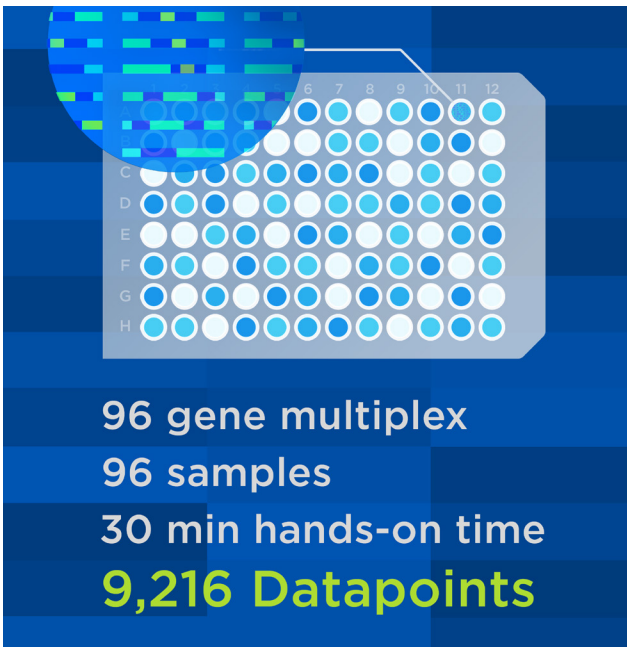
nCounter® PlexSet™ Reagents



Product Highlights

- Multiplex up to 96 custom probes across 96 samples to generate 9,216 data points per run
- Utilization of lysed cells eliminates the need for RNA purification and amplification
- Digital gene expression provides accurate reproducible results
- No upfront probe optimization required
- Only 30 minutes of hands-on time; no need for cDNA conversion*, amplification*, or replicates

** Single cell protocol requires amplification*

Multiplexing Re-imagined

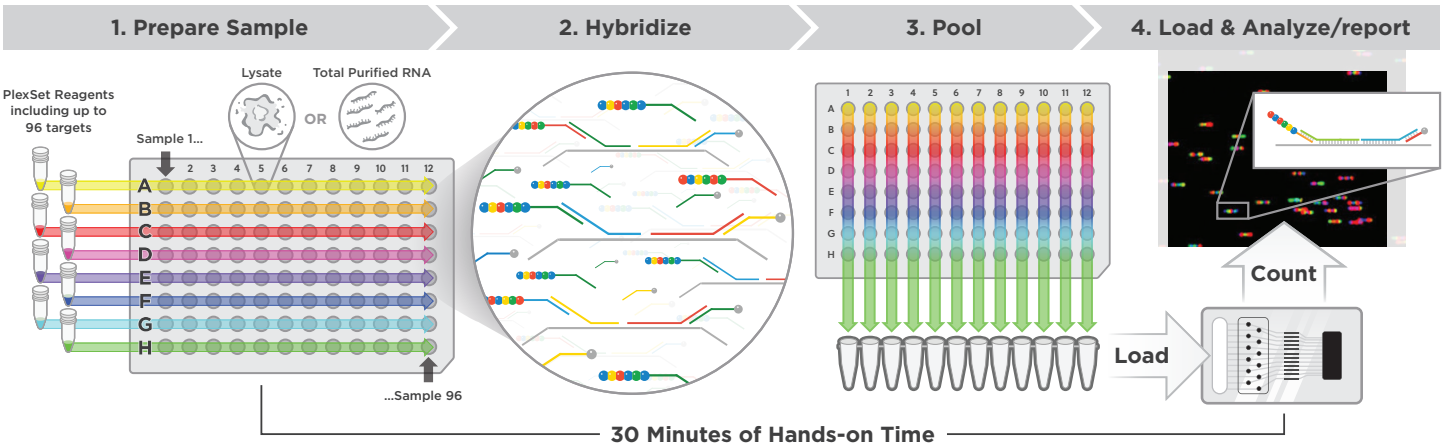
PlexSet technology enables multiplexed gene expression assays to be performed more efficiently and cost-effectively than ever before for projects ranging from 3 to 96 RNA targets. Unlike other gene expression technologies, PlexSet reagents enable researchers to reduce their hands-on time by eliminating the need for cDNA conversion, replicate utilization, or RNA purification. Similar to other nCounter assays, Plexset reagents provides a simple and robust method for multiplexing targets without the need to optimize probes or amplification conditions. The PlexSet reagents are based on proven nCounter technology utilizing molecular barcodes for highly multiplexed digital analysis.

