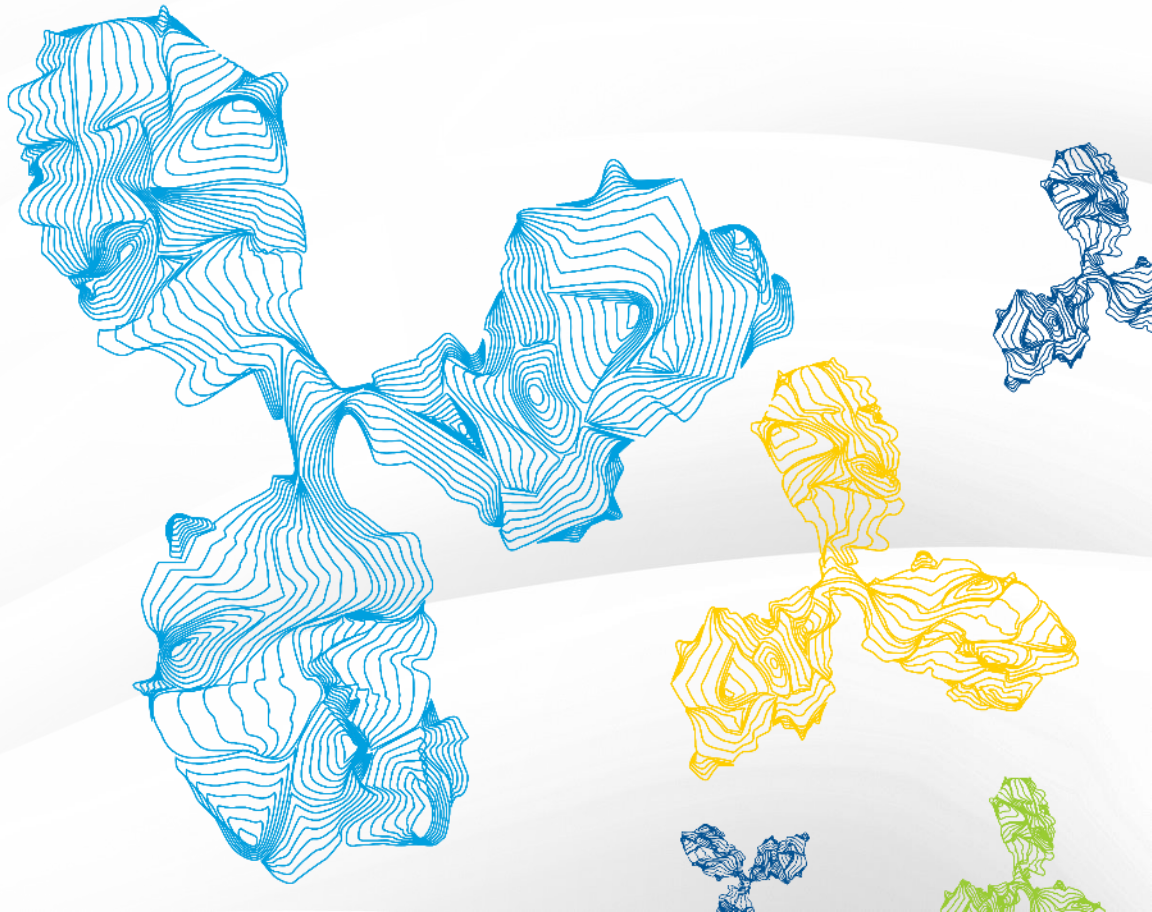
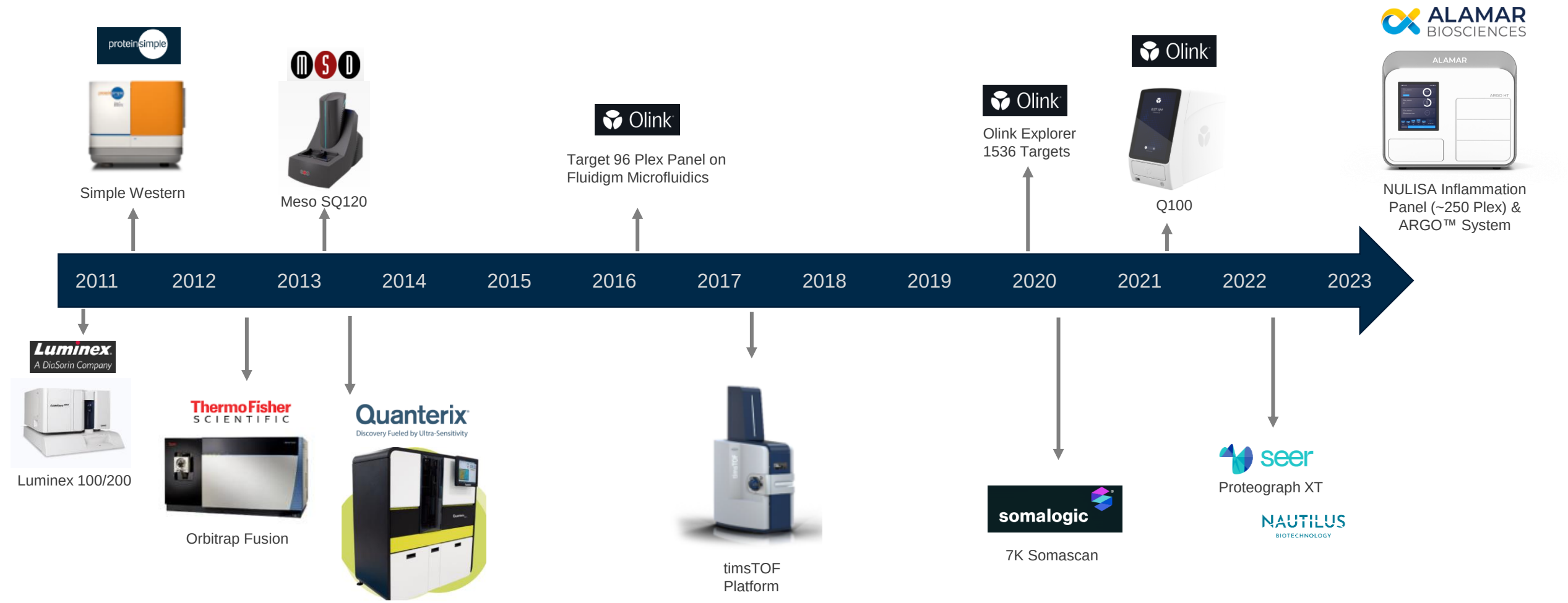
NULISA: Fully automated ultra-sensitive immunoassay platform for profiling fluid-based protein biomarkers

Doug Hinerfeld, Ph.D. Senior Director of Application Support

WACD
October 13, 2023



Proteomics: The Next Frontier in Life Science Tools and Diagnostics



Alamar Biosciences

Mission: Power precision proteomics to enable the earliest detection of disease

- **NULISA™ -** ultra-sensitive Attomolar (10^{-18}M) NGS enabled digital immunoassay platform for protein analysis from 1 – 1000's of plex
- **Attobody™:** bi-paratopic antibodies with picomolar affinity
- **ARGO™ -** Fully automated solutions to perform assay across a range of multiplex levels and applications from discovery to the clinic

Automated solutions for **ultra-sensitive** protein analysis across a range of **multiplex** level to detect **critical biomarkers** from discovery to the clinic.

Fast Facts

Founded
2018

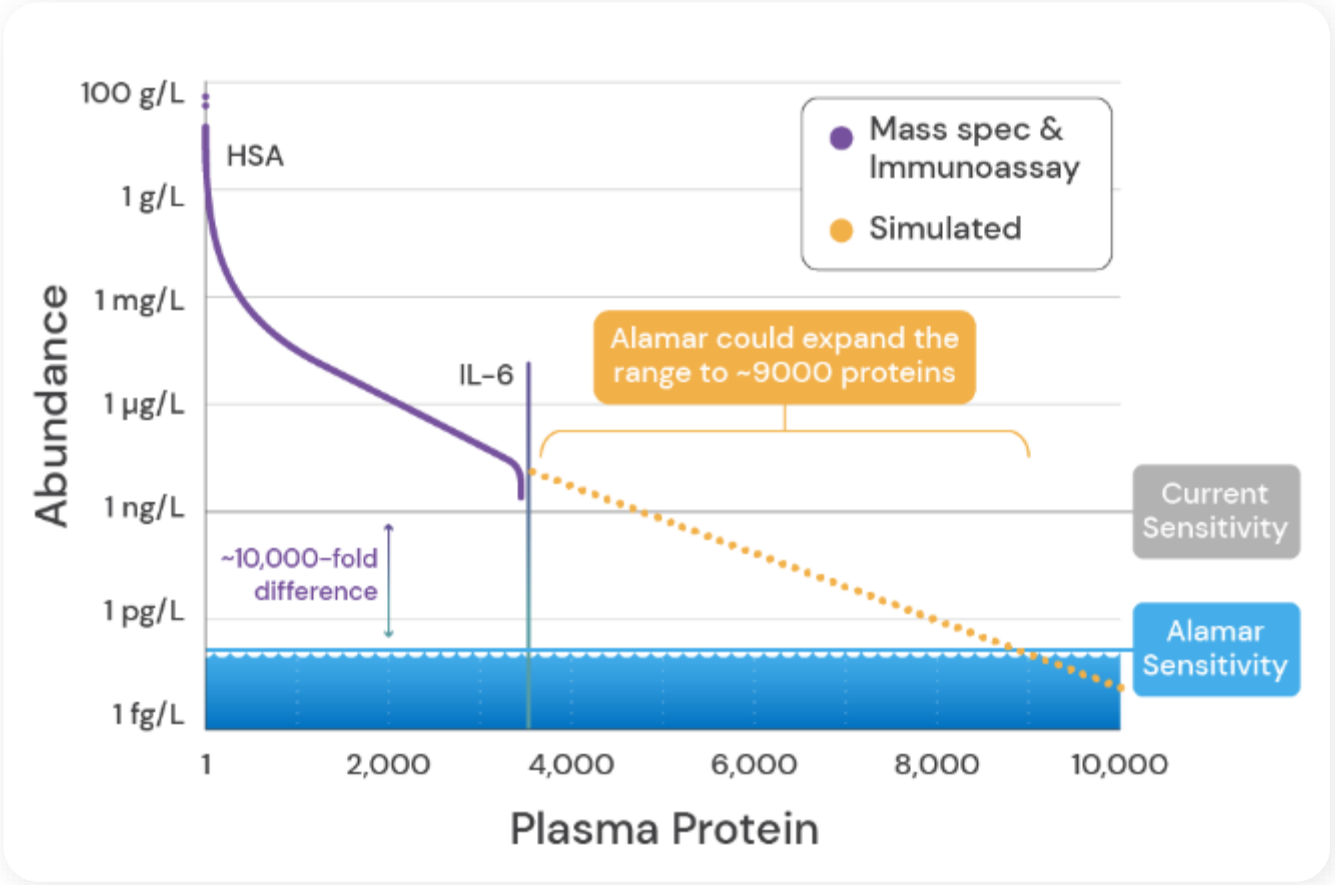
Team
70+ Full-Time Employees

Based
San Francisco Bay Area

Stage
Development

Funding
$120M Series A/B+

NULISA™ platform enables greater sensitivity and precision in high-plex proteomics



