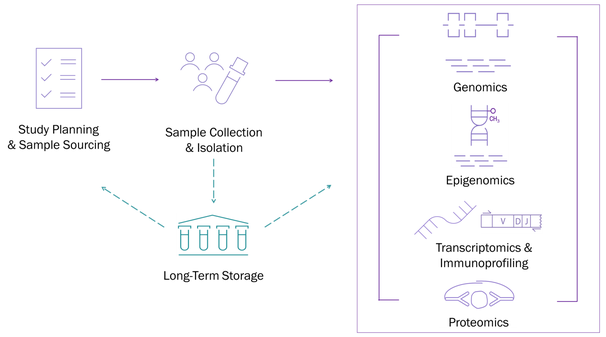
Harnessing the Power of Multiomics from Fresh and Fixed Patient Sample Characterization

Andrea O'Hara, Yang Han, Yongjun Fan, David Corney, Chris Mozdzierz, Haythem Latif, Ginger Zhou
GENEWIZ Multiomics & Synthesis Solutions from Azenta Life Sciences, South Plainfield, NJ

Abstract

The omics era has greatly expanded the repertoire of approaches available for researchers and clinicians to unravel the complexity underpinning human health: Next Generation Sequencing (NGS) approaches can characterize genomes, epigenomes, transcriptomes, and proteomes. The analyses are critical to assess in individuals both pre- and post-treatment during therapeutic development and early-stage clinical trials. Peripheral blood mononuclear cells (PBMCs) offer a non-invasive approach that, when combined with omics tools, can provide a near holistic view of immune processes across patient cohorts. Meanwhile, Formalin Fixed Paraffin Embedded (FFPE) tissues are a staple in clinical diagnostics and an ideal means to store archival tissue but can be difficult to work with in traditional NGS assays.

Here we detail workflows using both fresh and fixed patient samples to rapidly produce a diverse set of multiomics results including genomics, epigenomics, transcriptomics, and proteomics.



For fresh blood draws, this starts with automated sample handling and processing to ensure high viability and yield of PBMCs, along with simultaneous plasma separation and collection. Samples are then aliquoted and simultaneously processed for whole exome sequencing, single cell RNA sequencing, epigenetic characterization, and Olink biomarker analysis. For fixed tissues, FFPE blocks were serially sliced into various FFPE slides, with a single slide H&E stained. Individual slides were then utilized for genome, epigenome, single cell RNA-seq, and digital spatial profiling. Genome information was captured using hybrid capture based approaches followed by deep NGS on an Illumina platform and analyzed for a variety of variants and tumor mutational burden. DNA methylation was detailed using target capture probes targeting DNA methylation sites. Single cell approaches were applied to explore the transcriptome using 10X Genomics scRNAseq kit, and Digital Spatial Profiling (DSP) was done using the NanoString GeoMx® Whole Transcriptome Atlas and immunostaining.

With these robust workflows, all these datatypes can be produced within days of fresh or fixed sample receipt using minimal sample amounts. High throughput integrative omics workflows, as described here, drive greater insights in human health, allowing for a rapid combined approach to address the biological questions at hand.

Conclusions

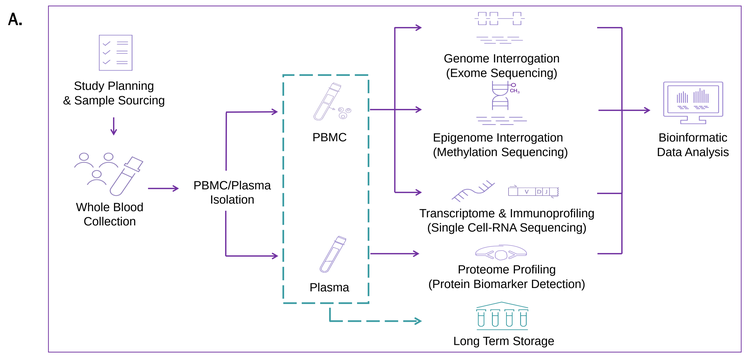
- Complex multiomics information can be collected from a single sample, including genomics, transcriptomics, epigenomics and proteomics investigation.
- Integrated analysis allows for deeper insights into post- treatment response and biomarkers for future discovery.
- Multiomics workflows can be modified to accommodate a variety of input sample types, allowing for flexible sample processing.
- Cutting-edge technologies such as scRNAseq and Spatial Profiling allow for deep exploration of the heterogenous tissues.

