

Evaluation of Microarray Sensitivity for DNA Profiling

Joo-Woon Lee*

Chemistry - School of Liberal Arts and Sciences, Korea National University of Transportation
Chungju, Chungbuk 380-702, Korea. *E-mail: jwoonlee@ut.ac.kr

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INTRODUCTION

DNA microarray technology has become a powerful tool for the investigation of gene expression, transcription analysis, disease screening, diagnostics and nucleic acid sequencing.¹⁻³ As reviewed by Xu and Lam,⁴ various immobilization technologies have been developed through either in situ photosynthesis or mechanical spotting. In general, mechanical spotting methods can be categorized by interaction between probes and surfaces, which includes physical absorption, non-specific surface interaction, and covalent attachment. Mechanical spotting based on covalent interaction, in particular, has become a dominant tool for the production of DNA microarrays. The superiority of covalent immobilization is its diverse choice of surface and linker chemistry and thus enables the attachment of a variety of probes as well as nucleic acids. Linkers and additives in accordance to the surface chemistry should be considered to improve probe immobilization. Linkers are critical because steric hindrance between probes and surfaces could affect the performance of probes against targets significantly. Carbon chains or random nucleic acids have been added to the probe to provide sufficient space between solid surface and active sites of probes. Glycerol is one of common additives for spotting solution because its intrinsic viscosity prevents rapid evaporation of spotting solution, resulting enhanced immobilization. Although the technological evolution has been extremely rapid, optimizing the various steps in a microarray experiment is still very labor intensive and time consuming.^{5,6}

Many researchers are now relying on commercially available microarray products not only because of cost and time efficiency but also the quality of surface functionalization.⁷ A large numbers of vendors including Asper Biotech, Schott Nexterion, TeleChem, Corning, and Erie Scientific are currently offering glass slides coated with various functional groups. However, there has been few

report⁸ to compare the performance of a number of commercial microarray slides in concert with linkers, additives, or DNA concentrations, systematically along with hybridization condition. Therefore, the primary objective of this study is to evaluate currently used commercial microarray slides based on surface chemistry with two types of linker modifications for the attachment to the surfaces in order for optimal microarray spotting and hybridization. Selection criteria are based on a spot intensity and a signal-to-noise (S/N) ratio when DNA probe binds to the slide and stained or hybridized with target DNA. The effect of glycerol concentrations on spot variability and size was also explored according to the type of slide coatings and linkers. At the end, optimum concentrations for probe and target DNAs were examined. With these experiments, an optimum arraying protocol for DNA profiling can be achieved and any incompatibilities of certain combinations can be avoided for future microarray production.

EXPERIMENTAL

Two sets of experiments were conducted to evaluate different types of surface chemistry on commercial microarray slides in respect to DNA probes for immobilization and to establish optimum DNA concentrations for spotting and hybridization for DNA profiling. Four types of slides were first tested based on the functional groups that are coated on the surfaces of each slides including epoxy (Schott Nexterion[®] slide E, denoted as NexE), aldehyde (Nexterion[®] slide AL, denoted as NexAL), aminosilane (3-aminopropyl trimethoxysilane) (Asper Biotech Genorama[®] SA slide, denoted as SA), and the aminosilane with an additional linker (1,4-phenylene diisothiocyanate) (Genorama[®] SAL slide, denoted as SAL). DNA probes are oligonucleotides that consist of a 13 mer segment with either a primary amino functional group positioned at the 5'-end with a C6 spacer arm (denoted as amino-linker) or an I-

linker™ (Integrated DNA Technologies) attached to the 5'-end of the oligomer (denoted as I-linker), which was attempted as an amino substitute for surface attachment.

