A fluorescence microscopy image of a tissue section, likely a developing embryo, showing cellular structures. The image is dominated by a large, curved, dark structure on the left side, possibly a blood vessel or a duct. The surrounding tissue is densely packed with cells, many of which are stained with various fluorescent dyes, appearing in shades of purple, red, and green. The overall image has a dark, almost black background, which makes the fluorescent signals stand out prominently.

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

biotechne® | ACD™

# Combining detection of small regulatory RNA with target RNAs

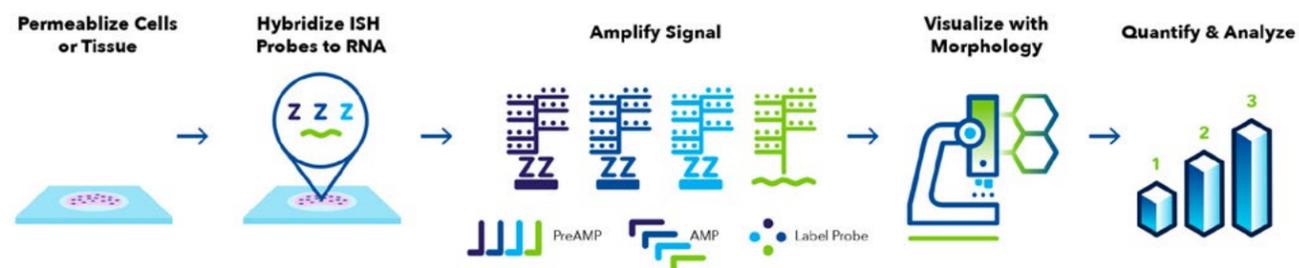
RNAscope™ technology is an advanced *in situ* hybridization assay that allows for the visualization of single-molecule gene expression directly in intact cells/tissues with single cell resolution. Detection of small RNAs (smRNA) requires a robust, highly specific and sensitive assay with quantifiable 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 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.

