Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 315 (2004) 30–37
BBRC
www.elsevier.com/locate/ybbrc

Dextran sulfate provides a quantitative and quick microarray hybridization reaction

Wei-Chi Ku, Wai Kwan Lau, Yu-Tien Tseng, Chi-Meng Tzeng, and Sung-Kay Chiu*

Department of Research and Development, U-Vision Biotech, Inc., Taipei, Taiwan, ROC

Received 5 November 2003

Abstract

Microarray technology is a powerful tool to speed up genomics study, yet many technical aspects need to be improved. The hybridization reaction of microarray experiments is carried out for 16 h or overnight in order to obtain reasonably strong signals for analysis in the presence of high salt buffer, like SSC. However, the quantitative aspect of microarray hybridization has seldom been investigated. In this study, we showed that higher overall signals from hybridization were achieved in a buffer system containing dextran sulfate, which can accelerate the kinetics of reaction by increasing the local concentration of the reactants. The dextran sulfate containing hybridization solution increases the reaction 4-fold (median) for cDNA microarray and 29-fold for oligonucleotide microarray. More importantly, the solution also provides a quantitative hybridization reaction, where the hybridization signals are proportional to the abundance of transcript added. The enhancement in the kinetics of hybridization is due to both dextran sulfate and formamide present in the solution, but the effect is not due to the higher temperature used during the reaction. With a slightly longer reaction time the hybridization reaction with the solution allows the detection of hybridization signals from rare transcripts that is not possible with regular hybridization buffers. With appropriate washing, the enhancement of kinetics by the solution does not increase the background signals at all, allowing higher signal-to-noise ratios to be achieved.

Keywords: Hybridization solution; DNA microarray; Quantitative changes; Acceleration; Dextran sulfate; Temperature

DNA microarray is a very powerful technology that allows parallel analysis of gene expression of hundreds to thousands of genes from cells or tissues treated differentially [1–3]. In general, DNA microarrays are made by spotting PCR products or pre-synthesized oligonucleotides on various derivatized glass surfaces or nylon membranes. Fluorescent labeled cDNAs prepared by reverse transcription from mRNA are used to hybridize to the immobilized DNA on the microarray. The signals of hybridization from two or more differentially treated tissues are quantitated by a laser confocal scanner. The genes that are overexpressed or repressed are sorted out and grouped by various clustering methods [1,4]. DNA microarray has widely been used in biological and medical researches, and the hybridization reaction is usually carried out overnight at the researcher's convenience. However, many technical improvements are needed, especially in the quantitative aspect and the speed of hybridization.

Hybridization is usually performed in one of the two popular buffer systems—5× SSC, 0.1% SDS, and 25% formamide (Buffer 1) at 42 °C or 3× SSC, 0.2% SDS (Buffer 2) at 65 °C. The time required for obtaining sufficiently high level of signals for analysis is usually 8–16 h—a time factor that limits the productivity for high throughput format studies. Many efforts have been made to reduce the hybridization time [5], but the high cost and demands for specific machines, however, are not applicable to many laboratories. Moreover, the intensities of the fluorescent signal from hybridized spots are generally assumed to be corresponding to the abundance of mRNA. However, inconsistency between the microarray data and the results of Northern blot raises doubts on the quantitative aspect of microarray data [6].

It has been known that dextran sulfate and polyethylene glycol, as molecular crowding reagents, can

* Corresponding author. Fax: +886-2-8912-1016.
E-mail address: david@u-vision-biotech.com (S.-K. Chiu).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.01.013

increase the local concentration of the interacting molecules [7,8]. In this study, we demonstrated that a hybridization solution containing dextran sulfate and formamide can increase the speed of hybridization, and it also provides a quantitative hybridization reaction for both cDNA and oligonucleotide microarrays. The accelerated hybridization kinetics shortens the time to 1 or 2 h without increase in background signals at all. From the result of this study, we believe that the dextran sulfate and formamide containing hybridization solution is therefore a better choice for microarray experiments.

Materials and methods

Chemicals and materials. Formamide and sodium dodecyl sulfate (SDS) were purchased from Calbiochem (Darmstadt, Germany). Dextran sulfate was from Sigma–Aldrich (St. Louis, MO, USA), and 20× SSC buffer (sodium chloride and sodium citrate) was from Amresco (Solon, Ohio, USA). As much as 6× SSPE buffer contains 0.9 M sodium chloride, 60 mM sodium dihydrogen phosphate, and 6 mM ethylenediamine tetra acetic acid (EDTA).

