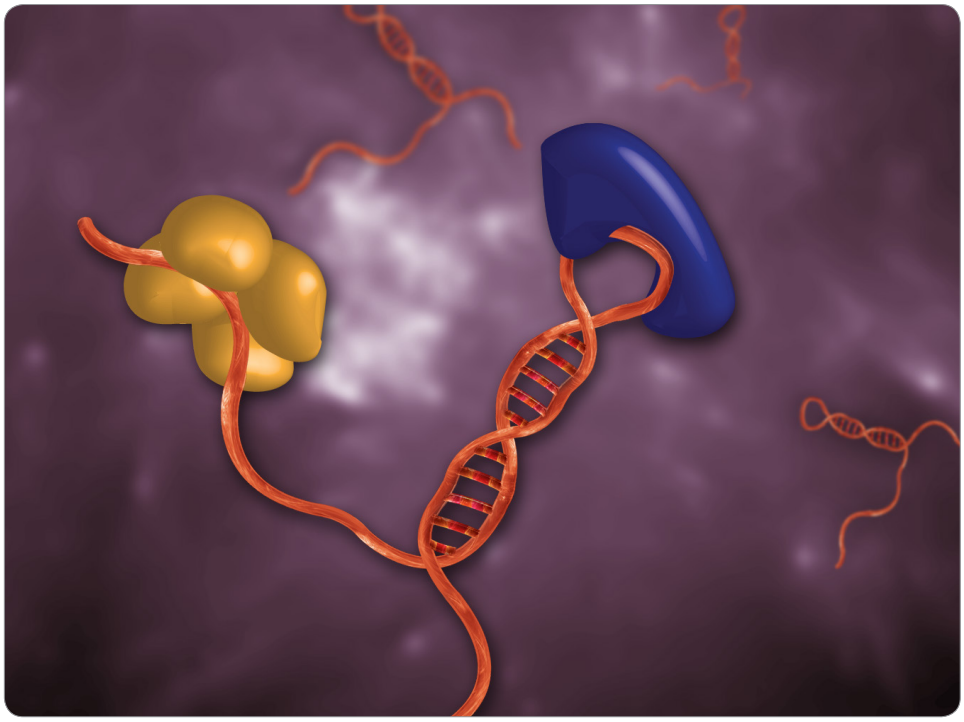
nCounter®
Custom lncRNA Assay



Product Highlights

- High precision, **digital quantification of lncRNAs**
- Analyze up to **800 RNAs in a single reaction**
- Quantify immunoprecipitated RNA directly with **no amplification**
- **15 minutes of hands-on time** for up **9,600 data points**
- **Compatible with FFPE,** crude cell lysates, and other challenging sample types
- **Analyze lncRNAs and mRNAs** in the same reaction

nCounter long non-coding RNA (lncRNA) Assay Overview

The **nCounter Custom lncRNA Assay** enables researchers to study focused sets of up to 800 lncRNAs with high precision and less hands-on time. Examples of lncRNAs (defined as RNAs > than 200 bases that do not encode proteins) have been known for decades. However, the recent advent of transcriptome-wide studies (based on tiling arrays and more recently sequencing) has shown lncRNAs to be far more pervasive than originally thought. Recent studies have shown that 10- to 20-fold more genomic sequence is transcribed to lncRNAs than protein-coding mRNAs[1]. LncRNAs can be categorized into multiple classes based their position relative to genes: Sense, Antisense, Bidirectional, Intronic, and Intragenic. Functional studies of lncRNAs are beginning to elucidate their importance as positive and negative regulators of gene expression. The precision and ease-of-use of nCounter lncRNA assays make them ideal for high-precision studies of lncRNAs across large sample sets or involving many experimental conditions.

Functional Role of lncRNA in Regulating Gene Transcription and Translation

There is a large body of evidence describing lncRNAs acting in *cis* or in *trans* to regulate gene expression[1]. Multiple modes of action have been described for lncRNAs including direct interaction with DNA (e.g., DHFR[2]) or mRNA (antisense binding) as well as interactions with a variety of proteins. Recent work suggests that an important mode of action for lncRNAs in epigenetic regulation is to serve as protein-binding scaffolds for complexes of chromatin binding and modifying proteins[3,4,5]. Examples of lncRNAs operating via this mechanism are XIST for X chromosome inactivation and H19 for maternal imprinting. Due to their role as epigenetic modifiers, lncRNAs have been shown to be important regulators of development, stem cell pluripotency and differentiation (**BOX 1**), and cancer (**TABLE 1**).

TABLE 1 lncRNAs Associated With Cancer

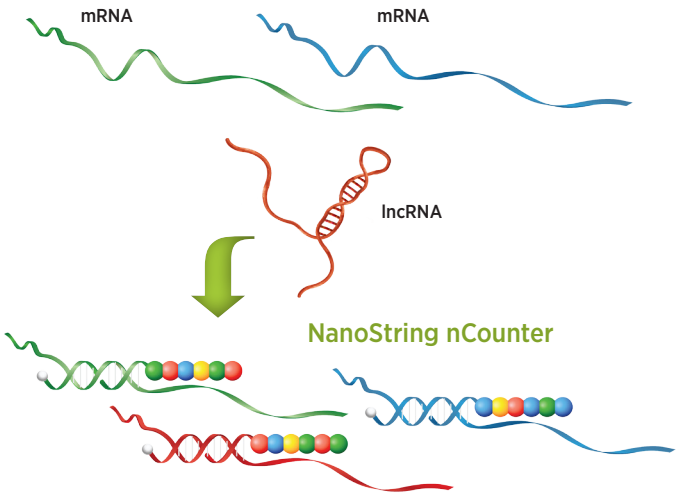
lncRNA	Observation
lincRNA-p21	Represses p53-dependent transcription via interactions with hnRNP-K
MALAT-1/Neat2	Implicated in metastasis of multiple cancers
HOTAIR	Implicated in breast cancer metastasis. Recent evidence of chromatin interactions
H19	Implicated in multiple cancers