- Best-in-class Attomolar (fg/ml) sensitivity increases the depth of measurable proteins
- Enabling detection of known biomarkers at lower levels
- Enabling discovery of novel biomarkers that are currently undetectable
- Enabling clearer definition of health baseline biomarker levels

NULISA™: the most sensitive immunoassay liquid biopsy platform

Unprecedented sensitivity

- Attomolar (fg/mL) level of detection
- Quantification of low abundance proteins
- Enables discovery of critical biomarkers

Widest dynamic range

- >12 logs dynamic range
- Quantification across biological range of protein levels
- In one reaction w/o dilution

Flexible, multiplex chemistry

- Single target or multiplex
- 100s to 1000s of targets in a single experiment
- Broad sample compatibility

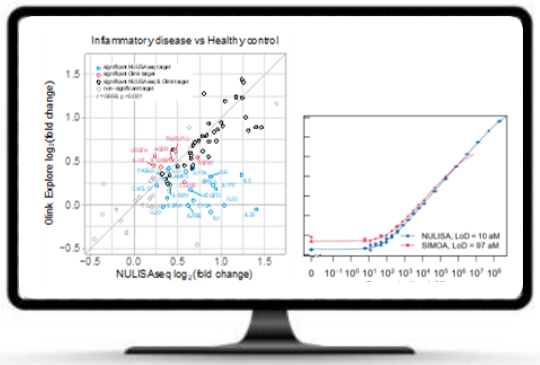
Fully automated

- Ease of use
- Minimal hands-on time
- High reproducibility

Precision proteomics at the **push of a button**

Full solution of instrument, reagent kits, and software

ARGO™ HT System



Consumables

Simple, robust, reproducible

Instrument

ARGO all-in-one instrument
w/ onboard qPCR

Software

From samples to insights

Scalability and Flexibility All-in-one instrument

Discovery

100s of markers with high sensitivity for *focused discovery* within known biology

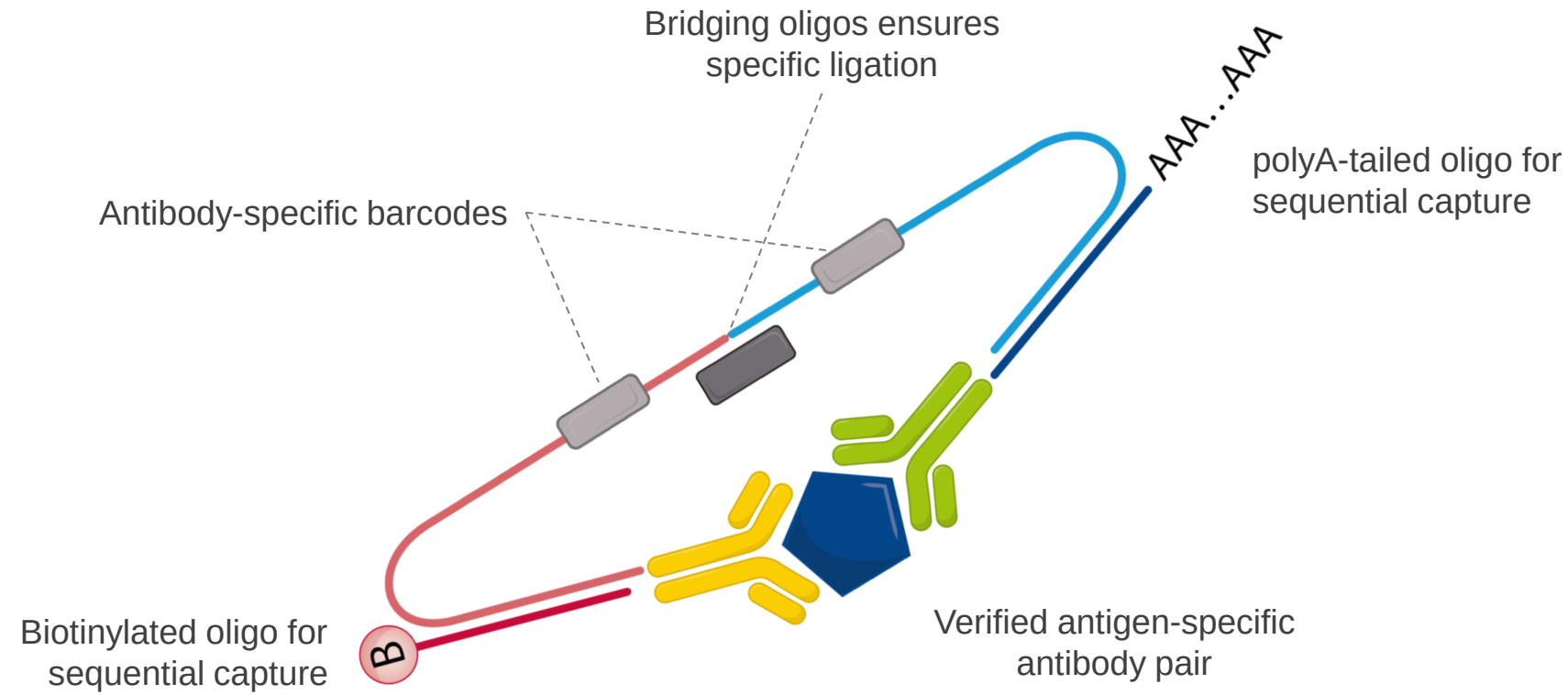
Translation

Multiplex *flexibility* to validate potential clinical targets with high sensitivity and precision

Clinical Biomarkers

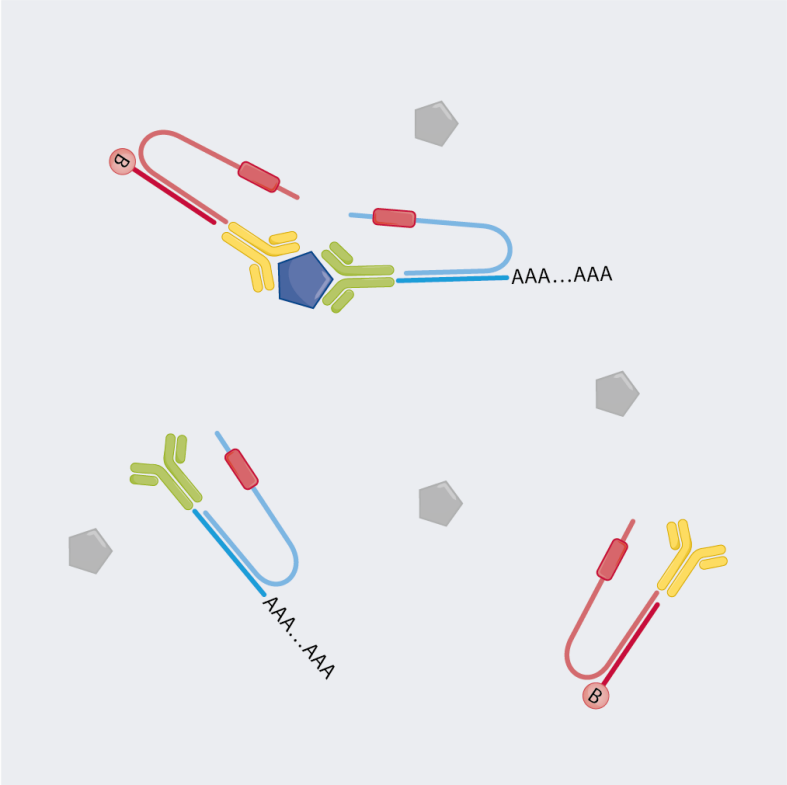
Single to low-plex qPCR-based assay kits for biomarkers and *future* diagnostics

NULISA Technology and how it works?

