# MOL231:
# Neuronal Gene Expression Analysis – Using a Novel In-Situ Hybridization Technique in an Adult Gastrotrich

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## Abstract

***Lepidodermella squamata*** or hairy belly as it is also called is a widely abundant, microscopic, and aquatic gastrotrich commonly found in lakes and ponds[1]. *L. squamata* exhibits a relatively simple central **nervous system** consisting of an anterior bilobed brain from which two lateral nerve cords extend towards the posterior end[2].

The purpose for this project was to use a novel In-Situ Hybridization (ISH) technique **– the hybridization chain reaction (HCR) –** to study proneuronal gene expression in *L. squamata* and visualize the expression pattern of neuronal genes in adult organisms.

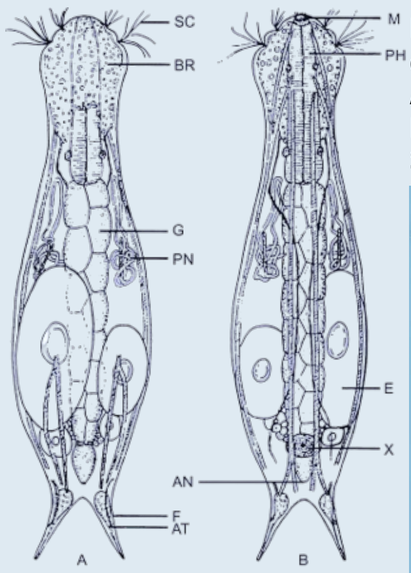



**Figure 1 –** Sketch of a *L. squamata* featuring: (A) dorsal view; (B) ventral view, AT= adhesive tubes, AN=anus, BR=brain, E=egg, F=furca, G=gut, M=mouth, PH=pharynx, PN=protonephridium, SC= sensory cilia[3].


**Figure 2 –** microscopic image of a *L. squamata* compared to a 20µm scale bar. Photo: Proyecto Agua cc-by-nc-sa [4]

## Hybridization Chain Reaction

Fixed animals rehydrated and permeabilized for the HCR probes to migrate the cells → Probes hybridized to their complementary mRNA sequences → Samples washed to remove unbound HCR probes, and then amplified with amplifier hairpins → Samples washed to remove excess amplifier → Samples treated with pre-antibody treatment and primary antibodies → Samples washed to remove excess primary antibodies, and then treated with secondary antibodies → Confocal laser scanning microscopy (Olympus FV3000) → Image processing (Fiji)

