miRNAscope™: a novel *in situ* hybridization technology for the detection of small regulatory RNAs with spatial and morphological context

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Introduction

microRNAs (miRNAs) are 18 to 22 nucleotide small regulatory RNA molecules that are expressed in a tissue, cell-type, and cell-state specific manner. Attempts at cataloging miRNAs have relied on small RNA sequencing approaches, microarray, and quantitative polymerase chain reaction (qPCR). While these methods deliver bulk expression levels, they do not provide detailed spatial information for miRNA expression. Additionally, existing small RNA ISH technologies suffer from poor reproducibility, specificity and sensitivity, extensive optimization at the level of sample pretreatment, target probe hybridization, and post-hybridization, consuming substantial quantities of specimen samples. This emphasizes the need for a technology that can reliably detect cell-type specific small RNA species in a tissue while maintaining high detection sensitivity and specificity.

To address this need ACD developed an RNA *in situ* hybridization (ISH) method known as miRNAscope™ . The miRNAscope™ ISH technology allows for detection of small RNAs, including miRNAs, with high detection sensitivity, specificity, and with single cell resolution. miRNAscope™ offers a "Ready-to-Use" small RNA detection ISH assay supplied with universal tissue pretreatment reagents and zero target probe optimization.

In this study, an RNA ISH assay was developed for the detection of small RNA species with unparalleled detection sensitivity and specificity, enabling morphological information with single cell resolution. The miRNAscope™ assay will improve the understanding of small RNA species in their native context and will elucidate their associated gene regulatory networks involved in health and disease.

miRNAscope™ Assay Applications and Key Features

- **Applications**
  - miRNA/siRNA/ASO tissue biodistribution
  - miRNA gain-of-function and loss-of-function studies
  - siRNA/ASO delivery mechanism, chemistries, short-term and long-term stability
- Key Features
  - Robust universal assay offering high sensitivity and high specificity
  - Universal assay conditions for all probes
  - Custom probe design to small RNAs between 17-50 nucleotides
  - Compatible with protein detection (ISH-IHC)
  - Compatible with FFPE, Fresh/Fixed Froze, Culture cell samples
  - Single-plex, chromogenic (Fast-RED) assay
- Platforms
  - miRNAscope™ HD Assay—Ready to Use reagents
  - miRNAscope™ LS Assay—fully automated with Ready to Use reagents

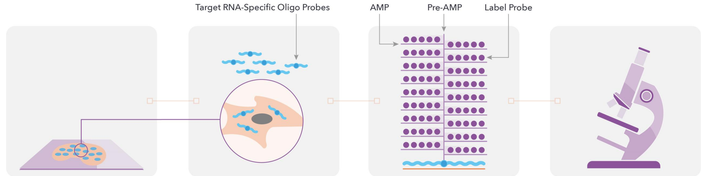

Figure 1: miRNAscope™ Assay procedure overview. Tissue sections are hybridized with miRNAscope™ specific-probes followed by signal amplification system to detect miRNAs and finally visualized using a standard bright-filed microscope.

Results

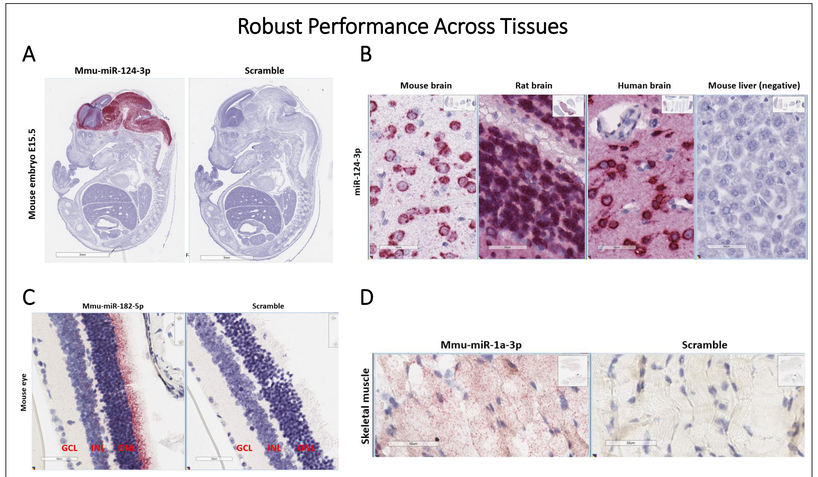

Figure 2: miRNA detection in various mouse tissues. **A)** Specific detection of Mmu-miR-124-3p in the central nervous system of FFPE mouse embryo E15.5. As a control, staining with a scramble probe demonstrates the specificity of the miRNAscope™ assay. **B)** Detection of the conserved miR-124-3p in FFPE normal adult mouse, rat and human brain tissues. The mouse liver, a known negative tissue, shows no miR-124-3p detection. **C)** Detection of the outer nuclear layer (ONL) specific miR-182-5p in FFPE mouse eye. As expected, negligible miR-182-5p was detected in the inner nuclear layer (INL) or ganglion cell layer. Staining with scramble probe demonstrates miRNAscope™ assay specificity. **D)** Visualization of the mouse skeletal muscle-enriched mmu-miR-1a-3p in FFPE quadricep tissue. As a negative control, tissue was stained with scramble probe demonstrating the specificity of this assay.