By enabling up to 8 samples to be processed in a single lane on the nCounter cartridge, PlexSet reagents increase sample-throughput 8-fold over standard custom CodeSets. PlexSet reagents are compatible with a wide range of sample types including total purified RNA from FFPE samples and cell lysates. They are suitable for a wide variety of project types and can be used in any biological system where gene expression analysis is of interest. NanoString's bioinformatics team designs custom probes based on gene-lists defined by the end-user or select from ~400 preselected pathway panels for varied biology and pathways. Widespread applications include cell screening, biomarker validation, drug screening, RNAi and CRISPR hit validation and phenotypic functional testing.

PlexSet Regent Details

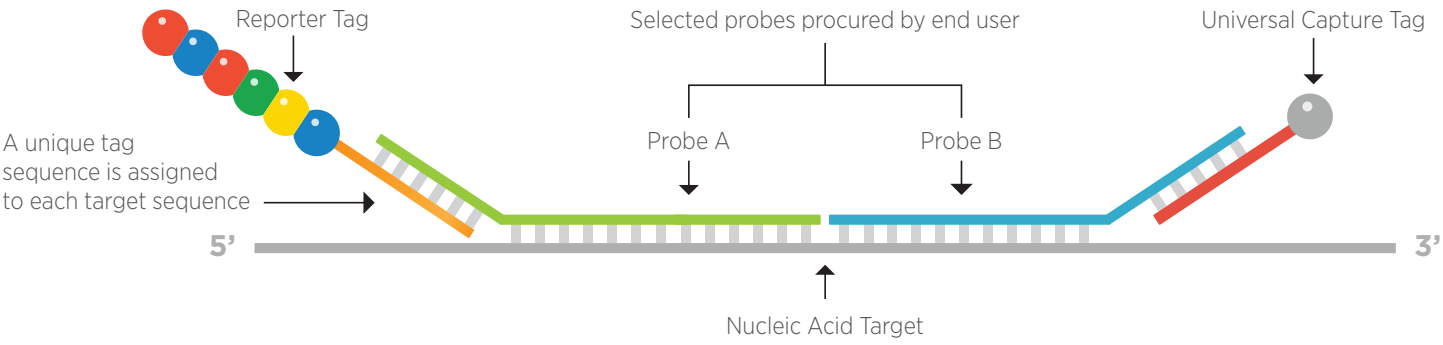
Minimum Input Material	50 ng of total purified RNA or 5,000 cells or 1 ng of RNA input with amplification
Hands-on Time	Approximately 30 minutes
Time to Results	24 Hours
Sample Types	• Total purified RNA (cells or tissues) • Cell lysates • Fresh frozen or FFPE-derived RNA • Amplified RNA (single cell)
Data Analysis	nSolver™ Analysis Software (Research Use Only)

Figure 1: nCounter PlexSet workflow



Samples are prepared by either lysing the whole cells or by purifying the total RNA from a variety of sample types. Add up to 96 unique samples on a 96-well PCR plate. Add PlexSet A-H mixes containing the unique barcodes, hybridization buffer, internal reference controls, and oligos for up to 96 custom targets across each row. Hybridize the plate overnight. Then pool each row vertically into the strip tubes and load the assay in to the nCounter system to generate the digital counts. Utilize nSolver Analysis software to analyze the research results.

Figure 2: Probe design



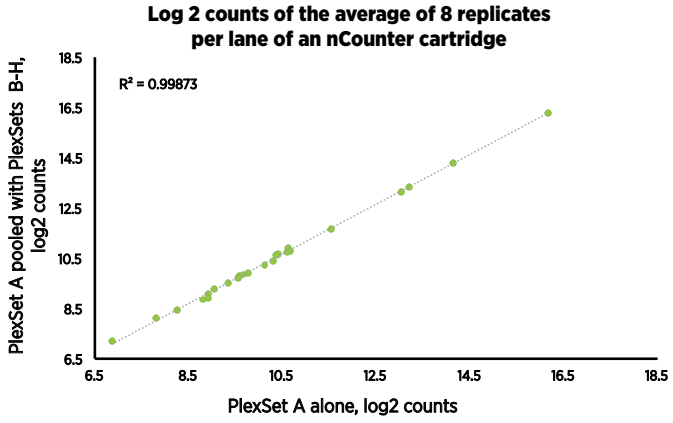
Unique probe design enables direct digital counting and multiplexing capabilities