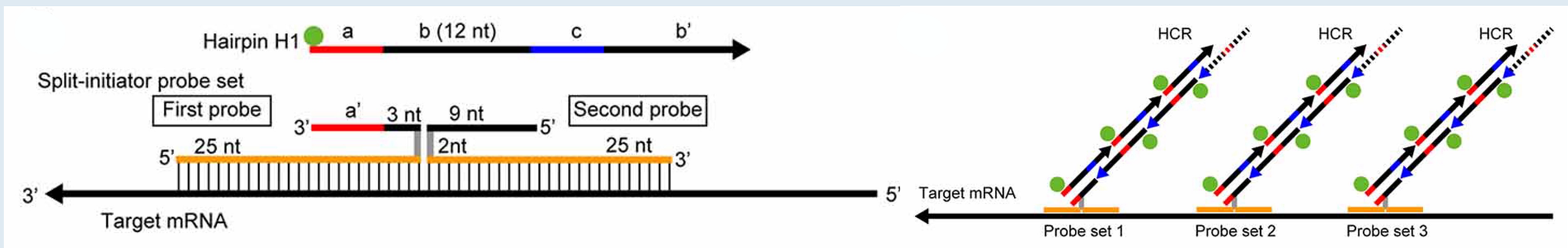
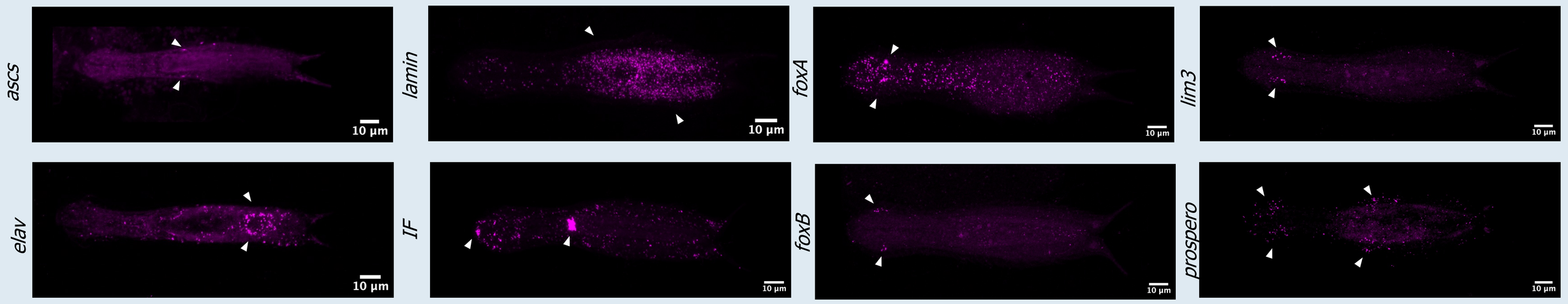
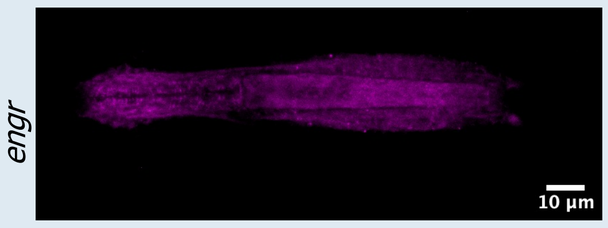
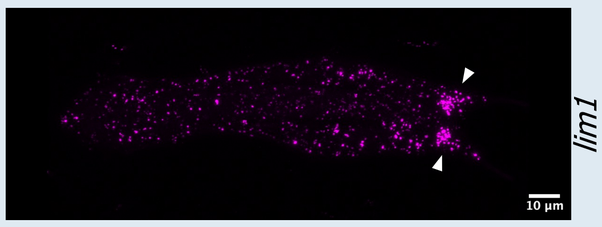



**Figure 3 –** Illustration of the Hybridization Chain Reaction[5].

## Results



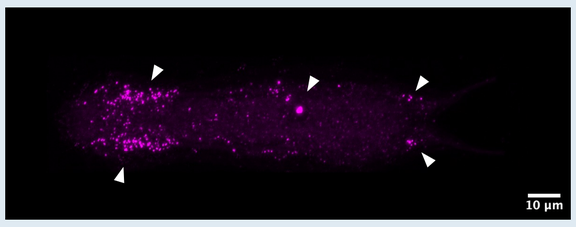




**Figure 3 -** HCR technique in *L. squamatas* for putative neuronal genes. Anterior=left, posterior=right.

## Discussion

**The HCR technique was successfully applied in *L. squamata* specimens.**

We did this using commercial reagents (buffers and probes purchased from Molecular Instruments[6]) and home made reagents (buffers according to the "Hybridization Chain Reaction(HCR) protocol V.1"[7] and probes designed using the in situ generator).

No specific gene expression pattern was identified for engrailed.

This could be due to the -20°C incubation step having been performed at RT, making the specimens less accesible.

**Gene expression was predominantly located in the brain and nervous system for *foxA, foxB, lim1, lim3,* and *prospero*.**

*IF* was expressed in the pharyngeal plug.
*Fer3* was expressed in the posterior located furca.
*Elav* and *lamin* were expressed around a developing egg and reproductive organ, respectively.

**Acknowledgements**
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