

**Introducing the  
RNAscope™ Plus  
small RNA-RNA  
Fluorescent Assay**

**biotechne®** | **ACD™**

# Combining detection of small regulatory RNA with target RNAs

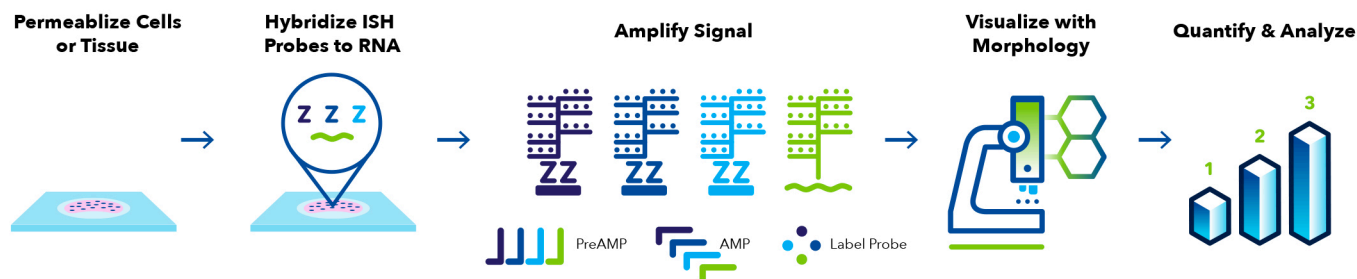
RNAscope™ technology is an advanced *in situ* hybridization assay that allows for the visualization of single-molecule gene expression directly in intact cells/tissues with single cell resolution. Detection of small RNAs (smRNA) requires a robust, highly specific and sensitive assay with quantifiable signal. The new RNAscope™ Plus smRNA-RNA Fluorescent Assay leverages the RNAscope patented core technology that enables signal amplification and background suppression for multiplex visualization of smRNA expression with target mRNAs in in formalin-fixed paraffin-embedded (FFPE) samples and fresh frozen samples.

The RNAscope Plus smRNA-RNA automated Leica or manual Fluorescent Assays use a proprietary method of *in situ* hybridization to simultaneously visualize one smRNA target microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), short interfering RNAs (siRNAs), or antisense oligonucleotides (ASOs) along with three RNA targets.

## Key Features

- Unlock the potential to detect small regulatory RNAs along with mRNA targets.
- Visualize ASOs, siRNAs, miRNAs, piRNAs and other elusive smRNA sequences with the signal boosting tyramide amplification system (TSA) chemistry.
- Interrogate smRNA biomarkers/therapeutics with cell-specific/morphology markers.
- Assess smRNA therapeutic delivery and biodistribution.
- Add a visual dimension to interrogate heterogeneous tissues and evaluate therapeutic efficacy.

## The new RNAscope™ Plus smRNA-RNA Fluorescent Assay workflow



**Figure 1. RNAscope Plus smRNA-RNA Fluorescent Assay workflow.** Starting with properly prepared samples, sections are first pretreated, and then smRNA and RNA-specific probes are hybridized to target RNAs. The RNAscope Plus smRNA-RNA Fluorescent Assay employs four independent signal amplification systems each using a different fluorophore. The assay can provide visualization of transcript expression as well as the positional relationship among four different genes within a cellular context. Single RNA transcripts for each target gene appear as punctate dots that are visible using a fluorescent microscope.

# Visualize Any RNA



Universal for virtually any gene and species



Specific and sensitive with performance reliability



Over 42,000 unique pre-designed probes available to select from



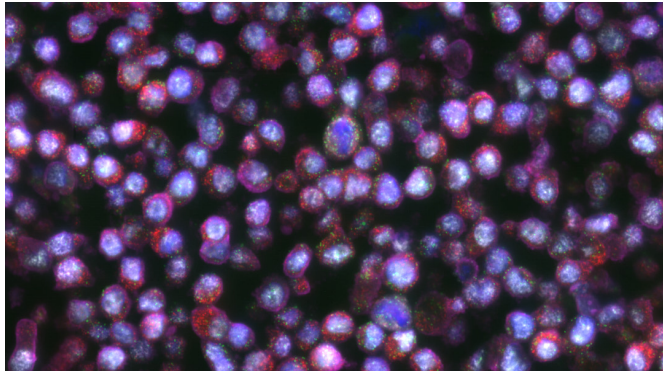
2-week turn around time for custom designs



