



nCounter® Rat miRNA Expression Assay Kit



Product Highlights

- **Detection of 423 rat miRNAs** from miRBase 17
- **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 sets**

nCounter® Rat miRNA Expression Assay Overview

The NanoString **nCounter miRNA Expression Assay Kit** allows investigators to profile a comprehensive set of 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 Rat miRNA Expression Assay Kit delivers accurate and sensitive expression profiling of over 400 rat miRNAs, providing comprehensive coverage of the miRBase database (17) and exceptional ease-of-use for miRNA expression analysis. The complete gene list for the Rat miRNA Panel CodeSet is available at www.nanostring.com.

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

nCounter Rat miRNA Sample Preparation Kit

The NanoString Rat 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 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 Rat 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. A Reporter Probe carries the fluorescent signal; a Capture Probe allows the complex to be immobilized for data collection. The protocol does not include any amplification steps that might introduce bias into the results.

More than 400 pairs of probes specific for a comprehensive set of miRNAs are combined with a series of internal controls to form the Rat miRNA Panel CodeSet. After hybridization of the Rat miRNA Panel 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.

Rat miRNA Assay Performance Data

To demonstrate the reproducibility of data generated via the nCounter Rat miRNA Expression Kit, we processed total RNA isolated from a flash-frozen whole rat liver per the nCounter miRNA Expression Assay Manual. 100ng total RNA per replicate was used for 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 400 miRNA species in rat liver total RNA were highly correlated between technical replicates.

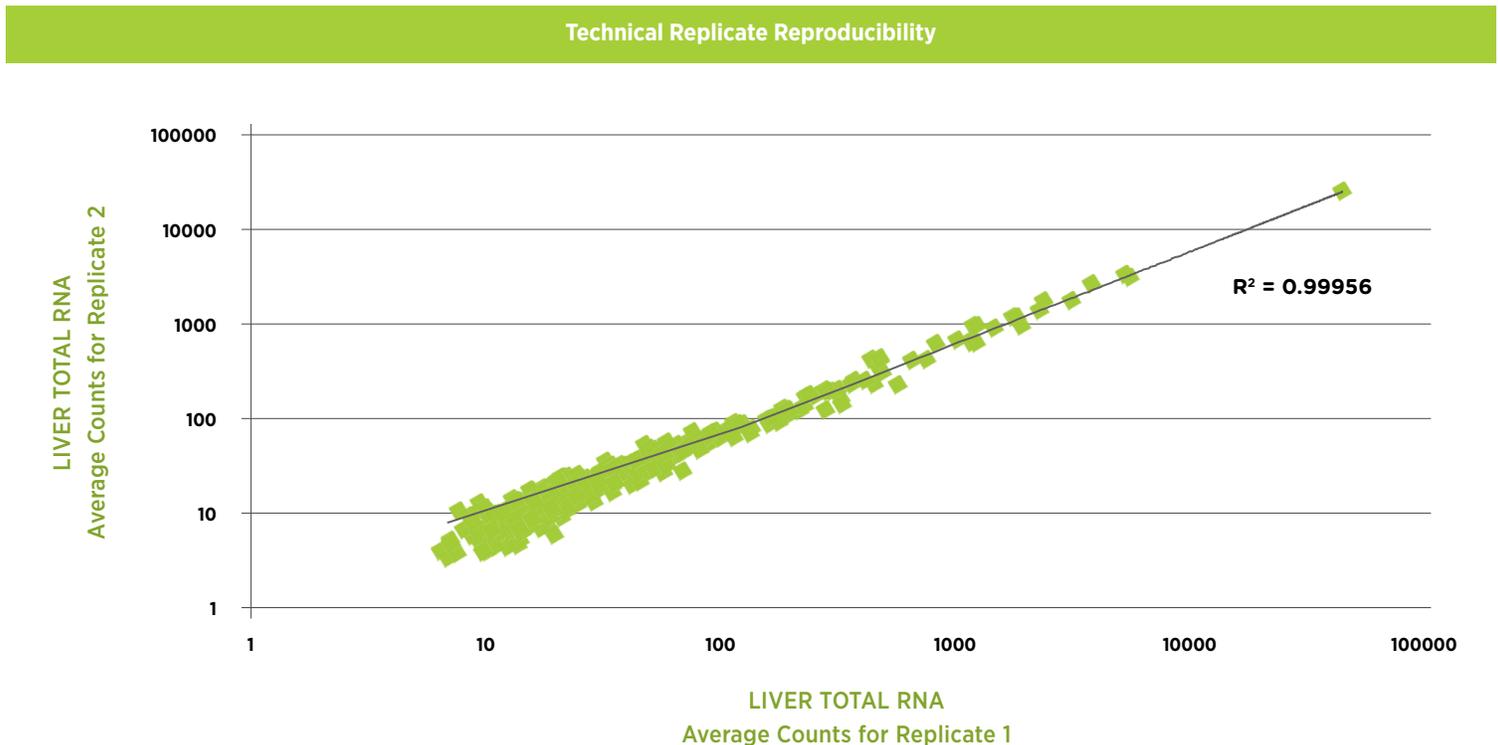
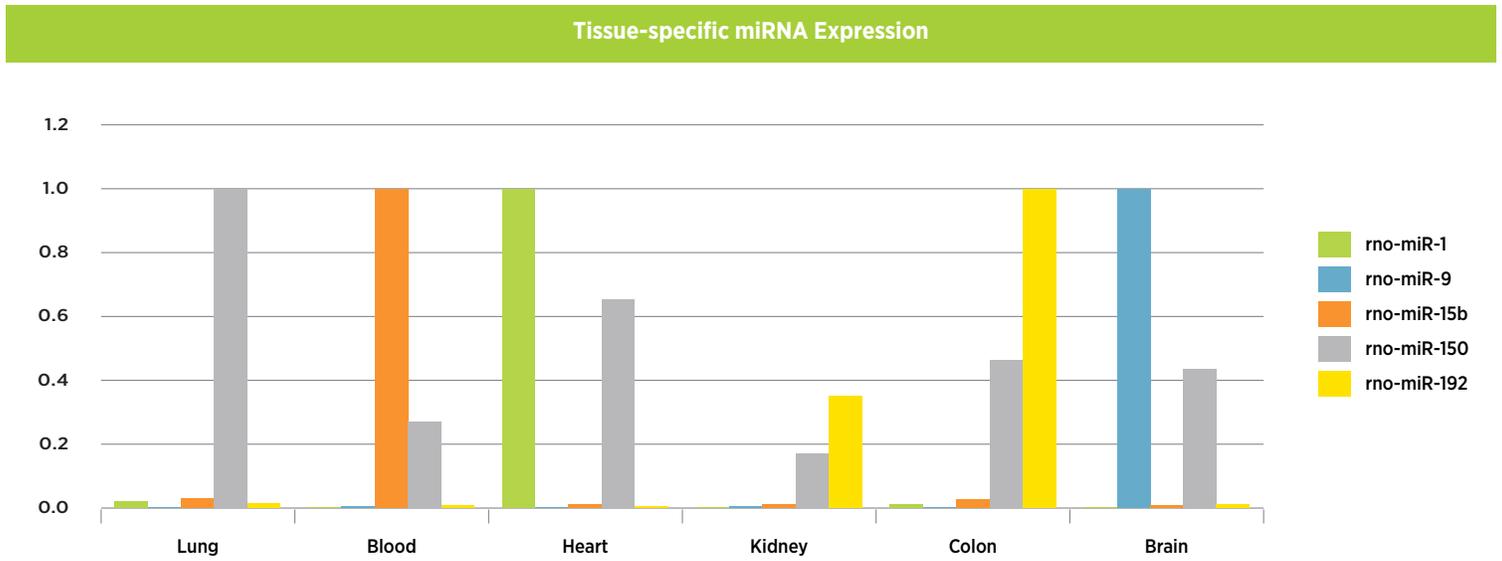


