nCounter® Analysis System

Grant Application Package

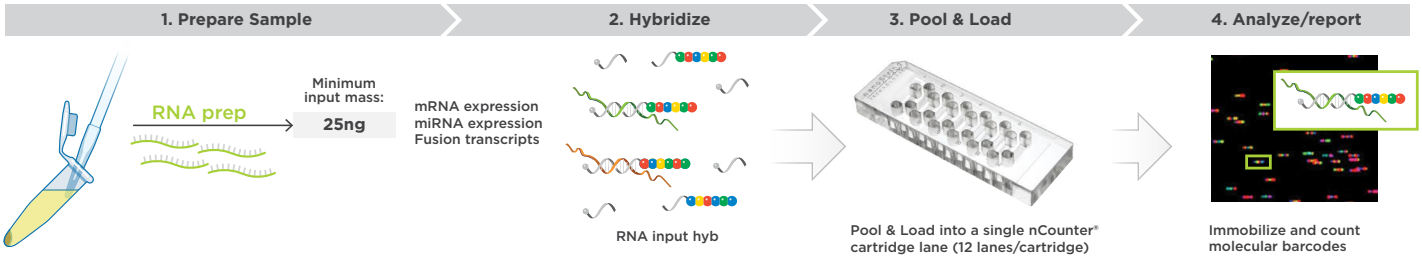
Platform Overview

The nCounter® Analysis System enables the profiling of 800+ mRNAs, microRNAs (miRNAs), and CNVs on one platform with high sensitivity and precision. The primary benefits of the platform are simplicity, reliability, and security. The workflow is simple, taking just 15 minutes of hands-on time and is mostly automated. Direct, digital counting of individual target molecules using the nCounter's patented molecular barcoding chemistry and single molecule imaging ensure a dynamic range of over 5 logs as well as highly reproducible results, eliminating the need for technical replicates. The assay does not require any reverse transcription or amplification, eliminating sources of potential error and bias and enhancing the reliability of the results. An additional advantage of nCounter chemistry is that the barcodes hybridize over a contiguous region of only 100 base pairs and there are no enzymatic steps, meaning that the assay is highly tolerant of difficult sample types such as FFPE and crude-cell lysates. The nCounter Pro system features advanced cyber security features such as an updated operating system (Windows 10 IoT), hard drive encryption (TPM 2.0), and data encryption (AES 256 and SHA-256) that enable parts of a 21 CFR Part 11 environment. All nCounter instruments and reagents are provided by NanoString Technologies and there are a wide variety of off-the-shelf panels and assays for human, mouse, rat, and non-human primate that enable research in oncology, immunology, and neuroscience.

Technology Overview

NanoString's nCounter technology is based on direct detection of oligonucleotides using color-coded molecular barcodes which are then used to individually count each of the target molecules. The probe pair consists of a Reporter Probe, which carries a fluorescent barcode on its 5' end, and a Capture Probe which carries a biotin on its 3' end, and each pair of probes hybridizes to a 100 base pair contiguous region on each target oligonucleotide. The barcodes consist of six different positions with each position one of four colors, creating a diverse number of different barcodes and allowing for multiplexing of 800+ targets in a single reaction tube.

The nCounter® Pro Analysis System consists of two separate instruments: the Prep Station and the Digital Analyzer. Purification and binding of the hybridized complexes is carried out automatically on the nCounter Prep Station. Magnetic beads derivatized with short nucleic acid sequences that are complementary to the Capture Probe and the Reporter Probes are used sequentially. First, the hybridization mixture is allowed to bind to the magnetic beads by the Capture Probe. Wash steps are performed to remove excess Reporter Probes as well as oligonucleotides that are not hybridized. After washing, the Capture Probes and Target/Probe complexes are eluted from the beads and are hybridized to magnetic beads complementary to the Reporter Probe. Wash steps are performed, and excess Capture Probes are washed away. Finally, purified Target-Probe complexes are eluted off and are immobilized on a streptavidin coated surface in flow cells (the cartridge) for imaging and counting. Imaging and counting are performed on the Digital Analyzer. Digital images are processed, and the barcode counts are tabulated in a comma separated value (CSV) format. NanoString's patented molecular barcodes allow for true, digital detection technology of individual target molecules.