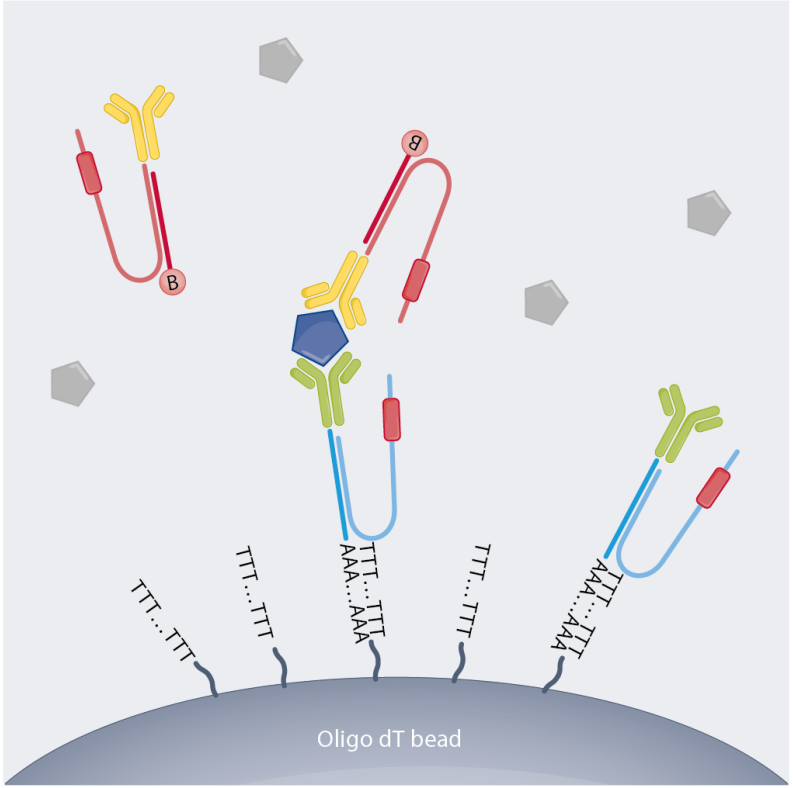


NULISA Workflow

- **Step 1:** in solution antibody – antigen immune complex formation

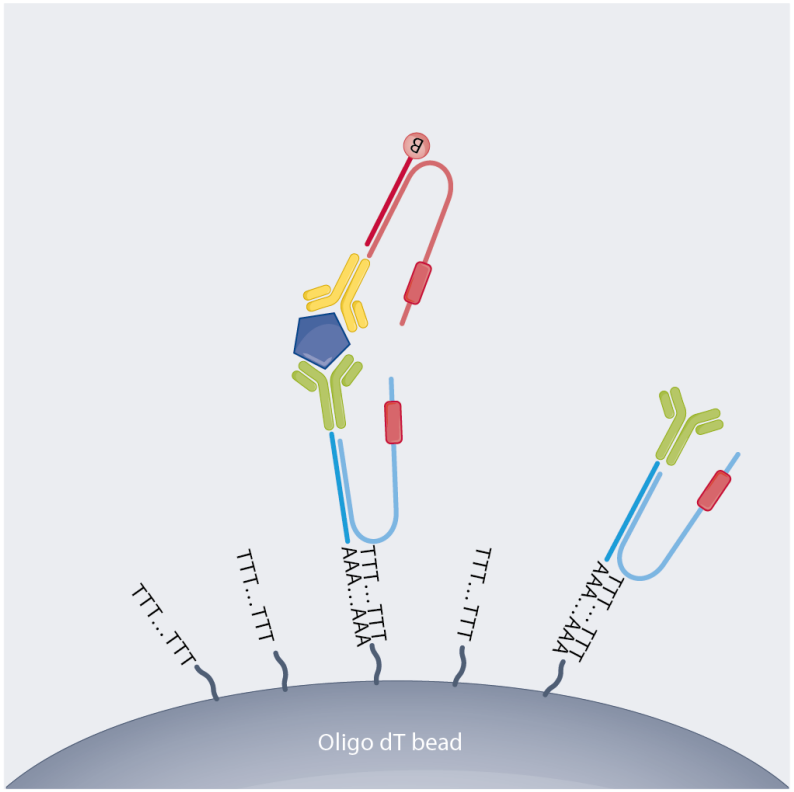


- **Step 2:** 1st bead-based capture – polyA-linked capture antibody on poly-dT beads

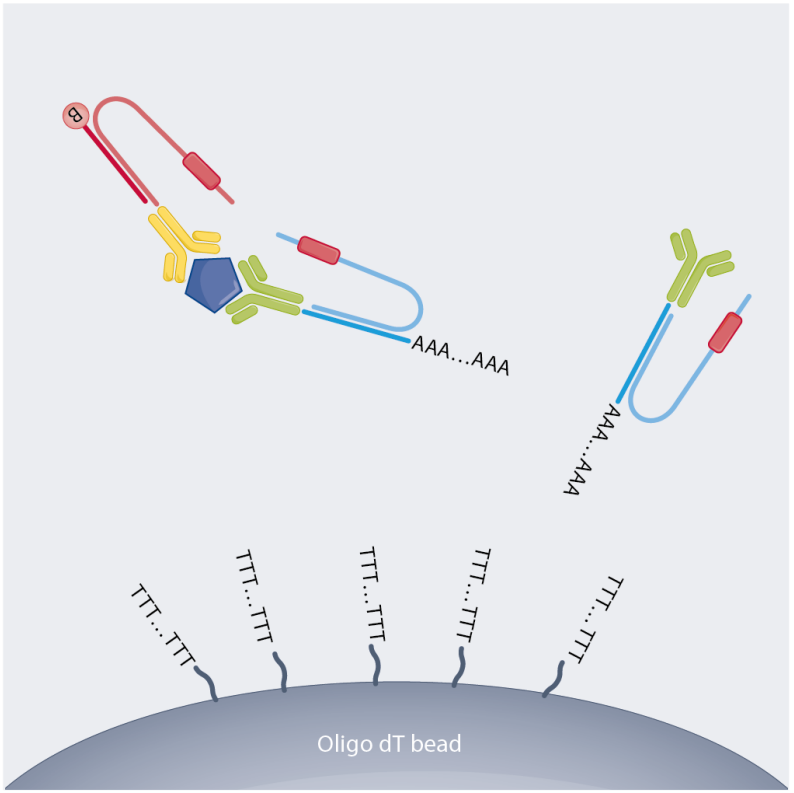


NULISA Workflow

- **Step 3:** wash – removal of all free detection antibodies, analytes and matrix; retention of specific analyte bound by capture antibody

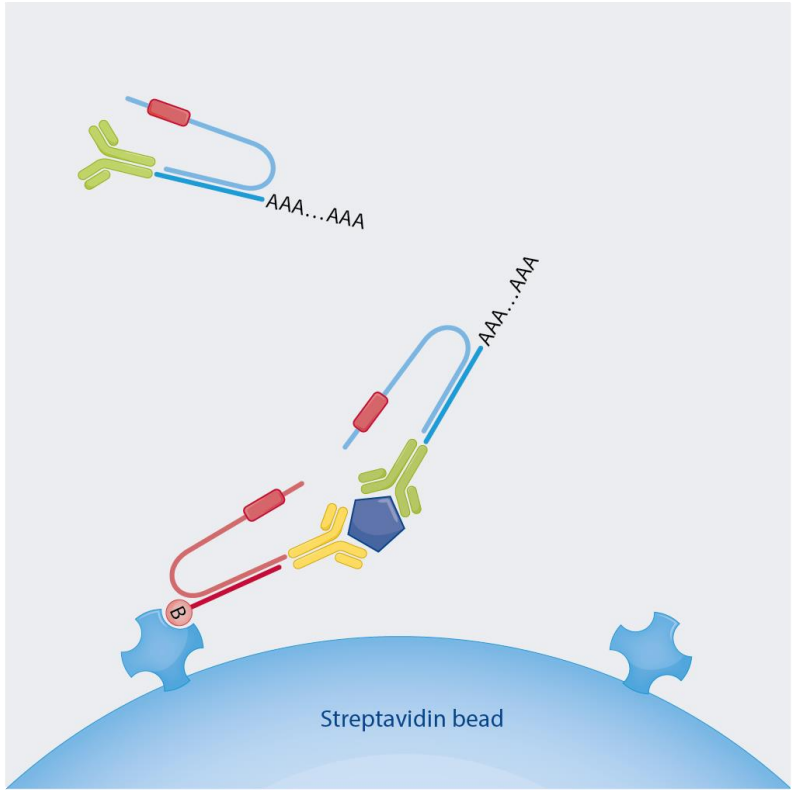


- **Step 4:** release of capture antibody/analyte complex

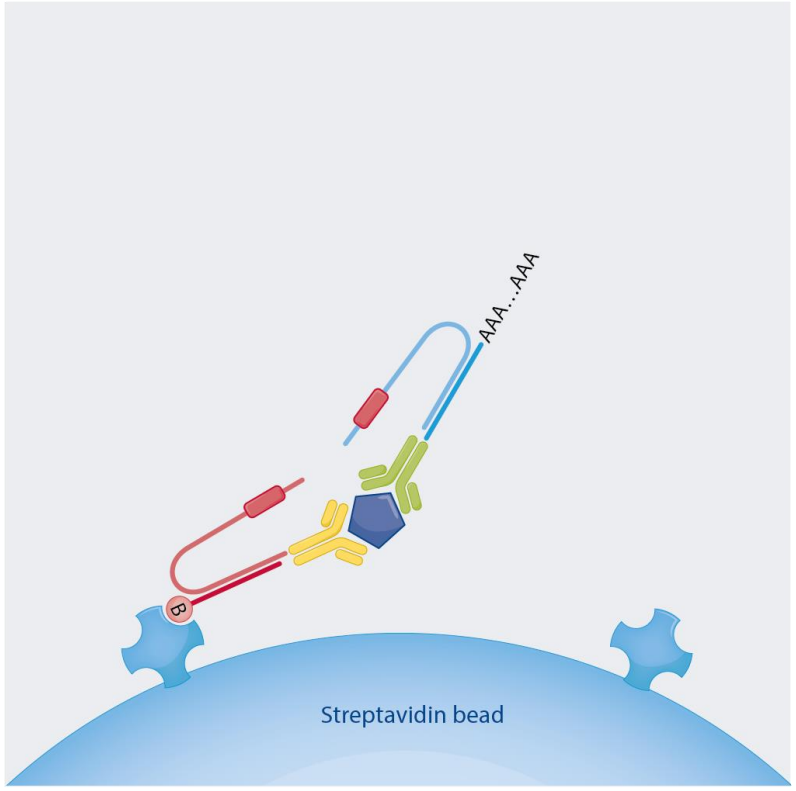


NULISA Workflow

- **Step 5:** 2nd bead-based capture via biotinylated oligo binding to streptavidin

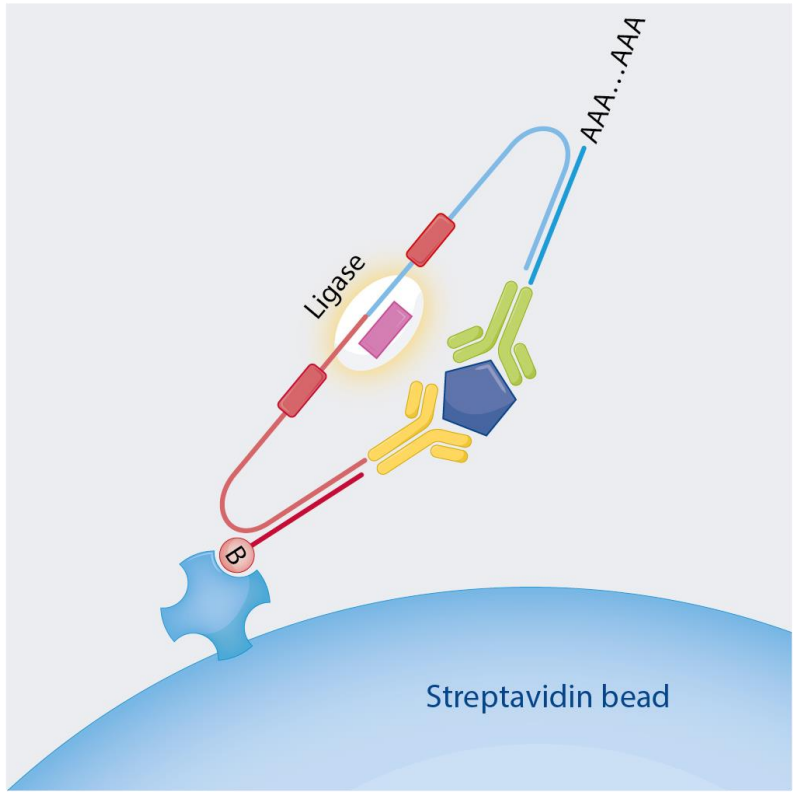


- **Step 6:** wash – removal of all antibodies and residual matrix not complexed with analyte and 2nd detection antibody

