# Deciphering the complexities of neurodegeneration and neuroinflammation with NanoString® gene expression profiling

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## Abstract

Neurodegenerative diseases represent a growing health concern and economic burden as the aging population increases and disease rates soar. Although early diagnosis and treatment have remained elusive, much progress has been made by applying molecular approaches, notably gene expression and proteomic profiling, to advance our understanding of disease at a mechanistic level.

To address the growing need for biomarkers, gene expression signatures, and novel drug targets in neurological disease, NanoString has collaborated with leaders in the field to develop novel and powerful gene expression tools. These tools bring the robustness and simplicity of the nCounter® system along with its expertly curated and data-driven panel development approach to areas such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), neuropathic pain, traumatic brain injury (TBI), and infections of the CNS.

The nCounter Neuropathology and Neuroinflammation gene expression panels were used to detect disease specific changes in gene expression within fresh frozen, formalin-fixed, paraffin-embedded (FFPE), and blood. Our results show robust performance regardless of sample type or post-mortem interval (PMI) and high concordance between fresh frozen and fixed samples. Marked changes in gene expression within key pathways were observed between normal and diseased patients, correlated with disease progression. Finally, our cell type profiling analysis enabled the measurement of changes in cell composition within specific regions of the CNS over various stages of disease. All data was generated in less than 24 hours from purified RNA to results using the nCounter Analysis System.

NanoString's goal is to bring the advanced multiplexed molecular profiling tools that have accelerated the field of cancer drug development including pathway mapping, predictive biomarkers, biology subtyping, mixed-cell deconvolution and others to help lead transformational progress in the important field of neuroscience.

## nCounter workflow

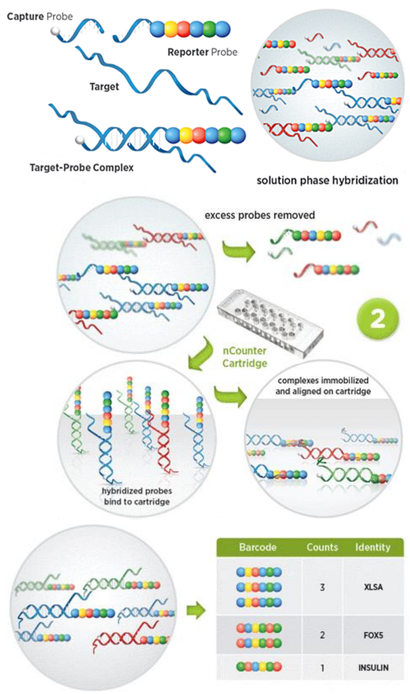
NanoString's nCounter Analysis System performs a highly multiplexed, digital quantification of up to 800 genes in a single reaction. This is achieved with the help of reporter codesets, which are color-coded "barcodes" specific for each gene.

Workflow consists of three major steps:

**1 Hybridization**
In the hybridization step, sample material is mixed with excess codeset and the codeset hybridizes to the mRNA target in solution.

**2 Purification**
Purification is carried out robotically which removes excess codeset and immobilizes the codeset/RNA complexes in the nCounter cartridge for data collection.

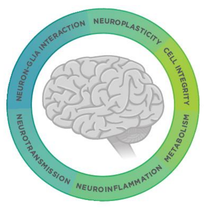
**3 Digital imaging**
CCD capture technology is used for data collection and digital images are processed and reporter probe counts are tabulated for data analysis using NanoStrings nSolver™ analysis software and advanced analysis modules.




| Feature | Specifications |
|---|---|
| Number of Targets | 770 (Human), 770 (Mouse) including internal reference genes |
| Standard Input Material (No amplification required) | 25 ng–300 ng |
| Low Input Material | As little as 1 ng with nCounter RNA Low Input Kit and Panel specific primer pools (sold seperately) |
| Sample Type(s) | FFPE-derived RNA, total RNA, fragmented RNA, PBMCs, whole blood/plasma, IPS cells |
| Customizable | Add up to 30 unique genes with Panel-Plus |
| Time to Results | Approximately 24 hours |
| Data Analysis | nSolver™ Analysis software |

