NanoString® Technologies, Inc.

nCounter® Analysis System

REV 3.1

Digital Genomics *for* Pathway-based Translational Research

Molecules That Count®

Gene Expression • miRNA Expression • Copy Number Variation • Single Cell

nanoString
TECHNOLOGIES®

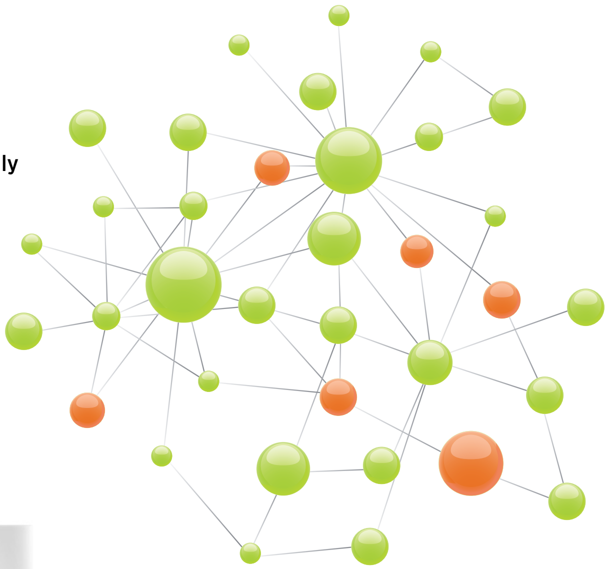
nCounter® Analysis System
Digital Quantification of Nucleic Acids

Digital Genomics *for* Pathway-based Translational Research

NanoString's nCounter® platform is a complete solution for digitally detecting and counting large sets of molecules in a single tube. For translational researchers studying pathways or validating hypotheses generated on discovery platforms, the nCounter Analysis System is *the* ideal solution.

The Era of Pathway-based Biology

- Pathways are now **the emerging unit of analysis for understanding biology**
- Entire pathways, or multiple pathways, **need to be interrogated quickly and easily**
- **Focus is on extracting meaning, not processing data**



nCounter® is *the* Ideal Tool

Digital	»	The nCounter platform can be used to **digitally study pathway biology or validate targets** identified by Next Generation Sequencing discovery experiments
Multiplexed	»	Multiple pathways can be directly interrogated **in a single tube without amplification***
Fast *and* Simple	»	Spend time on **advancing knowledge, not sample prep and data analysis**
Compatible	»	Study even **difficult sample types, including FFPE samples**
Flexible	»	View biology from multiple angles; **RNA, miRNA, DNA**

** Single Cell assay requires amplification prior to analysis on the nCounter System.*

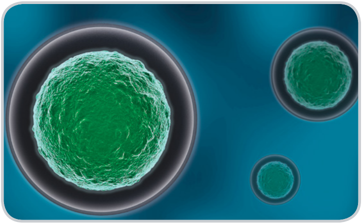
Innovation in Research *and* Translational Applications

NanoString's technology uses **color-coded molecular barcodes** that can hybridize directly to many different types of target molecules. It is ideal for a range of applications requiring **efficient, high-precision quantitation of hundreds of target molecules across a sample set. All nCounter Assays generate high-quality results from challenging samples types, including FFPE.**

RNA Analysis

Gene Expression Analysis

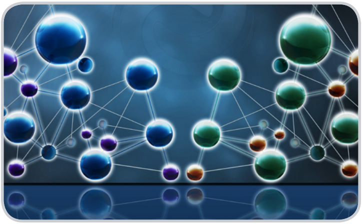
- Analyze up to 800 genes simultaneously
- No RT, no amplification, no enzymes
- Directly assay tissue, cell and blood lysates, and FFPE extracts in a simple workflow



Single Cell Gene Expression Analysis

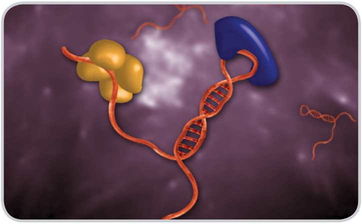
Single Cell assay requires reverse transcription and amplification prior to analysis on the nCounter System.

- Analyze multiple pathways for up to 800 genes
- Flexible format allows interrogation of exact gene number of interest
- Obtain single cell sensitivity while minimizing amplification



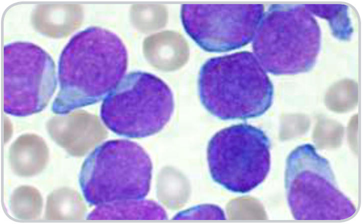
Plex2™ Expression Analysis

- Multiplex hundreds of targets and multiple samples in a single tube
- Customizable to study size
- Digital data in a high throughput format



lncRNA Expression Analysis

- High precision, digital quantification of lncRNAs
- Analyze up to 800 lncRNAs in a single reaction with no amplification
- Compatible with FFPE, crude cell lysates and other challenging sample types

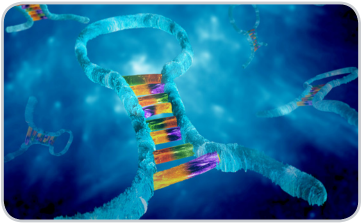


Leukemia Fusion Gene Analysis

- Profile a comprehensive set of fusion genes in different leukemia subtypes
- Includes probes for 11 wild-type translocation partners and 12 leukemia-related biomarkers
- 15 minutes of hands-on time per run

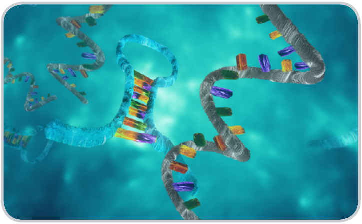
High-precision, Digital Quantification *of* Nucleic Acids

miRNA Analysis



miRNA Expression Analysis

- Multiplexed target profiling of miRNA transcriptomes in a single reaction
- miRNA discovery and validation on one platform
- High specificity - accurately distinguish between highly similar miRNAs



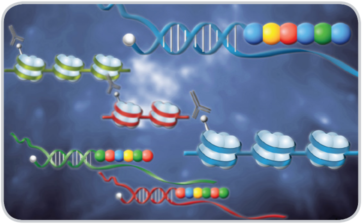
miRGE™ Expression Analysis

- Simultaneously profile miRNA and mRNA expression in a single reaction
- No RT, no amplification, fewer pipetting steps
- Profile FFPE samples and other difficult sample types

DNA Analysis

Copy Number Variation Analysis

- Multiplex up to 800 target regions in a single reaction
- Just 25-minutes of hands-on time per 12 samples
- High accuracy for multiallelic CNVs



ChIP-String Expression Analysis

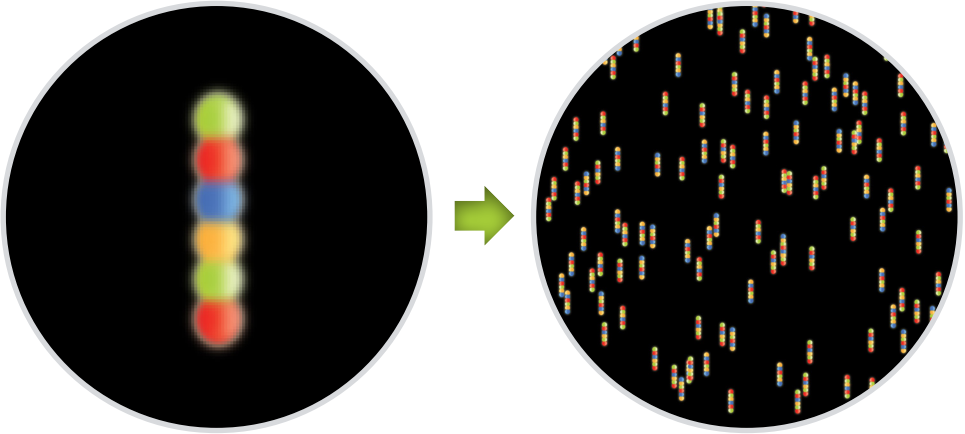
- Analyze up to 800 loci with 15-minutes of total hands-on time
- Excellent correlation with ChIP-Seq results
- No library prep or amplification required

Innovation in Chemistry

NanoString's **patented technology** used in the nCounter Analysis System is a **true digital detection technology** capable of **highly multiplexed, direct profiling of individual molecules in a single reaction without amplification*.**

Molecules That Count®

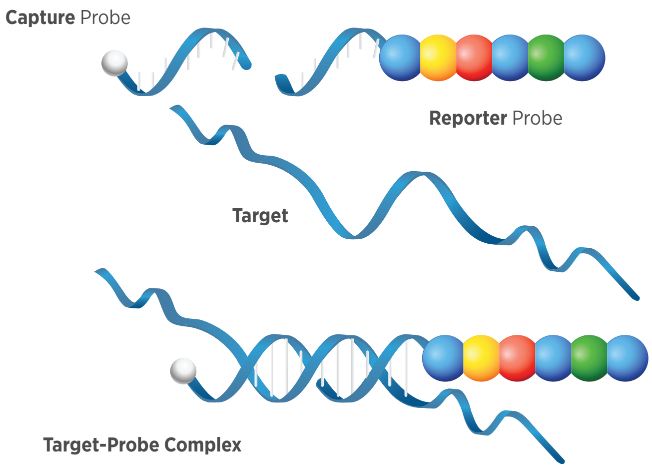
Each color-coded barcode represents a single target molecule. Barcodes hybridize directly to your target molecules and can be individually counted without the need for amplification* – **providing very sensitive digital data.**

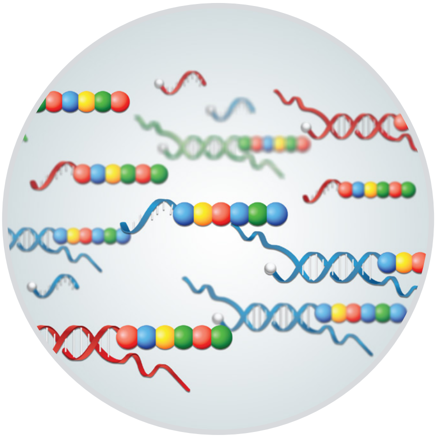


Single molecule barcodes, each attach to an individual nucleic acid.

