Integrated Multiomics Profiling of Lysates Using nCounter® Protein and RNA Panels for Comprehensive Oncology Insights

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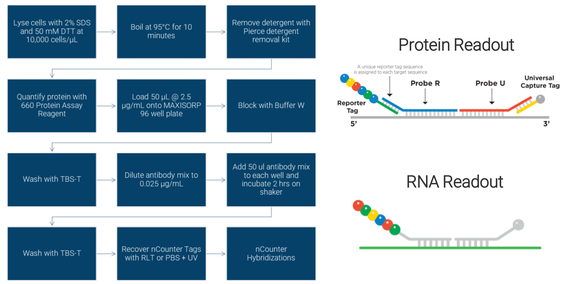
Introduction

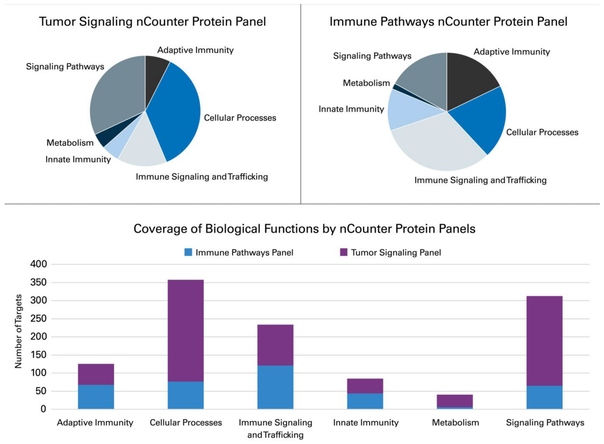
Multiomic approaches that integrate transcriptomic and proteomic measurements are critical for understanding complex biology in oncology, where pathway activity, drug response, and biomarker programs often require both RNA and protein context. The nCounter platform enables simultaneous quantification of RNA and protein targets from the same sample, reducing workflow variability while maximizing biological insight, building on the demonstrated translational and clinical utility of established nCounter mRNA assays (e.g., Prosigna® [FDA-cleared], Lymph2Cx, and Merck's Tumor Inflammation Signature [TIS]).

We evaluated the feasibility of lysate-based multiomics using an nCounter lysate workflow designed for streamlined cell line screening. As a proof of concept, 41 commercially available cell lysates were profiled, leveraging a stackable high-plex protein configuration composed of the nCounter Tumor Signaling Panel plus Immune Pathways (500+ Abcam RabMab monoclonal antibodies when stacked). The assay showed high reproducibility between replicates with a Pearson R ≥ 90%. We then applied orthogonal pathway perturbations in A-431 lysates (Calyculin A, pervanadate, and phosphatase treatment) to drive expected phosphorylation changes, providing a functional specificity check that phospho-epitope measurements respond in the anticipated direction in a multiplex format. To further demonstrate end-to-end workflow feasibility, we also tested unpurified lysate as direct input in an nCounter RNA assay using an internal 24-plex housekeeping RNA panel, enabling a straightforward path to paired 700-plex nCounter RNA + 550-plex protein readouts from the same lysate sample. The nCounter Lysate Assay provides a streamlined, reproducible way to screen hundreds of pathway-relevant proteins with demonstrated feasibility for direct RNA readout from the same lysate, thereby helping teams generate actionable multiomic insight faster while staying on a single, integrated nCounter workflow.