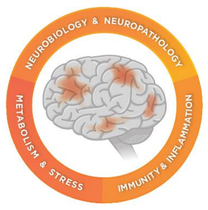
## Panel content and design

**nCounter Neuropathology Panel**

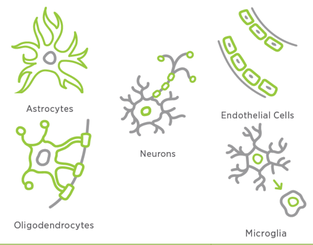



**Human & Mouse Panels**
**770 Genes**
**>20 Pathways**
**Cell Type Profiling**

**nCounter Neuroinflammation Panel**




**CNS-specific Cell Type Profiling**




**Peripheral Immune Cell Profiling**

| | |
|---|---|
| B-cells | Mast cells |
| $CD45^+$ cells | Neutrophils |
| CD8 T cells | NK CD56dim cells |
| Cytotoxic cells | NK cells |
| Dendritic cells | T-cells |
| Exhausted CD8 | $T_H1$ cells |
| Macrophages | $T_{reg}$ |

| | | | |
|---|---|---|---|
| Axon and Dendrite Structure | Compartmentalization & Structural Integrity | Adaptive Immune Response | Immunity & Inflammation |
| Neural Connectivity | | Cytokine Signaling | |
| Neuronal Cytoskeleton | | Inflammatory Signaling | |
| Tissue Integrity | | Innate Immune Response | |
| Autophagy | Metabolism | Microglia Function | |
| Carbohydrate Metabolism | | NF-kB | |
| Lipid Metabolism | | Autophagy | Metabolism & Stress |
| Oxidative Stress | | Carbohydrate Metabolism | |
| Transcription and Splicing | | Cellular Stress | |
| Unfolded Protein Response | | Lipid Metabolism | |
| Activated Microglia | Neuroinflammation | Angiogenesis | Neurobiology & Neuropathology |
| Cytokines | | Apoptosis | |
| Matrix Remodeling | | Astrocyte Function | |
| Myelination | Neuron-Glia interaction | Cell Cycle | |
| Trophic Factors | | DNA Damage | |
| Angiogenesis | Neuroplasticity, Development & Aging | Epigenetic Regulation | |
| Apoptosis | | Growth Factor Signaling | |
| Chromatin Modification | | Insulin Signaling | |
| Growth Factor Signaling | | Matrix Remodeling | |
| Transmitter Release | Neurotransmission | Neurons and Neurotransmission | |
| Transmitter Response and Reuptake | | Notch | |
| Transmitter Synthesis and Storage | | Oligodendrocyte Function | |
| Vesicle Trafficking | | Wnt | |

## Methods

Human and mouse samples obtained from the University of Pennsylvania Center for Neurodegenerative Disease Research were tested for use with the nCounter Human and Mouse Neuropathology Panels and analyzed with the nSolver Advanced Analysis 2.0 software.

Sample Types:
Mouse:
Whole brain hemispheres lacking cerebellum from 9-12 month old 5XFAD mice and wild-type littermate controls.

Human:
Alzheimer's disease: frontal cortex, hippocampus & visual cortex
Parkinson's disease: midbrain & hippocampus
Frontotemporal dementia (FTD): frontal cortex & whole blood

Sample Input:
Mouse:
Purified RNA from fresh-frozen tissue (50 ng)

Human:
Purified RNA from fresh-frozen (100 ng), ethanol-fixed & FFPE brain tissue (100 ng, adjusted for DV200)
Purified RNA from whole blood (100 ng)

Analysis: data were analyzed using nSolver Advanced Analysis 2.0.

## Robust performance on a variety of sample types

### Excellent correlation between fresh frozen and FFPE specimens

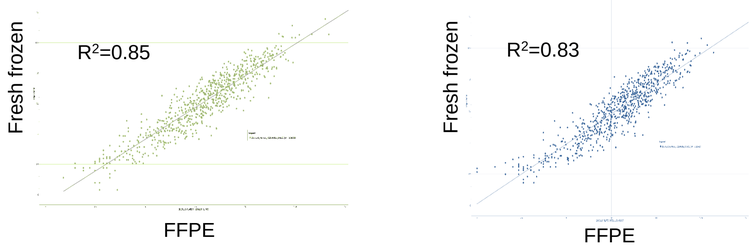



**FIGURE 1. Robust performance on FFPE tissue.** Patient-matched fresh frozen (FF) and formalin-fixed paraffin embedded (FFPE) samples were compared from 2 different patients. FF and FFPE samples in both cases were taken from opposite brain hemispheres. Top Left: hippocampus, low AD case. Top Right: Frontal cortex, high AD case.