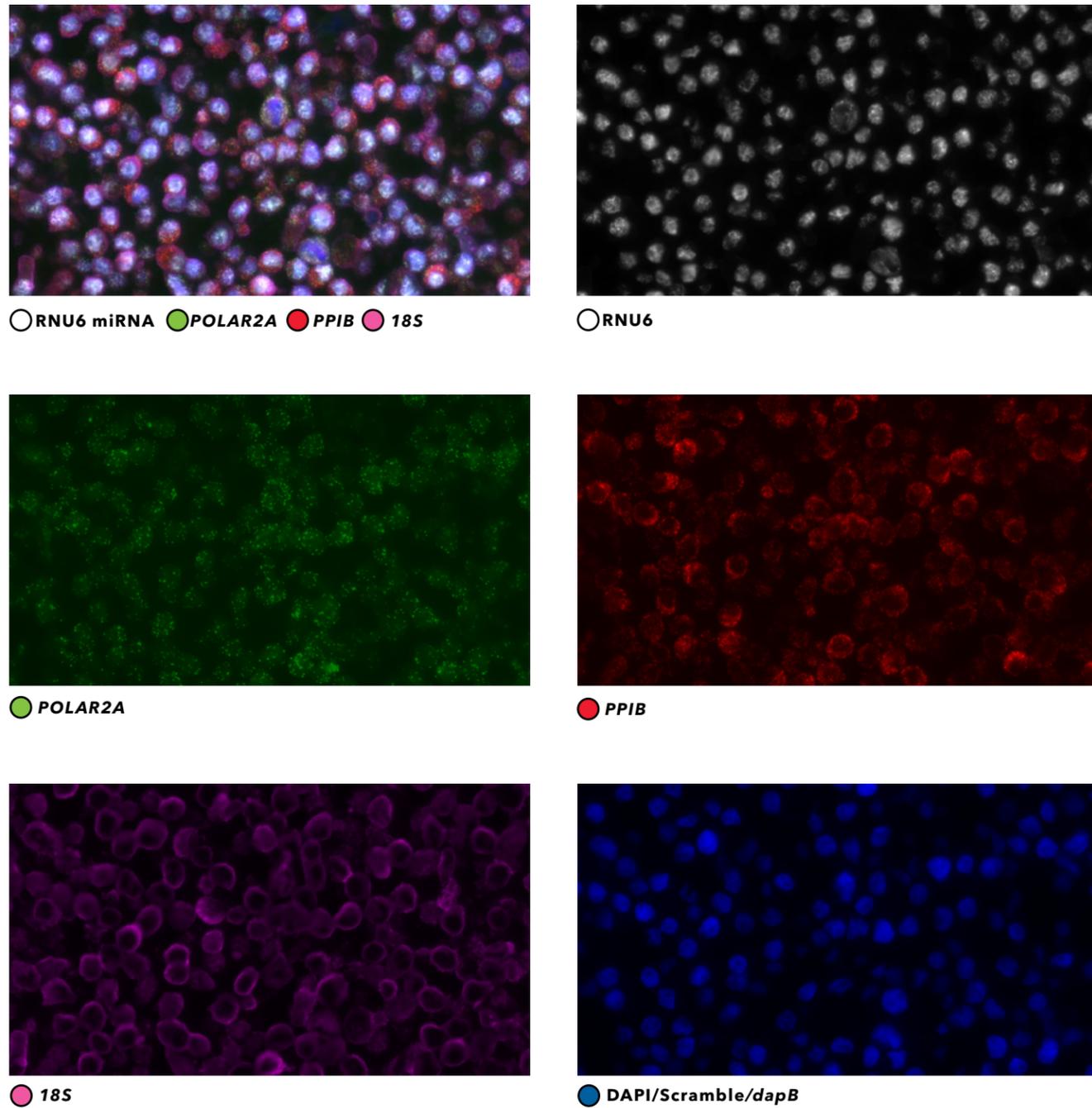
