n a n o S t r i n g

nCounter® miRNA Expression Assay Kit

miRNA Biology & Biomarker Development

Identify and validate miRNA biomarkers faster and more reproducibly than with RNA-Seq or qPCR-based platforms. Using the nCounter miRNA Expression Assay Kit, you can analyze hundreds of biologically relevant miRNAs in less than 24 hours with a simple protocol that does not rely on reverse transcription or amplification.

Product Highlights

- Reliably detect and quantitate the most biologically relevant human, mouse, miRNAs directly from FFPE, blood, or biofluids

- Skip laborious library prep and process your samples with less than one hour of hands-on time

- Experience unparalleled reproducibility and specificity with a dynamic range of five logs

- Receive publication-ready figures within 24 hours with robust, off-the-shelf data analysis solutions

Feature	Specifications
Number of Targets	• Human v3 assay: 827 • Mouse v1.5: 577 plus 33 murine-associated viral miRNAs
Sample Input - Standard (No amplification required)	100 ng
Sample Type(s)	FFPE, Blood, Biofluids, Cells
miRNA Sample Prep Reaction Volume	10 µL
Hybridization Reaction Volume	30 µL
Hands-on Time	45 Minutes
Time to Results	< 24 hours
Data Analysis	nSolver™ Analysis Software (RUO) or the ROSALIND® platform

nCounter miRNA Panel Overview

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that regulate gene expression of target mRNAs and have been implicated in the widespread control of critical biological processes such as proliferation, differentiation, and apoptosis. The relative abundance of stable circulating miRNAs associated with different disease states offers the potential for discovery of novel biomarkers and new biological insights.

The nCounter Human v3 miRNA expression assay kit contains miRNAs that have either been sequenced with high confidence and/or found to be clinically relevant (Figure 1) and an analysis of the cumulative sequenced reads of all mature human miRNAs found in the latest version v22 of miRBase (Figure 2) showed that the most abundant miRNAs are adequately covered in the human v3 assay.

nCounter miRNA Assay Preparation

The nCounter miRNA expression assay kit includes reagents for ligating unique oligonucleotide tags (miRtags) onto miRNAs of interest, allowing these small RNAs to be detected with great specificity and sensitivity. The miRtag ligation reaction (shown as Step 1 in Figure 3) can be performed in a background of total RNA. Sample preparation involves a multiplexed annealing of the specific tags to their target miRNA, a ligation reaction, and an enzymatic purification to remove unligated tags.

The hands-on time for the miRtag ligation step is 30 minutes, with a time of approximately two hours for the entire protocol. This innovative sample preparation method delivers tagged miRNAs ready for quantification using the nCounter Analysis System.
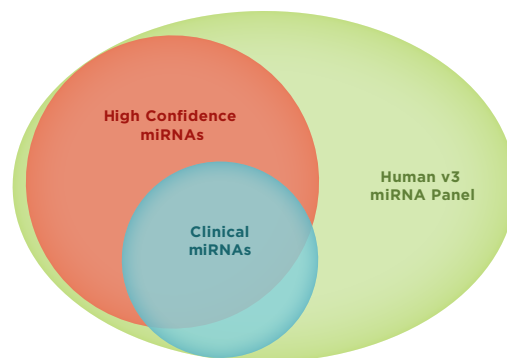

FIGURE 1: Coverage of the nCounter v3 Human miRNA expression assay kit compared to miRNAs that are sequenced with high confidence and/or found to be clinically relevant.
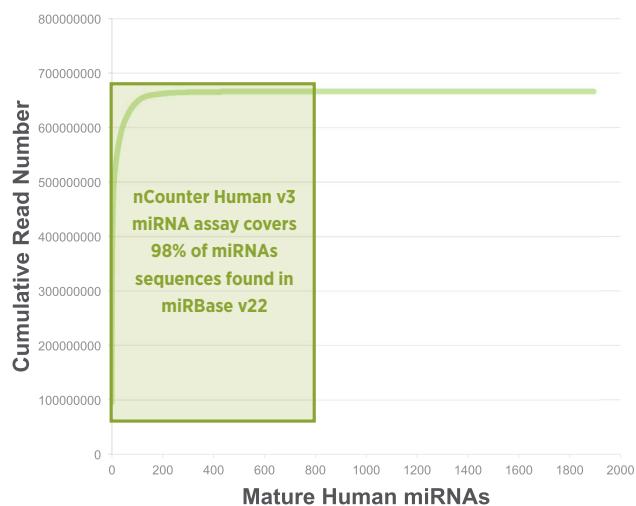

nCounter Human v3 miRNA assay covers 98% of miRNAs sequences found in miRBase v22

