Peptide Fractionation Simplified

Introduction

Peptide fractionation is a valuable and established tool in proteomic analyses by liquid chromatography-mass spectrometry (LC-MS). It allows complex proteomes to be analysed in greater depth. Samples often contain proteins in a very wide range of concentrations, proteins present at low concentrations may be masked in analyses. To achieve more comprehensive identification and quantification, the combination of a peptide fractionation orthogonal to the on-line LC-MS/MS step has proven to be particularly powerful.

PreOmics iST-Fractionation technology exploits dipole-moment and mixed-phase interactions between the peptides and the cartridge sorbent (patent pending). The three fractionation steps provide enhanced proteome coverage taking only ten minutes to perform in the lab - a reasonable compromise between proteomic depth and increased measurement time.

Material

Sample	Human plasma (2 µL) HeLa cell pellet ($6x10^5$ cells = 100 µg of protein)
iST 8x kit*	PreOmics, P.O.00001
8x3 reactions iST-Fractionation Add-on kit	PreOmics, P.O.00100 *Note* kit contains 3 fractionation buffers and additional plasticware
LC column	IonOptiks C18 length 250 mm, i.d. 0.075 mm, particle diameter 1.6 µm
LC solvent	Solvent A: 0.1 % formic acid Solvent B: 0.1 % formic acid in 80 % acetonitrile

*The iST-Fractionation Add-on kit must be used in conjunction with iST, iST-NHS or iST-BCT as it does not contain the cartridge.

Methods

Sample preparation

Replicate samples (n=3) were prepared as per the iST 8x protocol until the WASH step had been performed. Then the adapter containing the cartridge with peptides bound 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.

Instrument settings

- Samples were analysed using a nanoElute LC coupled to Bruker timsTOF Pro
- Data wase analysed using MaxQuant
- Injection volume 200 ng
- Oven temp 50°C MS conditions
- DDA PASEF, 60 minute gradient

LC Gradient - linear

Time (seconds)	Flow rate (µL/min)	%A
0	0.4	2.0
2460	0.4	17.0
3600	0.4	34.0
3780	0.4	95.0
4500	0.4	95.0

Results

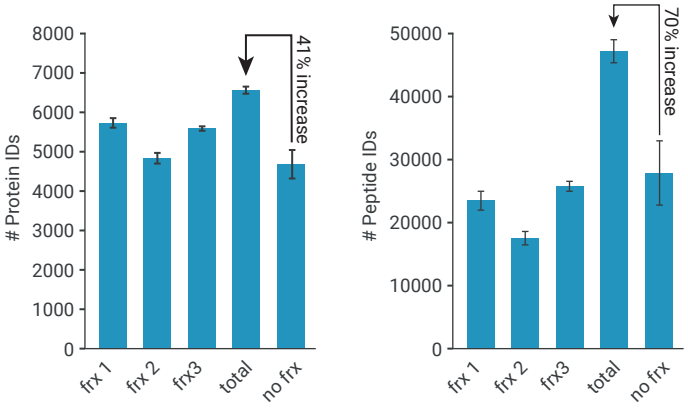

Figure 1 | Using 6x105 HeLa cells as starting sample. The left graph shows protein identifications for the three fractions each vs total proteins found for the three fractions combined vs the amount of protein identifications for an unfractionated sample, similarly the right graph shows the same information for the peptide identifications.

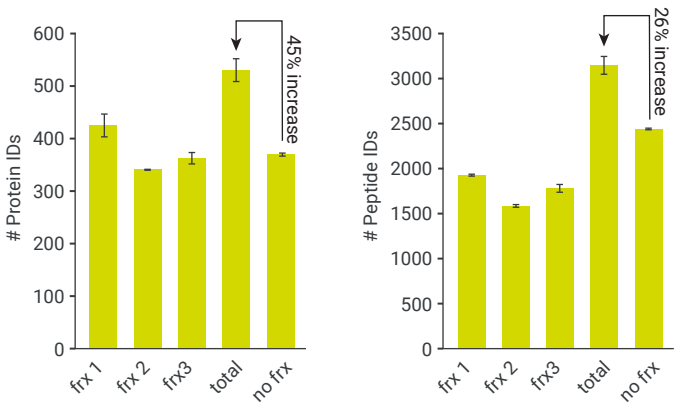

Figure 2 | Using human plasma (2 µL) as starting sample. The left graph shows protein identifications for the three fractions each vs total proteins found for the three fractions combined vs the amount of protein identifications for an unfractionated sample, similarly the right graph shows the same information for the peptide identifications.

Conclusion

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. For HeLa cells we saw an increase of 41% of protein identifications and 71% for peptide identifications. Using plasma there was a 46% increase in protein identifications and 27% increase in peptide identifications. The improvement in identifications is dependent on the sample matrices analysed and instrumentation available. Peptide fractionation is usually considered a challenging technique, typically carried out by expert users. However the iST-Fractionation Add-on kit makes this technique simple and reproducible.

Products

Product	Manufacturer	Product Code
8x3 reactions iST-Fractionation Add-on kit	PreOmics GmbH	P.O.00100
12x3 reactions iST-Fractionation Add-on kit	PreOmics GmbH	P.O.00101
96x3 reactions iST-Fractionation Add-on kit	PreOmics GmbH	P.O.00102