Performance of 12000 Feature Oligonucleotide Microarray Manufactured by *in situ* Electrochemical Synthesis of DNA

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Introduction

Oligonucleotide microarrays are becoming an increasingly common tool used by the research community to examine gene expression in biological samples. Scientists rely on data produced by microarray experiments to assess changes in gene expression levels among various experimental tissues and treatments. Biological experiments have a number of inherent variables making it imperative that the microarray platform be extremely reproducible, both to provide confidence in the data collected and to accurately identify small changes in gene expression patterns. Because the most interesting genes are often expressed at the lowest levels in the sample, it is equally important to use a sensitive and reproducible microarray system. There are numerous commercial sources of microarrays available to the research community based on a variety of deposition and synthetic technologies. Most of these platforms require the researcher to use a predefined set of oligonucleotide probes or cDNA sequences as well as significant lead-time to prepare a custom array. Most commercial microarrays require a large expense, as there is often a high cost associated with the purchase of system-specific capital equipment.

CombiMatrix Corporation's CustomArray™ is a high quality, completely customizable array format that provides a cost effective and timely solution for microarray users. Oligonucleotide probes can be defined and designed by the user and supported by the open source CombiMatrix probe design system at no additional cost. In addition, the platform takes advantage of equipment used for scanning microscope slide microarrays that avoids the necessity of purchasing additional equipment. The CustomArray™ is manufactured under stringent conditions and each and every array is quality controlled. The turn around time is typically less than thirty days.

This document describes the platform of the CustomArray™ platform in hybridization experiments using a complex biological sample.

Methodology

A 12,000 feature oligonucleotide array was designed by assembling a list of human genes of interest. The array also contained housekeeping genes, positive and negative control sequences for downstream analysis. Oligonucleotide probes for the entire microarray were designed in one step using a web-based probe design algorithm available at no charge to CustomArray™ users. The probe identification system was designed to find specific, compatible probes for hybridization, and used a range of heuristic including: tendency for hairpin formation, base frequency, randomness, and potential to cross-hybridize to multiple targets. Probes were designed to be 35 to 40 bases in length with Tm's falling within a 5ºC window. Probes were randomly distributed across the surface of the array to generate a layout file which was then used to direct the synthesis of oligonucleotides at individual sites on the array.

Nucleic acid samples were labeled using the Ambion Message Amp II kit according to the manufacturers instructions and hybridized to the arrays according to the protocol recommended by CombiMatrix.

Technology

CustomArray™ uses a specially modified "CMOS" semiconductor to direct the molecular assembly of a specific sequence of DNA bases in response to a digital command. Each feature on the array (a microelectrode) is digitally addressed to selectively generate acid, by means of an electrochemical reaction which, in-turn, controls the detritylation reaction during phosphoramidite synthesis. A software program is used to control the detritylation pattern applied on the chip during synthesis.

Fig 1a (left): 12000 feature CustomArray chip available in 1x3 inch format to fit most microarray scanners (e.g. Axon 4000B)

Fig 1b (right): Microarray synthesizer used to manufacture CustomArray chips

Performance

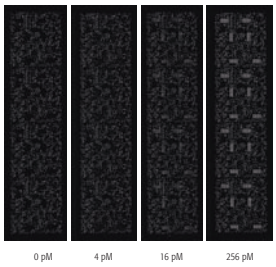

Fig 2a: Image comparing four 12,000 feature chips with the same amount of complex background sample (K-562 CML cell line biotinylated cRNA) with spike-in control transcripts at four different concentrations (0,4,16 and 256 pM). Control transcripts were added just prior to the hybridization. The array was designed with probes directed to the spike-in control transcripts as well as a variety of genes expressed by the K-562 cell line. Replicate probes were spaced across the array to allow measurement of the variability within the array (note obvious pattern on 256 pM array)

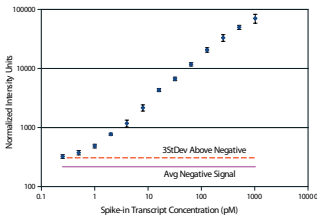

Fig 2b: Sensitivity and dynamic range as measured on the CustomArray 902 system. Three replicate arrays were hybridized at each spike-in concentration examined to assess the inter-array variability. Varying concentrations of spike-in cRNA control transcripts were combined with a constant amount (150 nM) of K-562 cRNA complex background such that final concentration of spike-in control transcripts would range from 0.25 to 1024 pM in the hybridization (mass ratio 1 in 600,000 to 1 in 150).

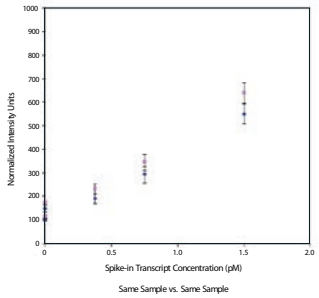

Fig 2c: The lower range of sensitivity and dynamic range as measured on the CustomArray 12K system. Varying concentrations of spike-in cRNA control transcripts were combined with a constant amount (150 nM) of K-562 cRNA complex background such that final concentration of spike-in control transcripts would range from 0.25 to 1024 pM in the hybridization (mass ratio 1 in 600,000 to 1 in 150). Replicate measurements (error bars) on two chips (red and blue) are shown. No difference in the upper end of the dynamic range was observed between 902 and CustomArray 12K.

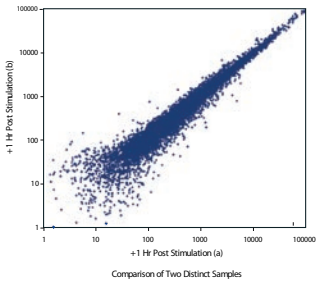

Fig 2d: Array to array reproducibility as measured on Fig 2c: The lower range of sensitivity and dynamic range as measured on the CustomArray 12K system. Varying concentrations of spike-in cRNA control transcripts were combined with a constant amount (150 nM) of K-562 cRNA complex background such that final concentration of spike-in control transcripts would range from 0.25 to 1024 pM in the hybridization (mass ratio 1 in 600,000 to 1 in 150). Replicate measurements (error bars) on two chips.

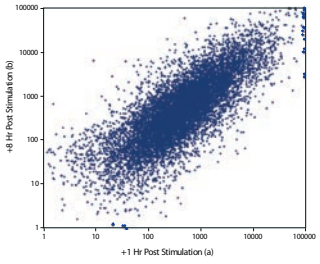

Fig 2e: Dynamic range of two different samples compared on one array. Human monocytes were stimulated with lipopolysaccaride and RNA harvested after 1 hr and 8 hr. Fold expression changes ranged up to 500 fold induction or repression.

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