# A Simple and Robust Method for Preparation of cDNA Nylon Microarrays

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## Abstract

DNA array technology has made remarkable progress in recent years and has become an indispensable tool in molecular biology research. However, preparing high-quality custom-made DNA arrays at a reasonable cost is still an important concern because we cannot abandon the use of DNA array systems designed for specific purposes. To address these problems, we here report the use of rolling circle amplification products of cDNA plasmids dissolved in 80% formamide as DNA probes immobilized on a nylon membrane. First, because formamide is practically non-volatile under ambient conditions and nucleic acids are easily dissolved in it, the use of formamide as a DNA solvent ensures that the DNA concentration of the solution will not change during arraying, which often takes several hours to a day depending on the number of DNA spots and arrays to produce. Secondly, the use of rolling circle amplification technology greatly reduced the labor needed to prepare the spotted DNA. The results in this study demonstrate that the introduction of these two modifications in preparation of nylon DNA array greatly improved its quality.

**Key words:** microarray; cDNA; nylon membrane; formamide; rolling circle amplification

DNA array technology[1,2] has made remarkable progress in recent years and the usefulness of the technology is now widely accepted.[3–5] Although various types of DNA arrays have become commercially available, the high cost is still concern. This concern becomes more serious as the density and the number of probes on DNA arrays increases. Even though the repetition of the experiments is quite critical for obtaining statistically significant data, the high cost of commercial DNA microarrays sometimes restricts the number of the experimental runs. To address this practical problem in DNA microarray experiments, Shena et al. developed a user-friendly DNA array preparation system based on mechanical spotting.[6] If the user can afford a set of DNAs to be spotted, this system makes it possible to prepare microarrays at a reasonable cost. In this approach, however, the variation in microarray quality is a serious drawback since the quality of microarrays produced by mechanical spotting varies considerably depending on various ambient parameters such as humidity and temperature. Although many ef-

forts have been made to resolve this problem, the best method of preparing a high-quality microarray in a robust manner is still a matter of concern.

In this context, we intended to generate a robust and cost-effective DNA array system using cDNA clones. Because Kazusa DNA Research Institute is widely known as a mammalian cDNA clone resource,[7–14] we decided to use these cDNA plasmids for microarray production. For immobilization of cDNA plasmids on a solid phase, nylon membrane is more suitable than other solid supports, such as glass and plastic, due to its high binding capacity. Spotted DNAs are spontaneously captured on nylon membranes and can be irreversibly bound onto it by UV irradiation.[15] In combination with a radioisotopic detection method, nylon DNA microarrays have been successfully used for highly sensitive gene expression profiling.[16–18] Furthermore, the recent emergence of nylon membranes with low fluorescent background might open the way to use the same platform also in a non-radioisotopic detection system.[19] Another advantage of the nylon microarray system over other solid-phase supports is its simplicity to implement because molecular biologists are quite familiar with hybridization experiments using nylon membranes with conventional RNA/DNA

blotting hybridization. Thus, we consider the value of the nylon microarray system has not diminished at all although it sounds rather old-fashioned at present.

## 1. Search for Solvent Most Suitable for DNA Spotting onto Nylon Membranes

The preparation of nylon microarrays is conventionally done by a mechanical spotting method using a commercially available robot. We considered that one of the most serious practical problems of this system was evaporation of the DNA solvent during spotting, which causes variation in the amounts of DNA delivered. Thus, we first searched for solvents most suitable for DNA spotting based on vaporization properties. To compare evaporation levels of various solvents commonly used for arraying, we prepared 8 kinds of solvents and left them in a 384-well PCR reaction plate (ThermoFast384 plate from AB gene, Epsom, UK) in three volumes (5 $\mu l$, 10 $\mu l$ and 20 $\mu l$, ten wells each) at room temperature for 16 hr. The residual volumes of solvents were then estimated (Fig. 1). The results clearly showed that the volume of 80% formamide solution (sample g) did not change at all even after we left aliquots of DNA solution in an open plate overnight under ambient conditions. Interestingly, absolute formamide absorbed ambient moisture vapor and thus its volume increased during this experiment (sample h). Since changes in the volume of DNA solution inevitably affects the amount of DNA delivered onto nylon membrane by mechanical spotting, 80% formamide appears to be the best solvent for the preparation of nylon DNA arrays, at least in this regard.

