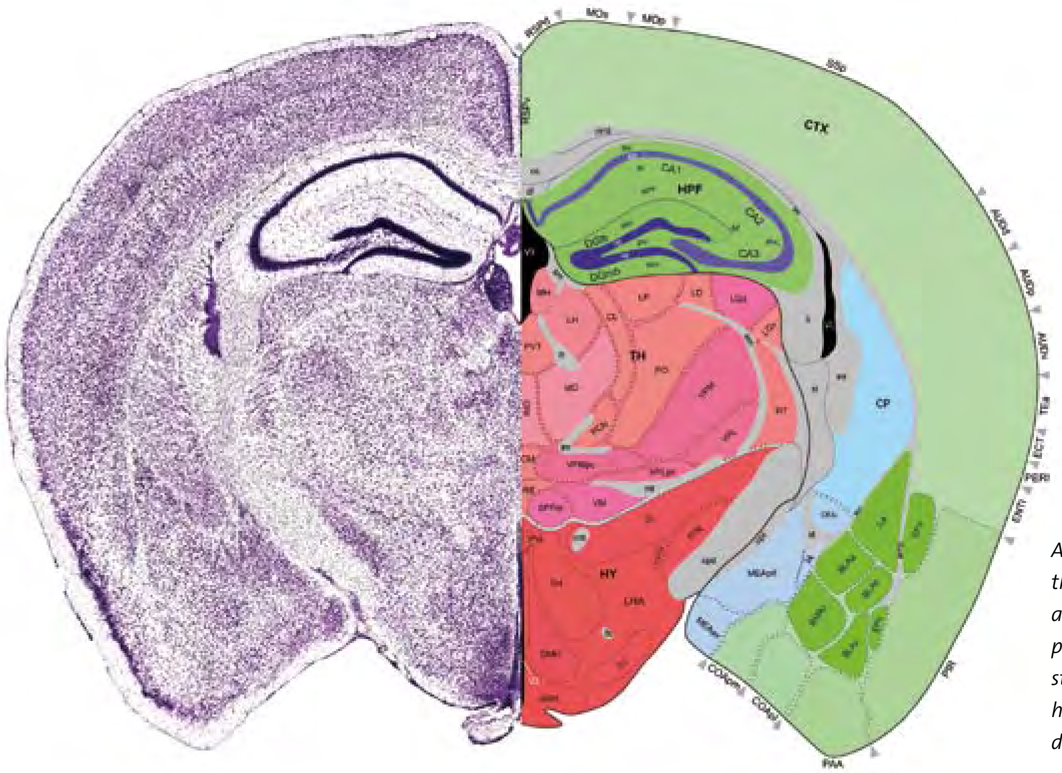

A composite image, taken from the Allen Reference Atlas, of a brain section in the coronal plane. The left half is a Nissl-stained image, and the right half is a graphic illustration delineating the brain structures.

Painting a picture of gene expression throughout the mouse brain

Scientists at the Allen Institute for Brain Science in Seattle, USA, have recently completed the Allen Brain Atlas, a three-dimensional map detailing the expression of over 21,000 genes throughout the mouse brain. This comprehensive, three-year study depended on five Tecan GenePaint™ systems for the automated in situ *hybridization of gene probes in hundreds of thousands of brain sections, providing patterns of expression for every gene down to the cellular level. The atlas is freely available at www.brain-map.org.*

The Allen Institute for Brain Science was launched in 2003, with the major aim of performing innovative basic research on cellular function in the brain and the key principle to make its data and tools freely available to the public as rapidly as possible. The Allen Brain Atlas, the institute's first project, was started in 2003 and completed in September 2006[1], and represented a comprehensive survey of every available, known gene in the genome. "The idea for the Allen Brain Atlas was originally defined when Institute founder and philanthropist Paul Allen convened a group of world class neuroscientists to answer the question 'What is the one thing that could make

Multiple assembly of slides containing brain sections into the flow-through chambers

the biggest difference in the field of brain science?’,” explained Kelly Overly, Ph.D., Research Alliance Manager at the Allen Institute.

