

Precision meets depth: Enrichment-based workflow enables quantitative in-depth proteome analysis in blood plasma

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INTRODUCTION

Blood is a rich source of disease-associated biomarkers, yet plasma proteomics is limited by the vast differences in protein abundance, which can obscure low-abundance signals. ENRICH technology employs bead-based enrichment to compress this dynamic range, enhancing the detection of low-level proteins in LC-MS analysis. Because enrichment can alter original concentration relationships, we developed a Controlled Quantitative Experiment (CQE) to benchmark ENRICH-iST against neat plasma in terms of proteome depth, reproducibility, and quantitative accuracy

MATERIALS

A controlled quantitative experiment was prepared by spiking K2EDTA bovine plasma (Neo Biotech) into pooled K2EDTA human plasma (Diaserve Laboratories) at seven defined volume ratios, ranging from 1:0 to 0:1, each in quadruplicate.

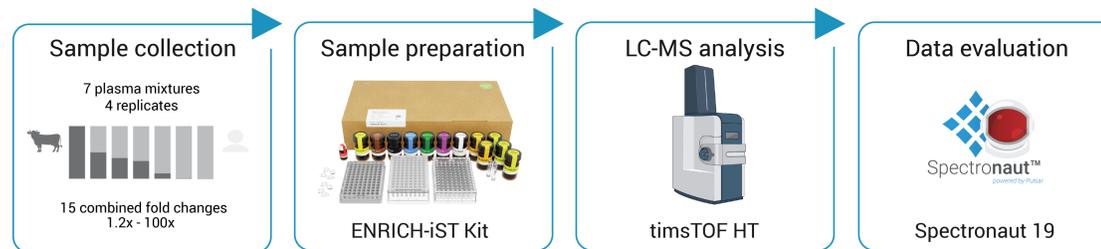
METHODS

Sample preparation: Neat plasma was prepared from 2 μ L input using the iST-BCT protocol (PreOmics). ENRICH-iST samples were processed from 20 μ L plasma on a KingFisher™ Flex using the ENRICH-iST 96x HT protocol, including enrichment, lysis, and digestion, followed by peptide cleanup.

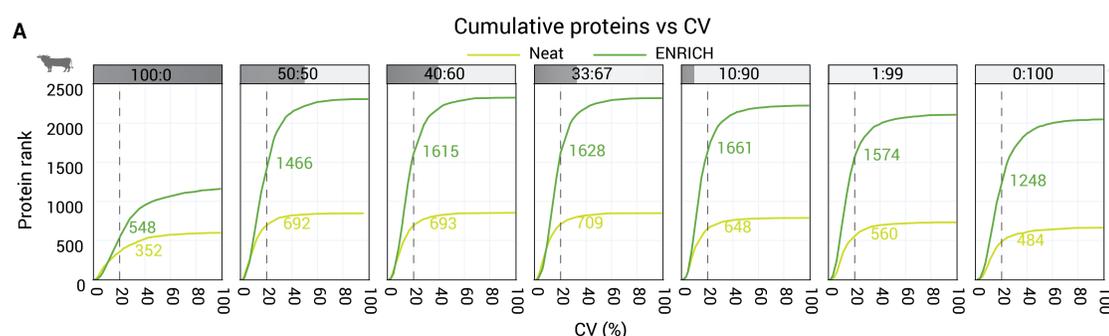
LC-MS analysis: 300 ng of peptides were analyzed using nanoElute® 2 HPLC (Bruker) equipped with an Aurora™ Ultimate CSI 25 x 75 C18 UHPLC column (IonOpticks), operated with a 30-min gradient and coupled to a timsTOF HT mass spectrometer (Bruker) in dia-PASEF® mode.

Data processing: Raw files were searched in Spectronaut® 19 against Pulsar generated spectra library for pure species. Observed fold-changes were calculated by combining different dilution conditions.

