



**Powered by  
RNAscope™**

Accelerate Gene  
Therapy Development

RNA and Vector DNA  
Visualization Tools for  
Biodistribution and  
Safety Analysis

# Visualize Biodistribution, Function, and Safety

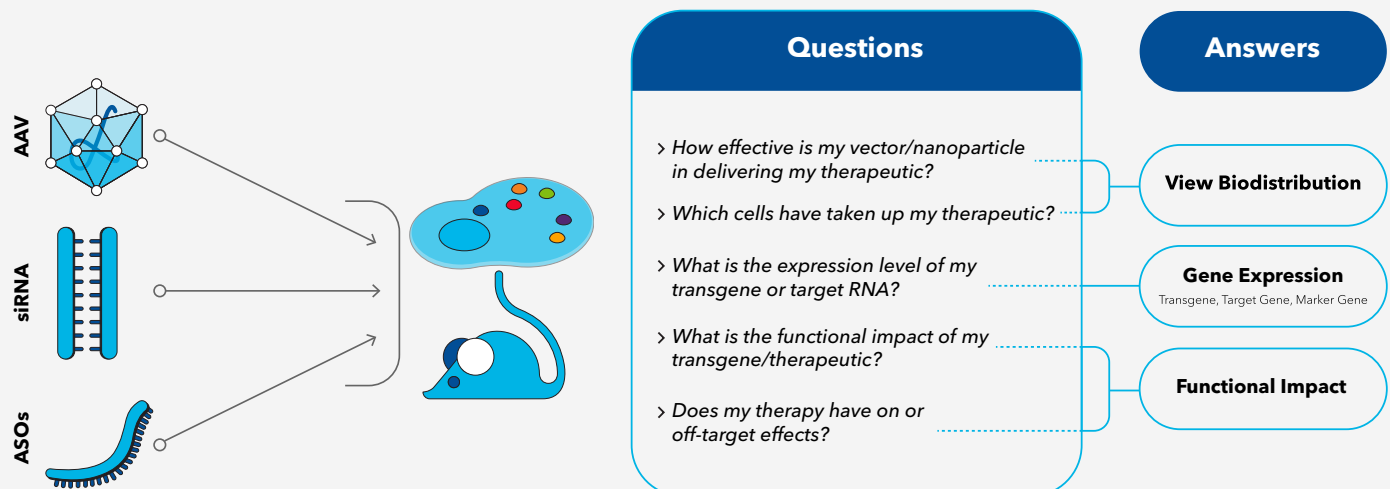
## Powerful Outcomes in Gene Therapy

- Guided by RNAscope™

RNAscope™ *in situ* hybridization (ISH) is the most preferred and trusted technology by leading pharmaceutical and biotech companies for evaluation of therapeutic biodistribution and cellular tropism in pre-clinical model systems. Quantify changes in gene expression and cellular function confidently, to optimize therapeutic efficacy and safety.

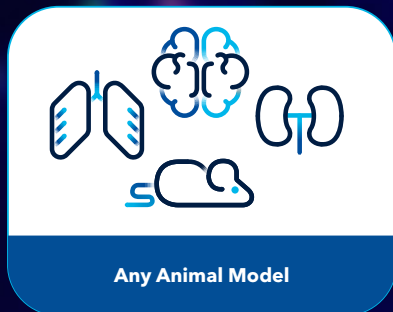
- **Visualize** tissue biodistribution and cellular tropism of viral vectors such as AAV, transgene and RNA therapeutics delivered by non-viral platforms with single-molecule sensitivity and sub-cellular resolution.
- **Select** engineered vectors and capsids for highest transduction efficiency.
- **Quantify** expression and persistence of transgenes including codon-optimized or gene-edited cargo.
- **Evaluate** therapeutic efficacy including RNA efficiency within targeted cells and tissues.
- **Improve** safety by evaluating dosing and identifying off-target events in relevant organs.

