Bioinformatics Solutions Inc.

TMT Quantification Analysis with PEAKS Online

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Aim:

To provide an overview of TMT (Tandem Mass Tag)-based quantification/data analysis in PEAKS Online.

Summary:

PEAKS Online supports TMT quantification of individual protein/peptides. A published data set [1] was used as a case study to demonstrate the capability of TMT-based data analysis in PEAKS Online.

Introduction:

TMT, also referred to as "tandem mass tag", is a MS/MS based quantification strategy using isobaric mass tags.[2] All TMT reagents are composed of 3 parts: a mass reporter group used as reporter ions for quantification in MS2/MS3, an amine reaction group that enables TMT reagents to react and binds to the N-terminus of a peptide or the side chain of a Lysine residue, and a mass normalizer that connects the mass reporter group and amine reactor group. The mass normalizer balances the masses of each intact TMT molecule; however, the mass reporter molecule (produced by fragmentation) has a different mass for each TMT tag. This allows the same peptide each with a different label among different samples to be comparable. During MS fragmentation (MS2 or MS3), the reporter ion would be cleaved, and the abundance of corresponding peptide will be shown as the reporter ion ratio. TMT quantification has great advantage in multiplexing (up to 18plex) for multi-condition comparison and have gained its popularity in proteomic quantification. PEAKS Online support TMT quantifications and direct visualization of results.

Study aims and background:

Instrumentation and the availability of data acquisition algorithms have greatly facilitated the multiplexing proteomic quantitation. In this study, the same samples were analyzed with three different data acquisition methods on Thermo Eclipse to demonstrate showing the new advantage of real-time search (RTS) acquisition strategy.

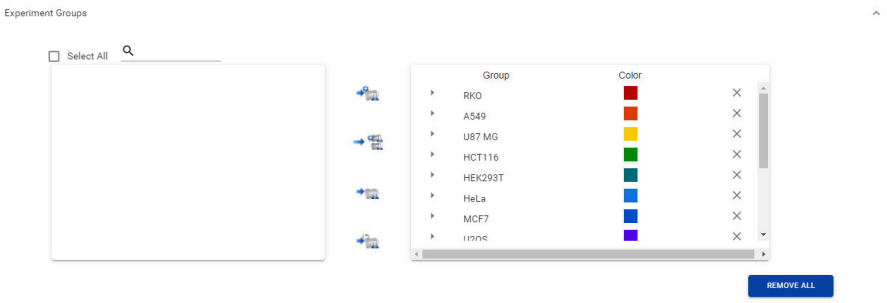

Fig 1. In one experiment, eleven groups were set as one cell line per group.

Experimental design:

Eleven human cell lines (RKO, A549, U87 MG, HCT116, HEK293T, HeLa, MCF7, U2OS, SUM159, PANC1, and Jurkat) were lysed, reduced, alkylated and then digested with LysC/Trypsin. The digested peptides were labelled individually with TMT reagents, mixed, and then fractionated by basic pH reversed-phase HPLC. Labelled peptides were pooled and fractioned by basic pH reversed-phase. Ninety-Six fractions were collected and consolidated into 24 samples, and 12 of them were analyzed on Eclipse.Three MS acquisition methods were used: high resolution MS2, synchronous precursor selection (SPS) MS3 and RTS-SPS-MS3.

Data analysis:

MS data (12 runs *3) was analyzed in Peaks Online using the built-in TMT-11 plex (CID/HCD) method in PEAKS Q (de novo assisted Quantification) module (Fig 2). For quantification part, auto normalization was applied (Fig 3), and spectrum filter of quality was set at 15.

Fig 2. Auto Normalization was selected for intra sample normalization, so the intensity of each channel was equal since same amount of samples from each cell line were mixed.

Sample Name	# MS Run	# MS1	# MS2	#PSM	#Peptides	#Sequences	#Proteins	#ProteinGroups	#PSM/#MS2
RTS90	12	63038	333536	101912	83114	80499	8993	8218	31%
SPS90	12	39224	236300	80660	68631	66653	8208	7457	34%
MS2	12	85476	385192	116477	90817	87759	9477	8687	30%

Table 1. Protein identification results of each acquisition strategy

Result comparison of deep proteome analysis between three different acquisition methods:

Out of three acquisition methods, MS2 method identified the most proteins followed by RTS-SPS-MS3 (RTS90) method with only 5% less protein identification (Table 1). This suggests the real time search strategy can greatly improve the efficiency of SPS 3 event by avoiding unidentifiable scans. The number of quantifiable PSM and protein groups are shown in table 2 and Fig. 3.

