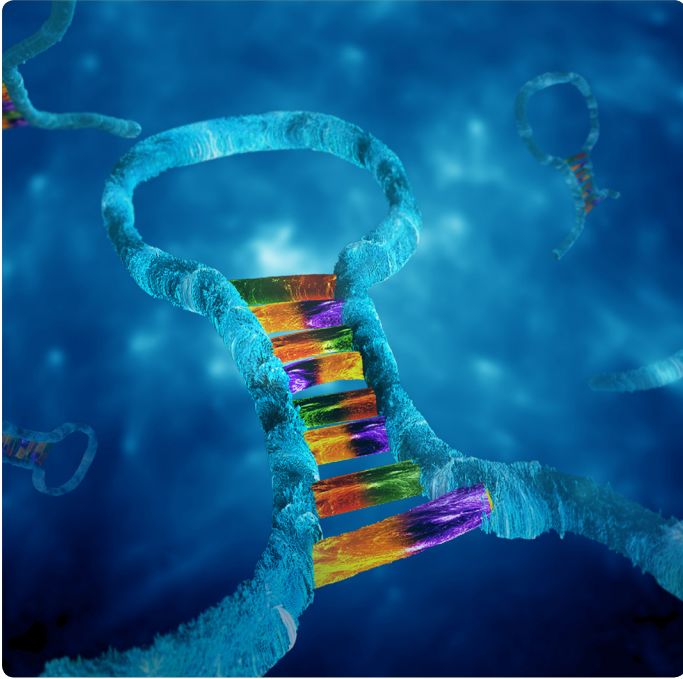


nCounter® Human v3 miRNA Expression Assay



Product Highlights

- 800 human miRNAs from miRBase v21
- Highly specific and sensitive miRNA profiling
- Fully automated target purification and data acquisition
- Direct digital detection without amplification
- Compatible with RNA derived from a variety of samples, including FFPE
- Choose from broad miRBase coverage panels or à la carte custom miRNA CodeSets

nCounter Human v3 miRNA Expression Assay Overview

The NanoString nCounter miRNA Expression Assay Kit enables investigators to profile miRNAs with a low false-positive rate and excellent specificity. This product requires minimal hands-on time and is 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 v3 miRNA Expression Assay Kit delivers accurate and sensitive expression profiling for 800 human miRNAs and provides exceptional ease-of-use for miRNA expression analysis. The complete gene list for the Human v3 miRNA CodeSet is available at www.nanostring.com.

À la carte custom miRNA CodeSets are available for performing larger validation studies on subsets of miRNAs included in the species specific miRNA Expression Assay Kits. Please contact sales@nanostring.com for further information.

nCounter Human v3 miRNA Sample Preparation Kit

The NanoString Human v3 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 800 human miRNAs, so that these short RNAs can be detected with great specificity and sensitivity by 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 v3 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. Reporter Probes carry the fluorescent signal, and Capture Probes immobilize these complexes for data collection.

Eight hundred pairs of probes specific for a set of miRNAs are combined with a series of internal controls to form the Human v3 miRNA CodeSet.

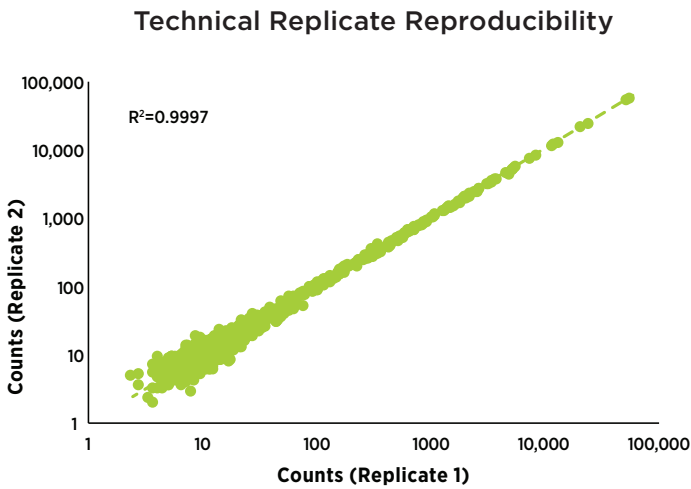
After hybridization of the Human v3 miRNA CodeSet with the tagged miRNA preparation, samples are transferred to the nCounter Prep Station where excess probes are removed and probe/target complexes are aligned and immobilized in the nCounter Cartridge. Cartridges are then placed in the nCounter Digital Analyzer for data collection. Each miRNA of interest is identified by the "color code" generated by six ordered fluorescent spots present on each Reporter Probe. The Reporter Probes on the surface of the cartridge are then counted and tabulated for each miRNA species.

