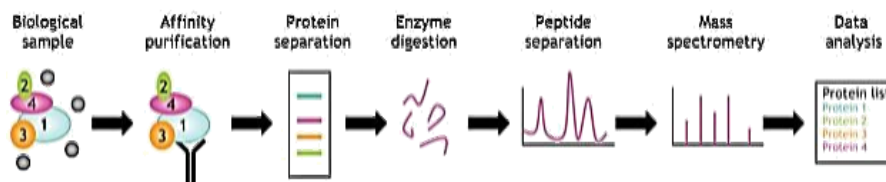


Mass Spectrometry-based Proteomics Workshop



Theme: MS data analysis, statistical & bioinformatics analysis

For discovery proteomics, global PTMs & protein complex study

4-day workshop

3rd floor Conference room
Building #1



- Section I: Basic Mass Spectrometry, proteomics case study and Hand-on LC-MS Analysis Day 1
- Section II: MS database searching by MaxQuant and Mascot Day 2
- Section III: Statistical analysis and Bioinformatics analysis, PTM, Day 3
- Section IV: Thermal Proteome Profiling, untargeted metabolomics/lipidomics Day 4

For more information about this workshop,
please contact Dr. Meng at 15010054101

Mass Spectrometry-based Proteomics Workshop

March 26, 2019

Day 1:

- 8:00 - 8:30 am:** **Registration**
room 108
- 8:30 - 9:00 am** **Opening ceremony and Introduction to Workshop and workshop agenda overview**
Center leader:
Sheng Zhang, Director, Cornell Proteomics and Metabolomics Facility
Third Floor Conference Room
- 9:10 - 10:00 am** **Experiment 1: Protein denaturing, reduction and alkylation of *E coli* Lysate samples for shotgun proteomics analysis:** Attendees divided into 3 groups. 2-3 people in sub-group for one sample
Instructors: Drs. Zhang, Meng, Feng Dan; Mrs Xia Bin
room 118, 120
- 10:10 - 11:00 am** **Lecture 1: Experimental design and sample preparation prior to MS analysis**
Instructors: Dr. Zhang for design and Dr. Meng for sample preparation
- 11:10 - 12:00 am** **Lecture 2: Basics of mass spectrometry (MS) & LCMS proteomics workflows**
Instructor: Dr. Zhang
Third Floor Conference Room
- 12:00am - 12:10pm** **Experiment 2: In solution trypsin digestion**
room 118,120
- 12:10 - 1:00 pm** **Lunch**
- 1:00 to 1:50 pm** **Lecture 3: Global quantitative proteomics and quantitative PTMs**
Sheng Zhang, Director, Proteomics and Metabolomics Facility
Third Floor Conference Room
- 2:10- 3:10 pm** **Experiment 3: Lab tour and LC-MS instrument demo:**
Attendees divided into 3 groups (20 min/tour rotation).
NanoLC-MS/MS for Ecoli lysate by DDA in 6600 TripleTOF: by Dr. Meng
TMT10plex or TMT11plex labeled sample analysis : by Dr. Zhang
First dimensional High pH RPLC-based fractionation: by Ms. Xia Bin
Third Floor Conference Room
- 3:20 to 4:20 pm** **Lecture 4: Protein Complex study by IP-pull down and cross linking MS**
Sheng Zhang, Director, Proteomics and Metabolomics Facility
Third Floor Conference Room
- 4:30- 5:10 pm:** **Experiment 4: Stop trypsin digestion & set up nanoLC-MS/MS analysis**
Overnight run for in-solution digested *E coli* samples (with 90-min gradient for each run)
7 samples on 6600, by *Dr. Meng* (attendees #1- #7)
7 samples on Orbitrap Fusion, by Dr. Zhang (attendees #8 - #13)
room 118,120

Mass Spectrometry-based Proteomics Workshop

March 27, 2019

Day 2:

8:30 - 9:15

Pending(Thermo)

9:15 -10:00

Pending(Sciex)

10:00 - 10:20 am:

Laptop computer setup

Each attendee needs to bring his/her own laptop, distribute individual MGF, PKL to each attendee by Instructor:

Third Floor Conference Room

10:20 - 10:50 am

Lecture 5: Introduction to Mascot-based MS database searching & data interpretation

Dr. Qingshi Meng

10:50 - 12:00 am

Experiment 5: Database searching practice for E coli samples – by Mascot 2.6

use the distributed MGF, PKL files using Mascot public search engine or local server