Figure 2 showcases the unique probe design for the PlexSet technology. Probes include both target-specific and tag specific sequences. These probes, based on NanoString's patented technology, enable direct, digital counting of individual molecules with color-coded molecular barcodes via fluorescent microscopy and allow for high-precision counting of up to 96 target molecules across a sample set in one reaction. The PlexSet chemistry is uniquely optimized to allow for up to 8 samples to be analyzed per nCounter cartridge lane. With this sample multiplexing capability, PlexSet reagents easily allow for hundreds of samples to be processed on a single nCounter instrument per day with sensitivity and specificity equivalent to standard nCounter gene expression chemistries.

Data from multiplexed samples and samples processed independently are highly correlated

Figure 3 shows 96-plex data from pooled samples is equivalent to 96-plex data from samples run independently. Not only does this validate the quality of data from PlexSet reagents, it also means that data from past studies with standard nCounter chemistry can easily be compared to new data generated with PlexSet reagents.

Figure 3: High correlation between multiplex and single plex samples

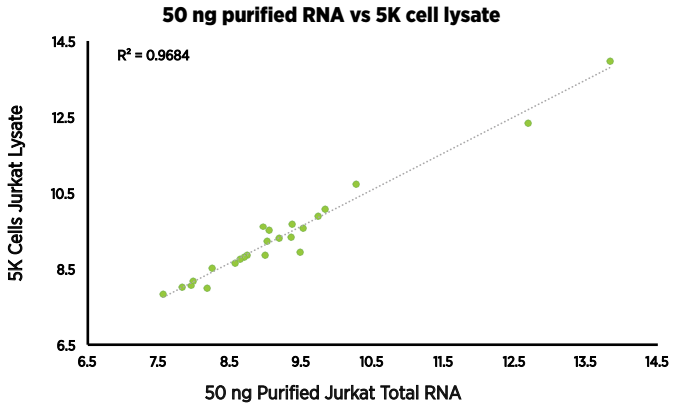


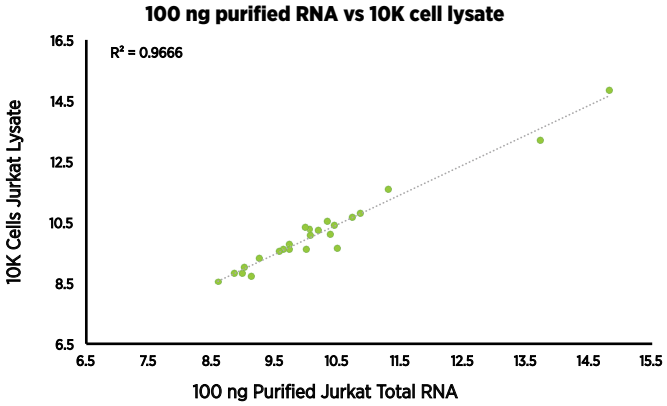
Lyse-and-Go protocol

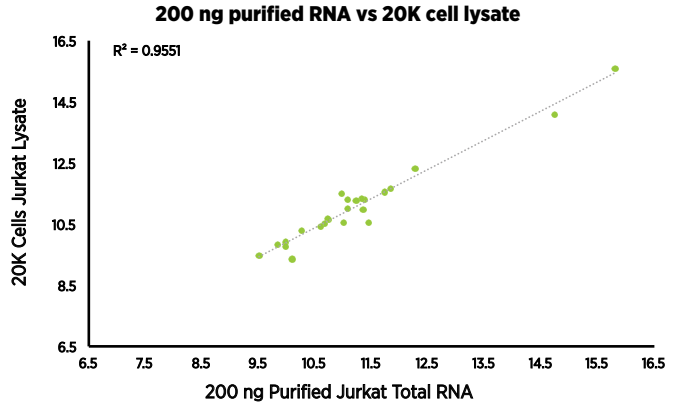
Directly use lysed cells as samples in the experiment to attain specific and sensitive gene expression analysis. The utilization of cell lysis helps researchers save time and resources. Figure 4 showcases three graphs demonstrating high concordance between 5,000-20,000 lysed cells and 50-200 ng total purified RNA from Jurkat cells using PlexSet technology.