Microarray production. Eureka Human Glass cDNA Microarray and Adipose cDNA Chip were manufactured by U-Vision Biotech, Taiwan. In brief, Eureka Human Glass cDNA Microarrays (1536 genes in triplcate and 4608 spots total) and Human Adipose cDNA Chip (350 genes in triplicate and 1050 spots total) were prepared from purified PCR products. In some of the experiments in the study (in Figs. 2 and 5), different subsets (172 and 15 genes, respectively) of the Human Adipose cDNA were used. The PCR products for microarray printing were prepared by standard methods and purified by ethanol precipitation. In the experiments using oligonucleotide microarrays, four hundred 60-mer oligonucleotides were designed individually with an in-house computer algorithm to minimize cross-hybridization, and synthesized by MWG-Biotech (Singapore), the PCR products and the oligonucleotides were resuspended in 50% DMSO, denatured at 95 °C for 5 min, and cooled on ice for 5 min before printed onto EasySpot Universal Microarrray Slides and EasySpot Oligonucleotide Microarray slides, respectively (U-Vision Biotech). The printed slides were immobilized at 42 °C overnight at high humidity. The slides were washed with 0.1% SDS twice and immersed in boiling double distilled water for 2 min to remove any unbound DNA. The slides were stored desiccated at room temperature.

RNA isolation and cDNA synthesis. Human embryonic kidney (HEK) cells were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Hyclone, Logan, UT, USA). Total RNA was isolated from 10^6 to 10^7 HEK cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and the mRNA fraction was purified using Qiagen mRNA Midi Kit (Qiagen, Hilden, Germany). Fluorescent cDNA was reverse transcribed from 2 to 4 μg HEK mRNA using 13 μM of random hexamer (Invitrogen). The reaction mixture was heated to 70 °C for 10 min, and placed at room temperature for 10 min to allow primer annealing. In one of the experiments of the paper, different amounts of *Arabidopsis thaliana* mRNA were added to the purified human mRNA before the next step. The mixture was added with 1× first-strand buffer, 10 mM dithiothreitol, 500 μM dNTP (dATP, dCTP, and dGTP), 200 μM dTTP, 0.2 U RNasin, and 13 U Superscript II (Invitrogen), 100 μM Cy3 or Cy5-dUTP (Amersham–Pharmacia Biotech, NJ, USA). Reverse transcription was carried out at 42 °C for 90 min, followed by adding 30 mM sodium hydroxide at 70 °C for 15 min to hydrolyze the RNA template, and neutralized with 30 mM hydrochloric acid. The unincorporated nucleotides were removed by using Qiagen QIAquick Nucleotide Removal Kit (Qiagen).

Hybridization, scanning, and analysis. The purified fluorescent cDNA was mixed with 20 μg poly(dA)n and 20 μg of human Cot-1 DNA (Invitrogen) and concentrated with a Microcon YM-30 Concentrator (Millipore, Bedford, MA, USA). Equal volume of either one of the 2× regular hybridization buffers (10× SSC, 0.2% SDS, with 50% formamide, or 6× SSC, 0.4% SDS) or the 2× hybridization solution developed in this study (containing 20% dextran sulfate, 0.2% SDS, 50% formamide, and 12× SSPE; this reagent is sold under the trade name EasyHyb Hybridization solution, U-Vision Biotech) was added into the concentrated cDNA mixture, which was then heated at 95 °C for 3 min and cooled down at room temperature for 20 min. Hybridization reactions using the two regular hybridization solutions were carried out at 42 and 65 °C, respectively, whereas using EasyHyb solution on cDNA microarray and oligonucleotide microarray hybridizations were at 52 and 42 °C, respectively. After hybridization, the arrays were washed with 2× SSC, 0.1% SDS at 42 °C for the oligonucleotide microarray, or at 52 °C for the cDNA microarray, for 5 min, followed by 0.1× SSC, 0.1% SDS at room temperature for 10 min, and finally 0.1× SSC for 1 min. The arrays were dried by centrifugation at 50*g* for 5 min. Detection of fluorescent signals was performed with a ScanArray 3000 (GSI, Watertown, MA, USA) and the quantitation of data was analyzed with ImaGene 4.1 (Biodiscovery, Marina del Rey, CA, USA).

Results and discussion

Dextran sulfate and formamide can increase hybridization signals

Volume-occupying reagents or molecular crowding reagents, such as polyvinyl alcohol, polyethylene glycol, and their derivatives, have been used to increase the interaction between biomolecules [8] including the association of two complementary DNA strands [7]. We, therefore, conducted a study on the ability of various volume-occupying reagents in accelerating the hybridization of DNA molecules in microarray experiments. Among those tested, dextran sulfate was the reagent that gave the best results in giving the highest signal, and lowest background signals (data not shown).

We went on to study the optimal concentration of dextran sulfate for giving the best results in hybridization. Solutions containing 5× SSC, 1% SDS, and different amounts of dextran sulfate were used to carry out hybridization reaction using 2 μg mRNA extracted from HEK culture cells onto the Eureka cDNA Microarrays (4608 spots) for 1 h. We also compared this with a control hybridization buffer (5× SSC and 1% SDS but without formamide), which was hybridized overnight (Fig. 1A). An increase in averaged hybridization signals from the microarray was observed when a higher concentration of dextran sulfate (from 0% to 10%) was used. Solutions containing dextran sulfate with concentration beyond 10% made the hybridization solutions too viscous for manipulation. The background signals from *A. thaliana* genes did not increase to any extent with increasing concentrations of dextran sulfate compared with the solution without any dextran sulfate (0%) and the control hybridization solution (O/N in Fig. 1B).

