## Session Outline

### Session One

Tuesday February 20th, 9am-4pm, lunch will be provided
Ender's 10th floor conference room (please bring ID, restricted access to building).

Day one of the course will focus on sample preparation and LC-MS analysis.

Participants will

- Be introduced to the necessary concepts and instructed on the steps of sample preparation and analysis by LC-MS. Theory and specifics of chromatography, mass spectrometry and data collection will be discussed.
- Extract diverse samples (cells, livers, brain tissue, plasma) and process them for LC-MS using different conditions
- Write LC-MS methods, prepare chromatography and samples, and run the LC-MS machines.

### Tentative Schedule

| Time | Task | Notes |
|---|---|---|
| 9:00-10:00am | LC-MS and sample prep introduction | Lecture will take place in BCH conference room (Ender's 10th floor) |
| 10:00am-12:00pm | Sample prep, part 1<br>Two groups | Cells, livers, brain tissue, plasma +/- Ellman's reagent and two-three extraction buffers will be prepared by different student groups |
| 12:00-01:00 pm | Lunch | Sample prep will continue without attendance |
| 01:30-3:00pm | Sample prep, part 2<br>Write methods, run LC-MS | LC column will be set up, participants will write method and prepare sample pools and LC-MS vials. Participants will start runs |

## Session Two

Thursday February 22nd, 1pm-5pm, coffee and snacks will be provided.
Ender's 10th floor conference room (please bring ID, restricted access to building).

Day two and three will focus on LC-MS data analysis and statistics. Targeted and untargeted metabolomics platforms will be compared. Participants will work on analyzing metabolomics data using proprietary software, R-code and MetaboAnalyst. We will also focus on data integration and interpretation. Discussion and extended Q&A will allow participants to expand what they have learned and practice or demo any further aspects of LC-MS metabolomics.

Participants will

- Be introduced to theory and practice of data analysis, chromatography peaks alignment, ion adducts, and isotopes.
- Be introduced to TraceFinder (TF) and Compound Discoverer (CD) and targeted vs untargeted LC-MS analysis
- Practice data analysis using TF.

## Tentative Schedule

| Time | Task | Notes |
|---|---|---|
| 1:00-1:30pm | LC-MS data analysis intro | Discussion of targeted vs untargeted LC-MS analysis<br>Introduction into peak quality and alignment |
| 1:30-2:00pm | Data analysis set up for targeted | TF for targeted analysis |
| 2:00-2:30 pm | Break | |
| 2:30-3:00pm | Data analysis set up for untargeted | CD for untargeted analysis (CD will run overnight) |
| 3:00-4:00pm | Continue with TF | Export data |

## Session Three

Thursday February 29th, 1pm-5pm, coffee and snacks will be provided.
Ender's 10th floor conference room (please bring ID, restricted access to building).

Participants will

- Demo and practice data integration and statistics using R-code and MetaboAnalyst
- Demo and practice preparation of data for publication using Prism and Illustrator
- Participants will be given a homework assignment: Data analysis, interpretation, visualization – communicate your results by preparing a short presentation that will be submitted via e-mail. More details in class.

## Tentative Schedule

| Time | Task | Notes |
|---|---|---|
| 1:00-1:30pm | Finalize CD untargeted data analysis | |
| 1:30-2:00pm | Finalize TF targeted data analysis. Export data, analyze with R-code | |
| 2:30-3:00 pm | Break | |
| 3:00-3:30pm | Statistics using Metaboanalyst and Prism. Data visualization using Illustrator | |
| 3:30-4:00pm | Q&A | |