Tissue-Based Gene Therapy Biodistribution Analysis Using RNAscope™ *In Situ* Hybridization

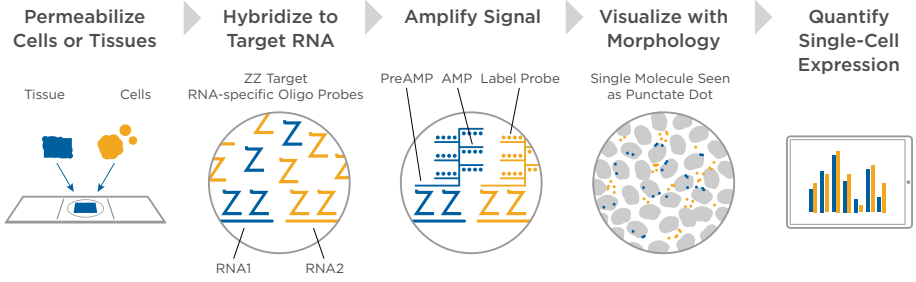
Targeted gene therapy has shown great promise for treating rare diseases, particularly those that arise from single gene defects. Techniques to introduce therapeutic transgenes include adeno-associated virus (AAV), lentivirus, or lipid nanoparticle (LNP) mediated delivery. While qPCR-based methods provide average values for copy number from extracted nucleic acids, the RNAscope *in situ* hybridization (ISH) technology provides morphology-based, cell-specific quantification of vector DNA and transgene mRNA.

Features

- Molecular detection of single-copy DNA vectors
- Codon-optimized transgene expression analysis
- Catalog probes to many commonly used AAV and lentiviral vectors and promoters
- Custom probes designed within 1-2 weeks

Benefits

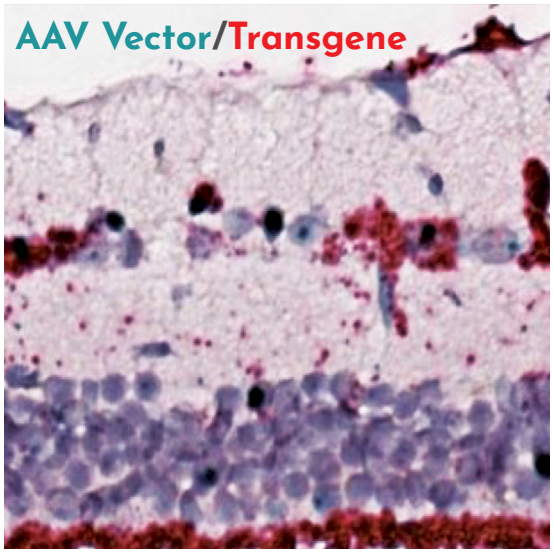
- Simultaneously visualize in vivo delivery of vector DNA and therapeutic transgene mRNA
- Identify cell tropism of AAV vector by multiplexing with cell-type markers
- Distinguish transgene from endogenous sequences, at the single nucleotide level
- Quantify AAV+ cell number and track persistence over time



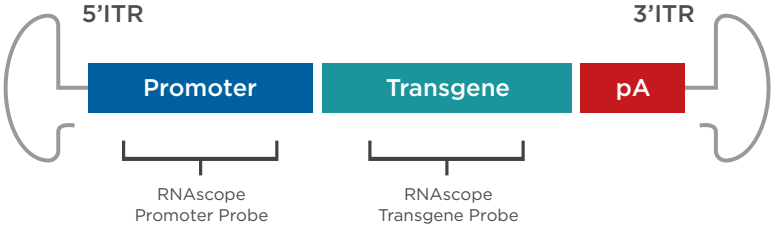
Morphology-Based Biodistribution of AAV Vector and Transgene

The RNAscope ISH technology is an ideal solution for detecting AAV vector DNA and therapeutic transgene mRNA expression within intact tissue morphology, addressing critical questions on tissue biodistribution, persistence, cellular tropism and vector promotor activity.