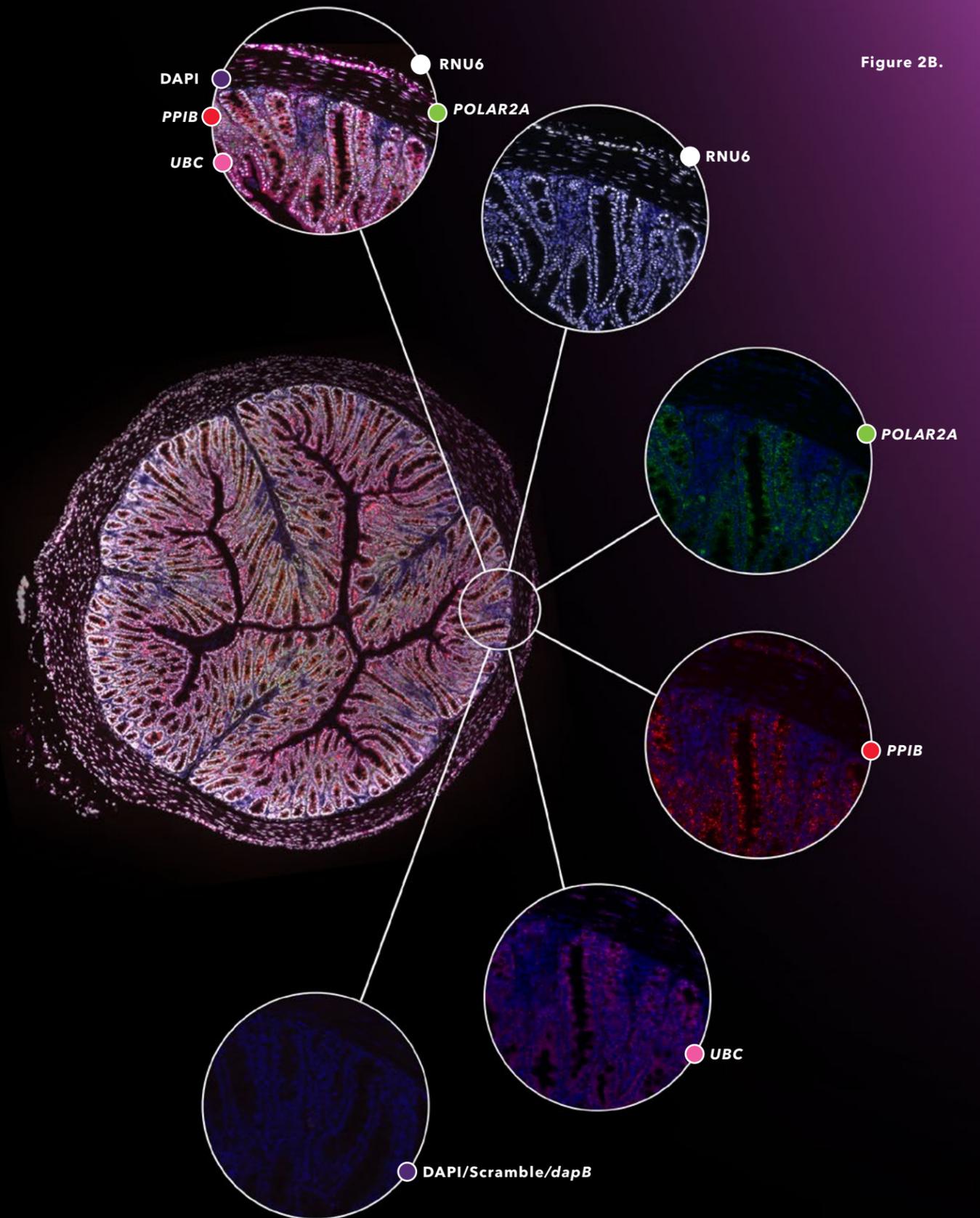