Utilizing nCounter for lncRNA Studies

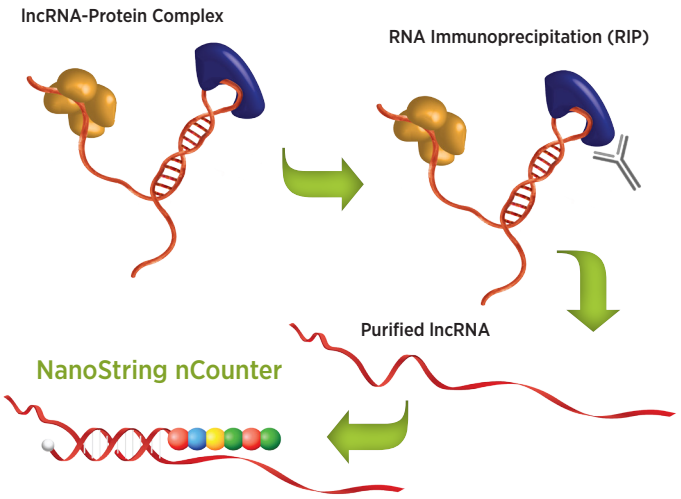
The nCounter lncRNA Assay is ideal for validation of lncRNA discoveries and other studies requiring a rapid, cost-effective method of screening hundreds of lncRNAs across large sample sets. It allows researchers to select up to 800 lncRNAs for analysis in a single multiplexed reaction, using the proven nCounter Analysis System in use today for mRNA, miRNA, and CNV analysis. nCounter's digital counting technology generates data with unparalleled precision and requires only 15 minutes of hands-on time to generate up to 9600 data points. Probes for lncRNAs and mRNAs can be combined in the same codeset to enable simultaneous analysis of both classes of RNA in the same reaction.

FIGURE 1 Utilizing the nCounter Custom lncRNA Assay

Study Expression of lncRNAs Along with mRNAs



Study Interaction of lncRNAs with Proteins



BOX 1 lncRNAs and Stem Cells

lincRNAs and Stem Cells

Long intergenic non-coding RNAs (lincRNAs) are a class of lncRNAs that are emerging as important regulators of pluripotency in stem cells. Hundreds of lincRNAs have been shown to be expressed in mouse and human ES cells[5,6]. Further, transcription of some lincRNAs have been shown to be controlled by pluripotency transcription factors and knockdowns of lincRNAs have shown to alter pluripotent state. A recent publication using the **nCounter Analysis System**[7] shows that lincRNAs implicated in maintaining pluripotent state physically bind to multiple chromatin regulatory proteins to affect shared gene expression programs.

For studies of lncRNA-Protein interactions via RNA immunoprecipitation (RIP), nCounter can quantify immunoprecipitated RNA directly with no amplification. Additionally, the assay is compatible with FFPE specimens, crude cell lysates, whole blood, and other challenging specimens. Minimal sample input and the ability to analyze lncRNAs and mRNAs simultaneously in the same reaction conserves precious samples. For more information on how nCounter facilitates efficient, high-performance studies of lncRNAs, visit www.nanostring.com.

References

1. Nagano and Frasier (2011) No-nonsense functions for long noncoding RNAs. *Cell* 145.
2. Martianov *et al.* (2007) Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript. *Nature* 445.
3. Rinn *et al.* (2007) Functional demarcation of active and silent chromatin domains in human HOX loci by non-coding RNAs. *Cell* 129(7).
4. Zhao *et al.* (2010) Genome-wide identification of polycomb-associated RNAs by RIP-seq; *Mol Cell* 40(6), 2010 December 22; 40(6)
5. Khalil *et al.* (2009) Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci USA* 106(28).
6. Guttman *et al.* (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* 223(7).
7. Guttman *et. al.* (2011) lincRNAs act in the circuitry controlling pluripotency and differentiation. *Nature* 477(7364).

nanoString TECHNOLOGIES®

System Performance

Description	Specifications
Maximum number of probes per CodeSet	800
Recommended size of target region submitted	> 200 bases
Recommended amount of starting material	100 – 500 ng of total RNA
Sample types supported	RNA Prepared by RIP, Total RNA, Cell Lysates in GITC, FFPE derived total RNA and PAXgene lysed whole blood
Synthetic spike titration correlation	> 0.95
Linear dynamic range	7 x 10^5 total counts
nCounter Prep Station throughput	12 samples / 2.5 hours
nCounter Digital Analyzer throughput	12 samples / 2.7 hours (up to 72 samples per day unattended running in continuous mode)

Ordering Information

Description	Quantity / Use	Part Number (P/N)
nCounter Custom lncRNA CodeSet	Custom	XT-GXA-P1CS-XXX
nCounter Master Kit (all reagents, sample cartridges, and consumables necessary for processing 48 or 192 assays)	48 Assays 192 Assays	NAA-AKIT-048 NAA-AKIT-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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