An Activated GOPS-poly-L-Lysine-Coated Glass Surface for the Immobilization of 60mer Oligonucleotides

By *Q. Wu*, *W. Ma**, *R. Shi*, *B. Zhang*, *X. Mao*, and *W. Zheng*

To explore a method for enhancing the immobilization and hybridization efficiency of oligonucleotides on DNA microarrays, conventional protocols of poly-*L*-lysine coating were modified by means of surface chemistry, namely, the slides were prepared by the covalently coupling of poly-*L*-lysine to a glycidoxy-modified glass surface. The modified slides were then used to print microarrays for the detection of the SARS coronavirus by means of 60mer oligonucleotide probes. The characteristics of the modified slides concerning immobilization efficiency, hybridization dynamics, and probe stripping cycles were determined. The improved surface exhibited high immobilization efficiency, a good quality uniformity, and satisfactory hybridization dynamics. The spotting concentration of 10 μmol/L can meet the requirements of detection; the spots were approximately 170 nm in diameter; the mean fluorescence intensity of the SARS spots were between 3.2×10^4 and 5.0×10^4 after hybridization. Furthermore, the microarrays prepared by this method demonstrated more resistance to consecutive probe stripping cycles. The activated GOPS-PLL slide could undergo hybridization stripping cycles for at least three cycles, and the highest loss in fluorescence intensity was found to be only 11.9 % after the third hybridization. The modified slides using the above-mentioned method were superior to those slides treated with conventional approaches, which theoretically agrees with the fact that modification by surface chemistry attaches the DNA covalently firmly to the slides. This protocol may have great promise in the future for application in large-scale manufacture.

1 Introduction

In recent years, oligonucleotide microarrays were favored by many researchers for their lower cost and higher specificity over DNA or cDNA microarrays. Oligonucleotide microarrays have been applied to genetic mutational scanning and polymorphism analysis [1–3], screening of disease and selecting antisense reagents [4], and monitoring of gene expression [5, 6]. In oligonucleotide microarray fabrication, the first problem that has to be faced and needs to be resolved is to immobilize or directly synthesize oligonucleotides on solid supports. Methods for immobilizing oligonucleotides mainly include physical adsorption and covalent linkage. Oligonucleotides that were bound to the surface by the former method were unstable and liable to be removed from the surface. Hence, according to Taylor et al. [7] the oligonucleotide immobilization by physical adsorption is gradually being substituted by methods that promote covalent linkage.

With the development of the DNA microarray technology, many reports on the modification of glass surfaces and the immobilization of oligonucleotides onto solid supports by covalent linkage have appeared [8–12]. In this paper, the process of slide surface chemistry based on conventional protocols for poly-*L*-lysine coating was improved [6, 13] in order to immobilize 60mer oligonucleotides by deposition technology. The oligonucleotides were covalently attached to the activated surface. Some assays, such as the immobilization efficiency and hybridization experiments were designed and carried out to compare the quality of the modified slides with conventional slides. At the same time, the modified slides were used in the preparation of microarrays to detect the SARS coronavirus.

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2 Materials and Methods

2.1 Materials

The main chemicals and solvents were purchased from Sigma-Aldrich (USA). Glass microscope slides (25 × 75 × 1 mm) were purchased from Darko (USA). Cy3-Amidite was obtained from Amersham Pharmacia (UK), whereas 60mer oligonucleotides and Cy3 labeled oligos were synthesized in our laboratory using an ABI 3900 DNA synthesizer (USA).

2.2 Preparation of the PLL (poly-Lysine) Surface

Poly-*L*-Lysine (PLL) coated glass slides were prepared according to the protocol of Brown's Lab of Stanford University [14].

2.3 Preparation of the Activated GOPS-PLL Surface

The glass slides were washed in 1 M NaOH, 1 M HCl, ddH_2O and methanol in turn. The cleaned slides were put into a freshly prepared solution of 1.5 % GOPS (3-glycidoxypropyltrimethoxysilane) and 95 % ethanol for 50 min,

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then dipped in 95 % ethanol for 1 min to remove the excessive silane and dried at 80 °C in a vacuum oven. The GOPS-modified slides were covalently coupled with PLL according to the protocol [14]. The GOPS-PLL-coated slides were placed at room temperature for 2 hours into an activation solution which included 0.2 % 1,4-phenylenediisothiocyanate (PDITC), 10 % pyridine and 89.8 % N,N-dimethylformamide, rinsed with methanol and acetone twice, respectively, dried at 45 °C, and finally stored in a dark closed box under dry conditions.

