Application Note

Introducing the novel miRNAscope™ *in situ* hybridization assay for the robust detection of microRNAs and other small RNA targets with spatial resolution

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INTRODUCTION

The RNAscope™ *in situ* hybridization (ISH) technology provides a powerful method to detect gene expression within the spatial and morphological tissue context. The proprietary probe design strategy in combination with the advanced signal amplification enables highly specific and sensitive detection of the target RNA. This robust high signal-to-noise technology allows for the detection of gene transcripts at the single molecule level with single-cell resolution and can further expand our understanding of gene expression in cell lines and tissues samples.

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs that can regulate the expression of mRNAs post transcriptionally either via translational repression or mRNA degradation. Dysregulation of miRNA expression is observed in various diseases and altered physiological states. Genes encoding the miRNA are significantly longer than the mature miRNA and are transcribed into large pri-miRNA which are processed into pre-miRNA and finally single-stranded mature miRNA which are approximately 18-22 nucleotides long.

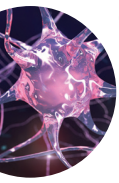
This document details the applications of the new miRNAscope assay in detecting a wide range of miRNAs across different samples for the most popular research areas.

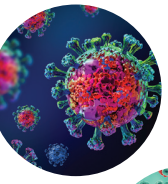
LIMITATIONS OF CURRENT TECHNOLOGIES IN DETECTING miRNAs

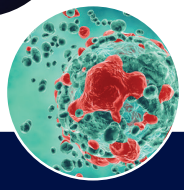
The current most commonly used tools for miRNA detection rely on RNA sequencing methods, microarray, and quantitative polymerase chain reactions (qPCR). While these methods deliver bulk expression levels, they do not provide detailed spatial information for miRNA expression. Although, ISH-based miRNA detection methods that provide spatial context do exist, they demonstrate significant shortcomings in their performance:

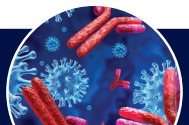
- Low sensitivity
- Poor signal-to-noise ratio
- Require extensive optimization for different probes and sample types
- Poor reproducibility

There thus remains an urgent need for a technology that can reliably detect cell type-specific small RNA species in cells and tissue while maintaining high detection sensitivity and specificity. **We have developed the breakthrough miRNAscope Assay, an advanced *in situ* hybridization assay that allows for highly specific and sensitive visualization of small RNA species expression with single cell resolution.**









THE NEW miRNAscope ASSAY CAN DETECT

- **miRNAs and other short noncoding RNAs** that regulate cellular processes
- **siRNAs** and monitor their delivery and biodistribution
- **Antisense oligonucleotides (ASOs)** and visualize their biodistribution

THE NEW miRNAscope ASSAY WORKFLOW

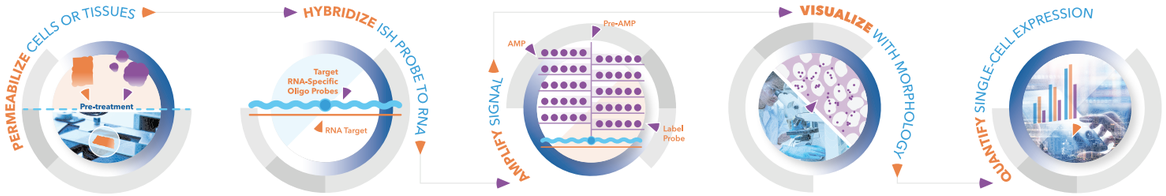


Figure 1. miRNAscope™ Assay procedure overview: Tissue sections are hybridized with miRNAscope specific-probes followed by signal amplification and finally visualized using a standard bright-filed microscope.

THE miRNAscope ASSAY CAN DETECT miRNAs IMPLICATED IN CANCER AND DISTINGUISH BETWEEN MULTIPLE MEMBERS FROM THE SAME FAMILY