RTS-SPS-MS3		SP3-MS3		MS2	
Peptide-Spectrum Matches	78682	Peptide-Spectrum Matches	76235	Peptide-Spectrum Matches	89571
Peptide sequences	68788	Peptide sequences	66789	Peptide sequences	75343
Protein groups	8360	Protein groups	7795	Protein groups	8661

Table 2. Quantification results of each acquisition strategy

Expression difference of proteins between different cell lines and the quantitation difference between different acquisition methods:

MS2 acquisition method in TMT would provide more quantifiable peptides, but the accuracy is lower than the MS3 method. [3] An example of protein quantification results is shown below (Fig 4). Two proteins were further analyzed for TMT quantification comparison between three acquisition methods. To eliminate bias, the same unique peptides from the specific protein were used in all three acquisition methods.

Human RelA-associated inhibitor(iASPP) is a regulator involved in apoptosis and transcription. By inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa-B) and Specificity protein 1 (SP1), it blocks the transcription of HIV-1 virus. Of 11 human cell lines, SUM159 showed the highest abundance of iASPP. However, the abundance ratio of iASPP in SUM159 resulted from three acquisition methods shows significant differences (Fig 5): respectively, 21.9 and 17.1 in RTS-SPS-MS3 and SPS-MS3. In MS2 acquisition method, the ratio was only 4.8. Similar trend could be found in HCT and Hela cell lines. The quantification result showed that MS3 TMT could reveal a more accurate ratio between channels.

Another example shown is Serine/threonine-protein kinase B-raf (BRAF). BRAF plays a vital role in mitogenic transduction signals from the cell membrane to the nucleus. BRAK activates the Mitogen-Activated Protein (MAP) kinase signal transduction pathway by phosphorylating MAP2K1 and is involved in a wide range of cancers: lymphoma, lung cancer, colorectal cancer, etc. In MS2 TMT acquisition, the abundance ratio between Jurkat and A549 is less than 3. However, in both MS3 TMT acquisitions, the ratio between two cell lines is around 6 (5.7 and 6.6). (Fig 6)

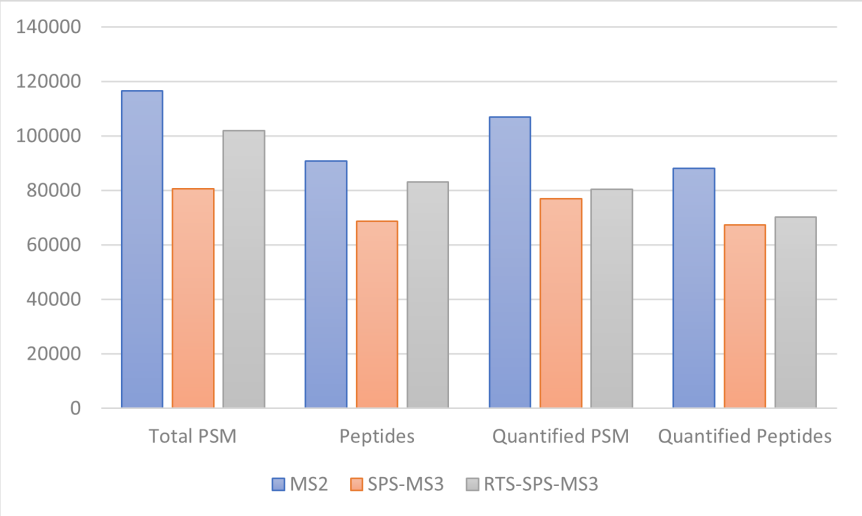

Fig 3. Comparison of identified/ quantified PSM and peptides number between three acquisition strategies.

Conclusions:

PEAKS Online can support complex TMT proteomic quantification analysis.