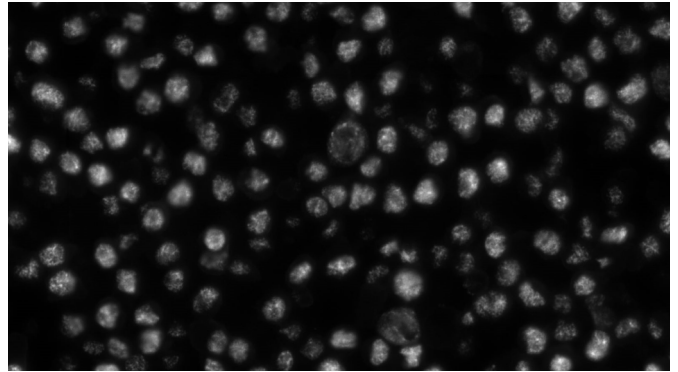
Ease of use with ready to use formulations

# The new RNAscope™ Plus smRNA-RNA Fluorescent Assay demonstrates robust detection of positive control miRNA and RNA targets in HeLa cells and mouse colon FFPE tissue

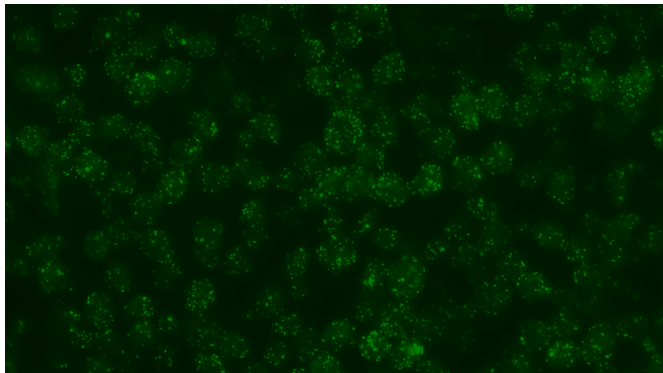
Figure 2A.