## Unlock Answers to Critical Questions



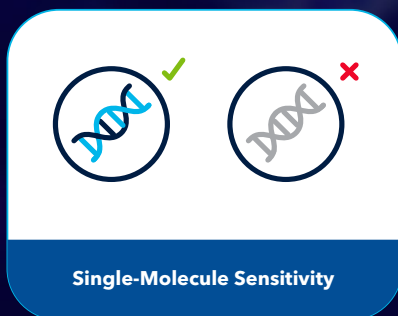
# Deep Insights to Make Informed Decisions

## Biodistribution



Optimize the targeting efficiency of your therapeutic using any preclinical animal model. Easily measure biodistribution, cellular tropism and transduction efficiency across multiple tissues of interest with high specificity.

## Gene Expression



Evaluate the performance of your engineered cargo *in vivo*. Quantify the expression and persistence of your transgene or therapeutic target with single-molecule sensitivity.

## Functional Impact

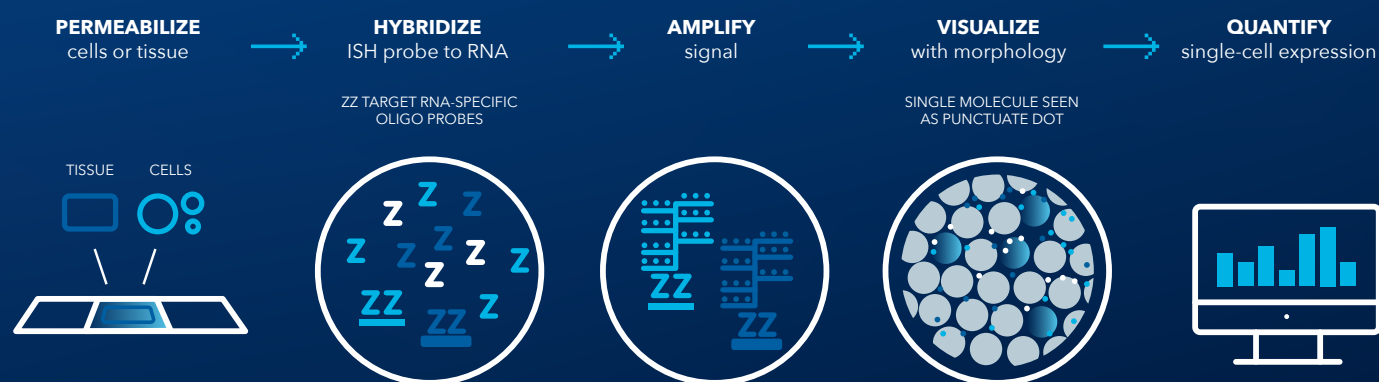


Demonstrate the functional efficacy and safety of your therapeutic in disease models. Visualize changes in targeted cells and tissues in spatial context using both protein and RNA biomarkers. Monitor off target events by visualizing biodistribution of the therapy in various organs.

# RNAscope Technology Overview

## Specific and Sensitive Automated Workflow Delivering Robust Performance You Can Trust

RNAscope is the gold-standard for RNA ISH and offers a specific and sensitive solution to visualize RNA at single cell and single molecule resolution. Gain spatial information on the biodistribution of the AAV vector and transgene mRNA in cells and tissues. Evaluate efficacy and safety of the oligonucleotide therapy by simultaneously detecting the antisense oligo (ASO) or siRNA along with the target transcript and the cell markers.



**Fig. 1 RNAscope Assay Workflow.** Tissue sections or cells are permeabilized, hybridized with the double ZZ probes in tandem, followed by signal amplification and detection with a chromogenic or fluorescent readout.

### Expert Probe Design with Proven Performance for Gene Therapy

*Trusted technology used in >8,000 publications with multiple ordering formats to choose from.*

**We offer ultimate flexibility in RNAscope probe design. Easily design probes specific to your proprietary therapeutic for use in any animal model system.**

Catalog Probes

Made-to-Order Probes

Exclusive Probes

FDA strongly recommends biodistribution studies to characterize engineered products (1). The RNAscope *in situ* hybridization assay is the most powerful spatial method available within the methods provided.

# Expert Probe Design

Known as the gold standard for RNA ISH, RNAscope delivers industry-leading sensitivity with unrivaled specificity by design. With seasoned expertise in custom probe design for cell and gene therapy applications, we can deliver probes that are uniquely designed to your therapeutic candidates for optimal results.