Two of each SA and SAL slides were printed with a spotting solution containing either amino- or I-linker DNA probes at a 50 μM concentration in 3x SSC (saline-sodium citrate) buffer solution, where glycerol concentration varied from 0% to 30% with 10% increment. For each of the eight total spotting solutions, ten replicate features were spotted on each slide with a spacing of 300 μm on an automated microarrayer (house-built arrayer at University of Texas at Austin) at 70% relative humidity. The NexE and NexAL slides were then spotted using two of each. The spotting solutions for them were the same as with the SA and SAL slides above, yet the DNA concentration was reduced to 15 μM as recommended by the manufacturer. All of each slides were stained using the Molecular Probes Invitrogen® DNA staining kit to assess the compatibility of the slides with the kit. They were then scanned using the GenePix® 4000B scanner with excitation at a 532 nm and a PMT gain of 400. Spots were analyzed based on the median intensity at 532 nm by subtracting background intensity (F532 Median - B532).⁹

Based on the results from the above experiments, the epoxy and aldehyde coated NexE and NexAL slides were further examined and compared along with another slide variation - the TeleChem ArrayIt® SuperEpoxy (denoted as AItE) and SuperAldehyde (denoted as AItAL) slides to optimize DNA profiling conditions in terms of a DNA concentration in both spotting and hybridization solutions. Three of each slide types were spotted with two identical arrays on each slide, with each array containing three blocks. Each one of the blocks was a set of twenty replicate features containing the same I-linker DNA probe in 3x SSC buffer at a concentration of 10, 15, or 20 μM , respectively, where 10% of glycerol was contained only for the epoxy coated NexE and AItE slides. With this spotting setup a total of 24 arrays and 1440 features were printed across the DNA concentration gradient and slide variation. DNA immobilization, washing, and blocking were conducted freshly prior to hybridization.

All of each type slides were hybridized with the complementary 5' Cy3 labeled DNA in 3x SSC and 0.1% SDS (sodium dodecyl sulfate) buffer at a concentration of 10, 15, or 20 μM respectively. This provided a gradient in which each concentration could be used to hybridize with one of each of the slide types for a total of 40 features over two arrays for each spotting concentration for each slide. The slides were allowed to hybridize overnight using a

cover slip method in slide cases in a hot water bath kept at 42 °C. They were then removed and washed with post-hybridization wash solutions according to the manufacturers' protocols for each slide type. After completion of washing, the slides were analyzed with the same instrumental measurement as described above. Once it became apparent that the aldehyde slides were exhibiting unusually large background intensities at higher hybridization DNA concentrations, another post-hybridization wash was conducted in a more rigorous manner in an attempt to reduce background. These results were also analyzed to derive a full diagnostic on the optimum spotting and hybridization DNA concentrations for each slide type.

RESULTS AND DISCUSSION

The first set of experiment was designed to test the performance levels of commercial microarray slides based on surface chemistry. Basically, slides from two vendors coated with four different functional groups were spotted with DNA probes with two different end groups in various concentration of glycerol in spotting buffer. Staining kit was employed to quantitate the level of DNA probe immobilized on each surfaces. As summarized in Fig. 1, epoxy (NexE) and aldehyde (NexAL) coated slides from Nexterion® greatly outperformed aminosilane (SA) and its isothiocyanate-derivative (SAL) coated slides from Genorama® regardless of glycerol content or types of linkers. In addi-

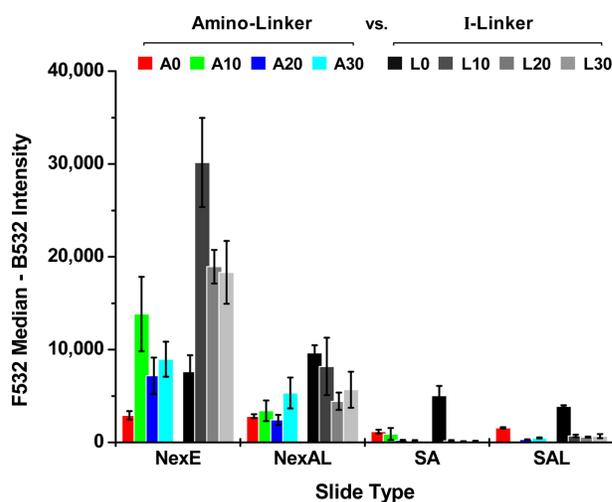


Fig. 1. Quantitative analysis of median intensity at 532 nm by subtracting background intensity (F532 Median - B532) of each stained slides that were spotted with amino- and I-linkers at various concentrations of glycerol. Numbers next to the linkers (A and I) in legend stand for glycerol concentrations of 0%, 10%, 20%, or 30%, respectively and error bars represent the standard deviation (SD).

