**Agenda**

**The Center for Environmental & Systems Biochemistry’s**

**NIH Resource Center for Stable-Isoptope Resolved Metabolomics**

**(RC-SIRM) Hands-On SIRM Workshop on**

**Experimental Design and Methods**

**July 24, 2017 – August 4, 2017**

**Lee T. Todd Building, University of Kentucky**

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**Monday July 24, 2017-Day 1**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building - Orientation-

Continental breakfast will be provided

Provide Overview of Week. Present Laboratory Rules and OSHA Rules and Personal Protective Equipment. Assignment of participants into two Groups

9:00 – 9:30 Introduction to SIRM - Led by Dr. Teresa Fan,

Room 570 Lee T. Todd Building

9:30 – 10:30 Instruction on calculating formulae for enriched media and treatments-

Led by Dr. Teresa Fan, Room 570 Lee T. Todd Building

10:30 – 11:00 Demonstration of metadata spreadsheet for tracer experiment –

Led by Teresa Cassel, Room 570 Lee T. Todd Building

**Group 1**

11:00 – 11:30 Demonstration of cell culture tracer experiment –

Led by Dr. Teresa Fan

11:30 – 11:45 Break

11:45 – 12:15 Demonstration of tissue grinding and methanol rinse/tissue quenching for extraction – Led by Teresa Cassel

**Group 2**

11:00 – 11:30 Demonstration of tissue grinding and methanol rinse/tissue quenching for extraction – Led by Teresa Cassel

11:30 – 11:45 Break

11:45 – 12:15 Demonstration of cell cultures tracer experiment – Led by Dr. Teresa Fan

**Afternoon**

12:15 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch will be provided

1:00 – 4:30 Step by step demonstration and participant practice of cell culture harvest and quenching – Led by Dr. Teresa Fan

4:30 – 5:30 Demonstration of time course nutrient medium sampling –

Led by Dr. Teresa Fan

5:30 – 6:00 Guided practice of metadata entry into template and recap/questions –

Led by Dr. Teresa Fan, 570 Lee T. Todd Building

**Tuesday July 25, 2017-Day 2**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building - Watch the extraction video tutorial

Continental Breakfast will be provided

9:00 – 12:00 Step by step demonstration and participant practice of polar/non-polar extraction of cell lysates – Led by Dr. Teresa Fan

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:00 – 5:00 Step by step demonstration and participant practice of polar fraction aliquot and lipid reconstitution – Led by Dr. Teresa Fan

5:00 – 5:30 Demonstration of lyophilization of polar fractions –

Supervised by Dr. Richard Higashi

5:30 – 6:15 Guided practice of metadata entry into template and recap/questions –

Led by Dr. Teresa Fan, Room 570 Lee T. Todd Building

**Wednesday July 26, 2017-Day 3**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building

Discussion of lyophilization theory and practice - Led by Dr. Richard Higashi

Continental breakfast will be provided

9:00 – 10:00 Step-by-step demonstration and participant practice of media extraction –

Led by Dr. Teresa Fan

10:00 – 10:15 Break

10:15 – 12:30 Step by step demonstration and participant practice of protein extraction – Led by Dr. Teresa Fan

**Afternoon**

12:30 – 1:30 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:30 – 4:30 Step-by-step demonstration and participant practice of protein quantification by BCA – Led by Dr. Teresa Fan

4:30 – 5:30 Guided practice of metadata entry into template and calculation of protein concentration from BCA data –

Led by Dr. Teresa Fan, Room 570 Lee T. Todd Building

5:30 – 6:00 Recap - Questions

**Thursday July 27, 2017-Day 4**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building

Continental breakfast will be provided

Participants will be oriented to the metadata spreadsheet for their solo practice plates

9:15 – 12:00 Overview NMR data analysis: isotopomers and their quantification -

Led by Dr. Andrew Lane, Room 570 Lee T. Todd Building

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:00 – 2:00 Participants solo practice of cell culture harvest and quenching

2:00 – 4:00 Participants solo practice of polar non-polar cell extraction

**Friday July 28, 2017 -Day 5**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building

Continental breakfast will be provided

9:00 – 11:30 Participants solo practice of polar fraction aliquot and lipid reconstitution

11:30 – 12:00 Guided practice of metadata entry into template –

Led by Dr. Teresa Fan Room 570 Lee T. Todd Building

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Bio Pharm Complex

Lunch is provided

1:15 – 5:00 Step by step demonstration and participant practice of NMR data analysis –Led by Dr. Teresa Fan, Room 570 Lee T. Todd Building