### No effects of PMI on gene detection

### Robust performance in human blood

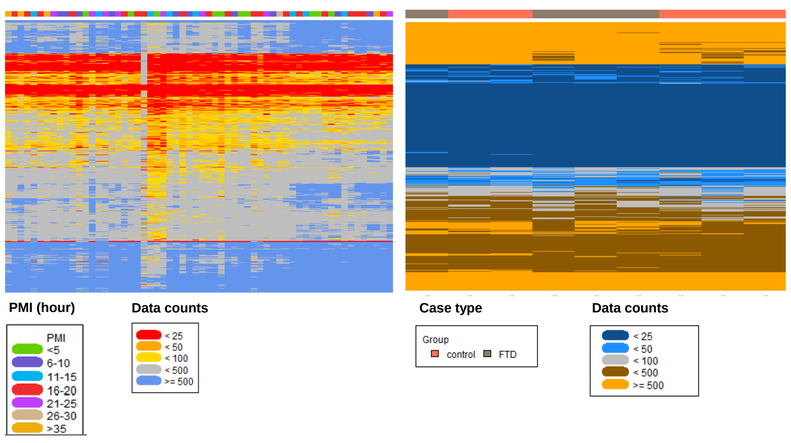



**FIGURE 2. NanoString platform performance in association with post-mortem interval (PMI) and in blood samples.** Left: Unbiased clustering reveals that samples do not cluster based on PMI. Robust counts can be seen in all PMI groups ranging from <5- >35 hours PMI. FFPE brain specimens. Right: Raw data obtained from whole blood samples demonstrate that 63% of all genes included in the neuropath panel were detected above limit of detection in blood samples from healthy control and frontotemporal dementia patients. n=5 controls, n=4 FTD patients.

### Work directly on cell lysates and crude FFPE extracts

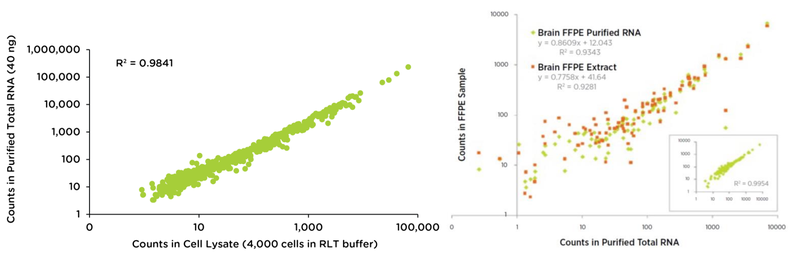



**FIGURE 3. Flexible sample input.**
Left: Multiplexed gene expression analysis on NanoString platform using 4000 cells lysed in RLT buffer (Qiagen) reveals strong correlation as compared to 40 ng purified total RNA. Right: Correlation between brain FFPE extract (orange) and brain FFPE purified RNA (green) to purified RNA from frozen brain tissue. (Inset) Correlation between brain FFPE extract and purified RNA from brain FFPE tissue.

## Distinguish between normal and disease state

### Mouse Neuropathology panel reveals robust genetic differences between wild-type mice and mice modeling Alzheimer's disease

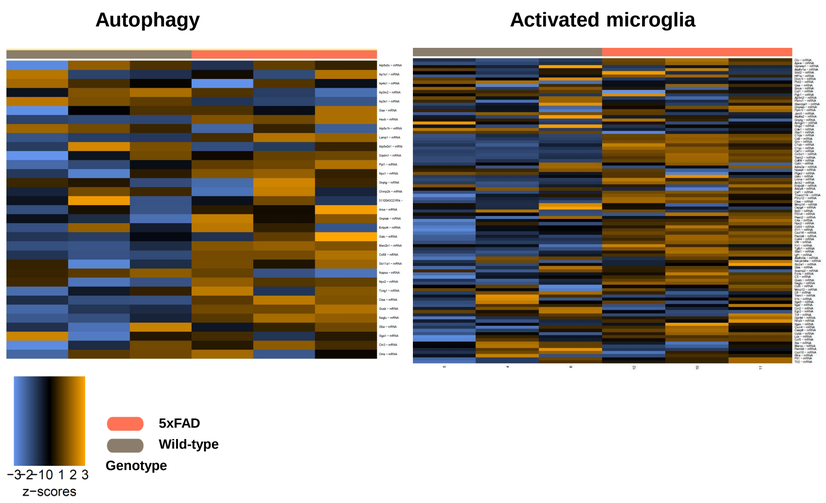



**FIGURE 4. Panel content distinguishes control from diseased tissue.** Unbiased clustering reveals robust differential expression for autophagy and activated microglia pathways. (left) 5XFAD mouse model of Alzheimer's disease show increased autophagy. (Right) Increased microglia activation in 5xFAD mice compared to wild-type controls. N=3 mice per group.