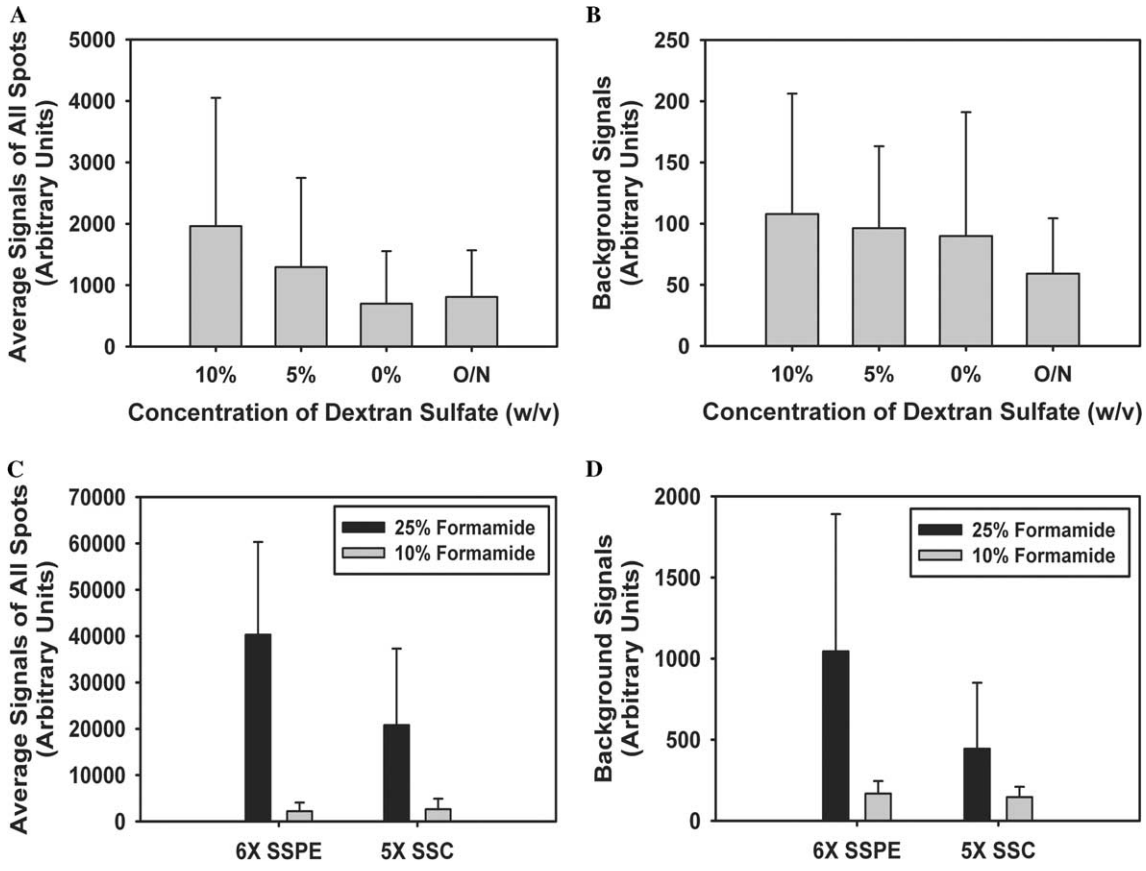

Fig. 1. Optimization of the enhancing components in the hybridization buffer. Hybridization buffers containing 5× SSC, 1% SDS, and various amounts of dextran sulfate from 0% to 10% were used in the hybridization reactions between labeled cDNA mixture and Eureka cDNA microarrays (A,B). The hybridization was carried out at 65 °C for 1 h except the control reaction (containing 5× SSC and 1% SDS), which was carried out overnight (O/N). The signals of hybridization from all the spots on a single array were summated and divided by the total number of spots to generate the average signals of all spots, and the ranges of the signals were displayed as above each of the bar (like an error bar) (A,C). The average background signals from *Arabidopsis* genes were also calculated (B,D). In (C,D), the averaged hybridization signals and the averaged background signals were shown in the reactions using the same hybridization buffers except replacing one component at a time, 6× SSPE or 5× SSC, and 25% or 10% formamide.

The average background signal levels from our microarray system were around 100–200 arbitrary units of fluorescence signal.

We also tested the effect of several concentrations of formamide on the signals of hybridization. The hybridization solution containing 25% formamide produced much higher signals of hybridization than that containing 10% formamide, disregard of the buffer used, either 6× SSPE or 5× SSC (Fig. 1C), although 6× SSPE was clearly a better buffer than 5× SSC. Even though the background or non-specific signals from the plant genes using 25% formamide buffer were higher than those using 10% formamide (Fig. 1D), they could be eliminated by the use of 0.1% SDS. By varying the concentration of SDS, we found that 0.1% SDS produced a better result than 1% SDS because the latter concentration removed the background created by the 6× SSPE, and eliminated the formation of bright speckles formed on the microarrays with using 1% SDS (data not shown). After the optimization of all components, the quick hybridization solution contained 10% dextran sulfate, 25% formamide, 0.1% SDS, and 6× SSPE, and we named this solution EasyHyb hybridization solution.

