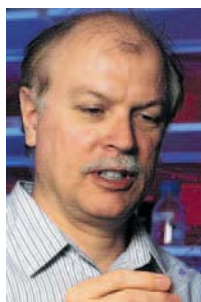


New Visions for Genomic Research



Figure 1: The GenePaint™ thermo-rack module for up to 48 hybridization chambers.



Tecan, in co-operation with Professor Gregor Eichele of the Max-Planck Institute for experimental Endocrinology (Hannover, Germany), has

developed a system that allows automated *in situ* hybridization (ISH). Ten-fold higher throughputs compared to manual processing and improved reproducibility open new doors in functional gene analysis.

HUGO remains a driving force in functional genomics

A large effort over a relatively short period of time resulted in the sequencing and mapping of the human genome. While this achievement provides a great leap towards unraveling the cause of many diseases and their possible treatments, the DNA sequence alone is not sufficient to reach this goal.

The gap between the primary nucleotide sequence and the expression and function of genes still remains a challenge. The next big challenge is to discern which specific parts of the genome code for proteins, the tissue and temporal expression of proteins, and the function of both genes and their expressed proteins. Functional genomics is helping to resolve these tough challenges, and the use of automated systems is as important in functional genomics as it has been in the sequencing of the human genome.

***In situ* hybridization: simple and automated**

ISH is a very promising technique for analyzing the expression and function of genes in different tissues. In order to establish a database of gene expression patterns in different tissues, Professor Gregor Eichele of the Max-Planck Institute for experimental Endocrinology in Hannover, Germany collaborated with Tecan in developing an automated system that works quickly and accurately. When compared with other

techniques this method has a significant advantage as it shows both the expression patterns of genes in tissues and also provides a three dimensional location of the expressed gene at the cellular level. This information is vital to addressing many questions that functional genomics poses.

Professor Eichele states: *“When analyzing various tissues from different organisms, from plants through to humans, we see that certain genes are only expressed in specific cells of the tissue. For example, an oncogene in tumors may only be expressed in a few of the tumor cells. This technique can thus be used as a diagnostic tool, particularly in cases where the sequence of a gene alone does not provide enough information about the disease or condition. Therefore, over the coming years we will need to analyze the expression patterns of thousands of genes.”*

Professor Eichele explains that the potential possibilities that ISH offers is often limited by the number of labor intensive and very time consuming steps. This produces an



Figure 2: Tecan's GenePaint™.

In situ hybridization with GenePaint™ (Figure 2)

- Fully automated system for *in situ* hybridization.
- Additional module based on Tecan's pipetting robot.
- Stable mounting of tissue sections in hybridization chambers.
- Incubation of the sections under moist, nuclease-free conditions.
- Exact and efficient liquid handling.
- Minimal reaction volume (90µl net volume).
- Exact, quickly attained incubation temperatures between 10°C and 65°C.
- Constant moistening of tissues.
- Reliable incubation times and conditions.
- 10-fold increase in throughput compared to manual processing.
- User-friendly operation with Gemini software.

urgent need for an automated system that allows the analysis of thousands of genes.

Principles of GenePaint™

The combined efforts of Professor Eichele and Tecan have resulted in GenePaint™. This system can perform automated ISH in tissue sections. The basis of the system is a Tecan pipetting robot that performs all necessary liquid handling steps, moves plates etc and incubates samples. More than 20 different reagents are pipetted during this complex process. However, the 'real' innovation of GenePaint™, the Hybridization Chamber, is integrated into the pipetting robot. This chamber consists of a microscope slide and a glass plate that is mounted with thin spacers and pressed together. This assembly makes up the extremely thin chamber. Mounted in a temperature-controlled rack (see Fig 1), GenePaint™ can process a total of 48 chambers in one run. Depending on the size of the robot, several thermo-racks can be mounted on the workbench, allowing parallel processing of samples.

Hybridization of the complementary probe is carried out at 55°C and the excess hybridization probe is then removed by washing the slide at 62°C. Temperature control is vital for successful hybridization and the exact temperature has to be maintained constantly throughout these steps.

"Highly precise automated process"

Following hybridization, detection of the bound probe can be carried out using non-radioactive methods. The necessary incubation and washing steps are also performed automatically in the hybridization chambers. Both freeze- and paraffinated tissue sections may be processed with the GenePaint™ System. Paraffinated sections have to be de-paraffinized before the process.