We next compared the immobilization efficiencies of DNAs dissolved in 80% formamide and 20 mM EDTA; 0.1 N NaOH[17,18,20] and 20 mM EDTA; or 60% glycerol and 20 mM EDTA. A phagemid pBluescript II SK(+) (Stratagene, La Jolla, CA, USA) was prepared by High Purity Plasmid Midiprep System (Marligen Biosciences, Inc., Ijamsville, MD, USA) and used for this test. This vector plasmid DNA was dissolved at a concentration of 0.1 $\mu g/\mu l$ of three kinds of solvents (with 0.05% xylene cyanol for visualization of the spots) and spotted onto a positively charged nylon membrane (Biodyne B, from Pall Corp., East Hills, NY, USA) stuck on a slide glass using a GeneTac RA1 microarrayer (Genomic Solutions Inc., Ann Arbor, MI, USA) by using solid pins (200 $\mu m$ in diameter) with a spot spacing of 500 $\mu m$. After spotting, the nylon membrane was dried *in vacuo* at 80°C, and then UV-irradiated after wetting the membranes by soaking them in distilled water. The membrane array prepared in this way was hybridized with the ^{33}P-labeld vector plasmid DNA (Fig. 1B). The spot intensity in 80% formamide was the highest and most robust (coefficient of variation is lowest) among the three solvents. The hybridization signal at the center of the spot in 80% formamide was on average twofold stronger than the others
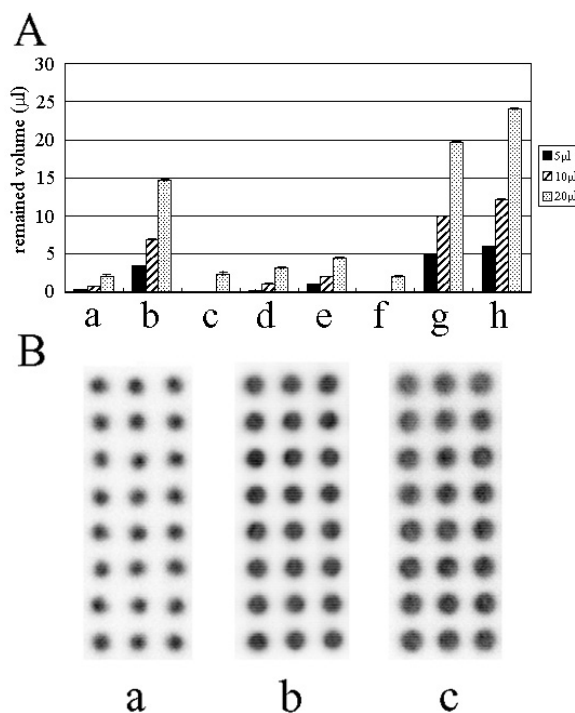