Formation of Oligo Arrays on Two Kinds of Surfaces

The probes were printed onto an activated GOPS-PLL surface and PLL surface, respectively, by a Cartesian Pixsys 5500 robot (Cartesians, USA). The microarrays were re-hydrated above the 3 × SSC solution and placed into a beaker at 100 °C to snap dry, then UV cross-linked at 90 mJ/cm^2 for 30 s using a Bio-Rad UV cross-linker (Bio-Rad, USA). The two printed kinds of slides were stored in the dark at room temperature until used for hybridization.

Detection of Immobilizing Efficiencies

A 26mer oligonucleotide probe FA1 fluorescently labeled with 3'Cy3 (see Tab. 1) of different concentrations (1 μmol/L–80 μmol/L) was printed onto the two kinds of slides. After 3 hours of printing, the slides were scanned with the Packard ScanArray Lite Scanner (GSI Lumonics, USA). The slides were then rinsed using the washing solution (2.5 mmol Na_2HPO_4; 0.1 % SDS) and ddH_2O twice respectively, and were scanned again.

Hybridization of the Oligo Microarray

The microarrays printed with different concentrations of the FA2 probe were hybridized with FA3 target (see Tab. 1), and scanned under the conditions of 90 % laser power and 70 % PMT.

Stripping Procedure

After the hybridization with the fluorescently labeled FA3 target, the slides were immersed in boiled stripping buffer (2.5 mmol Na_2HPO_4, 0.1 % SDS) for 15 min, then rinsed in ddH_2O and dried. This procedure was repeated until the FA3 target was completely removed before the next hybridization reaction.

Preparation of an Oligo Microarray of the SARS Coronavirus

The method for the preparation of 60mer SARS-CoV oligo microarrays was the same as that for the hybridization of the oligo microarray above. There were 12 SARS-CoV oligonuclotides, 2 positive control probes, 2 negative control probes and blank controls (The oligonuclotide sequences

Table 1. Sequences of the oligonucleotides.

Oligo name	Length bp	Sequence from 5' to 3'
FA1	26	TTTTTTTTGTTTGGCTGGTGTGGATC-Cy3
FA2	60	TTTTTTGATCCACACCAGCCAAACGATCCACACCAGCCAAACGATCCACACCAGCCAAAC
FA3	18	CY3-GTTTGGCTGGTGTGGATC
SARS01	60	TCGTGAGCTCAATGGAGGTGCAGTCACTCGCTATGTCGACAACAATTTCTGTGGCCCAGA
SARS02	60	TGTGTGTTTGCCTATGTTGGCTGCTATAATAAGCGTGCCTACTGGGTTCCTCGTGCTAGT
SARS03	60	ACTTCAGTCTTTACAAGTGTGCGTGCAGACGGTTCGTACACAGGTTTATATTGCAGTCAA
SARS04	60	CCAGCTTCACGAGAGCTATCTGTCACATTCTTCCCAGACTTGAATGGCGATGTAGTGGCT
SARS05	60	ATTAGTGATGAAGTTGCTCGTGATTTGTCACTCCAGTTTAAAAGACCAATCAACCCTACT
SARS06	60	GTGAGCTTCGTCCAGACACTCGTTATGTGCTTATGGATGGTTCCATCATACAGTTTCCTA
SARS07	60	TGCTGTAGACATTAATAGGTTGTGCGAGGAAATGCTCGATAACCGTGCTACTCTTCAGGC
SARS08	60	GTTTCTACAGGTTAGCTAACGAGTGTGCGCAAGTATTAAGTGAGATGGTCATGTGTGGCG
SARS09	60	TTTATCACCCGCGAAGAAGCTATTCGTCACGTTCGTGCGTGGATTGGCTTTGATGTAGAG
SARS10	60	GGTGGTTATTCTGAGGATAGGCACTCAGGTGTTAAAGACTATGTCGTTGTACATGGCTAT
SARS11	60	TAGCGGAGGTGGTGAAACTGCCCTCGCGCTATTGCTGCTAGACAGATTGAACCAGCTTGA
SARS12	60	TGCTCCAAGTGCCTCTGCATTCTTTGGAATGTCACGCATTGGCATGGAAGTCACACCTTC
Neg1	60	GTAACGTTAAGAGACTACCATTGCACATGCCCTAAGAACAGGTACAATAGAGTAGGTACA
Neg2	60	GTATTAATTATTGCTAGCTGATCATACCACGTTAGTCGTTAAGCATGCATGCAGCTAGGA

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are listed in Tab. 1). The method for the preparation of oligonuclotides used was according to Wu et al. [15] and these oligonuclotides were printed onto a 12 × 12 microarray, each probe with 6 spots. The SARS samples were throat swabs and gargling fluids of SARS patients from 302 hospitals in Beijing. The samples labeled by means of the RD-PCR method were applied to the microarray hybridization [16].