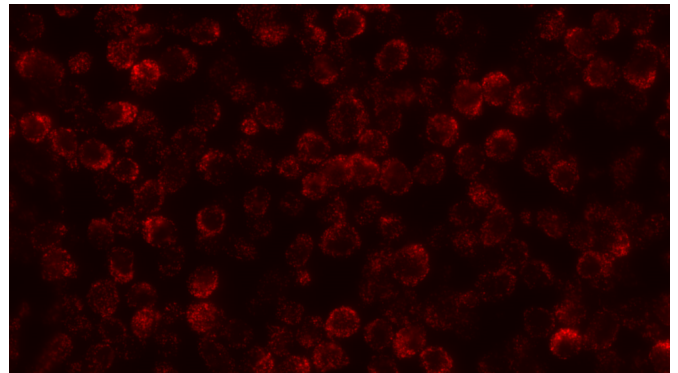
○ RNU6 miRNA ○ POLAR2A ○ PPIB ○ 18S



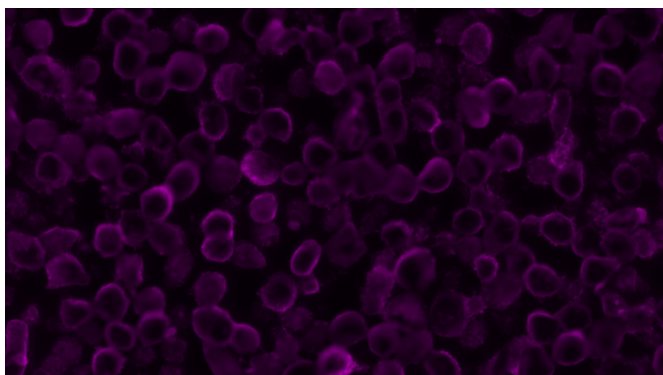
○ RNU6



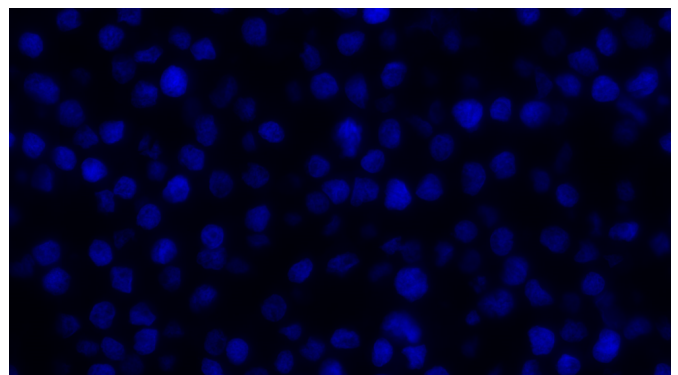
○ POLAR2A



○ PPIB

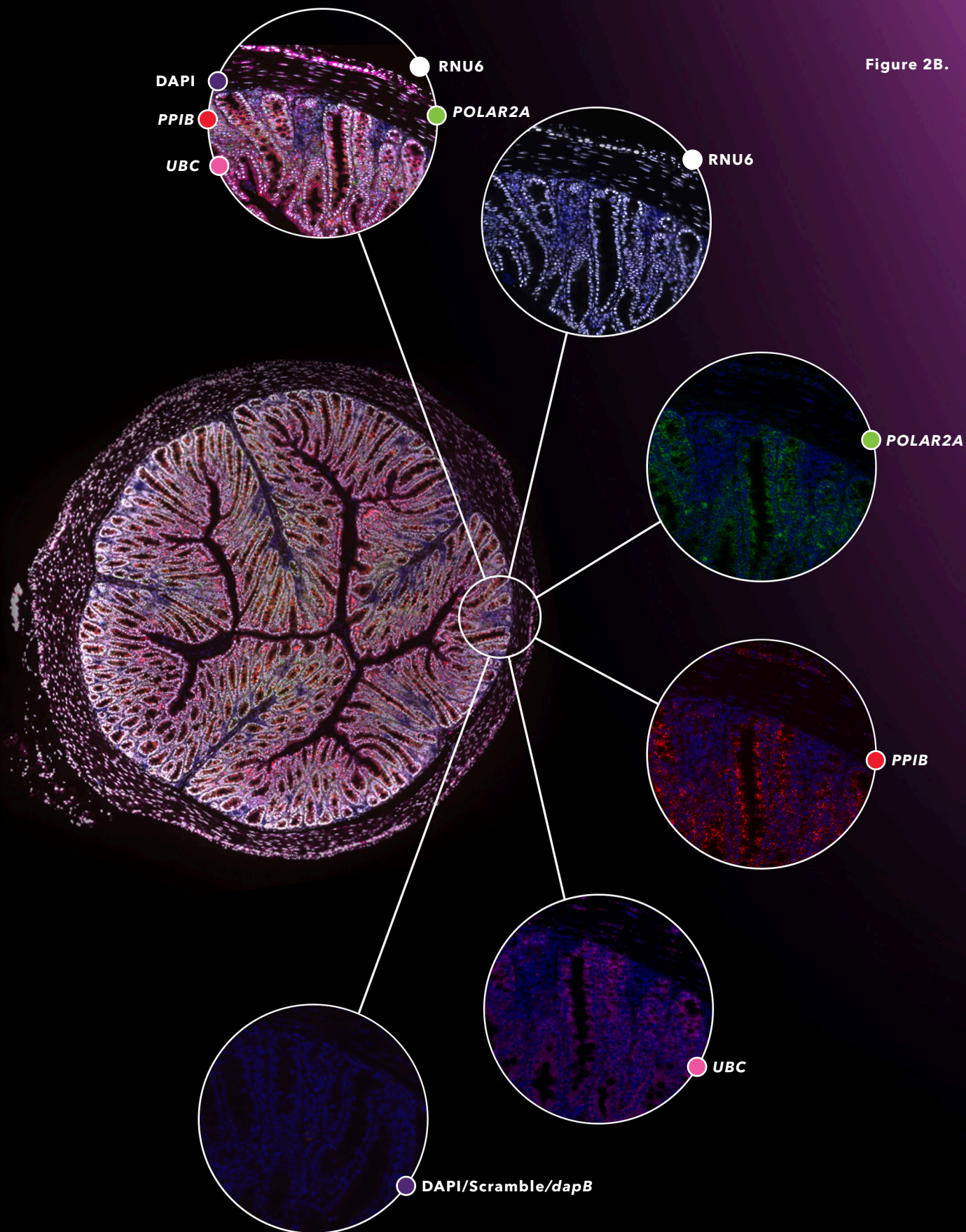


○ 18S



○ DAPI/Scramble/dapB

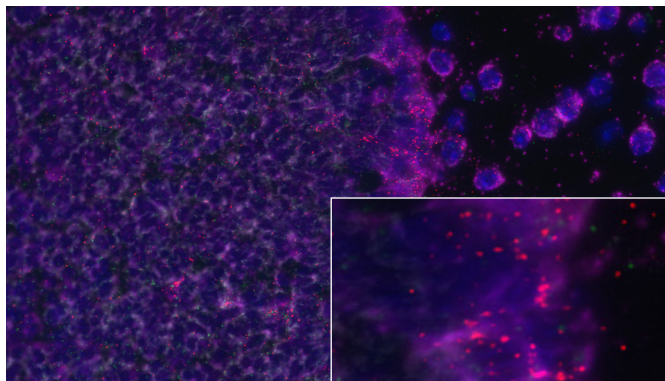
Figure 2B.



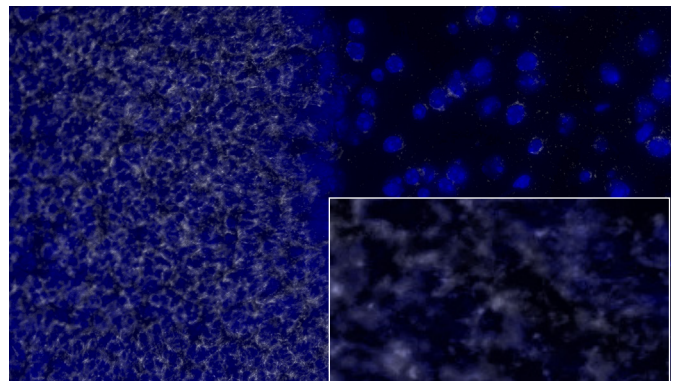
**Figure 2. RNAscope™ Plus smRNA-RNA assay performance with positive control probes.