For both raw data files generated from 6600 QTOF and Orbitrap Fusion

Instructors: Drs. Meng, Zhang, Xia Bing

12:00 - 1:00 pm

Lunch

1:30 pm - 2:00pm

Lecture 6: Introduction to MaxQuant-based MS database searching

Dr. Zhang, Cornell Proteomics and Metabolomics Facility

Third Floor Conference Room

2:00pm - 3:30pm

Experiment 6: MaxQuant searching practice for protein IDs and LFQ of E coli samples

For raw data files generated from Orbitrap Fusion, exporting results into Excel

Distribute individual raw files to each attendee

3:30 to 4:00 pm

Experiment 7: MaxQuant Database search practice: for SILAC and iTRAQ/TMT samples

Distribute individual raw files from Orbitrap Fusion to each attendee

4:00 to 4:50 pm

Experiment 8: MaxQuant Database search practice: LFQ for IP pulldown samples and quantitative PTMs

Instructors: *Sheng Zhang, Dr. Meng and Xia Bing*

Distribute individual raw files from Orbitrap Fusion to each attendee

4:50- 5:10 pm

Lecture 7: How to understand global search report from MaxQuant & Mascot results

Instructors: *Dr. Zhang*

4 Supplementary documents for understanding MaxQuant, Mascot, PD 2.2.

5:10 - 5:40 pm

Experiment 9: Summary of the full workshop group results for Ecoli shotgun analysis

Distribute individual excel files (result for individually prepared *Ecoli* sample) to each attendee

Instructors: *Sheng Zhang and Dr. Meng*

Mass Spectrometry-based Proteomics Workshop

March 28, 2018

Day 3:

- 8:30 - 9:00 am:** **Lecture 8: Statistical analysis of global proteomics: how to determine DEPs**
 1) By Excel;
 2) Perseus software;
 3) EasyFit Program
 Instructor: *Sheng Zhang*
- 9:00 - 9:45 am** **Experiment 10: Perseus statistical practice: MQ-TMT data with 2-sample t-test**
 Instructor: *Sheng Zhang, Dr. Meng*
- 9:40 - 10:30 am** **Experiment 11: Perseus statistical practice: MQ-SILAC data with ANOVA test**
 Instructor: *Sheng Zhang, Dr. Meng*
- 10:30 - 11:15 am** **Experiment 12: Perseus statistical practice: MQ-Label-free data with 2-sample test**
 Instructor: *Sheng Zhang, Dr. Meng*
- 11:15 - 12:00 am** **Experiment 13: Perseus statistical practice: TMT6plex data with 1-sample t-test**
 Instructor: *Sheng Zhang, Dr. Meng*
- 12:00 - 1:00 pm** **Lunch**
 Biotech Bldg., room 130
- 1:30 – 2:00 pm** **Lecture 9: Bioinformatics analysis (GO/KEGG enrichment) of global proteomics data sets using Clusterprofiler in R language and DAVID 6.8, Protein-protein interaction network by String 10.0**
 Instructor: *Dr. Meng*
- 2:00 - 3:15 pm** **Experiment 14: Bioinformatics analysis practice**
 Instructors: *Drs. Meng and Zhang*
- 3:15 - 3:35 pm** **Lecture 10: Web-based bioinformatics tools to make plots/figures for scientific presentations**
 Instructor: *Dr. Zhang*
- 3:35 - 4:00 pm** **Experiment 15: Practice for making plots using online tools**
 Instructors: *Drs. Meng and Zhang*
- 4:00 - 5:30 pm** **Lecture 11: PTM (1)**
 phosphorylation, acylation, ubiquitination, glutathionylation, nitration,...

Mass Spectrometry-based Proteomics Workshop

March 29, 2019

Day 4:

- 8:30 - 9:15 am** **Lecture 11: PTM (2)
Glycosylation**
- 9:15- 10:00 am:** **Lecture 11: Thermal Proteome Profiling (TPP) for protein-ligand & protein-protein interactions**
Instructor: Sheng Zhang
- 10:10- 10:50 am:** **Lecture 12: Experimental Design and Workflow for Untargeted metabolomics**
Instructor: Sheng Zhang
- 11:00 - 11:30 am** **Lecture 13: Untargeted lipidomics**
Instructor: Sheng Zhang
- 11:30 - 12:00 pm** **Summary of workshop and issue of certificate**
- 12:00 - 1:00 pm** **Lunch**