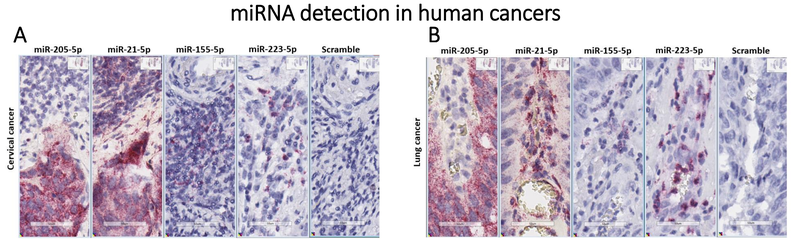

Figure 3: Detection of commonly studied miRNAs in human cervical and lung cancer. miRNA detection in **A)** human cervical and **B)** lung FFPE tissues. Two miRNAs, human miR-205-5p and miR-21-5p, were observed at high levels in cervical and lung cancer tissues in a cell-type specific manner. The myeloid-enriched miR-223-5p miRNA was detected in high levels in a subset of cells with a strong nuclear stain. A lymphoid-specific miRNA, miRNA-155-5p, was found to be present at lower levels in densely populated regions of the cancer tissues. As a negative control tissues were stained with scramble probe demonstrating the specificity of the miRNAscope™ Assay.

miRNAscope™ ISH-IHC Co-detection and Assay Controls

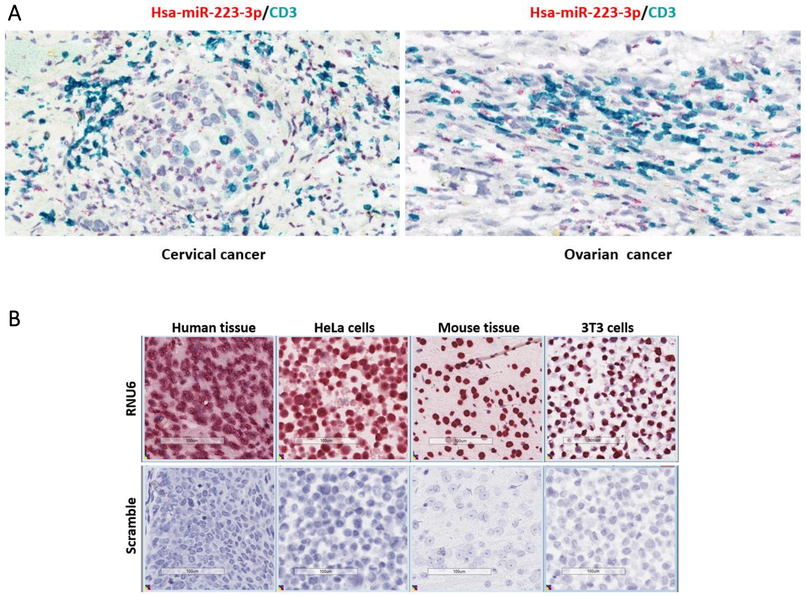

Figure 4: Sequential miRNAscope™ ISH-IHC and Assay Controls. **A)** Sequential ISH-IHC staining was performed on human cervical and ovarian cancer tissues. The myeloid-specific miRNA, hsa-miR-223-5p, and T-cell marker CD3 were detected on the same tissue section. ISH and IHC staining shows mutually-exclusive localization. **B)** miRNAscope™ Assay will offer universal positive and negative control probes. Strong nuclear RNU6 signal is observed in a variety of tissues including human, mouse, Hela, and 3T3 tissues/pellets. A universal scramble probe demonstrates the specify of this assay across species and samples.

Summary

- The miRNAscope™ Assay is a robust RNA in situ hybridization assay enabling the detection of small RNAs in tissues with spatial and morphological context at single cell resolution. Key applications include: miRNAs, siRNA, ASO, and other short RNA sequences (17-50nt). The assay will be a single-plex chromogenic (Fast-RED) assay compatible with the Manual platform or the fully automated Leica platform.