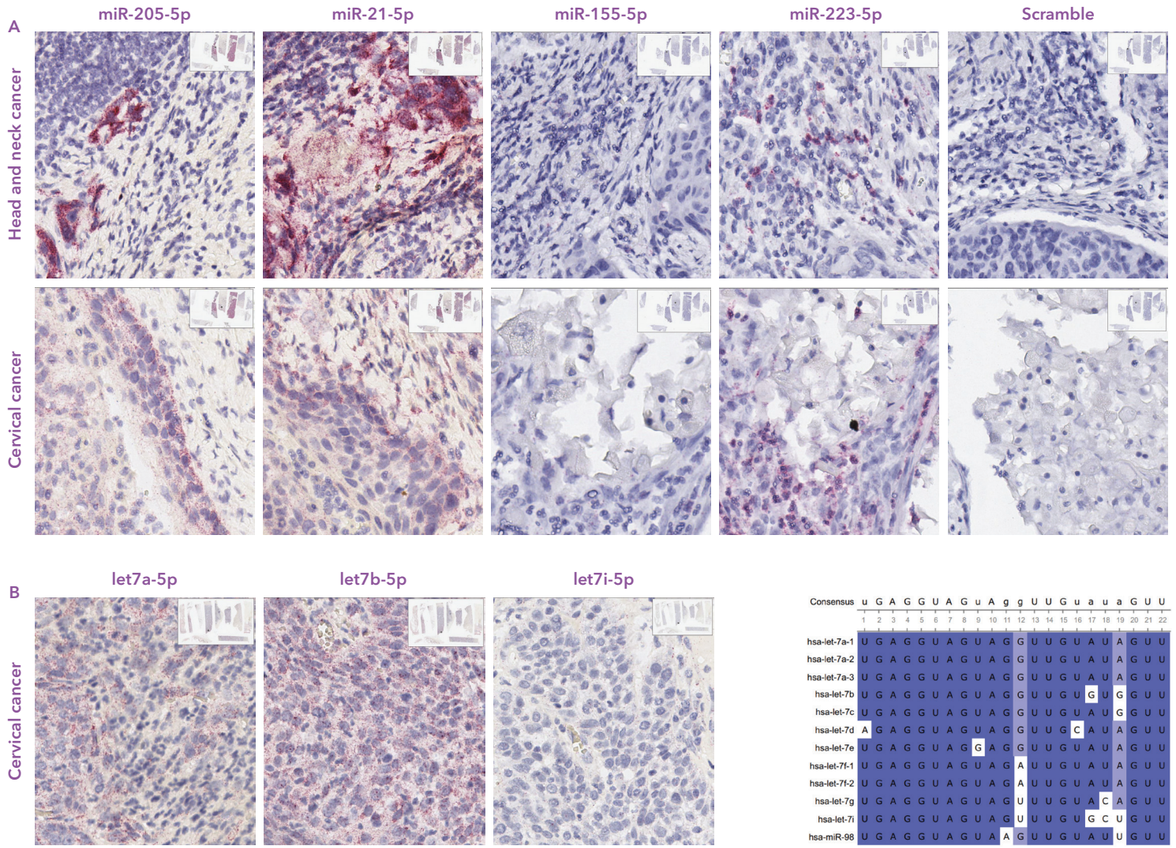


Figure 2. Detection of miRNAs in solid tumors: A, red punctate dots indicate positive signal for target miRNAs in head and neck tumor and cervical tumor. B, detection of let7a, let7b and let7i miRNAs in cervical cancer tumor.