## Highlights:

- Design to any exogenous DNA vector or RNA therapeutic down to as little as 17bp in length with industry-leading sensitivity and specificity.
- Probes with single molecule sensitivity at sub-cellular resolution to detect if your therapeutic is in the correct cell type or trapped in the interstitial spaces.
- Detect any cargo including sequence or codon-optimized human transgenes, CRISPR/Cas9, guide RNAs, or other regulatory non-coding RNAs.
- Differentiate endogenous RNAs from any animal model system including non-human primates.

# Proprietary Target Sequences

- Exclusive probes program available to protect your proprietary sequences such as therapeutic ASO's, siRNAs, etc.
- We employ a rigorous data encryption workflow to protect your sequence information.
- Seamless and stringent legal framework to safeguard your proprietary research data.

# Automation Compatible Solutions



RNAscope, RNAscope Plus smRNA-RNA, BaseScope, and miRNAscope kits are all available for use with the fully automated BOND RX research stainer by Leica Biosystems automating the permeabilize, hybridize and amplify steps of the RNAscope workflow.



Automate RNAscope ISH probes on the Roche DISCOVERY ULTRA system. RNAscope and BaseScope probes can be purchased from ACD, and used in combination with Roche chromogens, fluorophores, and bulk reagents for fully automated assays.



Now available from Bio-Techne, the Lunaphore COMET™ delivers an automated end-to-end spatial biology workflow for hyperplex protein detection using off-the-shelf unconjugated primary antibodies.

# Simplify AAV-Based Therapeutic Development

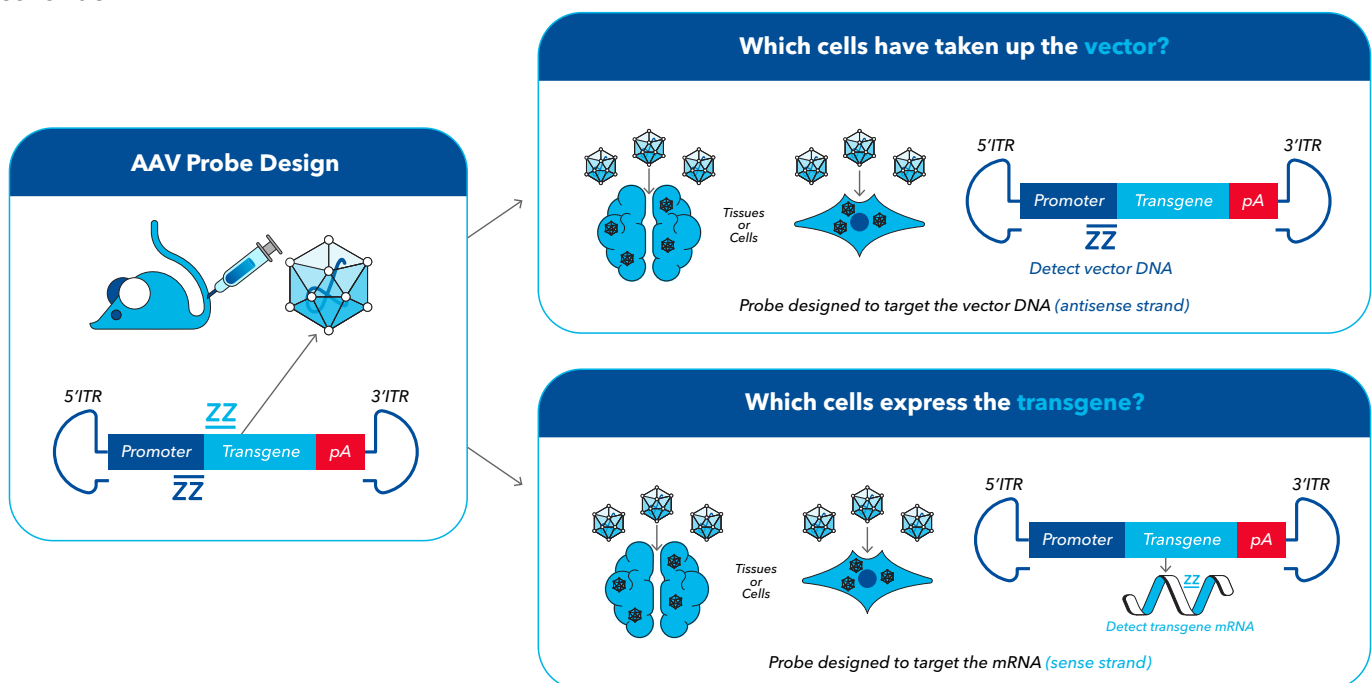
## Morphology-Based **Biodistribution** of AAV Vector and Transgene

The RNAscope ISH technology is an ideal solution for detecting AAV vector DNA and therapeutic transgene mRNA expression with morphological context, addressing critical questions on tissue biodistribution, cellular tropism, and vector performance in vivo. Screen for transduction efficiency of capsid or vectors designed to escape the immune response.

