

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

### Introduction

The diagnosis and treatment of neurodegenerative and other neurological disorders is hampered by the difficulty in accessing affected areas of the brain. Disease diagnoses are often based on post-mortem confirmation of disease pathology or medical imaging during disease progression. While early diagnosis and treatment remain elusive, major strides forward are possible with advanced molecular biology methods such as gene expression analysis and profiling.

These advanced techniques, however, are hampered by large sample input requirements, a lack of robust data analysis pipelines, and poor reproducibility with challenging sample types. Highly sensitive and robust technology beyond traditional methods are necessary to accurately analyze brain tissue (fresh frozen [FF] or formalin-fixed paraffin-embedded [FFPE]), blood samples, or cerebrospinal fluid (CSF) to detect disease specific changes in gene and protein expression, cell composition, and cell function.

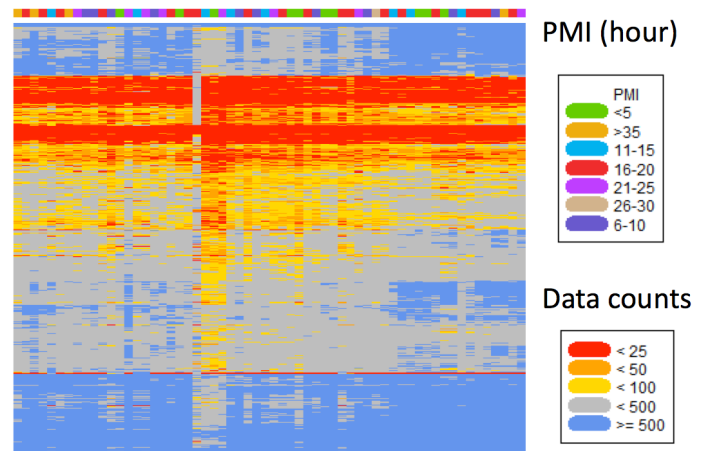
To address this challenge, NanoString developed the nCounter® Neuropathology and Neuroinflammation Gene Expression Panels that feature expertly curated and annotated gene content specific to neurodegeneration and neuroinflammation. Developed for research of neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, neuropathic pain, traumatic brain injury, and CNS infections, the panels allow comprehensive 770-plex gene expression analysis of relevant pathways, processes, and cell types with nSolver™ Analysis software.

In this application note, we demonstrate robust and reproducible results from limited and challenging sample types and investigate changes in immune and neuronal cell composition and function in AD and PD patient samples and mouse models.

### Results

#### Robust performance regardless of post-mortem interval or sample type

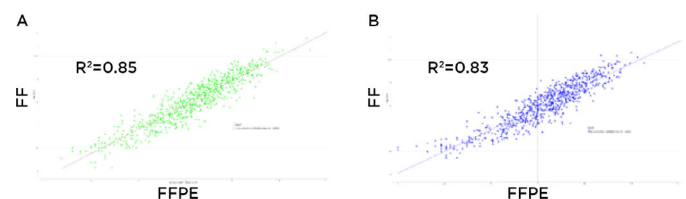
The use of post-mortem tissues from autopsies plays an important role in the research of neurological disorders.<sup>1</sup> However, collection of such tissue is invariably associated with a time delay, or post-mortem interval (PMI). As the PMI varies between samples and is known to impact RNA integrity and resulting gene expression,<sup>2</sup> we used the Neuropathology Panel to assess NanoString performance across FFPE brain specimens from multiple brain regions, including midbrain, frontal cortex, visual cortex, and hippocampus. Samples were obtained from AD and PD patients and normal controls. No clustering was observed based on PMI with robust gene expression detection in all PMI groups (Figure 1). These results indicate that the PMI does not affect NanoString gene expression data.



**FIGURE 1** Neuropathology Panel gene detection across various PMI.

FFPE tissue was obtained from AD and PD patients and normal controls from multiple brain regions. No clustering based on PMI was observed.

In addition to PMI, samples are often prepared or preserved with a variety of methods, specifically FF and FFPE. To confirm robust performance with these sample types, gene expression was measured on matched FF and FFPE samples from two human samples. High correlation was observed between FF and FFPE from a low AD hippocampal sample ( $R^2=0.85$ , Figure 2A) and a high AD frontal cortex sample ( $R^2=0.83$ , Figure 2B).



**FIGURE 2** Correlation between FF and FFPE from AD patients.

High correlation was observed between FF and FFPE from (A) a low AD hippocampus sample and (B) a high AD frontal cortex sample using the Neuropathology Panel.