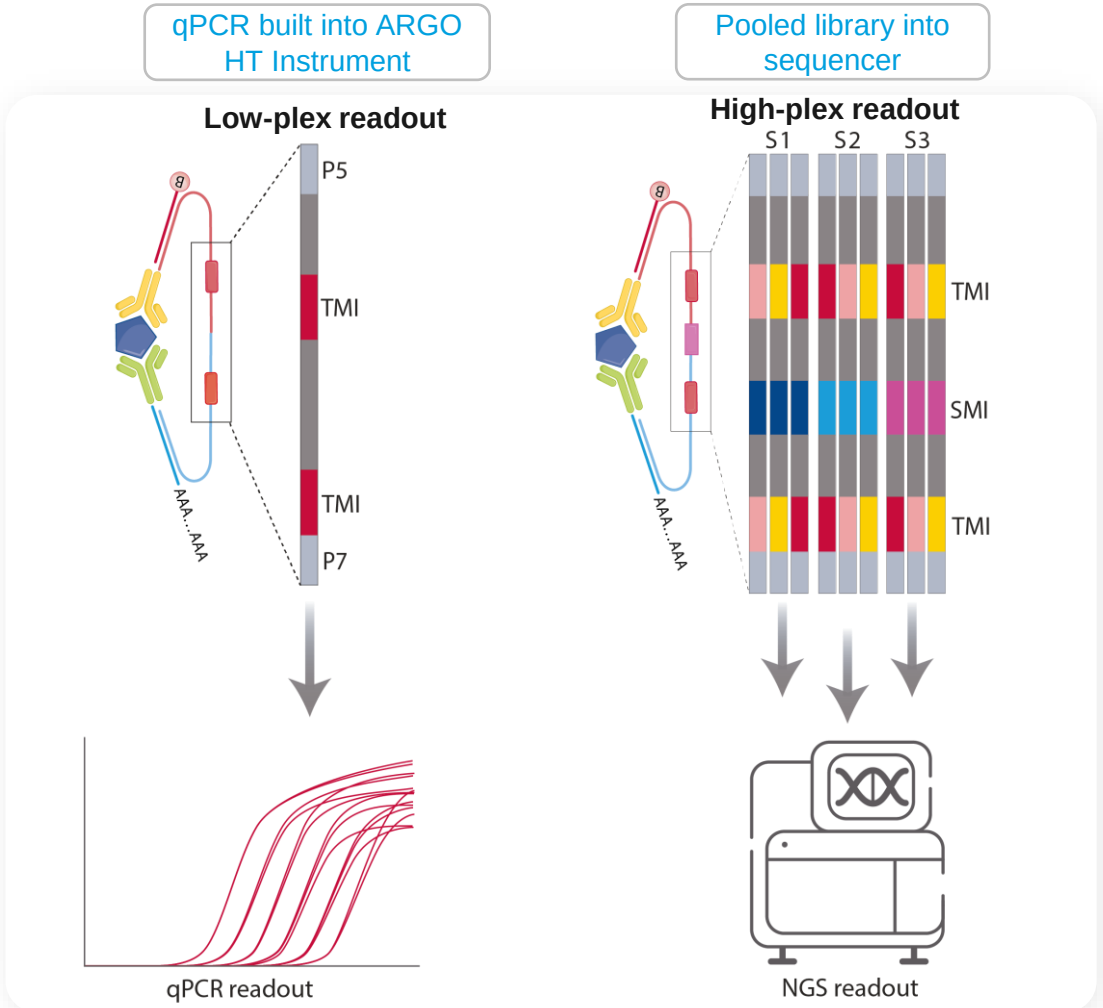


NULISA Workflow

- **Step 7:** ligation of specific bar-coded oligos

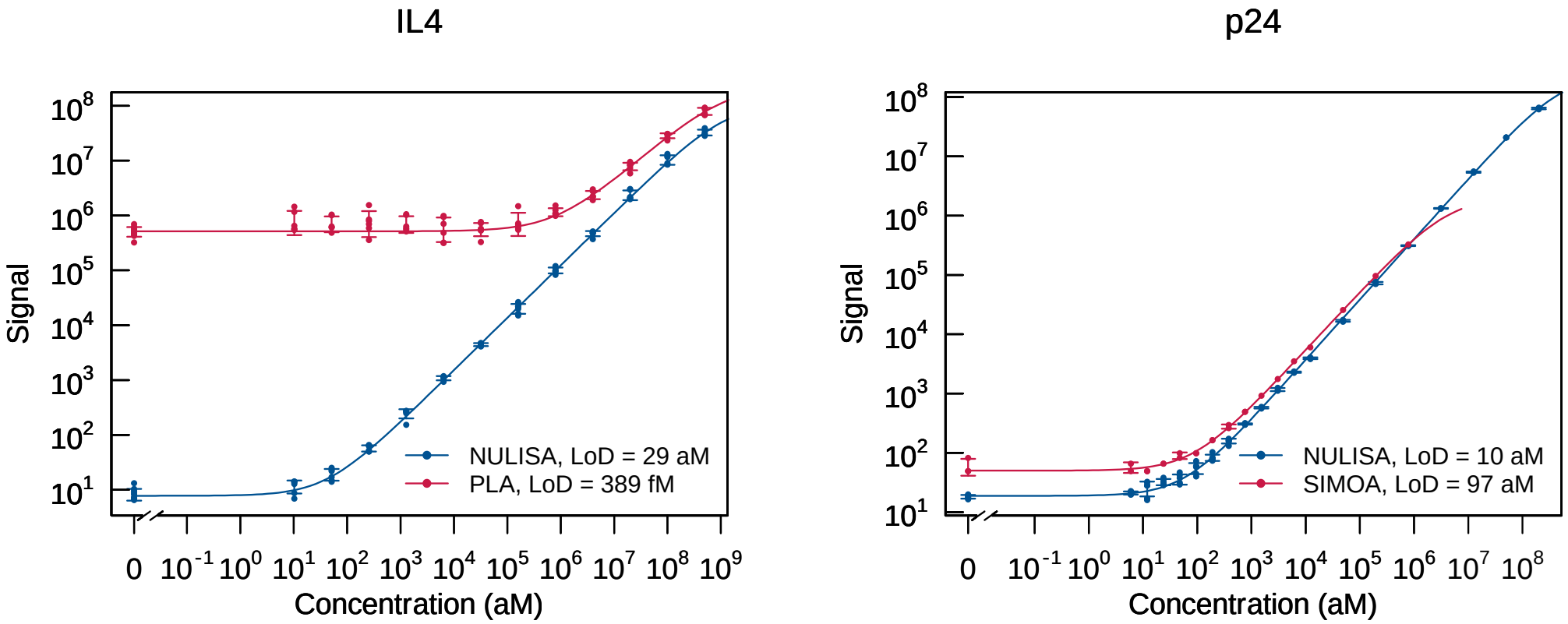


- **Step 8:** wash, then qPCR or NGS



NULISA achieves the highest sensitivity with the same antibodies using proprietary assay background suppression

IL-4 and HIV p24 NULISA using the same antibodies: NULISA vs PLA and SIMOA

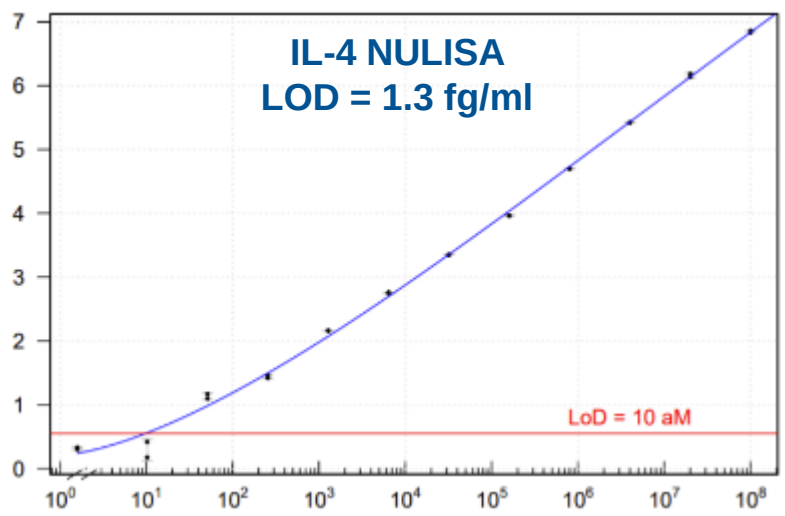


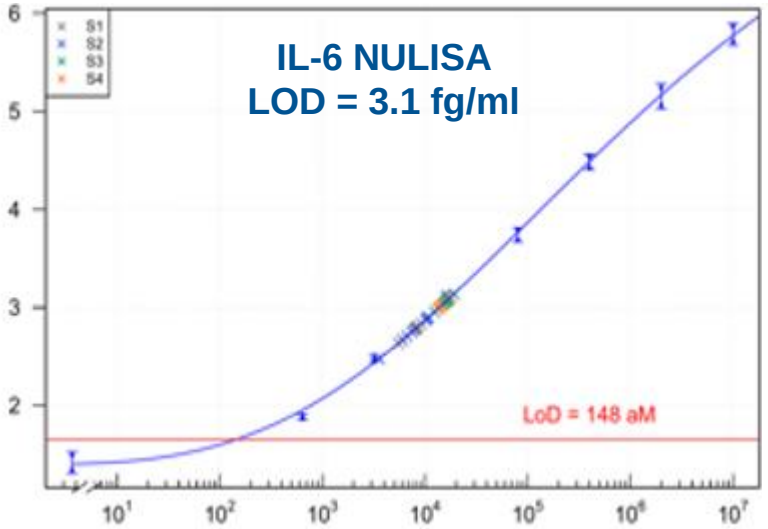
NULISA lowers the limit of detection and increases the quantifiable dynamic range with less sample