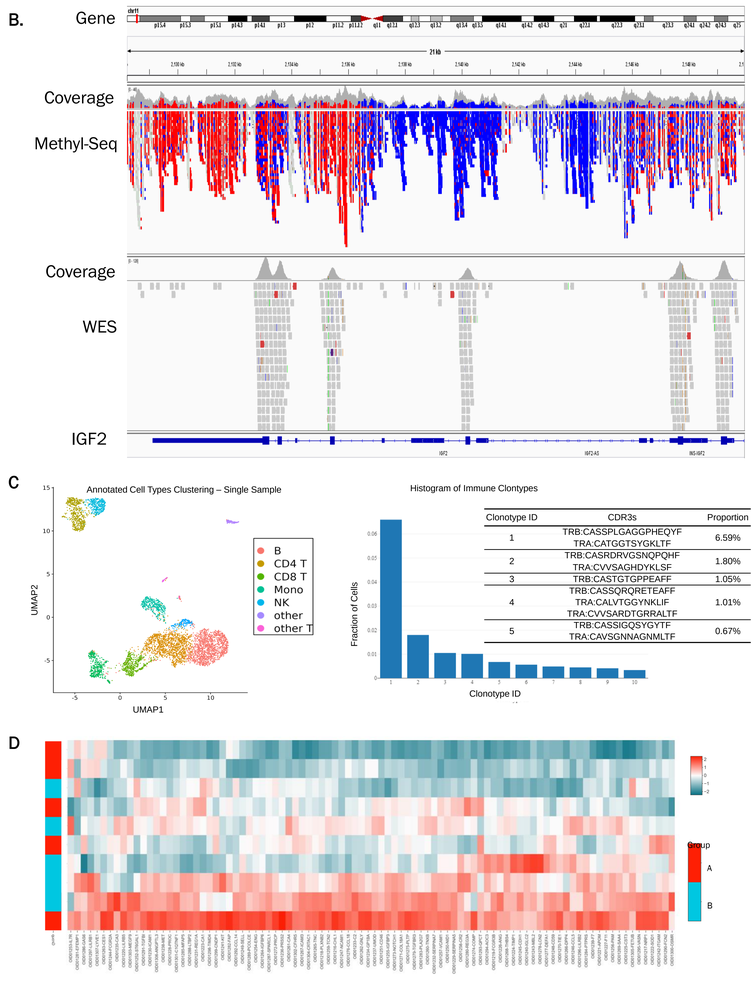
GENEWIZ® From Azenta Life Sciences

Visit us at Booth #933

Scan to download or learn more

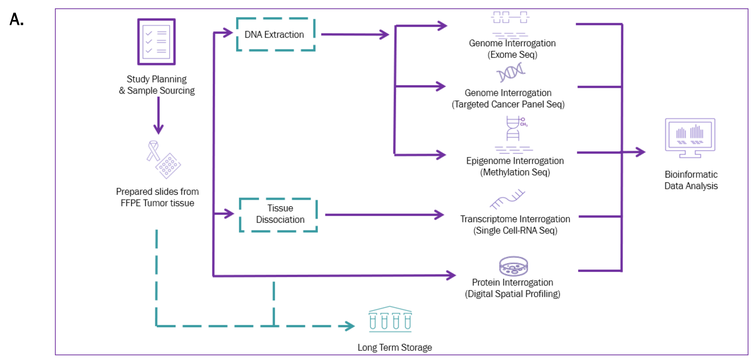
Results





Clonotype ID	CDR3s	Proportion
1	TRB:CASSPLGAGGPHEQYF TRA:CATGGTSYGKLTF	6.59%
2	TRB:CASRDRVGSNQPQHF TRA:CVVSAGHDYKLSF	1.80%
3	TRB:CASTGTGPPEAFF	1.05%
4	TRB:CASSQRQRETEAFF TRA:CALVTGGYNKLIF	1.01%
5	TRB:CASSIGQQSYGYTF TRA:CAVSGNNAGNMLTF	0.67%