nCounter Lysate Workflow



Stackable Panel Content with up to 550 Targets



Profiled 41 Cell Lines from Diverse Backgrounds

Tissue of origin	Cell line	Cellosaurus ID	Disease
Blood / Bone marrow	CCRF-CEM	CVCL_0207	Acute lymphoblastic leukemia
	HEL	CVCL_0001	Erythroleukemia
	HL-60	CVCL_0002	Acute promyelocytic leukemia
	HL-60 + DMSO	CVCL_0002	Acute promyelocytic leukemia
	HL-60 + PMA	CVCL_0002	Acute promyelocytic leukemia
	K562	CVCL_0004	Chronic myelogenous leukemia
	Molt-4	CVCL_0013	Acute lymphoblastic leukemia
	RPMI-8226	CVCL_0014	Multiple myeloma
	THP-1	CVCL_0006	Acute monocytic leukemia
Blood / Lymph node	Daudi	CVCL_0008	Burkitt lymphoma
	NK-92	CVCL_2142	Natural killer cell lymphoma / leukemia
	Raji	CVCL_0511	Burkitt lymphoma
	U-937	CVCL_0007	Histiocytic lymphoma
Bone	U2OS	CVCL_0042	Osteosarcoma
Brain	U-251MG	CVCL_0021	Glioblastoma
Breast	MCF-7	CVCL_0031	Breast adenocarcinoma
	MDA-MB-231	CVCL_0062	Breast adenocarcinoma
	MDA-MB-468	CVCL_0419	Breast adenocarcinoma
	SK-BR-3	CVCL_0033	Breast adenocarcinoma
Cervix	HeLa	CVCL_0030	Cervical adenocarcinoma
	SiHa	CVCL_0032	Cervical squamous cell carcinoma
Colon	Caco-2	CVCL_0025	Colorectal adenocarcinoma
	HCT116	CVCL_0291	Colorectal carcinoma
	HT-29	CVCL_0320	Colorectal adenocarcinoma
Umbilical vein endothelium	HUV-EC-C + VEGF	CVCL_2959	Endothelial cell (normal)
Kidney	A498	CVCL_1056	Renal cell carcinoma
	Caki-1	CVCL_0234	Clear cell renal cell carcinoma
Liver	HUH7	CVCL_0336	Hepatocellular carcinoma
Lung	H1975	CVCL_1511	Lung adenocarcinoma (NSCLC)
Nervous system	SH-SY5Y	CVCL_0019	Neuroblastoma
Pancreas	ASPC1	CVCL_0152	Pancreatic ductal adenocarcinoma
	PANC-1	CVCL_0480	Pancreatic ductal carcinoma
Prostate	LNCaP clone FGC	CVCL_0395	Prostate adenocarcinoma
	PC-3	CVCL_0035	Prostate adenocarcinoma
Skin	SK-MEL-28 + IFN alpha	CVCL_0526	Melanoma
	A-431	CVCL_0037	Epidermoid carcinoma
	A-431 + Antarctic Phosphatase	CVCL_0037	Epidermoid carcinoma
	A-431 + Calyculin A	CVCL_0037	Epidermoid carcinoma
	A-431 + EGF	CVCL_0037	Epidermoid carcinoma
	A-431 + Pervanadate	CVCL_0037	Epidermoid carcinoma
	A-431 + UV	CVCL_0037	Epidermoid carcinoma

Table 1 | Cell line panel profiled for lysate assay development and benchmarking. Table lists the human cell lines used in this study, grouped by tissue of origin, with associated disease annotation and Cellosaurus (CVCL) identifiers to ensure unambiguous cell line provenance and cross-study traceability. The panel spans hematologic and solid tumor lineages and includes selected perturbation/stimulation conditions (e.g., HL-60 ± DMSO/PMA and A-431 derivative conditions) to support performance assessment across diverse biological backgrounds.