The three-year project mapped the expression of over 21,000 genes using an automated *in situ* hybridization (ISH) platform consisting of five GenePaint systems, Tecan’s innovative platform for a variety of automated ISH, fluorescence ISH (FISH) and immunohistochemistry applications. “The automated GenePaint platform was a core part of the project,” Kelly said. “To put it in context, one of our scientists worked in a traditional academic research laboratory prior to joining the atlas project and he mapped just ten genes in five years!”

The Allen Institute originally chose GenePaint because of the previous success that Dr Gregor Eichele, one of the scientific advisory board members, had experienced with the platform. “We scaled up the application that Dr Eichele had set up at the Baylor College of Medicine in Houston, Texas,” explained Paul Wohnoutka, Director, Atlas Operations. “We began with two Tecan GenePaint systems and, over a nine-month period, built this up to five systems. It was very important that we had a sufficiently high throughput platform to meet our demands, and the GenePaint systems seemed to be the best available option. Another critical factor is the quality of the data, which has been sufficiently high for the scientific community to accept. The platform is also very easy to use and we have found it to be really reliable.”

Each gene has been individually mapped out across a series of 16 to 20 sagittal brain sections (25 µm thick) taken from fresh-frozen mouse brains (56 day-old, male C57BL/6J mice). In addition, a subset of the genes was also mapped across a series of approximately 60 coronal brain sections. The institute’s molecular biology laboratory designed and developed the probes for every gene of interest and these were automatically hybridized to the brain sections using the Tecan GenePaint systems.

“We simply place the slides onto the GenePaint systems for automated labeling and staining of the sections,” said Kelly. “We used existing ISH protocols that we adapted for the the GenePaint system.”

Each GenePaint processing cycle takes between 22.5 to 23 hours to run a batch of 192 slides so, with five systems, the researchers can process nearly 1,000 slides in parallel every day. The hybridized slides are then taken to the institute’s automated imaging facility, a series of microscopes equipped with digital cameras and driven by software programs that allow numerous slides to be loaded onto the microscope for individual imaging. The microscopes’ autofocusing algorithms can identify where each tissue section is on the slide and then take a series of images from each section at high magnification (allowing cellular-level resolution). Each series of pictures is then stitched together to produce a high resolution image of the entire tissue section. One specific cross-section of the brain is a composite of 100 images on average, up to a high of 140 images; these composite images can be freely viewed using the on-line Allen Brain Atlas application. The atlas includes functions for zooming in or out of the images as well as the Brain Explorer visualization tool, which provides computationally reconstructed three-dimensional images of individual gene expression patterns throughout the whole brain.

Assembled flow-through chamber with slide carrying brain tissue sections after in situ *hybridization (ISH). The tissue sections show particular genes (purple precipitation) in specific areas of the mouse brain*

"Since its completion in 2006, we have continued to enhance the atlas with the aim of making it increasingly useful to the scientific community," Kelly added. "Our software developers and bioinformatics teams are developing more browsing and analysis tools to allow researchers to readily find those pieces of information that are most relevant to their work, or to identify biologically relevant patterns that might only be seen with such a comprehensive collection of data. We are also continuing our annotation of the data, and all five of the GenePaint systems are still being used for ongoing projects."

The atlas website receives approximately 10 million hits per month and a number of peer-reviewed research papers have already been published that use data from the atlas, including a study of human memory[2] and another study that looked at glial cell differentiation and potential risk factors for multiple sclerosis[3].

The Allen Institute has started to apply the GenePaint systems to additional projects. "Now that we have this great automated platform and infrastructure for high throughput ISH, we are applying it to a diversity of collaborative research projects with outside institutions, thus further helping the scientific community," Kelly said. "We are also beginning some work on a particular part of the human brain, the neocortex, and are exploring opportunities to help advance research on brain diseases and disorders, such as autism, epilepsy and schizophrenia."

References
1. Lein ES et al. (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature 445: 168-176
2. Papassotiropoulos A et al. (2006). Common Kibra alleles are associated with human memory performance. Science 314: 475-478
3. Dugas JC et al. (2006). Functional genomic analysis of oligodendrocyte differentiation. J Neurosci 26: 10967-10983

Four chamber racks containing a total of 192 flow-through chambers on a Freedom EVO 200. Eight pipetting tips are used to pipette various reagents, buffers and wash solutions onto the slides at precisely programmed intervals, allowing the ISH procedure to be performed in an automated fashion.