**Figure 1**. Selection of the most suitable solvent for DNA spotting on nylon membrane. **A. Comparison of evaporation of various solvents commonly used for arraying.** Ten each of these solvents were placed in a 384-well PCR reaction plate overnight. After 16 hr, residual volumes were estimated by pipetting the solvents using an autopipetter and averaged volumes of each solvent with error bars are shown in a bar chart. The overnight air temperature varied between 21.3°C and 24.6°C and humidity between 30% and 42%. **a**, 1 × TE (10 mM Tris HCl/1 mM EDTA, ethylenediamine-N,N,N′,N′-tetraacetic acid)/4% glycerol; **b**, 50% dimethyl sulfoxide/0.5 × TE; **c**, 0.4 N NaOH/0.15 M NaCl; **d**, 0.4 N NaOH/0.15 M NaCl/4% glycerol; **e**, 0.4 N NaOH/0.15 M NaCl/10% glycerol; **f**, 3 × SSC (standard saline citrate, 1 × SSC = 0.15 M NaCl/0.015 M sodium citrate); **g**, 80% formamide/20 mM EDTA; **h**, 100% formamide. **B. Comparison of immobilization efficiencies using three different kinds of solvents.** The pBluescript II SK(+) was labeled with $[\alpha^{33}$-P]dCTP by using a RadPrime DNA Labeling System (Invitrogen Corp., Carlsbad, CA, USA), and used for hybridization after removing free nucleotides with a spin column (ProbeQuant G-50 Micro Columns, Amersham Biosciences Corp.). Hybridization was performed at 68°C overnight in 2 ml of PerfectHyb solution (TOYOBO Co., Ltd., Osaka, Japan) with this ^{33}P-labeled vector plasmid DNA in a small bag ($10^4$ cpm/$\mu l$). After hybridization, the membrane was washed twice in 2 × SSC containing 1% sodium dodecyl sulfate (SDS) at 68°C for 15 min, and then twice in 0.1 × SSC containing 1% SDS at 68°C for 30 min. This washing condition was applied to all of the nylon microarray hybridization experiments in this paper. After washing, the membrane was completely dried *in vacuo* and exposed to Imaging Plate BAS-SR0813 (Fuji Photo Film Co., Ltd., Tokyo, Japan) for 2 hr. Hybridization signals were digitized using a microarray scanner (FLA-8000, Fuji Photo Film Co., Ltd.) with 20-$\mu m$ resolution, and quantified using an array data analyzing software, ArrayGauge and image analyzing software, ImageGauge (Fuji Photo Film Co., Ltd.). **a**, 80% formamide/20 mM EDTA; **b**, 0.1 M NaOH/20 mM EDTA; **c**, 60% glycerol/20 mM EDTA. Quantification of the signal intensities is shown in Table 1.

**Table 1.** Comparison of hybridization signals of DNAs spotted with aqueous NaOH solution, 60% glycerol, and 80% formamide.

| Spotting solution | Highest signal intensity (PSL/pixel)[1] | Radius of spot signal (μm)[1] | Integrated signal intensity (PSL)[1] |
|---|---|---|---|
| 0.1 M NaOH | 0.061 [0.022] | 280 [19] | 83 [26] |
| 60% Glycerol | 0.058 [0.015] | 350 [29] | 119 [26] |
| 80% Formamide | 0.129 [0.032] | 233 [16] | 126 [29] |

1) The averages derived from 50 spots (5 spots from 5 pins × 2 sets) are shown. The numbers in square brackets indicate standard deviations.
The signal intensities are expressed in PSL unit as described in the FLA-8000 instruction manual.

(Table 1). This means that using 80% formamide as a solvent improves the sensitivity of nylon membrane arrays.

Moreover, the 80% formamide solvent had an advantage over other solvents besides non-volatility and reinforcement of signal intensity; the resultant spot size of DNA in 80% formamide on nylon membrane was smaller than those in either 0.1 M NaOH or 60% glycerol (Table 1). When spotter pins with a diameter of 200 μm were used, the 80% formamide solution gave hybridization signal spots whose diameter was 233 μm on average whereas the 0.1 M NaOH solution and the 60% glycerol solution gave hybridized spots of 280 μm and 350 μm, respectively. The compactness of DNA spots using the 80% formamide solution would make it possible to prepare nylon DNA microarrays with a higher density than before. When we arrayed cDNA plasmids dissolved in 0.1 M NaOH, the distance between spots is routinely kept to at least 500 μm to avoid overlapping of hybridization signals of adjacent spots. According to our preliminary experiment, the formamide system would enable us to increase the spot density of nylon microarray by reducing the spacing to 450 μm when using ^{33}P or ^{35}S for target labeling. This allowed us to spot 4800 probes within an area of 2 cm × 5.5 cm.

