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Lab Note

Microarray partition using a recycled marker pen and neutral balsam

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Microarrays are very powerful tools for both basic research and clinical studies [1]. There are a variety of types of microarrays, e.g. DNA microarrays, protein microarrays, carbohydrate arrays, tissue microarrays [2], etc. They have been widely applied for numerous applications from gene expression monitoring [3], single nucleotide polymorphism analysis [4] to protein–protein interaction profiling [5], protein–small molecule interaction analysis [6], etc.

The most well-recognized microarrays usually carry thousands to millions of probes on a single array. To save cost, time, and labor, it is a good practice to create sub-arrays on a single standard substrate slide in a layout compatible with the standard multi-channel pipette, thus facilitating high-throughput operation. As to effectively analyze multiple samples on a targeted microarray, the sub-arrays must be physically partitioned to prevent cross-contamination.

To effectively partition sub-arrays on a single slide, several types of incubation frames have been developed, e.g. Frame-Seal™ incubation chamber (Bio-Rad, Hercules, USA), SmartGrid™ (CapitalBio Corporation, Beijing, China), and Whatman® FAST slide incubation chambers (GE Healthcare, Bethesda, USA). Usually, these frames are adhesive on one side. They could be fastened and stuck to the microarray and could be easily removed after incubation. However, these chambers are not reusable and the number and layout of the chamber is fixed. If the chamber is not properly handled, leaking will occur and thus cause cross-contamination. To increase the reusability of the chamber, non-adhesive chamber coupled with a clipping toolkit is also available, e.g. the ArraySlide chamber and ArraySlide gasket (Gel Company, San Francisco, USA). Another available partition method is to imprint pre-patterned wax using a mold by a device named SlideImprinter (Gel Company). However, this device and the accessories are expensive.

Here, we described a simple method for hydrophobic partition of flexible number of sub-arrays on a single microarray using a recycled marker pen and hydrophobic neutral balsam as ink. A recycled marker pen was cleaned by ethanol to remove all the colorful ink until the tip turned white and then air-dried. Neutral balsam was chosen as ink because it could form rigid and water-repellent barrier on glass slide when dried as shown in immunohistochemistry experiments. The neutral balsam solution was freshly prepared before partitioning by mix neutral balsam (Shanghai Yiyang Instrument Corporation, Shanghai, China) with xylene (China National Medicines Corporation LTD, Beijing, China) at a ratio of 5 : 1 (v/v). As shown in **Fig. 1**, the procedure of partitioning is as follows: (i) design and print a layout exactly the same size of the standard glass slide as guidance and stick it to the back of the microarray to be partitioned; (ii) dip the marker pen in the neutral balsam solution and draw lines according to the guidance and let it air dry. After partitioning, the microarrays could be subjected to traditional microarray fabrication and the following experiments. Results showed that up to 48 sub-arrays could be partitioned on a single slide (**Fig. 1**) and the solution in all the sub-arrays could be well separated by the water-repellent neutral balsam barrier.

To evaluate the feasibility of our partitioning method, its compatibility with the two major types of microarray, i.e. DNA microarray and protein microarray, was tested. We first constructed a DNA microarray with 24 identical sub-arrays (**Fig. 2**), which were hydrophobically partitioned by neutral balsam. DNA oligos were dissolved at a concentration of 10 μM in 50% dimethyl sulfoxide and printed on aldehyde-derivatized glass slides (CapitalBio) by a SmartArray™-48 microarrayer. A Hex-labeled oligo was spotted at the bottom of each sub-array as landmark. The microarray was incubated at 37°C in a humidity chamber overnight for probe immobilization, immersed in a newly prepared 0.3% $NaBH_4$ solution for 5 min to block the free aldehyde groups, and washed with 0.2% sodium dodecyl sulfate (SDS) for 5 min. The microarray was dried by spinning and stored desiccated in a black box at room temperature. The samples for hybridization were polymerase chain reaction (PCR)-amplified by a Cy5-conjugated universal primer and plasmids as