1

HYBRIDIZE





solution phase hybridization

Hybridization

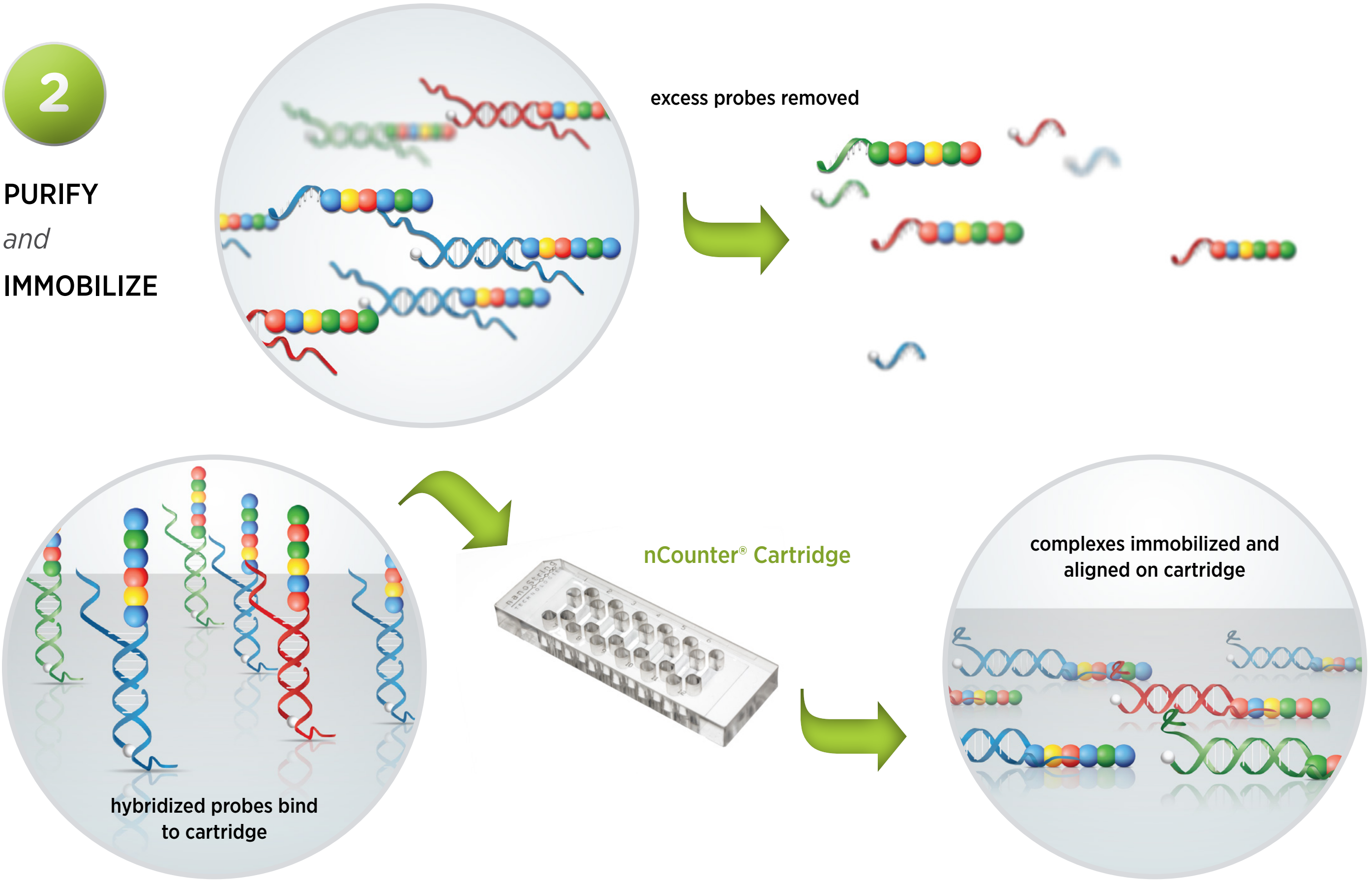
Barcoded probes hybridize directly to a target molecule in solution. The **Reporter Probe** carries the signal and the **Capture Probe** allows the complex to be immobilized for data collection.

** Single Cell assay requires amplification prior to analysis on the nCounter System.*

1 Molecule = 1 Count

2

PURIFY
and
IMMOBILIZE

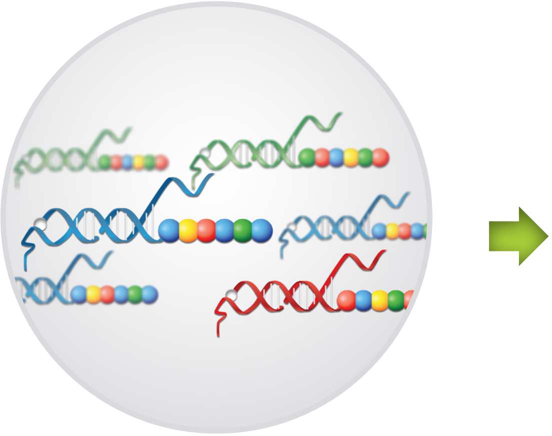


Sample Processing

After hybridization, samples are transferred to the nCounter Prep Station where excess probes are removed and probe/target complexes are bound, immobilized and aligned on the nCounter Cartridge.

3

COUNT



Barcode	Counts	Identity
🔵🟡🔴🔵🟢🔵 🔵🟡🔴🔵🟢🔵 🔵🟡🔴🔵🟢🔵	3	XLSA
🔴🔵🟡🟢🔴🟢 🔴🔵🟡🟢🔴🟢	2	FOX5
🟢🔴🔵🟡🟢🔴	1	INSULIN

Digital Data Acquisition

Sample cartridges are placed in the nCounter Digital Analyzer for data collection. Barcodes are counted and tabulated for each target molecule.

Fully Automated, Multi-application System

The **nCounter® Analysis System** delivers a complete and **cost-effective solution** for detecting and counting large sets of target molecules in a **high-performance, user-friendly workflow.**

Simple, fast, and cost-effective quantitation of hundreds of targets in virtually any sample with the PUSH OF A BUTTON.

Just **add sample** and **press start.**

- **Delivers 10,000s of precise data points every day**

  Digital detection of target molecules and **high levels of multiplexing** means **no compromise between data quality and data quantity.**

- **Straightforward, high-quality data with rapid results**

  Data output files include the target identifier and count number along with a **comprehensive set of internal controls** that enables each assay to be **highly quantitative and reproducible.**

- **Sample type flexibility**
- **Ready-to-use reagents**
- **Intuitive, easy-to-use touchscreen user-interface**
- **Manufactured under GMP/ISO 13485**

Instrumentation

Prep Station

Automated fluidic instrument purifies target-probe complexes and immobilizes complexes for digital detection

Digital Analyzer

Fluorescent reader counts barcodes and exports data in an easy-to-use format

Reagents

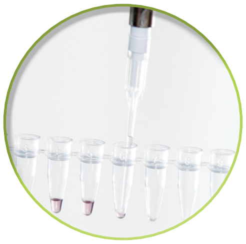
Master Kit

Consumables and reagents for sample processing - ready-to-load, require no additional preparation

CodeSets

Custom or pre-designed sets of barcoded probes pre-mixed with a comprehensive set of system controls

Simplified, User-friendly Workflow

	1 Hybridization	2 Sample Processing	3 Digital Data Acquisition
Only **15 Minutes** of Total Hands-on Time			
Process	**Set Up Hybridization** Add buffer, CodeSet and sample into a strip tube and hybridize overnight.	**Set Up Prep Station** Place the strip tube onto the automated **nCounter® Prep Station** with reagents and consumables from the nCounter Master Kit.	**Set Up Digital Analyzer** Take the cartridge from the nCounter Prep Station and place it into the **nCounter® Digital Analyzer** for direct digital counting.
Hands-on Time	**5 minutes**	**5 minutes**	**5 minutes**
	Day 1	**Day 2** (automated)	**Day 2** (automated)

Process Data *via* nSolver™ Analysis Software

Import nCounter data into nSolver to **quickly and easily QC, normalize, and analyze.**

The nCounter Analysis System's multiplexing capabilities and ease-of-use workflow produce 10,000s of data points per day.

10,000s of Data Points per Day

Sample Multiplex	# of Genes per Run		Samples per Day		Data Points per Day
Standard (1-Plex)	**800** genes	X	**48** samples	=	**38,400**
Standard (1-Plex)	**800** genes	X	**96** samples	=	**76,800***
nCounter® Plex^2 (2 samples per lane)	**400** genes	X	**192** samples	=	**76,800***
nCounter® Plex^2 (4 samples per lane)	**200** genes	X	**384** samples	=	**76,800***

All throughput calculations assume 4 runs per Prep Station per day.

** Requires 2 Prep Stations*

nCounter® Gene Expression CodeSets

nCounter® Gene Expression CodeSets offer a cost-effective way to analyze the expression levels of **up to 800 genes simultaneously,** with **high precision and accuracy**. The system can **directly assay tissue and blood lysates** as well as **Formalin-Fixed Paraffin-Embedded (FFPE)** extracts in a simple workflow that requires only **15 minutes of total hands-on time** per reaction.