Taking all these results into consideration, we concluded that 80% formamide had superior properties as a spotting solution to other conventional solvents. In practice, we decided to employ 80% aqueous formamide containing 20 mM EDTA/0.05% xylene cyanol as a DNA solvent for spotting onto nylon membranes.

## 2. Nylon Microarrays Using TempliPhi-Amplified DNA as a Probe

Variation in concentrations of cDNA plasmids to be arrayed is another concern because cDNA plasmid yields vary widely among cDNA despite the labor-intensiveness of the plasmid preparation. Since hybridization signals sensitively reflect the amounts of cDNA plasmids immobilized on a solid support, it was obviously preferable to keep the concentrations of DNA probes as constant as possible. In addition to yield variation, since our cDNA plasmids were routinely prepared from small-scale bacterial culture of recombinant clones in a robotic system,

the total amounts of cDNA plasmid thus recovered were relatively small. Thus, we needed to devise a simple and robust method to prepare thousands of cDNA plasmids at constant concentrations and in sufficient quantities for arraying. To achieve this, we applied an enzymatic amplification of these cDNA plasmids; although polymerase chain reaction (PCR) was frequently used for the preparation of cDNAs to be spotted, we used a bacteriophage φ29 DNA polymerase-based rolling circle amplification (RCA) system[21–23] in this study termed "TempliPhi reaction" by the supplier. We considered the TempliPhi reaction to be more suitable for the preparation of cDNA plasmids than PCR for the following reasons: (1) although the yields of PCR products considerably depend on the size and the sequence of cDNA inserts, the TempliPhi reaction is less sensitive to these parameters than PCR[23–26]; (2) since the TempliPhi reaction is an isothermal amplification method, tens of thousands of the amplification reactions could be performed in parallel without any special instruments like the thermal cycler needed for PCR; (3) since the TempliPhi reaction continues until enzymes and substrates for DNA polymerization are consumed, the final concentration of the TempliPhi products is nearly constant in terms of weight, regardless of the size and the sequence of cDNA plasmids, at the saturation level only if the initial amounts of cDNA plasmids subjected to the TempliPhi reaction are large enough to reach the saturation level.[23,25–27]

Because the PCR amplification efficiency is known to depend on the size of the target region, optimization of the reaction conditions is required for each clone. Thus, a size-independent amplification method like the TempliPhi reaction is extremely desirable for our purpose because cDNA clones maintained at the Kazusa DNA Research Institute are enormously long inserts, which sometimes causes inefficient PCR amplification when using a universal vector primer set for a particular clone. It must be noted that the TempliPhi reaction also amplified the vector region in the cDNA plasmids. This did not cause any serious problems since the DNA-binding capacity of the nylon membrane was high enough to retain all the spotted TempliPhi reaction products and since the background hybridization signal originating from the vector portion was essentially negligible. In addition, we found that, at least when using a nylon membrane as a

support, the purification step of the TempliPhi reaction products can be omitted. When using a glass support, redundant primers inhibit the binding of the PCR products or TempliPhi products and sufficient polynucleotide for probe DNA is not retained. Because nylon support has a high capacity to bind sufficient polynucleotides for probe, redundant primers are not a problem. The probable background noise originating from reaction components is also not a concern, as shown in Fig. 2. In some cases, PCR produces by-products which may cause undesirable background noise; this is another reason why purification of PCR products is needed. With the TempliPhi reaction, by-products are negligible only if sufficient template DNA exists as a circular form.

To assess the TempliPhi product probe, 94 kinds of PCR and TempliPhi products from human cDNA plasmids (gene names used here are available upon request) were arrayed on the same nylon membrane with two control DNAs, pBluescript II SK(+) and $\lambda$-fragment in pSP64 poly(A), and hybridized with ^{35}S-labeled human brain mRNA.[16] The triplicate membranes were hybridized as described in the next section. Reliability of obtained signals was assessed by ArrayStat (Imaging Research Inc., St. Catharines, Ontario, Canada), and corrected signals from two kinds of probes were compared after subtraction of background noise and median-normalization. Signal intensity of these two probe sets was comparable and showed good linear relation; the correlation coefficient between two probe sets was 0.955.