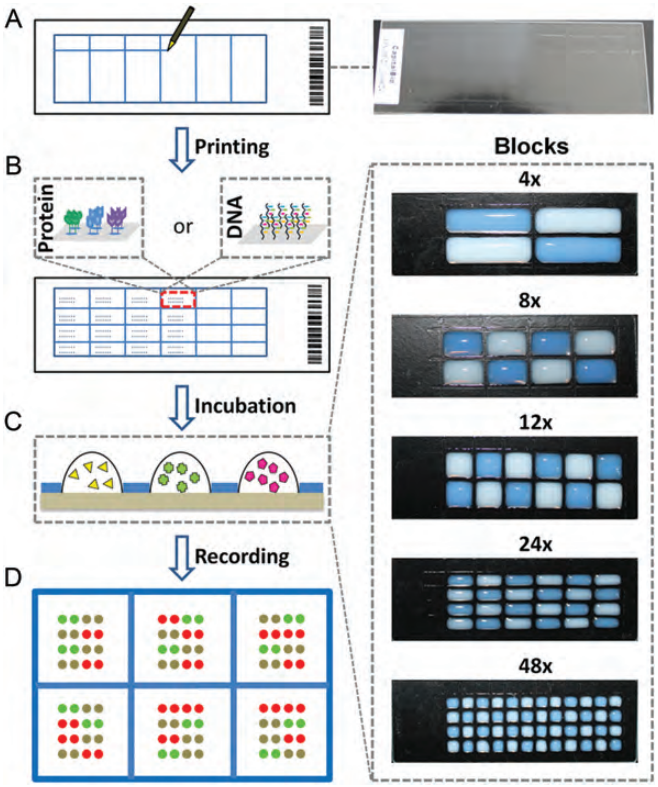

Figure 1 Hydrophobically partition sub-arrays on a microarray

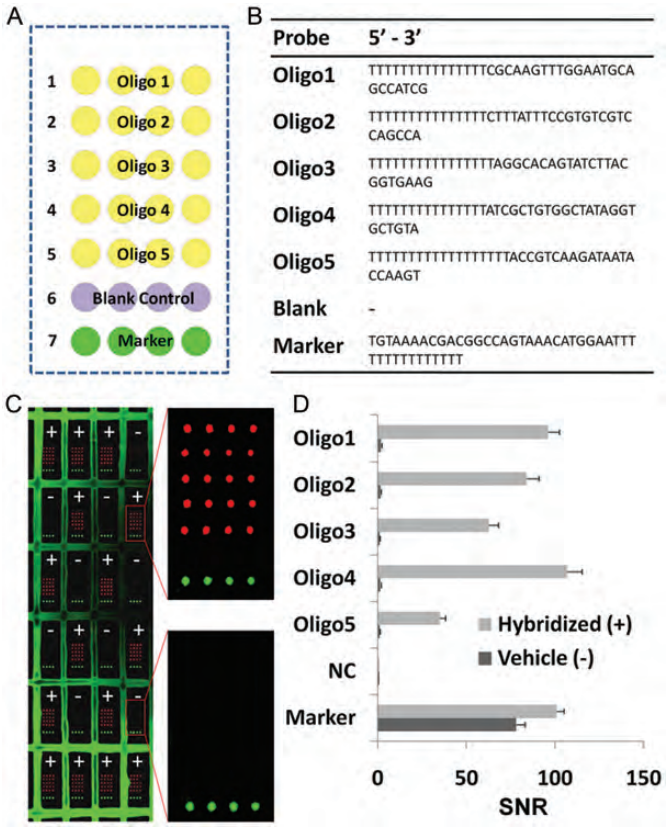

Figure 2 DNA microarray with 24 hydrophobic partitioned sub-arrays (A) The layout of the sub-arrays. (B) The sequences of the probes. (C) The overall hybridization image. (D) The SNR of the five probes tested.

templates. Ten microliters of hybridization mixture was prepared per sub-array, which contained 200 ng of each Cy5-labeled PCR products, 3× sodium chloride-sodium citrate buffer (SSC), 5× Denhardt's reagent (Sangon Biotech, Shanghai, China). The hybridization mixture was heated for 5 min at 95°C and chilled on ice immediately for 3 min. The selected sub-arrays were loaded with the prepared hybridization solution and the other sub-arrays were loaded with the hybridization solution without DNA. The hybridization was carried out at 55°C in a humidity chamber for 3 h, and then washed at 42°C by 0.2× SSC/0.2% SDS and 0.2× SSC. After spinning dry, the microarray was imaged with a GenePix™ 4200A Scanner (Molecular Devices, Sunnyvale, USA). The results showed that only the sub-arrays hybridized with the Cy5-labeled PCR products had expected signals. The signal-to-noise ratio (SNR) of the sub-arrays hybridized with Cy5-labeled PCR products was significantly higher than that of the vehicle controls [**Fig. 2C,D**]. These results indicated that our partitioning method could effectively prevent cross-contamination among the adjacent sub-arrays, and the dry neutral balsam on the microarray did not interfere with DNA hybridization.