Figure 2B.



**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.

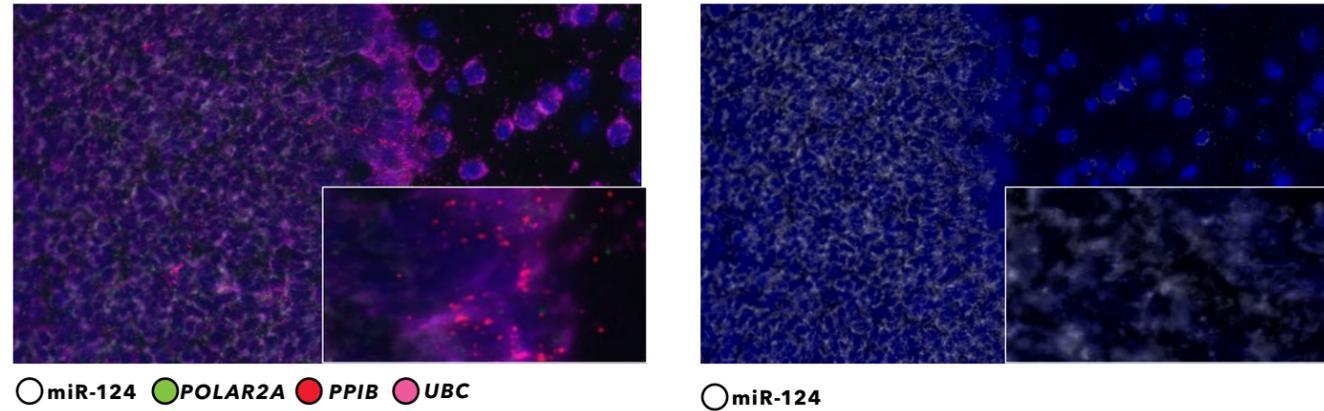
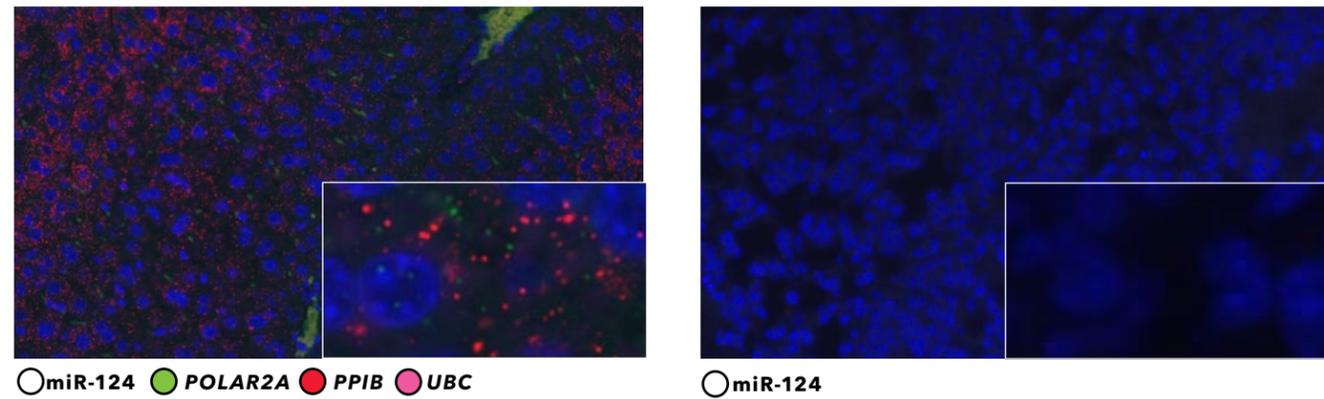


