

# Determination of Cellular Localization of ISH Patterns

## How the Gaido group determines cellular localization of ISH patterns.

Anatomical gene expression on 10 micron cryosections of the fetal testis is determined by examining the fluorescent ISH signal by itself and in combination with a fluorescent nuclear stain (DAPI). Invariably, ISH signal is associated closely with the periphery of nuclei, and this observation is used to assign cell type expression.

Initially, seminiferous cords and interstitium are identified by the spheroid DAPI pattern of the cords. Moving from the center of the seminiferous cord to its exterior wall, gonocytes, Sertoli cells, and myoid cells are encountered. Gonocyte nuclei have a lighter DAPI staining pattern and are larger compared to Sertoli cell nuclei.

The first nuclei encountered just exterior to the gonocytes and lining the periphery of each cord are Sertoli cell nuclei. Generally, Sertoli cell nuclei are arranged in a single layer around the entire cord; however, tangential cord sections contain clusters of several Sertoli cell nuclei. Just exterior to Sertoli cell nuclei are found myoid cell nuclei. Occasionally, a flattened nucleus indicative of myoid cells is observed. Invariably, ISH signal is associated closely with the periphery of nuclei and sometimes over nuclei, and this observation at 40X magnification (as well as overall pattern at 10X magnification) is used to assign cell type expression.

Specific criteria for identifying expression in each seminiferous cord cell type:

### **Gonocyte**

- 1) Expression found in the central portion of seminiferous cords at 10X.
- 2) No expression seen surrounding Sertoli cell nuclei at 40X.

### **Sertoli cell**

- 1) Expression seen surrounding Sertoli cell nuclei at 40X.
- 2) Like Sertoli cell cytoplasm, expression signal may extend into the center of the seminiferous cord and surround germ cell nuclei. However, Sertoli-specific expression may show a negative halo around gonocyte nuclei at 40X, but this observation must be made for several gonocyte nuclei to conclude negative expression in gonocytes.

### **Myoid cell**

- 1) Expression pattern at the periphery of seminiferous cords at 10X magnification.
- 2) At 40X magnification, expression associated with nuclei just exterior (away from the seminiferous cord center) to Sertoli cell nuclei.

**Leydig cell**

1) Because Leydig cells are embedded in interstitium with a relatively homogeneous cellular pattern, Leydig cell expression is identified by colocalization with a fluorescent *Cyp17a1* probe.