** Positive control targets, miR-RUN6, *PPIB*, *POLAR2A* and *UBC/18S* were detected across different sample types, **A**, HeLa cells, **B**, FFPE mouse colon tissue. *DapB* probe was used along with a scramble miRNA probe as a negative control. Nuclei were counter-stained with DAPI.

## Tissue-specific detection of miR-124 in mouse brain tissue using the RNAscope™ Plus smRNA-RNA Fluorescent Assay

Figure 3A.

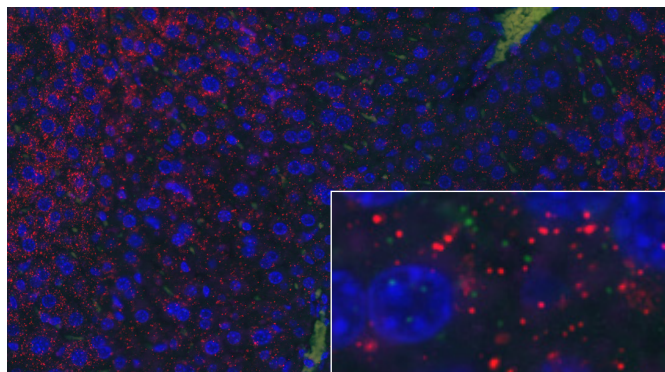


○ miR-124 ○ POLAR2A ○ PPIB ○ UBC

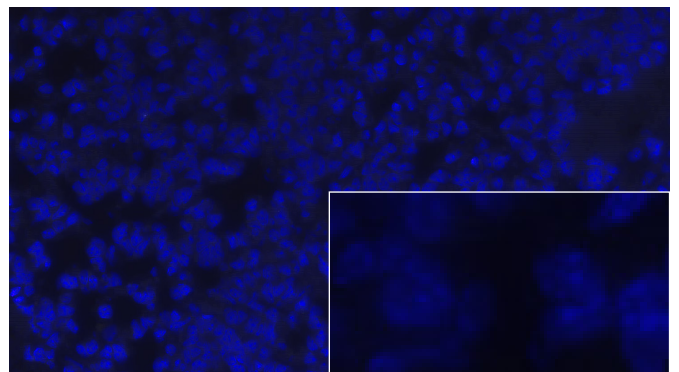


○ miR-124

Figure 3B.



