

INTRODUCTION

There is a profound need in establishing a defined plasma proteomic baseline of a healthy population to establish normal reference ranges for quantifiable peptides. High throughput MS data acquisition coupled to a fast bioinformatics tool for real-time analysis and QC reporting is essential. We investigate 4D proteomic profiles of 200 individuals by employing the followings: 1) automated sample preparation; 2) timsTOF Pro dia-PASEF (Parallel Accumulation Serial Fragmentation) and PASER platform; 3) dia-PASEF MS acquisition and implementation of automated LCMSMS quality controls; 4) novel library building algorithm to calibrate and normalize CCS (collisional cross-sectional) values; 5) CCS-enabled DIA-NN for neural networks search algorithm for analyzing data. This is the first in-depth study of 4D plasma profiling in a healthy population.

METHOD

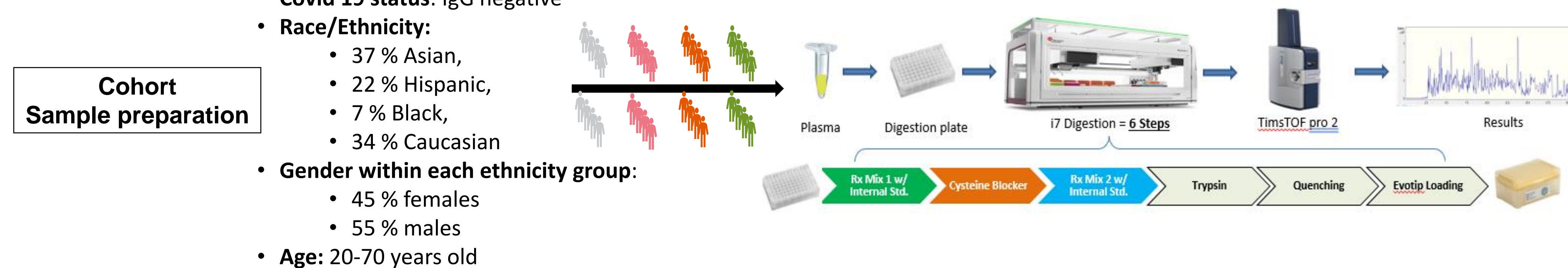
Development of workflow was carried out using a pool of plasma from healthy individuals. Protein denaturation, reduction, alkylation, digestions, and desalt were performed on a Beckman i7 automated workstation. Peptide samples were injected onto a PepSep column attached to an Evosep One coupled to a Bruker timsTOF Pro mass spectrometer. The CCS-enabled DIA-NN data analysis tool was used to normalize in real-time and allow for correct batch effects across thousands of runs. The workflow was being validated on plasma samples from 200 healthy individuals enrolled in the Coronavirus Risk Associations and Longitudinal Evaluation (CORALE) Study and run robustness was evaluated based on quality control runs of Hela cell tryptic peptides and predigested plasma (n=5) as an end-to-end workflow controls.

RESULTS

Baseline cohort design

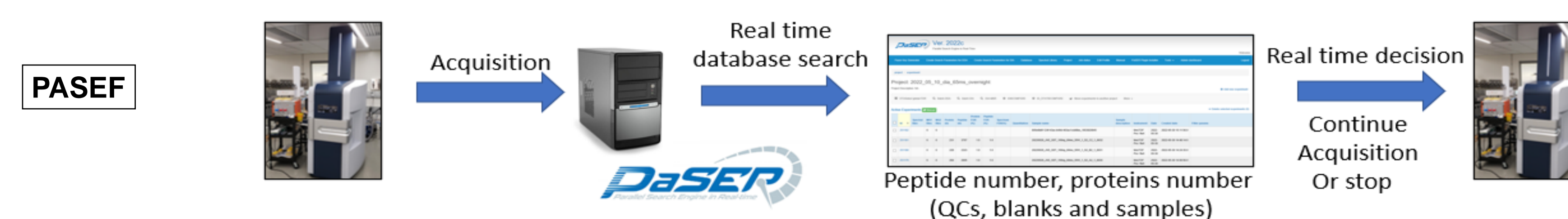
- **Covid 19 status:** IgG negative
- **Race/Ethnicity:**
 - 37 % Asian,
 - 22 % Hispanic,
 - 7 % Black,
 - 34 % Caucasian
- **Gender within each ethnicity group:**
 - 45 % females
 - 55 % males
- **Age:** 20-70 years old