THE miRNAscope ASSAY DEMONSTRATES ROBUST DETECTION OF miRNAs EXPRESSED IN THE BRAIN

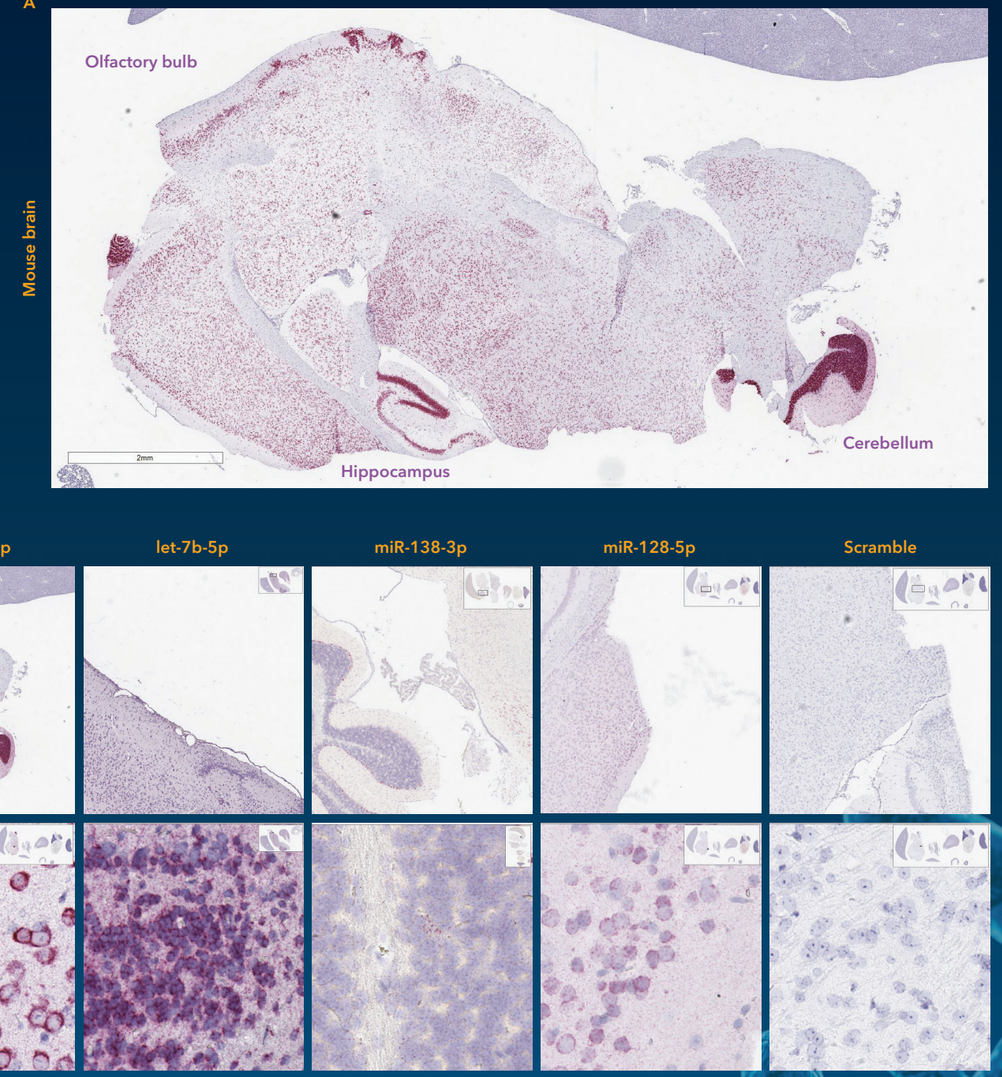


Figure 3. Detection of miRNAs in neuronal development and homeostasis: A, miR-124 expression is observed throughout the mouse brain. B, cell-specific expression of different miRNAs in the mouse brain tissue.

THE miRNAscope ASSAY CAN IDENTIFY TISSUE-SPECIFIC AND DEVELOPMENTAL STAGE-SPECIFIC EXPRESSION OF miRNAs

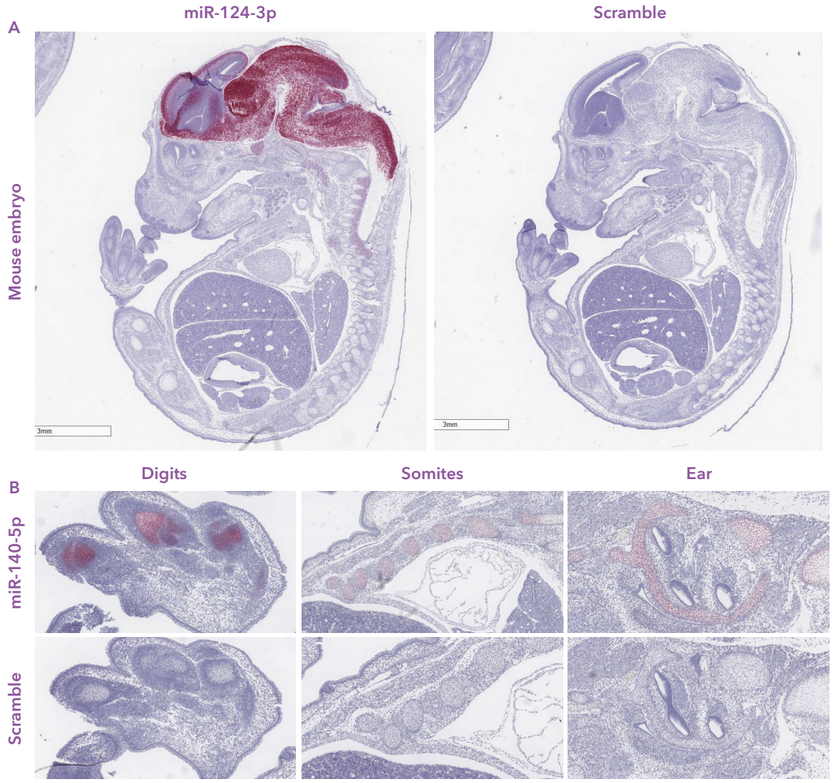


Figure 4. **Detection of miRNAs in developing mouse embryo:** A, positive miR-124 expression in the developing mouse brain and other parts of the nervous system. B, expression of miR-140 in the developing cartilage tissue of the mouse embryo.

