

nCounter[®] Human miRNA Expression Assay Kit

The nCounter Human miRNA Expression Assay Kit

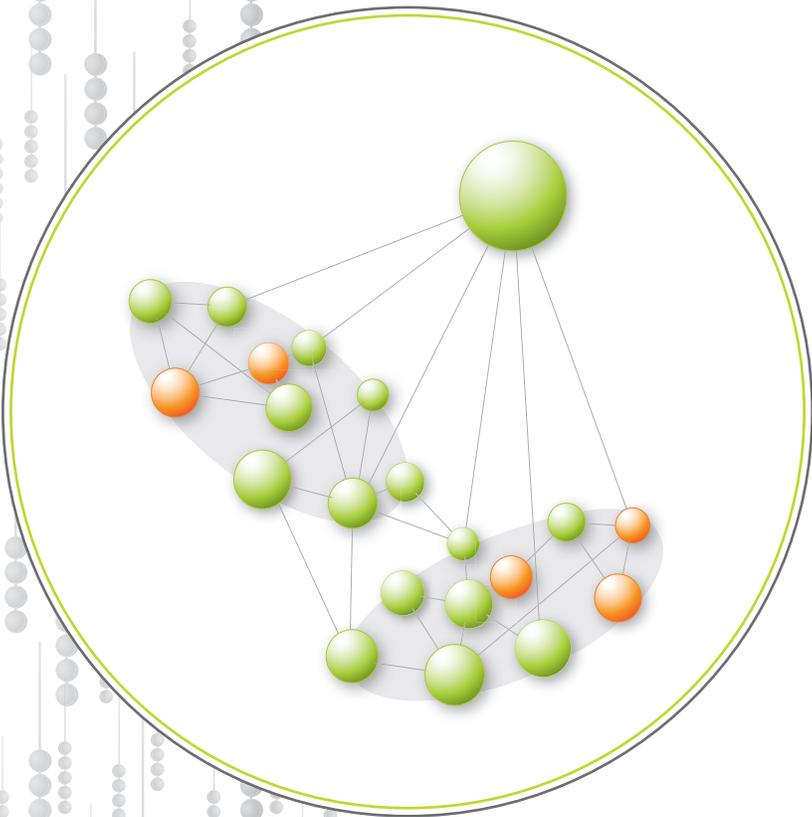
- Over 700 human and human-associated viral miRNAs from miRBase v. 14
- Highly specific and sensitive miRNA profiling
- Fully automated target purification and data acquisition
- Direct digital detection without amplification
- All reagents and consumables included

nCounter miRNA Assay Overview

The Nanostring nCounter miRNA Expression Assay Kit allows investigators to profile a comprehensive set of human miRNAs with superior specificity and sensitivity and with lower cost than microarrays. This is the first and only product capable of highly multiplexed, direct digital detection and counting of miRNAs in a single reaction without amplification.

MicroRNAs (miRNAs) are a class of small, non-coding RNA that regulate gene expression of target mRNAs via post-transcriptional gene silencing. These short RNAs have been implicated in the widespread control of critical biological processes such as proliferation, differentiation, and apoptosis.

Due to their central role in developmental processes, perturbations in miRNA expression patterns can lead to pathological conditions, including carcinogenesis. Much recent work has focused on investigating the promise of miRNA expression signatures as prognostic indicators of disease states.



The Nanostring nCounter Human miRNA Expression Assay Kit delivers accurate and sensitive expression profiling for more than 700 human and human-associated viral miRNAs and is sourced to the latest version of the miRBase database (v14) and provides exceptional ease-of-use for miRNA expression analysis. The complete gene list for the human miRNA CodeSet is available at www.nanostring.com.

nCounter® Human miRNA Sample Preparation Kit

The NanoString Human miRNA Sample Preparation Kit allows researchers to analyze miRNA expression using the automated nCounter Analysis System workflow. The kit provides reagents for ligating unique oligonucleotide tags onto more than 700 human and human-associated viral miRNAs, allowing these short RNAs to be detected with great specificity and sensitivity with the nCounter Analysis System.

Sample preparation involves a multiplexed annealing of specific tags to their target miRNAs, a ligation reaction, and an enzymatic purification step to remove unligated material. Sequence specificity between a miRNA and its synthetic sequence tag is ensured by careful, stepwise control of hybridization and ligation temperatures. Control RNA included in the nCounter Human miRNA Sample Preparation Kit allows for the monitoring of ligation efficiency and specificity throughout each step of the reaction. This innovative sample preparation method delivers tagged miRNAs ready for analysis using the nCounter Analysis System.

nCounter® Analysis System

After miRNA sample preparation, researchers use the nCounter Analysis System to obtain expression data. The nCounter Analysis System delivers direct, multiplexed measurement of miRNA gene expression, providing digital readouts of the relative abundance of hundreds of transcripts simultaneously. The system is based on target-specific probe pairs that are hybridized to the sample in solution. The protocol does not include any amplification steps that might introduce bias into the results. The Reporter Probe carries the fluorescent signal; the Capture Probe allows the complex to be immobilized for data collection.