As opposed to post-mortem FF or FFPE samples, analysis of blood or CSF permit studies on living subjects. We also tested the performance of the Neuropathology Panel on peripheral blood samples from five healthy control and four frontotemporal dementia (FTD) patients. Robust performance was observed with 63% (486/770) of all genes in the panel above the limit of detection, which is as expected considering many that many panel targets are CNS tissue specific.

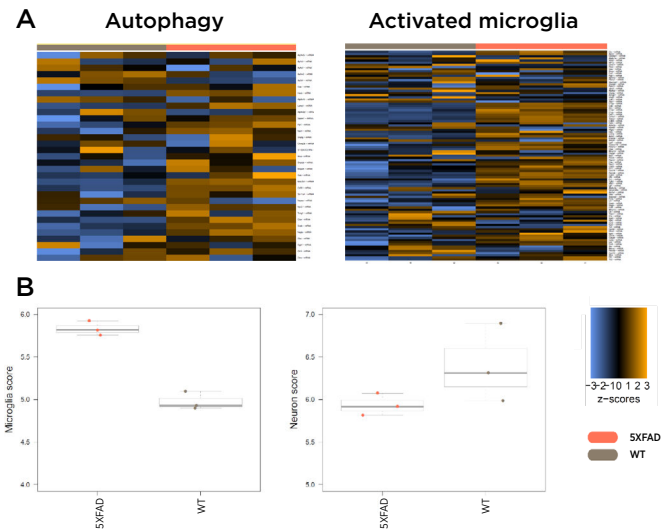
Together, this data confirms compatibility of NanoString gene expression profiling with the most common sample types acquired in neuroscience research.

## Deciphering the complexities of Neurodegeneration and Neuroinflammation with NanoString gene expression profiling

### Gene and cell profiling in a murine AD model and post-mortem brain

Disruption of cellular homeostasis is observed in the hippocampus of AD patients.<sup>9</sup> With performance well validated, the Neuropathology Panel was first used to interrogate genetic and cellular changes in whole brain samples from Wild Type (WT) and 5x familial AD (5XFAD) mice, which recapitulate the major amyloid pathology features of AD.<sup>3</sup> Compared with WT mice, 5XFAD mice exhibited increased autophagy and microglia activation (Figure 3A). Consistent with increased microglia activation, a nearly 2-fold increase in microglia cells was observed in 5XFAD mice (Figure 3B, left). Reduced neurons were also seen in these mice (Figure 3B, right) confirming the known phenotype of this model.<sup>4</sup> These results are consistent with findings from human AD (Figure 4).<sup>5</sup>

Similar to the 5XFAD mice, we observed significantly increased microglia in high AD patient samples compared with normal controls and patient samples with less severe AD (Figure 4A). These results are consistent with microglia activation in pathological states<sup>9</sup> (Figure 3A,B) and are also consistent with Iba1 immunohistochemistry from the same specimens previously collected. Accordingly, increased expression of astrocyte markers is evident in high AD (Figure 4B). A reduction in neurons was also revealed in the high AD hippocampus (Figure 4C).

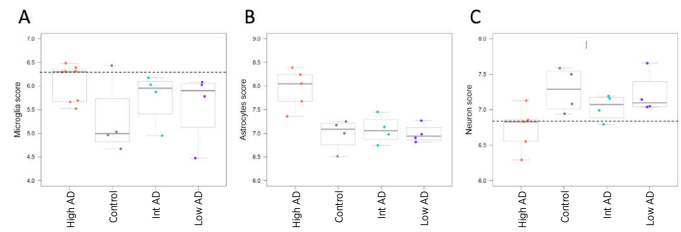


**FIGURE 3** Gene and cell profiling of 5XFAD mice.

Interrogation of whole brain hemisphere samples revealed increased autophagy (A, left panel) and activated microglia (A, right panel) in 5XFAD mice compared with WT mice. 5XFAD mice also exhibited an increased presence of microglia cells (B, left panel) and reduced neurons (B, right panel) when compared with WT mice.

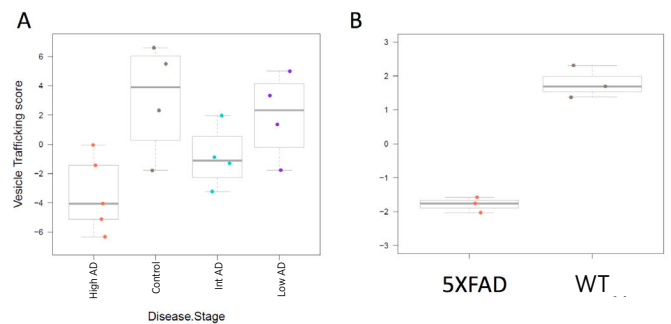
### Vesicle trafficking alterations in AD

In addition to alterations in immune cell composition, changes in vesicle trafficking are observed in both human AD and murine AD models. Profiling samples from high AD patients (staged according to the “ABC score”<sup>6</sup>), we observed reduced expression of genes involved in vesicle trafficking when compared with controls (Figure 5A). Reduced vesicle trafficking was also observed, to a lesser extent, in both low and intermediate AD patients (Figure 5A). A similar reduction was seen in whole brain samples from 5XFAD mice when compared with WT mice (Figure 5B).