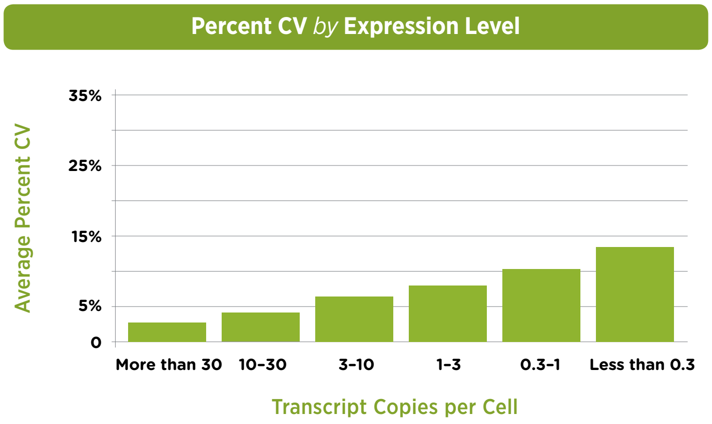
Gene Expression Panels

Many off-the-shelf Panel Kits are currently available which include CodeSets targeting genes associated with inflammation, immunology, and cancer as well as a reference gene CodeSet for aiding in reference gene selection. **Each Panel Kit arrives in your lab QC'd and ready-to-use** with all the reagents and consumables you need to perform the assay.

Custom CodeSets

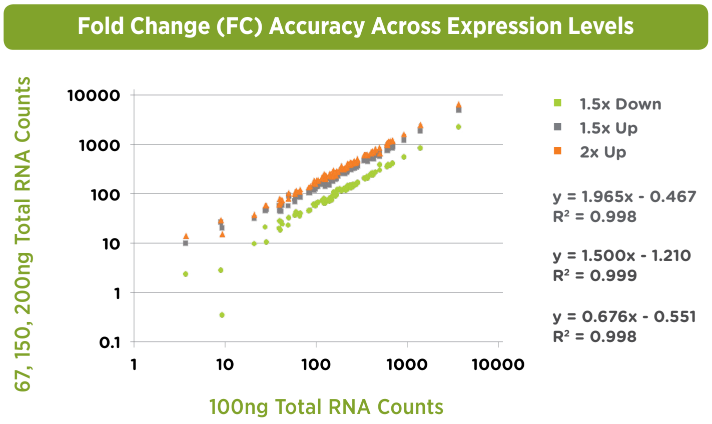
NanoString also offers the ultimate in flexibility by designing CodeSets to your specifications for any organism and gene set that you wish to analyze. Each customer receives a free consultation with our CodeSet design team.

Precision Across a Large Range of Expression



Genes were grouped by level of expression and percent CV was calculated for each group. **Many genes are expressed at less than one transcript per cell and can be measured with less than 15% CV, allowing for the system to quantitate 2-fold changes or less.** Precision increases with expression levels, allowing for quantitation of less than 1.2 fold changes in many cases.

Fractional Fold Change



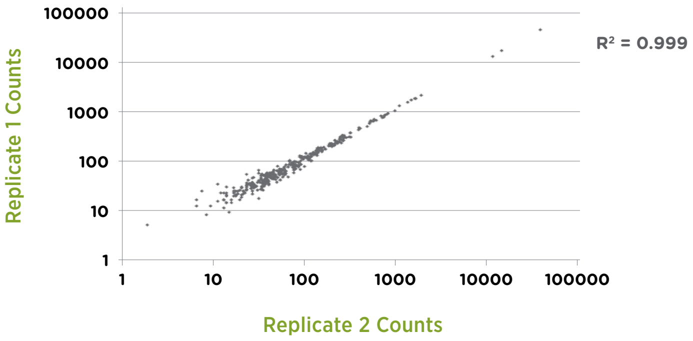
The amount of input material was varied to demonstrate the nCounter assay's ability to detect fractional fold changes across all levels of expression. **Plotted here are the counts from total RNA input amounts of 67ng, 100ng, 150ng, and 200ng of the same sample.** The 100ng reaction was used as the baseline value on the x-axis against which the others are plotted. The slopes correlate closely with the expected values of 0.67, 1.5 and 2 which indicate the assay's ability to detect fractional fold changes in both directions across all levels of expression.

Sample Flexibility *without* the Sample Prep

With protocols starting with **100ng of total RNA, raw cell or blood lysate, or Formalin-Fixed Paraffin-Embedded (FFPE) extracts**, **nCounter Gene Expression CodeSets** offer the ability to study large numbers of genes while conserving precious specimens, all with **excellent performance.** This means significantly **less bench-time, lower expense, and faster time to study completion.**

Sensitivity, Reproducibility and Dynamic Range

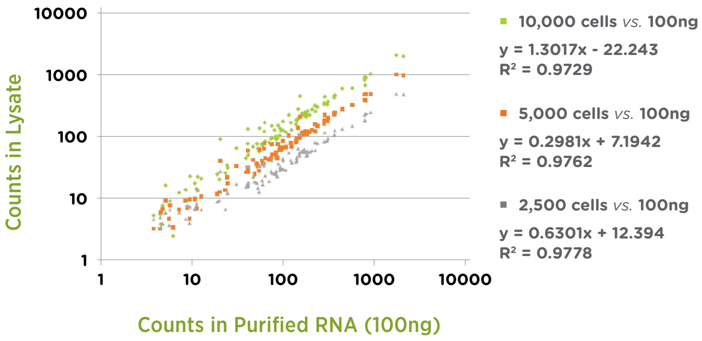
nCounter Assay Technical Replicates



Gene counts from two technical replicates plotted against one another demonstrate the assay reproducibility over a wide dynamic range (10–50,000 counts). One total RNA sample was split into two separate hybridization reactions and processed independently on the nCounter Analysis System. In this experiment, 75 counts is equal to a concentration of approximately 1 copy per cell. This data illustrates the high level of sensitivity and precision of the assay even at very low levels of expression.

Cell Lysate with Matched Total RNA

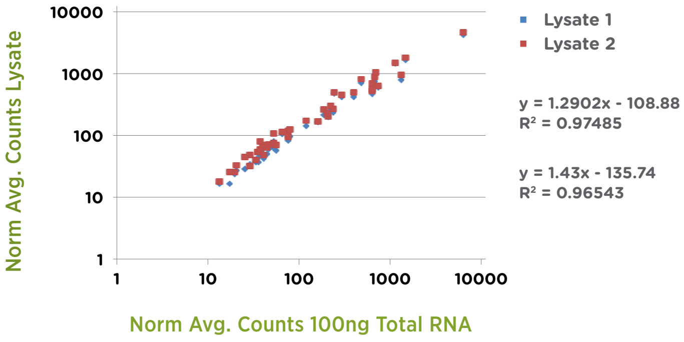
High Correlation – Crude Lysates & Purified RNA



Raw cell lysate was input directly into the nCounter Analysis System hybridization reaction using approximately 2,500, 5,000 and 10,000 cells in lysis buffer and compared to 100ng of purified total RNA as plotted on the x-axis. Results were highly correlated with one another and demonstrate that comparable data can be achieved with either protocol.

Matched Whole Blood Lysate and Total RNA

Correlation of Matched Total RNA and Blood Lysate

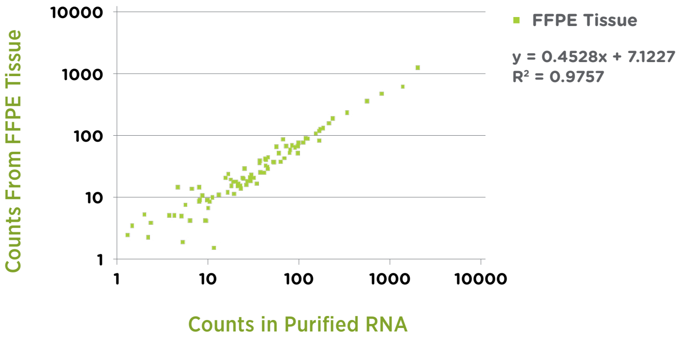


PAXgene™ lysed whole blood replicates were input directly into the hybridization reaction and compared to a matched purified total RNA sample as plotted on the x-axis. Results demonstrate that high quality data can be obtained by using PAXgene lysed whole blood.

PAXgene is a trademark of QIAGEN.

FFPE Samples

High Correlation – FFPE-derived and Purified RNA



FFPE-derived purified total RNA was input directly into the hybridization reaction and compared to a matched purified total RNA from fresh tissue on the x-axis. Results demonstrate that high quality data can be achieved from FFPE samples.

nCounter® Single Cell Gene Expression Assay

The **nCounter® Single Cell Gene Expression Assay** greatly expands the types of studies that can be analyzed on the nCounter Analysis System. **Entire biological pathways and custom gene signatures can now be studied** for single cells and the flexibility of the single tube assay allows interrogation of the exact gene number of interest.

