# KILO-TO-GIGA DNA MICROARRAY FOR CONVERSION INTO HIGH-DENSITY PROTEIN MICROARRAY ON DEMAND

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## ABSTRACT

We describe the development of simple and versatile high-density DNA microarrays (1 million spots/cm$^2$) that can be converted into *in situ*-synthesized protein microarrays on demand for global proteome analysis. To accomplish this, we adopted an integrated approach using high-throughput technologies including microemulsion PCR for single-molecule amplification on microbeads [1], on-chip capillary electrophoresis for sorting DNA-bound functionalized beads [2], and microreactor array technology for self-assembled molecular lithography [3]. The concept developed here is expected to markedly enhance the application of protein microarray to the study of functional proteomics.

**KEYWORDS:** DNA array, Microreactor array, Cell-free protein synthesis, Water-in-oil microemulsion

## INTRODUCTION

DNA microarrays can allow parallel genetic measurements in massive numbers and thus are a key technology for global analysis of genome content and gene expression profiling. Although, several methods for building microarrays have been developed, two have prevailed. In one type, DNA arrays are constructed *in situ* by photolithography and solid-phase DNA synthesis. Affymetrix pioneered in this field by developing a GeneChip. In another method, DNA arrays are generated by spotting and physically attaching DNA templates to a solid substrate using robotic printing technologies. However, the challenge to date has been to overcome two major issues: increasing array element density by at least an order of magnitude, coupled with an exponential cost reduction relative to pre-existing approaches. Next challenge in the post-genomic era is obviously requires the inclusion of a key feature in microarray technology that can facilitate a direct connection between individual DNA sequence information and their protein products and thus back again at the whole-genome level.

To meet these requirements, we previously reported a self-assembling and spotter-free arraying approach based on intaglio printing to generate high-density DNA-linked protein microarrays. In this paper, we report on the inclusion of microemulsion technology, which can load millions of DNA variant molecules individually and in parallel onto a microbead carrier and then convert them into a particle to which thousands of copies of identical DNA variants are bound. Further inclusion of our original work on system for transferring and arraying single-molecule-amplified bound beads into a microreactor array chip makes this approach very simple and robust for achieving high-density DNA-linked protein microarrays [4].

## EXPERIMENTAL

Water-in-oil (w/o) emulsion-droplets were generated by our optimized protocol entailing a simple stirring process using a vortex mixer and without the aid of a magnetic stirrer bar. An aqueous solution of the PCR mixture containing magnetic microbeads (2.8 μm), extremely diluted two kinds of template cDNA (0.035 nM) and two-types of fluorescent (Cy5 and FITC)-labeled PCR-primers specific to each template cDNA was stirred into an oil-surfactant mixture to create a microemulsion with ~$10^8$ aqueous droplets per 25 μl of solution (Fig. 1B). The emulsion PCR was carried out under the following conditions: 95°C for 5 min, 70 cycles of 94°C for 30 sec, 66°C for 30 sec, 72°C for 3 min, and 72°C for 7 min. After PCR cycles, the microdroplets were broken by the addition of breaking buffer (300 mM Sodium acetate, 10% Triton X-100 in isoproponol) followed by vortexing for 10 sec. The PCR product-coated beads were washed by using magnetic separation.

For microfabrication of microcapillary electrophoresis chip, microchannels of 100 μm depth and width were engraved by deep reactive ion etching on a 20×20 mm quartz plate (Fig. 2B). Reservoir tanks were constructed using polymer rings glued directly on the reservoir holes. Electrodes were set into the reservoirs of the sample and electrophoresis for bead containing solution was performed in phosphate-buffered saline (PBS, pH 7.4) for 1 min on a microscope stage for observation. Movies of migrating PCR-amplified DNA-coated beads and/or PCR primer-coated beads were recorded using a charge-coupled device (CCD) camera. The measurement of bead electrophoretic mobility (EPM) was estimated by subtracting electroosmotic flow (EOF) mobility from electromigration.

For microfabrication of microreactor array (μRA) chip, microarrays that were 5 μm in diameter, 6 μm in depth, and with a 5 μm step size were fabricated from 0.5-mm-thick silica glass ($SiO_2$) using photolithography and dry etching methods. The actual diameters of the chambers were measured using a scanning electron microscope (XL30 ESEM-FEG, Phillips) (Fig. 3A). Separated PCR product-coated beads were then arranged and self-assembled into the microreactors at one-to-one indexing with the assistance of a magnetic field applied by sliding a permanent magnet horizontally under the chip for a few minutes. Fluorescent records were captured under a microscope (A1R/A1, Nikon) equipped with a chilled CCD camera. The intensity was recorded using a 40× or 20× objective lens and the appropriate filter sets. Analyses were performed using laboratory-generated software.

## RESULTS AND DISCUSSION

In order to demonstrate the high-density microarray concept, first an optimized conditions of stirring process (9,000 rpm speed for 30 min at 4°C) for preparing microemulsions was fixed which generated very small droplets with a mean diameter of 7 µm and managed to maintain a single target DNA molecule and a single bead in individual droplets (Fig. 1). As shown in Fig. 1 (C and C'), two of the aqueous compartments contained a green-dye coated single magnetic bead that were labeled by binding to FITC fluorescence attached primer-generated PCR products.

