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Visualize gene expression and splice junctions at the single-cell level using the RNAscope® and BaseScope™ *in situ* hybridization assays

Summary

The nervous system consists of numerous specialized cell types that remain to be fully characterized at the molecular level. Due to the high degree of structural and functional heterogeneity and the intricate spatial organization of these cells, it is of special importance to analyze gene expression within the morphological and spatial tissue context. The RNAscope® *in situ* hybridization (ISH) assay enables highly sensitive and specific gene expression visualization within the nervous system. BaseScope™ is a ISH assay that allows visualization of splice junctions between adjacent exons and/or retained introns in order to characterize alternative splicing events in cells and tissues. This application note will highlight case studies for both ISH assays in the nervous system:

- *In situ* detection of G-protein-coupled receptors (GPCRs) in the adult mouse brain using the RNAscope® ISH assay
- *In situ* detection and visualization of EGFRvIII splice variant in human glioblastoma using BaseScope™ ISH assay
- *In situ* detection of circular RNAs in the developing mouse brain using BaseScope™ ISH assay

In situ detection of G-protein coupled receptors (GPCRs) in the adult mouse brain using the RNAscope® ISH Assay

Raising antibodies to G-protein coupled receptors (GPCRs) can be challenging due to difficulties in obtaining suitable antigen accessibility since GPCRs are often expressed at low levels and are very unstable when purified (Hutchings *et al*. 2010 (1)).Here we show examples for the detection of:

- Dopaminergic Receptors
- Cannabinoid Receptor
- Cholinergic Receptor
- Opioid Receptors

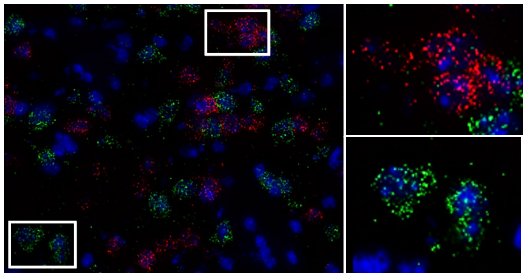


FIGURE 1. Detection of Dopamine Receptor D1 (*Drd1*, Red) and Dopamine Receptor D2 (*Drd2*, Green) in mouse brain striatum using the RNAscope® Multiplex Fluorescent assay on fresh frozen tissue samples. Delineated areas are shown as higher magnification insets. Cells are counterstained with DAPI.

Figures 1-4 provide both fluorescent and chromogenic images for four types of GPCRs - readily detected as mRNA targets by the RNAscope® assay - in hippocampal and striatal normal mouse brain areas: Dopaminergic Receptors D1 and D2 (*Drd1* and *Drd2*), Cannabinoid Receptor 1 (*Cnr1*), Opioid Receptor μ (*OPRM1*), δ (*OPRD1*), and κ (*OPRK1*), and Cholinergic Receptor Muscarinic 3 (*Chrm3*).

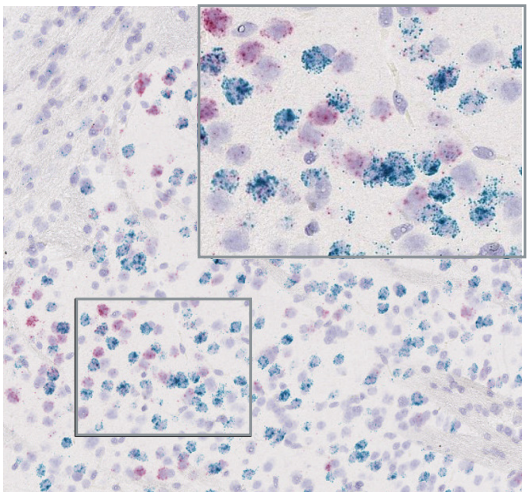


FIGURE 2. Detection of Cannabinoid receptor 1 (*Cnr1*, Red) and Dopaminergic receptor D1 (*Drd1*, Green) in normal mouse brain striatum using the RNAscope® 2.5 HD Duplex Chromogenic assay on FFPE tissue samples. The delineated area is shown as higher magnification inset. Cells are counterstained with hematoxylin.

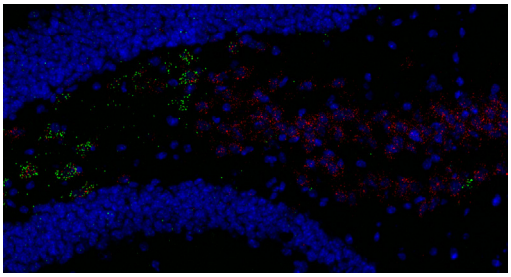


FIGURE 3. Detection of Cholinergic Receptor Muscarinic 3 (*Chrm3*, Red) and Dopamine Receptor D2 (*Drd2*, Green) in normal mouse brain hippocampus using the RNAscope® Multiplex Fluorescent assay on fresh frozen tissue samples. Cells are counterstained with DAPI.