Figure 1. A. Schematic of liquid biopsy multiomics sample processing workflow. Briefly, venipuncture whole blood from donors were collected in heparin tubes and shipped to Azenta Laboratories for PBMC and plasma isolation within 24 hrs of blood draw. **B. Data visualization of whole genome methylation sequencing and whole exome sequencing.** DNA was isolated from frozen PBMC pellets. Exome panel hybrid capture libraries were sequenced to ~100X raw coverage, data were analyzed using Sentieon DNAScope pipeline. For Methyl-Seq, whole genome enzymatic Methyl-Seq was sequenced to ~30X raw coverage. Methyl-Seq data was aligned and analyzed using Bismark. BAM files were loaded to IGV to show coverage and alignments. Methyl-Seq tracks were colored by IGV Bisulfite CG mode. **C. Single cell whole transcriptomic sequencing and immune profiling of one representative sample.** Cryopreserved PBMCs were thawed, counted and processed for single cell RNA-seq (left) and VDJ libraries capturing the T-cell repertoire (right). Histogram and table of the most abundant T-cell clonotypes in the sample. D. **Protein Biomarker Assay shows patient specific profiles.** Plasma was isolated from 10 donor whole blood samples and subsequently analyzed by Olink Target 96 Cardiometabolic protein panel. After data QC, primary data analysis and normalization to Normalized Protein eXpression (NPX) units, expression values of select proteins were plotted as a heatmap, generated by Olink Insights. Grouping information of 10 samples were shown on the left.



B.

Top Mutations - Colon

SYMBOL	Variant	Allele Depth	Freq.	Consequence	IMPACT
FOXA1	14:38061742-C>T	8,811,337	0.602	Missense	MODERATE
KMT2D	12:49434685-CCTT>C	685,874	0.560	Inframe Deletion	MODERATE
PIM1	6:37139100-T>G	11,751,273	0.520	Missense	MODERATE
NUP93	16:56855472-C>G	743,794	0.517	Missense	MODERATE
BARD1	2:215632255-CA>TG	11,481,144	0.499	Missense	MODERATE
HLA-C	6:31239378-T>G	821,711	0.464	Missense & Splice Variant	MODERATE
HLA-C	6:31239577-A>C	16,911,314	0.437	Missense	MODERATE
SH2B3	12:111884608-T>C	13,851,058	0.433	Missense	MODERATE
ERBB2	17:37880220-T>C	1,452,472	0.246	Missense	MODERATE
MGA	15:42042023-A>G	2,361,452	0.161	Missense	MODERATE
TP53	17:7578529-A>G	1,239,203	0.141	Missense	MODERATE
POLD1	19:50912061-G>C	801,119	0.130	Missense	MODERATE

Top Mutations – Lung

SYMBOL	Variant	Allele Depth	Freq.	Consequence	IMPACT
TP63	3:189526115-G>A	9,011,112	0.552	Missense	MODERATE
HLA-C	6:31237162-C>G	10,091,135	0.529	Missense & Splice Variant	MODERATE
DDR2	1:162724424-G>A	605,680	0.529	Missense	MODERATE
HLA-A	6:29910358-C>G	17,671,729	0.494	Missense	MODERATE
BARD1	2:215632255-CA>TG	819,792	0.492	Missense	MODERATE
PRDM1	6:106555110-G>A	10,951,024	0.483	Missense	MODERATE
LRP1B	2:141032087-GC>CT	850,790	0.482	Missense	MODERATE
RBM10	X:47044749-GC>G	637,178	0.219	Frameshift	HIGH
RBM10	X:47038537-C>A	854,185	0.178	Stop Gained	HIGH
MED12	X:70354590-G>A	780,162	0.172	Missense	MODERATE
NRAS	1:115256529-T>A	1,885,353	0.158	Missense	MODERATE
SLC34A2	4:25667797-C>T	1,511,234	0.135	Missense	MODERATE
KEL	7:142640662-G>T	1,098,161	0.128	Missense	MODERATE
TEK	9:27202833-C>A	861,110	0.114	Missense	MODERATE
FAM175A	4:84391470-A>G	1,669,202	0.108	Missense	MODERATE
GRIN2A	16:10032190-C>A	1,271,143	0.101	Missense	MODERATE