## Assess pathway dysregulation and monitor disease progression

### Alzheimer's-associated neurotransmission deficits

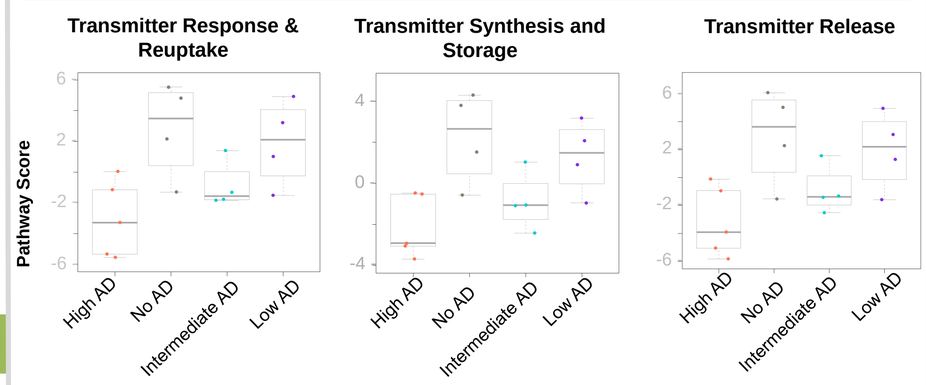



**FIGURE 5. Conserved disturbance in neurotransmission pathway scores.** Pathway score using nSolver Advanced Analysis software reveals decreased score in association with High AD for transmitter response and uptake (left), transmitter synthesis and storage (center), and transmitter release (right). Progressive changes are evident in accordance with disease severity.

### Conserved changes in AD and PD

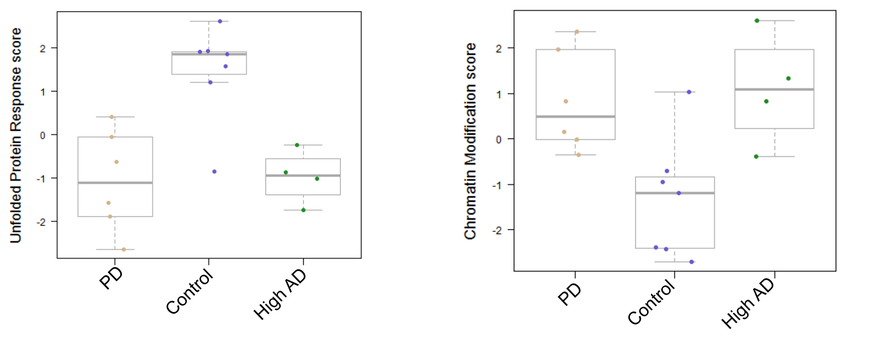



**FIGURE 6. Conserved changes in AD and PD pathway scores.** AD hippocampus and PD hippocampus in late stage of disease are compared to control patients. Left: Unfolded protein response pathway score is reduced in both AD and PD. Right: Chromatin modification pathway score is elevated in both AD and PD.

### Monitoring disease progression in the AD hippocampus

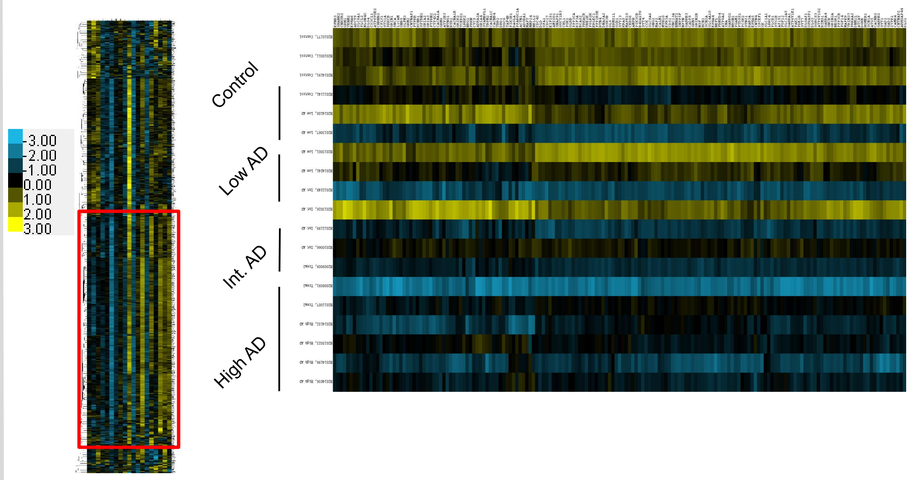



**FIGURE 7. Neuropathology panel content includes relevant genes that are altered in accordance with disease progression.** Rapid identification of a subset of genes that change in accordance with disease progression: highest expression seen in control patients with a progressive increase corresponding to increasing disease severity. Hippocampal FFPE specimens from high AD; Intermediate AD; low AD and normal controls; ABC neuropathological staging criteria.