FIGURE 2: The nCounter Rat miRNA Expression Assay allows for differential detection of tissue-specific miRNAs.



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 examined both global miRNA expression in a number of tissues and utilized the Let7 family of miRNAs (Table 1) to test sequence specific detection.

Expression for a select set of miRNAs is shown for the panel of tissues assayed (Figure 2). Each miRNA is shown normalized to the highest count value observed for that particular miRNA in the tissue panel. Each rat tissue produced a distinct miRNA expression profile, e.g. rno-miR-1 is highly expressed in heart, 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.

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, indicating

that the nCounter miRNA Assay can accurately distinguish between highly similar miRNAs with great sequence specificity.

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

miRNA	Sequence
rno-let-7a	ugagguaguagguuguauaguu
rno-let-7b	ugagguaguagguuguguguu
rno-let-7c	ugagguaguagguuguauuguu
rno-let-7d	agagguaguagguugcauaguu
rno-let-7e	ugagguaggagguuguauaguu
rno-let-7f	ugagguaguagaauuguauaguu
rno-let-7i	ugagguaguaguuguugucuguu

TABLE 2: Low cross-hybridization between miRNAs of the Let7 family demonstrates the superior specificity of the nCounter Rat miRNA Assay.

nCounter Probes	miRNA Target						
	Let7a	Let7b	Let7c	Let7d	Let7e	Let7f	Let7i
Let7a	100%	-	7%	1%	13%	2%	-
Let7b	-	100%	3%	-	-	-	-
Let7c	-	2%	100%	-	-	-	-
Let7d	3%	-	1%	100%	3%	-	-
Let7e	3%	-	-	-	100%	1%	-
Let7f	3%	-	-	-	1%	100%	-
Let7i	-	-	-	-	-	-	100%

System Performance

Description	Specifications
Number of targets	423 Rat miRNAs
Recommended amount of starting material	100 ng purified total RNA
Sample types supported	Rat 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 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 4 mRNA housekeeping controls 5 non-mammalian miRNA spike-in probes

Ordering Information

Description	Quantity / Use	Part Number (P/N)
nCounter Rat miRNA Expression Assay Kit	12 assays	GXA-RMIR-12
	24 assays	GXA-RMIR-24
	48 assays	GXA-RMIR-48
	96 assays	GXA-RMIR-96
nCounter À la carte Custom miRNA Sets	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

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