5:00 – 6:00 Presentation from data processing to biochemical interpretation: Part I –

Led by Dr. Teresa Fan, Room 570 Lee T. Todd Building

6:00 – 6:30 Recap and Questions

**Saturday July 29, 2017 Day 6**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building - Roundtable Discussion

Continental breakfast will be provided

9:15 – 12:00 Group 1 goes with Dr. Richard Higashi for IC-FTMS & Nanospray Acquisition Demonstrations

9:15 – 12:00 Group 2 goes with Dr. Andrew Lane for NMR Acquisition Demonstration

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:15 – 4:00 Group 2 goes with Dr. Richard Higashi for IC-FT MS & Nanospray Acquisition Demonstrations

1:15 – 4:00 Group 1 goes with Dr. Andrew Lane for NMR Acquisition Demonstration

4:00 – 4:30 Both Groups: Recap and Questions - Meet in Room 570 Lee T. Todd Building

**Sunday July 30, 2017 Day 7**

**Morning**

11:00 – 12:45 Independent practice NMR data analysis – optional session

**Afternoon**

1:00 – 5:30 Load bus for lunch and tour of Buffalo Trace Distillery, Frankfort KY

6:00 – 8:00 Independent practice NMR data analysis – optional session

**Monday July 31, 2017 -Day 8**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building – Overview on targeted and untargeted chromatography FTMS: example of IC-FTMS and its data processing (lecture and /or video –

Led by Dr. Richard Higashi and/or Dr. Marc Warmoes

Continental breakfast will be provided

9:00 – 12:00 Step by step demonstration and participant practice of IC-FTMS targeted data processing - Dr. Marc Warmoes

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:00 – 3:00 Participants solo practice of protein extraction

3:00 – 5:30 Participants solo practice targeted data processing of IC-FTMS

**Tuesday August 1, 2017 -Day 9**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building

Continental breakfast will be provided

9:00 – 12:00 Participants will do targeted data processing of IC-FTMS

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:00 – 3:00 Participants solo practice of BCA protein analysis

3:00 – 4:00 Participants solo practice targeted data processing of IC-FTMS

4:00 – 5:30 Presentation from data processing to biochemical interpretation: part II - Dr. Teresa Fan

**Wednesday August 2, 2017 -Day 10**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building

* Introduce PC inhibitor and query affected pathways.
* Give the assignment. Provide media data.
* Led by Dr. Teresa Fan

9:00 – 12:00 Use pivot tables of 13C glucose traced experiment

* NMR and ICMS data from 2016 Panc1.
* Participants are doing pathway analysis.

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:15 – 3:45 Nucleotide and amino acid metabolism – integrating pathways – glutamine - example of targeted analysis using direct infusion FT-MS –

Led by Dr. Richard Higashi

4:00 – 6:00 Pathway Tracing using isotopomer and isotopollogue distribution data

* Part III.
* Led by Drs. Teresa Fan and Andrew Lane, Room 570 Lee T. Todd Building

**Thursday August 3, 2017-Day 11**

**Morning**

8:00 – 9:00 Meet in Room 570 Lee T. Todd Building

Data Repository at DRCC – Led by By Dr. Hunter Moseley

Continental breakfast will be provided

9:00 – 10:00 Participants solo practice pathway analysis from pivot tables of 13C glucose traced experiment (2016 or 2017 data depending on participant’s advancement)

10:00 – 12:00 Pathway Tracing using isotopomer and isotopollogue distribution data – Part IV –

Led by Drs. Teresa Fan and Andrew Lane, Room 570 Lee T. Todd Building

**Afternoon**

12:00 – 1:00 Lunch - Room 570 Lee T. Todd Building

Lunch is provided

1:00 – 2:00 Andrew Lane Flux Modeling

2:00 – 3:00 Zhen Qi Specific examples of flux modeling

3:00 – 3:15 Break

3:15 – 4:15 Jinze Liu Omics Integration

4:30 – 5:30 Ice Cream Social – Room 570 Lee T. Todd Building

**Friday August 4, 2015-Day 12**

**Morning**

8:00 – 8:30 Meet in Room 570 Lee T. Todd Building –

Continental breakfast will be provided

8:30 – 11:00 Assessment of student pathway analysis

11:00 – 1:00 Recap and lunch

**Evening**

5:30 pm Meet in the Lee T. Todd Building Atrium for transport to dinner with the symposium speakers