## Integrated content and analysis enable neuronal and immune cell type profiling

### Cell profiling in the brain confirms neuropathology

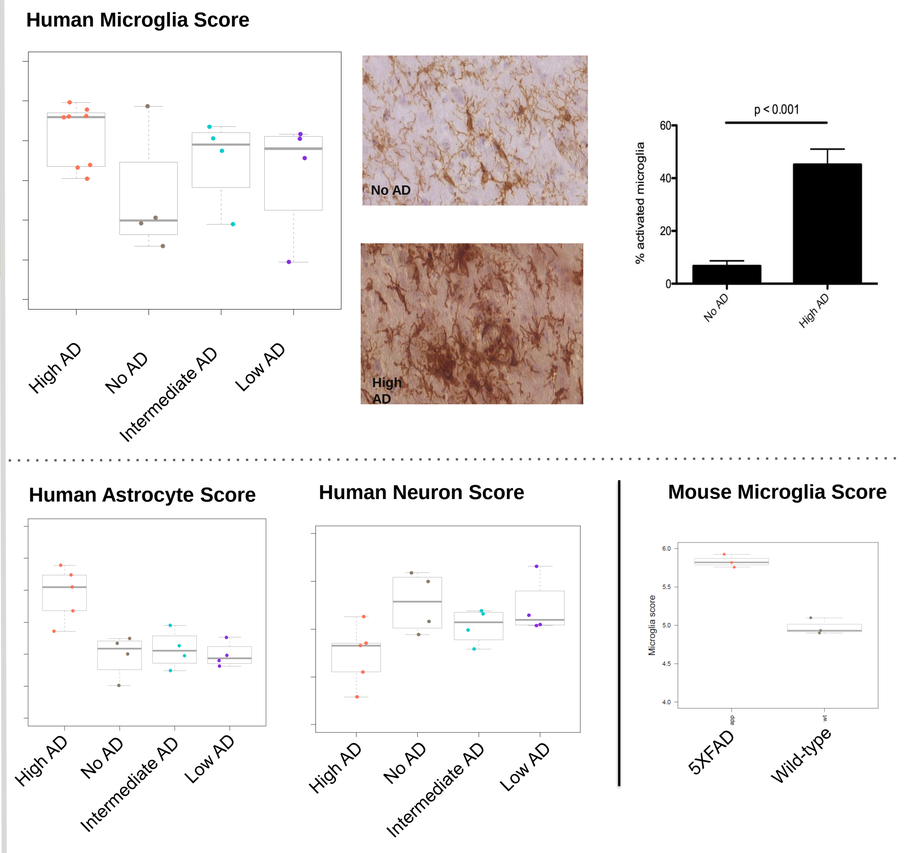



**FIGURE 8. Neural cell profiling.** Top Left: Cell type profiling using nSolver Advanced Analysis software reveals increased microglia score in association with High AD. Top Right: Iba1 immunohistochemistry demonstrates microglia activation in the human AD hippocampus. Quantification reveals a significant increase in activated microglia correlating with cell profiling data. Calculated as percent of total microglia. Student's t test. Bottom Left: Cell profiling in AD cases reveals increased astrocyte scores and reduced neuron scores, consistent with known gliosis and neurodegeneration. Similarly, cell profiling analysis of 5XFAD mouse tissue reveals increased microglia scores in line with human data.

## Conclusion

- nCounter Analysis System and new Gene Expression panels provide researchers with a powerful yet simple tool for accelerating research in Neurodegenerative Diseases and Neuroinflammation.
- Data driven panel design and panel content developed to cover neurodegenerative diseases broadly, enable discovery of targetable biology that is consistent across diseases.
- nCounter gene expression panels show robust and reproducible performance on a variety of sample types, including human post-mortem tissue.
- Panel content allows rapid identification of biology that distinguishes between normal and disease state and with Mouse and Human versions the panels enable seamless translational research.
- Novel cell profiling feature enables cell type profiling for key neuronal and immune cell types, measuring relative abundance.
- Integrated panel content and advanced analysis modules allow for rapid and deep data insights.

## Acknowledgements

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