nCounter Instruments

nCounter Pro

The nCounter Pro Analysis System consists of two separate instruments: the Prep Station and Digital Analyzer. The Prep Station is the automated liquid handling component of the nCounter Pro. It processes samples post-hybridization to prepare them for imaging and counting on the Digital Analyzer. Prior to placing samples on the Prep Station, samples are hybridized according to the nCounter protocol. On the deck of the Prep Station, hybridized samples are purified and subsequently immobilized in the sample cartridge for imaging and counting. The Digital Analyzer collects data by taking images of the immobilized Reporter Probes in the sample cartridge with a CCD camera through a microscope objective lens. Images are processed internally, and data output files include the target identifier and count number along with a comprehensive tally of internal controls that allows each assay to be quantitative. The resulting data file can be distributed using a variety of methods and is easily integrated with commonly used data analysis and visualization software packages.

nCounter Pro Prep Station & Digital Analyzer

nCounter SPRINT Profiler

The nCounter® SPRINT Profiler is an all-in-one benchtop nCounter system that combines the function of the Prep Station and the Digital Analyzer into one instrument. Hybridized samples are loaded into a SPRINT cartridge, where a series of microfluidic chambers purifies and immobilizes the fluorescent Reporter Probes and readies them for imaging. Images are processed, and output files include gene names along with associated count numbers.

nCounter SPRINT Profiler

nCounter Data Analysis

The ROSALIND® Platform

ROSALIND is a cloud-based software platform for life science research that enables scientists to analyze and interpret differential gene expression data without the need for bioinformatics or programming skills. The platform is always up to date: ROSALIND operates in a browser, eliminating the need to download software updates continually. ROSALIND makes analysis of nCounter data easy, with guided modules for quality control, normalization, pathway analysis, cell type profiling, differential expression, and gene set analysis. ROSALIND is free of charge for nCounter customers; a free account can be created at www.rosalind.bio/nanostring.

nSolver™ Analysis Software

nSolver Analysis Software is a free, on-premises integrated analysis software platform for storage, custom QC, and custom normalization of nCounter data. Generate highly customized exports, basic statistical outputs, and publication-quality figures quickly and easily with no incremental cost or bioinformatics expertise. nSolver output data, provided in the form of a simple CSV file, is extremely easy to both share and store due to the small file size. nSolver data is compatible with many standard third-party analysis programs including Ingenuity Pathway Analysis, Partek Genomics Suite, BioDiscovery Nexus Copy Number and Advaita iPathwayGuide. For deeper insights into your data, nCounter Advanced Analysis is a free, wizard-based add-on to the nSolver Analysis Software based on robust R statistics. The nSolver Advanced Analysis module enables an enhanced examination of experimental trends, identification of pathway-specific responses, and profiling of cell populations in an intuitive and sharable HTML report.

Data Analysis Service (DAS)

NanoString's Data Analysis Service (DAS) provides researchers with the opportunity to leverage NanoString's team of scientists and bioinformaticians to provide meaningful and insightful analysis of your data quickly. This is a highly consultative process that includes a one-on-one review of results with an expert highly trained in analyzing data produced by the nCounter platform. Also offered are highly comprehensive, interactive reports for the PanCancer IO 360™ and Breast Cancer 360™ Panels. Proprietary signature analysis for the TIS, PAM50 and LST gene signatures can be added to any standard DAS project on an a la carte basis.

nCounter CodeSets & Consumables

nCounter CodeSets

nCounter CodeSets are pools of color-coded molecular barcodes and capture probes designed for specific projects. In addition to target-specific probes, a comprehensive mix of control probes are added to each CodeSet for quantification, QC, and data normalization. Custom CodeSets and fixed content panels are available for analysis of mRNA, miRNA, lncRNA, and DNA.

nCounter Instrument Consumables

The nCounter Master Kit provides all the consumables and reagents you need to perform any nCounter assay on the nCounter Pro System. With each Master Kit order, you receive nCounter Cartridges, Prep Plates, and a Prep Pack, which includes racked tips and foil piercers, 12 sample strip tubes, strip tube caps, tube sheaths, cartridge well seals and hybridization buffer. Master Kits are available in packs for 12, 48 or 192 samples.

