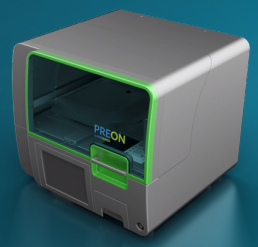
PreON - TMTpro 16 plex



Introduction

The use of tandem mass tagging (TMT) for relative quantitation in proteomics workflows is well established. The number of different mass tags can vary from 2 to 16. Chemical labeling reagents have specific handling and compatibility requirements to achieve good labeling efficiencies. In this information sheet we demonstrate the ability of the PreON automation to handle a proteomic chemical labelled methodology utilizing TMTpro 16 plex.

Material

Sample	Cell pellets from yeast (S.cerevisiae) 0,3 OD_{600} equal to 50 µg protein
iST-NHS PreON 96x	PreOmics, P.O.00080
TMTpro 16 plex	Thermo Fisher Scientific
Anhydrous acetonitrile	Applied Biosystems Cat. No 400060
Hydroxylamine	Sigma Aldrich, 467804

Methods

PreON information:

Method	iST-NHS
Sample type	Pellet
Digestion duration	1 hour
Number of samples	16
Amount of chemical labeling reagent	50 µg (10:1 ratio)
Split or complete protocol	Split protocol
Additional pauses	None

Figure 1 | PreON deck layout for TMTpro 16 plex compatible instrument

MS instrumentation: Thermo Orbitrap Fusion Lumos Tribrid Mass Spectrometer MS3 data acquisition

Data processing: MS Amanda

PREOMICS

Results

In Figure 2 the Pearson correlation coefficient R is depicted for the 16 corrected reporter ion intensities on peptide level. The results are highly reproducible, indicated by R values close to 1.

The overall accepted benchmark in the proteomics community for the TMT labeling is a total labeling efficiency greater than 98%, which is fulfilled by the PreON sample preparation automation with >99%.

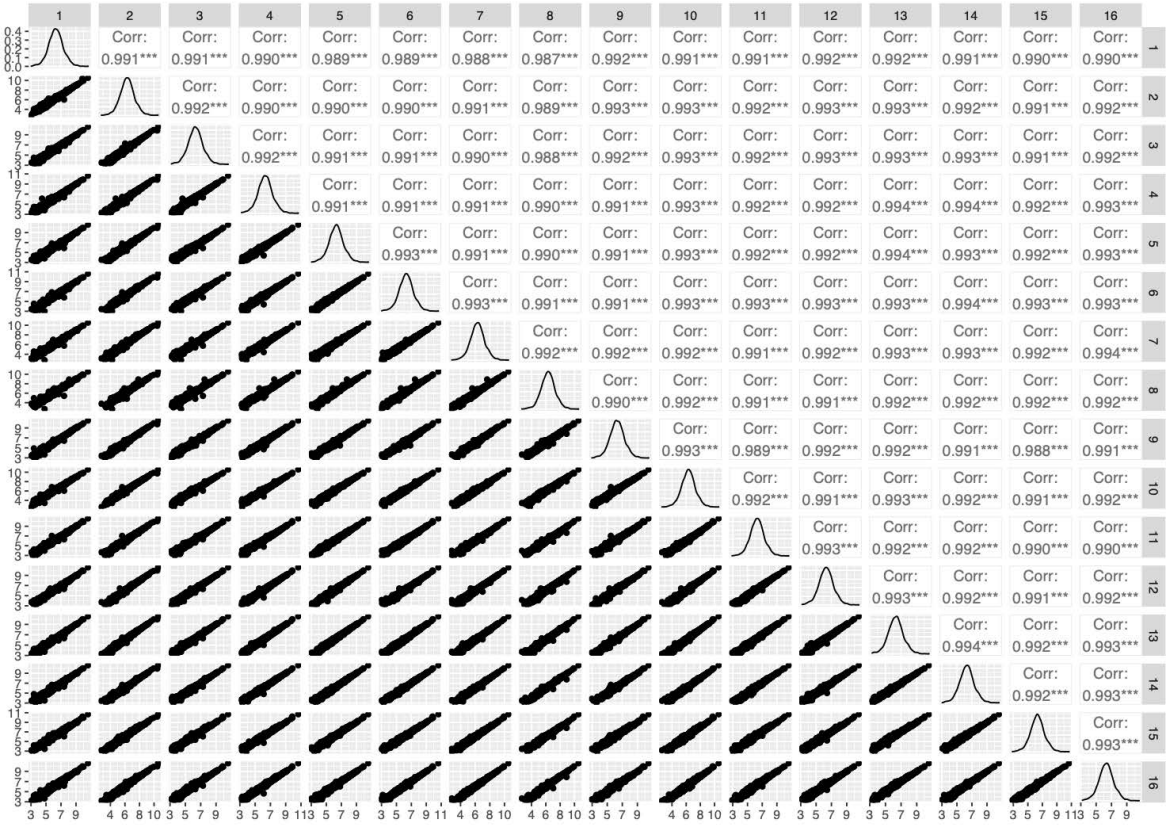


Figure 2 | Correlations of the 16 corrected reporter ion intensities at the protein level

Conclusion

The PreON system provides excellent labeling efficiency and is suitable for use with the TMTpro 16 plex workflow.