

Annotation Guidelines for Wholemout *In Situ* Hybridization

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Annotation

The expression patterns in the kidney were scored in the following categories:

- Ureteric tip
- Ureteric trunk
- Cap mesenchyme
- Rest of Interstitium
- Early tubule
- Late tubule
- Developing vasculature
- Ureter

For extra-renal signals, the scoring is broken down into bladder, urethra, testis, male associated reproductive structures, ovary, female associated reproductive structures, and adrenal glands. For genes with only one urogenital system (UGS) sample, the sex of the sample is specified (male, female).

Ureteric tip: Expression in the ureteric tip.

Ureteric trunk: Expression in the ureteric trunk. A gene that is expressed throughout the ureteric bud is scored as “present” for both ureteric tip and ureteric trunk.

Cap mesenchyme: a horseshoe pattern enclosing the ureteric tip.

The rest of Interstitium: a honeycomb pattern encasing the cap condensates or other non-epithelial pattern not in the cap mesenchyme or developing vasculature

Early tubule: Renal vesicles and (part of) S-shaped body. Superficial, dots with higher density than late tubule expression.

Late tubule: Renal tubules, tubules in developing nephrons beyond S-shaped body stage. Some with curly tubule shape, others are dots deeper in the kidney with far lower density than early tubules.

Developing vasculature: All vasculature expression patterns including major blood vessel, peritubular capillary, glomerular tufts, and invading developing vasculature into the S-shaped body. etc.

Ureter: Ureter epithelium or mesenchyme.

Testis: Anywhere in the testis.

Ovary: anywhere in the ovary

Male associated reproductive structures: mesenchyme and epithelium in the male reproductive system except the testis.

Female associated reproductive structures: mesenchyme and epithelium in the female reproductive system except the ovary.

Urethra: Signals inside or on the surface of the urethra.

Bladder: Signals inside or on the surface of the bladder.

Note:

1. Annotation is performed with the sample under microscope. The images are captured to best represent the in situ hybridization pattern but the 2-D representation may not be able to convey all the 3-D information in the sample due to the complex structure and in situ hybridization signals of the E15.5 uregenital system.
2. There are several technical limitations to the whole-mount in situ hybridization approach that may affect accurate scoring of a gene's detailed expression pattern leading to either false negative or positive scores. These limitations should be borne in mind when reviewing this data. For example, strong superficial reaction within a tissue may obscure internal expression domains resulting in false negative or false positive assignments particularly where a gene is expressed broadly within superficial cell populations.
3. Low-level ubiquitous expression is difficult to distinguish from background activity inherent in the methodology after long periods of detection (typically greater than 24 hours).
4. Trapping of probe in luminal compartments (for example the ureter) may lead to erroneous signals in these structures (typically greater than 24 hours). The scoring attempts to be as accurate as possible within the boundaries of the technical limits of WISH. Other approaches such as section in situ hybridization (SISH) and gene expression profiling can overcome some of these limitations and are an important complement to WISH.