Figure 4: High correlation between cell lysate and corresponding purified total RNA

Log 2 counts of the average of 8 replicates per lane of an nCounter cartridge







Preselected Pathway Panels

Expertly curated panels covering ~140 biological pathways and fields of interest for human, mouse, and rat samples. Each panel contains 90 genes chosen to comprehensively cover each pathway involved in the topic. All necessary controls and reference genes are included in each panel. Customize by adding additional genes of interest to the panels (or omitting genes that are not of interest). By utilizing PlexSet reagents with these panels up to 96 samples can be run in a single nCounter run. For more information, please visit: **www.nanostring.com/plexset**

Adherens Junctions	Cancer Driver Genes	Chromatin-remodeling Complexes	Fatty Acid Metabolism
Adipose Differentation and Maintenance	Cancer Stem Cell Pathways	Common Signaling Pathways	Fatty Liver Disease
Allergic Response	Cardiovascular Disease	Cytokines and Chemokines	Fibrosis
Alzheimer's Disease	CD4+ T-cell Differentation	Cytokines	Focal Adhesion Signaling
AMPK Pathway	Cell Cycle	Cytoskeleton Regulation	Gap Junctions
Androgen Receptor Target Genes	Cell Death	Detoxification	Glucocorticoid Signaling
Angiogenesis	Cell Migration	Diabetes	Glycosylation
Angiogenic Signaling	Cell Stress and Toxicity	DNA Damage Repair	GPCR Pathways
Antigen Presenting Cell	Cell-Matrix Interaction	DNA Damage Signaling	Growth Factors
Apoptosis	Cellular Basis of Aging	Drug Transporters	Hedgehog Signaling
Atherosclerosis	Cellular Senescence	EGF and PDGF Signaling	Hematopoiesis
Autoimmunity	Cellular Stress	Endothelial Cell Function	Hippo Pathway
Autophagy	Chemokine Signaling	Epithelial Cell Junctions	Huntington's Disease
Blood Pressure Regulation	Chemotherapy Resistance	Epithelial to Mesenchymal Transition (EMT)	Hypoxia Signaling
Breast Cancer	Cholesterol and Lipoprotein Metabolism	Essential Amino Acid Metabolism	Immune response to bacterial infection
Calcium Signaling	Chromatin-modifying Enzymes	Estrogen Receptor Signaling	Immune response to fungal infection

Preselected Pathway Panels (continued)

Immune Response to tumors	Lymphoma	Oncology Drug Targets	Stem Cell Transcription Factors
Immune response to viral infection	MAPK Pathway	Osmotic Stress	Stem Cells
Immune Tolerance	Mesenchymal Stem Cells	Osteogenesis	Telomere Replication and Maintenance
Immunotoxicity	Metastasis	Osteoporosis	TGF-b Pathway
Induced Plurpotent Stem (IPS) Cells	Mitochondrial biogenesis and function	Oxidative Stress	TGF-b Target Genes
Inflammasome Complexes and Regulation	Molecular Toxicology	p53 Pathway	Th1 and Th2 Responses
Inflammatory Cytokine Signaling	mTOR Pathway	Parkinson's Disease	Th17 Response
Insulin Signaling	Multiple Sclerosis	Phagocytosis	Tight Junctions
Interferon Signaling	Myc Target Genes	Phase II Drug Metabolism	TNF Signaling
IO Lymphocyte Activity	Necrosis	Phosphatases	Toll-Like Receptor Pathway
IO Myeloid Activity	Neurotransmitter Receptors	PI3K-Akt Pathway	Transcription Factors
Irritable Bowel Disease	Neurotrophin Signaling	Polycomb and Trithorax Complexes	Type I Interferon Signaling
Kidney Toxicity	NF-kB Pathway	Polycomb and Trithorax Target Genes	Type II Diabetes
Learning and Memory	NF-kB Target Genes	Prostate Cancer	Ubiquitination
Leukemia	Nitric Oxide Signaling	Protein folding	Unfolded Protein Response
Liver Cancer	Non-essential Amino Acid Metabolism	Retinoic Acid Signaling	VEGF Pathway
Liver Toxicity	Notch Pathway	Sepsis	WNT Pathway
Lung Cancer	Notch Target Genes	Skeletal Myogenesis and Myopathies	WNT Target Genes
Lymphocyte Activation	Obesity	Stem Cell Signaling	Wound Healing

