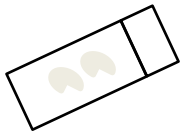
Preparation for in-situ Hybridization

- Tissue dissection and 4% paraformaldehyde fixation
- OCT-embedding (tissue sections) or methanol dehydration (wholemount tissue)
- DIG-Labeled antisense RNA probe Preparation (RNA quality should be checked on agarose gel just before the experiment)
- Solutions, reagents and DEPC water Preparation according to the platform instructions
- Instrument's RNAse-Treatment (Performed by the core facility personnel)
- Cryosections or wholemount hydration process previous to *in situ* hybridization protocol



22 hours (day 1)

- Fixation
- Proteinase K
- Acetylation
- Prehybridization
- Hybridization: DIG-labeled Probe

<u>Overnight stringent washes</u>:

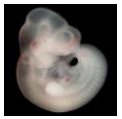
- 5xSSC
- SSC/Formamide
- TNE Buffer wash
- 2xSSC
- 0.2xSSC

Minimum 9 hours (day 2)

- Blocking
- Anti-DIG-AP antibody Incubation
- Post antibody washes
- AP Substrate Incubation
- Post-detection washes
- Fixation

Mounting and Imaging

Automated *in situ* hybridization on sections



- Proteinase K
- Fixation
- Prehybridization

3 hours (day 1)

Hybridization: DIG-labeled Probe *(outside the instrument)* — Overnight

<u>Stringent washes</u>:

- Post-Hybridization buffer
- Maleic Acid buffer
- Blocking

5 hours (day 2)

Anti-DIG-AP antibody Incubation *(outside the instrument)* — Overnight

Post-antibody washes — 4 hours (day 3)

One step post-antibody wash *(outside the instrument)* — Overnight

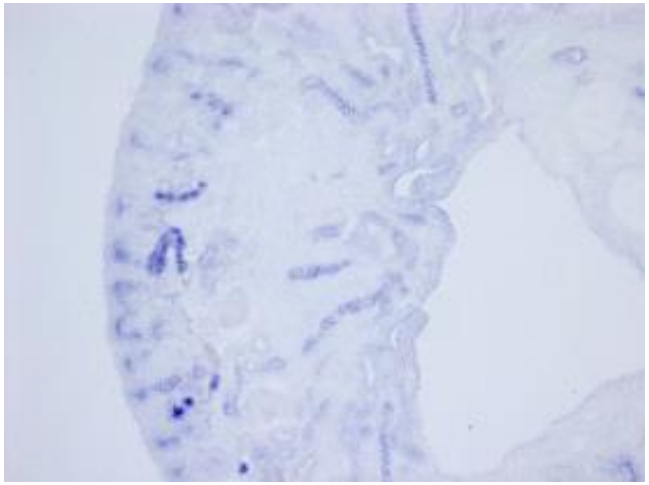
- AP Substrate Incubation
- Post-detection washes
- Fixation

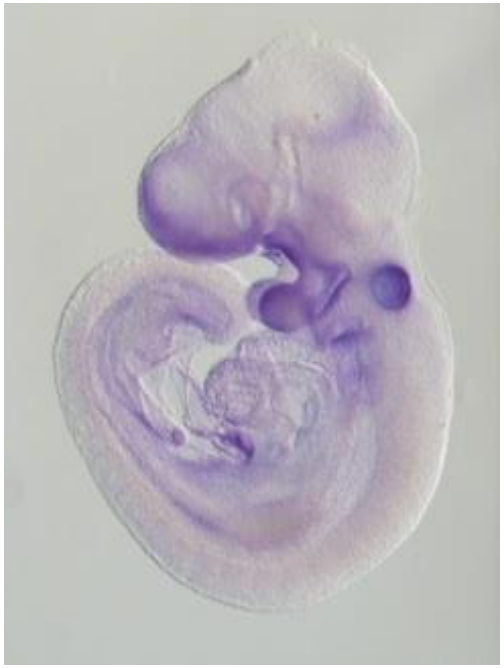
About 5 hours (day 4)

Ethanol dehydration/hydration

Mounting and Imaging

Semi-automated whole-mount *in situ* hybridization: 95% of steps are automated





Results obtained using our Tecan GenePaint system and automated/semi-automated mRNA detection protocol: mWFDC2 RNA detection by *in situ* hybridization on Tecan GenePaint system (Metanephric kidney sections and whole mount E9.5 mouse embryo).