

Accelerate Gene Therapy Development with Powerful Visualization Tools for Biodistribution and Functional Analysis

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

Visualize Therapeutic Biodistribution and Function

- **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.

More Informative than qPCR

Morphology-based biodistribution is extremely important in the field of therapies involving gene editing. While qPCR-based methods provide average values for copy number from extracted nucleic acids, the RNAscope technology provides morphology-based quantification.

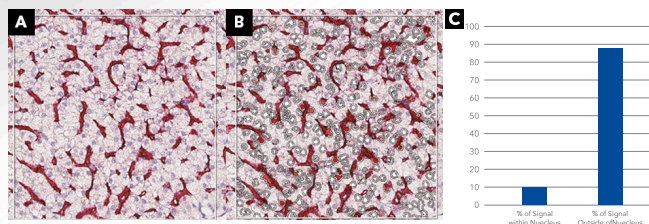


Fig 1. RNAscope Uniquely Enables Visualization of Cellular Uptake and Cellular Tropism. (A) AAV vector is primarily sequestered in the interstitial space. (B) HALO® (Indica Labs) overlay identifies AAV-positive nuclei in red and AAV-negative nuclei in white. (C) Quantification of % of signal detected within the nuclear boundaries vs signal located outside of the nucleus.

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

Automated RNAscope Assay Workflow

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.

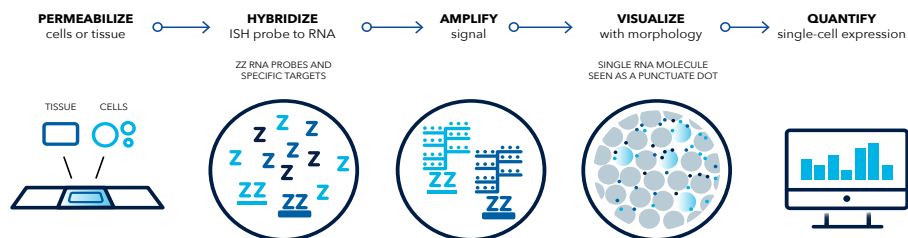


Fig 2. RNAscope Assay Workflow. Tissue sections or cells are permeabilized, hybridized with the double ZZ probes in tandem, followed by signal amplification and detection with a chromogenic or fluorescent readout.

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 of tissue biodistribution, persistence, cellular tropism and vector promoter activity.

- **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.

Visualize and Quantify small RNA Delivery and Function

Synthetic oligonucleotides like siRNAs and ASOs precisely target genes to control their expression. With RNAscope you can visualize small regulatory RNAs (smRNAs) alone, or along with its mRNA targets. Assess oligonucleotide therapy delivery, biodistribution, and interrogate smRNA biomarkers along with cell specific biomarkers.

- miRNAscope™ Assay allows visualization of ASOs or miRNA or siRNA and other nucleic acid targets between 17-50 nucleotides.
- RNAscope™ Plus smRNA-RNA Assay enables visualization of one small RNA (ASO, miRNA, siRNA) plus up to three mRNA markers.

Professional Assay Services

Need to get your results faster? Outsource your study to RNAscope experts at assay services.



Contact us for Free a Project Consultation

Speak to a specialist to get up and running quickly with your next RNAscope project.

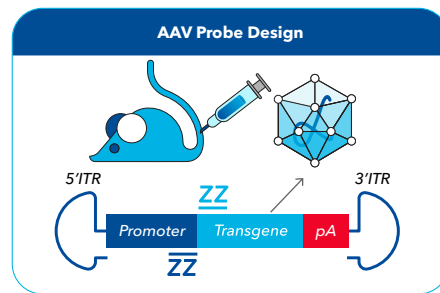
(1) S12 Nonclinical Biodistribution Considerations for Gene Therapy Products; Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration; Center for Drug Evaluation and Research (CDER); Center for Biologics Evaluation and Research (CBER) May 2023; ICH-Safety

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3A.



3B.

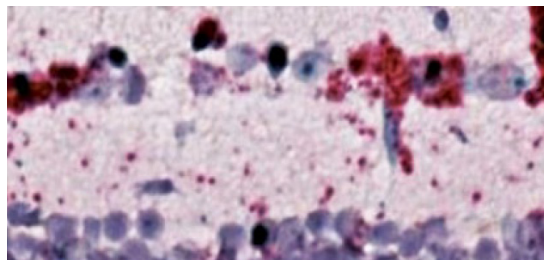
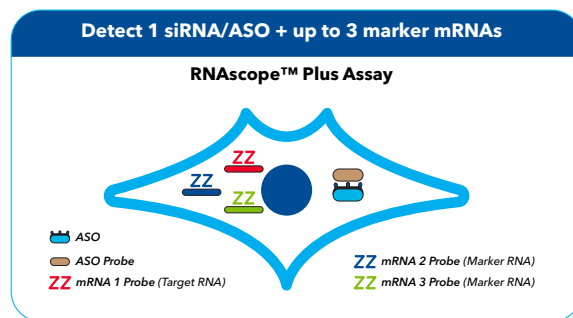


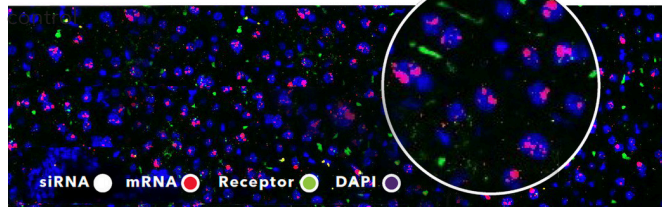
Fig 3A. Schematic Representation of The Gene Therapy Probe Design Strategy. RNAscope probes can be designed to target unique regions within the promoter of the AAV vector DNA or the transgene of a viral construct.

Fig 3B. RNAscope Assay to View Biodistribution. AAV-treated non-human primate retina stained with the RNAscope LS Duplex ISH assay to detect the promoter sequence from the AAV vector (green) together with a GFP transgene (red).

4A. RNAscope Plus Assay



4B. Mouse liver tissue PBS



Mouse liver tissue Treated (10mg/kg)

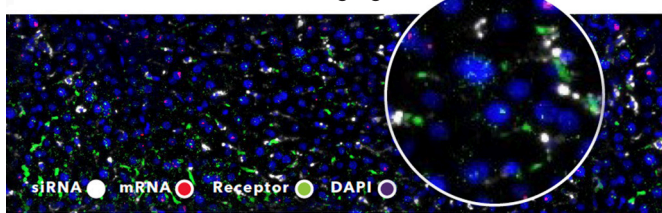


Fig 4A. Schematic Representation. The RNAscope Plus smRNA-RNA Assay. **Fig 4B. Detection of Small RNAs and Target RNAs.** The assay leverages the patented core technology that enables signal amplification and background suppression for multiplex fluorescent visualization of one small RNA with up to three mRNAs in formalin-fixed paraffin-embedded (FFPE) and fresh frozen samples.

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