Reducing Carryover Contamination During Microarray Printing

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Introduction

Contact printing of microarrays is typically performed with quill pins that have microchannels that serve as reservoirs to hold the sample. The standard volume of the reservoir is 250 nl, with each spot consisting of 600 pl, allowing up to 400 spots to be printed per dip. It is often unnecessary to spot the entire volume of the pin reservoir, so the pins must be washed to completely remove any residual sample prior to uptake of a new one. Thorough washing is especially important when the substance printed is strongly hydrophobic and binds to the surfaces of the pin reservoir. The washing procedure must be stringent enough to eliminate the remaining sample to avoid carryover contamination of the new sample.

The cleaning procedure usually consists of cleaning the pins with distilled water and removing the wash water from the pins with vacuum. Repeating this protocol at least three times greatly reduces the chances of carryover contamination.

The BioOdyssey™ Calligrapher™ miniarrayer is equipped with a static wash bath, a flow-through wash bath, and a vacuum system. We developed a protocol to optimize use of these features to eliminate sample-to-sample carryover during printing. Here we describe the process used to optimize wash parameters to eliminate sample carryover when printing either oligonucleotides or proteins, and present the optimized results.

Methods

Printing Oligonucleotides

To measure the effect of pin washing on sample carryover, successive rows of a solution containing 50 µM unmodified 60-mer oligonucleotide sample or 1x printing buffer alone were printed on two UltraGAPS slides (Corning) from a 384-well source plate using the BioOdyssey Calligrapher miniarrayer equipped with four quill pins. The instrument was programmed to print from a well containing sample, the washes were performed, and then buffer only was printed. This process was replicated eight times.

After printing and drying, the immobilized oligonucleotide was UV-crosslinked at 450 mJ/cm^2 and hybridized at room temperature for 60 min with a Cy3-labeled random 9-mer oligonucleotide probe. Slides were first washed at room temperature with a solution containing 2x SSC and 0.1% SDS for 10 min and then for 3 min with 0.2x SSC alone. Subsequently, the slides were scanned with a laser scanner with the photomultiplier tube (PMT) set such that the maximum signal intensity of the oligonucleotide spots was less than saturation (65,000).

Optimized Oligonucleotide Wash Program

The wash program protocols for testing carryover of oligonucleotides consisted of the following series of washes with distilled water and removal by vacuum:

Prewash
Step 1: Wash 2, 6 sec
Step 2: Vacuum, 2 sec

Cycle Wash (5 Cycles)
Step 1: Wash 1, 6 sec
Step 2: Wash 2, 2 sec
Step 3: Vacuum, 1 sec

Postwash
Step 1: Wash 1, 6 sec
Step 2: Wash 2, 4 sec
Step 3: Vacuum, 3 sec

Printing Proteins

A similar approach was used to check for carryover on a protein microarray. Rows of anti-IL-1 capture antibody were printed, a wash program performed, and rows of 1x printing buffer only were printed on two FAST slides (Schleicher & Schuell BioScience). After capturing the target protein, recombinant IL-1, the protein was detected by incubating the slide first with a solution containing a biotin-labeled anti-IL-1 antibody and then with Cy5-labeled streptavidin.

Optimized Protein Wash Program

The wash program protocol for proteins consisted of the following:

Prewash
Step 1: Wash 2, 6 sec
Step 2: Vacuum, 2 sec

Cycle Wash (5 Cycles)
Step 1: Wash 2, 6 sec
Step 2: Wash 1, 2 sec
Step 3: Vacuum, 1 sec

