EXPRESSION OF MATURE microRNAS, miR-1287-5p AND miR-658 IS UPREGULATED IN DIABETIC KIDNEY DISEASE (DKD) TISSUES

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. One such example is Diabetic Kidney Disease (DKD) and mechanisms underlying progression of DKD to End Stage Kidney Disease (ESKD) are not fully understood. In a recent study by Eiichiro Satake *et.al.*, authors identified a signature of 17 miRNAs and 6 axon guidance pathway proteins that were robustly associated with an increased 10-year risk of ESKD in Type 1 and Type 2 diabetes. Using miRNAscope *in situ* hybridization assay, localization and levels of two exemplar miRNAs, miR-1287-5p and miR-658 were detected in tissue affected by DKD but not in normal kidney tissue.[1]

The miRNAscope™ Assay is an 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 (**FIGURE 1**). 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 spatial analysis of miRNA expression.

miRNAscope™ ISH ASSAY FOR DETECTION OF MATURE miRNAS

- High sensitivity and specific detection of small RNA molecules with morphological context and spatial resolution
- Rapid probe design to target almost any small RNA markers (17-50nt) in any species

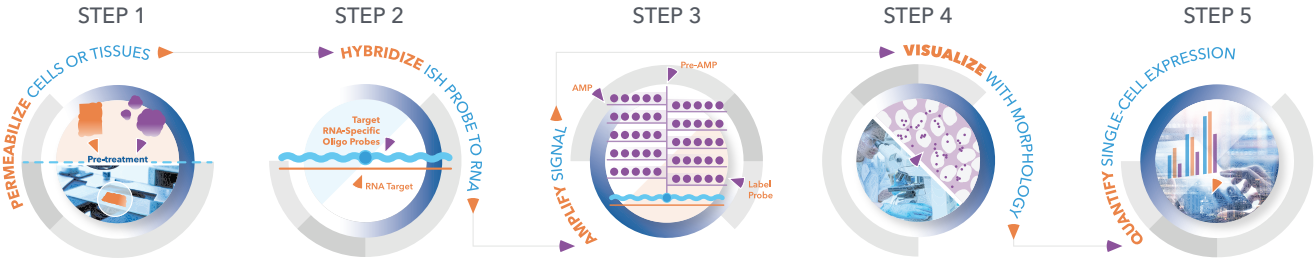

FIGURE 1. miRNAscope assay overview. Schematic of the workflow for the miRNAscope *in situ* hybridization methods used for the detection of miRNAs. FFPE kidney tissue sections were hybridized with miR-1287-5p and miR-658 probes followed by signal amplification to detect miRNAs. Finally, visualization of morphology and quantification was performed using a standard bright-field microscope.

miR-1287-5p AND miR-658 EXPRESSION WAS DETECTED IN DKD KIDNEY PATIENT SAMPLES BUT NOT IN NORMAL KIDNEY TISSUES USING miRNAscope ASSAY

The miRNAscope results demonstrate that the expression of miR-1287-5p and miR-658p is upregulated in human DKD kidney samples (**FIGURE 2**). The DKD kidney samples demonstrated expression of miR-1287-5p and miR-658 visualized as punctate red dots in the cells. Normal kidney tissues showed no expression of the miRNAs indicating a potential correlation between DKD and increased expression of miR-1287-5p and miR-658. The expression was further quantified using visual scoring system. **TABLE 1** depicts the scoring and quantification of percent positive cells expressing miR-1287-5p and miR-658 in normal and DKD kidney samples.

SAMPLE TYPE	miR-1287-5p SCORE; (% RANGE OF POSITIVE CELLS)	miR-658 SCORE; (% RANGE OF POSITIVE CELLS)
Human normal kidney 1	0; (0%)	0; (0%)
Human normal kidney 2	0; (0%)	0; (0%)
Human normal kidney 3	0; (0%)	0; (0%)
Human DKD kidney 1	1; (26-50%)	1; (1-25%)
Human DKD kidney 2	1; (1-25%)	1; (1-25%)
Human DKD kidney 3	1; (26-50%)	1; (1-25%)

TABLE 1. Quantification of percent positive cells expressing miR-1287-5p and miR-658 in normal and DKD kidney samples. Percentage of cells positive is scored visually based on number of cells with >1 dot/cell and binned into categories (i.e., 0%, 1-25%, 26-50%, 51-75%, 76-99%, 100%). Visual scoring was performed to assign a single score to a sample as, 0: No staining or <1 dot / cell; 1: 2-10 dots/cell no or very few dot clusters; 2: 11-20 dots/cell and/or <25% dots clusters or 3: >20 dots/cell and/or >25% dots clusters.