Over 700 pairs of probes specific for a comprehensive set of miRNAs are combined with a series of internal controls to form the Human miRNA CodeSet. After hybridization of the Human miRNA CodeSet with the tagged miRNA preparation, samples are transferred to the nCounter Prep Station where excess probes are removed and probe / target complexes are aligned and immobilized in the nCounter Cartridge. Cartridges are then placed in the nCounter Digital Analyzer for data collection. Each miRNA of interest is identified by the “color code” generated by six ordered fluorescent spots present on the Reporter Probe. The Reporter Probes on the surface of the cartridge are then counted and tabulated for each miRNA species.

Human miRNA Assay Performance Data

To demonstrate the reproducibility of data generated via the nCounter Human miRNA Expression Kit, we prepared Ambion® Human total Reference RNA per the nCounter miRNA Expression Assay Manual, using 100ng total RNA per replicate sample preparation. Raw data was normalized to internal positive spike 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 1).

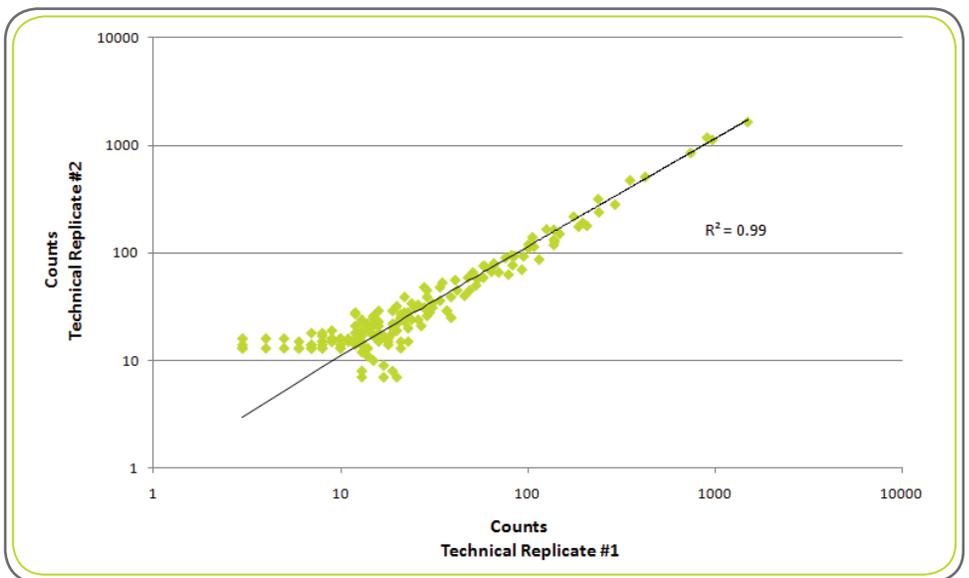


Figure 1: Counts for over 700 miRNA species in commercially available human total RNA were highly correlated between technical replicates.