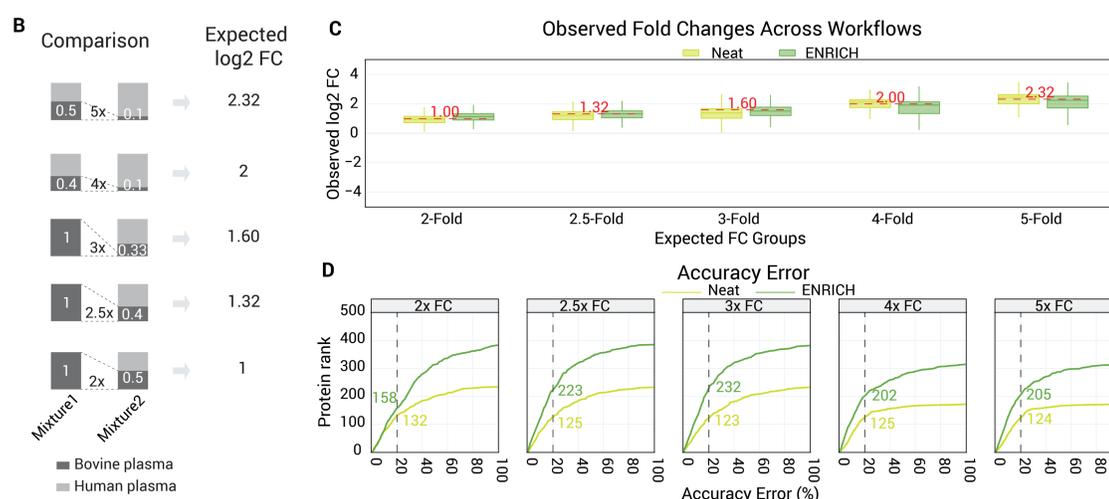
RESULTS



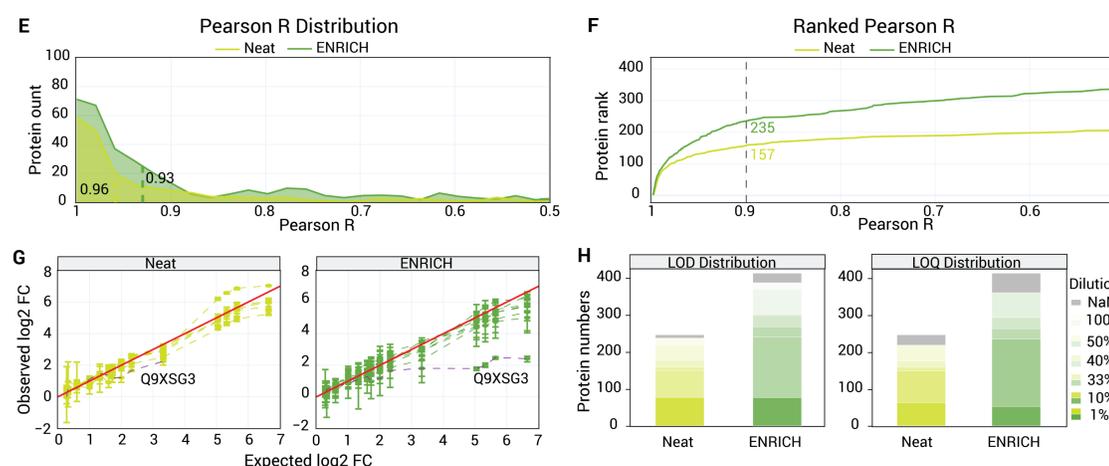
Experimental design of the CQE using the ENRICH-iST workflow.



Enhanced proteome coverage and precision with ENRICH-iST in CQE study. A) ENRICH-iST compressed the dynamic range of the plasma proteome, increasing the number of precisely quantified proteins (CV <20%) by 2- to 3-fold compared to neat plasma, depending on the mixing ratio and species. These results highlight the high repeatability of the ENRICH-iST workflow relative to neat plasma.



High accuracy demonstrated by ENRICH-iST. B) Quantified proteins from two different mixtures were combined to calculate the expected fold change. C) Both ENRICH-iST and neat plasma samples demonstrated high accuracy and strong comparability. D) ENRICH-iST and neat plasma showed comparable performance within the high-accuracy error range (<20%).



High linearity delivered by ENRICH-iST. E) Proteins quantified by both ENRICH-iST and neat plasma showed a strong linear correlation. F) With ENRICH-iST, more proteins were quantified among those with a Pearson correlation coefficient >0.9. G) Eight FDA-approved biomarker orthologs exhibited strong fold-change linearity in both workflows, with only one low-abundance outlier. H) Matrix-matched calibrated LOD/LOQ showed that ENRICH-iST achieved higher sensitivity and linearity, detecting and quantifying 1.7x more proteins than neat plasma, even at low dilution levels.

KEY TAKEAWAYS

Superior depth and reproducibility: ENRICH-iST consistently quantified more proteins than neat plasma across all dilutions with CVs <20%, delivering robust reproducibility and enhanced proteome coverage.

Preserved quantitative accuracy and linearity: ENRICH-iST maintained fold-change accuracy and showed higher linearity, enabling substantially more proteins to meet stringent quantitative criteria.

Extended proteome coverage with retained sensitivity: ENRICH-iST quantified 1.7x more proteins across the dilution series and preserved sensitivity even at low dilution levels, supporting reliable detection of low-abundance biomarkers.

CONTACT & MORE



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Conflict of Interest Disclosure
Abreha, M. is employed by PreOmics Inc.
Hu, Z., Limm, K., Wurzenberger, X., Boateng, G., and Kulak, N.A. are employed by PreOmics GmbH.

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