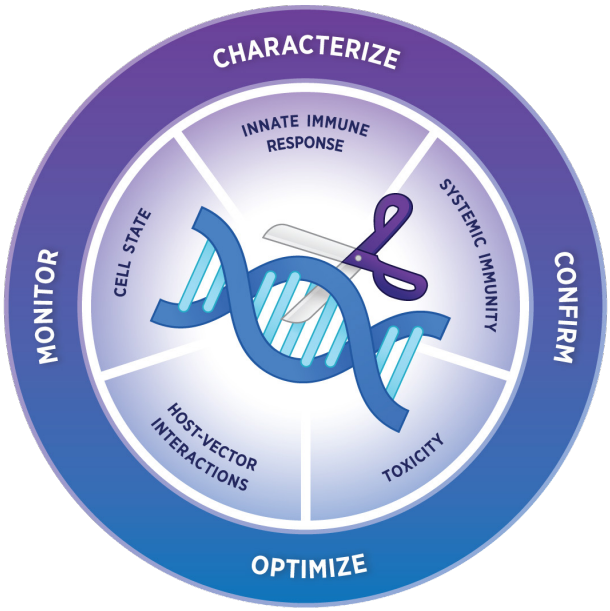
nCounter® Gene Therapy Optimization Panel

Gene Expression Panel

Vector Development • Viral Manufacturing • Treatment Monitoring

Explore innate and adaptive immune responses, and address critical questions related to host-vector interactions. Understand toxicities resulting from gene therapy treatments. Standardize the entire gene therapy process from vector development and viral manufacturing to post-treatment monitoring.



Product Highlights

- Directly profile 800 genes across 40 pathways
- Study processes known to impact gene therapy development and manufacturing
  - Cell State
  - Host-Vector Interactions
  - Innate Immune Response
  - Systemic Immunity
  - Toxicity
- Understand factors influencing optimal gene therapy development and manufacturing
- Monitor for toxicities
- Quantify the presence and relative abundance of different immune cell types present during therapy
- Customize to incorporate gene therapy specific biology, enabling parallel monitoring of therapy-based biology and host response biology
- Generate data in 24 hours with less than 30 minutes hands on time and simple data analysis

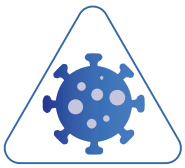
Feature	Specifications
Number of Targets	800 (Human and Mouse), including 12 internal reference genes for data normalization.
Sample Input - Standard (No amplification required)	25-300 ng
Sample Input - Low Input	As little as 1 ng with nCounter Low Input Kit and Primer Pools (sold separately)
Sample Type(s)	Blood, biopsies, xenografts, cultured cells/cell lysates, FFPE-derived RNA, total RNA, fragmented RNA
Customizable	Add up to 55 unique genes with Panel-Plus
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Analysis Software (RUO), Advanced Analysis for cell profiling, ROSALIND® platform

Gene Therapy Optimization Panel Applications Across Gene Therapy Development

Vector Development

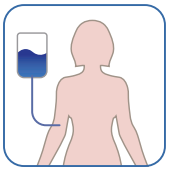
- How is the immune system responding?
- What host factors could influence effectiveness?
- Are there toxicities?

Viral Manufacturing



- What are the optimal conditions for viral manufacturing?
- What cell signaling events can be correlated with critical product qualities?
- What media conditions could enhance viral production?
- What is the optimal viral harvest time?
- Are there any off-target effects?

Treatment Monitoring



- Is the treatment working?
- Are there toxicities?
- How is the immune system responding?
- Are there any off-target effects?
- Are there any signs of adverse events?

Panel Themes

The Gene Therapy Optimization Panel includes annotations across 5 functional themes related to gene therapy development and manufacturing. Pathway coverage is outlined in the table below.