Postwash
Step 1: Wash 2, 6 sec
Step 2: Wash 1, 4 sec
Step 3: Vacuum, 3 sec

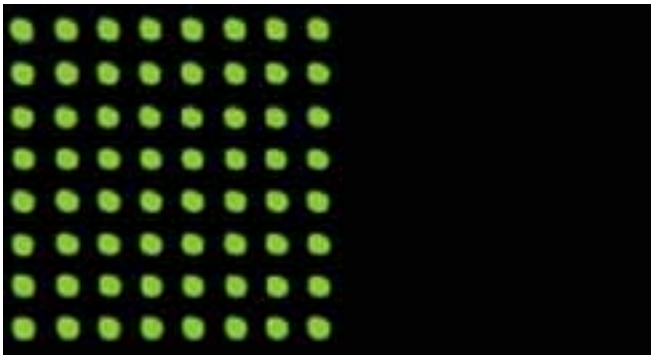

Fig. 1. Image of an 8 x 8 grid generated by one pin for the oligonucleotide carryover assay. Oligonucleotide (left) followed by buffer alone (right) was printed twice on duplicate UltraGAPS slides. Crosslinked oligonucleotide was detected by hybridization to a Cy3-labeled probe. Image is the average result of duplicate slides.

Results

In order to arrive at an optimal wash sequence, several initial tests were performed while varying the time of washing, the numbers of wash cycles, and the vacuum time. Following each assay, the slide was scanned and visually assessed for carryover. A key component of these tests was the proper adjustment of spot intensity by lowering the PMT so that all spots fall within the linear range of the instrument.

The hybridization results for one of the pins used to perform the DNA carryover experiment using the optimized wash protocol are shown in Figure 1. Rows printed with hybridized synthetic oligonucleotide are shown on the left, while spots printed with printing buffer alone are on the right, each in duplicate. No carryover of sample into the buffer controls was detected.

The results of the ELISA-type sandwich assay used to perform the protein carryover experiment, as described in Methods, are shown in Figure 2 for one pin. The detected antibody is shown on the left, while spots printed with printing buffer alone are on the right, each in duplicate. As in the previous experiment using oligonucleotides, no carryover was found for the buffer control spots when printing proteins.

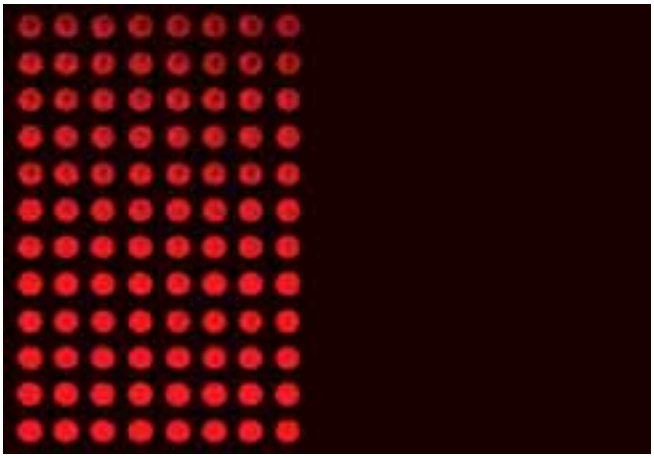

Fig. 2. Image of an 8 x 12 grid generated by one pin for the protein carryover assay. Arrays containing a capture antibody (left) followed by buffer alone (right) were printed twice on duplicate FAST slides. Protein was captured and then detected using a biotin-labeled antibody and Cy5-labeled streptavidin.

Discussion

The success of a microarray experiment is dependent upon many factors. One important factor is the contamination that could occur due to incomplete cleaning of the quill pins between samples during printing of the arrays. As the pins move from well to well, contamination can occur, mixing samples and producing heterogeneous spots on the array. In a typical gene expression analysis experiment, two different cDNA populations differentially labeled with Cy3 and Cy5 dyes are hybridized to the target DNA molecules printed on the slides. The signals resulting from contaminated spots will not be specific due to hybridization of invalid targets, leading to inaccurate results. Carryover in protein arrays is also a cause of concern. High-affinity antibodies can bind to minute amounts of antigen, leading to false positive signals.

The results shown here demonstrate that the BioOdyssey Calligrapher miniarrayer is capable of printing DNA and protein spots that may subsequently be used in hybridization or ELISA-type experiments with low amounts of carryover and nonspecific binding. Results from experiments conducted on arrays free of carryover contamination will have superior accuracy and reproducibility.

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