tion, I-linker showed ~2 fold higher intensity than amino-linker in general, however the chemical information about the I-linker was not disclosed by the vendor. Comparing median intensity subtracted with background, the NexE slides showed highest intensities for both the amino- and I-linkers and the NexAL slides showed the second highest intensities at half that of the NexE slides. While, the Genorama[®] SA and SAL slides showed the lowest intensities across the board even though the DNA concentration in spotting solution was ~3 fold higher than that of the Nexterion[®] slides. Hence, these results above indicate that aminosilane (SA) and its isothiocyanate-derivative (SAL) do not provide strong covalent interaction with both amino- and I-linker modified DNA probes.

Next, the influence of glycerol was explored. Epoxy coated NexE slides exhibited significant signal enhancement of 2~3 fold by addition of glycerol in spotting solution. The trend of signal enhancement was consistent in both linkers on NexE slides and the glycerol concentration was not critical within the range of 10~30%. It was supposed that the high viscosity of glycerol prevents the evaporation of aqueous solution and thus boosts the covalent interaction between DNA probe and functional groups on the slide surfaces. However glycerol had little effect on the signal intensities on aldehyde coated NexAL slides, and conversely reduced their intensities greatly on both SA and SAL slides. Therefore, the favored condition for NexAL, SA, and SAL slides is at 0% of glycerol with both linkers. These contradictory results imply that glycerol as an additive for spotting solution should be thoroughly evaluated in line with the types of slides and vendors.

In order to determine slide compatibility with staining process, signal-to-noise (S/N) ratio was investigated. In accordance with the intensity results, the Nexterion[®] slides exhibit superior performance with higher S/N ratios of 100 to 700 fold and the I-linker provided higher S/N ratios than those of the amino linker as a whole. Considering the I-linker outcome, it is observed that glycerol content greatly reduces the S/N ratios for the SA and SAL slides, yet increases them for the NexE and NexAL slides. This similar tendency for the effect of glycerol content on the S/N ratios is also observed for the slides spotted with the amino-linker.

While in respects of spot morphology,¹⁰ all of the slides were affected by glycerol content in the spotting solutions. A large loss of circularity and conformity was observed in the NexAL slides and this similar observation was also found in the NexE slides, yet to a less degree. The both SA and SAL slides showed a loss of conformity along with a

drastic drop in intensity in the glycerol spots. Another characteristic that was inspected was the diameter of the spot features. Even though 100 μm size of pins were used, it was suspected that the variations in spot size would be generated due to hydrophilic/hydrophobic property at the surface of slides. The spots on the SA slides were all in the low 100 μm range and exhibited little variation despite the varying glycerol content and linker type. The SAL slides also did not alter the size in relation to glycerol content or linker variation, yet had much larger features than all other slides at a diameter near 200 μm . The spot sizes on both NexE and NexAL slides were around the 100 μm range and they seldom showed any trends in size in accordance with glycerol or linker.

In terms of a functional group for the both DNA probes, the epoxy coating variation of NexE slide outperformed in every aspects in compared with the aldehyde, primary amine, and isothiocyanate functionalities at the surfaces of NexAL, SA, and SAL slides. The I-linker provided higher overall results than the amino-linker. Negative effects of the glycerol did not increase in proportional to the content, but instead just required any amount of it to be present, preferably on the NexE slide. In counting all of the characteristics observed in the stained slides, therefore, it can be extractable from the results that the optimum slide and probe modification for DNA profiling is the epoxy-coated NexE slide at 10% glycerol content in the spotting solution with the I-linker. If other linkers to be used, however, the aldehyde-coated NexAL slide may be an alternative, as the intensities for the both linkers are quite strong at no glycerol content.

