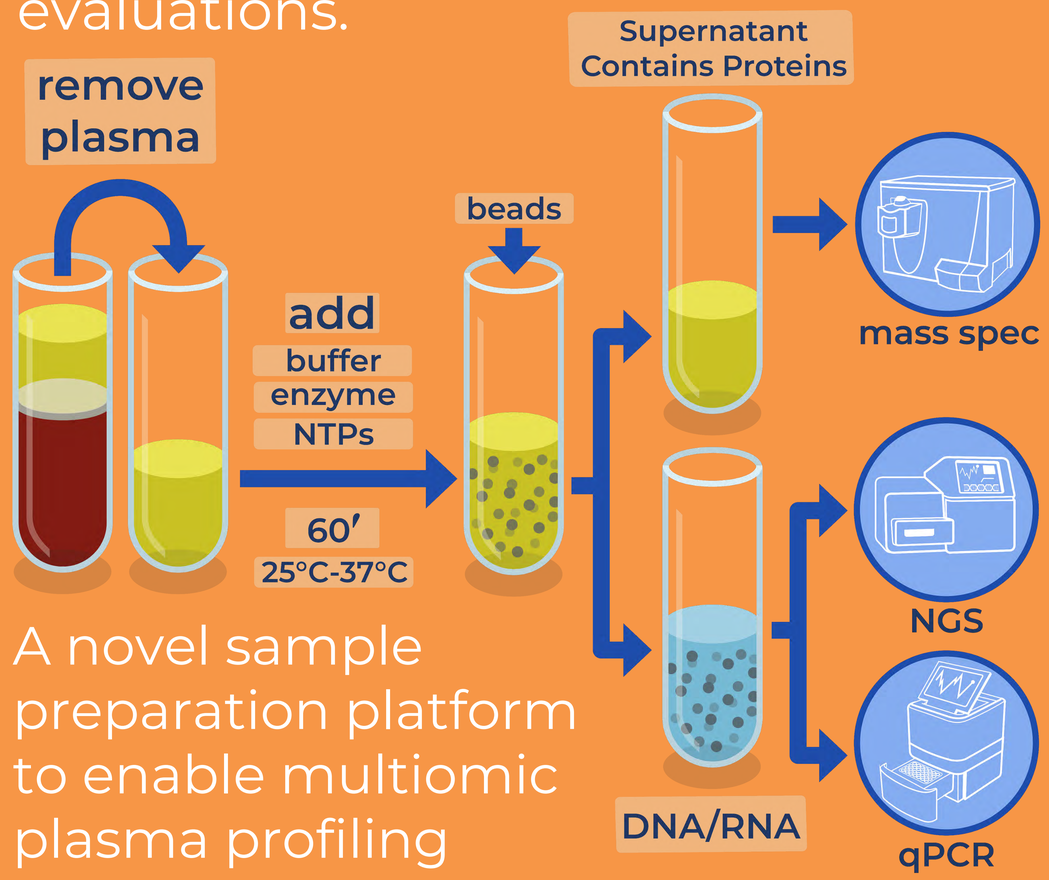
simplSEQ enables multiomic profiling from a single **plasma** sample.

A highly efficient, **non-destructive**, methodology to extract **nucleic acids** from plasma while enabling **proteomics** and additional –omics evaluations.



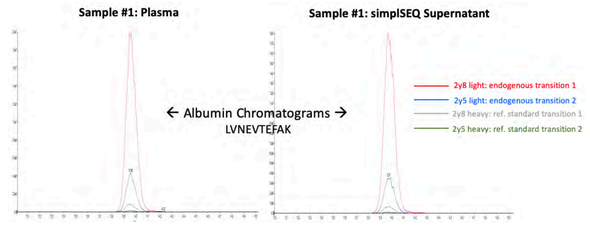
A novel sample preparation platform to enable multiomic plasma profiling

Brandon Young[1], Annie Moradian[2], Esthelle Hoedt[2], Susan Mockus[2], Baiju Parikh[3], John Spinosa[1]

[1]simplSEQ, [2]Precision Biomarker Laboratories at Cedars-Sinai, [3]InterpretBIO

Results

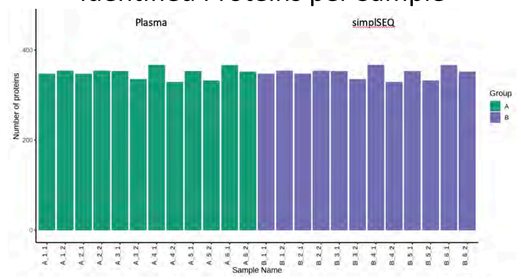
Mass Spec MRM on PBL's 60-protein Health Surveillance Panel (HSP)



Albumin chromatograms demonstrate protein integrity and recovery after simplSEQ processing.