Reproducible Results between Cell Line Replicates

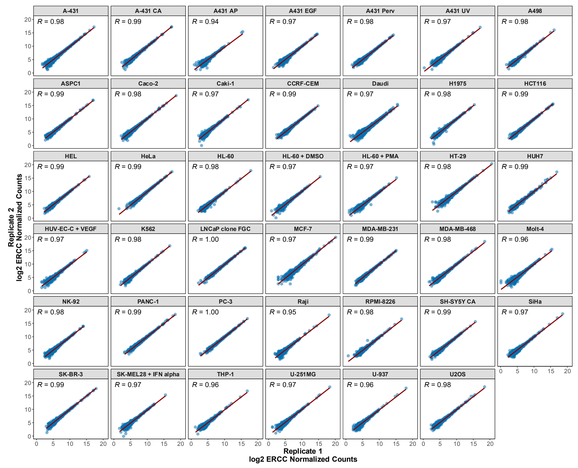


Figure 1 | Technical reproducibility across 41 lysates profiled with the nCounter Lysate assay. Each lysate was run in duplicate using the nCounter Lysate Assay and a high-plex protein panel (~550 antibody targets). Raw counts were sample-to-sample normalized using internal ERCC controls and then log2-transformed. Replicate 1 values were plotted against replicate 2 values. Pearson correlations were consistently high (R ≥ 0.90), demonstrating strong assay reproducibility.

Phospho-specific Targets Detected and Demonstrate Specificity

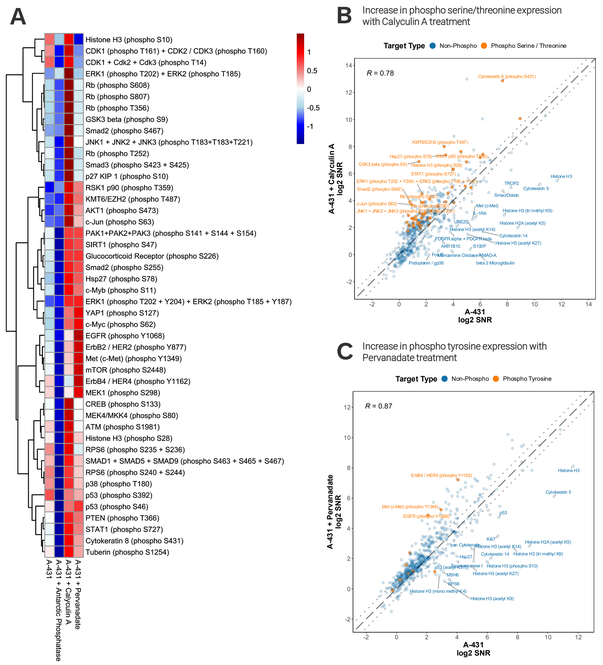


Figure 2 | Perturbation controls produce expected phospho-epitope shifts in A-431 lysates (nCounter ~550-plex), supporting phospho-antibody specificity. A-431 control lysate and matched treatments (Calyculin A, pervanadate, and post-lysate Antarctic phosphatase) were profiled with the nCounter high-plex protein panel (~550 antibody targets). Calyculin A inhibits PP1/PP2A (Ser/Thr phosphatases) and increases Ser/Thr phospho targets, whereas pervanadate inhibits protein tyrosine phosphatases and increases phospho-tyrosine targets; Antarctic phosphatase reduces phospho-antibody signal via global dephosphorylation. (A) Heatmap of phospho targets shown as z-scored log2(SNR) (SNR computed using internal IgG negative controls). (B–C) Target-wise correlation plots of log2(SNR) for A-431 control (x-axis) versus Calyculin A or pervanadate (y-axis); phospho targets (orange) shift above y = x black dashed identity line. Dotted gray line represents a ±1.5X change from y = x. .

Lysates Compatible with the nCounter RNA Assay for a Complete Multiomics Workflow