FIGURE 2: Cumulative mature human miRNA sequencing reads represented in the latest version v22 of miRBase. The nCounter Human v3 miRNA expression assay kit covers 98% of the miRNAs represented in miRBase v22.
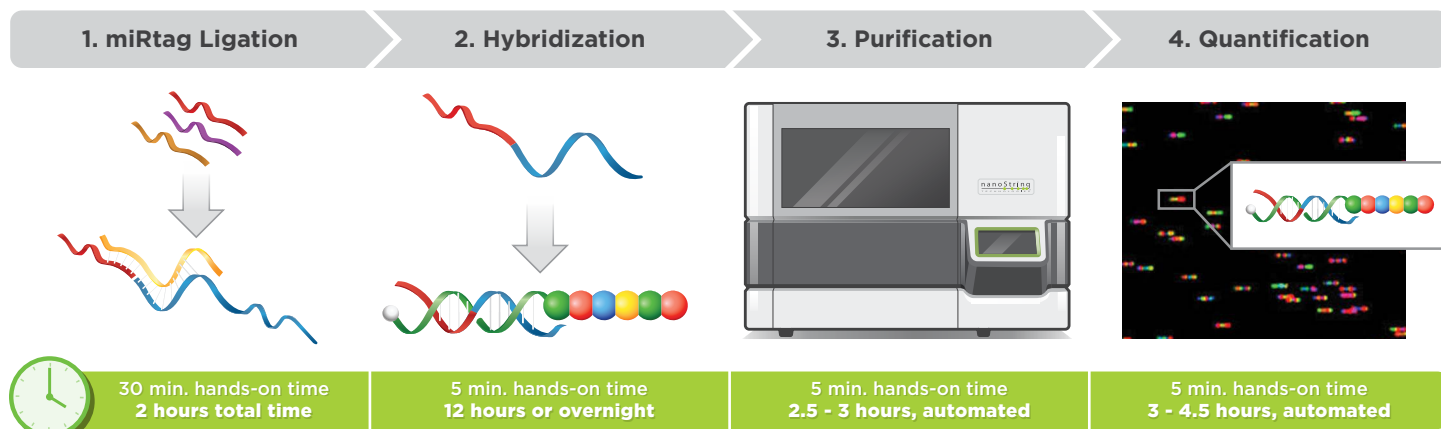
nCounter Workflow for miRNA

1. miRtag Ligation	2. Hybridization	3. Purification	4. Quantification
30 min. hands-on time 2 hours total time	5 min. hands-on time 12 hours or overnight	5 min. hands-on time 2.5 - 3 hours, automated	5 min. hands-on time 3 - 4.5 hours, automated

FIGURE 3: Workflow schematic for miRNA profiling using the nCounter system

nCounter Analysis System

After the ligation of miRtags to the targeted miRNAs, reporter and capture probes are added and hybridized to the tagged miRNAs overnight (shown as Step 2 in Figure 3) in a simple workflow that only takes four pipetting steps and 5 mins of hands-on time. After the overnight incubation, only 10 mins of hands-on time are required to set-up an automated purification step (to remove unbound probes) and facilitate digital counting of barcoded miRNAs (shown as Steps 3 and 4 in Figure 3). The nCounter miRNA expression assay kit can be run on any nCounter platform: the nCounter Analysis System or the SPRINT Profiler. Results are available next-day and can be turned into publication quality figures in minutes.

nCounter miRNA Kit Performance

To demonstrate the reproducibility of data generated via the nCounter miRNA Expression kit, we prepared Ambion® Human total Reference RNA per the nCounter miRNA Expression Assay Manual, using 100 ng total RNA per replicate sample preparation. Raw data were normalized to internal positive spike-in controls present in each reaction to account for minor differences in hybridization and purification efficiencies. Counts for individual probes in technical replicates within a cartridge were highly correlated between sample preparations (R^2 > 0.99, Figure 4A). Similarly, a comparison of nCounter data generated after preparation of total RNA from FFPE tissue and matched frozen (non-FFPE) tissue also showed highly significant concordance (R^2 > 0.95, Figure 4B).

Replicate experiments using the mouse v1.5 miRNA expression assay kit were compared (Figure 5A) and found to be highly correlated. Expression for a select set of miRNAs is shown for the panel of tissues assayed (Figure 5B). Each miRNA is shown normalized to the highest count value observed for that miRNA in the tissue panel. Each mouse tissue produced a distinct miRNA expression profile, e.g. mmu-miR-122 is highly expressed in liver, but minimally detected in other tissues. These data demonstrate the ability of the nCounter miRNA Assay to faithfully quantify the unique miRNA signature of a given tissue or cell type.