**FIGURE 4** Relative abundance of key cell types involved in AD.

Neuropathology Panel interrogation of human FFPE samples from AD patients and normal controls revealed increased presence of (A) microglia and (B) astrocytes and (C) reduced presence of neurons. Dotted line indicates the mean value in high AD.



**FIGURE 5** Interrogation of vesicle trafficking in human AD and a murine AD model.

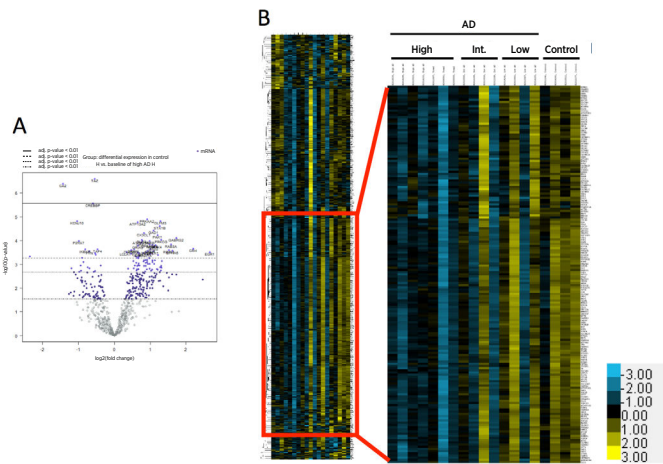
(A) The Neuropathology Panel revealed reduced expression of genes involved in vesicle trafficking in hippocampal samples from high AD patients when compared with controls and low/intermediate AD patients. (B) A similar reduction in gene expression in whole brain hemisphere samples from 5XFAD mice was seen compared to WT mice.

### Biomarkers of disease progression and neurotransmission deficits in AD

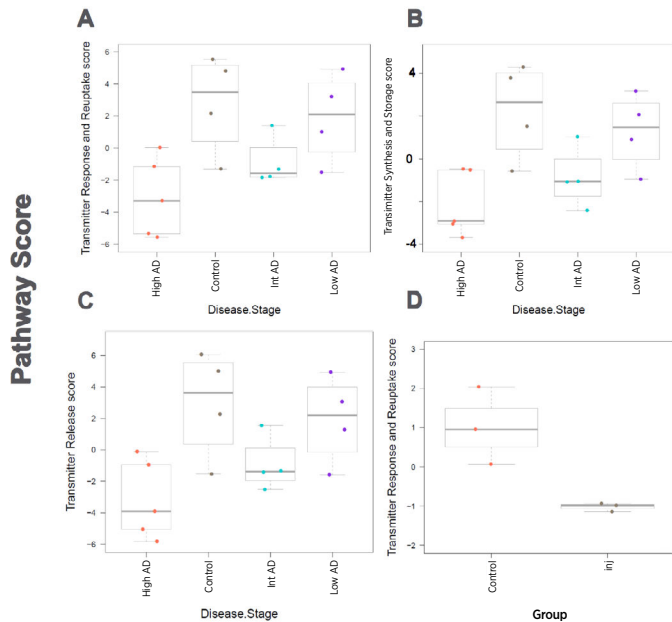
We next assessed the correlation between gene expression and disease progression in hippocampal FFPE samples from high AD patients and normal controls as the hippocampus is one region earliest affected by neuropathological hallmarks of AD.<sup>7</sup> Differentially expressed genes in the AD samples were observed when compared with normal controls (Figure 6A). We also identified a subset of genes that change in accordance with disease progression with highest expression in controls and a progressive decrease corresponding with increasing disease severity (Figure 6B). This broad profiling data highlights how the content in the Neuropathology panel can be used for biomarker discovery in addition to cell and functional profiling.

Deeper analysis of the hippocampal gene expression data with pathway scoring revealed conserved deficits in neurotransmission in samples from high AD patient samples compared with normal controls. Moderate deficits were visible in intermediate AD patient samples, while low AD patient samples were comparable to controls (Figure 7A-C). Consistent with the results obtained from human samples, interrogation of whole brain samples from 5XFAD mice injected with insoluble human tau recapitulated deficits in neurotransmitter response and reuptake (Figure 7D). These results demonstrate further conserved changes in both human AD and the corresponding murine model.