These results indicate that TempliPhi products are available as microarray probes with sufficient quality, at least as much as PCR product. It is not easy to obtain sufficient quantity of PCR products. In fact, although the amplification reaction was performed with the extension time of 5 min here, two genes were not amplified at all and three genes were inefficiently amplified, whereas all of the clones were correctly amplified by the TempliPhi reaction. Taking the simplicity of reaction devices into account, we concluded that TempliPhi products are superior to PCR products as DNA array probes.

## 3. An Appropriate Hybridization System for Nylon Microarray with Trace of Hybridization Solution

When using mRNA as a target, 200 ng of mRNA per nylon microarray (75 mm $\times$ 25 mm) is routinely labeled with [$\alpha^{35}$-S]dCTP ($>$37 TBq/mmol from Amersham Biosciences Corp., code#SJ1305) or with [$\alpha^{33}$-P]dCTP ($>$92.5 TBq/mmol from Amersham Biosciences Corp., code#AH9905) by reverse transcription primed by oligo-dT as described.[16] After purification using a QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany), labeled target mixture was hybridized with a nylon microarray in PerfectHyb Hybridization Solution (TOYOBO Co., Ltd., Osaka, Japan), whose volume was
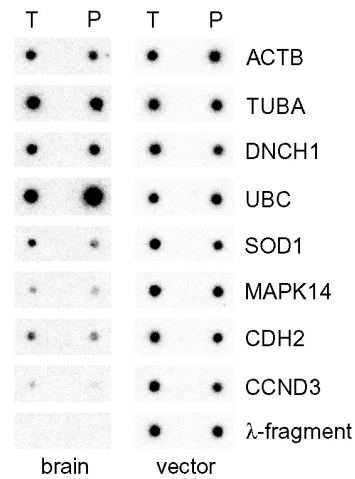


**Figure 2**. Evaluation of TempliPhi products as probes for nylon microarray. Human cDNA plasmids were subjected to DNA amplification with a TempliPhi DNA amplification kit (Amersham Biosciences Corp., Piscataway, NJ, USA). TempliPhi reaction was performed in a 384-well PCR reaction plate (twintech PCR plate 384, Eppendorf AG, Hamburg, Germany) following the supplier's instructions with slight modifications. For reaction templates, 5 ng of cDNAs were used as template in 10 $\mu$l of reaction volume. Amplification reaction was performed at 34°C overnight. After heat inactivation of enzymes, the reaction mixture was dried, and the residue was dissolved in 5 $\mu$l of 80% formamide/20 mM EDTA/0.05% xylene cyanol, and spotted after heat denaturation (at 95°C for 5 min). PCR was performed using TaKaRa LA Taq (Takara Bio Inc., Ohtsu, Shiga, Japan) under the following reaction conditions: template, 10 ng; reaction volume, 50 $\mu$l; PCR primers, 1 pmole/$\mu$l of M13 universal forward and reverse primers (for $\lambda$-fragment, an SP6 primer and a specific primer whose sequence is AACAGCTATGACATGAATAC were used); thermal cycling conditions, denature for 30 sec, annealing for 30 sec and extension for 5 min, 25 cycles. After confirming PCR products by agarose gel electrophoresis, 40 $\mu$l of reaction mixture was dried and the residue was dissolved in 5 $\mu$l of the aqueous formamide solution described above, and spotted after heat denaturation (at 95°C for 5 min). In this experiment, we used plasmid DNAs of internal and external control genes for microarray probes. ACTB, beta-actin; TUBA, alpha-tubulin; DNCH1, cytoplasmic dynein heavy chain; UBC, ubiquitin C; SOD1, Cu/Zn superoxide dismutase; MAPK14, mitogen-activated protein kinase 14; CDH2, N-cadherin; CCND3, cyclin D3; $\lambda$-fragment, 1648-bp fragment from $\lambda$ phage (nucleotide from 23,130 to 24,777, acc.#02459) cloned into pSP64 poly(A) vector (Promega Corp., Madison, WI, USA). T, TempliPhi products; P, PCR products. The membrane was hybridized with ^{35}S-labeled human brain mRNA or vector DNA (pBluescript II SK(+)). After washing and drying, the hybridized membrane was exposed to Imaging Plate for a week (mRNA) or overnight (vector DNA). As clearly shown, no hybridization signal was detected from either the TempliPhi or PCR products of $\lambda$-fragment despite of the presence of sufficient DNA shown by vector hybridization, indicating signal intensity is not affected by reaction components. Notice that PCR fragments hybridize with vector DNA because they were amplified by a pair of universal primers and contain the sequences derived from the vector. The signal intensity from the PCR products is nearly equal to that from TempliPhi products. This means the immobilized quantity of the PCR products was larger than that of the TempliPhi products.