- **Characterize** tissue biodistribution, cellular tropism, and transduction efficiency of your vector at single-cell resolution in conjunction with cell-type specific markers.
- **Measure** abundance of AAV+ cells in target tissues and track vector persistence over time.
- **Quantify** RNA expression of any vector cargo including sequence or codon-optimized human transgenes, CRISPR/Cas9, guide RNAs, or other regulatory non-coding RNAs.
- **Demonstrate** therapeutic efficacy within vector transduced cells and tissues.
- **Easily Scale** from small animal models to non-human primates with the capability to easily distinguish human transgenes from homologous host transcripts.

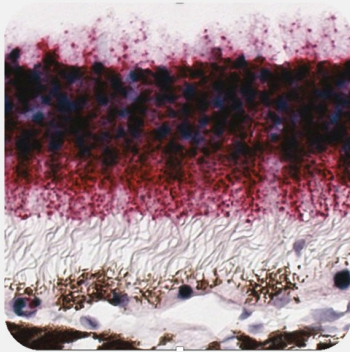
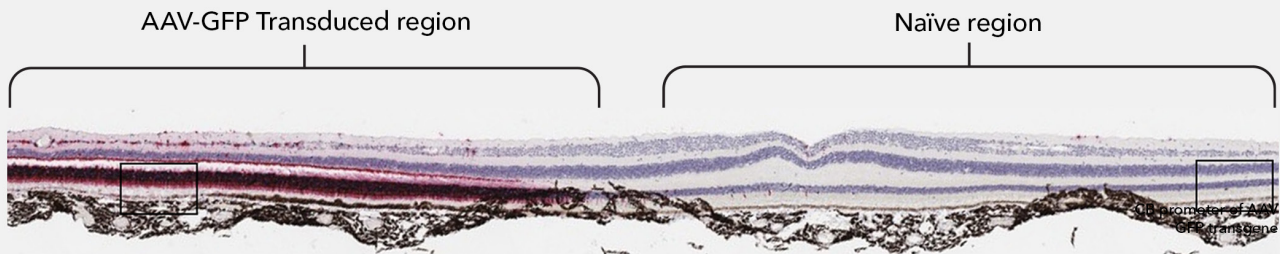
## Representation of the Gene Therapy Probe Design Strategy

RNAscope probes can be designed to target unique regions within the promoter of the AAV vector DNA or the transgene mRNA of the viral construct.



# Visualize the **AAV vector** DNA and the **Transgene mRNA** on the Same Slide.

Image of the Naïve and Transduced Region of the Mouse Retina



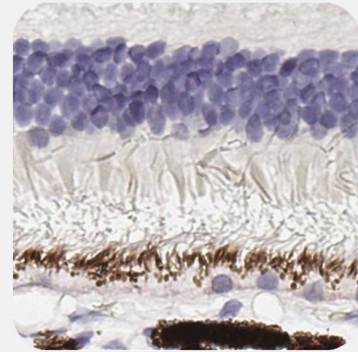
OPL (Outer plexiform layer)

ONL (Outer nuclear layer)

R&CL (Rod and cone layer)

