Technical Note

UHN Microarray Centre

Advalytix SlideBooster™ Hybridisation Station improves hybridisation kinetics for oligonucleotide arrays

- Advalytix SlideBooster™ hybridisation station was compared to our standard hybridisation method for cDNA and oligonucleotide microarrays
- Oligonucleotide microarrays hybridised using the SlideBooster™ had improved signal intensity compared to standard hybridisation methods

Introduction

In a typical microarray experiment, hybridisation occurs within the 20-100 µm gap between the microarray and glass coverslip. Fluor-labelled DNA fragments move within this gap by diffusion, a relatively slow process considering large DNA strands may have to diffuse several centimeters in order to reach the complementary array feature. As a result, low signal-to-noise ratios are possible as only a fraction of the molecules present in the labelled sample may bind to their complimentary DNA features[1]. If the system has not reached its equilibrium state during the 16-18 hour hybridisation incubation, poor reproducibility of microarray data will be likely.

Olympus Advalytix has developed a novel agitation technology based on surface acoustic waves (SAW) to agitate the samples during hybridisation. The SlideBooster™ is a hybridisation station that uses SAW agitation to increase signal-to-noise ratio, improve slide-to-slide reproducibility, enhance homogeneity across the array and can reduce incubation time[1].

The goal of this study was to evaluate the SlideBooster™ hybridisation station and compare the microarray results with those obtained following the UHNMAC standard hybridisation protocol (as described in the Aminoallyl (Indirect) Labelling protocol).

Method

10 µg Human Universal Reference RNA (HURR) and 10 µg HeLa total RNA were labelled, with cyanine3 and cyanine5, respectively, following the UHNMAC Aminoallyl (Indirect) Labelling protocol. Fluor-labelled cDNA was pooled and hybridised to arrays from the same production batch. The arrays were divided into two groups for the hybridisation incubation; one group was placed on the SlideBooster™ hybridisation station (16 hours, 37°C) and the other was placed in plastic hybridisation boxes containing DIG Easy Hyb (for humidity), wrapped in plastic wrap and placed in a dry air incubator (16 hours, 37°C). This experiment was performed using cDNA (UHNMAC Hum19K) and oligonucleotide (UHNMAC Hum34.6K) arrays.

Results

Oligonucleotide arrays hybridised in the SlideBooster™ had higher signal intensity, less background signal, and enhanced homogeneity across the array when compared with the arrays hybridised by the standard method (Figure 1). For the cDNA microarrays, the results from arrays hybridised in the SlideBooster™ hybridisation station were only slightly better compared to those hybridised by the standard method (data not shown).

Discussion

The Advalytix SlideBooster™ hybridisation station did improve the signal intensity, enhance overall signal homogeneity across the array and reduce background for the Hum34.6K oligonucleotide arrays. Further evaluation of the SlideBooster™ showed that other oligonucleotide arrays benefitted from the SAW agitation technology.

We speculate that increased hybridisation efficiency for the oligonucleotide arrays was due, in part, to the small size of the spotted oligos (70-mer) and the fact that oligonucleotide arrays have more specificity than cDNA arrays. Arrays processed on the SlideBooster™ hybridisation station have the fluor-labelled DNA move across the entire array surface, increasing the chances of complementary sequence hybridisation.

In addition to the benefit of improved signal-to-noise, the SAW technology may also allow for less sample RNA to be labelled, by increased detection sensitivity, and for hybridisation incubation times to be shortened. The issue of reduced incubation time has not yet been evaluated.

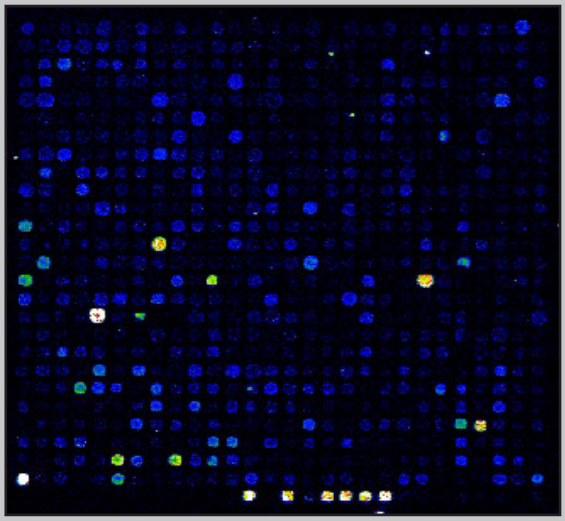
Conclusion

For oligonucleotide microarrays in particular, we recommend hybridisation incubations to be performed on the SlideBooster™ hybridisation station instead of the standard hybridisation method. Use of the SlideBooster™ hybridisation station is available through the UHNMAC Expression Service.

References

1. Advalytix website: http://www.advalytix.com/sb401800__138.htm

A



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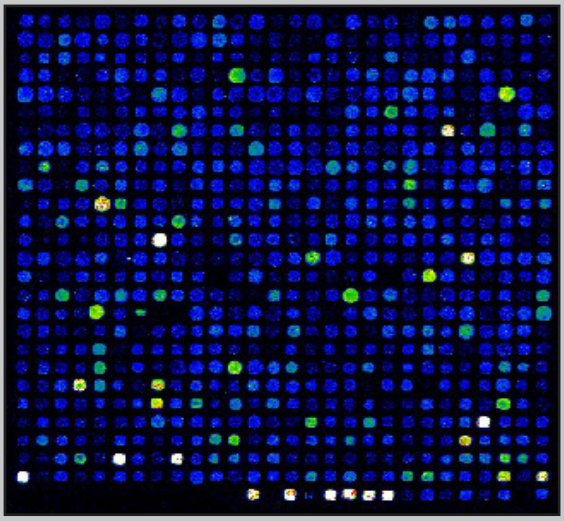

Figure 1. A subarray of the UHNMAC Hum34.6K oligonucleotide array that was hybridised in a plastic hybridisation chamber following the standard UHNMAC hybridisation method (16 hours at 37°C; image A) compared to a subarray from the same production batch hybridised in the SlideBooster™ hybridisation chamber for 16 hours at 37°C (image B). Hybridisation of oligonucleotide arrays in the SlideBooster™ results in images with improved signal intensity and reduced background. Both slides were hybridised with cyanine3-labelled HURR cDNA and cyanine5-labelled HeLa cDNA and scanned on the ScanArray4000 (PE) scanner (laser power 100, PMT 77).