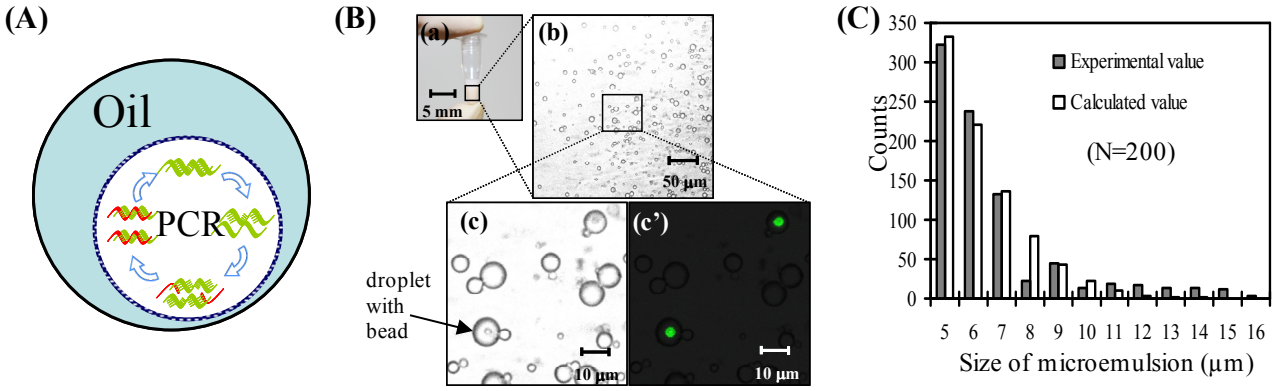


*Figure 1: Water-in-oil microemulsion PCR result. (A) Schematic representation of PCR in droplet. (B) Photographs of w/o microemulsion. Experimental microscopy bright field (b, c) and fluorescence (c') images of microemulsion. A single-bead containing microemulsion is highlighted by an arrow. (C) The heterogeneous size distribution of microemulsions is plotted with a high consistency with calculated values.*

An equal probability of no molecules during single-molecule distribution in microemulsion may negate the presumed ability of high-density arrays. Therefore, it required separating the functionalized (PCR product-bound) and non functionalized (PCR primer-bound) beads because of the efficiency of successful PCR in droplets was only nearly 3-5%. For this purpose, we microfabricated a laboratory-made microcapillary electrophoresis chip and separated DNA-bound functionalized beads from nonbound beads based on difference of migration during electrophoresis (Fig. 2B). A high distinct electrophoretic mobility (EPM) was observed for DNA-bound beads and they were separated successfully from nonbound beads (Fig. 2C).

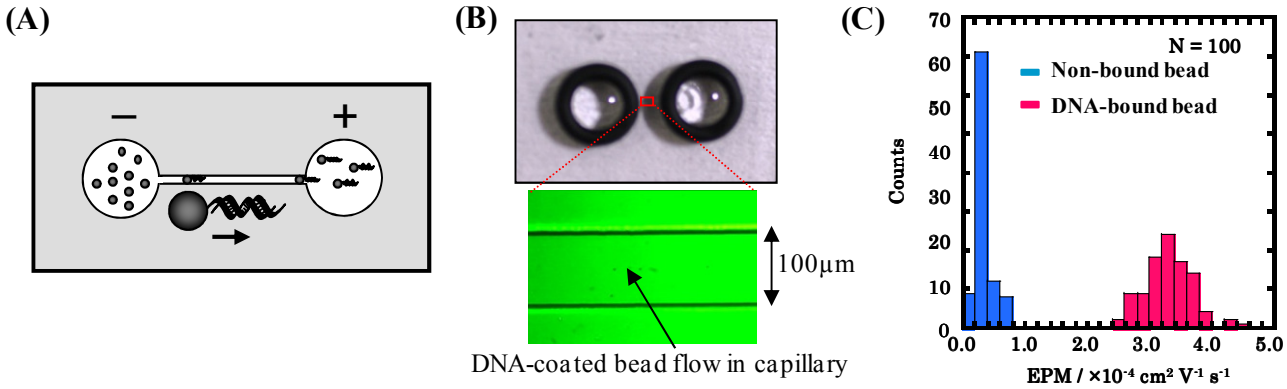


*Figure 2: On-chip separation of functionalized (PCR-product-bound) and non functionalized (primer-bound) magnetic beads using microcapillary electrophoresis. (A) Schematic representation and (B) photographs of devise. An experimental microscopy fluorescence image of a microcapillary during separation is also shown. (C) Electrophoretic mobility histograms of functionalized and nonfunctionalized beads.*

Finally, for high-density arraying, DNA-bound beads self-organized into the microchambers of the microreactor array chip. To induce arrangement, a droplet of the aqueous solution with beads was spotted onto the chip, followed by the insertion of single beads into single wells using a magnetic field applied by sliding a permanent magnet horizontally under the chip (Fig. 3A). Two oligonucleotide primers labeled with FITC and Cy5 and specific for one allele (Fig. 3B) generate two types of DNA-bound magnetic bead, which were confirmed by gel electrophoresis (Fig. 3B) and then successfully arrayed in a high-density format (Fig. 3C), demonstrating a high bead-filling ration near 99% and thus makes our concept very simple and robust for achieving high-density molecular self-assembling DNA microarrays which in turn applicable for 'decoding' genetic information into functional information (generation of protein microarray) and thus can uncover which bead type, containing a particular sequence variant, is present in each well of the microreactor array.