More Genes

Analyze multiple pathways for up to 800 genes – flexible format allows interrogation of exact gene number of interest; same workflow when analyzing anywhere from 20 to 800 genes

Single Cell Sensitivity

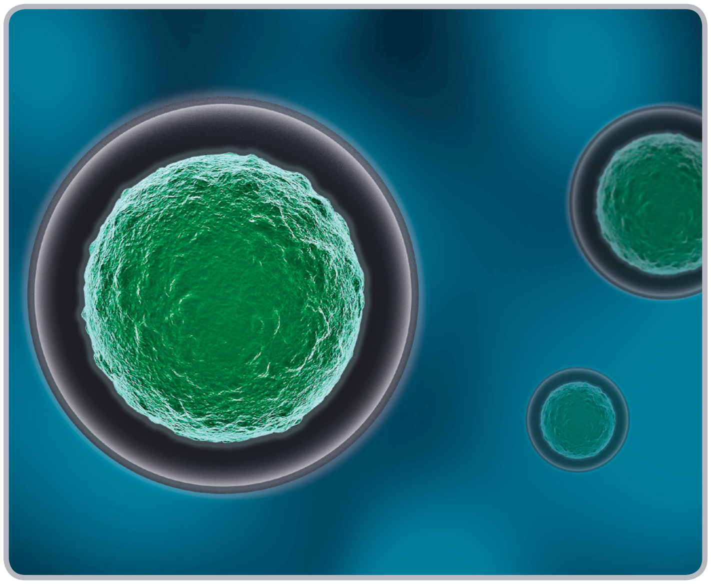
Obtain single cell sensitivity while minimizing amplification – single tube assay provides a simple workflow & utilizes the entire sample

Digital Counting

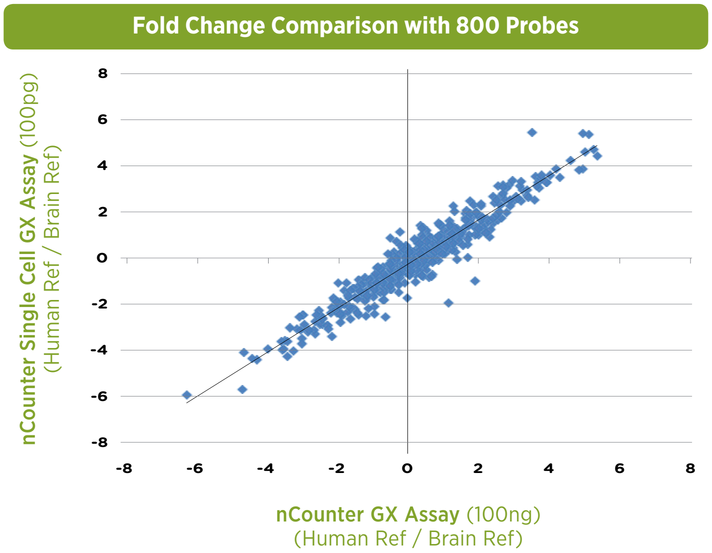
Single molecule counting minimizes data noise – determine fractional fold changes

High Throughput

Obtain 10,000s of single cell expression values every day



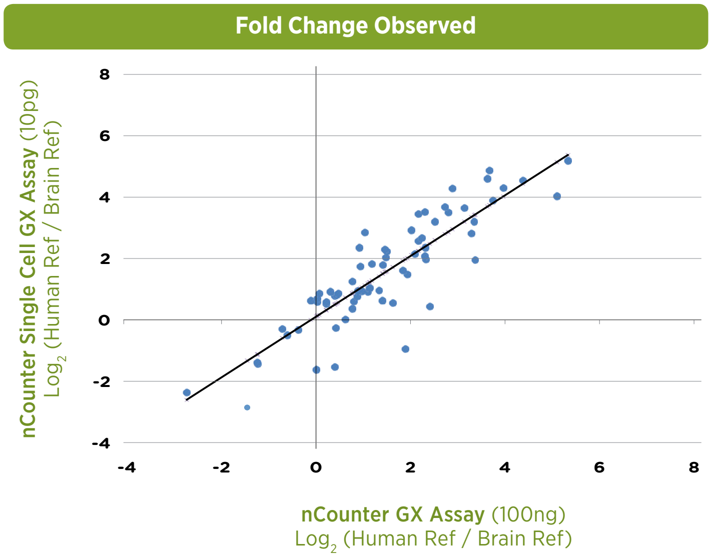
Up to 800 Targets from a Single Cell



Fold Change Comparison with 800 Probes

nCounter Single Cell Expression Assays enables detection of up to 800 genes. Fold changes observed between Human Reference and Brain Reference total RNA with 800 probes. Values from standard nCounter Gene Expression assays with 100ng total RNA as input (x-axis) are shown *vs.* fold changes observed with 100pg total RNA input material prepared according to the nCounter Single Cell Gene Expression Protocol (y-axis).

High Correlation of Fold Changes



Fold Change Observed

nCounter Single Cell Expression Assays enable sensitive detection of fold changes at the single cell level. Fold changes observed with probes using standard GX assay input of 100ng on the x-axis and 10pg, an amount of RNA equivalent to a single cell, prepared according to the nCounter Single Cell Gene Expression Protocol on the y-axis.

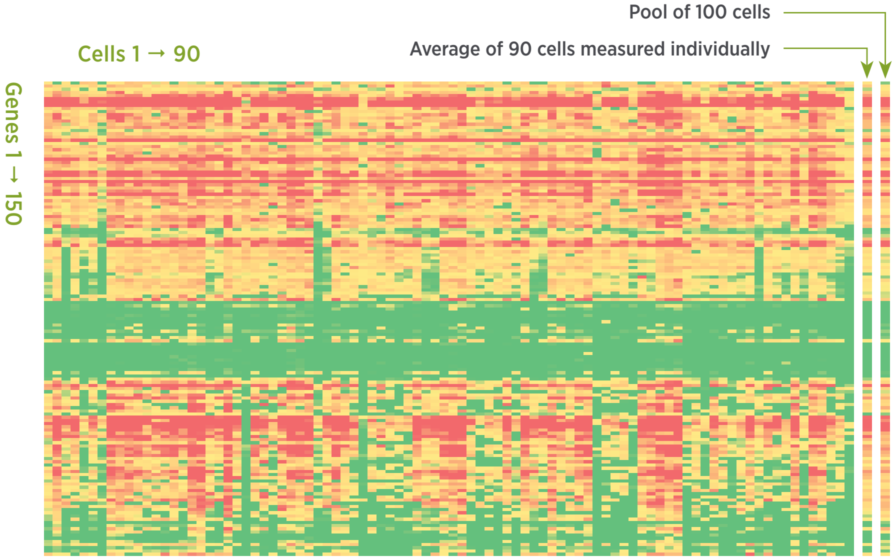
One Cell – Up to 800 Genes

The ability of the **nCounter® Single Cell Gene Expression Assay** to **analyze up to 800 genes in a single tube** eliminates the need for sample partitioning and minimizes the number of amplification cycles. The digital single molecule read-out **provides highly reproducible results and allows researchers to generate 10,000s of digital expression values per day.**

Make Every Cell Count

See the differences in single cell gene expression profiles that may be hidden when looking at larger cell populations. Cells were isolated and sorted using the BD FACSJazz™ Cell Sorter and then analyzed on the nCounter platform after amplification using NanoString's **Multiplexed Target Enrichment** protocol. As seen in **FIGURE 1,** gene expression profiles from individual cells were highly heterogeneous, demonstrating that **individual cells within a seemingly homogenous cell population can have distinct expression profiles.**

The average gene expression profile constructed from 90 individual cells is equivalent to the expression profile observed from a 100-cell pool, highlighting that **expression profiles from cell populations are the composite of the individual expression profiles of cells within that population.** These data highlight the **excellent reproducibility and linearity** of the digital nCounter detection technology for Single Cell analysis.



H9 cells sorted with BD Biosciences FACSJazz™ Cell Sorter.

FIGURE 1: Counts observed after amplification with the input amount indicated for individual probes in a 150-plex CodeSet are shown with robust expression (>250 counts) in red, moderate expression (50–250 counts) in yellow and minimal expression (<50 counts) in green. nCounter Single Cell Expression Assays identify distinct gene expression profiles in single cells. Data is shown for 90 single cells, the average of 90 individual cells, and a triplicate average of a 100-cell pool.

FACSJazz is a trademark of BD Biosciences.

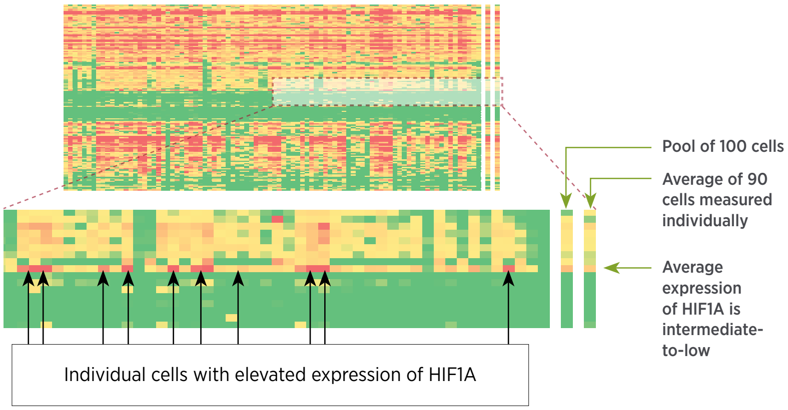


FIGURE 2: Single cell gene expression analysis allows for identification of genes with elevated expression in only a small subset of samples.

Get More Data From Every Cell

Single cell gene expression data can highlight new genes or pathways that are unremarkable when analyzing pooled cells. As seen in **FIGURE 2**, the 100-cell expression value of the highlighted gene is intermediate, which could result in it being eliminated from further study. However, when examined at the single cell level, this gene is seen to be highly expressed for a small percentage of cells (~5% to 10%); a finding that could potentially indicate a significant biological role for this gene, meriting further analysis of this gene and its associated gene expression pathway. This example **highlights the importance of using a more comprehensive gene set when designing Single Cell experiments,** as genes that may have seemed unremarkable in prior analysis with pooled samples may be of great interest when assayed at the single cell level.

