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We use this protocol and it's working

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Abstract

Here we describe proteomics experiment with isolated mitochondrial extracts at the Proteome exploration laboratory (PEL) at Caltech by Baiyi Quan, Jeff Jones and Tsui-Fen Chou in collaboratin with Livia Hecke Morais and Sarkis Mazmanian.

Sample Preparation

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1. Proteins extracted from mitochondria are reduced using a final concentration of 5 mM of TCEP (tris(2-carboxyethyl)phosphine) at room temperature for 10 min.
2. The proteins are further alkylated using a final concentration of 20 mM of CAA (chloro-acetamide) at room temperature for 15 min.
3. A 25 ul aliquot of the lysate is acidified using 2.5 ul of 12% phosphoric acid. Using a pH test paper to make sure solution pH< 2.
4. Combine 165 ul of the S-trap buffer (90% MeOH with 100 mM triethylammonium bicarbonate) with the acidified lysate.
5. Transfer the combined colloid into the S-trap micro (Protifi, NY).
6. Centrifuge the S-trap micro at 4000 g for 1 min at room temperature.
7. Using 150 ul of the S-trap buffer to wash the S-trap and centrifuge at 4000 g for 1 min. Repeat the step two more times.
8. Add 20 ul of 100 mM triethylammonium bicarbonate containing 20 ug of Trypsin into S-trap micro. Allow the incubation stay overnight (14-16 hr) at 37 C.
9. After overnight incubation, directly adding 40 ul of the 50 mM triethylammonium bicarbonate and centrifuge at 4000 g for 1 min.
10. Add 40 ul of 2% formic acid in water to the S-trap, and centrifuge at 4000 g for 1 min.
11. Add 40 ul of 50% acetonitrile in water to the S-trap, and centrifuge at 4000 g for 1 min.
12. Speedvac the sample.
13. The sample is reconstituted in 20 ul of 2% acetonitrile and 0.2% formic acid in water and 500 ug of the peptide is used for LC-MS/MS analysis.

LC-MS/MS

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1. An aliquot of sample containing 500 ug of the peptides is subjected to the LC-MS/MS analysis. The sample is separated on an Aurora UHPLC Column (25 cm × 75 µm, 1.6 µm C18, AUR2-25075C18A, Ion Opticks) using an Easy-nLC 1200 liquid chromatography system. The gradient settings follows **Table 1**.

Time	Duration	Flow (nl/min)	%B
0:00	0:00	350	3
1:00	1:00	350	3
73:00	72:00	350	19
101:00	28:00	350	29

121:00	20:00	350	41
124:00	3	350	95
131:00	7	350	98

Table 1. LC gradient for the sample

Mobile Phase A: 0.2% formic acid, 2% acetonitrile, and 97.8% water.
Mobile Phase B: 0.2% formic acid, 80% acetonitrile, and 19.8% water.

1. The sample is analyzed on a Thermo Q-Exactive HF mass spectrometer using a data-dependent acquisition method. Detailed parameters of the scans are listed in **Table 2**.

Global settings	
Ion source type	NSI
Spray voltage	2000 V
Ion transfer tube temperature	300 C
Polarity	Positive
MS1 scan settings	
Resolution	60000
AGC target	3e6
Maximum IT	15 ms
Scan range	375-1500 m/z
MS2 scan settings	
Resolution	30000
AGC target	1e5
Maximum IT	45 ms

Loop count	12
Isolation window	1.2 m/z
NCE	28
Spectrum data type	Centroid
Fixed first mass	100 m/z

Table 2. MS settings for the run.

Proteomic Data analysis

3 1. The raw data generated by mass spectrometer is analyzed using Proteome Discoverer 2.5. The data is searched using the mouse proteome achieved from UniprotKB on 10/26/2020 (swissprot + trembl). The processing and consensus workflow are set according to **Figure 1** and **2**. The parameters are listed below in **Table 3**. All the parameters that are not mentioned are left defaulted.

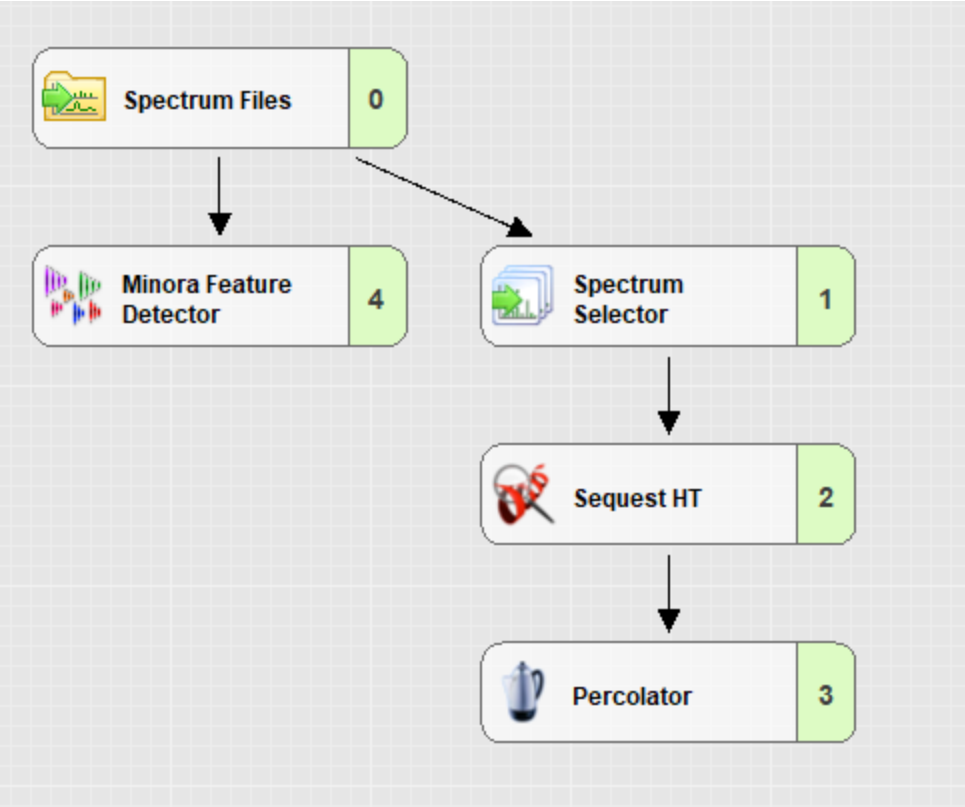

Figure1. Processing workflow of the PD analysis.

