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Fluorescence in situ hybridization (FISH)

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ABSTRACT

Fluorescence in situ hybridization (FISH) mRNA

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Protocol status: Working
We use this protocol and it's working

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1 Mouse brain sections were prepared, removed from cryoprotectant solution, and washed three

times in tris-buffered saline (TBS) at room temperature.
Sections were incubated with hydrogen peroxide (ACD) for 15 min at room temperature, washed several times in TBS, and then mounted to Superfrost slides.
Sections were allowed to dry for 10 min and a hydrophobic barrier (PAP pen, Vector Labs) was created around the tissue.
Tissue was incubated in 50% EtOH, then 70% EtOH, then 100% EtOH for 5 min each.
Sections were rehydrated in TBS for several minutes, digested withProteaseIV (ACD) for 25 min at room temperature, and rinsed twice with TBS before proceeding to the RNA Scope Multiplex Fluorescent v2 assay (ACD).

2 The RNA Scope Multiplex Fluorescent v2 assay was conducted according to the manufacturer's instructions, with all incubations taking place in a humidified chamber at 40 °C.
Two 5-minute washes in excess RNA Scope Wash Buffer (ACD) took place between each incubation in sequential order: probes (2-hours), AMP1 (30 min), AMP2 (30 min), AMP3 (15 min), HRP-C1/2/3 (15 min), TSA Cy3 (30 min), HRP blocker (30 min), HRP-C1/2/3 (15 min), and TSA Cy5 (30 min).
Samples were washed twice more in RNA Scope Wash Buffer, then twice more in TBS.
Samples were then blocked and immunostained for tyrosine hydroxylaseas.
After immunostaining, samples were mounted in Fluoromount G and stored at 4 °C for up to 1 week before imaging.