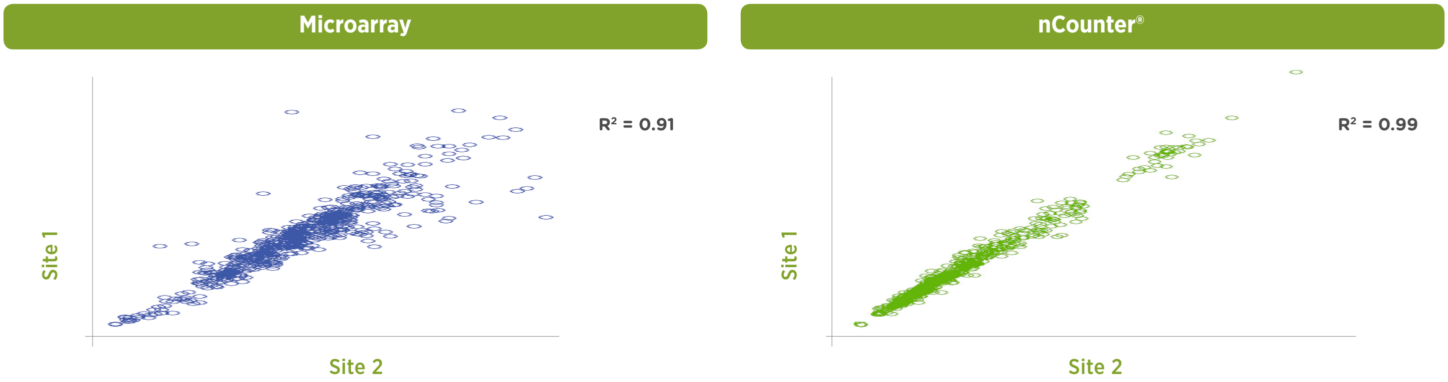
nCounter® Copy Number Variation CodeSets

The **nCounter® Copy Number Variation (CNV) CodeSets** provide **high accuracy and reproducibility** for studies of all sizes. **Achieve better results faster and with less effort.**

Product Highlights

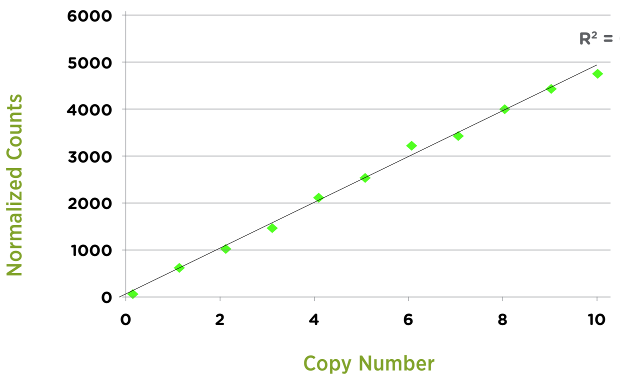
- Multiplex **up to 800 target regions in a single reaction**
- **High accuracy for multiallelic CNVs**
- **Excellent site-to-site reproducibility** ($R^2 = 0.99$)
- Just **25 minutes of hands-on time per 12 samples**

Superior Site-to-Site Reproducibility



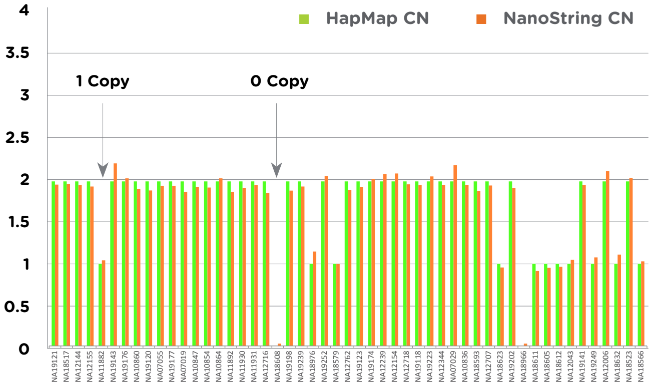
As with all nCounter assays, the reproducibility of the nCounter CNV assay is excellent. These scatter plots show the correlation of CNV data run in 2 separate sites by separate users on a microarray platform and nCounter respectively. **$R^2 = 0.99$ for nCounter compared to 0.91 for microarrays.**

Linearity



Linear response to increasing copy number. A synthetic DNA oligo was titrated at concentrations that are representative of varying copy numbers. Normalized counts are shown on the Y-axis, copy number is shown on the X-axis. **The number of molecules counted increases linearly with a linear increase in copy number.**

Accuracy



High Concordance with microarray data. A comparison of copy number calls between NanoString and microarray data for a single genomic region in 50 HapMap samples (Coriell Cell Repositories). Integer copy number values are shown relative to reference sample NA10851. **The nCounter CNV assay was 100% accurate for 0, 1 and 2 copies in this region across all samples.** Overall accuracy for the entire study (100 samples – 20 regions) was > 94%.

High-quality Data in a Simple Workflow

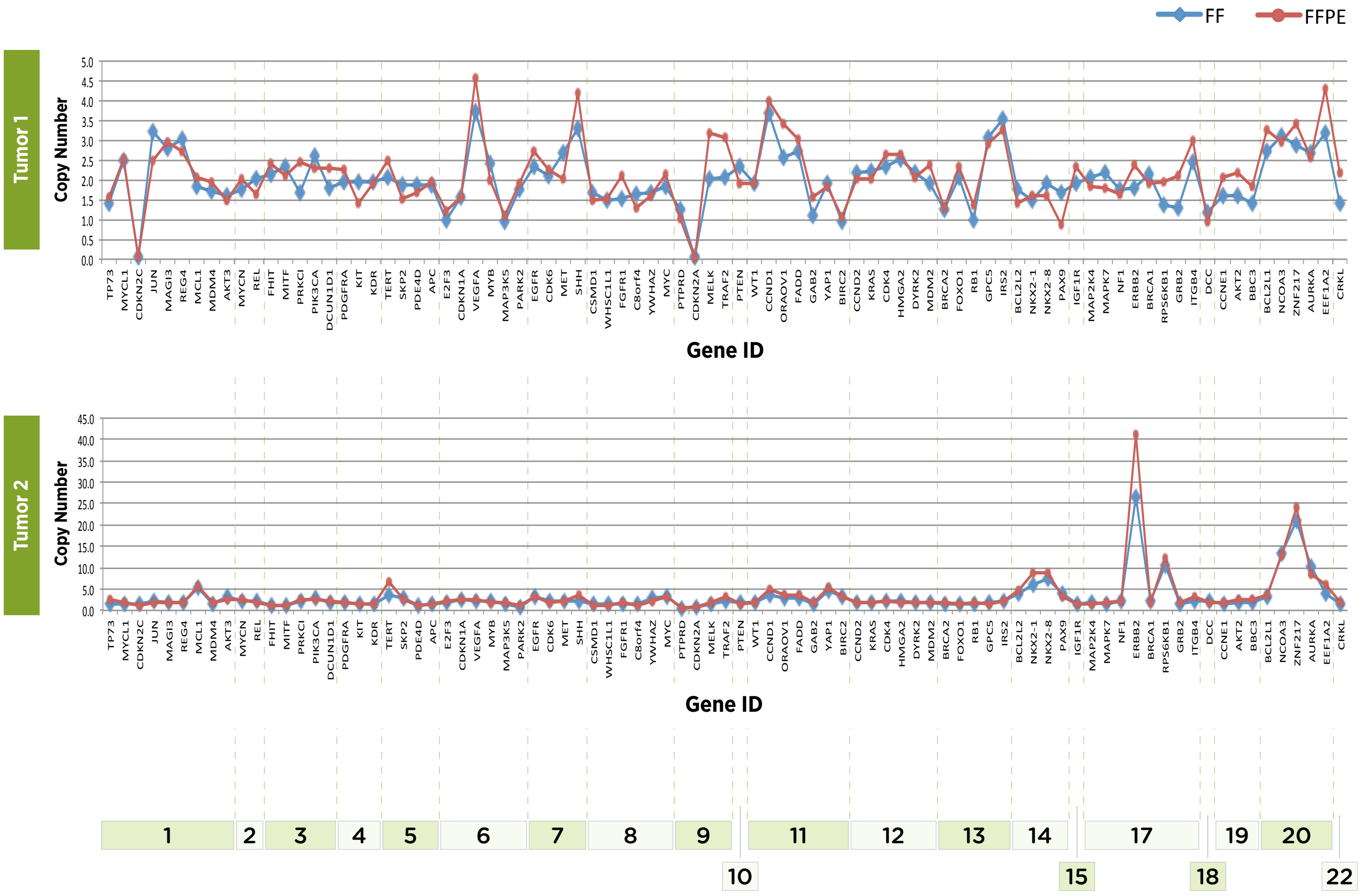
The nCounter CNV Assay workflow **requires minimal hands-on time.** In less than 25 minutes, you can set up 12 reactions with **no technical replicates required. Simply fragment your DNA and set up the hybridization – the rest is automated.**

Efficiently Screen 1,000s of Samples

NanoString's multiplexing capacity makes the most of your DNA by allowing you to **assay up to 800 loci in a single tube**, with as little as 300ng of starting material. With nCounter Custom CNV CodeSets you can screen hundreds of samples against hundreds of CNVs every week. Also based on the CNV workflow is the **nCounter® v2 Cancer CN Assay** - an assay that enables **rapid, cost effective analysis of 87 genes associated with cancer.**

Correlation of Copy Number Calls for Matching Fresh Frozen *and* **FFPE Samples** *with the* **nCounter® v2 Cancer CN Assay**

Copy number for genes included in the v2 Cancer CN Assay CodeSet for 2 matched pairs of Fresh Frozen (FF) and FFPE tumor samples. The data was analyzed using diploid reference samples that matched the sample type as closely as possible. For the fresh frozen tissue, we used the cell line NA10851 as a control and for the FFPE samples we used the Cancer CN FFPE Reference data set (provided by NanoString with the v2 Cancer CN Assay kit). DNA was fragmented by Alu1 digestion and the input amount was 300ng.



nCounter® Panel Products *for* Gene Expression, miRNA and CNV

NanoString's pre-made panels are available for a number of important pathway and research areas. All panels are created with input from customers and current research topics and are updated regularly.

nCounter® Panel Products

- Pre-made panels available to ship next day
- Single Cell Panels: Adapted for use with Single Cell applications. Panels include primer pools for multiplex target enrichment
- Virtual Pre-designed Panels: Collections of gene lists that have designs ready for manufacturing. Requires a minimum of 48 reactions to build with some additional delivery lead time
- Panel pricing is based on total gene number and reaction size, and are available in all standard sizes of 12 reactions or more