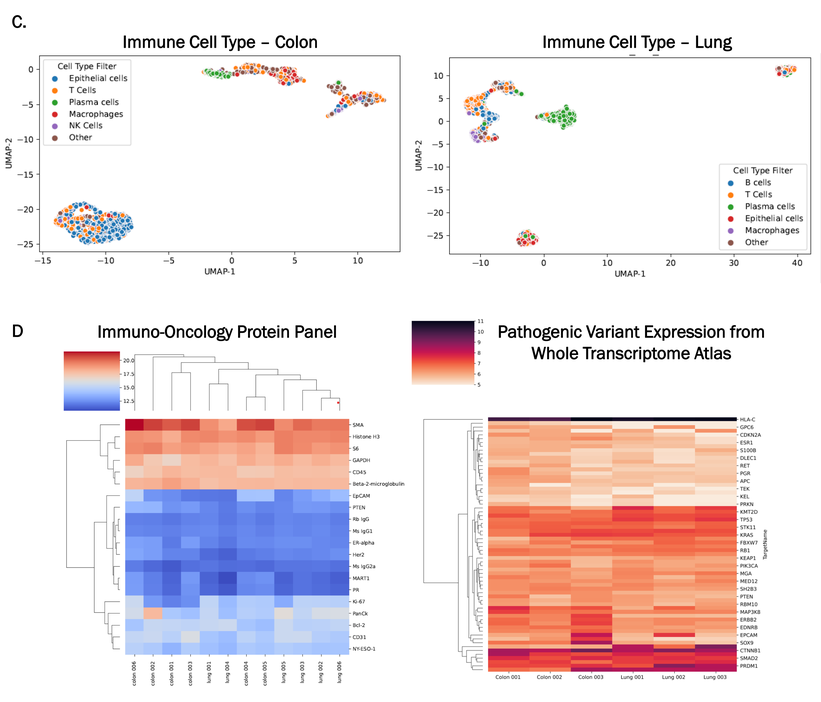


Figure 2. A. Schematic of FFPE multiomics sample processing workflow. FFPE archived tumor lung and colon blocks were sectioned into 25µm slides for studies. **B. Pan-cancer panel sequencing and targeted methylation sequencing elucidate sample specific signatures for colon (left) and lung (right) cancer samples.** DNA was isolated from 10µm FFPE. Cancer panel library was sequenced to ~2000X raw coverage and analyzed using Sentieon TNseq pipeline. Target Methyl-Seq using TWIST Methylome Panel with ~120x raw coverage was aligned and analyzed using Bismark. BAM files were loaded to IGV to show coverage and alignments. Methyl-Seq tracks were colored by IGV Bisulfite CHG mode. All mutations with a frequency of >10%, of moderate or high impact consequence are shown. **C. Single-cell gene expression results identify common tissue and immune cell types within lung and colon FFPE samples.** Samples were dissociated and single-cell sequencing was processed using the 10x Fixed RNA workflow. UMAP clustering of cells for colon (left) and lung (right) samples, identify tissue cells along with infiltrating immune cells in the tumor microenvironment. **D. GeoMx DSP protein panel and whole transcriptome atlas heatmap results on colon and lung cancer FFPE samples.** NanoString GeoMx Human Whole Transcriptome Atlas (WTA), Human Protein Core and Pan-Tumor panel were performed for spatial profiling. Clustering expression analysis of Immuno-Oncology protein panel (left) and mutated genes of interest (right), as described in B.