Figure 3B.



**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

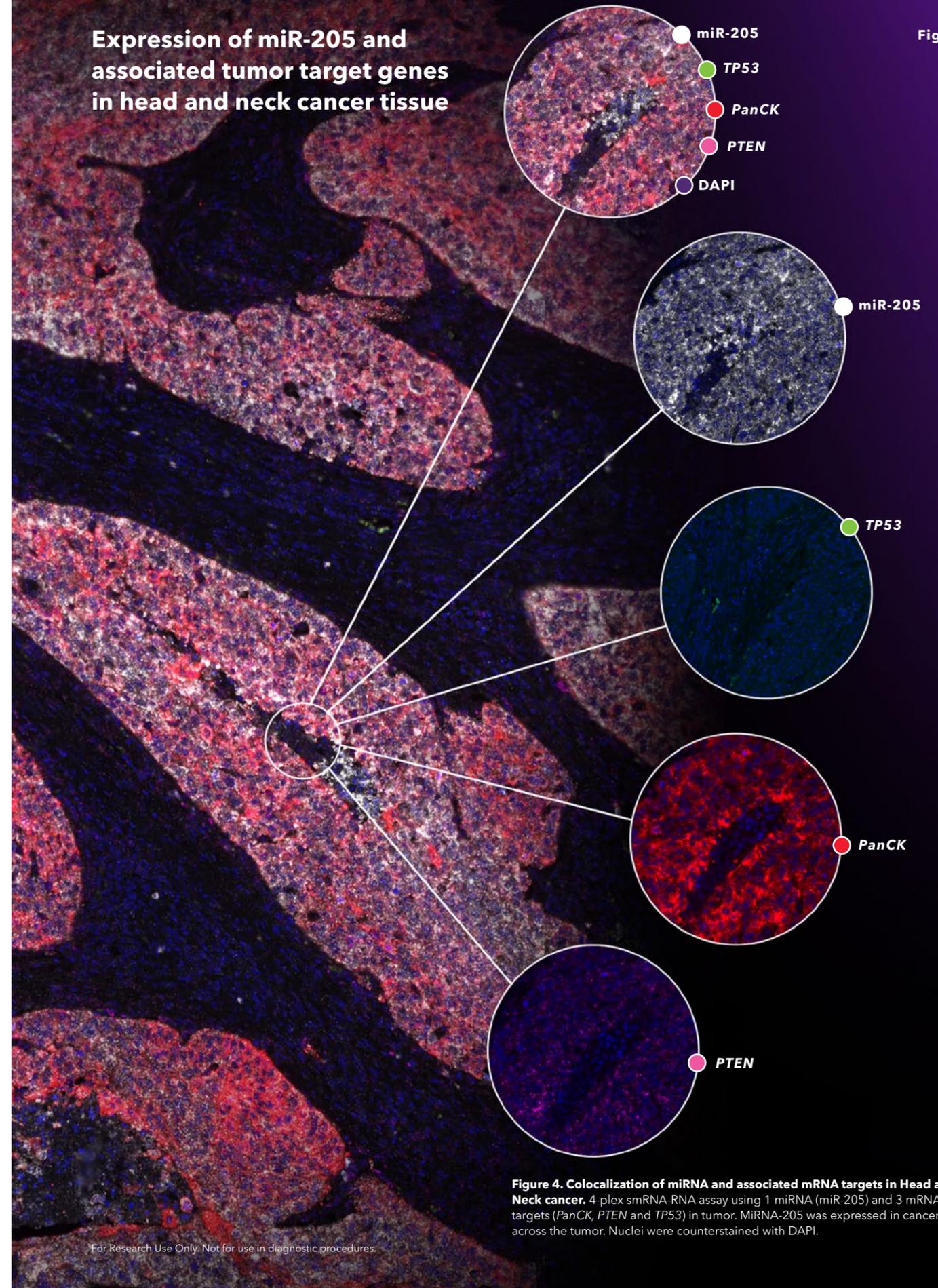


Figure 4.