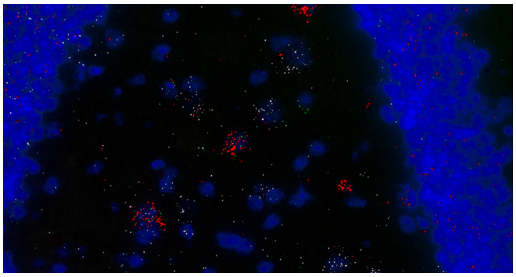


FIGURE 4. Detection of three distinct Opioid receptors in normal mouse brain hippocampus using the RNAscope® Multiplex Fluorescent assay on fresh frozen tissue samples: Opioid Receptor μ (*OPRM1* , *Green*), δ (*OPRD1* , *Red*), and κ (*OPRK1* , *White*). Cells are counterstained with DAPI.

In situ detection and visualization of EGFRvIII splice variant in human glioblastoma using BaseScope™ ISH assay

The epidermal growth factor receptor (EGFR) is overexpressed in a variety of human epithelial tumors, often as a consequence of gene amplification, including primary glioblastoma (GBM). Tumors with EGFR gene amplification frequently contain EGFR gene rearrangements, with the most common extracellular domain mutation being EGFRvIII, which results from an in-frame deletion of exons 2–7. About 50% of GBM patients with EGFR amplification harbor EGFRvIII. However, EGFRvIII can also be present independently of EGFR amplification.

In this case study, made-to-order BaseScope™ probes were used to detect expression of EGFR and EGFRvIII in glioblastoma tissue demonstrating that BaseScope™ assay can be used successfully to :

- Analyze exon junction in FFPE sample
- Visualize low to overexpression of a specific target
- Differentiate expression of wild type and splice variant
- Localize co-expression of wild and mutant target in tissue
- Demonstrate heterogeneous expression within a tissue sample

BaseScope™ assay probe design for EGFRvIII detection

The BaseScope™ assay is a more recently developed product from ACD, based on the same platform of proven and established RNAscope® technology. It enables detection of exon junctions in FFPE tissue with morphological context by using only one ZZ probe uniquely designed on the specific exon junction of interest.

4 BaseScope™ probes have been designed to detect specific exon junctions, as shown in figure 5. Probes E1-E2 and E7-E8 are specific to EGFR, while probe E8-E9 detects both EGFR and EGFRvIII and probe E1-E8 will detect specifically EGFRvIII. 7 samples were analvzed to determine their EGFRvIII status.

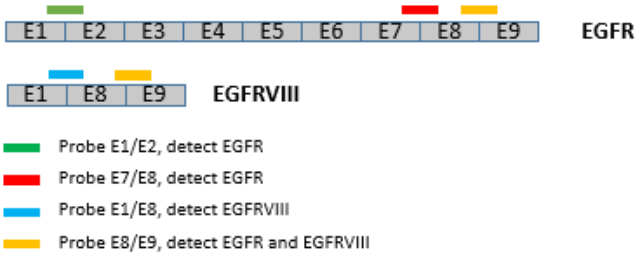
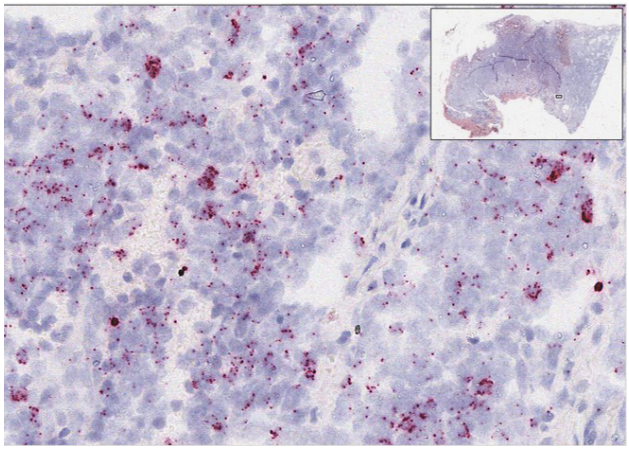
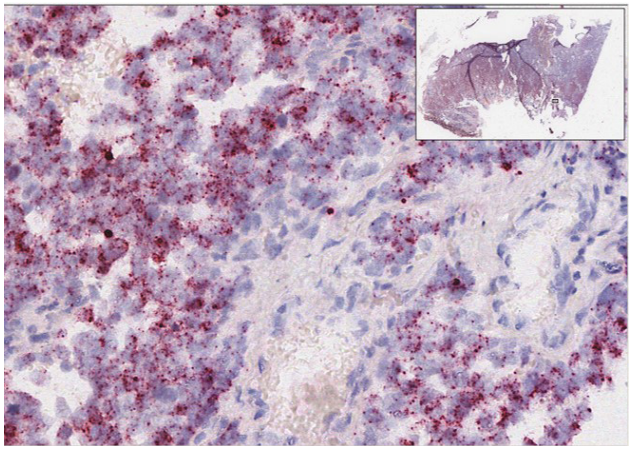