200–500 $\mu$l (typically 2.5–5.0 $\times$ $10^4$ cpm/$\mu$l), at 68°C overnight. In the case of using $^{35}$S-labeled target mixture, dithiothreitol was added to the hybridization solution at a final concentration of 40 mM in order to reduce the background noise of the nylon membrane.

At this stage, an appropriate hybridization protocol for trace of hybridization solution was desired. We took advantage of conventional rolling bottle-type hybridizers to down-size the hybridization platform without sacrificing simplicity, because stirring of such a small volume of hybridization solution is difficult in a typical hybridization bag or in hybridizers for slide glass microarray. To achieve uniform hybridization in a small volume of probe solution, we devised some inexpensive tools. Hybridization was performed in a glass vial with a screw cap, the size of which fitted to that of the nylon microarray. On hybridization, a glass rod, the length of which was slightly smaller than the depth of the glass vial, was put into the vial to arrest the nylon microarray. This rod worked as a spreader of hybridization solution over the nylon microarray; hybridization solution was trapped between the glass rod and the nylon membrane by capillary action and was evenly spread over the nylon microarray when the vial was rotated. Multiple capped glass vials including glass rod, nylon microarray, and hybridization solution could be put into a single glass hybridization bottle for a conventional rotatory hybridizer. Except for the use of small hybridization vials, hybridization protocols were essentially the same as conventional membrane hybridization. This setup enabled us to hybridize many cDNA microarrays simultaneously using a small volume of hybridization solution (as little as 200 $\mu$l) in a robust and economical way.

## 4. Nylon Microarray Derived from Robotically Prepared cDNA Plasmids

For practical use, robotically prepared cDNA plasmid solutions should be directly used for template of amplification without precisely measuring DNA concentration. Since we constructed several kinds of mouse cDNA libraries as previously described[28] and hundreds of thousands of cDNA plasmids have been prepared by an automated plasmid extraction robot MFX9600 (TOYOBO Co., Ltd.), we thus directly applied the TempliPhi reaction to these cDNA plasmids and evaluated the nylon microarray using the reaction products as probes. Direct comparison of cDNA microarrays harboring plasmids and the TempliPhi products are shown in Fig. 3A. The TempliPhi probes evidently gave much stronger hybridization signals than the plasmid probe. Histograms of quantitative signal intensities are illustrated in Fig. 3B. When we used the TempliPhi products in place of plasmids as probes, the peak of the hybridization signal strength distribution was 2–3 times larger than that observed using the plasmid probe, indicating that their distribution was still broad. This is probably because the length of the cDNA inserts varies from clone to clone and the ratios of vector to total weight distribute within some range; however, we cannot abandon the possibility that the saturation of amplification was not achieved because of the small initial amounts of cDNA plasmids subjected to the TempliPhi reaction. In any case, the TempliPhi reaction enabled us to prepare higher concentrations of probes with much less labor than preparation of cDNA plasmids using a small quantity of cDNA plasmids (several nanograms). Because we could reamplify the TempliPhi products effectively, this enzymatic amplification method was considered to be most suitable for the preparation of probes for DNA array analysis, especially when tens of thousands of different probes must be prepared in parallel.