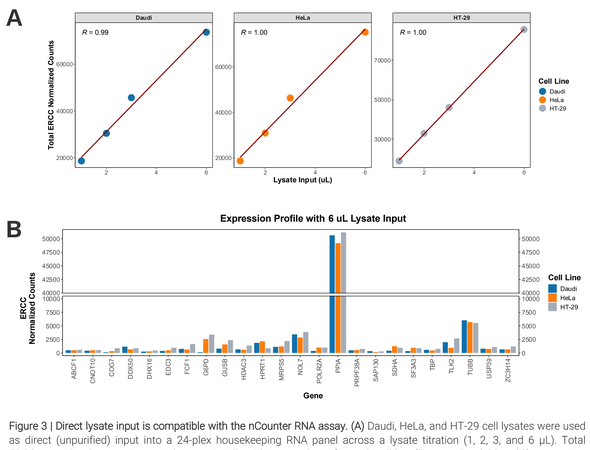
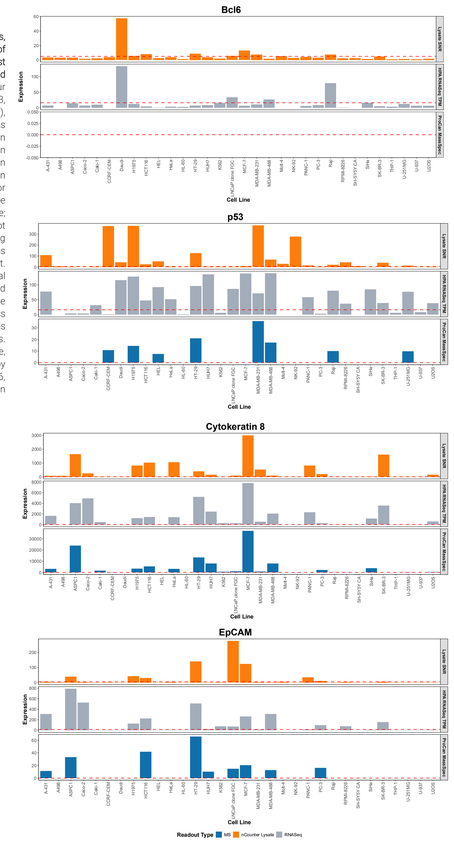


Figure 3 | Direct lysate input is compatible with the nCounter RNA assay. (A) Daudi, HeLa, and HT-29 cell lysates were used as direct (unpurified) input into a 24-plex housekeeping RNA panel across a lysate titration (1, 2, 3, and 6 µL). Total ERCC-normalized counts increased proportionally with input volume for each cell line (Pearson R = 0.99–1.00), supporting feasibility of running crude lysate directly in the nCounter RNA workflow. (B) Lysates were loaded directly into the nCounter RNA assay at 6 µL input. Bars show ERCC-normalized counts for each housekeeping transcript across the three cell lines, illustrating distinct expression patterns.

Targets Screened against Orthogonal RNA-Seq and Mass Spectroscopy Datasets

Figure 4 | With overlapping cell lines, cross-platform benchmarking of lysate protein signal against orthogonal mass spectrometry and RNA-seq references. For four representative targets (BCL6, p53, Cytokeratin 8, and EpCAM), nCounter lysate signal (SNR) was compared with orthogonal ProCan mass spectrometry protein abundance and Human Protein Atlas (HPA) RNA-seq expression across a panel of cell lines. For each target, bars show the three readouts side-by-side by cell line; missing bars indicate cell lines not present in the corresponding orthogonal dataset, or target was not detected in orthogonal readout. BCL6 illustrates a case where signal is observed in the lysate assay and supported by RNA-seq, while the corresponding ProCan mass spectrometry reference is unavailable for some cell lines. Horizontal lines in nCounter Lysate, RNA-Seq, and mass spectroscopy plots represent SNR = 5, tpm = 16, and mass spectroscopy protein abundance = 0, respectively.



Conclusion and Future Actions

1. High-plex lysate protein screening is feasible at scale
2. The lysate assay is highly reproducible: replicate lysate runs show consistently high concordance (Pearson R ≥ 0.90), supporting reliable quantitative screening.
3. Phospho antibodies demonstrate specificity with varying perturbations.
4. RNA readout is compatible with the lysate workflow: unpurified lysate can be used directly as input for an nCounter RNA assay.

Future Actions

1. Run a higher-plex RNA panel (beyond housekeeping) to enable richer pathway interpretation alongside the ~550-plex protein readout
2. Expand integrated multiomics analysis outputs (e.g., combined visualizations/paired analyte views) to translate lysate screening into interpretable biology.
3. Continue protocol optimization for lysate performance (e.g., binding/blocking/substrate variables) to further improve sensitivity across targets and sample types.

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