Additional protocols for the system include Fluorescence *in situ* Hybridization (FISH) and Immunostaining. The system can process up to 200 tissue sections simultaneously.

Gene expression in the mouse brain

Using gene expression in the mouse brain as an example we present here this novel method as well as some other results from Professor Eichele's lab.

As a first step, frozen sections of mouse tissue are applied to standard microscope slides, mounted in the GenePaint™ hybridization chambers and positioned in the GenePaint™ thermo-rack and several pre-hybridization steps are carried out (paraffin elimination, proteinase K digestion, etc). Hybridization then takes place with Digoxigenin-marked, gene specific riboprobes (several hours incubation at 50-55°C). Washing steps are carried out under stringent conditions at 55-64°C. Digoxigenin-specific antisera and the following amplification steps allow the

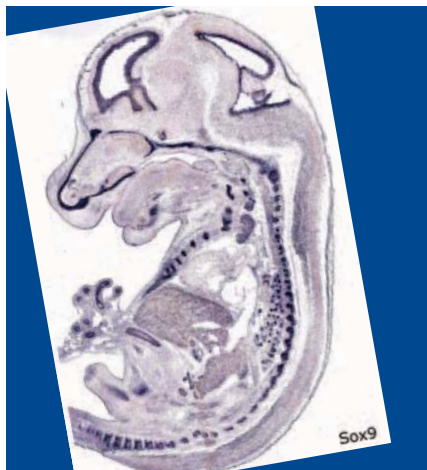


Figure 3: Expression pattern of the Sox9 gene in a mouse embryo (sagittal section).

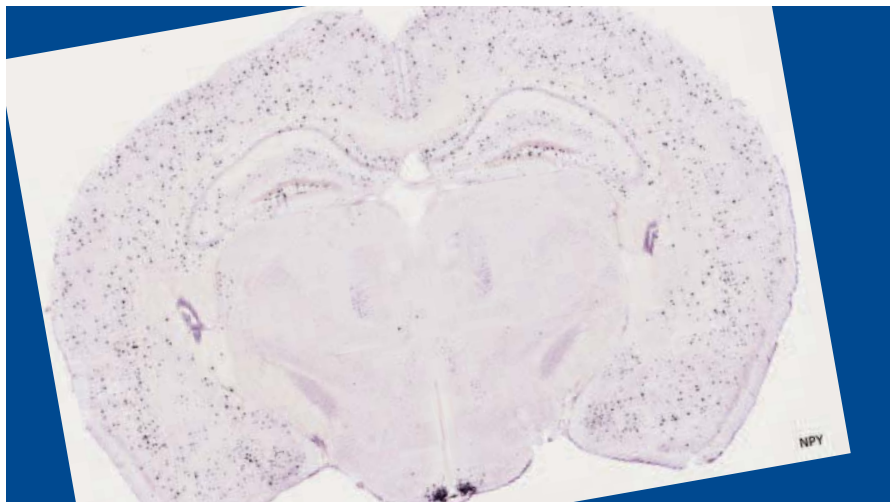


Figure 4: Expression pattern of the NPY gene in mouse brain (coronal section).

detection of riboprobes via a secondary detection system. For the detection of conjugated marker enzymes such as basic phosphatase or peroxidase, appropriate substrates are used.

The results are convincing as Figs 3 and 4 show.

The data presented has been published in Nature (Reymond et al. (2002) Human chromosome 21 gene expression atlas in mouse. Nature 420: 582-586). For more information please visit www.genepaint.org.

Professor Eichele also compared the GenePaint™ system to manual processing in his lab: *“For the manual processing of 20 slides, one lab technician is occupied constantly for almost 2 days. The process requires the operator’s attention almost every minute. Slides have to be dipped into different solutions, reagents have to be pipetted onto the slides, the slides have to be transferred to temperature controlled incubators, etc. Using the automated system, one person can process ten times as many slides in the same timeframe. The throughput is only limited by the number of GenePaint™ systems used. An additional benefit of the system is the great reproducibility of the results due to the highly precise automated process.*

“One person can process ten times as many slides in the same timeframe”

We also noticed that during manual processing many tissue sections are damaged while almost no slides were damaged using the GenePaint™ system. This damage is attributed to constant manipulation and removal of reagents from the slide”.

The judgment of an expert

Professor Eichele is extremely satisfied with the applications and the potential of the GenePaint™ system: *“This application opens up completely new possibilities in functional genomics. Both academic research institutions and pharmaceutical companies have a tremendous need for automated in situ hybridization!”*