Running samples on the nCounter SPRINT Profiler requires two types of consumables: the nCounter SPRINT microfluidic sample cartridge and the nCounter SPRINT Profiler Reagent Pack. The nCounter SPRINT Reagent Pack contains the bulk running buffers and reagents necessary to process 192 samples (16 runs, each run for up to 12 samples).

Training and support

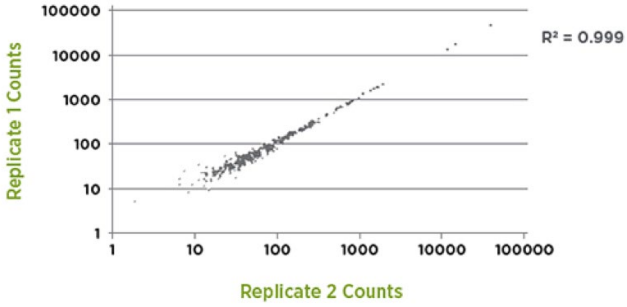
Upon purchase and acquisition of an nCounter system, NanoString provides on-site installation and calibration by a trained field engineer. Once the instrument has been qualified, a field application scientist provides a comprehensive introduction and training for the nCounter platform. Training covers routine use of the instrument, designing experiments, and performing data analysis. Additionally, the field application scientist will perform wet lab experiments on-site to ensure that the customer has hands-on experience operating the instrument and performing assays. Upon successful completion of training, the customer can access additional support regarding instrumentation, consumables, and software from NanoString's website or by contacting the NanoString Tech Support Team.

nCounter Sample Types & Supporting Data

nCounter assays can be used with a wide variety of different sample types such as purified total RNA, raw cell or blood lysates and formalin-fixed, paraffin-embedded (FFPE) tissue sections with no loss in precision. Even severely degraded RNA can be used, owing to the fact that the nCounter probe sets only hybridize to a 100 base pair contiguous region of the target oligonucleotide.

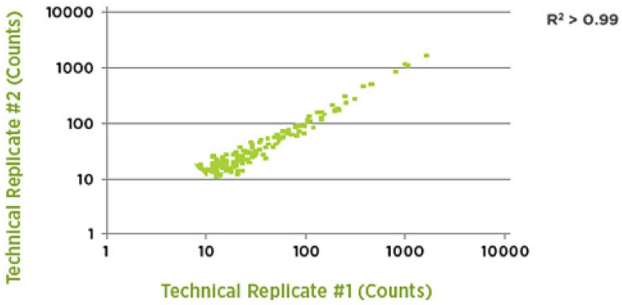
Gene Expression Profiling of mRNA

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 are 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 expression levels.



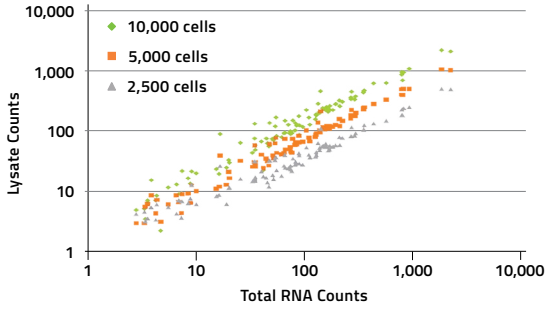
miRNA

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



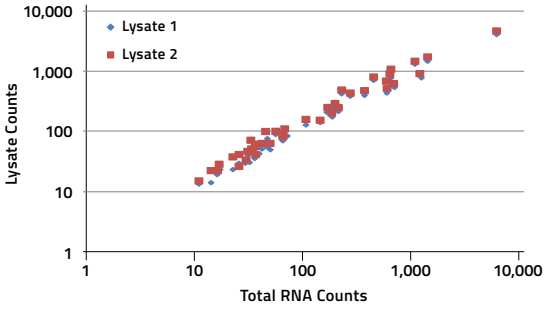
Crude Cell Lysates

Gene expression counts from three different cell lysate preparations made of 2,500, 5,000, and 10,000 cells were compared to those from 100 ng of purified total RNA. Results using cell lysates were highly correlated with that from purified RNA (R^2 > 0.97 for all three) and demonstrated that comparable data can be achieved with either protocol.