○ miR-124 ○ POLAR2A ○ PPIB ○ UBC



○ miR-124

**Figure 3. Specificity of RNAscope™ Plus smRNA-RNA staining.** 4-plex smRNA-RNA assay using 1 miRNA (miR-124) specific to brain and 3 mRNAs (*POLAR2A*, *PPIB*, *UBC*). **A)** Specific staining of *miR-124* across cerebellum of mouse brain along with other positive control probes, **B)** Mouse liver tissue showing absence of *miRNA-124* but presence of the positive control targets demonstrating specificity of the assay. Nuclei were counterstained with DAPI.

# Expression of miR-205 and associated tumor target genes in head and neck cancer tissue

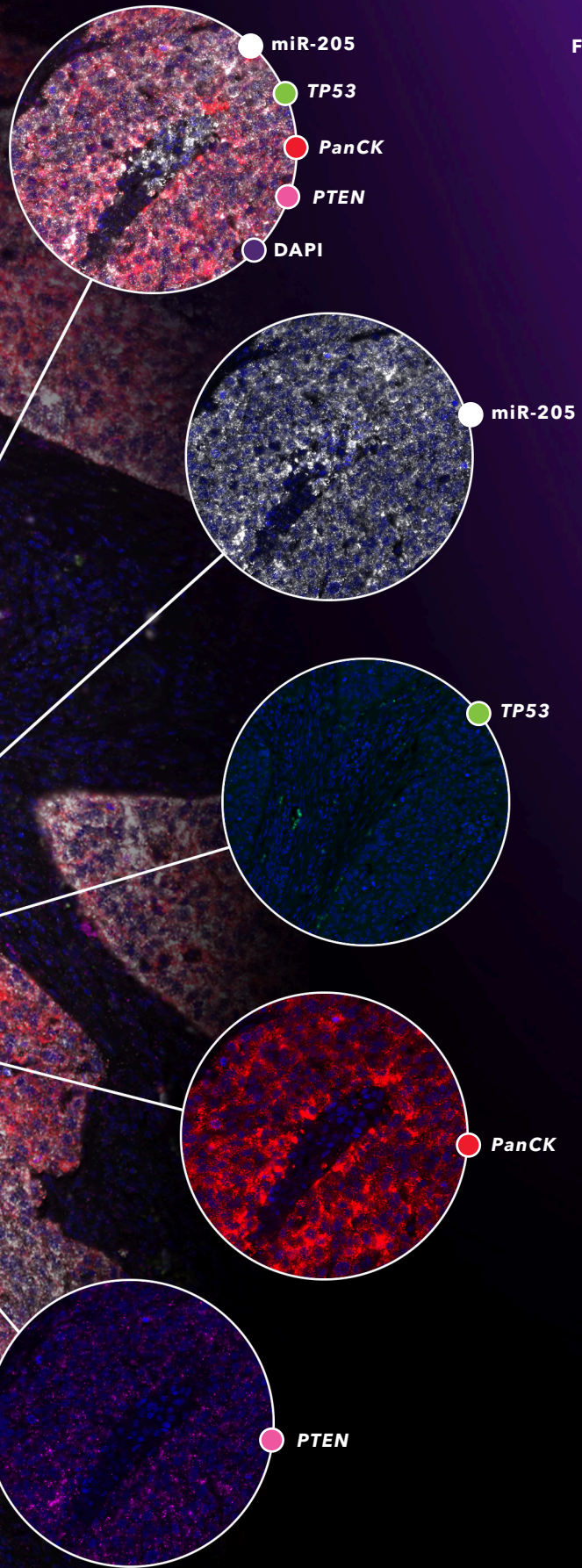


Figure 4.

**Figure 4. Colocalization of miRNA and associated mRNA targets in Head and Neck cancer.** 4-plex smRNA-RNA assay using 1 miRNA (miR-205) and 3 mRNAs targets (*PanCK*, *PTEN* and *TP53*) in tumor. MiRNA-205 was expressed in cancer cells across the tumor. Nuclei were counterstained with DAPI.

**Co-detection of brain specific miRNA and mRNAs using the new fluorescent Vivid dyes™**

Figure 5A.