3 Results

The oligonucleotide immobilization efficiency was evaluated on two platforms (PLL surface and activated Gops-PLL surface) by spotting the FA1 oligonucleotide at six different concentrations (ranging from 1 to 80 μmol/L) to yield a 12 × 8 microarray. The result is shown in Fig. 1. The fluorescence intensity on the activated Gops-PLL surface was higher than that of the PLL surface at each of the spotting concentrations. It was found that the optimum probe concentration would be 10 μmol/L, but not 20 μmol/L because the spotting concentration of 10 μmol/L was enough to satisfy the requirements of detection. Fig. 2 depicts the relationship between probe concentration and signal intensity following hybridization.

Oligo microarrays printed on an activated GOPS-PLL slide could undergo hybridization stripping cycles for at least three cycles. Images of hybridization are presented in Fig. 3 (A, B, C, D). Comparing with the first hybridization, the highest loss in fluorescence intensity on activated GOPS-PLL slides with different concentrations was only 11.9 %, while the loss on the PLL slides was obvious, which could be as high as 58.3 %.

The hybridization results of SARS clinical samples with our improved microarray are shown in Fig. 4. Several SARS

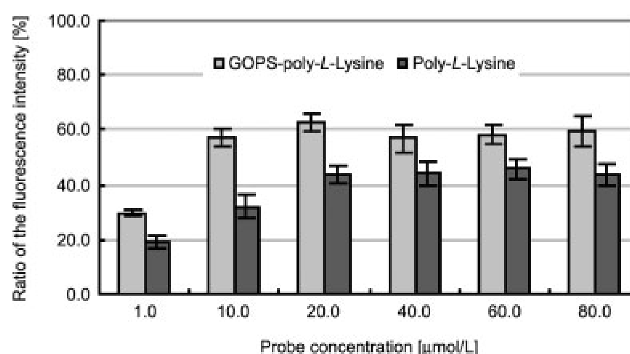


Figure 1. Oligonucleotide immobilization efficiency with different probe concentrations. The immobilization efficiency [%] is the ratio of the fluorescence intensity after washing and before washing.

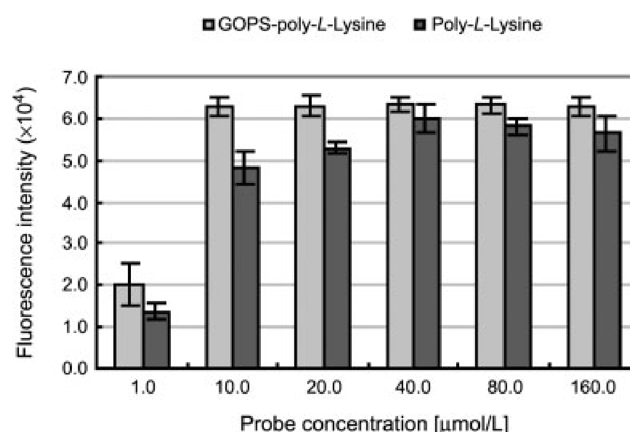
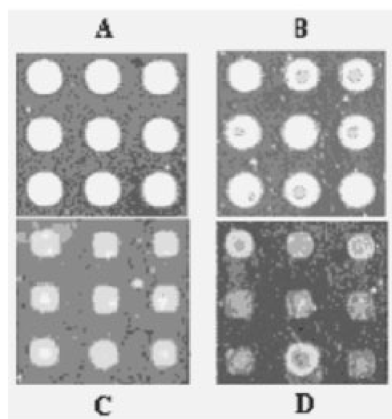


Figure 2. Hybridization with different probe concentrations. Slides printed with different concentration of FA2 probe (60mer TTTTTTGATCCACACCAGCCAAACGATCCACACCAGCCAAACGATCCACACCAGCCAAAC) were hybridized with FA3 target (18mer CY3-GTTTGGCTGGTGTGGATC).