Panel Kits Include

- **Pre-made CodeSet** for specific panel of interest
- **Master Kit** materials (cartridge, plate pack, prep pack)
- **Primer pools** (Single Cell)
- **USB drive:**
  - Unique Reporter Library File (RLF) for the specific panel
  - Product Data Sheet

nCounter® Panel Products

Gene Expression Panels

Description	No. of Genes
Human Cancer Reference	230
Human Immunology	594
Mouse Immunology	561
Human Inflammation	184
Mouse Inflammation	179
Human Kinase	519
Human Stem Cell	199
Customer Assay Evaluation	47

Single Cell Panels

Description	No. of Genes
Human Cancer Reference	233
Human Immunology	594
Mouse Immunology	561
Human Inflammation	171
Mouse Inflammation	180
Human Stem Cell	187
Customer Assay Evaluation	47

miRNA Discovery Panels

Description	No. of miRNAs
Human	800
Mouse	600
Rat	423
Fly (*D. melanogaster*)	184

Cancer CN Panel v2

Description	No. of Genes
CNVs commonly amplified or deleted in cancer	87

Human Karyotype Panel

Description	Regions
Human karyotype monitors gross chromosomal abnormalities	8 probes per arm

Other Pre-designed Panels

Description	No. of Genes
Human Angiogenesis	179
Human Breast Cancer-ER	196
Human p53 Pathway	102
Human Apoptosis	160
Human Cell Cycle	183
Human Jak-Stat Pathway	86
Mouse Cancer Genes	232
Mouse Stem Cell Genes	188

To see a complete list of genes in each panel, please visit **nDesign™ Gateway** at **www.nanostring.com/nDesign**

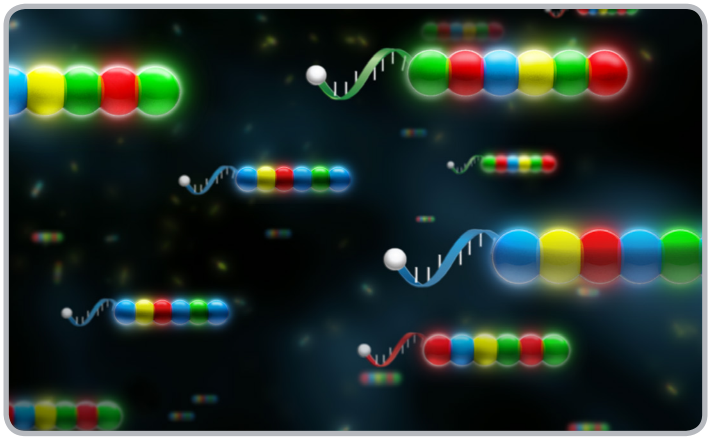
nanoString® TECHNOLOGIES

nCounter® Panel-Plus & CodeSet-Plus

nCounter® Panel-Plus & CodeSet-Plus add a **new level of flexibility to nCounter experiments.** Using the standard nCounter protocol and workflow, researchers can now **customize any off-the-shelf nCounter panel kit or custom CodeSet with the ability to spike-in up to 30 genes.** Plus products utilize **unique Reporter Probes exclusively formulated for use in Panel-Plus & CodeSet-Plus** and can be created upon receiving a list of your genes at the time of your order.

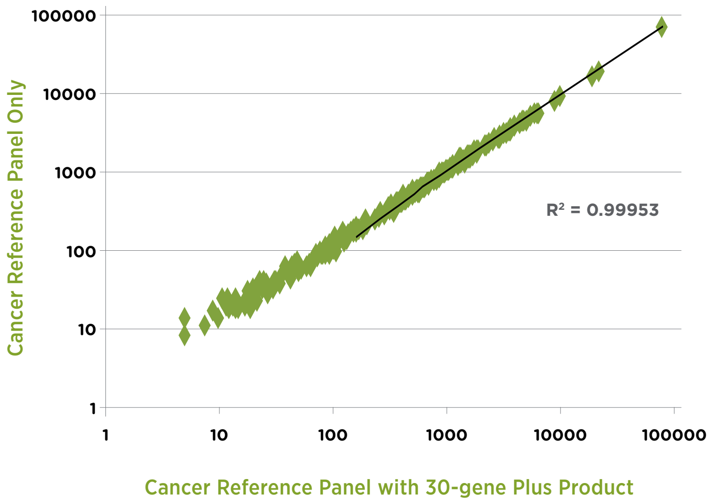
Product Highlights & Applications

- **30 unique Reporter Probes are universally compatible** with all nCounter Gene Expression and CNV Panels, including Single Cell application
- **Customize your experiment** by adding up to 30 of your genes related or unrelated to the panel content
- **Create unique Panel-Plus control sets** to spike-in when using different sample or cell types



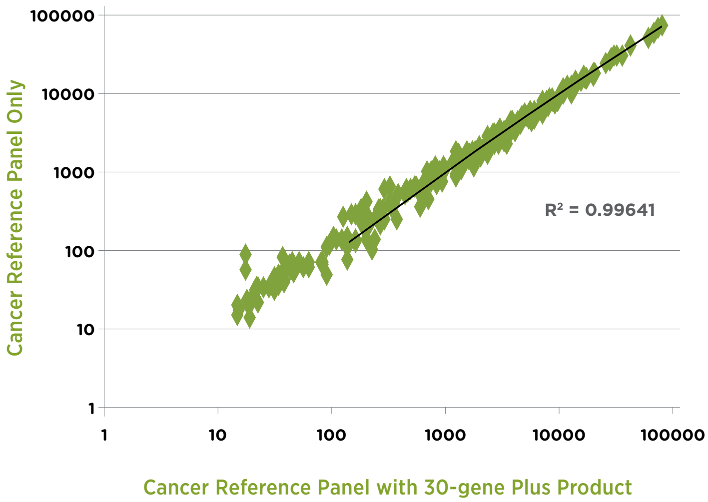
Performance of Panel-Plus Gene Sets with Pre-designed Panels

Gene Expression



Human Reference Total RNA (100ng) was hybridized with either nCounter Cancer Reference Panel (Original CodeSet) alone or nCounter Cancer Reference Panel with a 30-gene Panel-Plus (Original CodeSet with Plus). Assays were performed in triplicate for each condition tested and normalized to internal positive controls. Average count values for each triplicate measurement were determined and plotted for all nCounter Cancer Reference Panel probes in the Original CodeSet alone (y-axis) and Original CodeSet with Plus (x-axis).

Single Cell Gene Expression

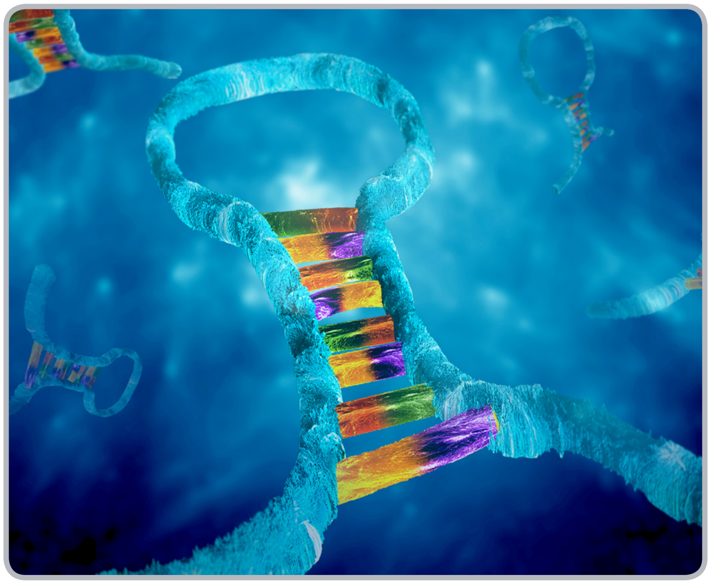


Human Reference Total RNA (100pg) was converted to cDNA and enriched via a Multiplexed Target Enrichment (MTE) with primers appropriate for downstream hybridization according to the nCounter Single Cell Expression Assay Protocol for Total RNA. Assays were performed in triplicate for each condition tested and normalized to internal positive controls. Average count values for each triplicate measurement were determined and plotted for all nCounter Cancer Reference Panel probes in the original CodeSet alone (y-axis) and Original CodeSet with Plus (x-axis).

nCounter® miRNA Expression Assays

nCounter® miRNA Expression Assays provide a **cost-effective** profiling solution capable of **accurately discriminating between miRNAs at single-base resolution**. Each CodeSet contains **a comprehensive collection of miRNAs** in a single, highly multiplexed assay.

Highly multiplexed, direct digital detection and counting of miRNAs in a single reaction without amplification.



Accurate Identification *with* High Specificity

At single-base resolution, the nCounter miRNA assay can accurately distinguish between highly similar miRNAs with great specificity. The innovative protocol is **highly sensitive and requires no amplification steps.**

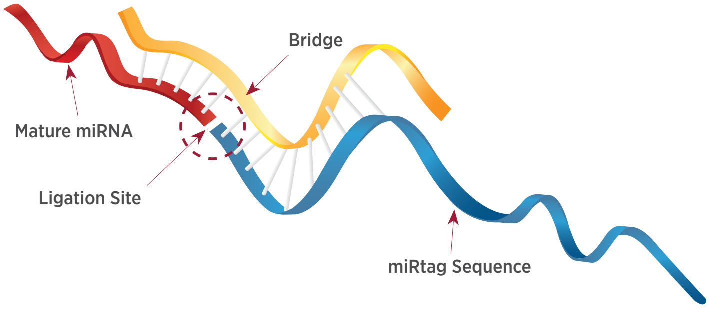
Comprehensive Transcriptome Coverage

Comprehensive CodeSets are available for human, mouse, rat and fly miRNA transcriptomes. High levels of multiplexing, digital counting, and the capacity to **process up to 96 samples per 24-hour time period*** provide **fast and cost-effective** access to thousands of data points per day.

