Deep mass spectrometry profiling of 10,000+ proteins in FFPE tissue reveals novel functional biology

SAPIENT

Jeramie Watrous, Joseph Capri, Kate Gallagher, Sejun Park, Tao Long, Jessica Weant, Mo Jain

Introduction

Formalin-fixed, paraffin-embedded (FFPE) tumor tissue represents the largest, most clinically annotated oncology resource available, yet the proteome – which provides the most direct readout of target abundance, pathway activation, immune contexture, and therapeutic response – has remained largely inaccessible in these samples at scale.

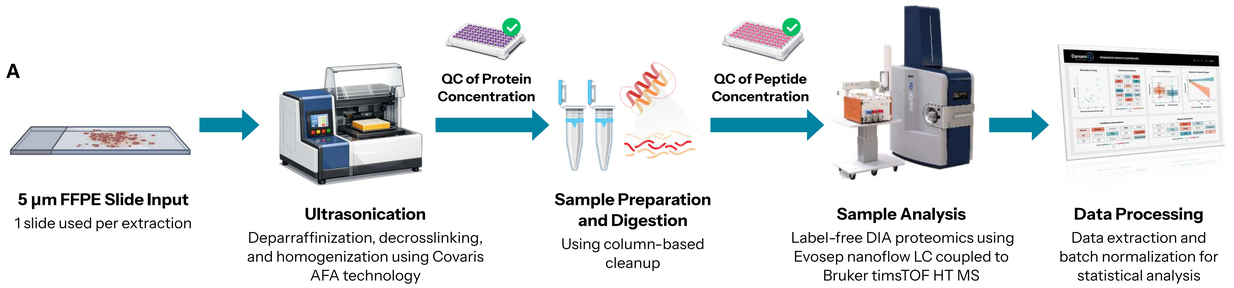
Fixation effects on protein structures largely limit profiling approaches to DNA and RNA sequencing or low-plex immunohistochemistry (IHC) which cannot quantify important functional tumor biology, assay specific protein isoforms, or uncover novel protein targets for therapeutic development.

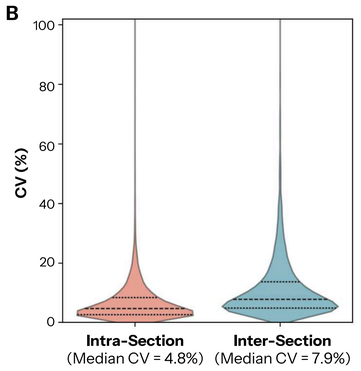
Herein we describe the development of a next-generation mass spectrometry-based FFPE proteomics workflow enabling the large-scale, quantitative measure of 10,000+ protein groups in a single 5 µm FFPE slide.

Methodology

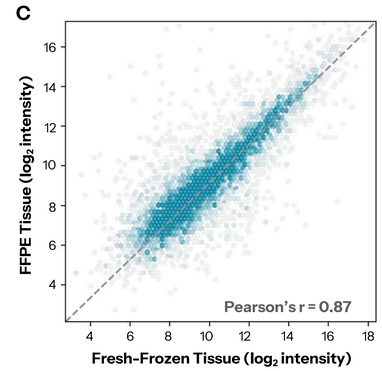
The FFPE proteomics workflow uses Covaris AFA® technology for ultrasonic deparaffinization and decrosslinking, followed by column-based cleanup and digestion for high-yield peptide recovery from minimal FFPE sample input. Processed samples are assayed via **label-free quantitative DIA proteomics analysis using Evosep™ nanoflow liquid chromatography coupled to Bruker timsTOF HT mass spectrometers (Fig A)**.

The workflow achieves **exceptional reproducibility across technical replicates and across sequential samples from a given FFPE block (Fig B),** as well as close reproducibility in proteins assayed and quantitation **relative to matched fresh-frozen tissue samples (Fig C).** Among the 10,000+ proteins measured are known oncogenes, approved drug targets, and emerging drug targets **(Fig D)**.

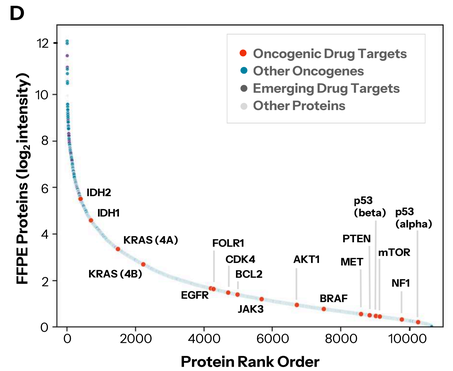




Technical median coefficient of variation (CV) is < 5% for repeat samples and < 8% across serial FFPE sections.



Greater than 0.8 correlation is observed between matched FFPE and fresh-frozen samples, with 97% protein overlap.



Proteins measured in FFPE tissue include more than 200 oncogenes and 500 approved drug targets.

