# nCounter® Canine IO Panel
Gene Expression Panel

Comparative Oncology • Immune Response • IO Therapeutic Development

Covering hundreds of genes involved in the immune response of canines to immuno-oncology therapeutics and designed with experts in canine comparative oncology, the nCounter Canine IO Panel is the only gene expression panel built to study the tumor and immune response to IO treatments in dogs. The panel takes advantage of tumor-specific coverage for the most common canine cancers to enable researchers to share data and access enhanced statistical power, potentially improving clinical trial success by translating findings from the treatment of spontaneous canine cancer to humans.
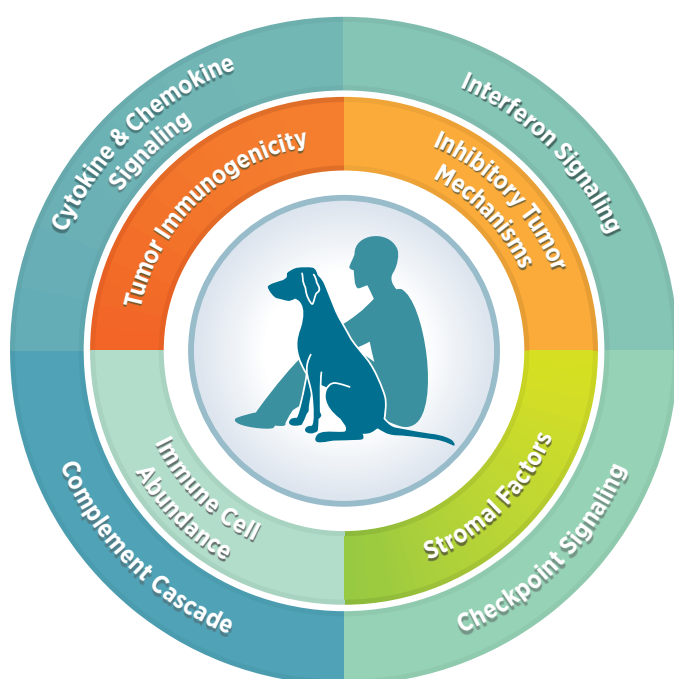
Product Highlights

- Profile 800 genes across 47 annotated pathways involved in canine immune response to IO treatments

- Overlapping content with NanoString's Human PanCancer IO 360™ and PanCancer Immune Profiling Panels provides a suite of panels for comparative studies

- PanCancer coverage with tumor specific content for top canine cancers including Melanoma, Osteosarcoma, Lymphoma, Urothelial Carcinoma, and Glioblastoma

- Easy to use nCounter system provides data in 24 hours with less than 30 minutes hands on time and simple data analysis

- Easy data sharing and collaboration with a common set of genes and a panel standard

Feature	Specifications
Number of Targets	800 (Canine), including internal reference genes
Sample Input - Standard (No amplification required)	25-300 ng
Sample Input - Low Input	As little as 1 ng with nCounter Low Input Kit (sold separately)
Sample Type(s)	Cultured cells/cell lysates, sorted cells, FFPE-derived RNA, total RNA, fragmented RNA, PBMCs, and whole blood/plasma
Customizable	Add up to 55 unique genes with Panel Plus
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Analysis Software (RUO) and the ROSALIND® Platform

Canine Comparative Oncology

One of the top challenges in cancer immunotherapy is the development of pre-clinical models that translate well to human immunity.[2] Many clinical trials for cancer fail due to efficacy and safety concerns not detected in preclinical studies with rodents. This is in part due to dissimilarities in the development of the immune system in rodents versus humans. Poor preclinical models mean that many crucial research questions are not addressed until human clinical trials, leading to high failure rates and increased development costs.

Comparative canine oncology is helping close this gap. Canines develop many tumors spontaneously, and the pathogenesis of canine cancers is comparable to that of human cancers. Unlike rodents, the development of the canine immune system is like that of humans. In fact, many drugs developed for humans are also effective in canines.

Drawing upon expertise in the fields of Immunology and Oncology, NanoString collaborated with a team of Veterinary Oncologists to select gene content for the Canine IO panel. Although the panel is built specifically for canines, relevant human gene content was included to allow for comparative studies.

Probe Design and Breed Compatibility

NanoString has successfully designed thousands of canine gene expression probes for custom codeset users and has deep expertise in optimizing probe designs across species. An important consideration when designing the Canine IO Panel was the selection of the reference sequence for the probes and its compatibility across a variety of breeds. Breeds particularly susceptible to cancer include, but are not limited to, the Rottweiler, Bernese Mountain Dog, Bouvier des Flandres, German Shepherd, Great Dane, Labrador Retriever, Bichon Frise, Boxer, and Golden Retriever. Each organism has a standard reference sequence, and in the case of domestic canines *(Canis lupus familiaris)*, the reference was based on an individual dog, Tasha the Boxer. For the Canine IO Panel, the Boxer Reference Sequence was considered in the context of a multi-breed whole genome sequence analysis and selected as the reference transcriptome for probe design due to its high frequency of use in the community and confidence in compatibility across all breeds.