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

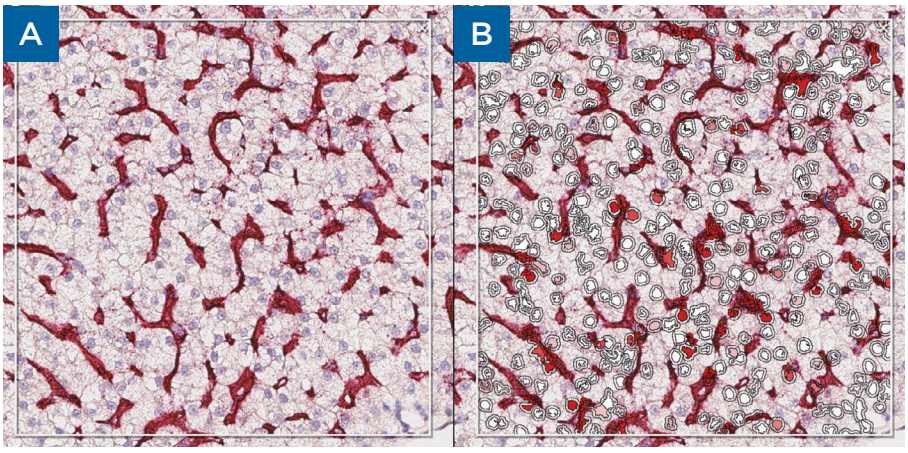


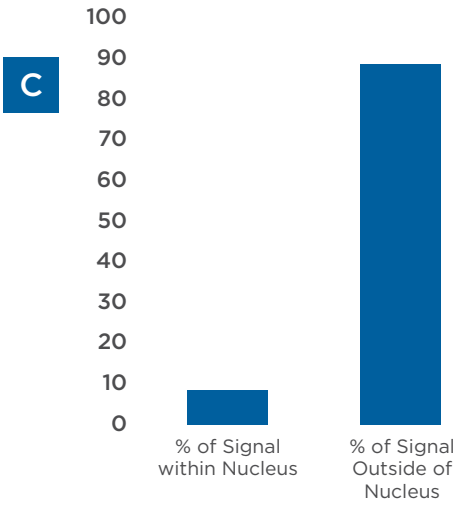
RNAscope probes can be designed to target unique regions within the promoter or the transgene of a viral construct.



Evaluate Subcellular Localization of Vector Delivery and Transgene Expression

The RNAscope assay enables single molecule detection of vector DNA and/or transgene mRNA in the tissue at the single-cell level, thus allowing for a high spatial resolution at both the cellular and subcellular level. Cell type-specific promoter activity and transgene expression heterogeneity can be assessed with the RNAscope assays, as can screening of vector delivery systems for cellular uptake efficiency and extracellular trapping of the vector.





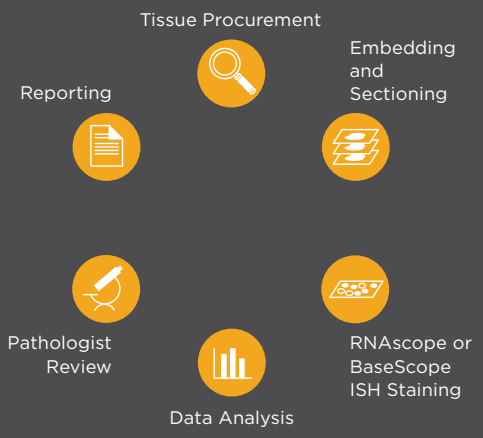
AAV-treated liver stained with the RNAscope 2.5 LS Red assay. (A) AAV vector primarily sequestered in the interstitial space. (B) HALO (Indica Labs) overlay identifies AAV-positive nuclei in red and AAV-negative nuclei in white. (C) Quantification of the percentage of signal detected within the nuclear boundaries (stained with hematoxylin) vs signal located outside of the nuclear boundaries.

Partner with ACD's Pharma Assay Services

- Trust your study to the experts in RNAscope and BaseScope™ ISH
- Have confidence in the science, data and research conclusions
- Receive actionable results in weeks rather than months

"We've had a great experience in working with ACD. Using their service team we are able to move faster through our testing for Phase I trial, we are very happy with the quality of data, thoroughness in the reports we receive and would highly recommend them for ISH assay development and implementation."

— Dr. Omar Kabbarah



Learn more about Pharma Assay Services at www.acdbio.com/PAS

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Learn more about the RNAscope technology for Gene Therapy Applications at: **www.acdbio.com/genetherapy**

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Doc #: MK 51-108/Rev B/Effective date 10/16/2019