** Requires 2 Prep Stations*

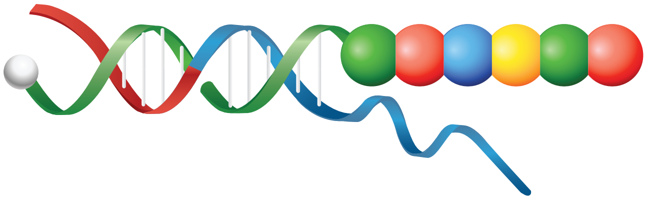
Elegant, Simple *with* Remarkable Results

miRNA Sample Preparation



To prepare a sample containing miRNA molecules for hybridization in the nCounter assay, proprietary DNA sequences called miRtags are ligated to the mature miRNAs through complementarity with sequence-specific bridging oligonucleotides (bridges). This multiplexed reaction leads to the highly precise, sequence-specific tagging of all miRNAs in a single tube. Excess tags and bridges are removed via a simple enzymatic step in the same tube. No further purification is required.

Sample Hybridization



The miRtagged mature miRNA is then hybridized to a probe pair in the standard nCounter gene expression assay workflow. The Reporter Probe carries the signal; the Capture Probe allows the complex to be immobilized for data collection. Hybridization occurs in one multiplexed reaction.

Data You Can Count On

With multiplexed target profiling of miRNA transcriptomes in a single reaction, the **nCounter® miRNA Expression Assays** deliver high levels of **sensitivity, specificity, precision, and linearity** for a wide range of research areas such as **oncology, neurobiology, developmental biology, and stem-cell research.** Assay performance is demonstrated below using commercially available total human RNA.

Single-base Resolution

miRNA Target

nCounter Probe	Let7a	Let7b	Let7c	Let7d	Let7e	Let7f	Let7g	Let7i
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%

The Let7 family of miRNAs contains several members differing in sequence by as little as a single nucleotide. Percent cross-hybridization for the Let7 probes was assessed by tagging and hybridizing individual Let7 family miRNAs in the presence of the comprehensive human miRNA CodeSet including probes to eight Let7 family members. The minimal cross-hydridization observed (with no probe exceeding 6%) indicates that the **nCounter miRNA assay can accurately discriminate similar sequence miRNAs with great specificity.**

nCounter miRNA Expression Assay *and* Sample Preparation Kits

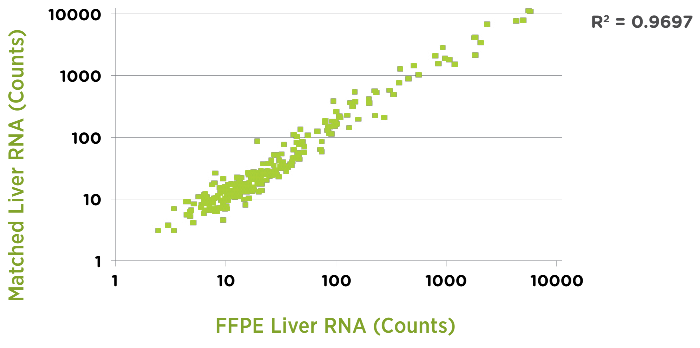
nCounter miRNA Expression Assay Kits contain a comprehensive collection of miRNAs derived from miRBase. The off-the-shelf kits **arrive in your lab with the highly multiplexed CodeSet and all of the reagents and consumables necessary to perform the assay.** Kits are available in 12, 24, 48, and 96 reactions sizes for Human, Mouse, Rat or Fly, offering flexibility for labs and projects of all sizes.

The nCounter miRNA Sample Preparation Kit provides reagents for ligating unique oligonuceotide tags onto miRNAs, **allowing these short RNAs to be detected with high specificity and sensitivity** with the nCounter Analysis System.

nCounter® À la carte Custom miRNA Sets are available for performing larger validation studies on subsets of miRNAs included in the species-specific nCounter miRNA Expression Assay Kits.

FFPE Samples

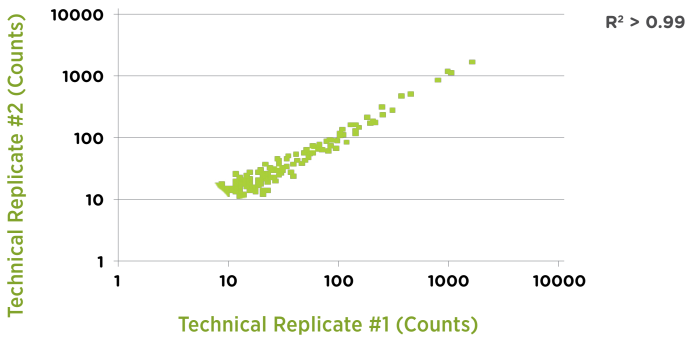
High Correlation – FFPE-derived and Purified RNA



Formalin-Fixed Paraffin-Embedded (FFPE) samples are difficult to analyze with many techniques due to RNA degradation. To demonstrate the ability of the miRNA assay to analyze degraded RNA samples, we tested FFPE-derived purified total RNA from liver samples against purified total RNA from matched fresh-frozen tissue using the nCounter Human miRNA Expression Assay Kit. **Counts for all detected miRNA species were highly correlated ($R^2 > 0.95$) between the two sample types.**

Assay Reproducibility

nCounter Assay Technical Replicates

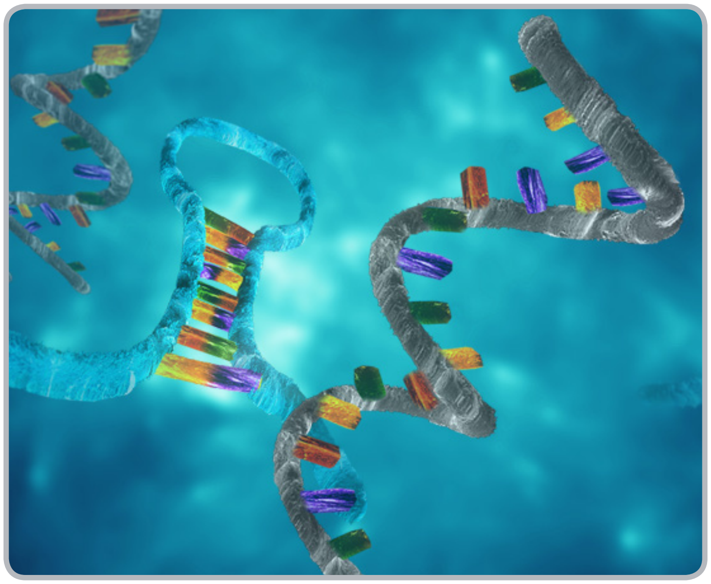


To demonstrate the reproducibility of the miRNA assay, we used the nCounter Human miRNA Expression Assay Kit to analyze commercially purchased human total RNA. The samples were prepared and run on the system separately. When plotted against one another, **the assay demonstrated very high reproducibility, with R^2 values of greater than 0.99.**

nCounter® miRGE™ Expression Analysis

The innovative **nCounter® miRGE™ Assay** is designed to provide a **single-tube, sensitive, reproducible and highly multiplexed method** for **simultaneously detecting subsets of both mRNAs and miRNAs.** It can be run on **total RNA isolated from any source, including Formalin-Fixed Paraffin-Embedded (FFPE) samples.**

No splitting of RNA samples into aliquots for two assays means that less sample can be utilized upfront for expression profiling of both miRNA and mRNAs.

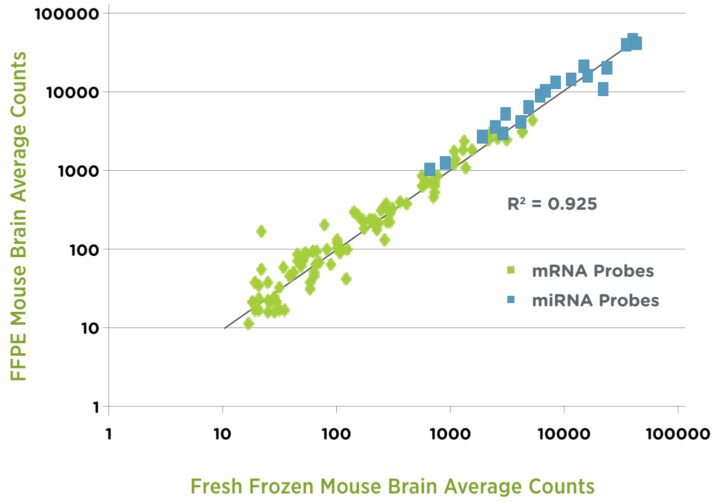


Simultaneous Profiling of miRNAs *and* mRNAs

Revolutionizing the workflow for profiling miRNA and mRNA expression, nCounter miRGE CodeSets allow for the **simultaneous profiling of both miRNAs and mRNAs in a single reaction.** Two assays in a single tube mean **less sample required and less preparation time.** nCounter miRGE CodeSets are **capable of profiling samples from FFPE extracts**, as well as other precious and difficult sample types.