**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 mRNA 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™**

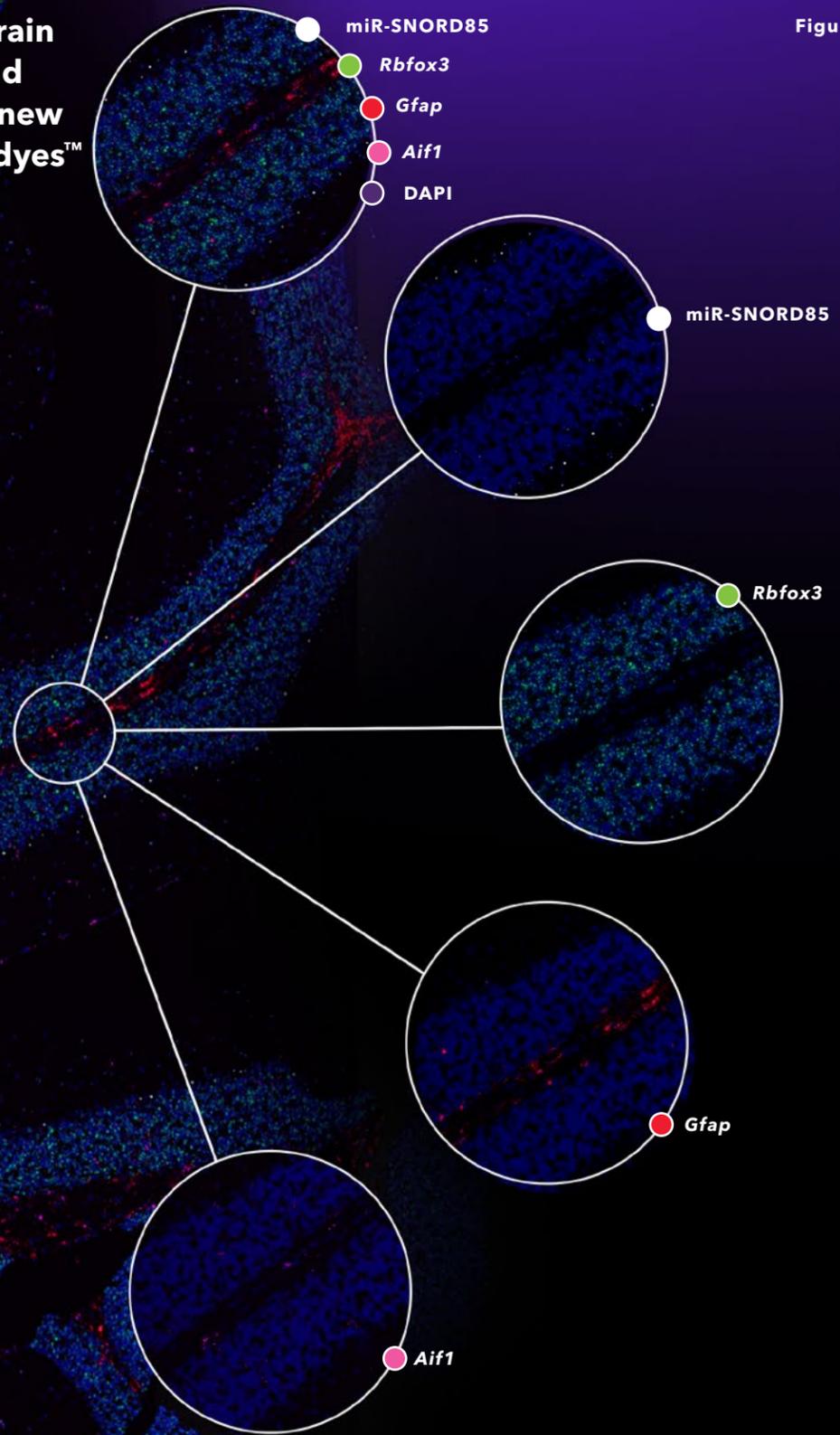


Figure 5A.