Boxer Reference Sequence

- The Boxer was the first dog to have its genome fully sequenced in 2004.

- The sequencing was conducted by a consortium led by Kerstin Lindblad-Toh of the Broad Institute.[†]

- The Boxer was chosen after analyses of 60 dog breeds due to the low amount of variation in its genome.

- A first draft of the dog genome was deposited into free NIH databases for public use in July 2004.[†]

- The NCBI reference genome used for the Canine IO Panel design was CanFam3.1

Analysis of Cross-Breed Probe Design Compatibility

- Plassais et al performed whole genome sequencing of 144 breeds, 54 wild canids, and 100 village dogs.

- Only 91 million variants were found by Plassais et al, and of those 1% were in exons.

- nCounter is robust to at least 5% sequence variability without any alteration in hybridization.

- No issues are therefore anticipated with using canine as a reference for other breeds.

Reference: [†] https://www.genome.gov/12511476/2004-advisory-dog-genome-assembled

The nCounter Canine IO Panel Annotations

The nCounter Canine IO Panel enables researchers to explore canine immune response to immune-oncology treatments.

Immune					Tumor		Microenvironment
Cytokine & Chemokine Signaling	Interferon Signaling	Checkpoint Signaling	Complement Cascade	Immune Cell Abundance	Tumor Immunogenicity	Inhibitory Tumor Mechanisms	Stromal Factors
305 Genes	47 Genes	67 Genes	33 Genes	52 Genes	74 Genes	94 Genes	101 Genes
Cytokine and Chemokine Signaling	Interferon Signaling	Costimulatory Signaling	Complement System		Antigen Presentation	Epigenetic Regulation	Angiogenesis
					DNA Damage Repair	Hypoxia	Matrix Remodeling and Metastasis
						TGF-beta Signaling	
						Wnt Signaling	

Immune Cell Profiling Feature

Genes included in the Canine IO Panel provide unique cell profiling data to measure the relative abundance of 14 different immune cell types. The table below summarizes each cell type represented by gene content in the panel, as qualified through biostatistical approaches and selected literature in the field of immunology. For more information on immune cell profiling please see the Danaher et. al. publication "Gene expression markers of Tumor Infiltrating Leukocytes."

Cell Type	Associated Canine Genes
B-cells	BLK, CD19, FCRL2, MS4A1, PNOC, SPIB, TNFRSF17
CD45	PTPRC
CD8 T Cells	CD8A, CD8B
Cytotoxic Cells	CTSW, GZMA, GZMB, GZMH, KLRB1, KLRD1, KLRK1, LOC102156836, NKG7, PRF1
Dendritic Cells	CCL13, CCL2, CD209, HSD11B1
Exhausted CD8	CD244, EOMES, LAG3, PTGER4
Macrophages	CD163, CD68, CD84, LOC476900

Cell Type	Associated Canine Genes
Mast Cells	CPA3, HDC, LOC100049001, MS4A2
Neutrophils	CSF3R, FCAR, LOC476396, LOC478984, S100A12
NK CD56dim cells	IL21R
NK cells	LOC490356, NCR1
T-cells	CD3D, CD3E, CD3G, CD6, SH2D1A, TRAT1
Th1 Cells	TBX21
Treg	FOXP3

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 easy. Genes included in the IO Canine Panel are organized and linked to various advanced analysis modules to allow for efficient data 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

Ordering Information

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

Product	Product Description	Quantity	Catalog Number
nCounter Canine IO Panel	Gene Expression CodeSet profiling (800 genes) 780 canine immune response genes + 20 internal reference controls	12 Reactions	XT-CIO-12
nCounter Canine IO Panel Standard	Standard containing a pool of synthetic DNA oligonucleotides that correspond to the target sequence of each of the 800 unique probe targets in the panel.	12 Reactions	PSTD-CIO-12
nCounter Canine IO Primer Pool	Primer pools for Low Input RNA profiling of 780 canine genes + 20 internal reference controls. Master Kit, RNA Low Input Kit & Panel CodeSet Required	12 Reactions	LOW-CIO-12
nCounter Analysis System Master Kit Reagents and Cartridges	Reagents, cartridges, and consumables necessary for sample processing on the nCounter Analysis System	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
Low Input RNA Reagent Kit	48 rxn kit for profiling from low sample input amounts	48 Reactions	LOW-RNA-48

Selected Panel References

1. Canter RJ et al. Characterization and Potential Applications of Dog Natural Killer Cells in Cancer Immunotherapy. Journal of Clinical Medicine. 2019; 8, 1802.

2. Chen D et. al. Top 10 Challenges in Cancer Immunotherapy. Immunity. 2020; 52 (1), 17-35.

3. Danaher P et al. Gene expression markers of Tumor Infiltrating Leukocytes JITC 2017

4. Plassais J et al. Whole Genome Sequencing of Canids Reveals Genomic Regions Under Selection and Variants Influencing Morphology. Nature Communications. 2019; 10:1489.

For more information, please visit nanostring.com

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