first row; *manA*, *pykF*, *pfkB*, *gapA*, and *eda*, in the second row; *zwf*, *pykA*, *gnd*, *glk*, and *tktB* in the third row; *eno*, *aceF*, *lpdA*, *gltA*, and *sdhC* in the fourth row. Information regarding *E. coli* gene sequence was obtained from the *E. coli* genome project home page at Wisconsin University (http://www.genetics.wisc.edu) and the open reading frames (ORFs) were amplified by the PCR. Primers for each coding sequence were designed by the primer design program that we developed (Fig. 3B).

It can be seen from Fig. 4 that the hybridization signals are very clear from the background. In this study, we examined only the operational efficiency, accuracy, and stability of our DNA microarrayer. The limiting step in the manufacture of the whole genome DNA chip is to obtain the ORFs by PCR, because of its high cost and intensive labor. We are currently amplifying the *E. coli* ORFs in the order of importance. When this is completed, it will be possible for the entire genome *E. coli* chip to be made for global gene expression monitoring.

CONCLUSIONS

The study of gene expression on a genomic scale is most obvious opportunity possibly to make a complete genomic sequences of the model organisms. The DNA chip makes it possible to measure the transcription levels of all genes at the same time [7]. Many research groups are willing to carry out research using a DNA chip, but the high price of microarrayer and scanner makes it difficult to do so. Compared with commercially available microarrayers, the building cost of the presently described DNA chip microarrayer is much more economical. An advantage of making our own microarrayer is the convenience to upgrade and repair the instruments at a low cost. We constructed a DNA microarrayer for a total cost of 23 million Korean Won. Several DNA chips like the *Saccharomyces cerevisiae* chip are starting to become available commercially. However, DNA chips of organisms which are not widely used will not be made commercially. These DNA chips of less commercial interests, such as *Clostridia* and *Rhodococcus* to name a few, can therefore be "home-made". Our main purpose of this research is to describe procedures which will allow the manufacture of microarrayers at a reasonable cost by any laboratory. Detailed microarrayer information and experimental procedures are also available in our laboratory homepage (http://che.kaist.ac.kr/~apbiot/).

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