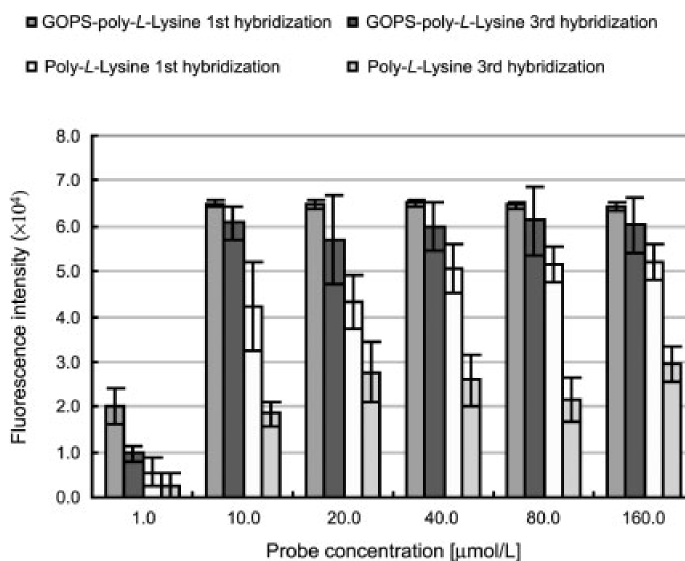


Figure 3. Three cycles of hybridization after stripping. A: GOPS-PLL slide, first hybridization; B: GOPS-PLL slide, third hybridization; C: PLL slide, first hybridization; D: PLL slide, third hybridization. Conditions: The slides were immersed in boiled stripping buffer (2.5 mM Na_2HPO_4 × 0.1 % SDS) for 15 min after each hybridization.

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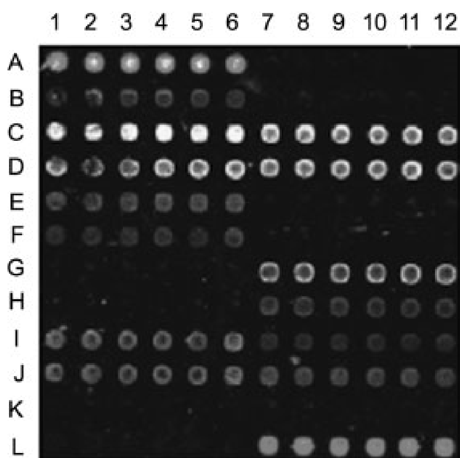

Figure 4. SARS oligonucleotide microarray hybridized with SARS patients' sample (12×12). The SARS patients' samples are labeled by means of the RD-PCR method. Spots of (A1–A7) and (L7–L12) are positive controls; spots of (A7–A12) and (L1–L6) are empty controls; spots of (B7–B12) and (K7–K12) are negative controls; others are SARS probes.

probe sites clearly demonstrated positive signals, whereas no signal was shown in the negative and blank control probe sites. The microarray was analyzed with QuantArray 2.0 software, and the following data were obtained. The spots are approximately 170 μm in diameter, and the mean fluorescence intensity of the SARS spots are between 3.2×10^4 and 5.0×10^4. The background fluorescence intensities were found to be only between 100 and 300. In comparison with the PLL surface microarray, our improved microarray had better sensitivity and homogeneity.

4 Discussion

In the development of the microarray technology, DNA or oligonucleotide probes were required to bond to the slide surface faster, more stable and more precise. Many researchers have introduced the chemical reactions on several supports and the linkage between DNA (or oligonucleotide) and the modified surface. The poly-*L*-lysine coated glass slide, prepared by protocols of Patrick Brown's Lab, was widely applied in cDNA microarray. The main mechanism of binding DNA onto the surface is physical adsorption. A specific binding probably results from an electrostatic interaction of the phosphate backbone of DNA and the positively charged amino surface. Moreover, DNA (usually PCR amplified material) was immobilized by ultraviolet (UV)-irradiation to form covalent bonds between the thymidine residues in DNA and the positively charged amine groups on the poly-*L*-lysine slides. A low oligonucleotide binding efficiency and a detection of non-uniform sensibility spots were observed [17].