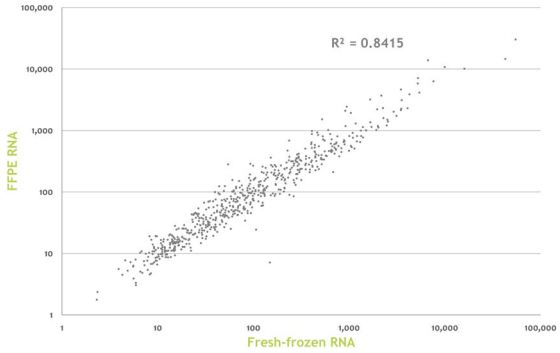


Whole Blood Lysates

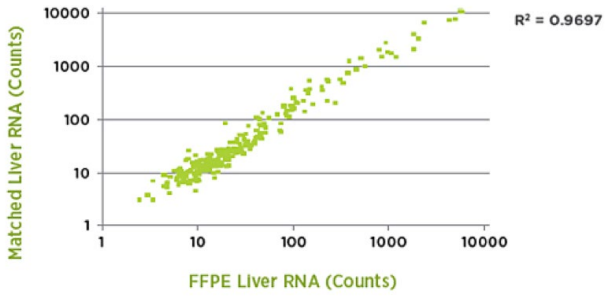
Gene expression counts from two PAXgene-lysed whole blood replicates were compared to that from 100 ng of matched purified total RNA. Results using blood lysates were highly correlated with purified RNA (R^2 > 0.96 and R^2 > 0.97) and demonstrated that high quality data can be obtained using PAXgene-lysed whole blood. (PAXgene is a trademark of Qiagen).



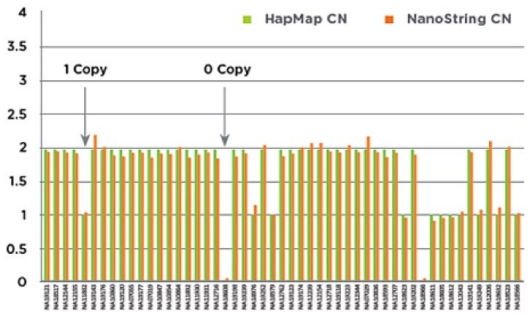
Formalin-Fixed Paraffin-Embedded Tissue (FFPE)
Gene expression counts from RNA derived from FFPE were compared to that from matched purified total RNA and the results were highly correlated with purified RNA (R^2> 0.84), demonstrating that high quality data can be achieved from FFPE tissue sections using the nCounter Analysis System.



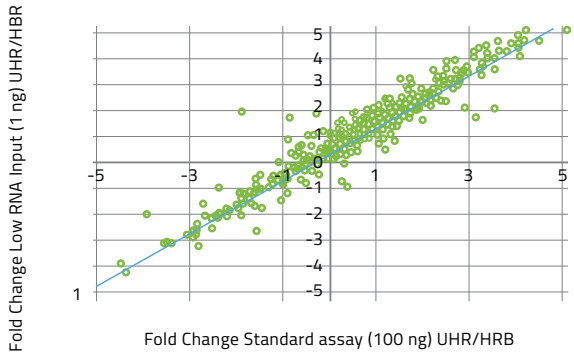
Analysis of miRNA from FFPE Samples
Formalin-Fixed, Paraffin-Embedded (FFPE) tissue sections are difficult to analyze due to RNA degradation. To demonstrate the ability of the miRNA assay to analyze degraded RNA samples, we compared gene expression counts of miRNA from purified total RNA from FFPE liver sections with that from 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.



Analysis of DNA Purified from FFPE Samples
This figure shows copy number counts for genes included in the nCounter Cancer CN Assay for two matched pairs of Fresh Frozen (FF) and FFPE tumor samples. The data was analyzed using diploid reference samples matching the sample type as closely as possible. For the fresh frozen tissue, we used 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 Cancer CN Assay kit). DNA was fragmented by AluI digestion using an input amount of 300 ng.



Analysis of Degraded and Low RNA Input Samples
The nCounter Low RNA Input Kit enables high quality gene expression profiling of up to 800 gene targets from as little as 1 ng of sample. The kit is optimized for use with RNA from FFPE tissue as well as crude cell lysates. Additionally, the kit can be utilized for the study of low expressing genes. The streamlined, user-friendly workflow and reliable results enable gene expression studies of low sample inputs or low expressing genes to be completed quickly and efficiently.