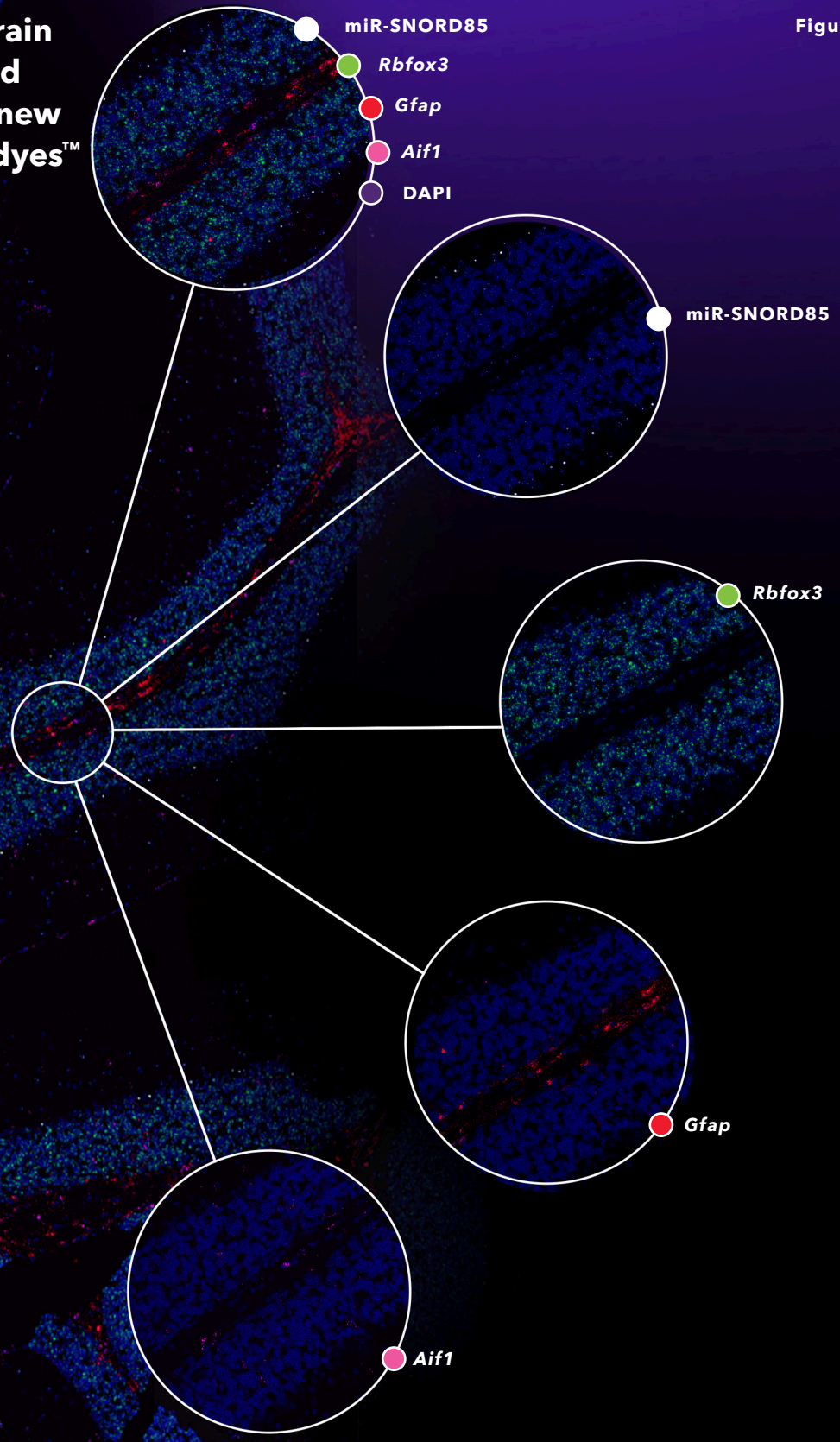