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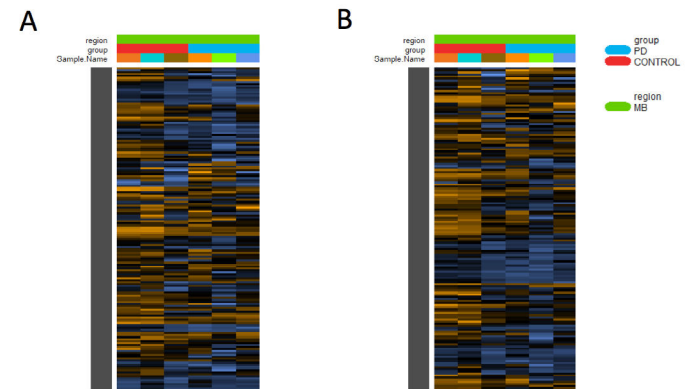
**FIGURE 6** Interrogation of gene expression and disease progression in the AD hippocampus. Using FFPE hippocampus samples, the Neuropathology Panel revealed numerous differentially expressed genes in AD (A) and gene expression changes associated with AD severity (B).



**FIGURE 7** Neurotransmission deficits associated with AD. Neuropathology Panel interrogation of human FFPE hippocampus samples revealed conserved deficits in neurotransmitter (A) response and reuptake, (B) synthesis and storage, and (C) release in high AD patients. (D) Mouse Neuropathology Panel interrogation of murine FFPE hippocampus samples from 5XFAD mice revealed neurotransmitter response and reuptake deficits similar to those observed in humans. Results are shown as pathway scores, which are calculated as the first principal component of the pathway genes' normalized expression. For a given pathway, principal component analysis scores each sample using a linear combination (a weighted average) of its gene expression values, weighing specific genes to capture the greatest possible variability in the data.

## Disease-specific changes in PD

Disease-specific changes in PD were also analyzed for comparison to the AD data set. Using FFPE midbrain samples, pathway changes in neurotransmitter release (Figure 8A) and vesicle trafficking (Figure 8B) were observed.

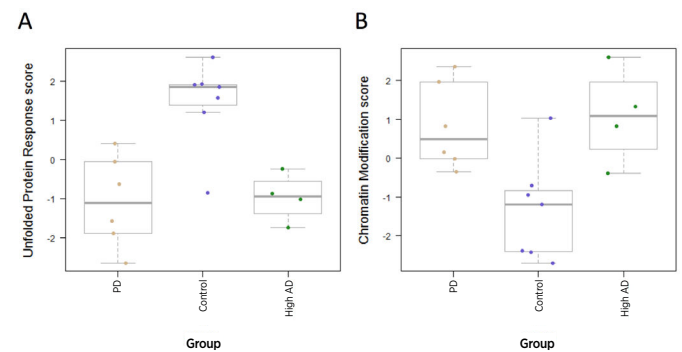


**FIGURE 8** Disease-specific changes in PD.

Neuropathology Panel interrogation of human FFPE midbrain samples from PD patients (Stage 3-4) revealed pathway changes corresponding to neurotransmitter release (A) and vesicle trafficking (B).

## Conserved changes in AD and PD

Accumulation of misfolded proteins and aberrant DNA methylation are associated with neurodegenerative diseases.<sup>10,11</sup> Using FFPE hippocampal samples from high AD, stage 6 PD, and normal controls, pathway scoring revealed similar changes between AD and PD with regards to unfolded protein response (Figure 9A) and chromatin modification (Figure 9B). These results are consistent with previous observations of conserved changes across AD and PD.<sup>12,13</sup>



**FIGURE 9** Conserved changes in AD and PD.

Neuropathology Panel interrogation of human FFPE hippocampus samples from high AD or stage 6 PD patients revealed similar changes in unfolded protein response (A) and chromatin modification (B) when compared to normal controls.

## Discussion

In this application note, experimental data demonstrates the performance of the Neuropathology Panels in both basic applications and disease-specific investigations. We observed robust performance irrespective of PMI and in FF, FFPE, and peripheral blood samples on the nCounter platform. Investigations on AD and PD mouse models and human samples demonstrated the applicability of the panel content and nSolver Advanced Analysis to these conditions. Results were consistent with previous observations highlighting key alterations in cellular composition and neuronal function associated with the disease state and disease progression. Additionally, we demonstrated the potential for biomarker discovery with gene expression profiling of samples across neurodegenerative diseases and disease states. The Neuropathology and Neuroinflammation Panels provide robust and reproducible results that help untangle the complex biology of neurological disorders.

## References

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**For more information about the nCounter Neuropathology Panels used in this application note visit, [nanosttring.com/neuropathology](https://nanosttring.com/neuropathology)**

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