Comparison of two different popular hybridization buffers and EasyHyb solution under their optimal conditions

We compared EasyHyb hybridization solution with the two commonly used hybridization buffers, 5× SSC, 0.1% SDS, and 25% formamide (Buffer 1) and 3× SSC, 0.2% SDS (Buffer 2) under their own optimal conditions on small-scale cDNA microarrays (see Fig. 2 legend for details), and only 1 h of hybridization was allowed for EasyHyb solution, but 16 h for the two buffers that were usually used for the equilibrium of the hybridization reaction. Quantitation of signals showed that EasyHyb hybridization solution yielded the highest average signal from all the spots and Buffer 1 ranked second (Figs. 2A–D). As shown in Fig. 1, EasyHyb solution increased the

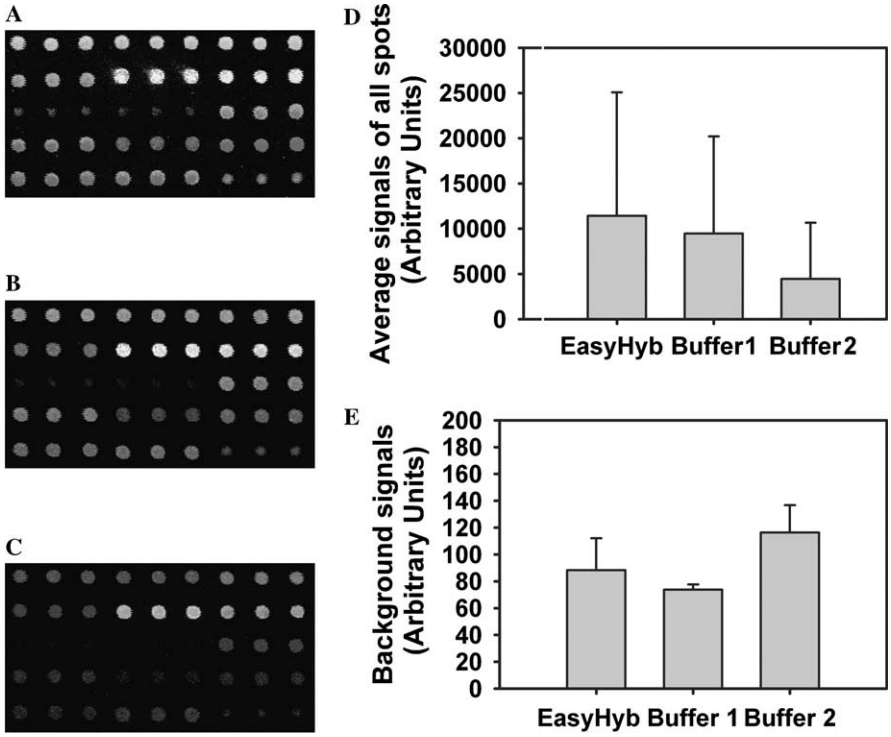

Fig. 2. EasyHyb solution gives higher signals than the other two regular hybridization buffers. Cy3-labeled cDNA of 11 genes was hybridized onto three 15-gene (in triplicate) microarrays with three different hybridization buffers. Spots of various intensities were obtained from (A) using EasyHyb hybridization buffer hybridized at 52 °C for only 1 h, (B) using 5× SSC, 0.1% SDS, and 25% formamide (Buffer 1) at 42 °C for 16 h, (C) using 3× SSC, and 0.2% SDS (Buffer 2) at 65 °C for 16 h. The power level was 90% and PMT 80%. (D) The average of signals from all the spots on each condition was plotted. (E) The background signals from different conditions were also plotted. The standard deviation of variation of signals from all spots was indicated above each bar. The data were from averages of spots from three slides hybridized with each buffer.

hybridization signals and it did not result in higher background signals than the other two conditions (Fig. 2E).

To investigate quantitatively the enhancement of kinetics of the EasyHyb solution in a larger scale, Eureka human cDNA microarrays with 1536 genes spotted in triplicate were hybridized with EasyHyb solution or Buffer 1 both for 1 h. EasyHyb solution enhanced the hybridization signal of almost all the genes on the array (Fig. 3). The median ratio of enhancement was 3.8 and the maximum was 50.8 (truncated from Fig. 3 for clarity). The average background signals from using EasyHyb solution and Buffer 1 were 204 ± 165 and 303 ± 126, respectively.

In contrast to the traditional Northern blots, the enhancement of hybridization kinetics with some volume occupying reagents usually increases the background and reduces the quality of hybridization [7,9,10], EasyHyb solution increases the signal 3- to 4-fold without increasing the background signals and thus achieves higher signal-to-noise ratios.

