Novel cartridge-based peptide fractionation enables highly reproducible in-depth proteome coverage

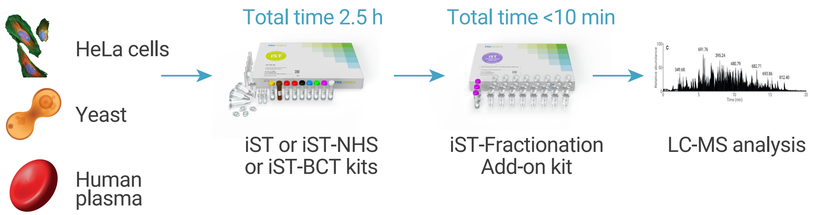
[1]Sebastian H. Johansson, [1]Katrin Hartinger, [1]Zuzana Demianova and [1]Nils A. Kulak
[1]PreOmics GmbH, Martinsried, Germany

Do you need to look deeper in the proteome? Are you doing biomarker discovery?

Smile! Peptide fractionation simplified

- An easy-to-use sample preparation kit suitable for all users
- Three fractions collected exploiting orthogonal separation
- Add-on to the existing robust and reliable iST technology

Materials & Methods



- **Input:** 6x10^5 of HeLa cells, 2 µL human plasma, 1mL of 0,6 OD_{600} yeast extract.
- **Workflow:** Replicate samples (n=3) were prepared using the iST protocol until the WASH steps had been performed. The adapter containing the cartridge with bound peptides was transferred to a fresh collection tube. FRACTION-1 (200 µL) was added to the cartridge and centrifuged (1.000 rcf, 1-3 min). The adapter with cartridge was transferred to a fresh collection tube and steps repeated with FRACTION-2 and FRACTION-3. All the fractions tubes were dried in a vacuum evaporator and resuspended in LC-LOAD.
- **LC-MS analysis:** Easy nLC 1200 coupled to LTQ Orbitrap XL or TimsTOF.
- **Data analysis:** MaxQuant software.

Results

For an additional ten minutes of hands-on sample preparation time, the iST-Fractionation Add-on kit provides a substantial increase in both protein and peptide identifications compared to unfractionated samples prepared using a standard iST kit alone.

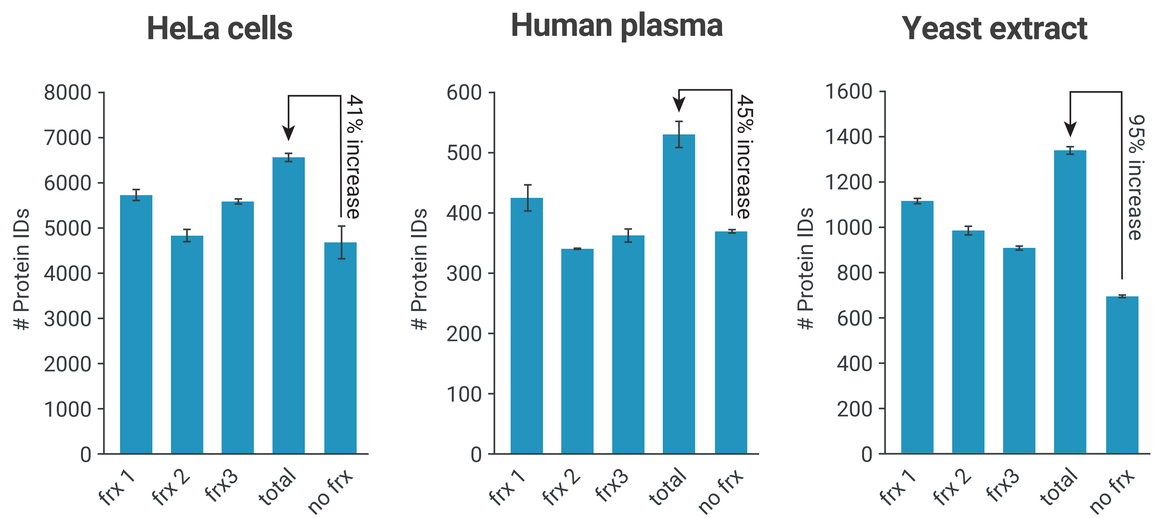


Fig. 1 | **Comparison of protein identifications in three different sample types with and w/o fractionation steps.** The protein identifications for the three fractions each vs total proteins found for the three fractions combined vs the amount of protein identifications for an unfractionated sample.

Discussion

- Workflow was evaluated with three sample types representing a model organism, cells and biofluids
- The three fractions were collected in <10 mins
- Gain of 41%, 45%, and 95% in proteome coverage using HeLa cells, human plasma, and Yeast, respectively

Key features

- Deeper proteome characterization
- Intuitive protein sample preparation workflow
- Out of the box solution
- Easily automated
- Adaptable to high-throughput assays