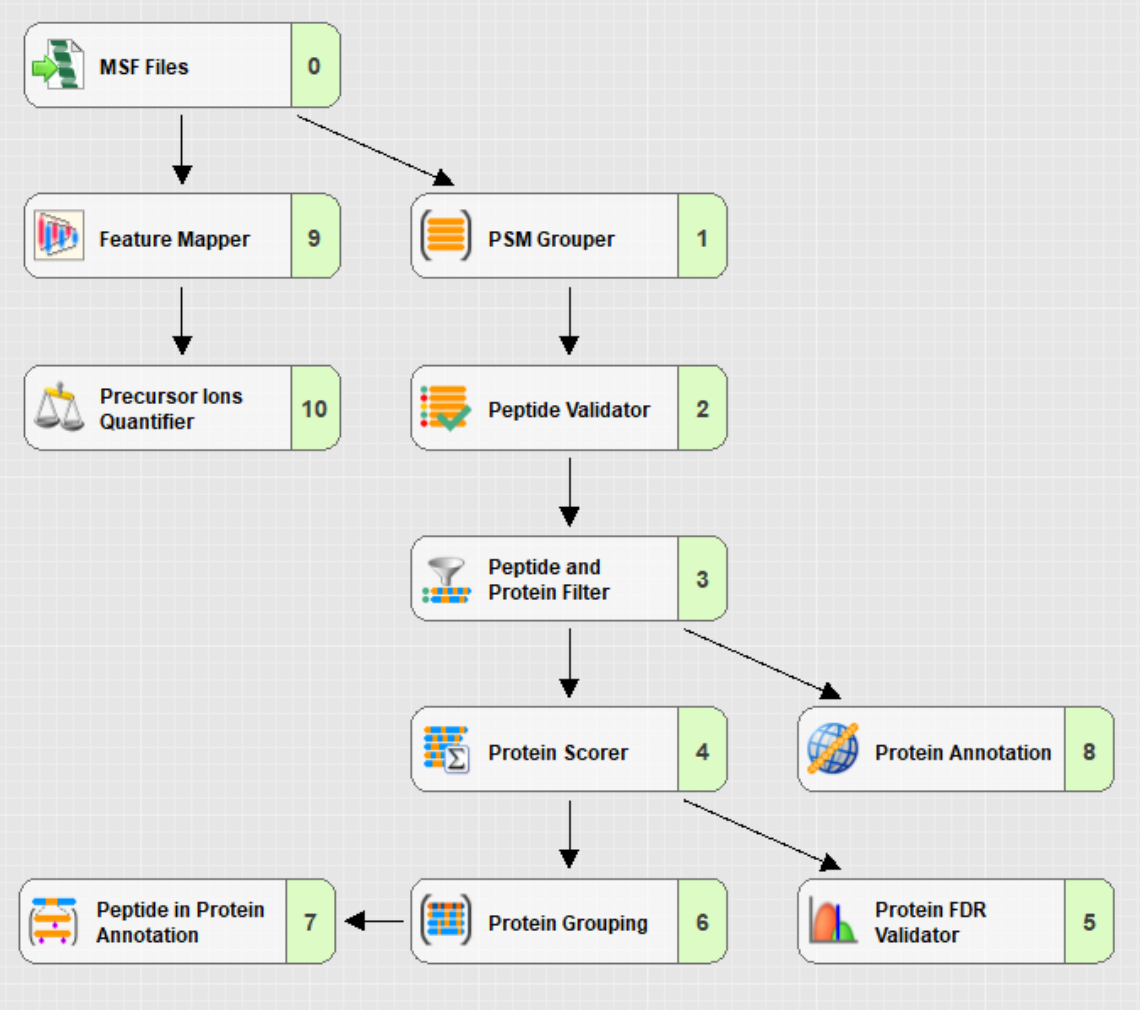

Figure
2. Consensus workflow of the PD analysis.

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SequestHT settings	
Enzyme name	Trypsin (Full)
Max. missed cleavage	2
Min. peptide length	6
Max. peptide length	144

Precursor mass tolerance	10 ppm
Fragment mass tolerance	0.02 Da
Max. equal modification	3
Dynamic modification	Oxidation/ +15.995 Da (M)
Dynamic modification (protein terminus)	Acetyl/ + 42.011 Da (N-Terminal)
Dynamic modification (protein terminus)	Met-loss/ - 131.040 Da (M)
Dynamic modification (protein terminus)	Met-loss+Acetyl/ - 89.030 Da (M)
Static modification	Carbamidomethyl/ + 57.021 Da (C)
Percolator	
Target/Decoy selection	Concatenated
Validation based on	q-Value
Target FDR (Strict)	0.01
Target FDR (Relaxed)	0.05
Feature Mapper settings	
Maximum RT shift (min)	2
Mass tolerance	5 ppm

Table 3. Parameters for PD searching

1. The protein list exported from PD results are further analyzed using R scripts based on the TidyProteomics package (jeffsocal.github.io/tidyproteomics/articles/overview.html).