

BCM SOP: Tissue sectioning

This protocol is based on previously published methods by this lab and modified by Dr. Cecilia Ljungberg (February 2015) to suit the current study.

All mouse tissue is prepared as fresh frozen sections. For tissue preparation, see “BCM SOP: Mouse Tissue Harvest and Processing for *In Situ* Hybridization”.

References:

- 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

A. Cryostat sectioning

The fresh frozen embryo and postnatal specimens are sectioned at 20 µm on Leica CM3050S cryostats. Sections from each specimen are collected on Superfrost Plus slides and organized as 8 sets with 4 sections per slide.

E16.5 and E18.5 sections are collected as intact torsos, ventral to dorsal. Postnatal lung sections are collected separately for different lobes, except sections for right upper and middle lobes, which are collected together.

E14.5 embryo sections (used for probe validation) are collected as 25 µm sagittal sections (only sections including lungs collected) and organized as 6 sets with 4 sections per slide.

B. Fixation, Acetylation, Dehydration

Following sectioning, the sections are allowed to air dry onto the slides at room temperature for a minimum of 30 min and then fixed and acetylated.

The acetylation is important to reduce non-specific probe binding to the tissue sections.

All steps performed on a Leica XL autostainer, except the acetylation which is performed manually.

1. Fix tissue in 4% paraformaldehyde (in PBS) for 20 min
2. Wash in PBS 5 min
3. Equilibrate slides briefly in 0.1 M triethanolamine (TEA)
4. Acetylate in two changes of freshly made 0.25% v/v acetic anhydride in 0.1 M TEA, 5 min each
5. Dehydrate tissue through a graded series containing 50%, 70%, 95% and 100% ethanol
6. Air dry slides, check quality of sections microscopically, then store slides at -80°C in boxes containing a drying agent and sealed with electrical tape