HIGH-PLEX PROTEOMIC CHARACTERIZATION OF PRECLINICAL MODELS WITH THE NULISAseq™ MOUSE PANEL 120

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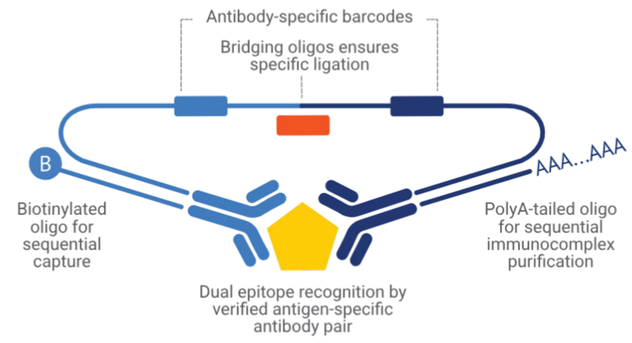
Abstract

Objectives: With the emergence of several preclinical models of neurodegenerative diseases including Alzheimer's Disease (AD), proteomic characterization of these models is needed to better understand their clinical relevance and utility in therapeutic evaluation. Along with amyloid plaques and neurofibrillary tangles, neuroinflammation is an underlying mechanism of disease progression and a multiplex proteomics approach is needed to track the temporal regulation of inflammatory and neurodegenerative processes as well as the degree to which these preclinical models recapitulate the biology of the human disease.

Methods: Previously, we developed a 250-plex Inflammation Panel and 120-plex CNS Disease Panel on the automated ARGO platform to profile key proteins such as IL6, phospho-Tau, and amyloid beta for profiling of biofluids in human studies. To provide a complementary approach for proteomic profiling in preclinical models, we have developed a 120-plex Mouse Panel for detection of cytokines, growth factors and other CNS disease targets. Plasma samples from different mouse strains were assayed at multiple input volumes. CSF and brain lysates were also evaluated. Dilutional linearity, reproducibility and overall detectability were assessed. Additionally, various preclinical models of AD and Traumatic Brain Injury were also evaluated.

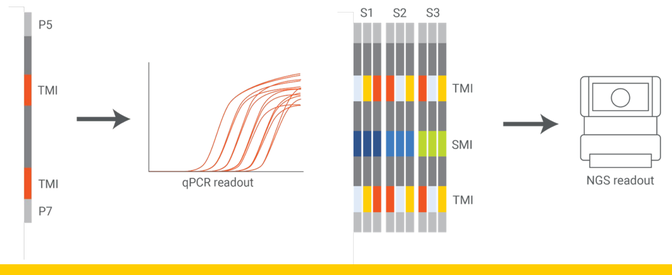
Results: Our results demonstrate robust performance with 5% median intra-assay CV and 97.4% detectability in mouse plasma samples from 5 common strains. Additionally, lowering sample input volume from 10uL to 5uL has minimal impact on overall detectability (>95%), reflecting high assay sensitivity. Dilutional linearity further demonstrated good target linearity with signal-to-noise ratio over 2 for 97% of the targets in the panel. The analytical validation along with the application of the panel across different AD models demonstrates the utility of the multiplex NULISAseq™ Mouse Panel for comprehensive characterization of neurodegenerative and inflammatory markers in preclinical models.

NULISA Leverages Oligo-Conjugated Antibodies and a Dual Capture and Release Mechanism for High Specificity