To further compare the performances of cDNA plasmid- and the TempliPhi product-based nylon microarrays, we carried out microarray experiments using $^{35}$S-labeled reverse-transcribed products of total RNAs derived from mouse J774.1 cells as a target at two concentrations. In this experiment, the microarray harbored 3161 probes of mouse cDNA and the list of their annotations is available upon request. As shown in Fig. 3C, when the concentration of the labeled targets reduced to half, the signal intensities also reduced to approximately half in both probes. However, when more closely examined, we found that the linearity of the signal response from plasmid probes was poorer than that from the TempliPhi product probes. Correlation coefficients of two conditions in logarithmic space were 0.94 for TempliPhi product probe and 0.84 for plasmid probe. In addition, ratios of unreliable signals compared to membrane background were much different. Among the total 3161 probe spots, 1150 in plasmid probes and 167 in the TempliPhi-product probes had signal intensity below the average of background noise plus 1.96 $\times$ standard deviation (5% significance by two-sided test, shown by arrows in Fig. 3C). Namely, in the TempliPhi-product probe set, about 95% of probe spots gave sufficient signal to be distinguished from background noise, while more than one-third of the target spots were buried in background fluctuation in the plasmid probe set. Even after these buried signals were removed from the calculation, the correlation coefficients in logarithmic space were 0.95 for the TempliPhi product probe set and 0.90 for the plasmid probe set. These results confirmed that the TempliPhi product probes gave much higher hybridization signals than plasmid probes, as anticipated from the results of the hybridization of the same DNA array with the labeled vector fragments.

## 5. Conclusion

The new protocol for preparation of nylon cDNA microarray — use of 80% formamide and preparation of the DNA probe by rolling circle amplification — was de-
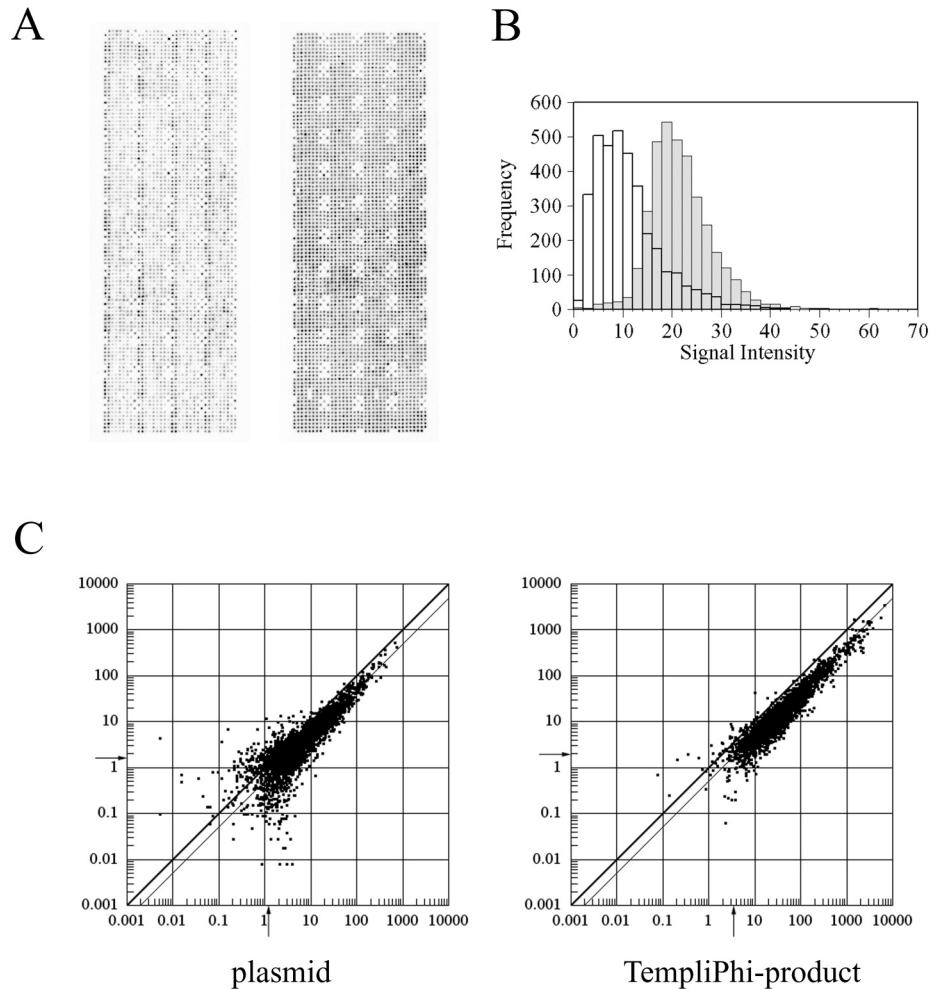
A