Ordering Information

Product	Product Description	Catalog Number	Unit
nCounter PlexSet-12 Reagent Pack	For custom Gene Expression analysis utilizing 12 genes and 96 samples per nCounter run. Each pack of reagents is sufficient for 2 nCounter runs or 192 assays and requires 24 reaction Master kit. Tier discounting is available for purchase of 4 packs or more (768 samples or more). PlexSet purchases only—does not include Master Kits.	PS-012-GX-192S (CS0)	192 Assays
nCounter PlexSet-24 Reagent Pack	For custom Gene Expression analysis utilizing 24 genes and 96 samples per nCounter run. Each pack of reagents is sufficient for 2 nCounter runs or 192 assays and requires 24 reaction Master kit. Tier discounting is available for purchase of 4 packs or more (768 samples or more). PlexSet only—does not include Master Kits.	PS-024-GX-192S (CS0)	192 Assays
nCounter PlexSet-48 Reagent Pack	For custom Gene Expression analysis utilizing 48 genes and 96 samples per nCounter run. Each pack of reagents is sufficient for 1 nCounter runs or 96 assays and requires 12 reaction Master kit. Tier discounting is available for purchase of 8 packs or more (768 samples or more). PlexSet purchases only— does not include Master Kits.	PS-048-GX-96S (CS0)	96 Assays
nCounter PlexSet-72 Reagent Pack	For custom Gene Expression analysis utilizing 72 genes and 96 samples per nCounter run. Each pack of reagents is sufficient for 1 nCounter runs or 96 assays and requires 12 reaction Master kit. Tier discounting is available for purchase of 8 packs or more (768 samples or more). PlexSet purchases only— does not include Master Kits.	PS-072-GX-96S (CS0)	96 Assays
nCounter PlexSet-96 Reagent Pack	For custom Gene Expression analysis utilizing 96 genes and 96 samples per nCounter run. Each pack of reagents is sufficient for 1 nCounter runs or 96 assays and requires 12 reaction Master kit. Tier discounting is available for purchase of 8 packs or more (768 samples or more). PlexSet purchases only— does not include Master Kits.	PS-096-GX-96S (CS0)	96 Assays
nCounter PlexSet Titration Kit-12	Kit is set up to perform titration for 12-gene format prior to processing samples with PlexSet-12 reagents in 96 sample format. Only one kit is required for the entire project. CodeSet only—does not include Master Kits	PS-GX-PTK-12 (CS0)	24 Assays
nCounter PlexSet Titration Kit-24	Kit is set up to perform titration for 24-gene format prior to processing samples with PlexSet-24 reagents in 96 sample format. Only one kit is required for the entire project. CodeSet only—does not include Master Kits.	PS-GX-PTK-24 (CS0)	24 Assays
nCounter PlexSet Titration Kit-48	Kit is set up to perform titration for 48-gene format prior to processing samples with PlexSet-48 reagents in 96 sample format. Only one kit is required for the entire project. CodeSet only—does not include Master Kits.	PS-GX-PTK-48 (CS0)	24 Assays
nCounter PlexSet Titration Kit-72	Kit is set up to perform titration for 72-gene format prior to processing samples with PlexSet-72 reagents in 96 sample format. Only one kit is required for the entire project. CodeSet only—does not include Master Kits.	PS-GX-PTK-72 (CS0)	24 Assays
nCounter PlexSet Titration Kit-96	Kit is set up to perform titration for 96-gene format prior to processing samples with PlexSet-96 reagents in 96 sample format. Only one kit is required for the entire project. CodeSet only—does not include Master Kits.	PS-GX-PTK-96 (CS0)	24 Assays

For more information, please visit nanostring.com

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