

Procedure for standardisation and normalisation of cDNA arrays

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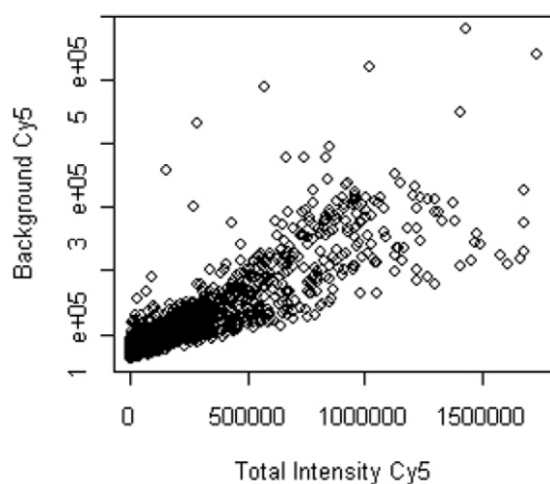
Introduction

Expression levels for large numbers of genes under different conditions can be measured by using microarrays. In livestock species often cDNA-arrays are used for this purpose. cDNA-arrays exhibit larger variability than oligo arrays and therefore require more care in order to reduce noise, standardise and normalise the data, and require some different statistical approaches for analysis because two samples are measured on the same slide, unlike in oligo-array technology

Normalisation procedure

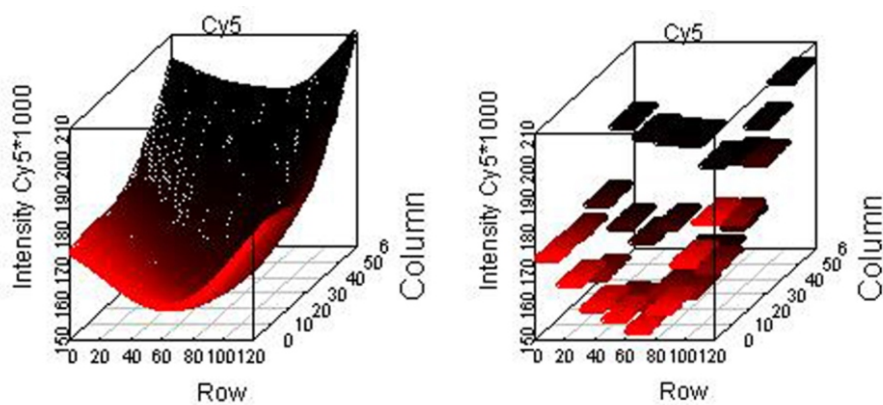
(1) Background correction using blank spots

The local background for each spot given by Analyzer 3.3 showed a clear correlation with spot-intensity ($r = 0.89$). Therefore blank spots were added to the slide in order to correct better for the back-ground introduced during scanning.

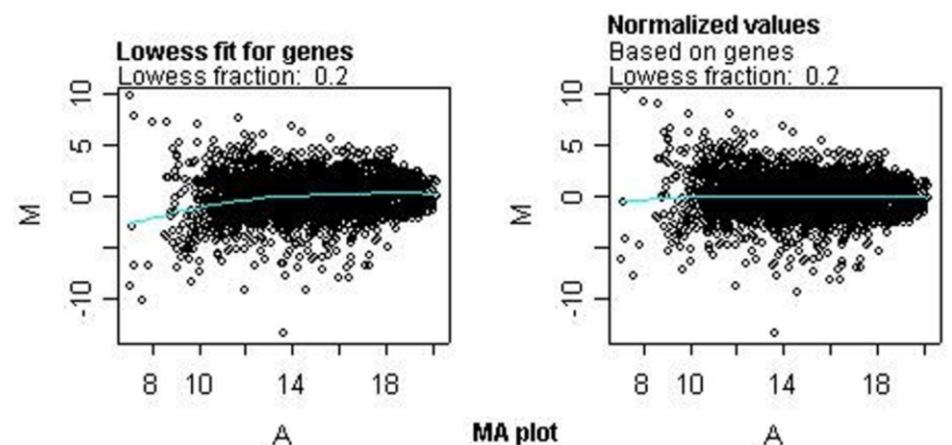


(2) Iteratively reweighted analysis

Before fitting the background on the blanks, those spots were scanned for outliers using iteratively reweighted analysis to allow for a robust background fit. The background was fitted by a spline function (left figure) or the median per patch (right figure).



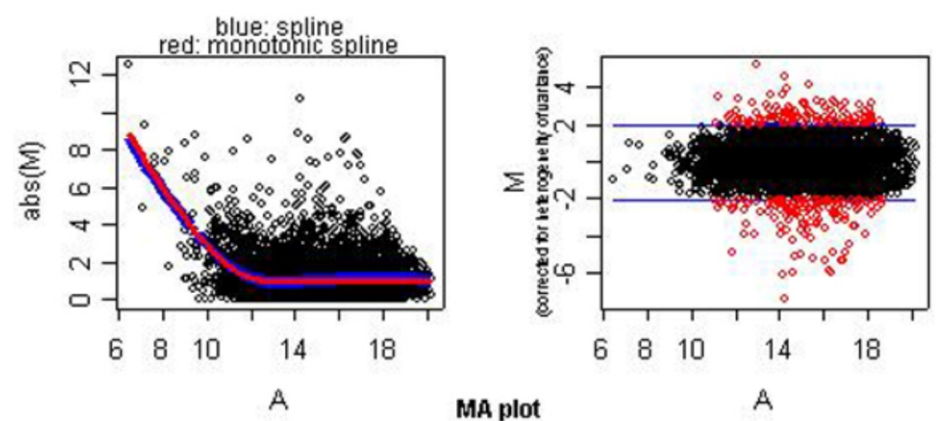
(3) Lowess fit for dye-bias on the ratio's with varying intensity



The $^2\log(\text{Cy5}/\text{Cy3})$ ratios (M-values) clearly differ by the intensity of the spot which is expressed by the A-values [$^2\log(\text{Cy5} * \text{Cy3})/2$]. Fitting a curve ensures dye-bias correction specific for low and high intensity spots.

(4) Identification of poor duplicated values

(5) Fitting a heterogeneous variance contour ...



for sample values to allow for decreasing variance with increasing intensity, used to provide weights for a weighted analysis and further comparison of significant ratio's (red spots) on different slides.

Summary

This poster explains the different steps which are required in a proper analysis of cDNA-microarray data, illustrated on a data set showing differences in gene expression levels between malabsorption syndrome infected and control chickens (Van Hemert et.al., accepted by Animal Biotechnology).