

Morphological Analysis of Zebrafish Embryos Using JB-4 Resin

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The zebrafish *Danio rerio* presents a unique opportunity for using forward genetics to decipher regulatory pathways central in early vertebrate development. We are using histological methods to better characterize mutant organ patterning defects. We have optimized protocols in our lab for orienting and embedding zebrafish embryos in JB-4 Plus plastic resin. Using JB-4 Plus plastic resin, we achieve excellent structural tissue preservation. We have also developed a protocol to use Hematoxylin and Eosin (H&E) staining protocols on JB-4 sections. For example, at 5 days post fertilization, we can clearly detect intact tissue structures such as the neural tube, notochord, gut tube and kidney region via an H&E stain (figure1).

We have also been able to examine mutant phenotypes. For example, a zebrafish mutant we are currently characterizing contains cysts in the pronephric (kidney) region. To characterize the initial cellular changes involved in cyst formation, we have examined renal cellular morphology via an H&E stain. We have observed that the defect appears in the tubular and ductal regions of the pronephros, showing an increased number of cells specifically in the cyst areas (figure 2).

In addition to examining cellular structures, we are interested in using histotechniques to further analyze RNA expression patterns. Because zebrafish are optically clear, we normally use whole mount *in situ* hybridization to analyze RNA expression patterns. To obtain better resolution and determine specifically in what tissues the RNA expression resides, we embedded embryos stained by *in situ* hybridization protocols in JB-4 resin. Shown in figure 3, embedding and sectioning does not remove the *in situ* stain. In this example, we can observe expression of *ptfla*, a marker known to highlight the hindbrain and exocrine pancreas [1]. By utilizing these histological techniques, we will be able to further our understanding of mutant phenotypes at the tissue level.

References

[1] E. Zecchin et al., Evolutionary conserved role of *ptfla* in the specification of exocrine pancreatic fates. *Dev. Biol.* 1, 174-84 (2003).

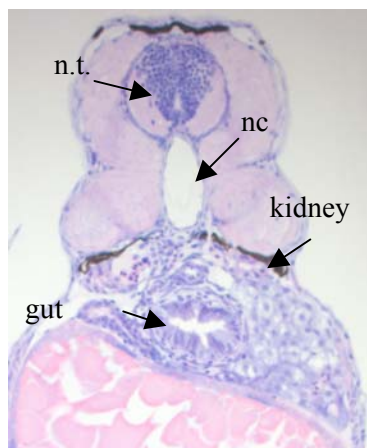


Figure 1: Transverse section (5micron) of a wildtype zebrafish embryo at 5 days post fertilization, stained with Hematoxylin and Eosin (mag=20x) . n.t.=neural tube; nc=notochord

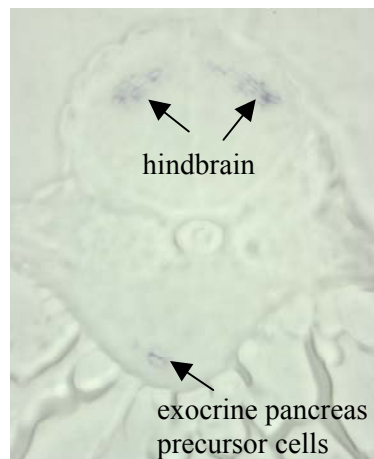


Figure 3: Transverse section (4micron) of a wildtype zebrafish embryo at 36 hours post fertilization. Whole mount RNA *in situ* hybridization with *ptfla* embedded in JB-4 resin, showing staining in the hindbrain and cells fated to become exocrine pancreas (mag=64x).

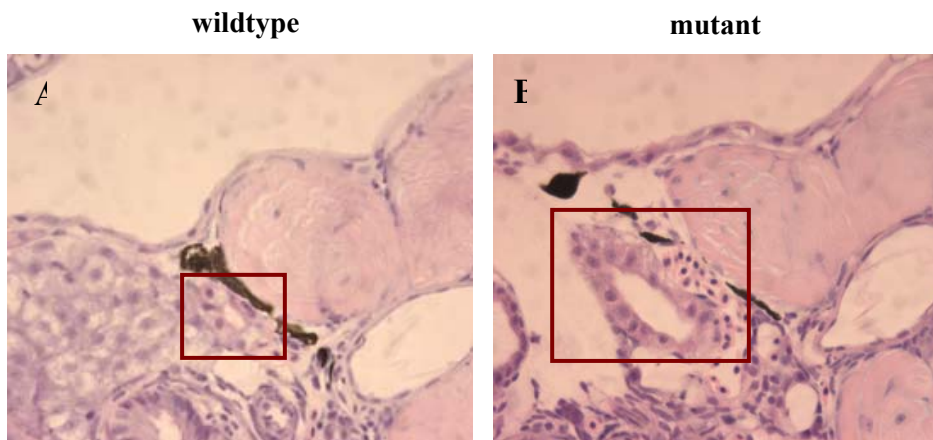


Figure 2: Transverse section (5 micron) of a wildtype and mutant zebrafish embryo at 5 days post fertilization, stained with Hematoxylin and Eosin (mag=64x) . **A:** Wildtype pronephric duct, composed of approximately 7 cells. **B:** locke mutant pronephric duct, composed of about approximately 14 cells.