Human v3 miRNA Assay Performance Data

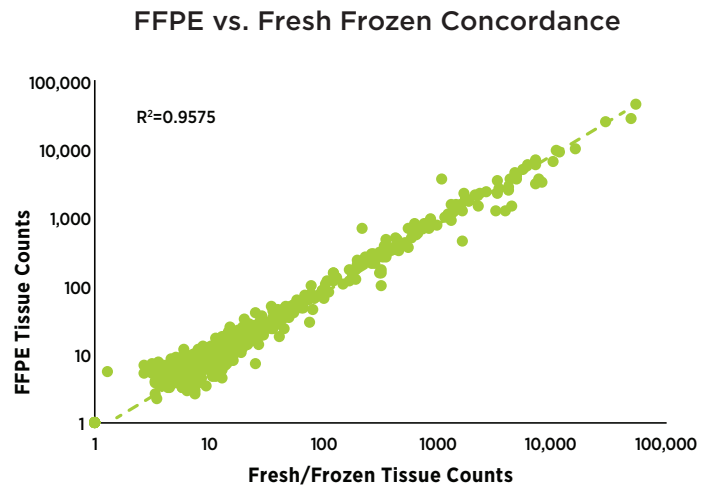
To demonstrate the reproducibility of data generated via the nCounter Human v3 miRNA Expression Kit, we prepared Ambion® Human total Reference RNA per the nCounter miRNA Expression Assay Manual, using 100 ng total RNA per replicate sample preparation. Raw data were normalized to internal positive spike 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).

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 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 which 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 5% cross-hybridization, indicating that the nCounter miRNA Assay accurately distinguishes between highly similar miRNAs with great specificity.



▲ **Figure 1:** Counts for 800 miRNA species in commercially available human total RNA were highly correlated between technical replicates.



▲ **Figure 2:** Counts for 800 miRNA species in RNA from FFPE liver and matched frozen liver samples were highly correlated.

miRNA	Sequence
hsa-let-7a	UGAGGUAGUAGGUUGUAUAGUU
hsa-let-7b	UGAGGUAGUAGGUUGUGUGGUU
hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU
hsa-let-7d	AGAGGUAGUAGGUUGC AUAGUU
hsa-let-7e	UGAGGUAGGAGGUUGUAUAGUU
hsa-let-7f	UGAGGUAGUAGAUUGUAUAGUU
hsa-let-7g	UGAGGUAGUAGUUGUACAGUU
hsa-let-7i	UGAGGUAGUAGUUGUGGCUUU

▲ **Table 1:** Sequences for miRNAs in the Let7 family are highly similar.

▼ **Table 2:** Low cross-hybridization between miRNAs of the Let7 family demonstrates the superior specificity of the nCounter Human v3 miRNA Assay.

		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%

System Performance

Description	Specifications
Number of probes	800 human probes
Recommended amount of starting material	100 ng purified total RNA
Sample types supported	Human purified total RNA
miRNA sample prep reaction volume	10 µL
Hybridization reaction volume	30 µL
Limit of detection	≤ 0.5 fM (~10 copies per cell)
Fold change sensitivity	> 2-fold change
Synthetic miRNA spike titration linearity	R ² ≥ 0.95
Linear dynamic range	2 x 10 ⁵ 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	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 Preparation Kit. Ligation Negative Controls (3) - Probes that recognize synthetic miRNA targets not included in the Sample Preparation Kit. mRNA Reference Controls (5) - Probes that recognize endogenous mRNA targets commonly expressed in tissues. Spike-in Controls (5) - Probes that recognize exogenous miRNA targets to monitor upstream RNA isolation/purification (optional).

Ordering Information

Description	Quantity / Use	Part Number (P/N)
nCounter Human v3 miRNA Expression Assay Kit	12 assays	GXA-MIR3-12
nCounter à la carte Custom miRNA CodeSets	192 assays	GXA-MICS-192
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

For more information, please visit nanosttring.com/products

NanoString Technologies, Inc.

530 Fairview Avenue North
Seattle, Washington 98109

T (888) 358-6266
F (206) 378-6288

nanosttring.com
info@nanosttring.com

Sales Contacts

United States us.sales@nanosttring.com
EMEA: europe.sales@nanosttring.com

Asia Pacific & Japan apac.sales@nanosttring.com
Other Regions info@nanosttring.com

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