Custom wheat microarray development for analysis of grain quality

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ABSTRACT

Many genes influencing wheat grain quality are expressed during seed development. Custom microarrays have been developed using data produced from previous SAGE (Serial Analysis of Gene Expression) analysis of the wheat genome. A 12K array was designed for each of two time points of the developing wheat grain (14dpa and 30dpa). The arrays contained genes that had shown statistical differences in expression between wheats of varying quality. In addition other genes of specific interest to the authors were included on the slide as were controls. An electrochemical detection system was used for recognition of hybridisation. This process of including only variable genes narrowed the number of data points to be analysed to a more manageable number. This system can therefore be used to analyse a larger number of varieties for genes of interest at a lower cost. This microarray tool should have wide application in wheat quality analysis.

INTRODUCTION

Microarray technology allows the simultaneous expression analysis of large sets of genes of known sequence (Schena *et al.*, 1995). CombiMatrix have developed an electrochemical detection system for oligonucleotide arrays (Ghindilis *et al.*, 2007; Roth *et al.*, 2006). The CombiMatrix system involves a semiconductor matrix of 12,544 individually addressable platinum microelectrodes on which different oligonucleotides can be simultaneously synthesised via digital control (Ghindilis *et al.*, 2007; Roth *et al.*, 2006). The electronics used for the oligonucleotide synthesis are subsequently utilized for the detection of redox active chemistries associated with hybridised target molecules (Ghindilis *et al.*, 2007; Roth *et al.*, 2006). Biotin bound target molecules are labelled with a streptavidin horse radish peroxidase (HRP), the array is exposed to the substrate tetramethylbenzidine (TMB) and hydrogen peroxide, oxidised TMB is reduced at the electrode surface which generates an electrochemical signal that is read with the ElectraSense™ microarray reader (Ghindilis *et al.*, 2007; Roth *et al.*, 2006).

Microarray analysis is an effective tool for plant functional genomics and has been successfully used to explore different aspects of the plant transcriptome (Close *et al.*, 2004; Pacey-Miller et al 2003; Potokina *et al.*, 2004; Potokina *et al.*, 2002; Sreenivasulu *et al.*, 2002; Sreenivasulu *et al.*, 2004; Watson and Henry, 2005).

The benefit of using the SAGE process to determine genes of interest for the Combimatrix array study is that no pre knowledge of the transcriptome is required. The SAGE study identified over 100,000 genes, a large proportion of which however were redundant or remained unchanged throughout experimental time periods. Never-the-less the data still requires processing which is expensive, time consuming and requires huge amounts of computer space. By using SAGE to determine which genes in the transcriptome were of interest due to their increased or decreased expression at various time points we can thus narrow the data points considerably and work more closely with a smaller set of data which we know will be of more interest, thus requiring less data manipulation and being more cost effective.

MATERIALS AND METHODS

Tissue was collected and RNA extracted from fifty wheat varieties at both 14 days and 30 days. CombiMatrix 12K Custom ElectraSense™ arrays were prepared based on genes of interest determined from SAGE (Serial Analysis of Gene Expression) library analysis collected from earlier experimentation as well as genes showing differential expression from Affymetrix data also collected in earlier experiments. Probes were designed based on the Tentative Consensus sequences (TC's) and singletons that were returned as perfect match hits to the LongSAGE tags.

Probes were designed based on these genes. There are 12,544 total features on a CombiMatrix 12k array. In addition to the probes for our genes of interest the chip contained control probes and some blank features. Spike in controls were included in the design to enable determination of the linearity of concentration versus signal.