Expanded from the results above, the functionalities at the surface of the NexE and NexAL slides have been further examined against the equivalent functionality variations by ArrayIt[®] (AItE with epoxy coating and AItAL with aldehyde coating). The DNA concentration gradients for both spotting and hybridization were examined using the same I-linker modified probe to determine optimum DNA profiling conditions according to equivalent functionality. The results are shown in *Figs. 2* and *3* for the epoxy coated NexE and AItE slides at 10% glycerol concentration and for the aldehyde coated NexAL and AItAL at no glycerol, respectively. In most cases, the epoxy coated slides demonstrated higher intensities than the corresponding aldehyde coated slides. Interestingly, the intensity on aldehyde slides decreased slightly as increasing the complementary DNA concentration in hybridization solution, yet it is assumed that the data extraction and analysis could be the source of error associated with spot mor-

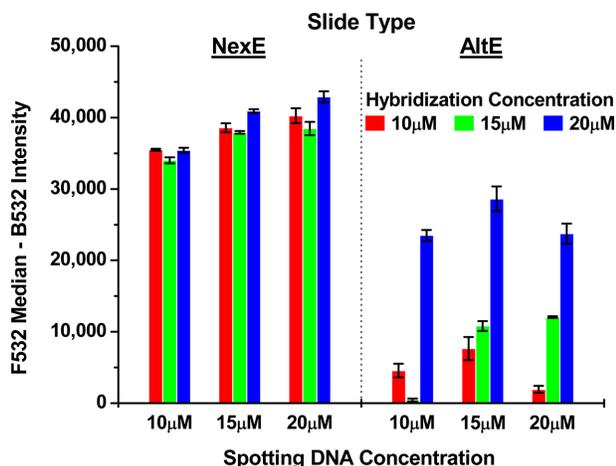


Fig. 2. Comparative analysis of intensity as a function of both I-linker DNA probe concentration for spotting and the complementary DNA for hybridization on epoxy coated Nexterion® slide E (NexE) and ArrayIt® SuperEpoxy slide (AItE) at 10% glycerol concentration. Error bars represent the standard deviation (SD).

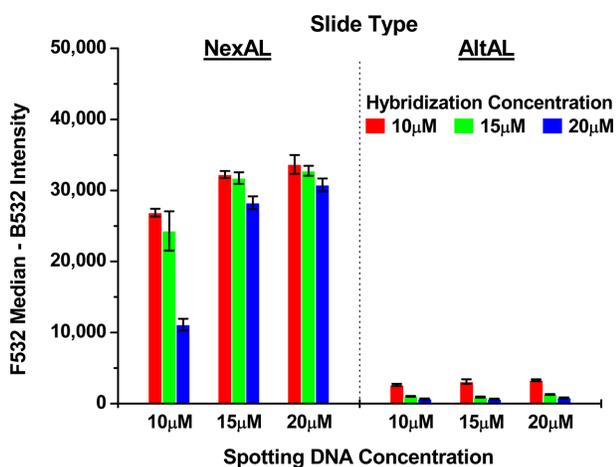


Fig. 3. Comparative analysis of intensity as a function of both I-linker DNA probe concentration for spotting and the complementary DNA for hybridization on aldehyde coated Nexterion® slide AL (NexAL) and ArrayIt® SuperAldehyde slide (AItAL) at no glycerol content. Error bars represent the standard deviation (SD).

phology rather than the efficiency of immobilization and/or hybridization.^{7,11} Based on a series of the diagnostic on optimum spotting and hybridization DNA concentrations for the surface chemistry of each slide type, it can be concluded that the epoxy slides are more favorable than the corresponding aldehyde slides in a full range on both spotting and hybridization concentrations, 10~20 μM.

