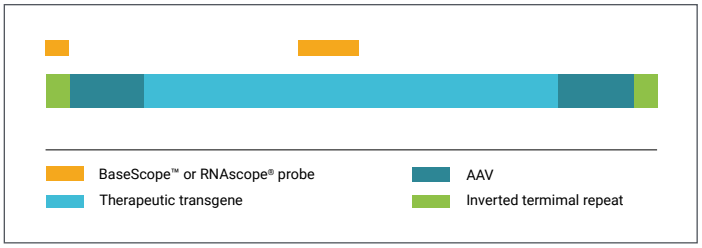
RNAscope™ ISH APPLICATION: GENE THERAPY BIO-DISTRIBUTION

DETECTION OF DELIVERY VECTOR AND THERAPEUTIC TRANSGENE EXPRESSION IN ANY INTACT FIXED TISSUE USING THE RNAscope™ AND BaseScope™ ASSAYS

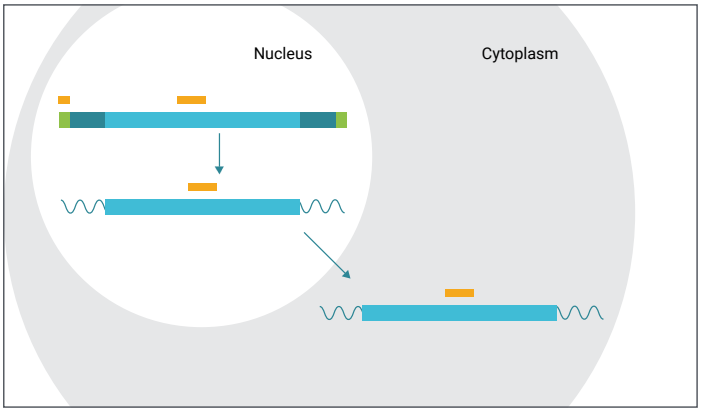
HIGHLY SENSITIVE & SPECIFIC

The RNAscope™ technology is a highly sensitive and specific RNA *in situ* hybridization (ISH) method for quantitative, tissue-based assessment of gene expression and addressing questions about therapeutic gene delivery vectors biodistribution and transgene expression. With regards to viral vectors such as adeno-associated virus (AAV) and transgene pharmacodynamics and pharmacokinetics, RNAscope™ ISH is an ideal method to:

- Monitor AAV tissue distribution and transgene expression in any animal model or human tissues
- Quantify AAV+ cell number in target and non-target tissues
- Obtain visual information about penetration of AAV from vasculature into the target tissue
- Quantify cell-specific transgene RNA expression over time to assess expression maintenance and transduced cell clonality
  - Distinguish uniform expression versus clonal populations with heterogeneous expression



AAV transgene structure and the RNAscope™ or BaseScope™ probe design

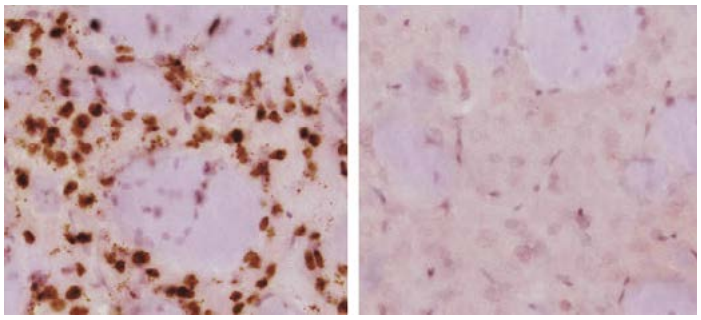


Schematic of AAV transgene detection in a cell with the RNAscope™ or BaseScope™ assay

SINGLE-MOLECULE DETECTION

The BaseScope™ and RNAscope™ assays use proprietary probe design and signal amplification technologies for specific, single-molecule detection of AAV vector DNA and transgene RNA, including the products of codon-optimized transgenes, in intact fixed tissue:

- Specific detection of AAV genomic DNA is achieved with probes targeting AAV inverted terminal repeat (ITR) sequence and anti-sense strand
- Detection of therapeutic transgene RNA without cross-hybridization to endogenous transcripts is achieved using proprietary probe design algorithms
  - A single nucleotide difference is sufficient for differential detection with the BaseScope™ assay



Detection of rAAV in injected striatum (left) and non injected striatum (right) using the RNAscope™ Assay

Learn more about RNAscope™ ISH for Gene Therapy Application | acdbio.com/genetherapy

RUN A PILOT STUDY WITH ACD's PHARMA ASSAY SERVICES (PAS)

- **Expert team** running and analyzing more than 10,000 slides per year
- **12 AAV probes** designed and in use; additional probes for any AAV genotype and transgene in two weeks
- **Four weeks** turn-around time from receipt of samples to results for typical projects
- **Board-certified** pathologist review
- **Quantitative** image analysis HALO™ Software

THE RNAscope™ ASSAY IN PUBLICATIONS ON GENE THERAPY

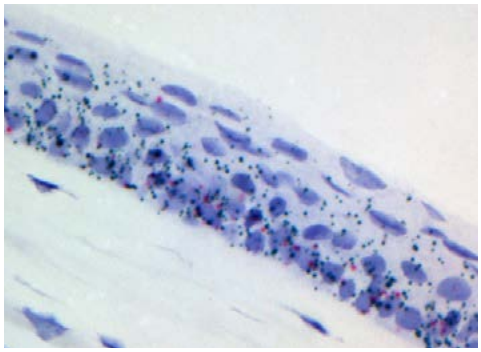
Detection of rAAV2/5 DNA in the rat striatum
Grabinski TM, *et al*. PLoS One (2015).

Impact of age and vector construct on viral-mediated gene transfer in rat brain
Polinski NK, *et al*. Mol Ther Methods Clin Dev (2016).

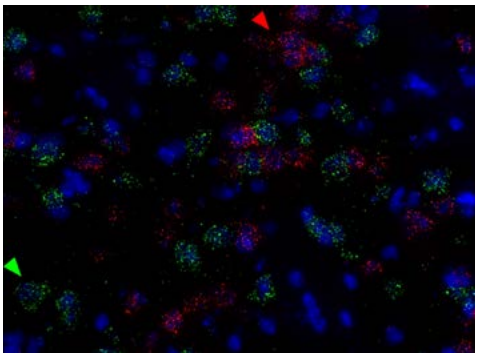
Silencing of the Huntington's disease gene with RNAi using AAV9-mediated delivery of microRNA
Keeler AM, *et al*. J Huntingtons Dis (2016).

Delivery of the secreted protein EPO to the liver via mRNAnanoparticle therapy
DeRosa F, *et al*. Gene Ther (2016).

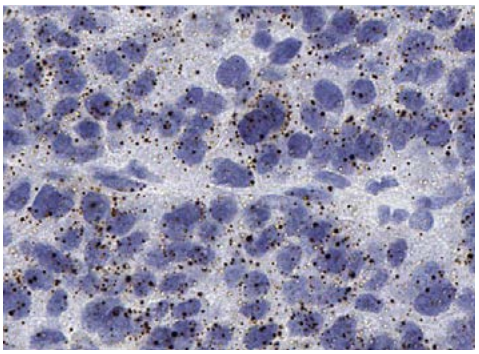
Silencing of ALS-associated gene SOD1 using microRNA-SOD1 delivered via rAAVrh10 in mice and nonhuman primates
Borel F, *et al*. Hum Gene Ther (2016).



Detection of *Wnt4* (red) and *Procr* (green) in the mouse retina



Detection of the dopaminergic GPCRs *Drd1* (red) and *Drd2* (green) in the striatum of the mouse brain



Detection of *Met* in human liver cancer

Learn more about ACD's Pharma Assay Services (PAS) | acdbio.com/services

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