Justification for Using Nanostring technology to Accelerate Your Research

High Reproducibility

The nCounter system is known for its high level of reproducibility and precision, as has been shown in various studies by independent research groups. Veldman-Jones et al. (2015) reported correlation scores >0.98 between technical replicates, irrespective of whether the RNA was derived from fresh cells or FFPE material. Similarly, Balko et al. (2012) investigated systematically the consistency between repeated nCounter measurements from breast cancer FFPE samples and found consistent R^2 values >0.99 (Supplemental material). In another study investigating molecular subgrouping of medulloblastoma, researchers looked at inter-operator variance and concordance between results obtained from the same sample sets at different processing sites and reported excellent correlation scores >0.97 (Northcott et al., 2012). nCounter panels include numerous controls that serve as indicators for technical variability present in the measurement of a particular sample.

High Level of Robustness, Even with Poor Quality Samples

There are many publications that clearly demonstrate gene expression analysis can be done reliably and accurately on FFPE material using the nCounter Analysis System, often where high failure rates are observed using RNASeq with the same material (Omolo et al., 2016; Lesluyes et al., 2016; Le Guellec et al., 2018). Sample quality requirements (i.e., RNA integrity numbers) are much higher for sequencing assays which can also be negatively affected by background contaminants, including chemical fixatives used in the FFPE process. In contrast, the short sequence (100 bp) required for nCounter probe detection and the fact that library preparation is not necessary results in a considerably better tolerance of the nCounter assay towards RNA quality. For example, Lesluyes et al. (2016) failed to reliably transfer an RNASeq-based signature for prediction of metastatic outcome in sarcomas from fresh frozen samples to FFPE tissue, as it was not possible to obtain sequencing results from more than 50% of the FFPE samples. Le Guellec et al. (2018) confirmed this experience in their study, where successful measurement of a particular signature using RNASeq occurred in only 17 out of 67 FFPE samples (25%), whereas all 67 samples (100%) were analyzed using the nCounter Analysis System.

Technology offers higher sensitivity than targeted RNASeq

While the correlation of expression level measurements between RNASeq and the nCounter platform is typically quite good overall (Leong et al., 2015), the concordance remains even for genes with low expression levels. Steijger et al. (2013) showed that the discrepancy is usually associated with small, short read numbers representing low abundant targets in RNA-Seq data sets and the failure of downstream analysis packages to robustly infer presence and expression of these genes. In contrast, counts for such low abundance targets are clearly removed from background noise levels when looking at nCounter data, demonstrating a higher sensitivity of the platform.

Faster Processing time

The total time from input sample to results on the nCounter platform is less than two days. In contrast, the amount of time to perform library preparation, QC/titration, and sequencing typically exceeds four days for RNASeq-based assays. The difference in actual hands-on time is even more pronounced, with 45 mins or less (only 15 minutes for a basic gene expression assay) required for the nCounter versus four hours or more for the average RNASeq run.

Simple Data Analysis

Data analysis of nCounter experiments can be performed easily. Data files are small and can be processed without sophisticated software tools, eliminating the need for extensive computing infrastructure or highly specialized bioinformaticians. NanoString offers an openly available, on-premises analysis software package (nSolver) for PC and Mac that further streamlines the QC and analysis steps. In addition, the ROSALIND Platform is available for intuitive data analysis in the cloud that can be easily shared and collaborated on.

Truly orthogonal validation technology

Because nCounter assays do not require reverse transcription, target amplification or library preparation, the nCounter is particularly well suited for orthogonal validation of candidate genes identified by sequencing, PCR, or array-based methods. An increasing number of studies employ the nCounter Analysis System for target validation (Sun et al., 2011; Smallridge et al., 2013; Bhargava et al., 2013; Sabo et al., 2014; Owonikoko et al., 2014; Graw et al. 2015).

Publications

The utility of the nCounter system is demonstrated in thousands of peer-reviewed publications, many in top journals. Visit www.nanostring.com/resources/publications to review the latest nCounter publications.

For more information, please visit nanostring.com

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