

BCM SOP: *In Situ* Hybridization

We use *in situ* hybridization to detect specific RNA expression within sections of mouse lung using non-radioactive digoxigenin- (DIG-) labeled probes. We use several signal amplification steps, including tyramide.

The *in situ* hybridization protocol uses a Tecan liquid-handling robot with GenePaint technology (Fig. 1) that was developed by Dr. Gregor Eichele at Baylor College of Medicine and the Max Planck Institute. Detailed protocols of the method have been published and are recommended for more detail:

- a) Yaylaoglu MB, Titmus A, Visel A, Alvarez-Bolado G, Thaller C, Eichele G. (2005) Comprehensive expression atlas of fibroblast growth factors and their receptors generated by a novel robotic *in situ* hybridization platform. Dev Dyn. 234:371-86.
- b) Carson J, Thaller C, Eichele G. (2002) A transcriptome atlas of the mouse brain at cellular resolution. Curr Opin Neurobiol. 12:562-565.
- c) The website www.genepaint.org



Fig. 1 Tecan EVO GenePaint liquid handling robot

In brief, slides carrying tissue sections are inserted into flow-through chambers, which are then placed into a temperature-controlled rack positioned onto a Tecan EVO

GenePaint liquid handling platform. All solutions are added using a computer-controlled liquid handling system. Water circulator baths that are regulated by the software that controls the liquid handling system control the temperature of both the slide racks and the solutions.

The following main steps are carried out by the Tecan EVO GenePaint robot in an automated manner:

- blocking steps required throughout the procedure
- washing steps required throughout the procedure
- proteinase K digestion
- paraformaldehyde fixation
- prehybridization
- hybridization
- stringency washes
- incubation with anti-DIG antibody coupled to peroxidase
- tyramide-biotin amplification reaction
- binding of streptavidin-alkaline phosphatase to biotin
- colorimetric detection of phosphatase moiety using BCIP/NBT reagents

A fully loaded machine can process 192 slides in 24 hours.

Following the *in situ* hybridization process the slides are disassembled from the Tecan flow-through chambers, rinsed in water and coverslipped in Hydro-Matrix mounting medium (Micro-Tech-Lab, Austria) using a Leica CV5000 automatic coverslipper.