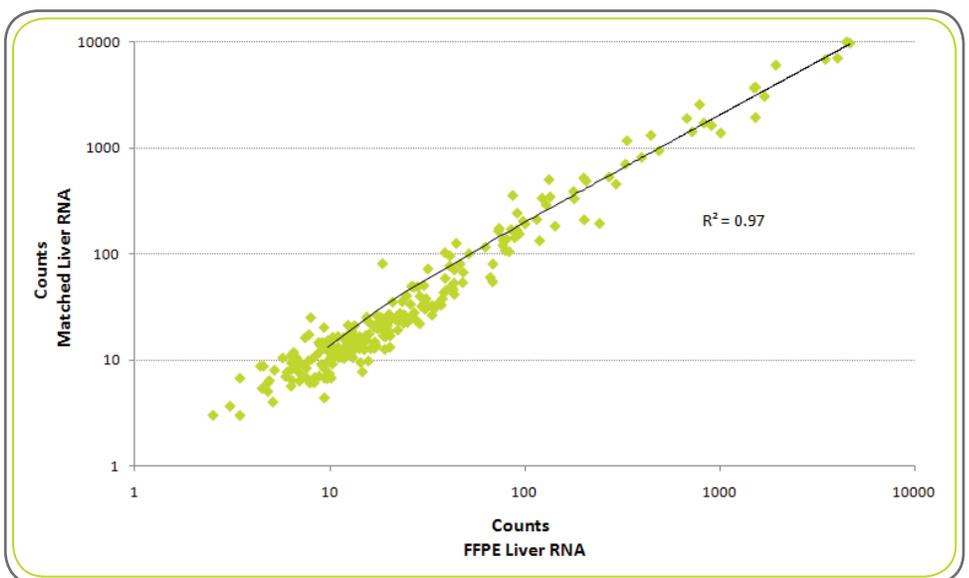


Figure 2: Counts for over 700 miRNA species in RNA from FFPE liver and matched frozen liver samples were highly correlated.

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 ($R^2 > 0.97$, Figure 2).

We were further interested in assessing the specificity of the small RNA sample preparation and hybridization, especially given that many miRNAs share highly

similar sequences. To do so, we utilized the Let7 family of miRNAs, many of whose members differ in sequence by only a single nucleotide (Table 1).

Individual synthetic Let7 miRNAs were prepared and analyzed per the nCounter 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). The majority of probes exhibited less than 1 percent cross-hybridization. No probe generated more than 6 percent cross-hybridization, indicating that the nCounter miRNA Assay can accurately distinguish between highly similar miRNAs with great specificity.

TABLE 1: SEQUENCES FOR miRNAs IN THE LET7 FAMILY ARE HIGHLY SIMILAR.

miRNA	Sequence
hsa-let-7a	ugagguaguagguuguauaguu
hsa-let-7b	ugagguaguagguugug <u>g</u> guuu
hsa-let-7c	ugagguaguagguuguau <u>g</u> guuu
hsa-let-7d	agagguaguagguugc <u>a</u> uaguu
hsa-let-7e	ugagguag <u>g</u> agguuguauaguu
hsa-let-7f	ugagguaguag <u>a</u> uuguauaguu
hsa-let-7g	ugagguaguag <u>u</u> uguacaguu
hsa-let-7i	ugagguaguag <u>u</u> ugug <u>c</u> uuuu

TABLE 2: LOW CROSS-HYBRIDIZATION BETWEEN miRNAs OF THE LET7 FAMILY DEMONSTRATES THE SUPERIOR SPECIFICITY OF THE nCOUNTER HUMAN miRNA ASSAY

		miRNA Target							
		Let7a	Let7b	Let7c	Let7d	Let7e	Let7f	Let7g	Let7i
nCounter Probe	Let7a	100%	-	4%	-	6%	2%	-	-
	Let7b	-	100%	1%	-	-	-	-	-
	Let7c	-	3%	100%	-	1%	2%	-	-
	Let7d	4%	-	-	100%	2%	1%	-	-
	Let7e	1%	-	-	-	100%	1%	-	-
	Let7f	5%	-	-	-	-	100%	-	-
	Let7g	-	-	-	-	-	1%	100%	-
	Let7i	-	-	-	-	-	-	-	100%

System Performance

Critical Specifications	
Number of Targets	664 Human miRNAs 82 Viral miRNAs
Recommended Amount of Starting Material	100 ng Purified Total RNA
Sample Types Supported	Human Purified Total RNA
miRNA Sample Prep Reaction Volume	10 μ L
Hybridization Reaction Volume	30 μ L
Limit of Detection	≤ 0.5 fM (~10 copies per cell)
Fold Change Sensitivity	> 2-Fold Change
Synthetic miRNA Spike Titration Linearity	$R^2 \geq 0.95$
Linear Dynamic Range	2×10^6 Total Counts
nCounter Prep Station Throughput	12 Samples < 2.5 Hours
nCounter Digital Analyzer Throughput	12 Samples/4 Hours (up to 72 samples per day unattended running in continuous mode)
Controls	6 Positive miRNA Assay Controls 8 Negative miRNA Assay Controls 5 mRNA Housekeeping Controls

Ordering Information

Description	Quantity/Use	P/N
nCounter Human miRNA Expression Kit	12 Assays	GXA-MIR1-12
	24 Assays	GXA-MIR1-24
	48 Assays	GXA-MIR1-48
	96 Assays	GXA-MIR1-96
nCounter Analysis System Includes the Prep Station and Digital Analyzer	1	NCT-SYS-120
Additional nCounter Prep Station	1	NCT-PREP-120
Additional nCounter Digital Analyzer	1	NCT-DIGA-120

NanoString Technologies, Inc.

530 Fairview Ave N, Suite 2000

Seattle, Washington 98109

(206) 378-NANO • (206) 378-6266

www.nanostring.com