Quantitative analysis of low abundant transcripts can also be possible with EasyHyb solution by allowing hybridization to increase from 4 to 8 h, so that the weak signals can be brought up to a significant level for quantitation instead of being discarded (data not shown). Alternatively, less mRNA samples can be used with a longer hybridization time. We determined that fluorescent-labeled cDNA derived from as little as

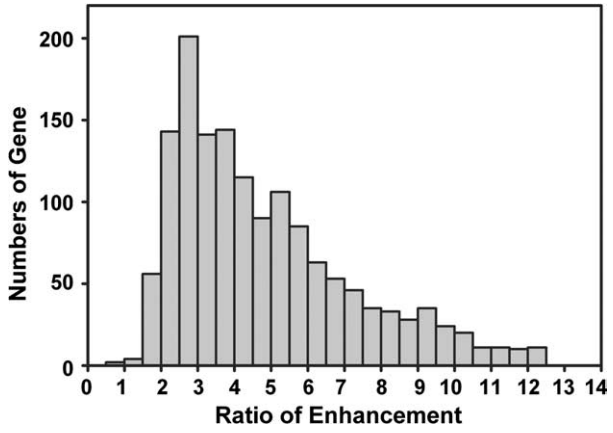

Fig. 3. Statistics of enhancement of hybridization signals by EasyHyb solution. Each of the two identical Eureka human glass cDNA microarrays, with 1536 genes was hybridized with Cy3-labeled cDNA from 2 μg HEK mRNA for 1 h, using either EasyHyb solution at 52 °C, or the regular hybridization buffer at 42 °C. The ratio of enhancement for each gene was calculated by dividing the hybridization signals from using EasyHyb solution by the hybridization signals from using the regular hybridization buffer. The outliners with the ratio of enhancement above 12 were truncated.

0.5 μg of mRNA is sufficient for hybridization (data not shown) while 2 μg is needed for the usual hybridization. The amount of precious RNA sample needed can be further reduced if indirect-labeling method is used in combination with EasyHyb solution (data not shown).

Hybridization is quantitative in the early phase

We also investigated the relationship between the change of fluorescent signals and the levels of transcripts in microarray experiments. To address the question, various amounts of five different *Arabidopsis* mRNAs, from 60 pg to 6 ng, were spiked into 2 μg HEK mRNA in the labeling procedure. The labeled cDNA molecules were hybridized individually onto several 50-gene human arrays, which also contained DNA spots of the 6 plant genes, with one being used as negative control, with EasyHyb hybridization solution at 52 °C for 1 h or Buffer 1 at 42 °C for 1 and 16 h.

After 16 h of hybridization with Buffer 1, the fluorescent signals increased rapidly as the amounts of spiked plant mRNA increased (Fig. 4C), but the rate of increase started to drop when the amount of mRNA added in the hybridization increased above 0.6 ng. In contrast, when EasyHyb solution was used, there was a proportional increase in fluorescent signals of the five plant genes with increasing amount of mRNA in the 1-h reaction (Fig. 4A), suggesting a quantitative relationship exists between the hybridized signals, and the amount of transcripts. Applying linear regression on the data set demonstrated that the R^2 values of all the lines were around 0.996. The high correlation coefficient suggests that the use of EasyHyb solution can yield consistent hybridization reactions in six separate arrays. The negative control gene (Ihc) and the blank control displayed low background signals.

A linear relationship was also observed in the 1-h reaction using Buffer 1 (Fig. 4B), albeit with lower signals. This indicates that saturation of signals occurs during the overnight hybridization. The signals from the 1-h hybridization using the EasyHyb solution were 3.4-fold (3.35 ± 0.35-fold) higher than those from the regular hybridization solution (Figs. 4A and B). This is consistent with the result from the experiment shown in Fig. 3.

The signal intensities from the spiked plant mRNA were in the similar range as those of the spots of human genes, suggesting the amount of the plant mRNA used was comparable with those of the human transcripts. Since the change in fold of signals can now be interpreted as the change in the amount of transcripts, it is an important improvement that EasyHyb solution should be used whenever quantitative analyses of the microarray data are needed.