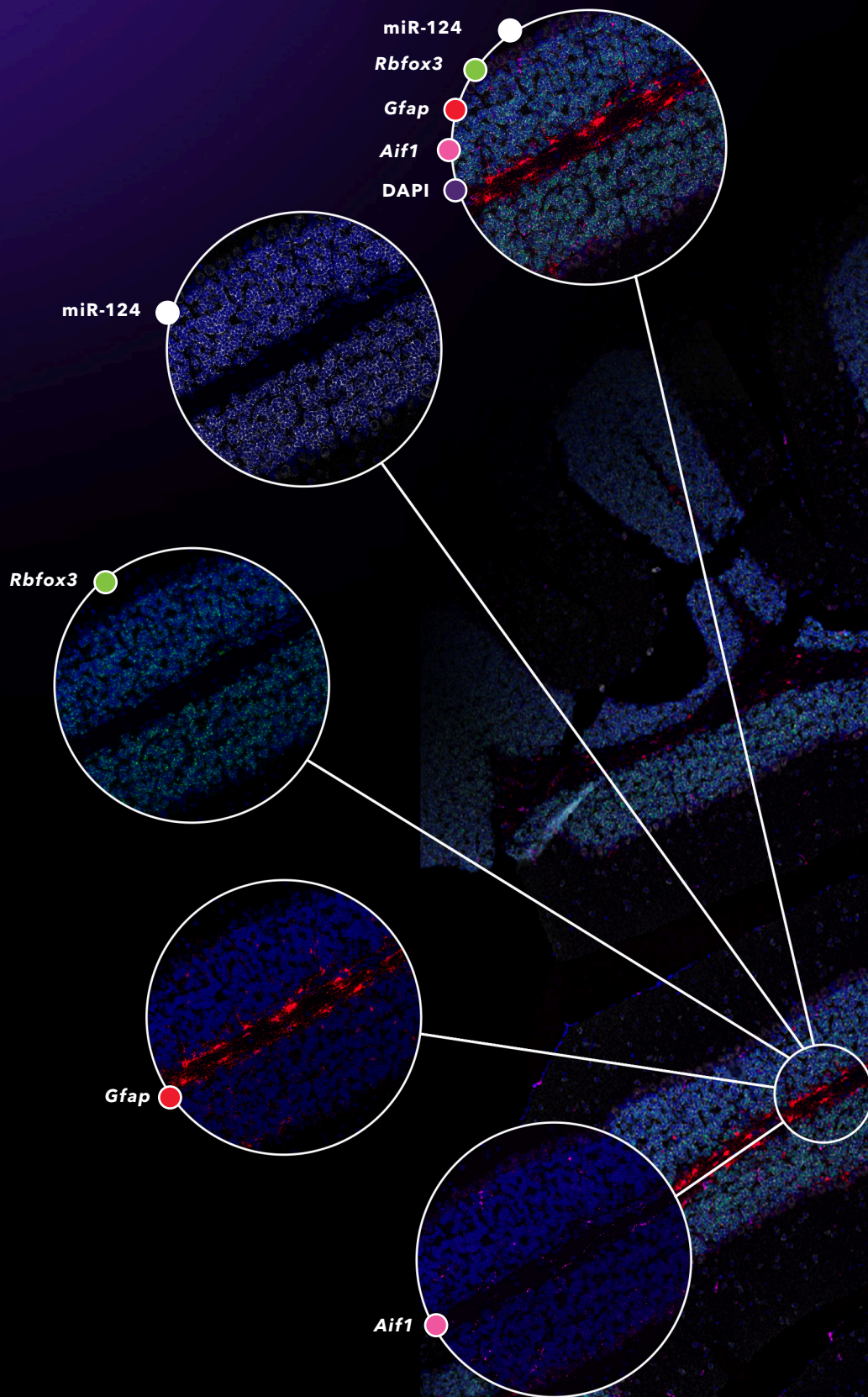