The target RNA was amplified and labelled with a Kreatech RNA ampULSe: Amplification and Labelling Kit for CombiMatrix arrays with Biotin ULS (Cat. no. EA-026; Kreatech Biotechnology, Amsterdam, The Netherlands). All steps were carried out as per the protocol. aRNA fragmentation was carried out according to the protocol and using fragmentation reagents from Ambion (Cat. no. AM8740; Ambion, Austin, TX, USA). Hybridization and electrochemical detection was achieved using the CombiMatrix Protocol for ElectraSense™ 12K Microarray Hybridization and Electrochemical Detection PTL007 and the CombiMatrix ElectraSense™ Detection Kit (Cat. no. 610027; CombiMatrix, Mukilteo, WA, USA). Arrays were stripped and rehybridised up to four times times each using the CombiMatrix ElectraSense™ Stripping Kit for 12K (Cat. no. 610029; CombiMatrix, Mukilteo, WA, USA) as per the CombiMatrix Stripping and Preparation of ElectraSense™ 12K Microarrays for Re-hybridisation protocol PTL003. An example of what a raw data scan looks like is given in Figure 1. The intensity scan is then transformed into a data table of intensity values.

Background correction and data normalisation on all scans was carried out by Emphron Informatics (Emphron, Queensland, Australia). The data from features for replicate probes was averaged.

RESULTS AND DISCUSSION

Chip quality parameters were determined three ways; the median of the correlation of a chip with every other chip, an Interquartile Range of the chip which ranks the chips and a correlation of the spike in controls. After normalisation the data points for each gene can then directly be compared between chips. Varieties of different milling qualities can be compared and differences in gene expression can be examined in an effort to find correlations between them.

The Electrosense detection method is extremely sensitive. We would however look more closely at the genes with a minimum of a ten fold difference in gene expression. Due to the large number of data points however it is practice to start with the highest fold change differences for the analysis which can be around 100 fold.

Genes of interest can be examined in multiple varieties and represented visually by graphing the expression level of individual genes against the particular parameter of interest, for example starch content (Figure 2).

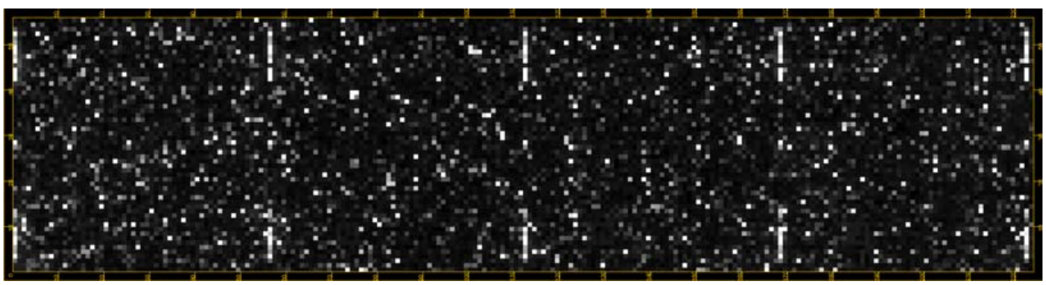

Figure 1. A scan of an Electrosense microarray. Brightness of spots is associated with levels of expression.

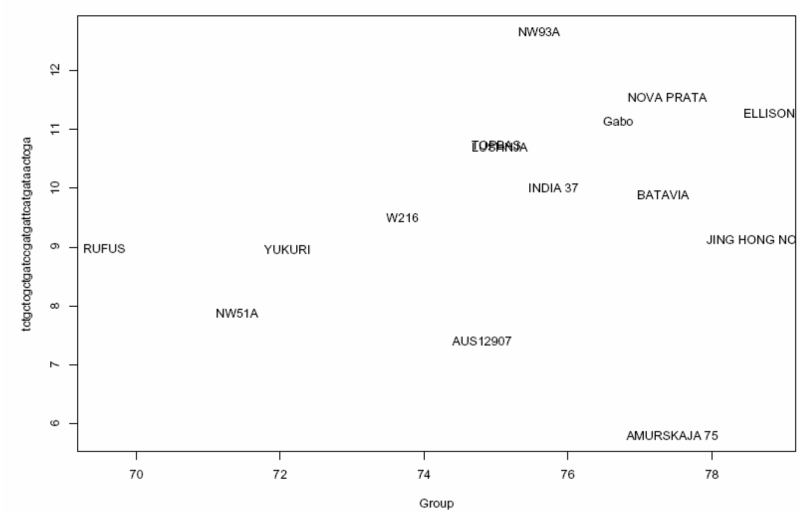

Figure 2. Expression of the gene of interest (y axis) is compared to starch content (x axis) for 15 different varieties

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