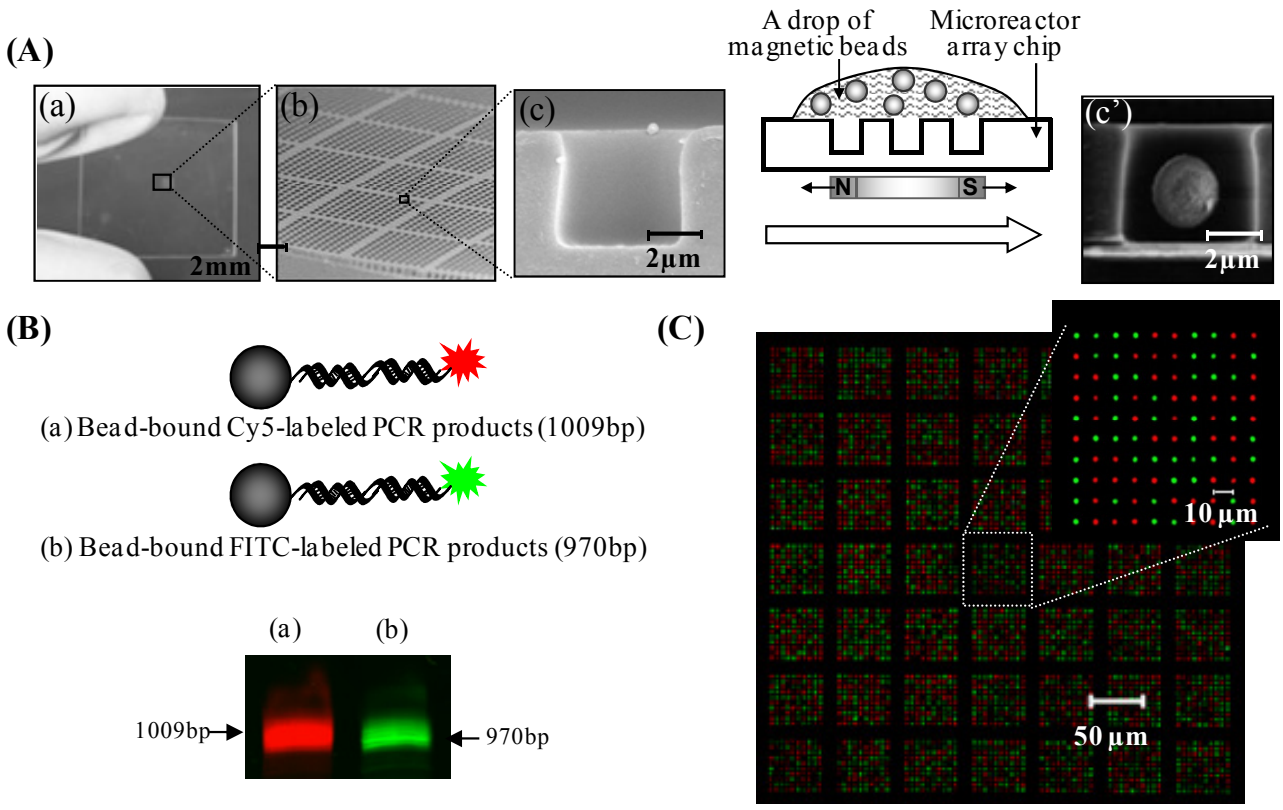


*Figure 3: Self-assembled high-density DNA microarray for conversion into protein microarray on demand. (A) Magnified photographs (a, b) and cross-sectional SEM image before (c) and after (c') filling with magnetic beads of fabricated microreactor array chip comprising 10,000 reactors/mm$^2$. A schematic of micrometer-sized paramagnetic beads filling the microreactor array chip is also shown. (B) Schematics of PCR amplified gene variant-bound microbead using microemulsion technology (top) and confirmation using polyacrylamide gel electrophoresis (bottom). (C) Experimental microscopy fluorescence images of self-assembled DNA-bound bead patterns using mixture of FITC- and Cy5-labeled genotypes.*

## CONCLUSION

A novel concept to generate versatile high-density DNA microarrays is demonstrated which can be converted to a protein microarray on demand using a simple step of cell-free protein synthesis. This feature makes this system ideal for high-throughput production of a highly diverse protein microarray for global proteome analysis.


## ACKNOWLEDGEMENTS

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