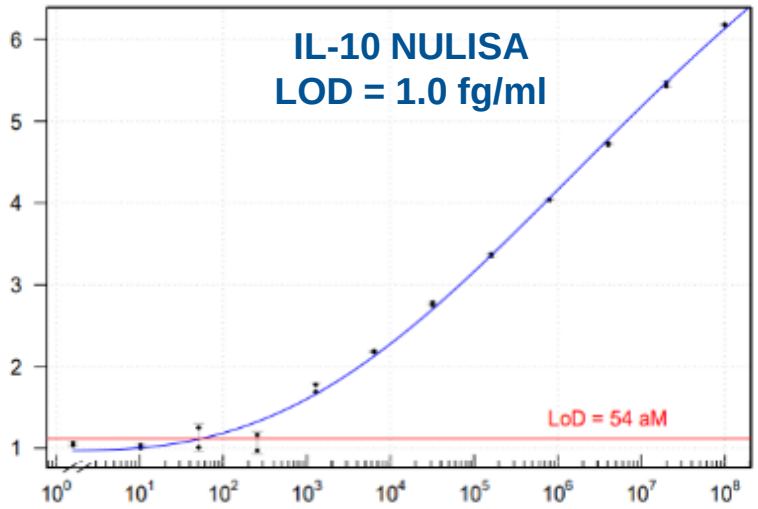
IL-4 assay using 100 pM capture/detection antibodies and 500 pM IL-4 at 5-fold dilution.

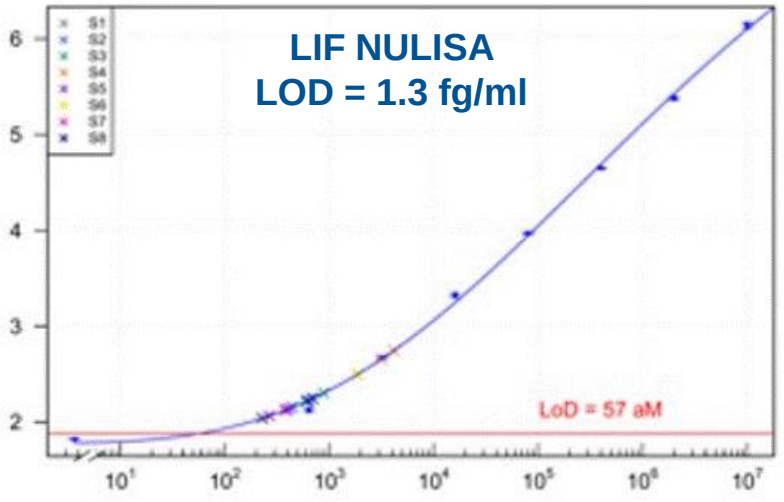
Source: https://www.biorxiv.org/content/10.1101/2023.04.09.536130v1

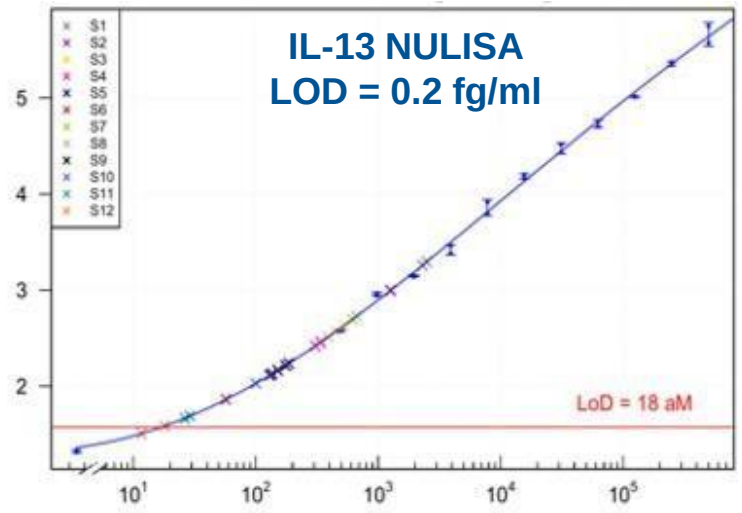
Routine and reliable attomolar (fg/mL) LOD for single-plex NULISA

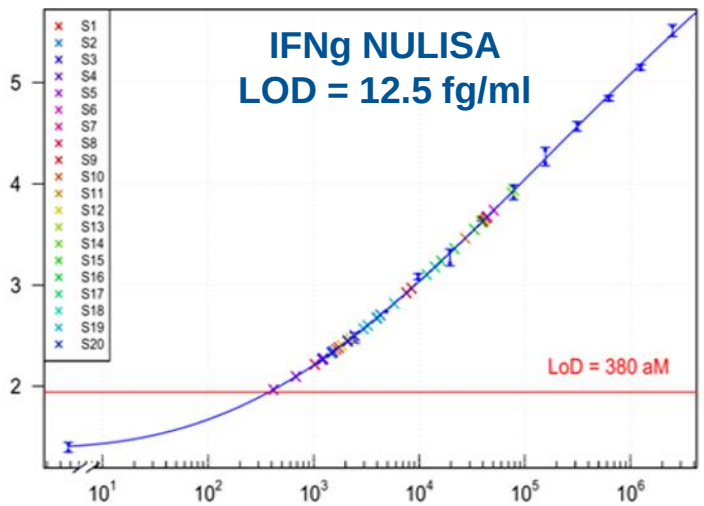






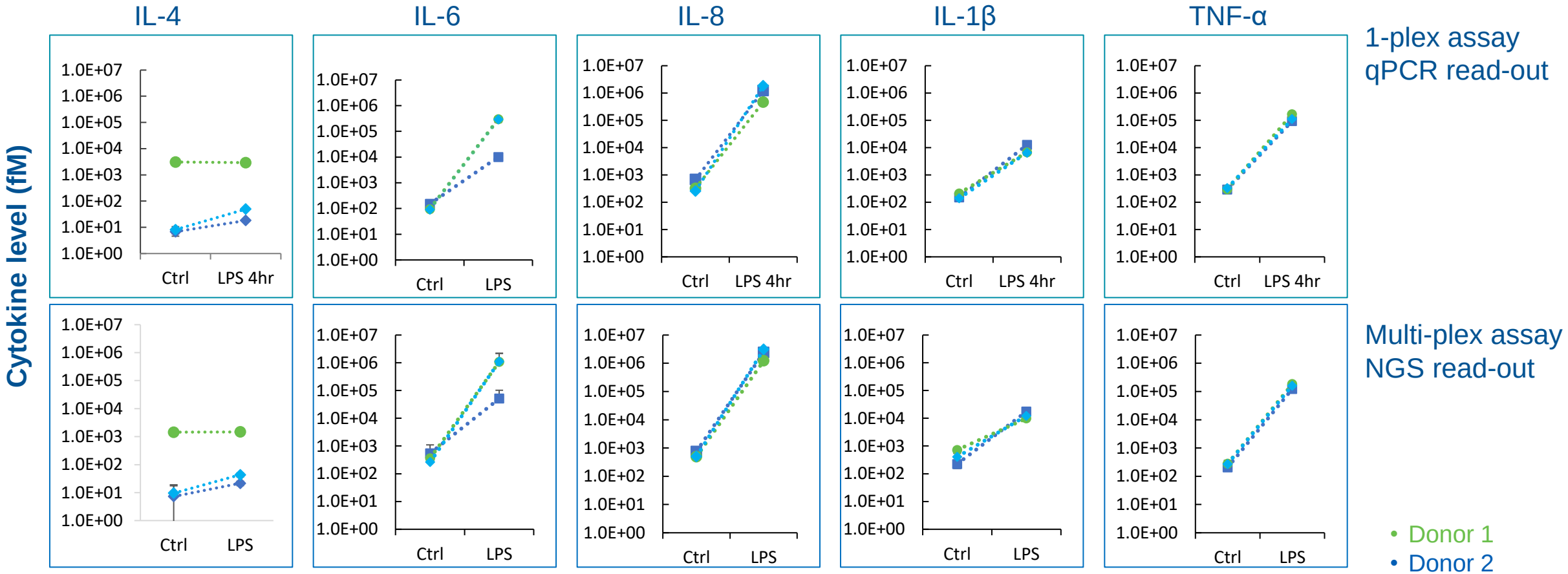






NULISA “sample-in-data-out” platform enables high precision analysis of single and multiplexed analytes

Concordance, high sensitivity and wide dynamic range for single-plex qPCR and multi-plex NGS NULISA readouts



Five cytokines from LPS stimulated whole blood samples from 3 healthy donors were assessed by NULISAseq or NULISA using 1.33 µL of plasma per assay

NULISAseq™ Inflammation Panel

Most complete coverage of cytokines and chemokines on the market

250+
biomarkers in 10µL

Attomolar sensitivity
(10 fg/mL)