CO-DETECTION OF TARGET miRNAs AND PROTEINS USING THE JOINT ISH-IHC WORKFLOW

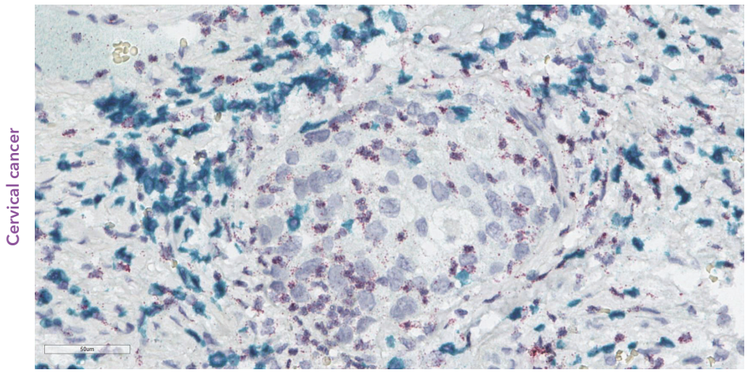


Figure 5. **Simultaneous detection of miRNA and protein in cervical cancer using miRNAscope ISH-IHC:** Myeloid cell-specific miR-223 visualized as red punctate dots in combination with CD3 protein in green.