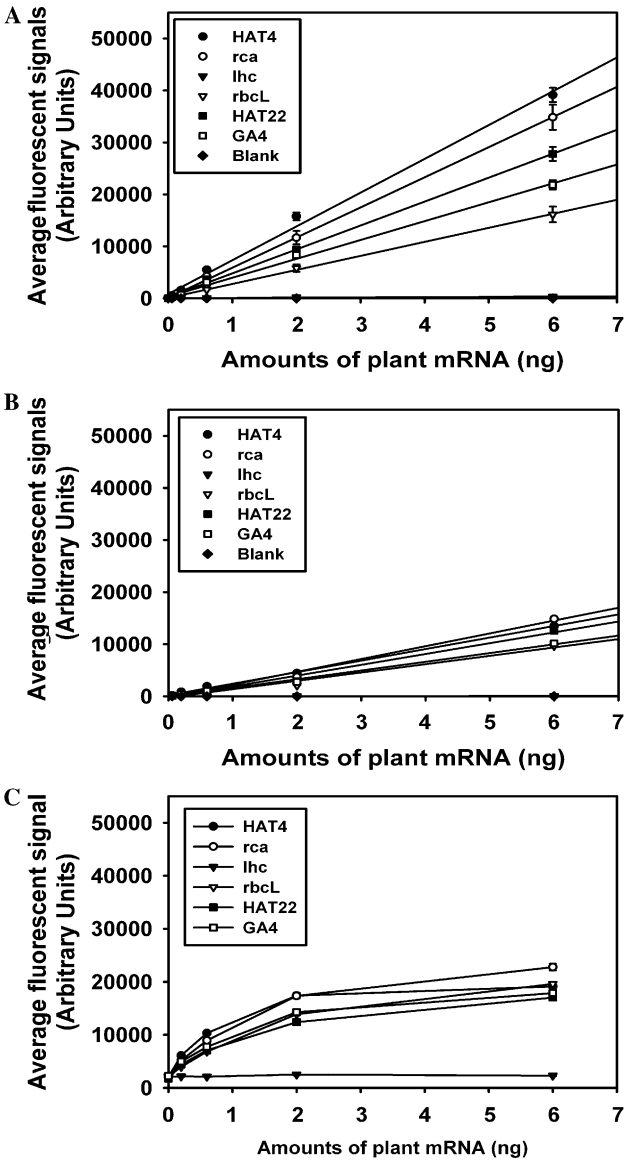

Fig. 4. Enhanced and quantitative signals from using EasyHyb hybridization solution. Different amounts (0 pg, 60 pg, 0.2 ng, 0.6 ng, 2 ng, and 6 ng) of five plant gene mRNAs (HAT4, rca, rbcL, HAT22, and GA4) were spiked in 2 μg HEK mRNA during cDNA synthesis procedure. The purified Cy3-labeled cDNAs were hybridized on 6 human cDNA microarrays, which also contained the five plant genes. A buffer control (50% DMSO or blank) and another plant gene DNA (lhc-1) were spotted as negative controls. (A) The signals from the 2-h hybridization using EasyHyb solution. (B) The signals from the 1-h hybridization reaction using Buffer 1 (5× SSC, 0.1% SDS, and 25% formamide). (C) The signals from the overnight (16 h) hybridization reaction using Buffer 1. Each line in (A,B) represented the linear regression of each plant gene. The standard derivations from three spots for each gene were shown as the error bars. In (B,C), the error bars are too small to read.

Enhancement in kinetics using EasyHyb solution is not due to higher temperature

Although we found an increase in the kinetics of hybridization using EasyHyb solution at 52 °C

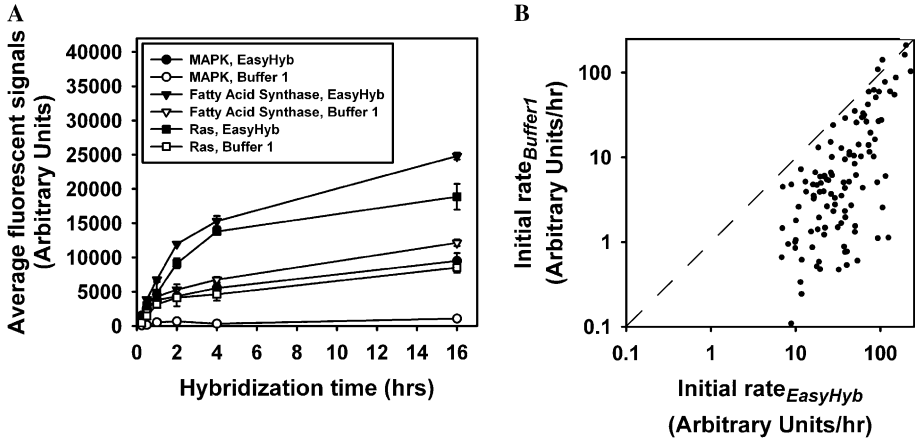

Fig. 5. EasyHyb hybridization solution increases the initial rate of hybridization and signals of almost all genes. Human cDNA microarrays with 172 genes were hybridized at 52 °C with either EasyHyb solution or 5× SSC, 0.1% SDS, and 25% formamide for different time periods before signal analysis. (A) The time courses of reaction were plotted for only three genes (MAPK, fatty acid synthase, and Ras genes) expressed at medium signal levels. The time points were 15 min, 30 min, 1, 2, 4, and 16 h. (B) Initial rate of hybridization (the slope from the time course) was plotted for every gene in the arrays. Spots with slower initial rate mean that the spots had lower signals. The error bars shown in (A) were the standard derivation from 6 spots in 2 arrays for each gene.

compared to Buffer 1 at 42 °C, we argued that the enhancement was not due to the difference in temperature used. We performed experiments using Cy3-labeled cDNA from human cells hybridized on 172-gene cDNA microarrays at 52 °C for both EasyHyb solution and Buffer 1. Hybridization reaction was terminated at different time points and the signals were quantified. The plot of the time course of reaction showed that all the genes examined increased in signals quickly in the first hour and the increase slowed down afterward (Fig. 5A, and data not shown). Both the initial rates of hybridization (the slope of first three time points) and the signals obtained at 16 h showed that EasyHyb solution increased both the initial rate of hybridization and the average hybridization signals when compared with Buffer 1 under the same temperature (black vs. open symbols in Fig. 5A). The scatter plot of the initial rates of hybridization from the two conditions showed that EasyHyb solution enhanced the hybridization of almost all the genes on the arrays compared to Buffer 1 (Fig. 5B).