Results

Proteomics analysis of FFPE samples has been **applied across diverse tumor types, including lung, gynecologic, breast, colorectal, and pancreatic cancers, as well as critical normal tissue** for differential protein expression analysis, enabling direct measurement of 10,000+ protein groups in a 5 µm FFPE slide or curl section.

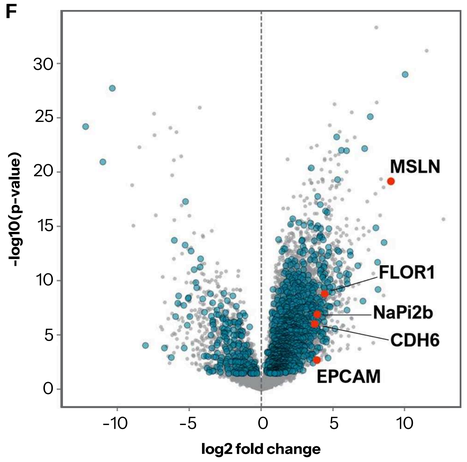
Proteomics data reveals **novel insights into functional tumor signaling, immune activation, and resistance mechanisms** with single protein and pathway-level resolution **(Fig E)**.

E

Pathway / Geneset	Category	Representative Detected Proteins in FFPE Colon Tumor
PI3K/AKT/mTOR signaling	Oncogenic signaling	AKT1, AKT2, AKT3, FOXO3, GSK3B, MTOR, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PIK3R2, PTEN, RICTOR, RPS6, RPS6KB1, RPTOR, TSC1, TSC2
MAPK / RAS–RAF–MEK–ERK signaling	Oncogenic signaling	ARAF, BRAF, GRB2, HRAS, KRAS, MAP2K1, MAP2K2, MAPK1, MAPK14, MAPK3, MAPK8, MAPK9, NF1, NRAS, RAF1, RPS6KA1, RPS6KA3, SHC1, SOS1
Receptor tyrosine kinase signaling	Oncogenic signaling	AXL, DDR1, EGFR, EPHA2, EPHB2, ERBB2, ERBB3, IGF1R, INSR, MET, PDGFRB
Cell cycle & proliferation (E2F / G2M / mitotic)	Oncogenic signaling	AURKA, AURKB, CCNB1, CDK1, CDK2, CDK4, CDK6, MCM2, MCM3, MCM4, MCM5, MCM6, MCM7, MKI67, PCNA, PLK1, RB1, TOP2A, TPX2
Apoptosis & anti-apoptotic regulation	Resistance mechanism	APAF1, BAK1, BAX, BCL2, BCL2L1, BCL2L2, BID, BIRC2, CASP3, CASP6, CASP7, CASP8, CASP9, CFLAR, DIABLO, FADD, MCL1, XIAP
DNA damage response & repair	Resistance mechanism	ATM, ATR, CHEK2, ERCC1, ERCC2, ERCC3, FANCA, LIG1, LIG3, LIG4, MGMT, MLH1, MRE11, MSH2, MSH6, NBN, PARP1, PMS2, RAD50, XRCC1, XRCC4, XRCC5, XRCC6
EMT & lineage plasticity	Resistance mechanism	ACTA2, CDH1, CDH2, COL1A1, COL1A2, COL3A1, FN1, MMP14, MMP2, MMP9, S100A4, SPARC, TAGLN, VIM, ZEB1, ZEB2
Antigen processing & presentation (MHC I/II)	Immune signature	B2M, CALR, CANX, CD74, ERAP1, ERAP2, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-DRB1, HLA-E, HLA-F, HLA-G, PSMB8, PSMB9, TAP1, TAP2, TAPBP
Interferon-γ / JAK–STAT immune signaling	Immune signature	GBP1, GBP2, GBP5, IFNGR1, IRF8, IRF9, ISG15, JAK1, JAK2, MX1, OAS1, STAT1, STAT2, STAT3
Cytotoxic immune effector & T-cell infiltration	Immune signature	CD3D, CD3E, GZMA, GZMK, LCK, LCP2, PRF1, ZAP70

Analysis of ovarian tumor samples and matched control tissues reveals differential expression of known targets as well as dozens of novel proteins and protein isoforms for tumor targeting. Through **selective tagging and enrichment of tumor cell surface proteins (SurfaceSeek™), we further identify a number of differentially expressed surface proteins** optimal for ADC or T-cell engager therapies **(Fig F)**.

F



FFPE proteomics analysis of ovarian tumor and matched normal tissue reveals differentially expressed protein targets, including both established (**orange**) and novel cell surface proteins (**blue**) for therapeutic development.

Conclusion

Sapient's next-generation FFPE proteomics method **unlocks previously inaccessible functional biology in FFPE samples, enabling novel biomarker discovery, faster target identification and validation, and mechanistic context** for accelerating drug development.

Learn more about FFPE proteomics

https://sapient.bio | discover@sapient.bio