Throughput and reproducibility-automated digestion workflow

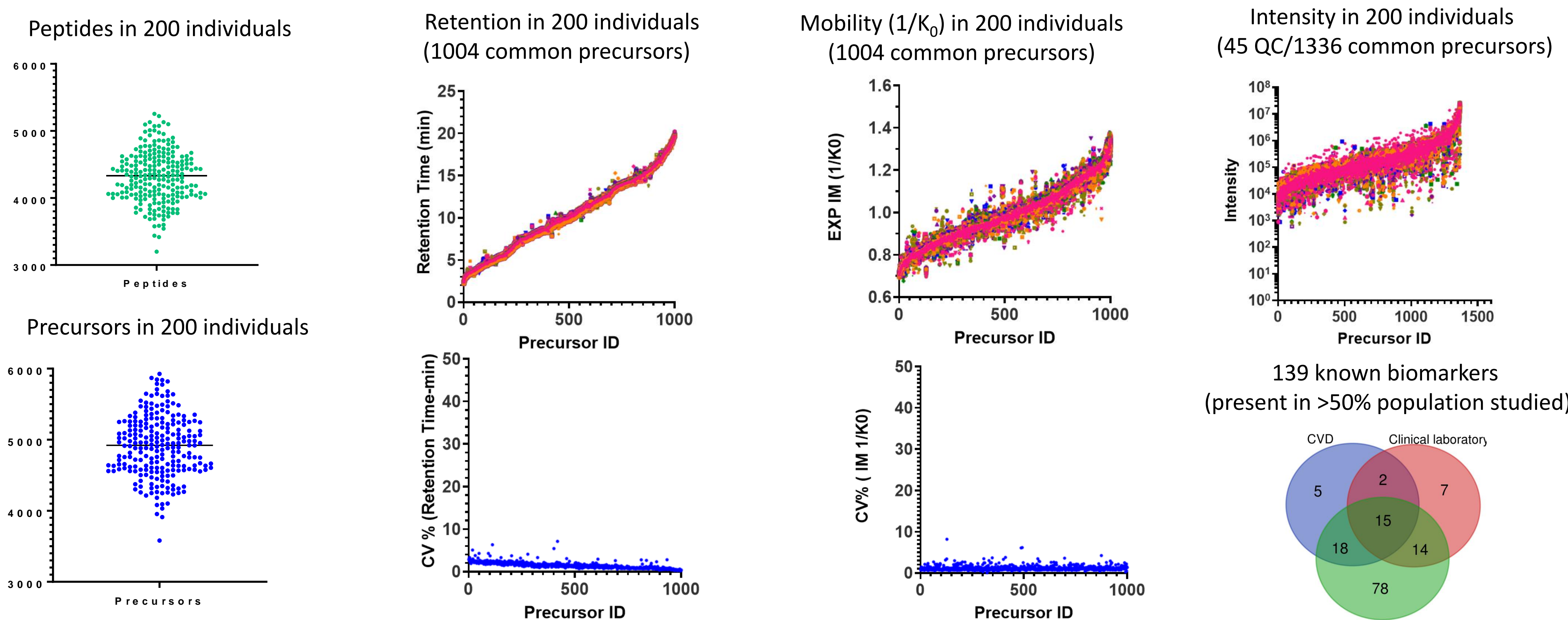


Healthy plasma was obtained from 200 COVID-19 negative individuals in the baseline population of the CORALE study who self-reported as healthy. Plasma protein denaturation, reduction, alkylation, digestion were performed using the Beckman i7 automated workstation (Beckman Coulter). Briefly, plasma proteins were denatured with L 2,2,2-trifluoroethanol (TFE), reduced by dithiothreitol and denatured. Samples were then alkylated iodoacetamide and digested by Trypsin. Digestion reactions were quenched with formic acid.

