SMP: Epigenetics

Project: Methylation Pattern Analysis

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Introduction

With an increasing amount of sequence data available in public databases and more than 500 ongoing sequencing projects, biological research has entered a new era. Researchers can easily access to complete genome sequences of more than hundred model organisms. This fact allows the design of an almost infinite number of complex experiments like gene expression profiling. New flexible tools are needed to ensure that these experiments can always be adapted to the rapid growing sequence information.
The GENIOM® plattform is an integrated instrument, providing microarray production, hybridization and detection in a single compact device. Microarray synthesis and hybridization is performed inside a microchannel chip - the DNA processor®. A microfluidic interface connects reservoirs containing synthesis chemicals and hybridization buffers to the DNA processor. The entire microfluidic system is kept in an inert argon atmosphere driving the fluids and providing superior reaction conditions for oligonucleotide synthesis. The fluidic path is a closed system eliminating the need of clean room atmosphere and humidity control during array production.
The DNA processor® is a three-dimensional microchannel chip subdivided into eight segments with individual fluid control - each of them serving as reaction carrier for in situ oligonucleotide synthesis and as hybridisation chamber. Thus, eight independent arrays result from one DNA processor. Each array may be loaded with a different sample which allows parallel hybridization of eight samples. Current density of each array is 6,000 features. Arrays of one DNA processor can be combined if your experiment demands higher density. Defining a complete DNA processor as one array results in 48,000 features.
The DNA processor is held in a cartridge for easy handling and auto-alignment into the instrument. A memory chip on the cartridge ensures correct identification and history tracking

Synthesis - To enable maskless light-activated synthesis geniom® one utilizes a digital micromirror device (DD) which is a grid of 786,432 hinge-mounted mirrors. Deflection of each single micromirror is addressed by software control. In geniom one the micromirror device is illuminated by a high pressure mercury discharge lamp to project light as defined spots onto the DNA processor®

Hybridisation - Amplified and labeled samples are easily injected with a syringe via a port at the instrument's front. Only 30µl of sample are required per array. Positive pressure transports the sample into the DNA processor® microchannels. As an alternative you can use febit's external hybridization unit which is suitable for most hybridization ovens. Performing hybridization externally and synthesizing the next DNA processor at the same time doubles sample throughput. Defined temperature conditions for hybridization are provided by a Peltier element. Hybridization time and temperature are easily programmed with the geniom® software. Hybridization buffers may either be obtained from febit or own buffers may be used. Wash cycles can be added to increase stringencyand optimize signal/noise ratio.

Detection - Different filters facilitate the use of standard fluorescence dyes including Cy3 and Cy5. Detection of dual labeling is possible by simply switching filters. Since geniom® technology uses a CCD camera pictures are taken in milliseconds. Multiple detection is available without considerable bleaching. For one 33µm x 33µm feature about 30 intensity read-outs result from the 8 megapixel CCD camera. Because location of each feature is predefined by the coordinates of corresponding micromirrors automated feature recognition replaces tedious manual spot finding. Signal levels are calculated from the read-outs and stored as raw data file in the geniom data base. Raw data can be analyzed either directly by geniom® software or exported for comparison to other technologies.

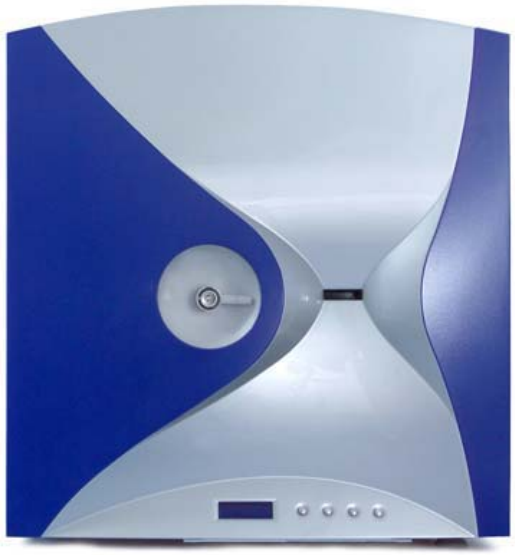

Fig 1: *GENIOM – the benchtop microarray facility.*

Results/Project Status

With its microchannel based reaction carrier the GENIOM platform represents a contained system with direct control over temperature and fluidic processes. Such an integrated system is perfectly suited for carrying out enzymatic reactions in a microarray format.
The so-called primer extension reaction is a prominent example of an application that makes use of the enhanced discrimination power of a polymerase enzyme. The polymerase is capable to distinguish nicely between a perfect matched and a mismatched DNA duplex structure.
Within SMP Epigenetic Profiling this enhanced discrimination power of a polymerase enzyme is employed to distinguish between a methylated and an un-methylated CpG island. Moreover, our goal is to quantify these methylation states
To be able to carry out polymerase reactions for analyzing CpG islands, a new synthesis chemistry has to be established on the GENIOM platform. That chemistry has to generate free 3'-hydroxyls that can be used by a polymerase for chain elongation. Therefore, the synthesis direction for fabrication of the oligonucleotide probes on the microarray has to be reversed. For the reversal of the synthesis direction we have selected a new generation of photolabile phosphoramidite reagents that were developed in Jörg Hoheisels group at the DKFZ. We have started to move this synthesis chemistry onto the GENIOM platform. First results of syntheses carried out with this chemistry revealed similar synthesis yields that have been achieved with the standard synthesis direction on the GENIOM platform before. Besides

providing the special synthesis chemistry reagents on a laboratory scale, next steps include the first simple primer extension reactions to verify the chain elongation reaction within the microchannel based reaction carriers of the GENIOM platform.

Lit.: ***1.*** *Baum M et al. Validation of a novel, fully integrated and flexible microarray benchtop facility for gene expression profiling. Nucleic Acids Res. 2003;31(23);e151.*