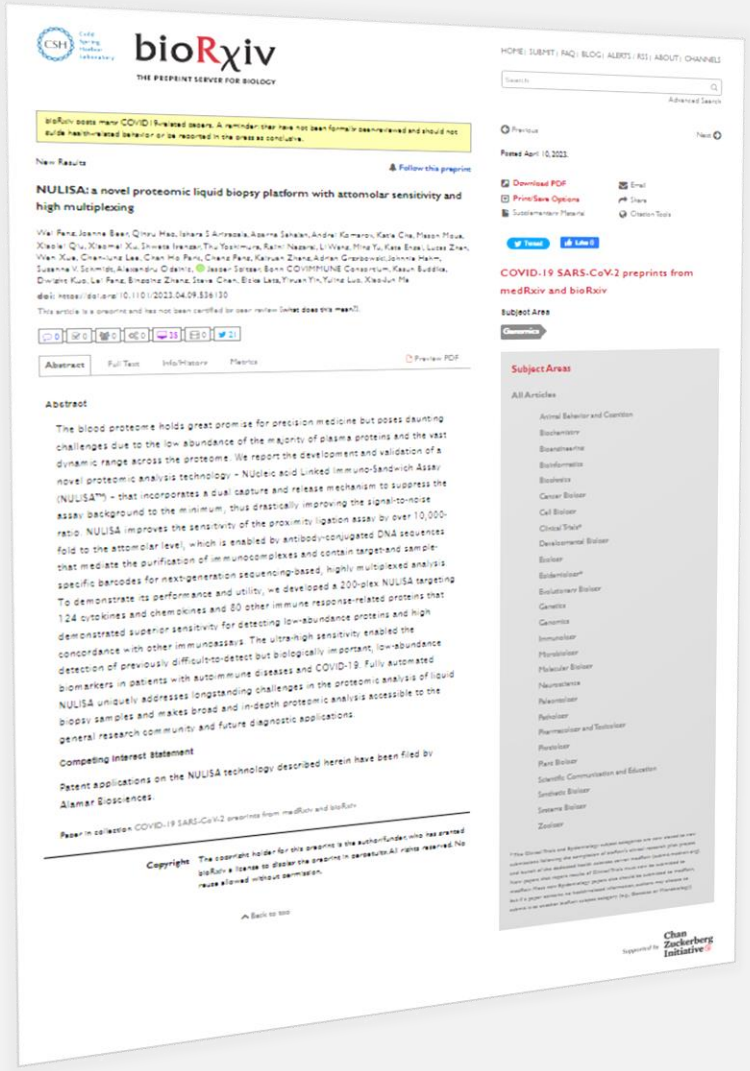
~12 logs
dynamic range without dilution

Highly reproducible
CV <10%

Customizable
Subset panels and panel+

TARGETS										
AGER	CCL23	CD83	CXCL13	GFAP	IL-13	IL-22	IL-9	MMP9	TAFA5	TNFSF10
AGRP	CCL24	CD93	CXCL14	GRN	IL-13RA2	IL-23A|EBI3	IRAK4	MPO	TEK	TNFSF11
ANGPT1	CCL25	CEACAM5	CXCL16	GZMA	IL-15	IL-23A|IL-12B	KDR	MUC16	TGFB1	TNFSF12
ANGPT2	CCL26	CHI3L1	CXCL2	GZMB	IL-15RA	IL-24	KITLG	NAMPT	TGFB3	TNFSF13
ANXA1	CCL27	CLEC4A	CXCL3	HAVCR1	IL-16	IL-27|EBI3	KLRK1	NCR1	THBS2	TNFSF13B
AREG	CCL28	CNTF	CXCL5	HGF	IL-17A	IL-2RA	KNG1	NGF	THPO	TNFSF14
BDNF	CCL3	CRP	CXCL6	HLA-DRA	IL-17A|IL-17F	IL-2RB	LAG3	NTF3	TIMP1	TNFSF15
BMP7	CCL4	CSF1	CXCL8	ICAM1	IL-17B	IL-32	LAMP3	OSM	TIMP2	TNFSF18
BST2	CCL5	CSF1R	CXCL9	ICOSLG	IL-17C	IL-33	LCN2	PDCD1	TLR3	TNFSF4
C1QA	CCL7	CSF2	EGF	IFNA1; IFNA13	IL-17F	IL-34	LGALS9	PDCD1LG2	TNF	TNFSF8
CALCA	CCL8	CSF2RB	EPO	IFNA2	IL-17RA	IL-36A	LIF	PDGFA	TNFRSF11A	TNFSF9
CCL1	CD200	CSF3	FASLG	IFNB1	IL-17RB	IL-36G	LIL-RB2	PDGFB	TNFRSF11B	TREM1
CCL11	CD200R1	CSF3R	FGF19	IFNG	IL-18	IL-37	LTA	PGF	TNFRSF13B	TREM2
CCL13	CD27	CST7	FGF2	IFNL1	IL-18BP	IL-3RA	LTA|LTB	PTX3	TNFRSF13C	VCAM1
CCL14	CD274	CTF1	FGF21	IFNL2;IFNL3	IL-18R1	IL-4	MDK	S100A12	TNFRSF14	VEGFA
CCL15	CD276	CTLA4	FGF23	IFNW1	IL-19	IL-4R	MERTK	S100A9	TNFRSF17	VEGFC
CCL16	CD3E	CTSS	FLT1	IKBKG	IL-1B	IL-5	MICA	SCG2	TNFRSF18	VEGFD
CCL17	CD4	CX3CL1	FLT3LG	IL-10	IL-1R1	IL-5RA	MICB	SDC1	TNFRSF1A	VSNL1
CCL19	CD40	CXADR	FLT4	IL-10RB	IL-1R2	IL-6	MIF	SELE	TNFRSF1B	VSTM1
CCL2	CD40LG	CXCL1	FTH1	IL-11	IL-1RL1	IL-6R	MMP1	SELP	TNFRSF21	WNT16
CCL20	CD46	CXCL10	FURIN	IL-12A|IL-12B	IL-1RN	IL-6ST	MMP12	SIRPA	TNFRSF4	WNT7A
CCL21	CD70	CXCL11	GDF15	IL-12B	IL-2	IL-7	MMP3	SLAMF1	TNFRSF8	
CCL22	CD80	CXCL12	GDF2	IL-12RB1	IL-20	IL-7R	MMP8	SPP1	TNFRSF9	