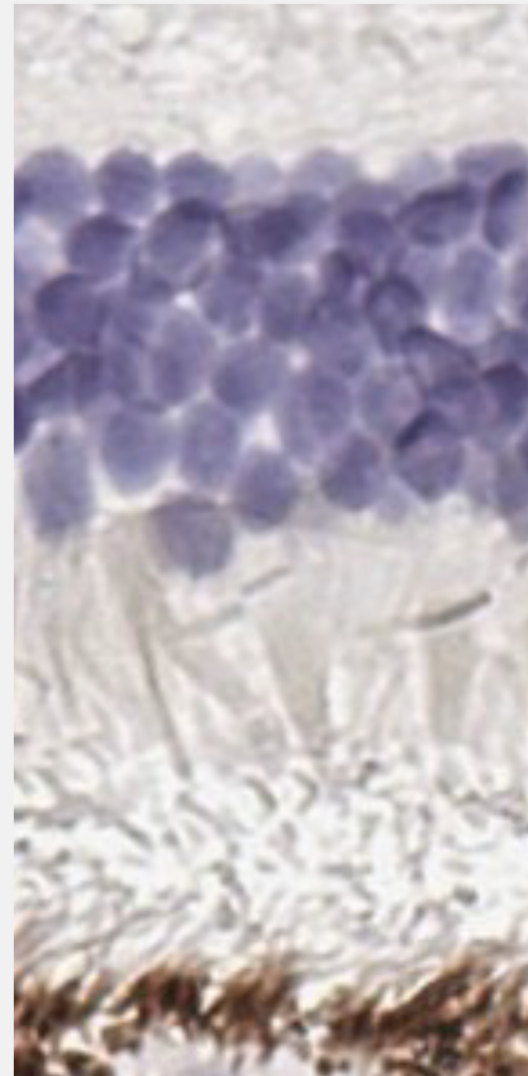
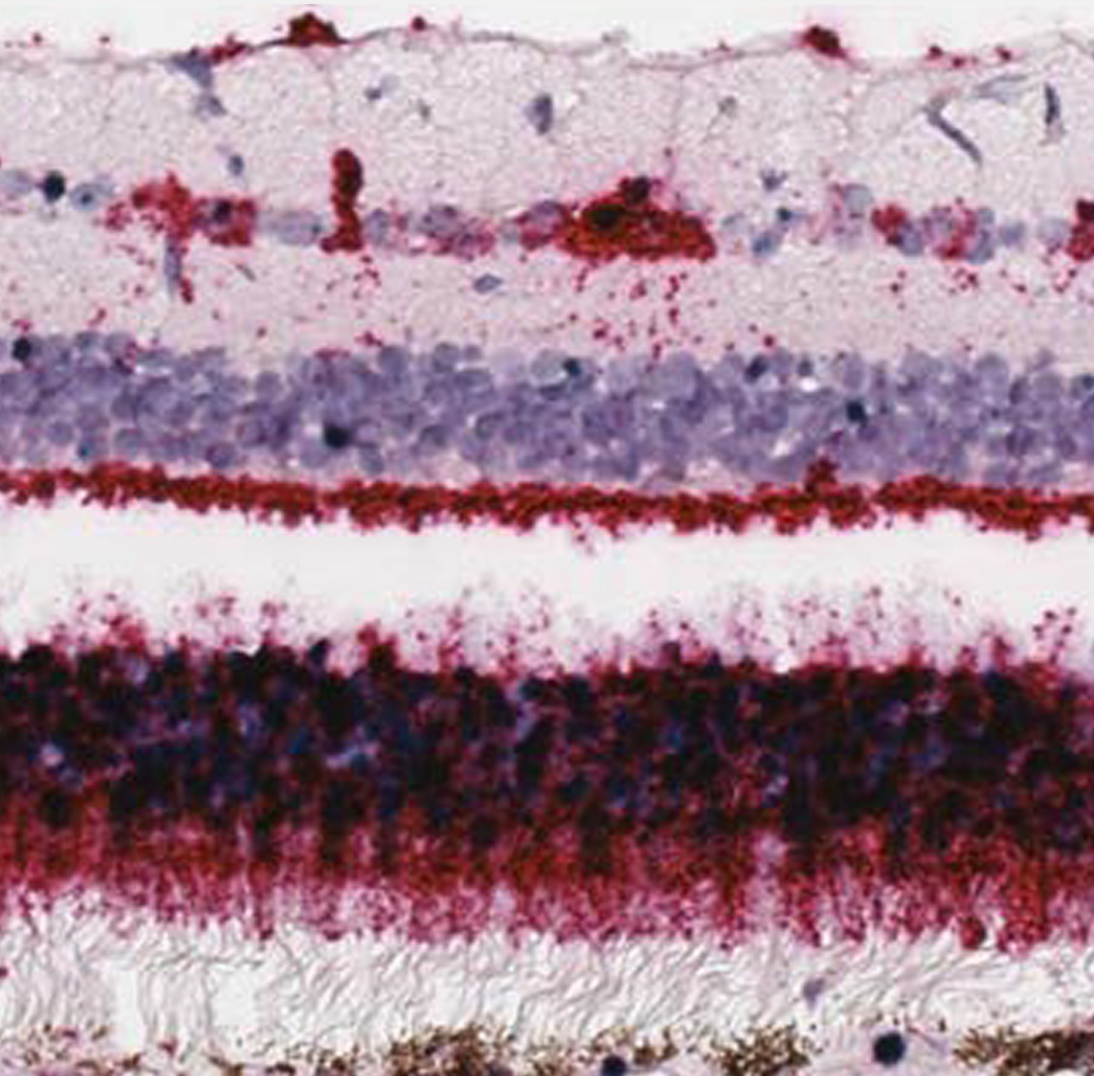
PE (Pigmented epithelium)