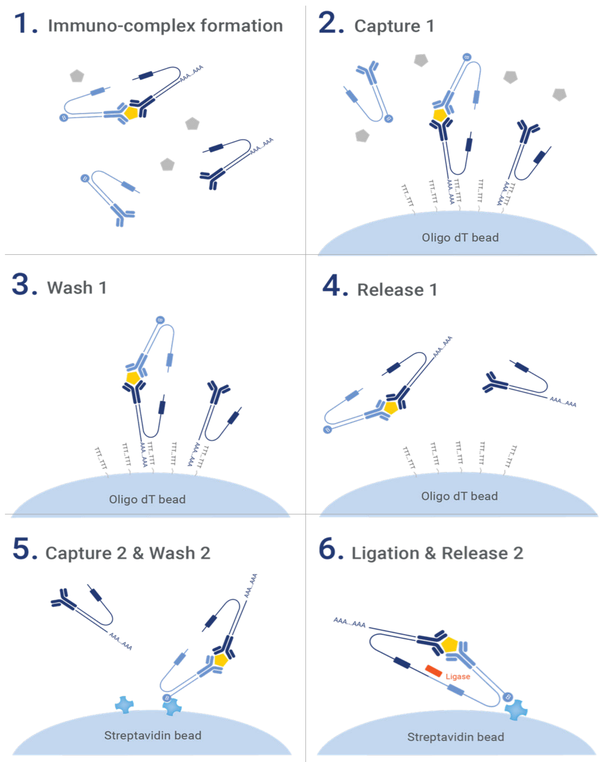


Low-plex readout: qPCR on ARGO™ HT

High-plex readout: Pooled library on Sequencer



NULISA is Fully Automated on the ARGO™ HT Platform for High-Throughput Multiplexed Proteomic Analysis



- Minimal Sample Input: ~12-25 µL
- Simple, automated workflow: <30 min hands-on time
- Singleplex analysis: On-board qPCR, <8 hrs
- Multiplex analysis: NGS-ready libraries
- High Throughput: Up to 258 samples in 3 x 96-well plates/day
- Integrated Data Analysis: Cloud-based analytics

NULISAseq™ Mouse Panel Enables Comprehensive Proteomic Profiling of Inflammation and Neurodegeneration in Preclinical Models

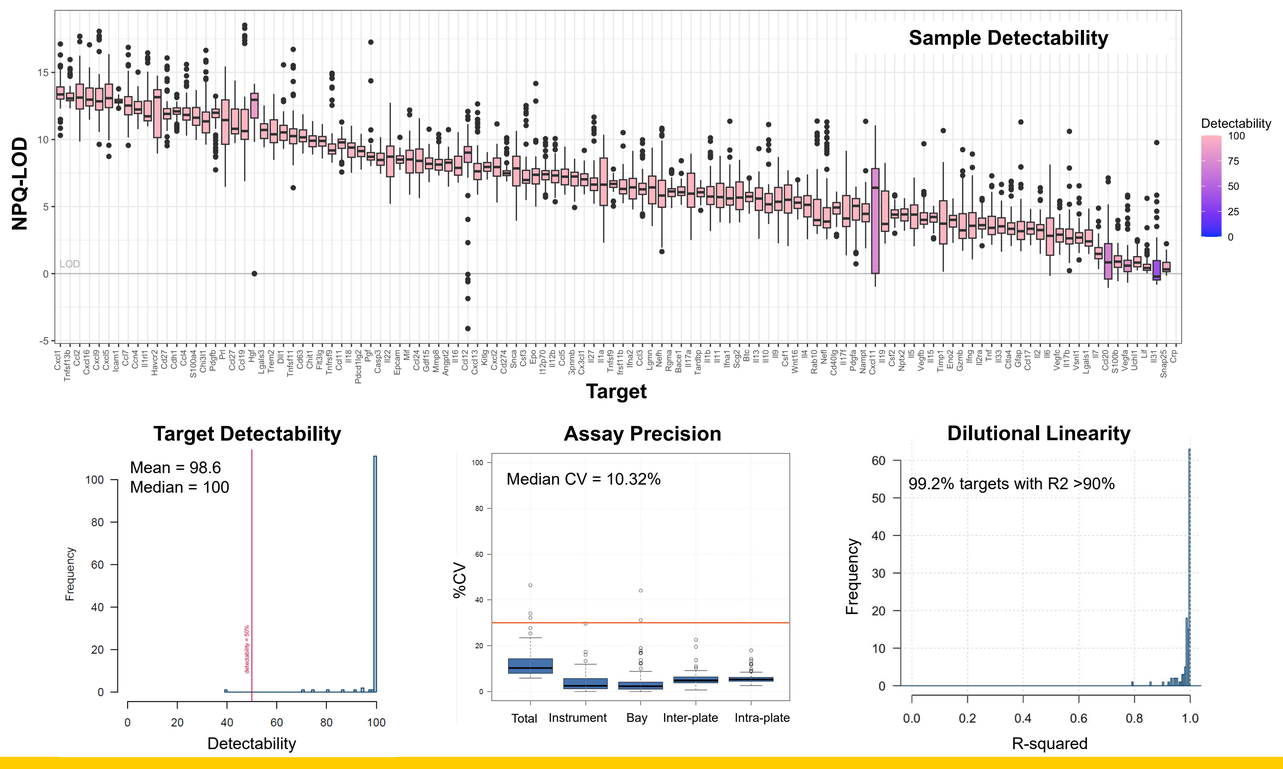
IMMUNOLOGY										
Cytokines					Chemokines			Regulatory/Signaling		Growth Factors
Crp	Il2	Il12B	Il19	Tnf	Ccl2	Ccl17	Cxcl1	Cd27	Lgals1	Csf1
Csf2	Il2Ra	Il12P70	Il22	Tnfrsf9	Ccl3	Ccl19	Cxcl2	Cd40lg	Lgals3	Csf3
Flt3L	Il4	Il13	Il27	Tnfrsf11	Ccl4	Ccl20	Cxcl5	Cd63	Lgmn	Eno2
Ifna1	Il5	Il15	Il31	Tnfsf9	Ccl5	Ccl24	Cxcl9	Chit1	Rab10	Gdf15
Ifna2	Il6	Il16	Il33	Tnfsf11	Ccl7	Ccl27	Cxcl11	Gzmb	Scg2	Hgf
Ifng	Il7	Il17A	Lif		Ccl11	Chi3L1	Cxcl13	Icam1	Vsnl1	Pgf
Il1A	Il9	Il17B	Mif		Ccl12	Cx3Cl1	Cxcl16			Vegfa
Il1B	Il10	Il17F	Nampt							
Il1Rl1	Il11	Il18	Tn13B							