Fresh Frozen *vs.* FFPE

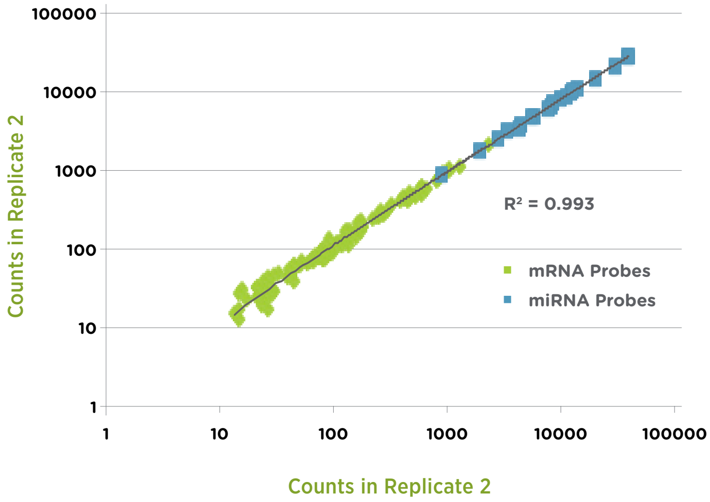
FFPE-derived and Purified RNA



Formalin-Fixed Paraffin-Embedded (FFPE) samples are difficult to analyze with many techniques due to RNA degradation. To demonstrate the ability of the miRGE assay to analyze degraded RNA samples, we tested FFPE-derived purified total RNA from brain samples against purified total RNA from matched fresh-frozen tissue. **Molecular counts of miRNAs and mRNAs correlate extremely well in total RNA purified from fresh frozen or FFPE-preserved matched tissue ($R^2 > 0.92$).**

Highly Reproducible Results

Mouse Lung Total RNA



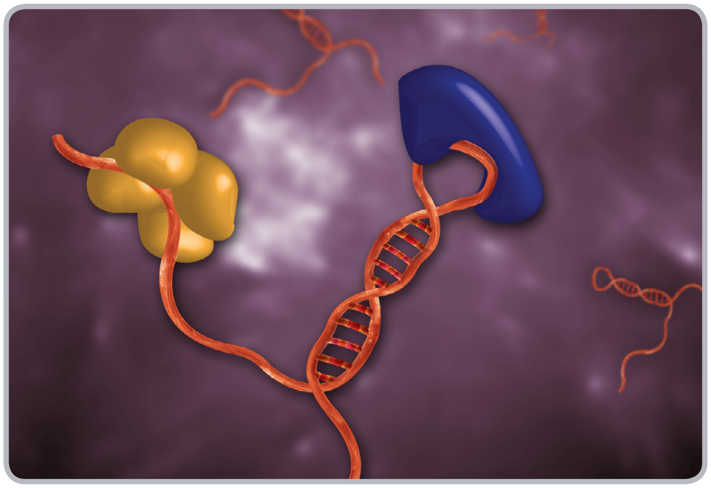
Counts for 20 miRNAs and 185 mRNAs species in mouse lung total RNA were **highly reproducible between technical replicates.**

nCounter® lncRNA Expression Assay

The **nCounter® Custom lncRNA Assay** enables researchers to study focused sets of **up to 800 lncRNAs with high precision and minimal hands-on time.**

Product Highlights

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

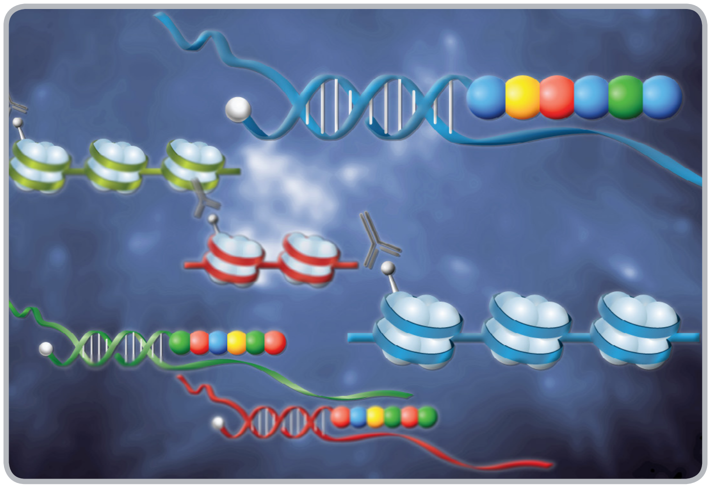


nCounter® ChIP-String Expression Assay

The **nCounter® Custom ChIP-String Assay** is a read-out method designed to **measure dsDNA fragments that have been enriched via various user-defined chromatin immunoprecipitation protocols.**

Product Highlights

- **Accurate differentiation and quantification of DNA** enriched by chromatin immunoprecipitation
- **Excellent correlation with ChIP-Seq results**
- Valuable biological insights through focused analysis
- Analyze **up to 800 loci with 15 minutes of hands-on time**
- **Results in 24 hours**
- **No library prep or amplification required**



nCounter® Plex²™ Expression Assay

The **nCounter® Plex²™ Assay** offers a cost-effective way to analyze expression levels of **hundreds of gene targets in multiple samples simultaneously in a single tube.** The system can directly assay tissue and blood lysates as well as Formalin-Fixed Paraffin-Embedded (FFPE) extracts in a **high-throughput workflow that is ideal for researchers with large numbers of samples.**

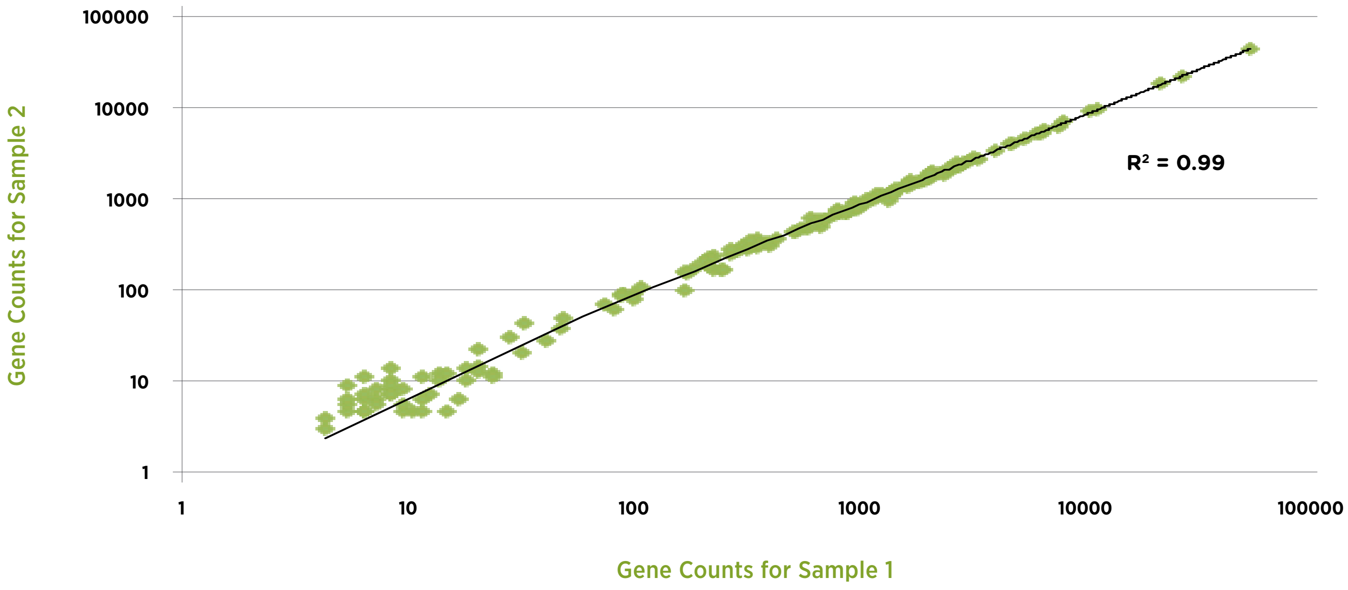
Product Highlights

- **Multiplex in 2-dimensions –** multiplex hundreds of targets and multiple samples in a single tube
- **Customizable to study size –** measure between 20–400 customizable targets in a single tube
- **Digital counting –** digital data in a high throughput format
- Directly assay **tissue extracts and whole-blood lysates, total RNA, cell lysates, and FFPE**
- Analyze up to **384 samples* per day**

** Requires 2 Prep Stations*

To determine assay precision when 4 multiple samples are profiled in a single tube, 4 samples derived from 2.5µl of Hela cell lysates were independently hybridized with 200 probe CodeSets each targeting the same 200 genes (each CodeSet contained 200 independent barcodes). After hybridization, the 4 samples were combined into a single tube for processing on the nCounter System. Technical replicate data were normalized and gene expression counts were then plotted to determine assay precision across all replicates. The plot below displays the correlation of 2 replicate samples. Comparisons between replicates showed **$R^2 \geq 0.99$**.

Assay Precision in Cell Lysates



nCounter® Custom CodeSet Design Process

The nCounter Custom CodeSet design process is completed in 4 EASY STEPS.

	1 Select Genes	2 Probe Design	3 Customer Review	4 Manufacture and Ship
Process Description	Submit your RefSeq IDs for **up to 800 target genes** to NanoString.	NanoString designs probes then **creates and sends a Design Report.**	Customer **reviews and approves Design Report.**	**NanoString manufactures and ships CodeSet** to customer.
Lead-time	Customer-defined	Custom GEx: **3 – 5 days** Custom CNV: **10 – 15 days**	Customer-defined	**3 – 5 weeks** (dependent on gene number and scale)

System Ordering Information

	Product Description	Unit(s)	Catalog Number
SYSTEM	**nCounter Analysis System** Includes both Prep Station and Digital Analyzer.	System	NCT-SYST-120
	nCounter Prep Station Automated liquid handler for processing assays.	Instrument	NCT-PREP-120
	nCounter Digital Analyzer Automated instrument for data collection.	Instrument	NCT-DIGA-120

nCounter® Prep Station

nCounter® Digital Analyzer

Contact Info

NanoString Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109

Contact Us

info@nanostring.com
Tel: (888) 358-6266
Fax: (206) 378-6288
www.nanostring.com

Sales Contacts

United States:	us.sales@nanostring.com
Europe:	europe.sales@nanostring.com
Other Regions:	info@nanostring.com

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