Real-time search results with PaSER



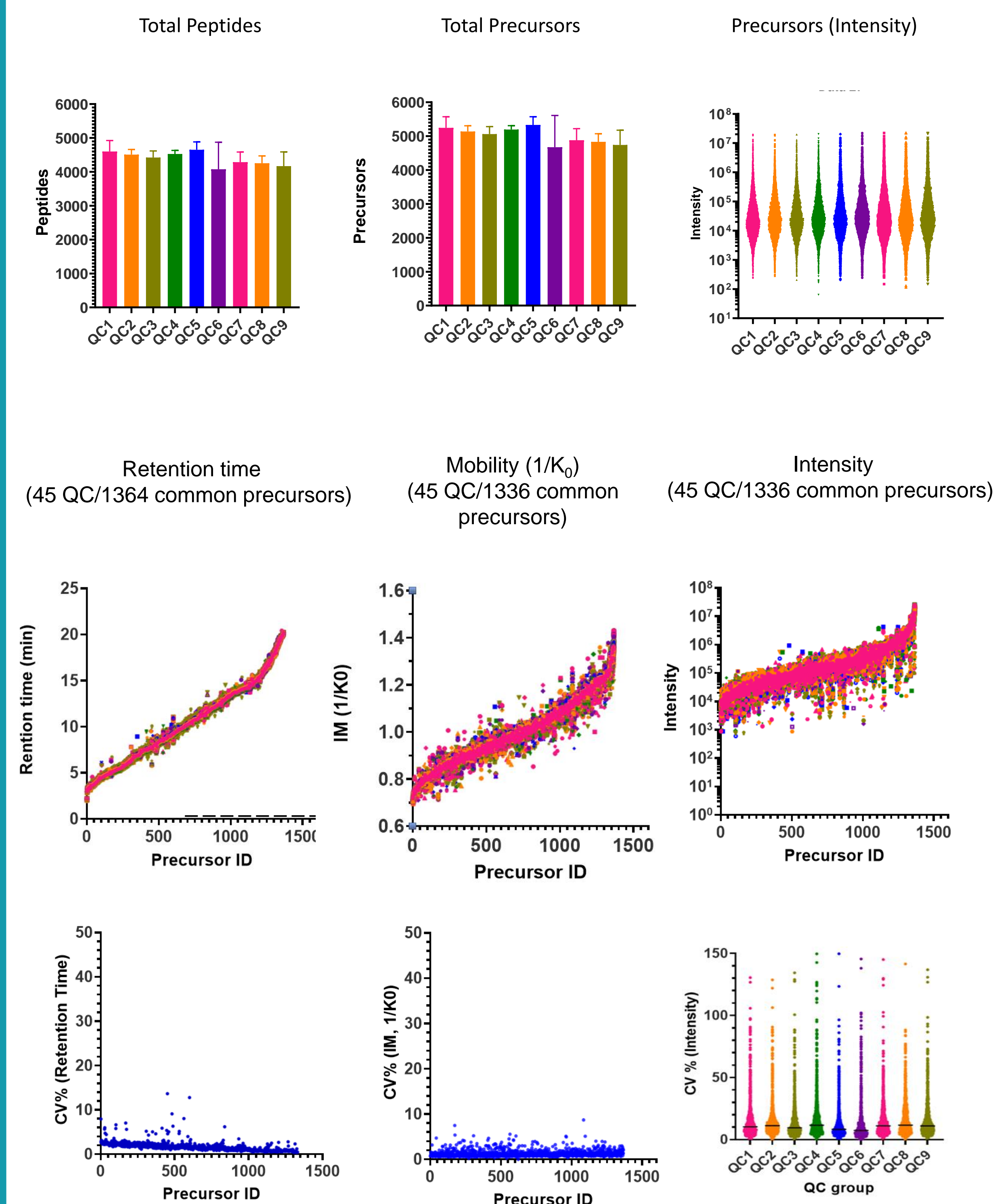
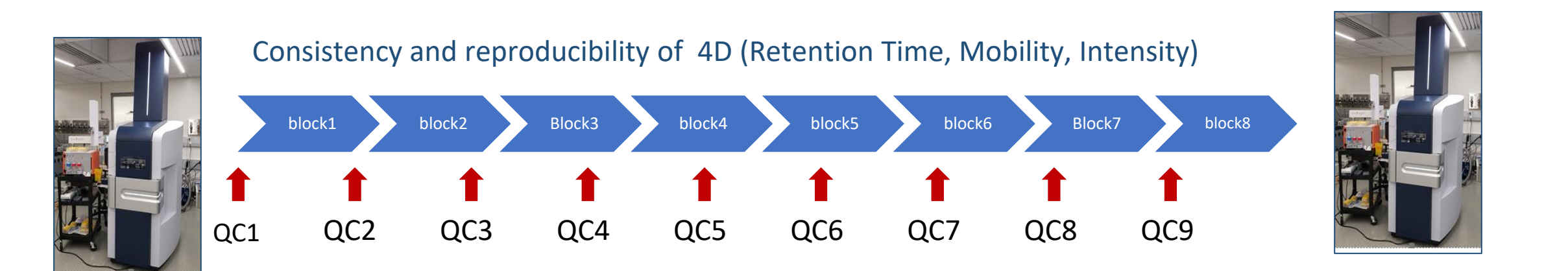
Coronavirus Risk Associations and Longitudinal Evaluation (200 CORALE-Baseline)



DIA (Data Independent Acquisition) data were analyzed using our improved CCS-enabled version of DIA-NN that calculates ion mobility-based scores for both target and decoy precursors and builds features for deep learning. We applied our complete 4D proteomic profiling workflow to plasma from 200 healthy individuals, allowing 4 D proteomic baseline profiles with tims TOF dia-PASEF MS methodology and novel data analysis and bioinformatic package (PaSER).

RESULTS

Implementation of Quality Control LC MSMS -system suitability QC (5 injections/QC)



Reproducibility was determined by 9 blocks of system suitability with replicating injections of 400 ng of a tryptic digest of plasma (n=5) with DIA-PASEF. PaSER analysis of 9 blocks of SST (45 samples) demonstrated good reproducibility: a) common precursors retention time; b) common precursors ion mobility (1/K0); c) common precursors intensities.

REFERENCES

CCS-Aware DIA Data Analysis. 2022 ASMS Poster, Robin Park¹, Tomasz Siwarga¹, Tharan Srikumar¹; Krieger, Jonathan¹; Qin Fu²; Patrick Garrett³; Christopher Adams¹; Dennis Trede¹; Gary Kruppa¹; John Yates³; Jennifer E. Van Eyk²; Rohan Thakur¹ (¹Bruker Daltonics, ²Cedars-Sinai Medical Center, Los Angeles, CA; ³Scripps Research, La Jolla, CA)

TIMScore: exploiting the CCS dimension for increased peptides and protein IDs. 2022 ASMS Talk, Chistopher Adams, Bruker Daltonics

NOVEL ASPECT

Development of a robust, precise, quantitative and automated 4D proteomic profiling workflow with dia-PASEF MS methodology and novel bioinformatics tools.