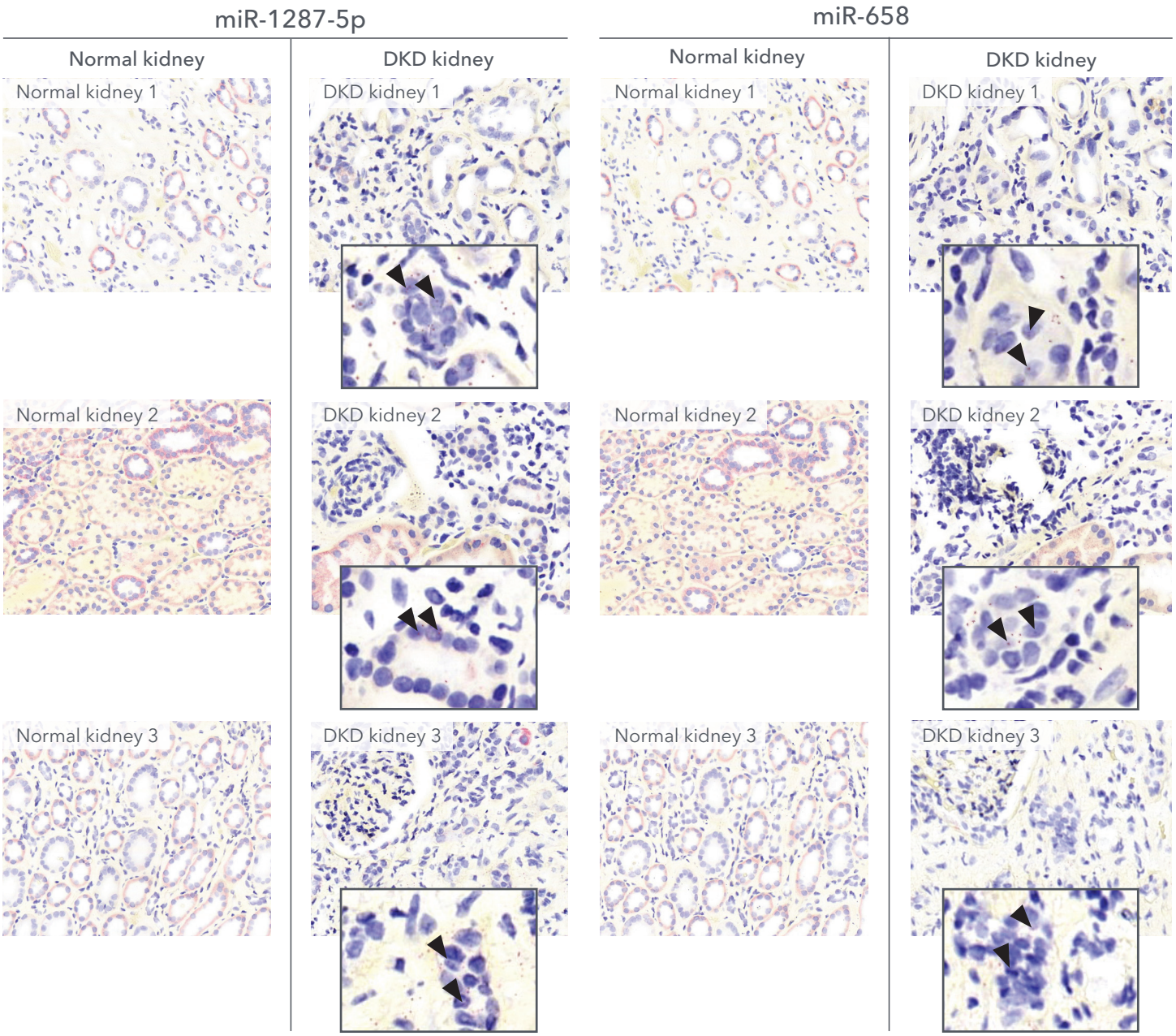

FIGURE 2. Expression of miR-1287-5p and miR-658 in normal and DKD human kidney samples. miRNAscope assay to detect miR-1287-5p using the SR-hsa-miR-1287-5p-S1 probe and detection of miR-658 using the SR-hsa-miR-658-S1 probe in normal and DKD kidney samples (3 samples each). Red punctate dots indicate positive staining marked by arrows.

In summary, this document demonstrates the capabilities of the miRNAscope assay for detection and visualization of miRNA targets in normal and diseased tissues. The miRNAscope assay provides a valuable tool to identify miRNA signatures in normal and diseased tissues with spatial and morphological context, and can be used for quantification of miRNAs in tissues. In this study we have detected cell specific expression of miR-1287-5p and miR-658 in normal vs DKD tissues at single cell resolution. This assay is also a powerful tool for assessing miRNA function by revealing the spatial organization of miRNA expressing cells and their role in disease progression. The miRNAscope platform thus provides a highly robust tool for identifying potential therapeutic RNA targets for DKD as well as other disease conditions.

REFERENCE

1. Satake *et al*., Comprehensive Search for Novel Circulating miRNAs and Axon Guidance Pathway Proteins Associated with Risk of End Stage Kidney Disease in Diabetes. Journal of the American Society of Nephrology. 32 (9) 2331-2351, 2021