NEUROLOGY			
Amyloid & Tauopathies	Movement: PD, HD, MS, ALS, Epilepsy	Injury & Vascular: TBI, Stroke	
Bace1	P-Snca-129 (P-αSyn)	Angpt2	Nefl
Nptx2	Rgma	Cdh1	Pdgfa
Ptau181	Snap 25	Epo	Pdgfb
Ptau217	Tardbp	Gfap	Timp1
Ptau231	Total Snca (αSyn)	Kitlg	Vegfb
Total Tau	Trem2	Mmp8	Vegfc
	Uchl1	Nefh	Wnt16

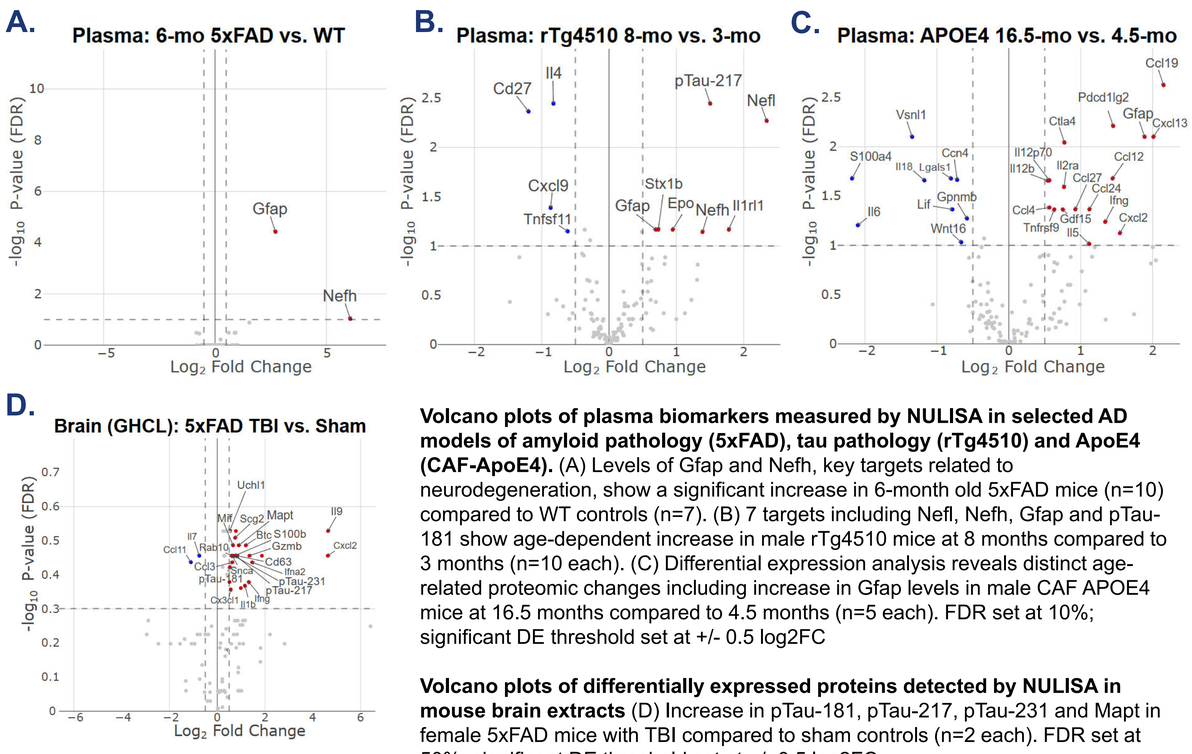
ONCOLOGY		
Differentiation and Proliferation	Immune Checkpoint	Cell Death
Btc	Cd274	Casp3
Ccn4	Ctla4	Gpnmb
Dll1	Pdcd1lg2	
Epcam	Tim3	
Prl		
S100A4		
S100B		