One interesting observation was that although the majority of spots were deviated from the diagonal line of the plot, the derivation was more prominent especially with the spots of lower signal level. This observation suggests that the low abundant transcripts were hybridized with a lower efficiency compared with the high abundant transcripts in Buffer 1, and EasyHyb solution enhances preferentially the hybridization of low abundant transcripts under the same temperature as Buffer 1.

Besides the above experiments, we have several points to support that the increase in the kinetics of hybridization by EasyHyb solution is not due to an increase in temperature. First, dextran sulfate alone, when it functions as a volume-occupying reagent [8], in the solution increases the chance of collision between the single-stranded DNA molecules even under the same temperature. Second, the conditions used for comparison are optimal for each hybridization buffer with their specific ionic strength, and amount of denaturants. Third, higher temperature does not necessarily lead to higher signals because the two DNA strands start to fall apart. Besides temperature, many other important parameters can also control the kinetics of hybridization, such as the length of the two hybridizing DNA molecules, the concentration of denaturants (formamide), and the ionic strength of the hybridization buffer, both of which can affect the extent of secondary structures of DNA [10,11].

EasyHyb solution can be used in oligonucleotide microarray

All the results of the above experiments were performed using DNA microarray printed with PCR products. We also studied whether the characteristics of EasyHyb solution were similar when it was used in oligonucleotide microarray. Six human 400-gene oligonucleotide microarrays were hybridized with a mixture of Cy5- and Cy3-labeled reversed transcribed human mRNA spiked with 5 different amounts of plant mRNA in the presence of EasyHyb solution, and another 6 microarrays in the Buffer 1 for 2 h. As shown in Fig. 6A, EasyHyb solution increased the hybridization reactions for a median of 29-fold in the Cy3 channel and a median of 48-fold in the Cy5 channel. The difference in ratios from the two channels was likely due to the higher errors from the weaker signals of Cy5 channel. The ratio of enhancement of hybridization on the oligonucleotide microarrays is much higher than that of cDNA microarray (3.8-fold), suggesting that the fluorescent-labeled