In this paper, similar results were obtained as illustrated in Fig. 1. Moreover, poly-*L*-lysine is such an innocuous chemical of rich amine groups that it is a good reagent for glass surfaces modified with amine groups. Our approach attempted to improve the method and a scheme is presented in Fig. 5. Firstly, the slides were silanized with GOPS after cleaning, and then the silane slides were coated with poly-*L*-lysine polymers, which made it possible for the poly-*L*-lysine to be covalently bound to the GOPS-modified glass, and subsequently the surface was activated by the homo-bi-functional cross-linker PDITC. Finally, the oligonucleotides were covalently immobilized to the modified surface.

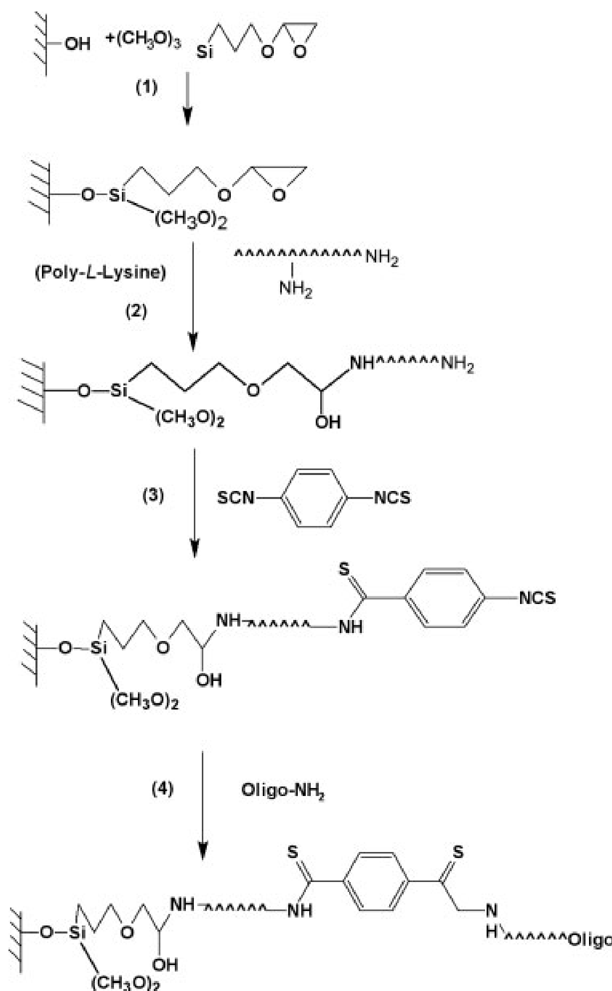

Figure 5. Scheme of oligonucleotides immobilized on slides by covalent linkage. Four steps were involved in this process: (1) the glass slides were silanized by GOPS, (2) the silanization surface was coated with poly-*L*-lysine covalently, (3) the GOPS-PLL surface was activated using PDITC, (4) oligonucleotides were attached onto the activated surface.

Firstly, the oligo loading capacity and the immobilization efficiency of the activated GOPS-PLL surface were examined. The results confirmed that both properties were superior to those of the PLL surface microarray as a consequence of the introduction of chemical PDITC causing more oligos to attach to the surface. Furthermore, the experiment of probe stripping revealed that the surface was more stable against high pH, salt and temperature. The results demonstrated that PLL was covalently bound to the surface as

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designed. A GOPS-PLL surface is a compact polymer layer in materials, which enhances not only the immobilization efficiency but also the stability. Therefore, in experiments of hybridization and the detection of the SARS coronavirus, the spots were more homogenic and sensitive. These results are shown in Fig. 2 and Fig. 4.

In conclusion, the conventional method of poly-*L*-lysine coating onto a microscope glass surface was modified to achieve a covalent immobilization of unmodified oligonucleotide on the glass surface. The oligonucleotides were linked stable, and the sensitivity of detection was improved. Satisfactory results were obtained in the experiments. A spotting concentration of 10 μmol/L was able to satisfy the requirements of detection. The spots were approximately 170 nm in diameter, and the mean fluorescence intensity of the SARS spots were in a range of 3.2×10^4 to 5.0×10^4 after hybridization, whereas the fluorescence intensities of the background were only between 100 and 300. Furthermore, the microarrays prepared by means of this method were more resistant to consecutive probe stripping cycles. The activated GOPS-PLL slide could undergo at least three hybridization stripping cycles, and the highest loss of fluorescence intensity amounted to only 11.9 % after the third hybridization. At the same time, the GOPS-PLL coating method is not complicated and not expensive when applied for large-scale manufacture. Further efforts will be taken so that such oligonucleotide microarray platform can be used in cDNA microarrays as well.