Pre-print on BioRxiv

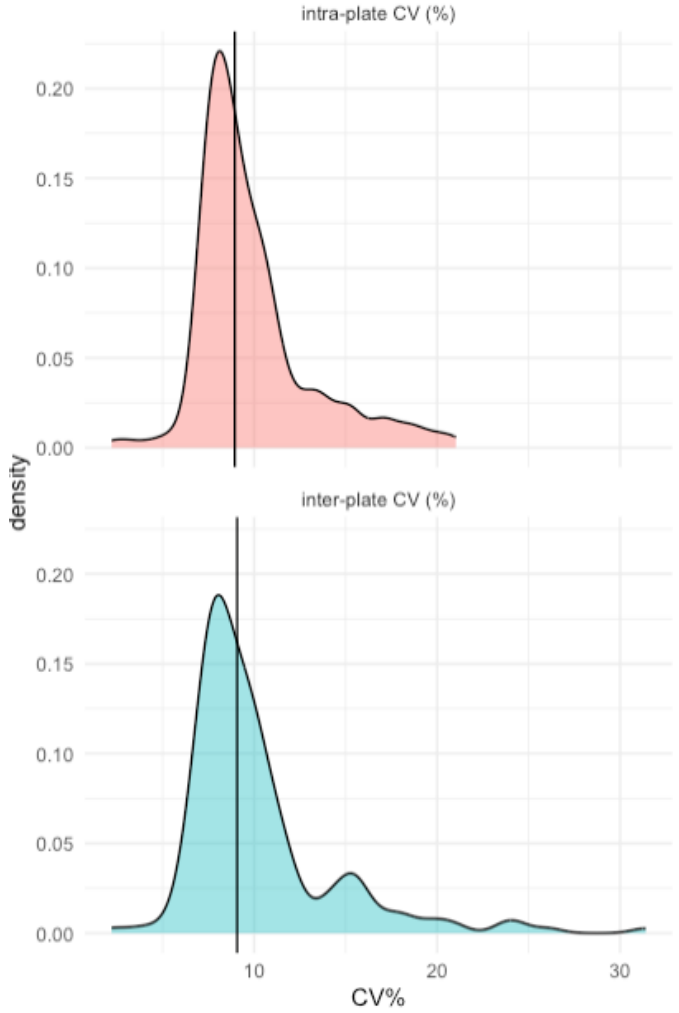


NULISA: a novel proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing

https://www.biorxiv.org/content/10.1101/2023.04.09.536130v2

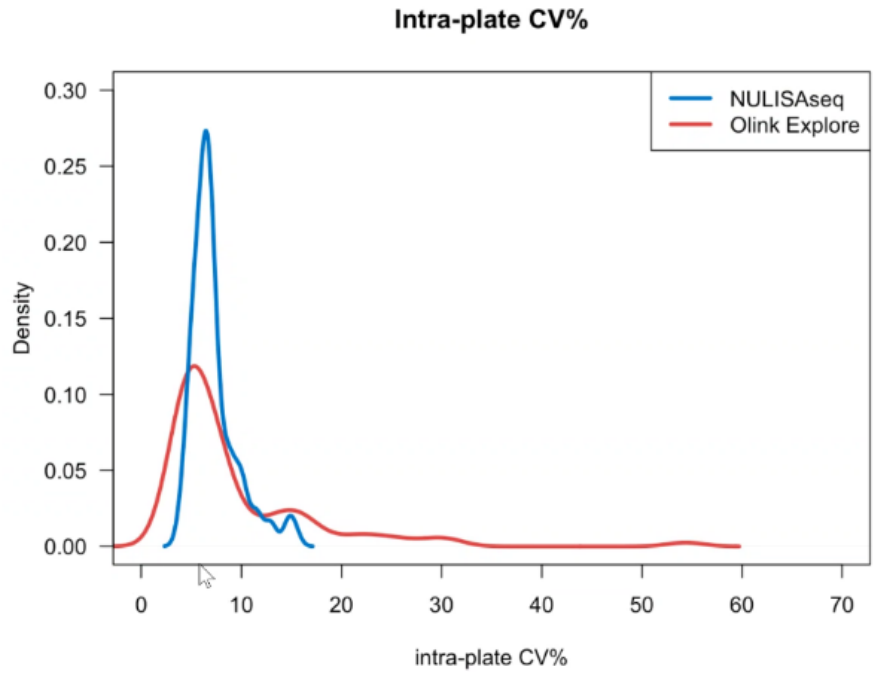
NULISA has tighter CV% versus Olink

200-plex NULISA
Intra- and inter-plate median CV within 10%

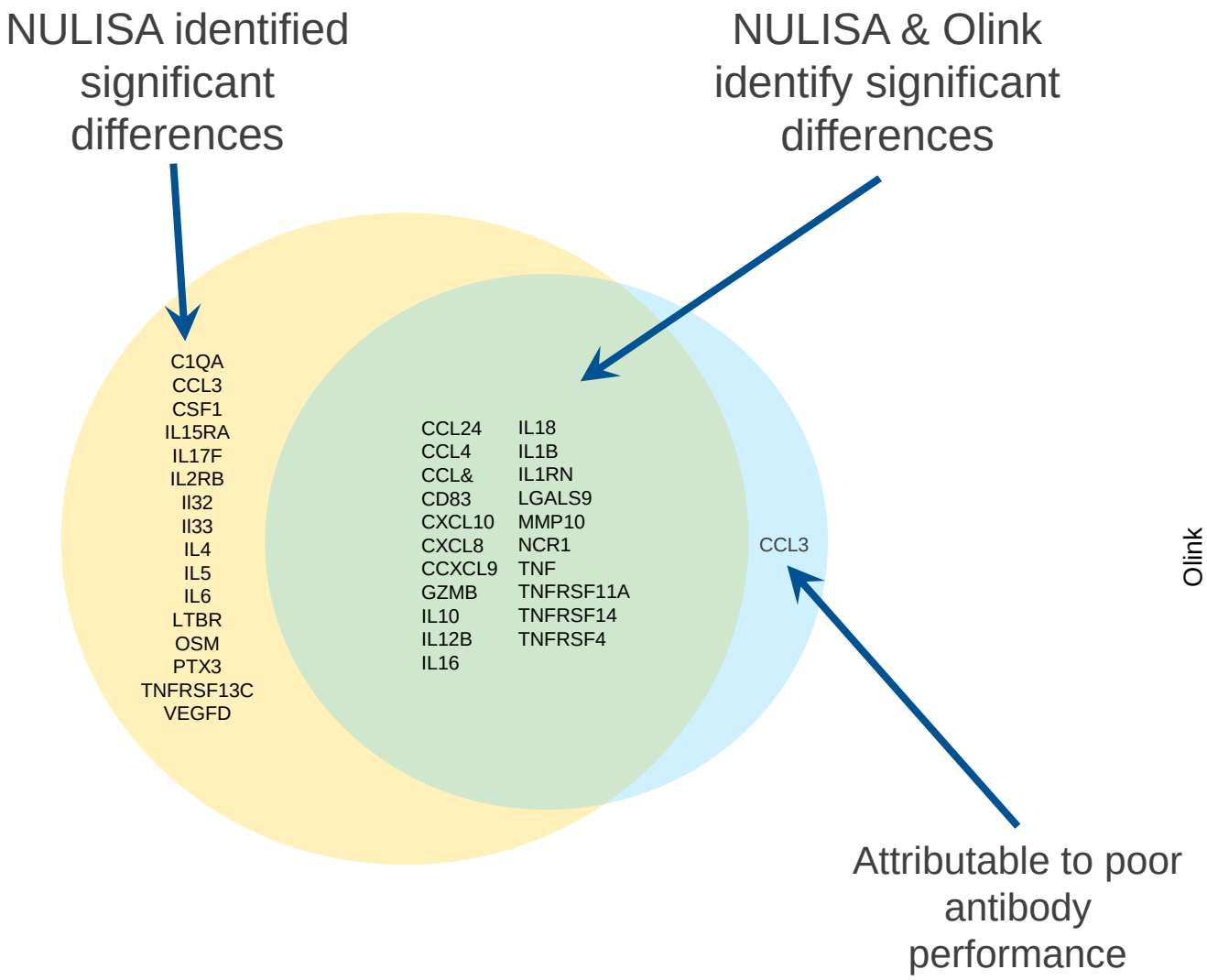


Intra-plate CV for NULISA and Olink for 92 shared targets in 159 plasma samples

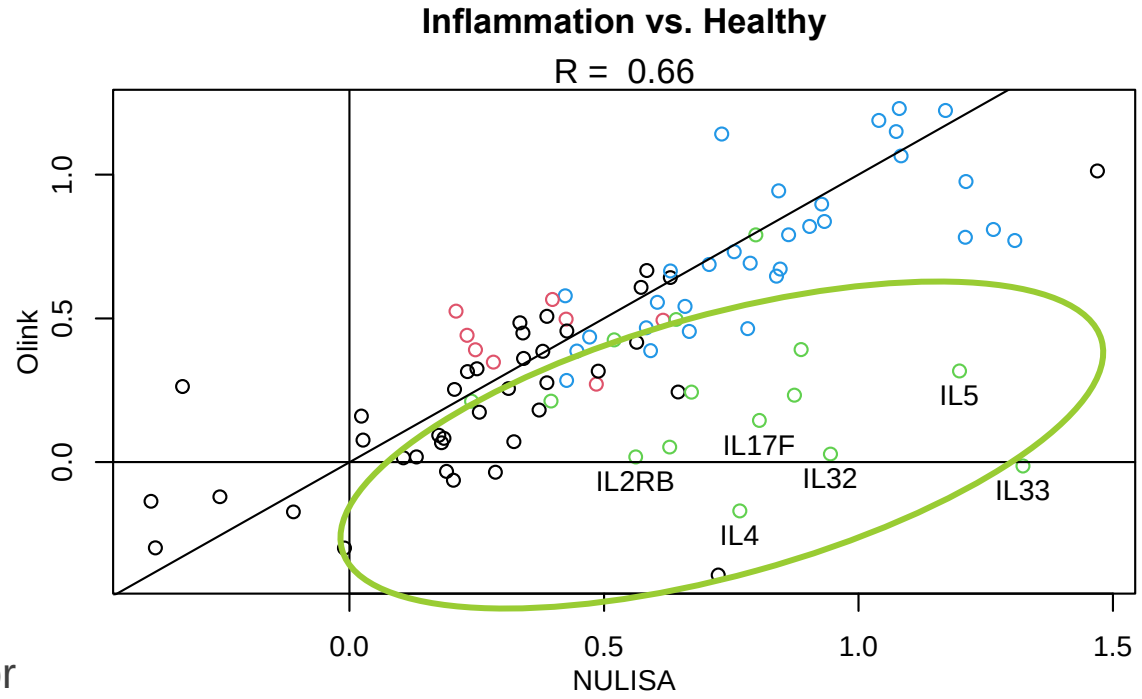
	CV% Plate 1 & 2 Average
NULISAseq	**7.3** (2.4) / **6.6** [4.1, 15.3]
Olink Explore	**9.4** (8.0) / **6.5** [2.6, 54.4]



NULISA shows greater detection of differential expression of key inflammatory markers versus Olink



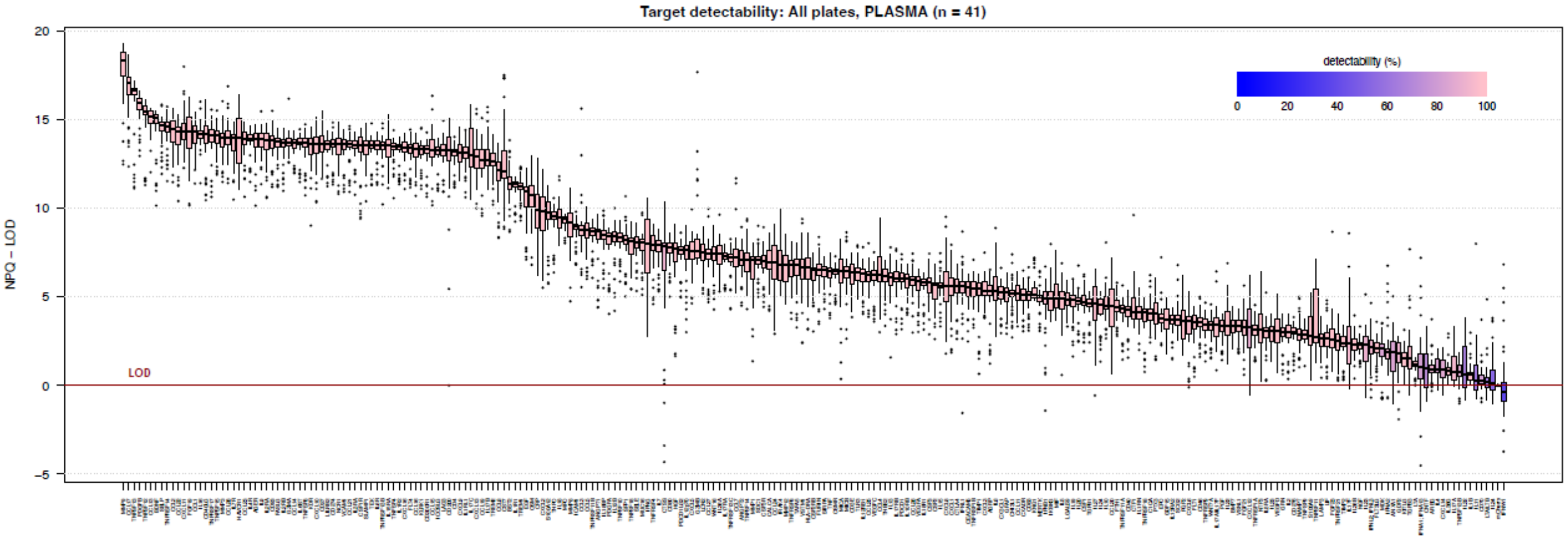
NULISA vs Olink fold-differences:
Inflammatory disease and healthy donor plasma



Feng, et al., (2023) BioRxiv

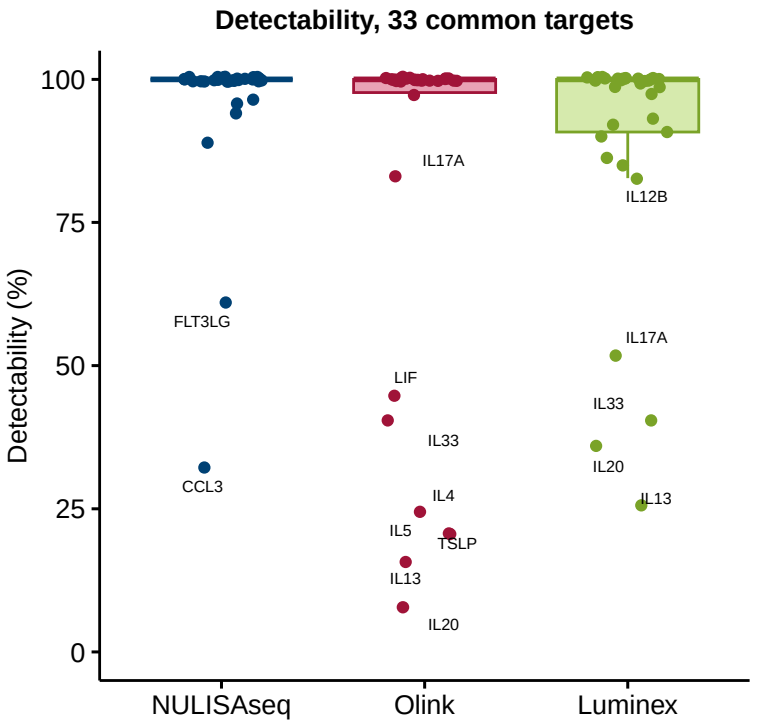
Detectability of NULISA Inflammation Panel in plasma

