

Analysis of three different fluorescent dyes for cDNA labelling in microarrays

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Conclusion

The three different dye pairs tested here exhibited similar sensitivities – the difference between the number of spots obtained was greater from one wavelength to the other of a dye pair than from one dye pair to another. Bleaching occurred for all dyes tested, also in the dark and most pronounced for the higher wavelength dyes. The signal-to-noise ratios generally were lower for the high wavelength dyes and although in the same range, the Oyster dye pair gave higher signal/noise ratios than the other dye pairs. Thus the new and relatively inexpensive Oyster dyes are suitable for microarray labelling and constitute an alternative to more established microarray dyes.

Materials and Methods

For all dye experiments a liver sample from one pig experimentally infected with the Gram-negative bacterium *Actinobacillus pleuropneumoniae* was compared to a liver sample from a non-infected pig.

An in-house Porcine immune focused Oligo Microarray (POM3) was used. POM3 contains 192 porcine oligoes <75mer, targeting mainly immune related porcine transcripts. This oligo array is spotted on Corning UltraGAPS, using a VersArray ChipWriter Pro.

Three different pairs of fluorescent dyes Oyster 550/650, Alexa 546/647 and Cy3/Cy5 (3DNA, Array 900 - expression array detection kits, Genisphere) were used in separate cDNA synthesis and labeling reactions. Hybridizations and washing were performed according to the manufacturers instructions.

Two slides were hybridized for each dyepair. Each slide was scanned 3 times, immediately after hybridization (first scan), after 1 month of storage in darkness and after 24 hours on the bench (12 hours of daylight and 12 hours of artificial light) using a CCD ArrayWoRxe auto (AppliedPrecision) set to different exposure times (0.3 for 595nm and 1.2 for 685nm).

Microarray image analysis was done using GenePixPro 6.0 (Axon) placing adaptive circle spots and using local background subtraction.

Evaluation was based on 7 genes each represented by 9 spots falling within the middle third of total spot intensity, selected from the Alexa dye experiments.

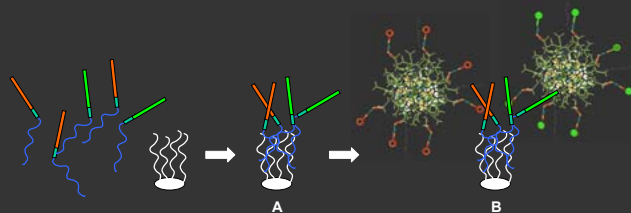


Figure 1. 3DNA 900 (Genisphere) is an indirect labelling kit where the primers used for the cDNA synthesis comprise a specific oligo sequence that can bind to one type of dendrimer carrying ~850 fluorescent molecules. **A:** Hybridization of cDNA to the array. **B:** Hybridization of cDNA specific oligo sequence on the primer.

Introduction

During the last decade transcriptome profiling, by simultaneous measurement of expression for thousands of genes in a single analysis, using microarrays, has become widely used.

To analyse gene expression, mRNA is extracted and transcribed into cDNA and the cDNA is labelled directly or indirectly by means of fluorescent dyes.

Problems with variation in data quality from microarray experiments are well known. These might result from suboptimal signal strength as a result of labelling method or type of fluorescent dye chosen.

Different dyes might exhibit different resistance to photobleaching depending on molecular structure of the fluorochrome used.

To assess variation due to the fluorescent dye chosen, three different dye pairs were tested for labelling of cDNA. Background fluorescence, signal-to-noise ratio, numbers of spots detected, and resistance to photobleaching between the three different dye pairs were evaluated.

Dye	Abs.	Emis.	No of spots detected	
			1.slide	2.slide
Oyster 550	555	574	686	643
Oyster 650	653	672		
Alexa 546	556	573	583	744
Alexa 647	650	665		
Cy3	550	570	642	724
Cy5	649	670		

Table. Absorption and emission wavelengths of the different dyes used in this study. Number of spots detected on two slides of each dye pair is listed. A spot is counted as detected if the signal intensity for either one of the wavelength minus the mean for the respective buffer spots is higher than zero.