THE miRNAscope ASSAY IS COMPATIBLE WITH DIFFERENT SAMPLE AND TISSUE TYPES

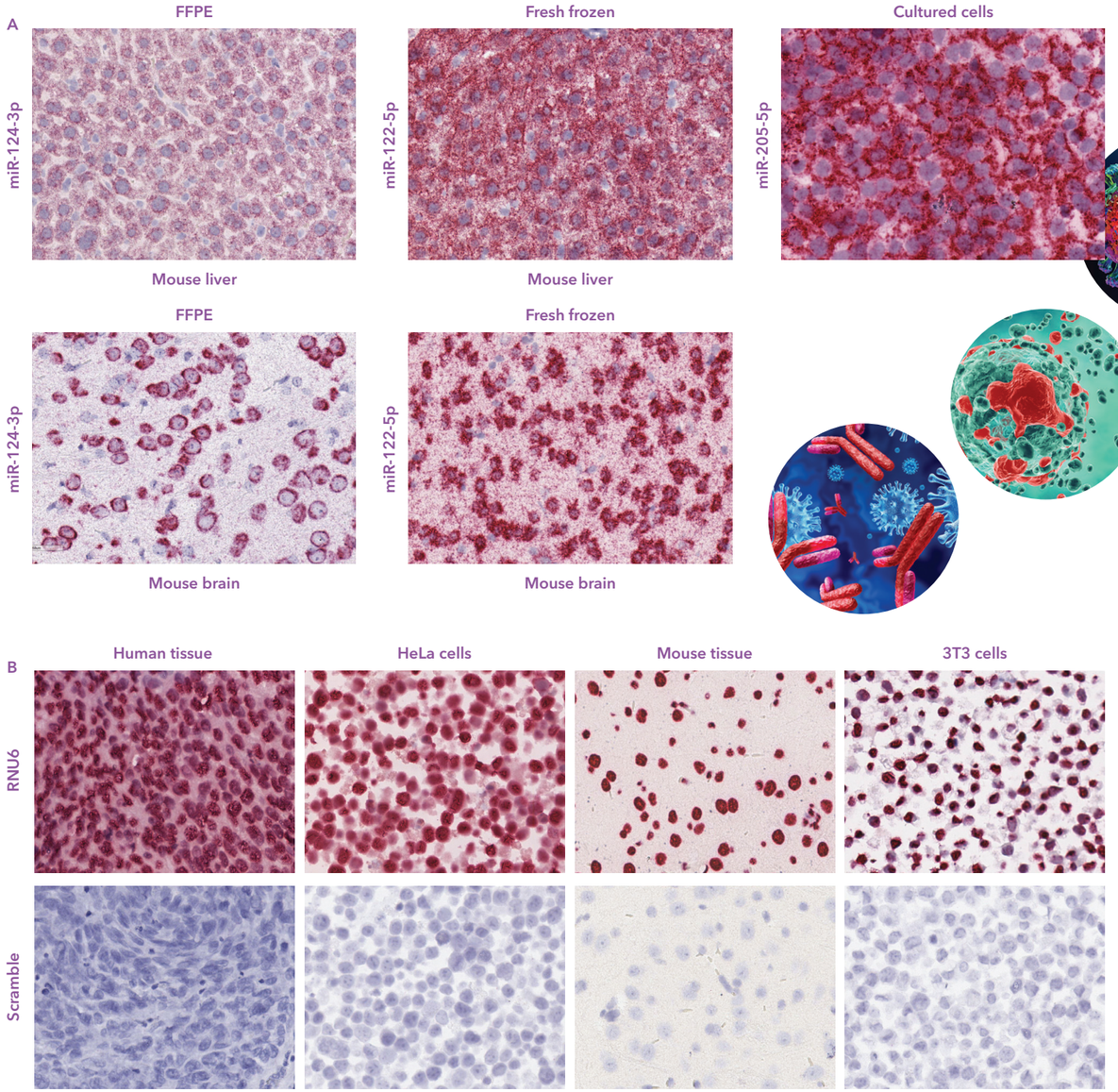


Figure 6. **miRNAscope assay is compatible with different sample types:** A, target miRNA detection in fresh frozen and FFPE samples from liver and brain tissues. B, strong positive signal detected using the universal positive control probe for the miRNAscope assay.

APPLICATIONS IN CANCER RESEARCH

Since miRNAs can efficiently regulate the expression of tumor suppressors as well as oncogenes, several miRNAs have been implicated in cancer initiation and progression. MiR-205 is upregulated in both cervical and ovarian cancer tissues. It promotes tumor invasion, migration and angiogenesis by activating the AKT signaling pathway. Similarly, miR-21 is also frequently upregulated in most gynecological malignancies including cervical and ovarian cancer. This oncogenic miRNA is upregulated by NFkB and the MAPK signaling pathway and helps in the dysregulation of programed cell death proteins. The miRNAscope assay can successfully distinguish between different members of the same miRNA family with similar sequences such as the let-7 miRNAs. In addition, there are several miRNAs such as miR-155, miR-223 and miR-126 that are upregulated in solid tumors that can be detected with high specificity using the miRNAscope assay (Figure 2).

APPLICATIONS IN NEUROSCIENCE

MiR-124 is one of the most abundantly expressed miRNA in the central nervous system and plays a role in neuronal development, maturation and survival. Similarly, miR-138p is expressed in the Purkinje cells and regulates cellular differentiation and maintenance. Let-7b regulates neural stem cell proliferation and differentiation by suppressing the expression of Ccnd1 and Txl. Another miRNA, miR-128 is significant in the development of the brain cortex and its aberrant expression can contribute to several neuropsychiatric conditions (Figure 3).

APPLICATIONS IN DEVELOPMENTAL BIOLOGY

Mir-140 has been demonstrated to regulate cartilage development in embryos and maintain tissue homeostasis. Downregulation of miR-140 expression leads to osteoarthritis-like changes in the cartilage. MiR-124 also plays a significant role in the developing nervous system by promoting the differentiation of progenitor cells to mature neurons (Figure 4).

The miRNAscope assay is also compatible with the *in situ* hybridization - immunohistochemistry (ISH-IHC) workflow which allows visualization of RNA and protein targets simultaneously. Co-detection of miRNA and protein targets can assist in expanding our understanding of cell-specific expression of pathologically relevant miRNAs (Figure 5). Similar to the RNAscope assay, the new miRNAscope assay is also compatible with fresh frozen samples, FFPE tissues and cultured cells (Figure 6A). The assay can be performed using a manual workflow as well as the Leica automated Stainer with equal efficiency.

Assessing sample RNA quality and specificity of signal detection necessitates the use of suitable positive and negative control probes. We have developed a universal positive control probe that works across different species and sample types. Scramble probes do not identify any mRNA or miRNA sequences and can be used as negative control probes for the miRNAscope assay (Figure 6B). Besides the miRNA probes highlighted here, made-to-order probes for any miRNA can be developed and delivered within 3 weeks.

SUMMARY

The new miRNAscope Assay is a RNA in situ hybridization assay enabling highly robust detection of miRNAs, siRNAs, ASOs, and other smaller RNAs (17-50nt) in tissues with spatial and morphological context at single cell resolution. Compared to the current tools for small-RNA analysis, the miRNAscope assay drastically improves the detection sensitivity and specificity of small-RNA species in various sample types across different tissues, thus filling a critical gap in RNA ISH technologies.

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