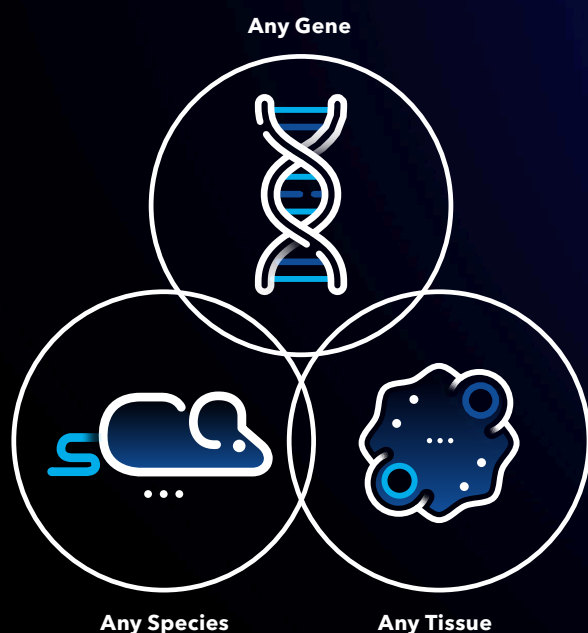
Figure 5B.



**Figure 5. Tissue specific expression of smRNAs, miR-124/miR-SNORD85 and cell specific mRNAs in mouse brain.** 4-plex smRNA-RNA assay using 1 miRNA (miR-124 /miR-SNORD85) and 3 cell type specific mRNAs (*Rbfox3*, *Gfap* and *Aif3*). **A)** Mouse cerebellum showing specific staining of miR-SNORD85 in the outer layer of the mouse brain cerebellum along with neuronal marker (*Rbfox3*), astrocytic marker (*Gfap*) and microglial marker (*Aif1*). **B)** Mouse cerebellum showing specific staining of miR-124a along with neuronal marker (*Rbfox3*), astrocytic marker (*Gfap*) and microglial marker (*Aif1*) using the new fluorescent vivid dyes. Nuclei were counterstained with DAPI.

# Product Information:

RNAscope Plus smRNA-RNA Reagent Kits		
Component	Part number (ACD, Bio-Techne)	Assay Compatibility
RNAscope™ Plus smRNA-RNA HD Reagents Kit	322785	For Manual workflow
RNAscope™ Plus smRNA-RNA LS Reagents Kit	322786	For Leica BOND RX workflow



Made-to-order RNAscope target probe can accommodate your specific needs:

If your gene of interest is not listed in our catalog, ACD can design and manufacture new in situ hybridization probes for you, we term these "RNAscope™ Made-to-order Target Probes"

## Recommended TSA dye combinations for a 4-plex assay

Fluorophores (Option 1)	Part number (ACD, Bio-Techne and Akoya Biosciences)	Recommended dilution range
TSA Vivid Fluorophore 520	323271	1:750-1:3000
TSA Vivid Fluorophore 570	323272	1:750-1:3000
TSA Vivid Fluorophore 650	323273	1:750-1:3000
Opal Polaris 780	FP1501001KT: Opal Polaris 780 Reagent Pack	TSA-DIG#: 1:750-1:3000 Polaris 780: 1:187.5-1:750

Reconstitute all TSA Vivid dyes with 100  $\mu$ L Dimethylsulfoxide (DMSO). Reconstitute Opal Polaris 780 with 300  $\mu$ L double distilled water (ddH<sub>2</sub>O)

Fluorophores (Option 2)	Part number (Akoya Biosciences)	Recommended dilution range
Opal 520	FP1487001KT: Opal 520 Reagent Pack*	1:750-1:3000
Opal 570	FP1488001KT: Opal 570 Reagent Pack*	1:750-1:3000
Opal 620	FP1495001KT: Opal 620 Reagent Pack*	1:750-1:3000
Opal 690	FP1497001KT: Opal 690 Reagent Pack*	1:750-1:3000

Reconstitute all Opals (except Opal Polaris 780) with 75  $\mu$ L Dimethylsulfoxide (DMSO). Reconstitute Opal Polaris 780 with 300  $\mu$ L double distilled water (ddH<sub>2</sub>O).

Fluorophores (Option 3)	Part number (Akoya Biosciences)	Recommended dilution range
Opal 520	FP1487001KT: Opal 520 Reagent Pack*	1:750-1:3000
Opal 570	FP1488001KT: Opal 570 Reagent Pack*	1:750-1:3000
Opal 690	FP1497001KT: Opal 690 Reagent Pack	1:750-1:3000
Opal Polaris 780	FP1501001KT: Opal Polaris 780 Reagent Pack	TSA-DIG#: 1:750-1:3000 Polaris 780: 1:187.5-1:750

Reconstitute all Opals (except Opal Polaris 780) with 75  $\mu$ L Dimethylsulfoxide (DMSO). Reconstitute Opal Polaris 780 with 300  $\mu$ L double distilled water (ddH<sub>2</sub>O).

# Where Science Intersects Innovation™

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**Contact Us**

Global [info@bio-techne.com](mailto:info@bio-techne.com) [bio-techne.com/find-us/distributors](http://bio-techne.com/find-us/distributors)

North America TEL 800 343 7475

Europe | Middle East | Africa TEL +44 (0)1235 529449

China [info.cn@bio-techne.com](mailto:info.cn@bio-techne.com) TEL +86 (21) 52380373

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