FIGURE 5. Schematic of BaseScope™ probe design for detection of EGFR and EGFRVIII

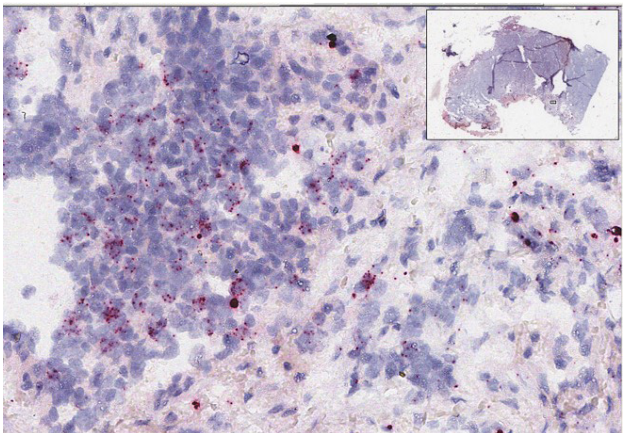
Probe E1/E2 (EGFR)



Probe E1/E8 (EGFRvIII)



Probe E7/E8 (EGFR)



Probe E8/E9 (EGFR & EGFRvIII)

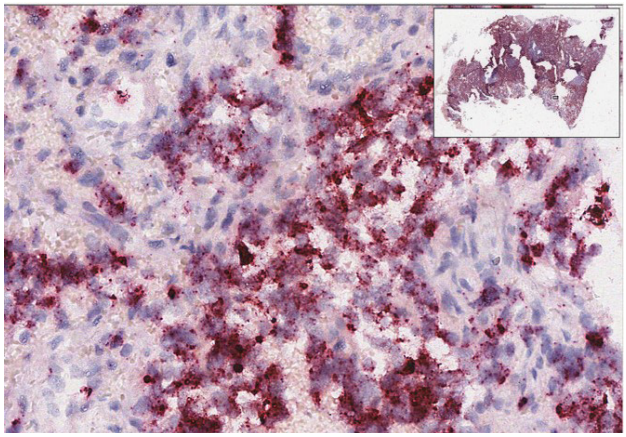


FIGURE 6. A WHO Grade IV glioblastoma case shows expression of EGFR and overexpression of EGFRvIII. Also, heterogeneous target expression within the tissue can be observed.

In situ detection of circular RNAs in the developing mouse brain using BaseScope™ Assay

Recently, a universal class of endogenous noncoding circular RNAs (circRNAs) has piqued the interest of multiple areas of research. In eukaryotes, these particularly stable single-stranded circular RNA molecules are generated by an alternative splicing mechanism that covalently links the 5' end of one exon with the 3' end of another exon. These highly conserved circRNAs are characterized by tissue- and developmental stage-specific expression patterns. More specifically, it was recently shown that circRNAs are particularly enriched in the brain with expression dynamics independent of the linear mRNA transcripts derived from the same gene. Although abundantly expressed in the nervous system, their function remains largely unknown. In addition, evidence has emerged for circRNA involvement in various diseases, including cancer, possibly by regulating gene expression levels through interaction with other molecules. The accurate detection and localization of circRNAs is pivotal to elucidate their biological functions especially given the fact that they could serve as putative clinical biomarkers.

Here, we highlight the anatomical localization and corresponding quantification of the linear mRNA and circRNA splice variants for the brain plasticity-related target *Dlgap1* in the hippocampus by detecting the mRNA-specific and circRNA-specific (head-to-tail junction) exon junction, respectively , in P1, P10 and P30 C57Bl/6J developmental mouse brains using BaseScope™ ISH assay.