Systemic Immune Response	Innate Immunity	Cell State	Toxicity	Host Vector Interactions
• Chemokine Signaling • Cytotoxicity • IL-1,2, 6, 17 Signaling • Immune Suppression Targets • JAK-STAT Signaling • Leukotriene and Prostaglandin Inflammation • Lymphocyte Trafficking • MHC Class I, II Antigen Presentation • NF-kappaB Signaling • Other Interleukin Signaling • T-cell Costimulation • TNF Signaling • Type II Interferon Signaling	• ALPK1 Signaling • Complement System • DNA Sensing • Glycan Sensing • Inflammasomes • Interferon Response Genes • NLR Signaling • Phagocytosis • RNA Sensing • TLR Signaling • Type I Interferon Signaling	• Autophagy • Glutamine Metabolism • Glycolysis & Glucose Transport • p53 Pathway • Senescence • Mitochondrial Metabolism	• Apoptosis • Oxidative Stress Response • Proteotoxic Stress • Tissue Toxicity	• Restriction Factors • Vector Entry

Immune Cell Profiling

Genes included in the Gene Therapy Optimization Panel provide unique cell profiling data to measure the relative abundance of 14 different immune cell types. The table to the right summarizes the genes included in each cell type signature, as qualified through biostatistical approaches and selected literature in the field of immunology.

Cell Type	Associated Human Genes	Associated Mouse Genes
B Cells	BLK, CD19, FAM30A, FCRL2, MS4A1, PNOC, SPIB, TCL1A, TNFRSF17	Blk, Cd19, Fcrlb, Ms4a1, Pnoc, Spib, Tcl1, Tnfrsf17
CD45	PTPRC	Ptprc
CD8	CD8A, CD8B	Cd8a, Cd8b1
Cytotoxic Cells	CTSW, GNLY, GZMA, GZMB, GZMH, KLRB1, KLRD1, KLRK1, NKG7, PRF1	Ctsw, Gzma, Gzmb, Klrb1, Klrd1, Klrk1, Nkg7, Prf1
Dendritic Cells	CCL13, CD209, HSD11B1	Ccl2, Cd209e, Hsd11b1
Exhausted CD8	CD244, EOMES, LAG3, PTGER4	Cd244a, Eomes, Lag3, Ptger4
Macrophages	CD163, CD68, CD84, MS4A4A	Cd163, Cd68, Cd84, Ms4a4a
Mast Cells	CPA3, HDC, MS4A2, TPSAB1/B2	Cpa3, Hdc, Ms4a2, Tpsab1, Tpsb2
Neutrophils	CEACAM3, CSF3R, FCAR, FCGR3A/B, FPR1, S100A12, SIGLEC5	Ceacam3, Csf3r, Fcgr4, Fpr1
NK Cells	NCR1, XCL1/2	Ncr1, Xcl1
NK CD56dim Cells	IL21R, KIR2DL3, KIR3DL1/2	Il21r, Kir3dl1/2
T Cells	CD3D, CD3E, CD3G, CD6, SH2D1A, TRAT1	Cd3d, Cd3e, Cd3g, Cd6, Sh2d1a, Trat1
Th1 Cells	TBX21	Tbx21
Tregs	FOXP3	Foxp3

Customization with Panel Plus

Customize your research project by adding up to 55 user-defined genes of interest with nCounter Panel Plus. Panel Plus capacity enables researchers to address content specific to their research areas of interest. Incorporate gene therapy specific biology to monitor therapy-based biology and host response biology in parallel. Measure unique viral vector expression or perform viral vector optimization.

nCounter® Pro Analysis System

The nCounter® Pro Analysis System provides a cost-effective automated solution for multiplex expression analysis of 800+ targets. The simple workflow requires just 15 minutes hands-on time and produces highly reproducible data in ~24 hours. No RT, amplification or technical replicates are required.

- Simple, streamlined, and automated workflow
- Exceptional reproducibility and performance
- Advanced cybersecurity controls
- Extensive panel menu
- Flexible custom solutions

nSolver™ Analysis Software

NanoString offers advanced software tools that address the continuous demands of data analysis and the need to get simple answers to specific biological questions easily. Genes included in the Gene Therapy Optimization Panel are annotated to allow for efficient analysis of relevant pathways.