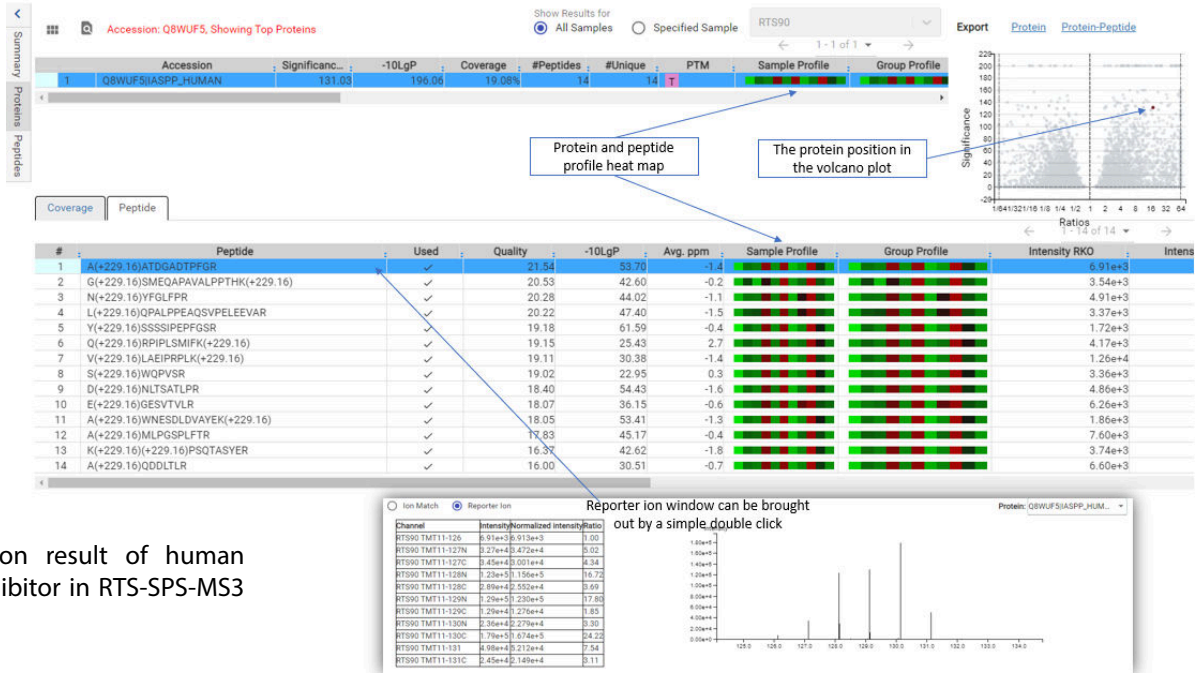

Fig 4. Quantification result of human RelA-associated inhibitor in RTS-SPS-MS3 experiment.

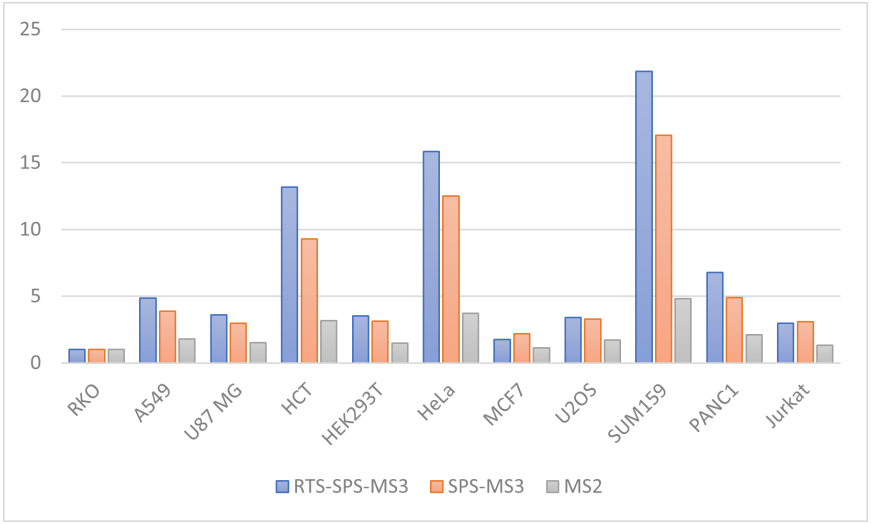

Fig 5. Abundance ratio of Human RelA-associated inhibitor in different cell lines. The abundance in RKO was set as 1.

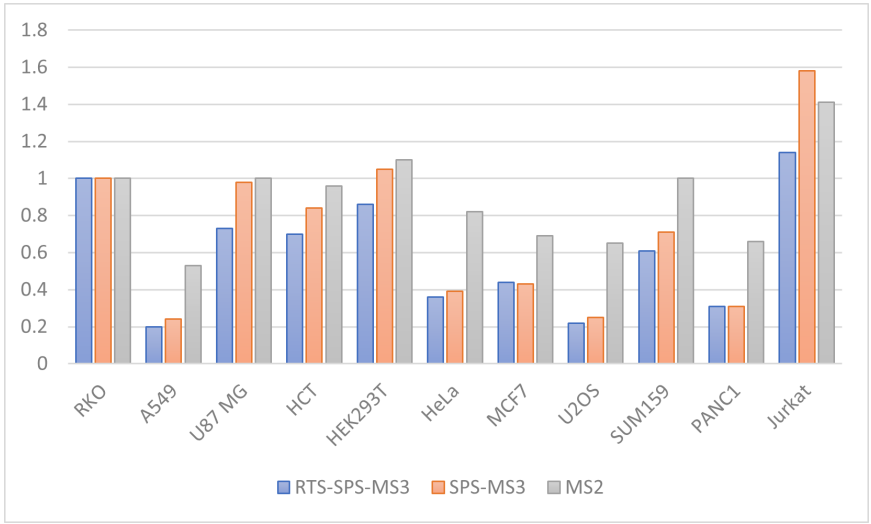

Fig 6. Abundance ratio of BRAF in different cell lines. The abundance in RKO was set as 1

References:

1. Thompson, A. et al. Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Analytical Chemistry 75, 1895–1904 (2003).
2. Yu, Q. et al. Benchmarking the orbitrap tribrid eclipse for next generation Multiplexed Proteomics. Analytical Chemistry 92, 6478–6485 (2020).
3. Ting, L., Rad, R., Gygi, S. et al. MS3 eliminates ratio distortion in isobaric multiplexed quantitative proteomics. Nat Methods 8, 937–940 (2011).