In another example, we fabricated a protein microarray with a glutathione S-transferase (GST)-tagged protein (0.5 μg/μl) as probe, bovine serum albumin (1 μg/μl) as the negative control, and a Cy3-labled secondary antibody (1.5 μg/μl, Jackson ImmunoResearh, West Grove USA) as landmark (**Fig. 3**), all of which were prepared in 25% glycerol and then printed on a epoxy-derivatized substrate slide (CapitalBio) by a SmartArray™-48 microarrayer. The microarray was incubated at 4°C overnight to ensure a maximum protein coupling on the microarray surface and stored under −20°C. The protein microarray was blocked with 1× phosphate-buffered saline with 0.05% Tween 20 (TBST) for 1 h at room temperature before incubation. Selected sub-arrays were loaded with an anti-GST antibody (Novagen, Gibbstown, USA. 0.25 μg/ml, diluted in 1× TBST) and the rest were loaded with an equal volume of 1× TBST; the binding was performed for 1 h at room temperature. The microarray was washed with 1× TBST for three times, 10 min each. A Cy3-conjugated secondary antibody (1.5 μg/ml, diluted in 1× TBST) was incubated on the whole microarray for 1 h at room temperature. The microarray was washed with 1× TBST for three times, 10 min each. After spinning dry, the microarray was imaged with a GenePix™ 4200A Scanner. The results showed that only the sub-arrays incubated with the Cy3-conjugated second antibody had expected signals. The SNR of the sub-arrays incubated with Cy3-conjugated PCR products was significantly higher than that of the controls [**Fig. 3C,D**]. These

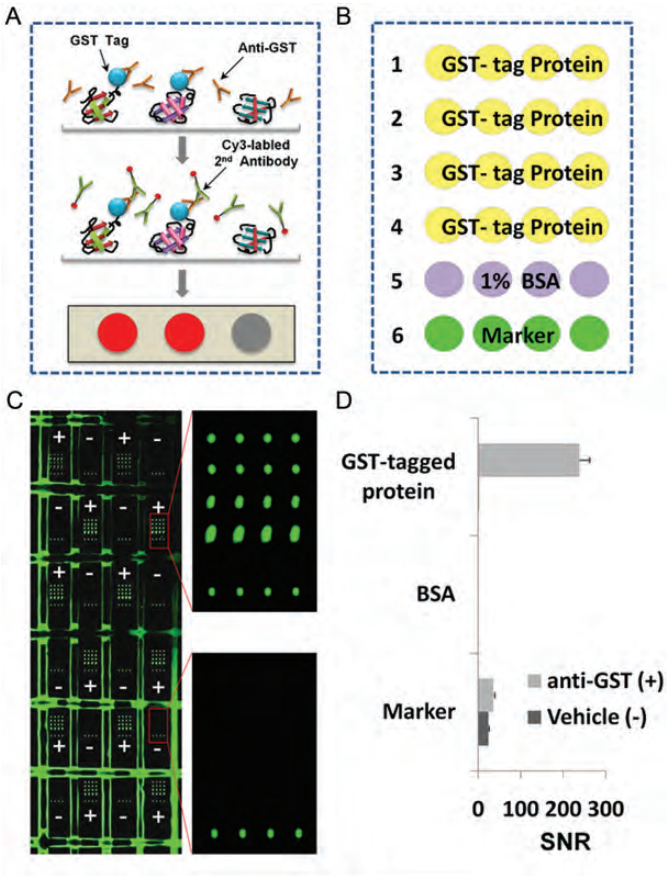

Figure 3 Protein microarray with 24 hydrophobic partitioned sub-arrays (A) The schematic of the experiment. (B) The layout of the sub-arrays. (C) The overall protein microarray image. (D) The SNR of the GST-tagged protein tested.

results indicated that our partitioning method could prevent cross-contamination among the adjacent sub-arrays, and the dry neutral balsam on the microarray did not interfere with protein–antibody interaction.

Taken together, we have developed a simple method for hydrophobic partitioning of sub-arrays on a standard microarray, using a recycled marker pen and neutral balsam as ink. The flexibility of this method lies in its ability of creating up to <48 sub-arrays, which could be, theoretically, any shape and layout, on a single microarray. DNA microarray and protein microarray experiments showed that our partitioning method is compatible with both these types of microarray experiments; the water-repellent barrier could effectively prevent cross-contamination from adjacent sub-arrays. Our method is not limited to DNA microarray and protein microarray; it could be generally useful for most of the microarrays whenever physical partitioning is required.

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