Choroid



**Transduced Region**

**Naïve Region**



# Streamline the Development of Oligonucleotide Therapies

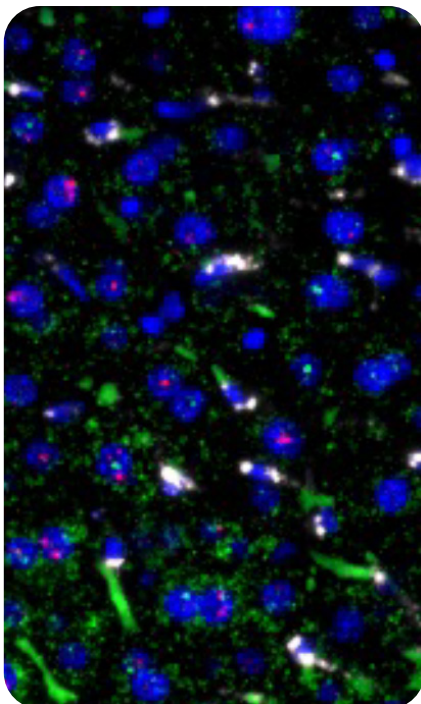
## Visualize and Quantify **Small RNA Delivery and Function**

Synthetic oligonucleotides like siRNAs and ASOs precisely target genes to control their expression. With RNAscope technology you can easily visualize both the biodistribution and function of your therapeutic oligonucleotide at single-cell resolution.

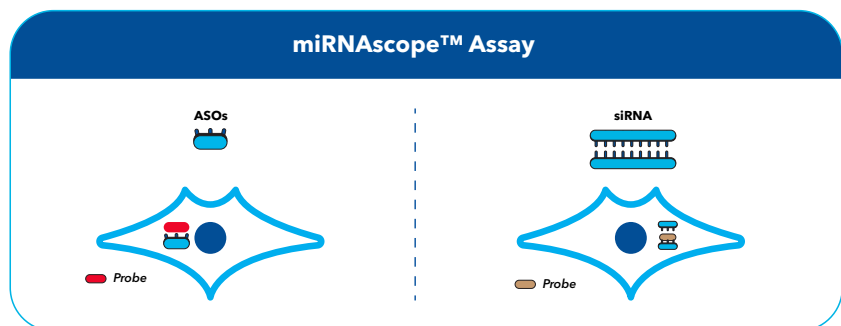
- **Assess *in vivo* biodistribution** - of the ASOs or small RNA and determine cellular uptake, and persistence over time.
- **Visualize** - 1 small RNA (ASO, miRNA, siRNA) + up to 3 mRNA targets in intact tissues.
- **Evaluate expression** - of the target gene and cell-specific marker genes with morphological context in heterogeneous tissues.
- **Safety and efficacy** - screen for potential toxicity and off-target effects.