We examined both global miRNA expression in several mouse tissues and utilized the Let7 family of miRNAs (Table 1) to test sequence specific detection. Individual synthetic Let7 miRNAs were prepared and analyzed per the nCounter human v3 miRNA assay protocol and the counts for all family members were examined. Counts for each Let7 probe in a single target assay were then expressed as a percentage relative to the perfect match probe in that assay (Table 2). Most probes exhibited less than one percent cross-hybridization, indicating that the nCounter human v3 miRNA assay accurately distinguishes between highly similar miRNAs with great sequence specificity.
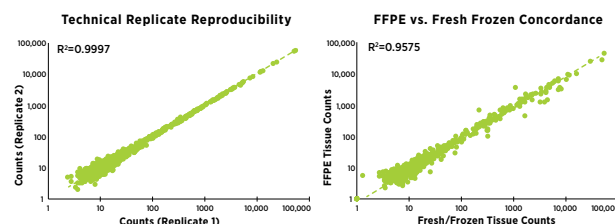

FIGURE 4: Human miRNA Kit Performance (A) Correlation between technical replicates of miRNA expression counts profiled using the human v3 miRNA expression assay kit. (B) Correlation between miRNA expression counts profiled in FFPE and fresh frozen tissue using the human v3 miRNA assay kit.
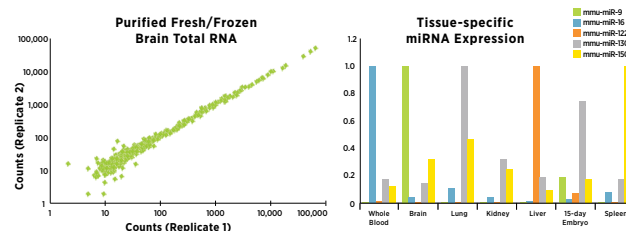

FIGURE 5: Mouse miRNA Kit Performance (A) Correlation between technical replicates of miRNA expression counts profiled in fresh frozen brain tissue using the mouse v1.5 miRNA assay kit. (B) Tissue-specific miRNA expression profiled in mouse using the mouse v1.5 miRNA expression assay kit.

miRNA	Sequence
hsa-let-7a	U G A G G U A G U A G G U U G U A U A G U U
hsa-let-7b	U G A G G U A G U A G G U U G U **G** U **G** G U U
hsa-let-7c	U G A G G U A G U A G G U U G U A U **G** G U U
hsa-let-7d	**A** G A G G U A G U A G G U U G **C** A U A G U U
hsa-let-7e	U G A G G U A G **G** A G G U U G U A U A G U U
hsa-let-7f	U G A G G U A G U A G **A** U U G U A U A G U U
hsa-let-7g	U G A G G U A G U A G **U** U U G U A C A G U U
hsa-let-7i	U G A G G U A G U A G **U** U U G U **G C U** G U U

TABLE 1: Sequences for miRNAs in the Let7 family are highly similar.

		miRNA Target							
		Let7a	Let7b	Let7c	Let7d	Let7e	Let7f	Let7g	Let7i
nCounter Probes	Let7a	**100%**	1%	5%	4%	17%	4%	-	-
	Let7b	-	**100%**	-	-	-	-	-	-
	Let7c	-	11%	**100%**	-	-	-	-	-
	Let7d	-	-	-	**100%**	-	-	-	-
	Let7e	-	-	-	-	**100%**	-	-	-
	Let7f	1%	-	-	-	-	**100%**	-	-
	Let7g	-	-	-	-	-	-	**100%**	-
	Let7i	-	-	-	-	-	-	-	**100%**

TABLE 2: Low cross-hybridization between miRNAs of the Let7 family demonstrates superior specificity of the nCounter human v3 miRNA expression assay.

System Performance

Description	Specifications
Limit of detection	≤ 0.5 fM (~10 copies per cell)
Fold change sensitivity	>2-fold change
Synthetic miRNA spike-in titration linearity	$R^2 \geq 0.95$
Linear dynamic range	2×10^5 total counts
Controls	Positive controls (6) – Probes that recognize synthetic mRNA targets included in the CodeSet at specified concentrations. Negative Controls (8) – Probes that recognize synthetic mRNA targets not included in the CodeSet. Ligation Positive Controls (3) – Probes that recognize synthetic miRNA targets included in the Sample Kit. Ligation Negative Controls (3) – Probes that recognize synthetic miRNA targets not included in the Sample Preparation Kit. mRNA Reference Controls (5 for Human Assay, 4 for Mouse Assay) - Probes that recognize endogenous mRNA targets commonly expressed in tissues. Spike-in Controls (5 for Human Assay, 3 for Mouse Assay) - Probes that recognize exogenous miRNA targets to monitor upstream RNA isolation/purification.

Ordering Information

Product Description	Quantity	Catalog Number
nCounter Human v3 miRNA Expression Assay Kit	12 Reactions	CSO-MIR3-12
nCounter Mouse v1.5 miRNA Expression Assay Kit	12 Reactions	CSO-MMIR15-12
nCounter Human v3 miRNA Sample Prep Kit	12 Reactions	Hu-MIRTAG-12
nCounter Mouse v1.5 miRNA Sample Prep Kit	12 Reactions	Mu-MIRTAG-12
nCounter a la carte Custom miRNA CodeSets	192 Reactions	GSA-MICS-192
nCounter MiRGE Codesets to Analyze mRNA & miRNA	48 Reactions	MIR-P1CC-48
nCounter Master Kit	Each	NAA-AKIT-012
nCounter Sprint Cartridge	Each	SPRINT-CAR-1.0
nCounter Sprint Reagent Pack	Each	SPRINT-REAG-KIT

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