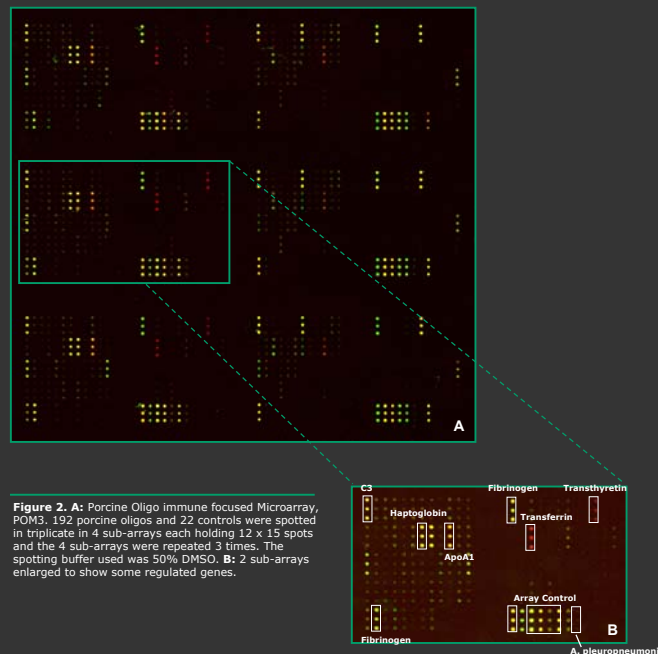


Figure 2. **A:** Porcine Oligo immune focused Microarray, POM3. 192 porcine oligos and 22 controls were spotted in triplicate in 4 sub-arrays each holding 12 x 15 spots and the 4 sub-arrays were repeated 3 times. The spotting buffer used was 50% DMSO. **B:** 2 sub-arrays enlarged to show some regulated genes.

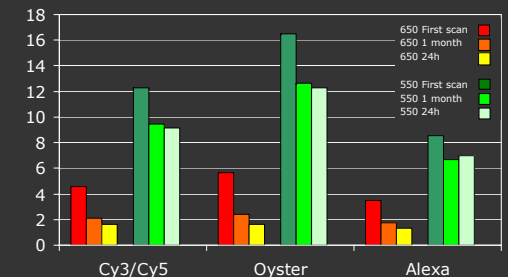


Figure 3. Signal-to-noise ratio based on 7 groups of genes, intensity for each spot was divided by the local background.

Results

Background fluorescence was uniform across the entire array and was not influenced by the fluorescent dye used for cDNA labelling.

Oyster demonstrated a higher signal-to-noise ratio at both wavelengths compared to the two others fluorescent dye pairs. It can be seen that

Oyster 550 had almost twice as high a signal-to-noise ratio as Alexa Flour 546 in the first scan. The high wavelength fluorophores were found to have a much less signal-to-noise ratio for all the tested dyes.

We found the relative rate of photobleaching to be similar, within the same wavelength, for the three dye groups (Figure 4). But there is quite a difference between the two channels in that the long wavelength dyes faint much faster than the short wavelength dyes.

We were surprised to find that 1 month in total darkness had such a large effect on the signal-to-noise ratio compared to the 24 hours on the bench. A possible explanation could be that ozone is an important photo-bleaching factor over time.

No correlation was found between number of spots detected and type of fluorescent dye used (Table 1). The number of spots detected ranged from 583 to 744 both found on the Alexa Flour 546/647 slides.

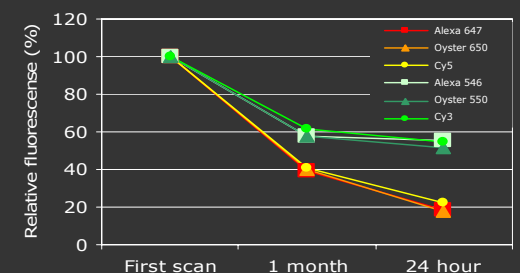


Figure 4. Rate of photobleaching for the different dyes. Relative fluorescence measured as mean signal intensity for the different dyes at three different times. Mean signal intensity measured at the initial scanning is set to 100%.