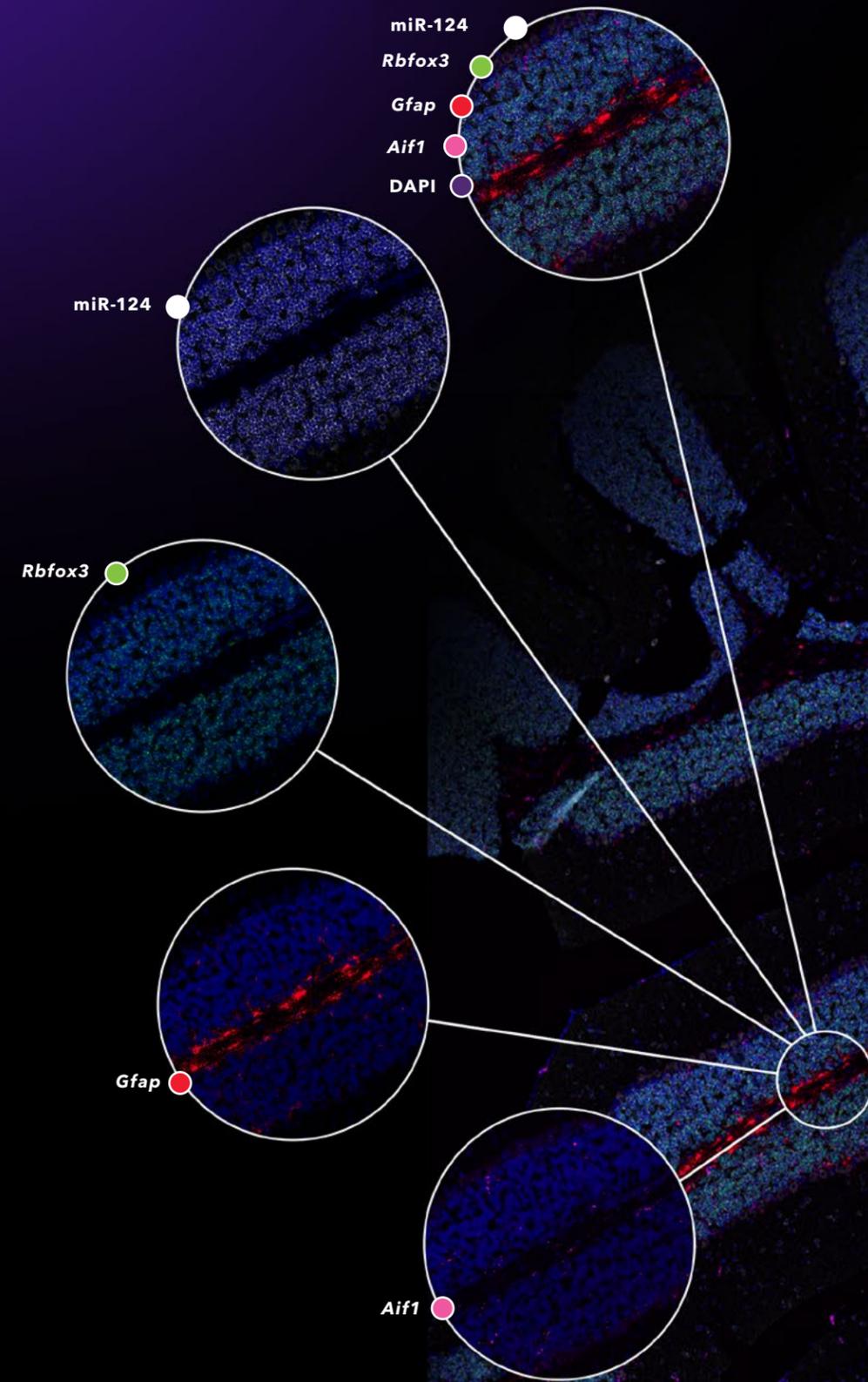
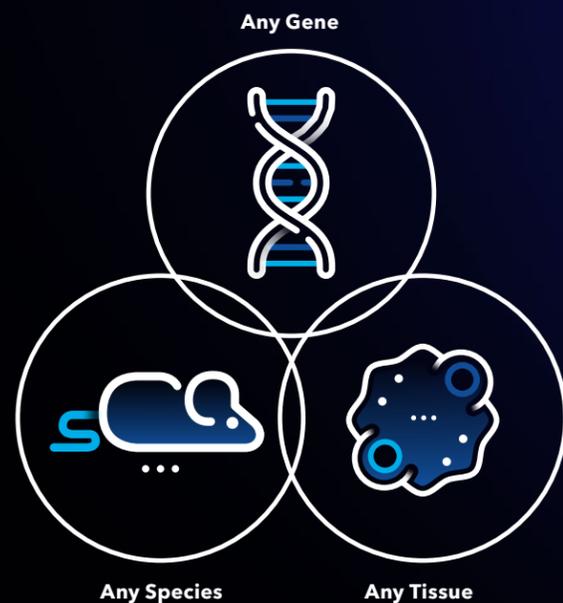


Figure 5B.

**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 *Aif1*). **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 µL Dimethylsulfoxide (DMSO). Reconstitute Opal Polaris 780 with 300 µL double distilled water (ddH2O)

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 µL Dimethylsulfoxide (DMSO). Reconstitute Opal Polaris 780 with 300 µL double distilled water (ddH2O).

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 µL Dimethylsulfoxide (DMSO). Reconstitute Opal Polaris 780 with 300 µL double distilled water (ddH2O).

# Where Science Intersects Innovation™

**Bio-Techne®** | R&D Systems™ Novus Biologicals™ Tocris Bioscience™ ProteinSimple™ ACD™ ExosomeDx™ Asuragen®



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