Analytical Characterization of NULISAseq™ Mouse Panel Shows Robust Performance

- Detectability: >95% in mouse plasma; >75% in mouse CSF
- High Assay Precision and Reproducibility: <25% intra and inter-plate CV (n=6 plasma samples; 2 instruments and 2 bays)
- High Dilutional Linearity: Mean R^2 value of 0.98 and a median R^2 value of 0.99 across all targets
- Strains:CD-1, ICR, SW, Balb/C, C57BL/6NTac
- Disease Models tested: AD, Aging, PD, TBI, RA, Obesity, Lupus, Cancer,Hemophilia, DMD, Rett, ALS, etc.



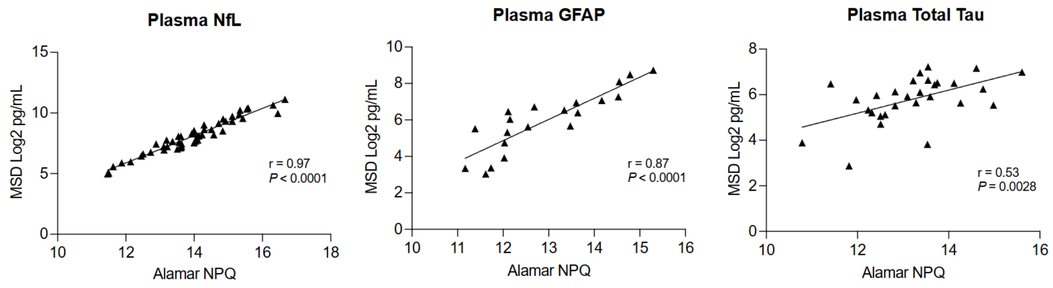
NULISAseq™ Mouse Panel Provides Deeper Insights into Disease Pathogenesis and Progression in Preclinical Alzheimer's and TBI Models



Volcano plots of plasma biomarkers measured by NULISA in selected AD models of amyloid pathology (5xFAD), tau pathology (rTg4510) and ApoE4 (CAF-ApoE4). (A) Levels of Gfap and Nefh, key targets related to neurodegeneration, show a significant increase in 6-month old 5xFAD mice (n=10) compared to WT controls (n=7). (B) 7 targets including Nefl, Nefh, Gfap and pTau-181 show age-dependent increase in male rTg4510 mice at 8 months compared to 3 months (n=10 each). (C) Differential expression analysis reveals distinct age-related proteomic changes including increase in Gfap levels in male CAF APOE4 mice at 16.5 months compared to 4.5 months (n=5 each). FDR set at 10%; significant DE threshold set at +/- 0.5 log2FC

Volcano plots of differentially expressed proteins detected by NULISA in mouse brain extracts (D) Increase in pTau-181, pTau-217, pTau-231 and Mapt in female 5xFAD mice with TBI compared to sham controls (n=2 each). FDR set at 50%; significant DE threshold set at +/- 0.5 log2FC

NULISA Shows Strong Correlations with Other Proteomic Platforms



Pearson Correlation Analysis demonstrated high correlation between plasma biomarkers measured with the NULISA Mouse Panel and MSD assays. Representative data for NfL and Gfap demonstrate r=0.97 and r=0.87 respectively. Correlation for Mapt was poor (r=0.53) likely due to differences in cross reactivity across species for MSD and NULISA assays. The NULISA Mouse Panel has been designed for detection of both human and mouse Tau isoforms.

NULISA™ Provides a Highly Sensitive and Multiplexed Solution for Proteomic Detection In Diverse Mouse Models

- The high sensitivity and multiplexing provided by the NULISA technology coupled with the high-throughput and automated workflows on the ARGO HT platform enable proteomic evaluation in low-volume plasma, CSF and brain samples from mouse preclinical models.
- The analytical validation and the application of the panel across different AD and TBI models demonstrates the broad utility of the multiplex NULISAseq Mouse Panel 120 for comprehensive characterization of neurodegenerative and inflammatory markers across preclinical models.

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