Analysis Modules available for Gene Therapy Optimization:

- Normalization
- Quality Control
- Individual Pathway Analysis
- Cell Profiling
- Differential Expression
- Gene Set Analysis
- Built-in compatibility for Panel Plus and Protein analysis

ROSALIND® Platform

ROSALIND is a cloud-based platform that enables scientists to analyze and interpret differential gene expression data without the need for bioinformatics or programming skills. ROSALIND makes analysis of nCounter data easy, with guided modules for:

Normalization / Quality Control / Individual Pathway Analysis Differential Expression / Gene Set Analysis \nCounter customers can access ROSALIND free of charge at https://www.rosalind.bio/nanostring

ROSALIND

Ordering Information

Gene Expression Panels arrive ready-to-use and generally ship within 24 hours following purchase.

Product	Product Description	Quantity	Catalog Number
nCounter® Human Gene Therapy Optimization Panel	800 genes, including 12 internal reference genes for data normalization. Codeset Only.	12 Reactions	XT-HSGTO-12
nCounter® Mouse Gene Therapy Optimization Panel	800 genes, including 12 internal reference genes for data normalization. Codeset only.	12 Reactions	XT-MSGTO-12
nCounter® Human Gene Therapy Optimization Panel Standard	Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the unique probe targets in the panel.	12 Reactions	PSTD-H-GTO-12
nCounter® Human Gene Therapy Optimization Primer Pool	Primer pools for the Human Gene Therapy Optimization Panel (for use with the Low RNA Input Kit)	12 Reactions	LOW-H-GTO-12
nCounter® Mouse Gene Therapy Optimization Panel Standard	Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the unique probe targets in the panel.	12 Reactions	PSTD-M-GTO-12
nCounter® Mouse Gene Therapy Optimization Primer Pool	Primer pools for the Mouse Gene Therapy Optimization Panel (for use with the Low RNA Input Kit)	12 Reactions	LOW-M-GTO-12
Low RNA Input Kit	Kit for use with all Low RNA Input Primer Pools	48 Reactions	LOW-RNA-48
nCounter® Analysis System Master Kit	Reagents, cartridges, and consumables necessary for sample processing on the nCounter Analysis Systems	12 Reactions	NAA-AKIT-012
nCounter® SPRINT Cartridge 1 Cartridge, 12 lanes	Sample Cartridge for nCounter SPRINT System	12 Reactions	SPRINT-CAR-1.0
nCounter® SPRINT Reagent Pack	nCounter SPRINT Reagent Pack containing Reagents A, B, C, and Hybridization Buffer	192 Reactions	SPRINT-REAG-KIT

Selected Panel References

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3. Srivastava, A. In vivo tissue-tropism of adeno-associated viral vectors. *Curr Opin Virol* **21**, 75–80 (2016).
4. Colomer-Lluch, M., Ruiz, A., Moris, A. & Prado, J. G. Restriction Factors: From Intrinsic Viral Restriction to Shaping Cellular Immunity Against HIV-1. *Frontiers in Immunology* **9**, (2018).
5. Goldstein, R. A. Skeletal Muscle Injury Biomarkers: Assay Qualification Efforts and Translation to the Clinic. *Toxicol Pathol* **45**, 943–951 (2017).
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7. Roberts, R. A. *et a*l. Translational Biomarkers of Neurotoxicity: A Health and Environmental Sciences Institute Perspective on the Way Forward. *Toxicological Sciences* **148**, 332–340 (2015).
8. Kirmaier, A., Krupp, A. & Johnson, W. E. Understanding restriction factors and intrinsic immunity: insights and lessons from the primate lentiviruses. *Future Virol* **9**, 483–497 (2014).

For more information visit nanostring.com/GeneTherapyOptimization

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