**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 23, 2018 – August 3, 2018**

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

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**Sunday July 22 arrive in Lexington**

**Afternoon**

4:30 – 6:30 Meet and greet of lab staff, PI’s and workshop participants – 789 South Limestone Street, Lee T. Todd Jr Building Atrium

**Monday July 23, 2018-Day 1**

**Morning**

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

 Continental breakfast will be provided

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

9:00 – 9:30 Overview of learning goals and expected workshop outcomes. Led by – Teresa Fan, 570 Lee T. Todd Jr Building

9:30-10:00 General Introduction to SIRM - Led by Dr. Teresa Fan,

 Room 570 Lee T. Todd Jr. Building

10:00 – 11:00 Introduction to SIRM and Panc-1 cells and experimental background, questions, and design- Led by Dr. Teresa Fan,

 Room 570 Lee T. Todd Jr. Building

11:00 – 12:00 Overview of experimental design: statistical considerations – Led by Dr. Hunter Moseley, Room 570 Lee T. Todd Jr. Building

**Afternoon**

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

 Lunch will be provided

1:00 – 2:00 Instruction on calculating formulae for enriched media and treatments-

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

2:00 – 3:00 Demonstration of metadata spreadsheet for tracer experiment –

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

**Group 1**

3:00 – 3:30 Demonstration of cell culture tracer experiment – Led by Dr. Teresa Fan

3:30 – 4:00 Demonstration of tissue grinding and methanol rinse/tissue quenching for extraction – Led by Teresa Cassel

**Group 2**

3:00 – 3:30 Demonstration of tissue grinding and methanol rinse/tissue quenching for extraction – Led by Teresa Cassel

3:30 – 4:00 Demonstration of cell culture tracer experiment – Led by Dr. Teresa Fan

4:00 – 4:30 Guided practice of metadata entry into template and recap/questions –

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

4:30 – 5:30 Demonstration of time course medium sampling – Led by Dr. Teresa Fan

**Tuesday July 24, 2018-Day 2**

**Morning**

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

 Continental Breakfast will be provided

 Watch the -quenching video tutorial

9:00 – 12:00 Step by step demonstration and participant practice of cell culture harvest and quenching– Led by Dr. Teresa Fan

**Afternoon**

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

 Lunch is provided

 Watch the extraction tutorial video

1:00 – 4:30 Step by step demonstration and participant practice of polar/non-polar extraction of cell lysates – Led by Dr. Teresa Fan

4:30 – 5:30 Step by step demonstration and participant practice of polar fraction aliquot and lipid reconstitution – Led by Dr. Teresa Fan

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

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

**Wednesday July 25, 2018-Day 3**

**Morning**

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

 Continental breakfast will be provided

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

9:00 – 10:30 Step by step demonstration and participant practice of protein extraction – Led by Dr. Teresa Fan

10:30 – 10:45 Break

10:45 – 12:30 Demonstration of protein extract dilution determination for BCA analysis –

 Led by Dr. Teresa Fan

**Afternoon**

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

 Lunch is provided

1:30 – 3:00 Step-by-step demonstration and participant practice of protein quantification by BCA – Led by Dr. Teresa Fan

3:00 – 3:15 Break

3:15 – 4:30 Step-by-step demonstration and participant practice of media extraction –

 Led by Dr. Teresa Fan

4:30 – 5:00 Demonstration of lyophilization of polar fractions –

 Supervised by Dr. Richard Higashi

5:00 – 6:00 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 Jr. Building

**Thursday July 26, 2018-Day 4**

**Morning**

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

 Continental breakfast will be provided

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

9:15 – 12:00 Participants solo practice of cell culture harvest and quenching

 Participants solo practice of polar non-polar cell extraction

**Afternoon**

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

 Lunch is provided

1:00-4:00 Overview NMR data analysis: isotopomers and their quantification -

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

**Friday July 27, 2018 -Day 5**

**Morning**

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

 Continental breakfast will be provided

9:00 – 12:30 Step by step demonstration and participant practice of NMR data analysis –Led by Dr. Teresa Fan, Room 570 Lee T. Todd Jr. Building

**Afternoon**

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

 Lunch is provided

1:30 – 4:30 Independent practice NMR data analysis

4:30 – 5:00 Break

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

 **Part I** – Led by Dr. Teresa Fan, Room 570 Lee T. Todd Jr. Building

6:00 – 6:30 Recap and Questions

**Saturday July 28, 2018 Day 6**

**Morning**

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

 Continental breakfast will be provided

 Overview of targeted and untargeted chromatography FTMS: example of IC-FTMS and its data processing (lecture or video)

 Led by Dr. Richard Higashi

9:00 – 12:00 Curation demonstration and participant practice of IC-FTMS targeted data processing - Dr. Jessica Kele Arruda Macedo

**Afternoon**

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

 Lunch is provided

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

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

5:30 – 6:30 Optional practice time for NMR or IC-FTMS data processing

**Sunday July 29, 2018 Day 7**

**Morning**

9:00 – 10:00Meet in Room 570 Lee T. Todd Jr. Building

 Continental breakfast will be provided

10:00 – 12:00 Roundtable Discussion

**Afternoon**

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

 Lunch is provided

1:00 – 2:00 Introduce participants to pathway analysis assignment. Provide complete SIRM data set from experiment using a PC suppressor.

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

2:00 – 4:00 Revisit the specific experimental design and impact on SIRM interpretation-

 Led by Dr. Teresa Fan, Richard Higashi, Andrew Lane, & Hunter Moseley,

 570 Lee T. Todd Jr. Building

**Monday July 30, 2018 – Symposium- Day 8**

Registration begins at 8:00 am

**Tuesday July 31, 2018 – Day 9**

**Morning**

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

 Continental breakfast will be provided

9:00 – 12:00 Participants practice targeted NMR or ICMS data or pathway analysis, depending on individual previous progress.

**Afternoon**

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

 Lunch is provided

1:00 – 3:30 Participants practice pathway analysis

3:30 – 4:00 Break

4:00 – 5:30 Presentation from data processing to biochemical interpretation:

 **Part II** – Led by Dr. Teresa Fan

5:30 – 6:30 Guided practice of metadata entry into template –

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

 (Provide participants with polar aliquot and BCA data)

**Wednesday August 1, 2018 -Day 10**

**Morning**

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

 Continental breakfast will be provided

9:00 – 12:00 Prepare pathway analysis presentations

**Afternoon**

12:00 – 1:00 Lunch - Room 570 -Lee T. Todd Jr. 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 isotopologue distribution data –

 **Part A** – Led by Drs. Teresa Fan and Andrew Lane,

 Room 570 Lee T. Todd Jr. Building

**Thursday August 2, 2018 -Day 11**

**Morning**

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

 Continental breakfast will be provided

 General introduction to statistics – Led by Dr. Hunter Moseley

9:00 – 10:00 Practical applications of statistics – Led by Dr. Hunter Moseley

10:00 – 12:00 Participant presentations and roundtable discussion of pathway tracing assignments

**Afternoon**

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

 Lunch is provided

1:00 – 4:00 Pathway Tracing using isotopomer and isotopologue distribution data –

 **Part B** – Led by Drs. Teresa Fan and Andrew Lane,

 Room 570 Lee T. Todd Jr. Building

4:00 – 5:00 Discussion of participants proposed experimental designs

5:00 – 6:00 Ice Cream Social – Room 570 Lee T. Todd Jr. Building

**Friday August 3, 2018-Day 12**

**Morning**

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

 Continental breakfast will be provided

8:30 – 11:00 Recap and workshop evaluations

11:00 – 1:00 Lunch/certificates/dismissal