Closely evaluating the strongest signals from each slide types, the epoxy-coated NexE slide by Nexterion® outperformed all of the others and the aldehyde-coated NexAL

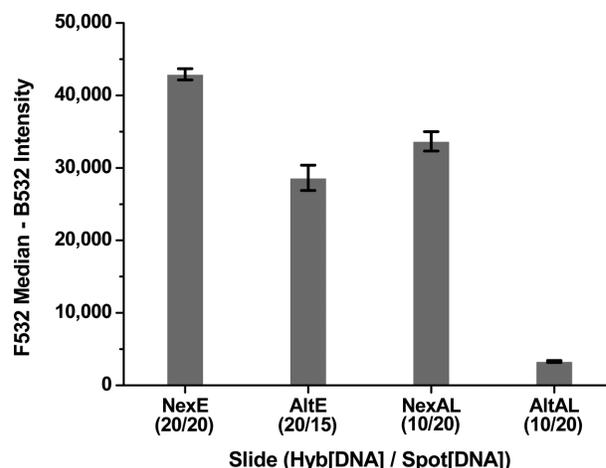


Fig. 4. Determined optimum spotting and hybridization DNA concentration ratios conditions for each slide type - epoxy coated Nexterion® slide E (NexE) and ArrayIt® SuperEpoxy slide (AItE) at 10% glycerol concentration, and aldehyde coated Nexterion® slide AL (NexAL) and ArrayIt® SuperAldehyde slide (AItAL) at no glycerol content. Error bars represent the standard deviation (SD).

had the second strongest, with the other epoxy-coated AItE slide by ArrayIt® not far behind as presented on Fig. 4. While the AItAL slide, the other aldehyde coating variant, did not perform well at all.

It was apparent that the background intensity increased as the hybridization DNA concentrations increased. It was also noticed that both NexE and NexAL slides exhibited greater background intensities than the ArrayIt® equivalents. The increase in background intensities as the hybridization DNA concentration increased was most drastically evident in the aldehyde slides, which became a mess at any concentration above 15 μM. Once it was perceptible that the aldehyde slides were not providing reliable data at higher hybridization concentrations, a second post-hybridization wash was conducted in a more rigorous fashion in an attempt to reduce background signal. The NexAL slide yielded a lowered background without serious loss of spot intensity, though the AItAL slide lost a great deal of spot intensity along with the background. Examining the S/N ratios, the trend appeared that the ratio decreased as the hybridization concentration increased. This pattern inversely mirrored that of the background intensity trend. It was also monitored that the epoxy slides generally provided the improved S/N ratios and the Nexterion® slides provided the better S/N ratios as a whole.

Taking into account all factors, selection of optimum DNA profiling condition is very dependable on the characteristics of application.¹² If low background is an impor-

tant factor, then the AItE would be ideal, however the NexAL would be the best if the DNA concentration in hybridization solution is limited. If neither of these factors are an issue, the NexE slide is the most versatile between the two brands tested, yet it is a bit of a tradeoff between a stronger spot signal or lower background signal.

CONCLUSIONS

According to the surface chemistry, four different functionality variants of commercial microarray slides have been examined by comparing the performance levels against two DNA probes with different end linkers at various glycerol concentrations. The epoxy coated NexE and aldehyde coated NexAL slides from Nexterion[®] greatly outperformed aminosilane (SA) and its isothiocyanate-derivative (SAL) coated slides from Genorama[®] regardless of glycerol content or types of linkers. The I-linker demonstrated higher overall intensities than the amino-linker in general and glycerol was just required to be present, only preferable for the epoxy coated NexE slide at the 10% content. The NexE and NexAL slides were further evaluated with the equivalent functionality variations (AItE and AItAL) by ArrayIt[®] in respect of the DNA concentration gradients for spotting and hybridization, in order to adequately optimize the array sensitivity for DNA profiling conditions compatible for each slides. Overall results appear that the epoxy equivalent NexE and AItE slides at 10% glycerol concentration have outperformed the corresponding aldehyde equivalent NexAL and AItAL at no glycerol, and the NexAL slide is some more favorable than the AItE. While this study is limited to particular commercial slides and the detailed chemical information is not disclosed by vendors, this present work successfully demonstrates that the optimum arraying protocols

could be applicable for future microarray spotting. Currently, microarraying factors such as array geometry, spot morphology, and target/probe concentration ratios are underway for protein profiling optimization.

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