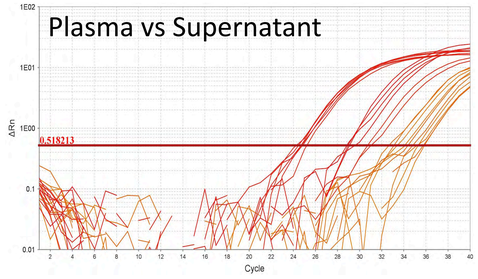
Mass Spec on PBL's Discovery Workflow (DIA)

Identified Proteins per Sample



The number of identified proteins between paired samples (plasma and simplSEQ supernatants) was similar, demonstrating the protein integrity and recovery after simplSEQ processing.

qPCR– comparison of free circulating NAs to cellular and exosomal NAs



Plasma (Red) vs lysed supernatant (Yellow). Plasma fraction that is tailed and attached to streptavidin beads compared to the attachment supernatant. The supernatant is removed and treated with a 0.1% IGEPAL to lyse cells and/or exosomes. This fraction was also tailed and bound to a new streptavidin beads (above). RNAse P Taqman assay used for RNA detection.

NGS – circulating DNA

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Coverage Depth	192	131	350	221	14	3	4
Coverage STDEV	171	326	615	175	12	20	9

Streptavidin beads were added directly into the AmpliSeq NGS protocol using a custom 37 gene panel. Samples 1-4 show high coverage depth for each marker or interest. Samples 5-7 show lower depth of coverage. The same samples corresponded to the higher Ct value samples in the qPCR reactions, In these 3 cases the plasma had precipitate and was more viscous which led to bead aggregation. Asking participants to observe an overnight fast before blood collection could minimize this effect.

Introduction

Multiomic profiling constitutes the next critical step in fully analyzing and elucidating biological processes underpinning disease progression and immune response. Traditionally samples are divided into equal parts for use in specific protocols for isolation of DNA, RNA and proteins. These protocols are optimized to remove the other components to increase data quality of downstream assays. Removal of these components, often using irreversibly damaging chemicals, provides lower yields due to decreased input amounts while also discarding the valuable material these specific kits are designed to remove. The simplSEQ enzymatic assay was designed to isolate and co-purify DNA and RNA without the need for proteases and chaotropic salts that damage proteins and metabolites. By utilizing this enzymatic approach in biological conditions the nucleic acids are separated from the other analytes without damage allowing for direct analysis.

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Methods

Seven unique donors provided blood samples in EDTA blood collection tubes. Tubes were centrifuged at 600 x g for 15 minutes at room temperature within 30 minutes of collection. Plasma was removed and placed into 2 tubes of equal volume. One tube was frozen for comparative analysis, the second tube was run according to the simplSEQ protocol. Buffers, dNTPs and enzyme were added directly to the plasma tube. The tailing assays were run for 3 hours at room temperature with StreptAvidin beads added into the reaction mixture for 30 minutes. The plasma was removed after magnetic separation of StreptAvidin beads and placed in a new tube for proteomics analysis at the Precision Biomarker Laboratories (PBL) of Cedars-Sinai.

Mass spec MRM: 20 µg of proteins from each sample were subjected to reduction, alkylation, followed by trypsin digestion at 1:10 ratio (enzyme:protein) for 16h at 37°C. The stable-isotope-labeled peptides, used as reference and normalization, were then spiked in each sample. Trypsin reaction was quenched using TFA. Each sample was desalted using SPE method. 2.5 µg of each digested and desalted samples were analyzed in triplicate using PBL's targeted assay: 30 min gradient on Thermo U3000 coupled with Sciex 6500+ Triple Quadrupole. PBL's unbiased protein discovery workflow consisted of trypsin digestion, desalting, followed by a 21-min gradient on Evosep and data independent acquisition on a ThermoFisher Exploris 480.

qPCR and NGS: Beads were washed 3 times and used in Reverse Transcription assays using SuperScript IV and random hexamers primers. The cDNA product was eluted from the beads using NaOH followed by AMPure XP bead clean up. Purified cDNA was then used for qPCR assays as well as full transcriptomic analysis. A targeted SNP panel using Ampliseq NGS assays was performed to generate DNA mutation data.

Conclusion

The Precision Biomarker Laboratories (PBL) of Cedars-Sinai generated identical results for both plasma samples, showing that the simplSEQ Nucleic Acid Isolation did not adversely affect the ability to perform protein mass spec analysis on the samples. The tailed nucleic acid attached to streptavidin beads were able to generate data for both DNA and RNA. This provides strong evidence that multiomics on a single sample is possible without the need to divide the sample into equal parts for analysis of each specific analyte of choice. In situations of rare and scarce samples this approach provides the opportunity for more in- depth analysis of all blood-based components.