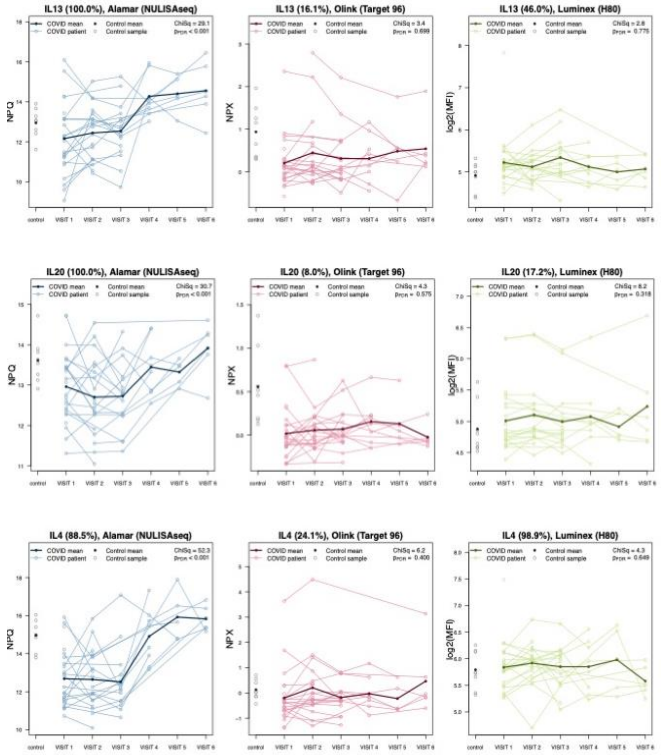
99.6% of targets were detectable in at least 50% of samples



Stanford Human Immune Monitoring Core Adopts NULISA and ARGO on performance and workflow automation

- NIH IMPACC Study of COVID-19 patients
- Three-way comparison between NULISAseq Inflammation Panel, Olink and Luminex.
- NULISAseq showed the highest detectability, which translated to biological insights not obtained from the other two platforms
- Feedback from this customer: **impressed with high sensitivity and fully automated workflow**
- Signed up as first beta customer.





"NULISA platform showed a higher sensitivity for a fair number of analytes and beyond the system's sensitivity, the fact that it comes packaged as an automated platform is attractive, especially given that PEA has historically been a complicated process."

NULISAseq™ CNS Disease Panel – *Available Q1 2024*

Targets (120 Plex)

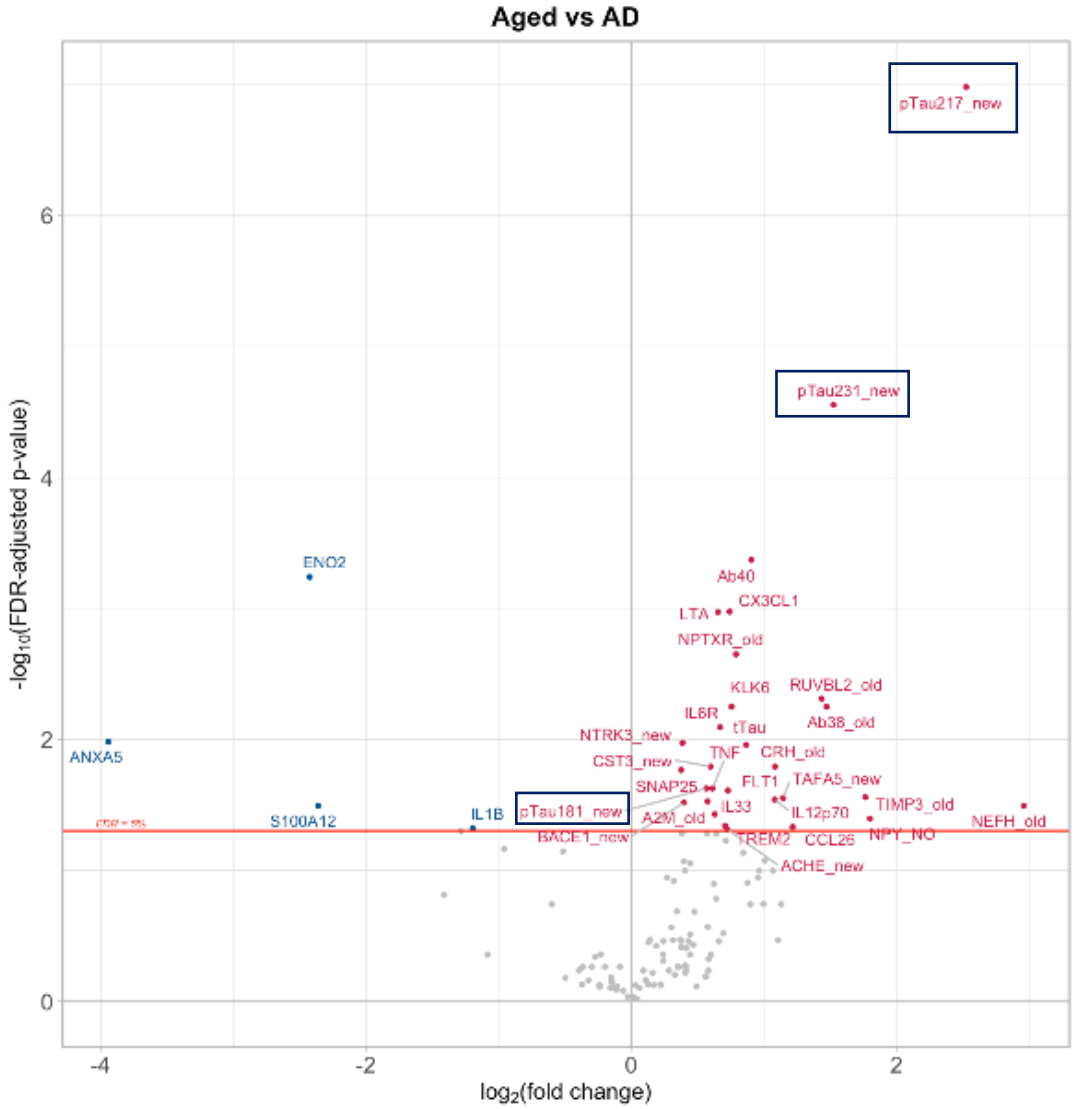
Amyloid Beta 38	GDI1	MCP1	PTN
Amyloid Beta 40	GDNF	MCP4	REST
Amyloid Beta 42	GFAP	MDC	RUVBL2
ACHE	GM-CSF	MDH1	S100A12
AGRN	GOT1	MIP1a/CCL3	S100B
ANXA5	Hemoglobin subunit Alpha	Mip1b/CCL4	SAA1
ARSA	ICAM1	MME	SFRP1
BACE1	IFN-gamma	Mesothelin	SFTPD
BASP1	IGF1	NCAM1	SLIT2
BDNF	IGFBP7	NEFH	SNAP25
CALB2	IL-1 beta	NF-L/NEFL	SNCAagg
CD40L/TNFSF5	IL-10	NGF	SNCAmono
CD63	IL-12p70	NPTX1	SOD1
CHIT1	IL-13	NPTX2	SQSTM1
CNTN2	IL-15	NPTXR	sTREM1
CRH	IL16	NPY	TAFA5
CRP	IL-17A	NRGN	TARC/CCL17
CST3	IL18	PARK7	TDP43
CX3CL1/Fractalkine	IL-2	PDGFRB	Tie-2/TEK
CXCL1/GROa	IL-33	PDLIM5	TIMP3
ENO2	IL-4	PGK1	TNF-a
Eotaxin	IL5	PLGF	TREM2
Eotaxin-3	IL-6	POSTN	tTau
FABP3	IL-6R a	PRDX6	UCHL1
FCN2	IL-7	PSEN1	VCAM1/CD106
FGF basic	IL8	p-TARDBP-409	VEGF R1
FOLR1	IL9	pTau181	VEGF R2
GAP43	IP-10	pTau217	VEGF-A
GDF15	KLK6	pTau231	VEGF-D

Designed with input from pharma and academic KOLs

Enabled by NULISA's combination of ultra-sensitivity and high-plex

Early-stage disease markers (pTau217, NFL, GFAP) and neuro-inflammation markers in single panel

CNS Disease Panel: Detecting key differences in plasma between age-matched and Alzheimer's Disease samples



Significantly up-regulated markers include:

- Phospho-Tau181
- Phospho-Tau217
- Phospho-Tau231

Significantly down-regulated markers include:

- S100A12
- ENO2

ARGO™ HT Instrument

Precision Proteomics at the Push of a Button

Simple, automated workflow
<30 min hands-on time

Integrated data analysis
Cloud-based analytics

Minimal sample input
10 – 20 µL

Single plex results <8 hrs
On-board qPCR analysis

Multiplex workflow
Outputs NGS-ready libraries

High throughput
Up to 288 samples in 3 x 96-well plates per day

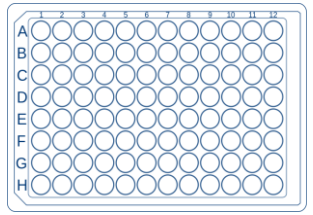
Proteomics at the **push of a button**

Enabling global access of proteomic analysis from sample to data

Fully **Automated** Workflow with **ARGO™ HT**

1
LOAD

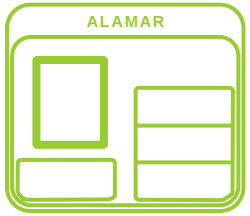
Simple, robust and reproducible



< 30 min hands-on time

2
RUN

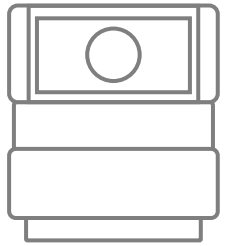
High throughput sample-to-results



< 8 hours

3
NGS

NextSeq2K (500m reads/ run)



~ 8 hours

4
ANALYZE

Cloud-based analytics

ARGO™ System **offers 3x faster and 20x simpler workflow solution**

1 2 3 4

5 6 7

7	>200 Step	3 Days
Instruments	Workflow	Sample to Result

Integrated qPCR System inside

NextSeq™ 2000

2	10 Step	1 Day
Instruments	Workflow	Sample to Result


ALAMAR
Experiment 0807-100805
1
Bay Unavailable
2
Bay Unavailable
3

Thank you for your attention

Contact:

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dhinerfeld@alamarbio.com

For more information
Visit www.alamarbio.com

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