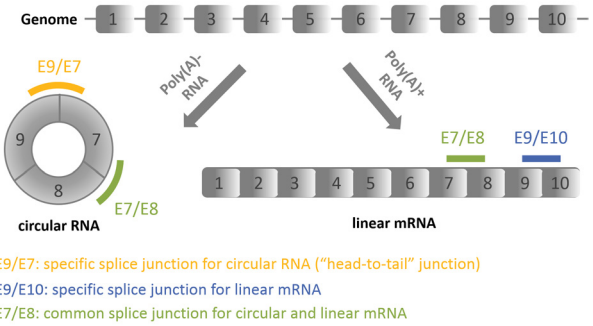


FIGURE 7. BaseScope™ probe design for *Dlgap1* circular RNA, linear mRNA and total RNA detection.

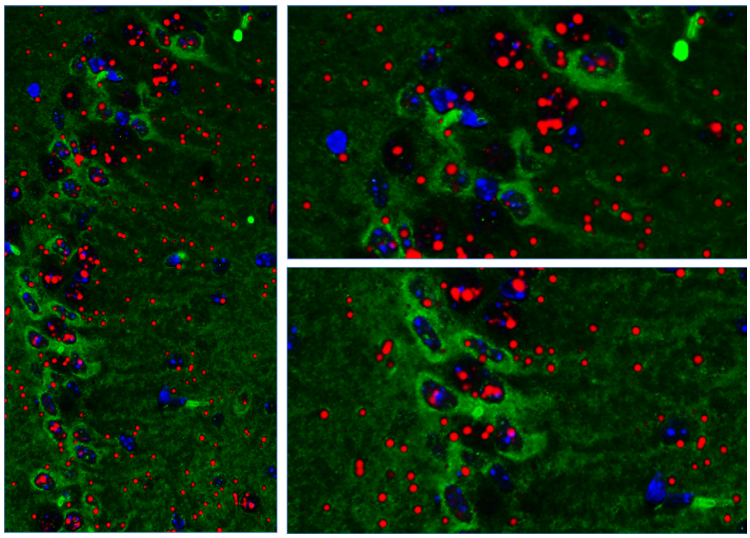


FIGURE 8. Combined ISH for *Dlgap1* circRNA and IHC for MAP2 protein as dendritic marker.

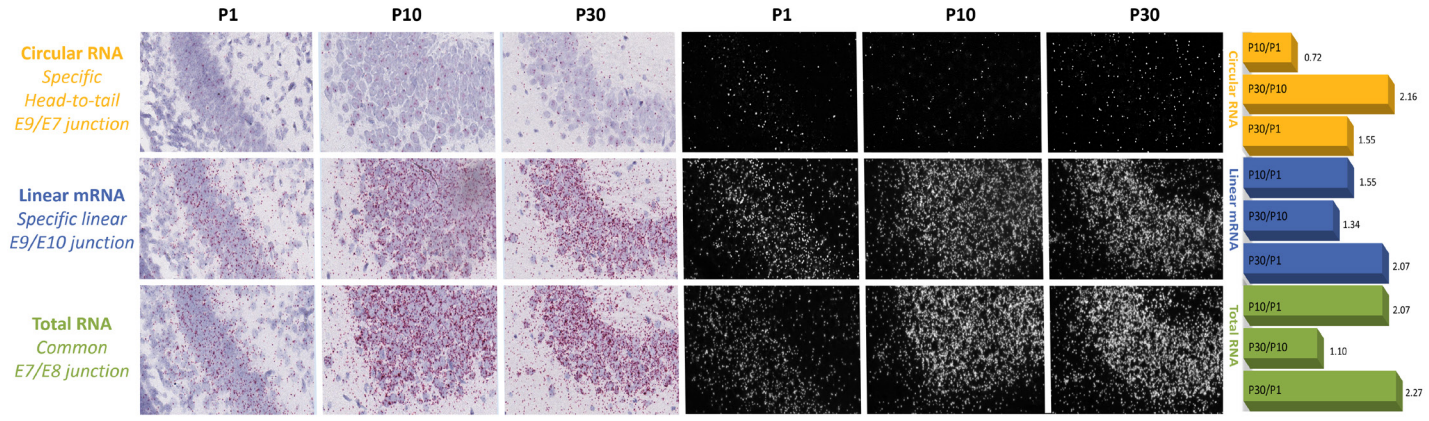


FIGURE 9. Highly sensitive and specific *in situ* detection and quantification of circular RNA, linear mRNA and total RNA for *Dlgap1* in C57Bl/6J developing mouse brain with focus on hippocampal CA3 using BaseScope™ assay.

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