Acknowledgements

The authors are grateful to Shiqing Zhang and Huiyong Zhang for their skillful technical support. This work was funded by National Nature Science Foundation of China (NSFC) Grant 39880032.

Received: December 7, 2004 [ELS 97]
Received in revised form: September 10, 2005
Accepted: September 16, 2005

References

[1] J. G. Hacia, F. S. Collins, Mutational analysis using oligonucleotide microarrays, *J. Med. Genet.* **1999**, *36*, 730–736.
[2] J. G. Hacia, B. Sun, N. Hunt, K. Edgemon, D. Mosbrook, C. Robbins et al., Strategies for mutational analysis of the large multiexon ATM gene using high-density oligonucleotide arrays, *Genome Res.* **1998**, *8*, 1245–1258.
[3] J. G. Hacia, Resequencing and mutational analysis using oligonucleotide microarrays, *Nat. Genet.* **1999**, *21*, 42–47.
[4] M. Milnern, E. M. Southern, Selecting effective antisense reagents on combinatorial oligonucleotide arrays, *Nat. Biotechnol.* **1997**, *15*, 537–541.
[5] H.-L. Kim, Comparison of oligonucleotide-microarray and serial analysis of gene expression (SAGE) in transcript profiling analysis of megakaryocytes derived from CD34+ cells, *Exp. Mol. Med.* **2003**, *35*, 460–466.
[6] S. Li, D. T. Ross, M. E. Kadin, P. O. Brown, M. A. Wasik, Comparative genome-scale analysis of gene expression profiles in T cell lymphoma cells during malignant progression using a complementary DNA microarray, *Am. J. Pathol.* **2001**, *158*, 1231–1237.
[7] S. Taylor, S. Smith, B. Windle, A. Guiseppi-Elie, Impact of surface chemistry and blocking strategies in DNA microarrays, *Nucleic Acids Res.* **2003**, *31 (16)*, e87.
[8] T. Strother, R. J. Hamers, L. M. Smith, Covalent attachment of oligodeoxyribonucleotides to amine-modified Si (001) surfaces, *Nucleic Acids Res.* **2000**, *28 (18)*, 3535–3541.
[9] N. Zammatteo, L. Jeanmart, S. Hamels, S. Courtois, P. Loutte, L. Hevesi et al., Comparison between different strategies of covalent attachment of DNA to glass surfaces to build DNA microarrays, *Anal. Biochem.* **2000**, *280*, 143–150.
[10] C. M. Halliwell, A. E. G. Cass, A factorial analysis of silanization conditions for the immobilization of oligonucleotide on glass surfaces, *Anal. Chem.* **2001**, *73*, 2476–2483.
[11] U. Maskos, E. M. Southern, Oligonucleotide hybridizations on glass supports: a novel linker for oligonucleotide synthesis and hybridization properties of oligonucleotides synthesized in situ, *Nucleic Acids Res.* **1992**, *20*, 1676–1684.
[12] M. A. Podyminogin, E. A. Lukhtanov, M. W. Reed, Attachment of benzaldehyde-modified oligodeoxynucleotide probes to semicarbazide-coated glass, *Nucleic Acids Res.* **2001**, *29*, 5090–5098.
[13] M. Schena, D. Shalon, R. W. Davis, P. O. Brown, Quantitative monitoring of gene expression patterns with a complementary DNA microarray, *Science* **1995**, *270*, 467–470.
[14] http://cmgm.stanford.edu/pbrown/protocols/1_slides.html
[15] Q. H. Wu, W. L. Ma, R. Shi, Q. Y. Guo, B. Zhang, Y. B. Song et al., Preparation of SARS 60mer oligonucleotide probes for microarray printing, *Guang Dong Medical J.* **2003**, *24*, Suppl (I).
[16] R. Shi, W. L. Ma, Q. H. Wu, B. Zhang, Y. B. Song, Q. Y. Guo et al., Design and application of 60mer oligonucleotide microarray in SARS coronavirus detection, *Chinese Sci. Bull.* **2003**, *48*, 1165–1169.
[17] P. L. Dolan, Y. Wu, L. K. Ista, R. L. Metzenberg, M. A. Nelson, G. P. Lopez, Robust and efficient synthetic method for forming DNA microarrays, *Nucleic Acids Res.* **2001**, *29 (27)*, 107.

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