## Probe Design Strategy for Detection of ASOs and siRNAs

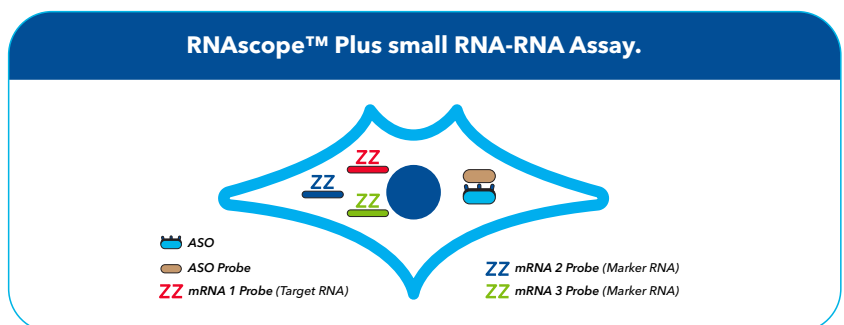
**Visualize synthetic and endogenous RNA sequences in tissue and cells.**



### Visualize ASO or siRNA tissue biodistribution

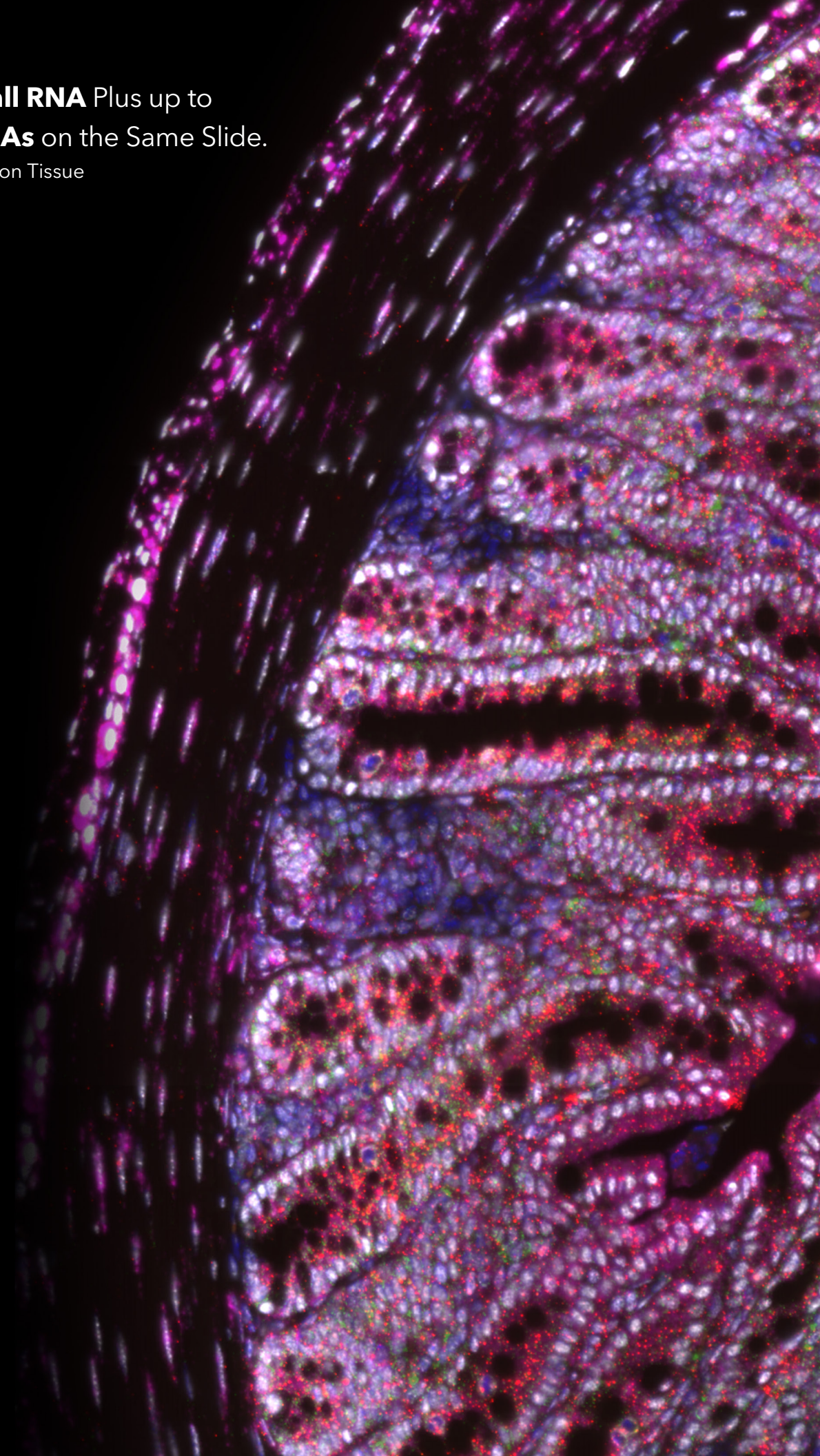


### Characterize ASO or siRNA cellular tropism and therapeutic efficacy





Visualize one **Small RNA** Plus up to  
Three **Marker RNAs** on the Same Slide.  
Image of the Mouse Colon Tissue





Julio D. Nieves,  
Associate Director, Imaging  
Adverum Biotechnologies

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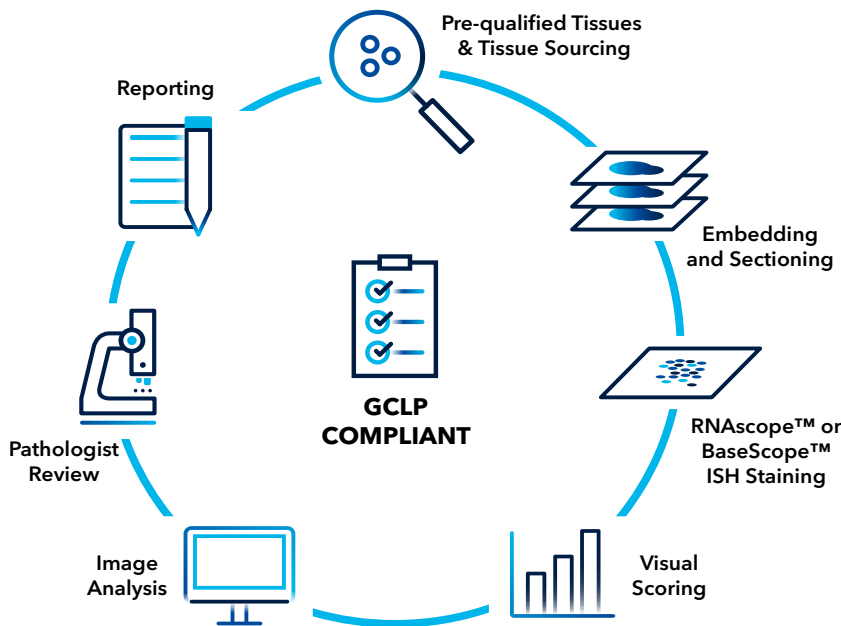
With RNAscope ISH we were able to detect the AAV vector down to a single copy. That's a level of sensitivity which is extremely important, and until now we haven't been able to really detect to this level using other conventional approaches."

# Professional Assay Services for Gene Therapy - Enabling Clinical Development

RNAscope Professional Assay Services provides flexible, scalable multi-omic services to help you achieve your research goals. Whether you are new to RNAscope technology or are a seasoned assay user, we offer expert end-to-end RNAscope services including early access to new RNAscope assay capabilities.

- **Established and Trusted Partner** - Save time with seamless execution of projects by experts who discovered the RNAscope technology for discovery, preclinical, clinical, and pilot studies, with highest quality data delivered.
- **Comprehensive** - Complete end-to-end services performed by experienced scientists and analyzed by a board-certified pathologist.
- **Convenient** - Access to pre-qualified tissue banks to source desired tissue types from trusted providers.
- **GCLP Compliant** - Standard operating procedures for sample tracking, data management, and data QC.

## End - to - End RNAscope ISH Assay Services



**Fig 2. Comprehensive Assay Capabilities.** End-to End and *à la carte* services for tissue sectioning, *in situ* hybridization (ISH) staining (chromogenic and fluorescent readouts), high resolution full slide scanning, scoring, and image analysis performed by an experienced team.

## RNAscope Professional Assay Services Workflow.

Quality processes and SOPs ensure reliability and confidence in the results.



# Where Science Intersects Innovation®

*(1) S12 Nonclinical Biodistribution Considerations for Gene Therapy Products; Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration; Center for Drug Evaluation and Research (CDER); Center for Biologics Evaluation and Research (CBER) May 2023; ICH-Safety*

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

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