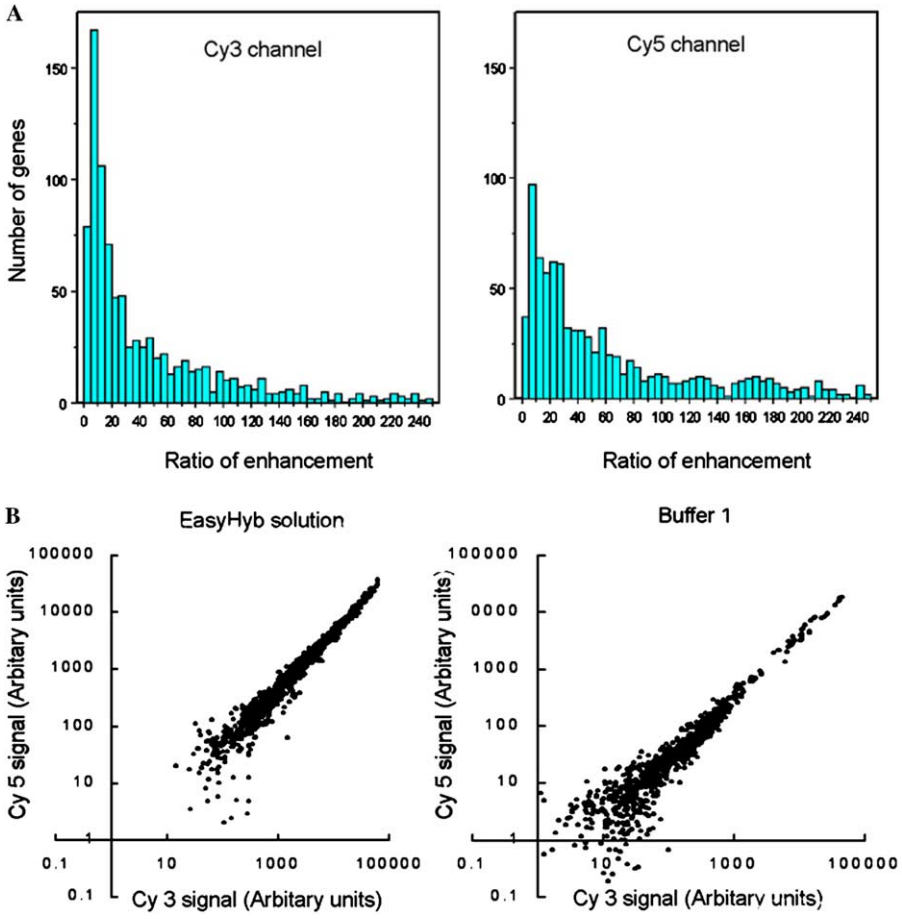

Fig. 6. EasyHyb solution can accelerate hybridization reaction on oligonucleotide microarray. (A) Distribution of the ratio of enhancement of hybridization reaction in both Cy5 and Cy3 channels. The hybridization reaction was carried out at 42 °C for 2 h using a mixture of Cy3- and Cy5 labeled reversed transcribed cDNA from HEK cells spiked with different amounts of *Arabidopsis* mRNA onto 12 human 400-gene (each gene in triplicate) oligonucleotide microarrays. The microarray also contains oligonucleotides representing the 6 *Arabidopsis* genes. The number of genes with the specified ratio of change was plotted against the ratio of increase in hybridization signals from using EasyHyb solution over Buffer 1. The median fold of increase in the Cy3 channel (left figure) is 29 whilst the median fold of increase in the Cy5 channel (right figure) is 48. (B) EasyHyb solution increases the signals from both Cy5 and Cy3 channels equally. Scatter-plots of the signals from the Cy 5 and the Cy3 channels were drawn using EasyHyb solution (left figure) and Buffer 1 (right figure).

target cDNA can have a higher chance to collide with the 60-mer oligonucleotides than the long PCR products (500–2 kbp long) in the EasyHyb solution. Moreover, EasyHyb solution did not increase preferentially the signals of either Cy5 or Cy3 because the scatter-plots of signals from both channels were tight and linear in both EasyHyb solution, and Buffer 1 (Fig. 6B). The linear and quantitative relationship between the signals of hybridization and amount of spiked plant mRNAs in the oligonucleotide microarray was also observed when EasyHyb solution was used (data not shown).

In conclusion, we showed that EasyHyb solution has two properties that are more superior to the two regular hybridization buffers (Buffer 1 and Buffer 2) that have been used by many researchers worldwide. First, EasyHyb solution enhances the kinetics of hybridization for 3- to 4-fold in the cDNA microarray and 29-fold in oligonucleotide microarray. Second, the solution would allow hybridization signals to be proportional to the amount of transcripts—a quantitative response. These two properties are very important in the microarray field because the solution can shorten the time in the overall microarray experiments, and it improves the quantitative aspect of the microarray hybridization.

Acknowledgments

The authors gratefully acknowledge our colleagues, Hsien-Ling Liu, Mandy Hsu, and Rong-Yih Yan, for their technical help and comments during the development of EasyHyb solution. We also like to thank Ching-Yu Tu for her technical help in the experiment of Fig. 6.

References

[1] D.J. Duggan, M. Bittner, Y. Chen, P. Meltzer, J.M. Trent, Expression profiling using cDNA microarrays, Nat. Genet. 21 (1999) 10–14.

[2] W.M. Freeman, D.J. Robertson, K.E. Vrana, Fundamentals of DNA hybridization arrays for gene expression analysis, Biotechniques 29 (2000) 1042–1055.
[3] N.L. van Berkum, F.C. Holstege, DNA microarrays: raising the profile, Curr. Opin. Biotechnol. 12 (2001) 48–52.
[4] V.J. Cheung, M. Morley, F. Aguilar, A. Massimi, R. Kucherlapati, G. Childs, Making and reading microarrays, Nat. Genet. 21 (1999) 15–19.
[5] C.F. Edman, D.E. Raymond, D.J. Wu, E. Tu, R.G. Sosnowski, W.F. Butler, M. Nerenberg, M.J. Heller, Electric field directed nucleic acid hybridization on microchips, Nucleic Acid Res. 25 (1997) 4907–4914.
[6] M. Taniguchi, K. Miura, H. Iwao, S. Yamanaka, Quantitative assessment of DNA microarrays-comparison with Northern blot analyses, Genomics 71 (2001) 34–39.
[7] R.M. Amasino, Acceleration of nucleic acid hybridization rate by polyethylene glycol, Anal. Biochem. 152 (1986) 304–307.
[8] P.E. Lavery, S.C. Kowalczykowski, Enhancement of recA protein-promoted DNA strand exchange activity by volume-occupying agents, J. Biol. Chem. 267 (1992) 9307–9314.
[9] G.M. Wahl, M. Stern, G.R. Stark, Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate, Proc. Natl. Acad. Sci. USA 8 (1979) 3683–3687.
[10] J.G. Wetmur, DNA probes: applications of the principles of nucleic acid hybridization, Crit. Rev. Biochem. Mol. Biol. 26 (1991) 227–259.
[11] B.L. McConaughy, C.D. Laird, B.J. McCarthy, Nucleic acid reassociation in formamide, Biochemistry 8 (1969) 3289–3295.