Application Note

Unlock miRNA expression profiles from FFPE samples using miScript® miRNA PCR Arrays

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miRNA expression profiling provides a valuable window into changes occurring in a biological system. Due to their stability and size, miRNAs can be readily extracted from FFPE samples using the miRNeasy FFPE Kit. Here, we use the miScript PCR System to profile miRNA expression changes in total RNA samples isolated from FFPE normal and tumor lung tissues.

Profile miRNA expression data in FFPE tissue

The miScript PCR System, in conjunction with the miRNeasy FFPE Kit, provides a sample-to-result solution for miRNA expression profiling using archived FFPE samples. The miScript PCR System consists of the miScript II RT Kit, miScript SYBR® Green PCR Kit, miScript Primer Assays and miScript miRNA PCR Arrays, and uses total RNA that contains miRNA as the starting material for cDNA synthesis (Table 1). RNA isolated from FFPE samples can be heavily fragmented, and formalin crosslinking can impair reverse transcription reactions. The miRNeasy FFPE Kit is specifically optimized to reverse crosslinking and efficiently release total RNA containing miRNA from tissues. miScript miRNA PCR Arrays have been organized into biological- and disease-focused panels using state-of-the-art bioinformatics algorithms and text mining tools. These panels profile the most relevant miRNA expression changes in total RNA from FFPE samples (Figure 1), with highly reproducible, reliable results (Figure 2).

Table 1. FFPE tissue recommendations

Step	Starting materials/conditions
Formalin fixation/ paraffin embedding	Use tissue less than 5 mm thick. Fix in 4–10% neutral-buffered formalin with a maximum fixation time of 24 hours.
Total RNA preparation from FFPE tissues	Use sections 5–20 µm thick, or multiple sections with combined thickness no greater than 40 mm and surface area <250 mm^2. Amount of FFPE tissue required depends on amount of tissue embedded and RNA quality. RNA quality will vary due to fixation and storage conditions.
RNA input for cDNA conversion	Use 125 to 250 ng per sample for pathway-focused arrays and 250 to 500 ng for whole miRNome.

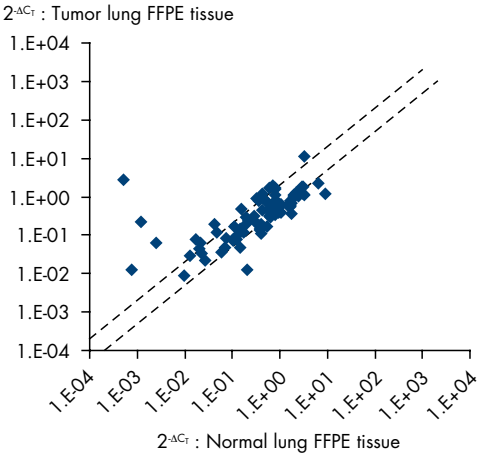

Figure 1. miRNA expression changes identified in tumor lung tissue. A scatter plot of $2^{-\Delta C_T}$ values shows significant differences in mature miRNA expression levels between normal and tumor lung tissue. These include miRNAs whose expression levels have been previously shown to be up-regulated (hsa-miR-9-5p, hsa-miR-18a-5p, hsa-miR-210-3p) and down-regulated (hsa-miR-126-3p, hsa-miR-143-3p) in lung cancer (1–4). For this experiment, 5 µm FFPE lung tissue sections were purchased from Asterand, and total RNA was prepared using the miRNeasy FFPE Kit. Using the miScript II RT Kit with miScript HiSpec Buffer, 125 ng RNA was reverse transcribed, and 0.5 ng cDNA was added to each well of the Human miFinder miScript miRNA PCR Array.

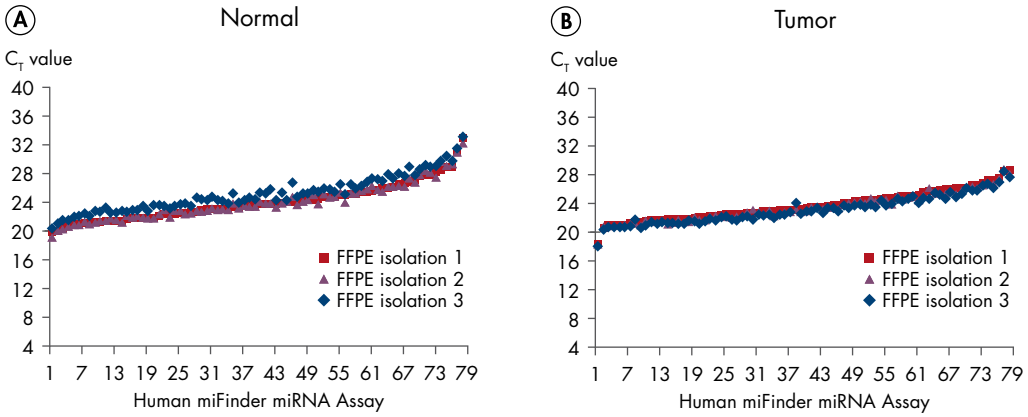

Figure 2. miScript FFPE Kit and miFinder miScript miRNA PCR Array consistently provide highly reproducible results. The raw data presented for one normal **A** and one tumor **B** FFPE lung tissue donor source demonstrates the high reproducibility that can be achieved using the miRNeasy FFPE Kit in combination with the miScript PCR System and miScript miRNA PCR Arrays. Each isolation was from a different section from the same donor, with the normal and tumor tissues coming from different donors.

Reference

1. Liu, C.G., et al. (2004) An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. Proc. Natl. Acad. Sci. USA 1. **101**, 9740.
2. Crawford, M., et al. (2009) MicroRNA 133B targets pro-survival molecules MCL-1 and BCL2L2 in lung cancer. Biochem. Biophys. Res. Commun. 2. **388**, 483.
3. Hayashita, Y., et al. (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 3. **65**, 9628.
4. Yanaihara, N., et al. (2006) Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 4. **9**, 189.

Ordering Information

Product	Contents	Cat. no.
miRNome miScript miRNA PCR Array	miRNome panels of miRNA assays	Varies
miScript miRNA PCR Array	Pathway- or disease-focused panels of miRNA assays	Varies
Custom miScript miRNA PCR Array	Custom panels of miRNA assays	Varies
miRNeasy FFPE Kit (50)	Columns, plasticware and reagents for 50 preps	217504
miScript SYBR Green PCR Kit (200)	Reagents for 200 x 50 µl PCR reactions	218073
miScript Primer Assay (100)	miRNA-specific primer for 100 x 50 µl PCR reactions	Varies*
miScript II RT Kit (12)	Reagents for 12 x 20 µl cDNA synthesis reactions	218160

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