B



C



plasmid                                    TempliPhi-product

**Figure 3**. Evaluation of microarray derived from robotically prepared plasmid cDNAs. **A. Effect of DNA amplification system.** Using a mouse cDNA set, nylon microarrays were prepared in two probe types: robotically prepared plasmids (left) and TempliPhi products (right). Ten microliters of cDNA plasmid solution from MFX9600 (containing typically 200–500 ng of plasmid) were evaporated and re-dissolved in 5 $\mu$l of 80% formamide solution as described in the text. For reaction templates of TempliPhi amplification, 10 $\mu$l of 1/50-diluted cDNA plasmid solution from MFX9600 (typically 4–10 ng of cDNA plasmid) was dried and used as template in 10 $\mu$l of reaction volume. Amplification reaction was performed at 34°C overnight. After heat inactivation of enzymes, the reaction mixture was dried and re-dissolved in 5 $\mu$l of 80% formamide solution. Arraying was performed as described in the text. The resultant nylon microarrays were stored in a desiccator at room temperature until use. The nylon membrane prepared in this way was examined by vector hybridization using ^{35}S-labeled pBluescript II SK(+) DNA. Signals from TempliPhi-products are apparently clearer and stronger than those from robotically prepared plasmids. **B. Statistics of spot signals.** Signal intensities of vector hybridization shown in panel A were quantified and are shown by histogram. Intensity is presented as PSL units. Open bars, signals from robotically prepared plasmid probes; filled bars, signals from TempliPhi product probes. Intensity distribution was improved by TempliPhi amplification of probe cDNA plasmids. Notice that distribution of the signal intensity from TempliPhi-products still has a width caused by the variation of the ratio of vector/insert length. **C. Mouse cDNA nylon microarrays were hybridized by ^{35}S-labeled authentic mouse mRNAs at two concentration levels.** Total RNA from mouse macrophage cell culture strain J774.1 was prepared using TRIzol Reagent (Invitrogen Corp.). Twenty micrograms of the mouse total RNA was labeled with [$\alpha$[35]-S]dCTP by reverse transcription primed by oligo-dT, purified, and used for hybridization. A result from plasmid probes is plotted in logarithmic scale of PSL unit in the left panel, and a result from TempliPhi product probe is plotted in the right panel. In both panels, the horizontal axis represents the result at a half input of labeled mRNAs compared to the vertical axis. Thus, if the nylon microarrays have linear response on input mRNAs, each plotted point ranges on a line of y = 1/2 x, shown in each panel by the thin oblique line. Thick oblique lines indicate y = x. Arrows indicate the level of background noise (average plus 1.96 × SD). Spot signals below these arrows are discarded. Among 3161 spots, 1150 spots were discarded in the microarray with plasmid probes and 167 with TempliPhi product probes.

veloped. This protocol greatly improved the quality of nylon DNA arrays as demonstrated in this study. More importantly, this new protocol was even simpler than conventional ones. Although the hybridization signals were radioisotopically detected in this study, it might be possible in the future to detect them by a non-isotopic method without loss of sensitivity.[19]

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