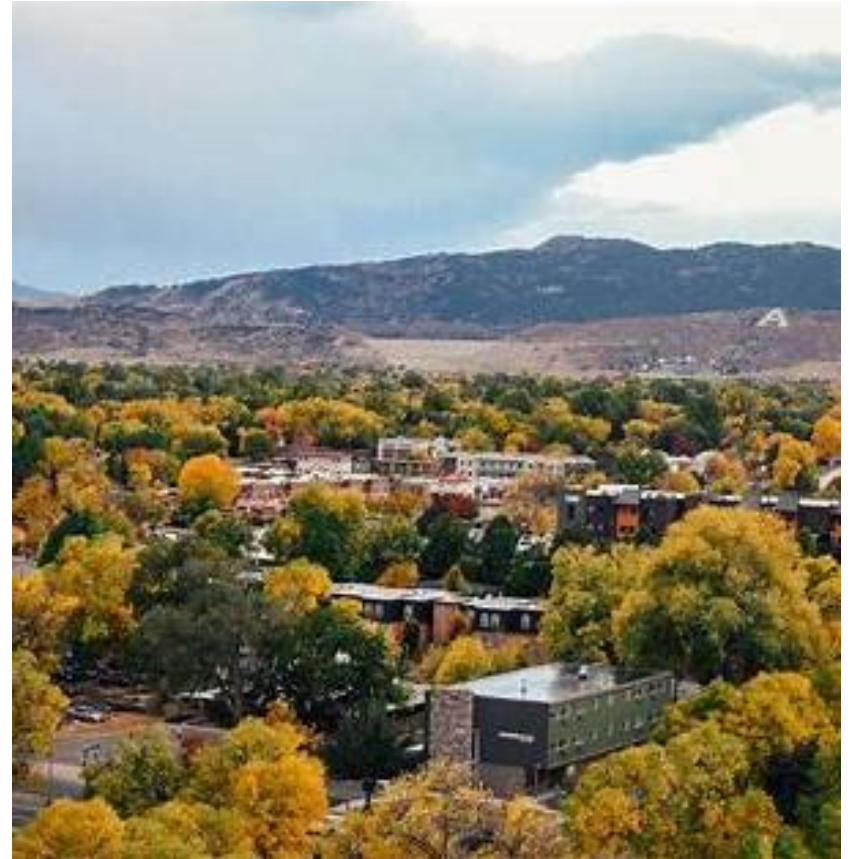
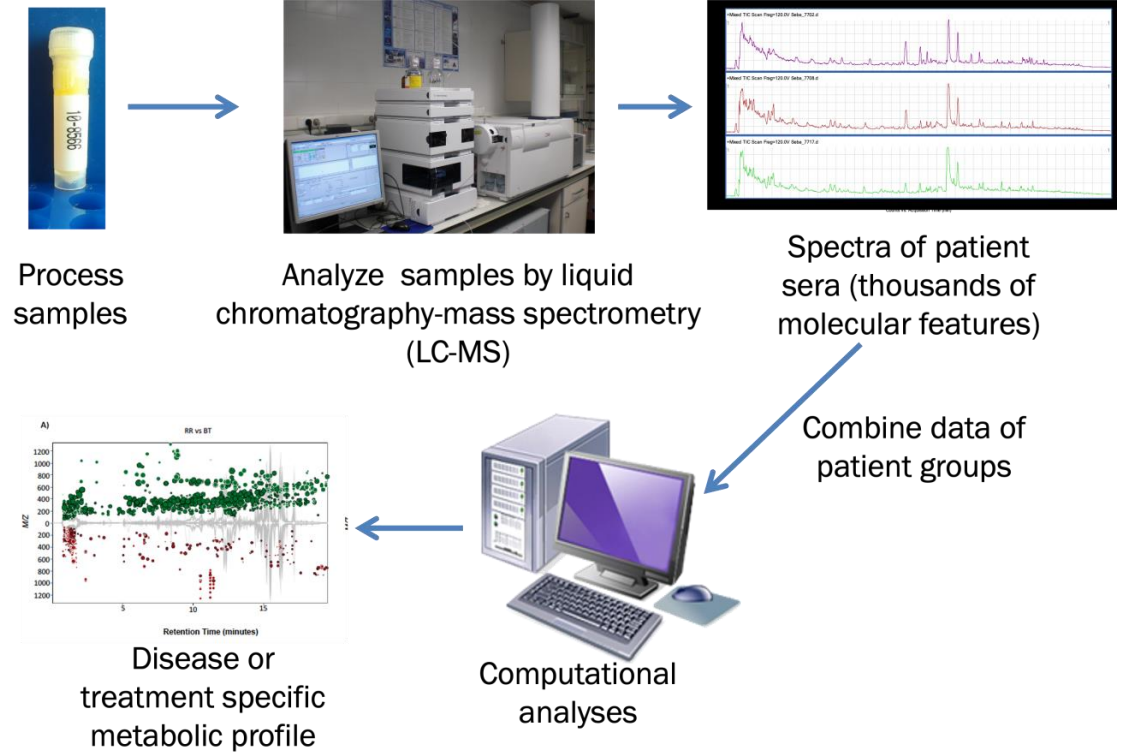


Urine and Serum Biomarker Analyses in Cohort B samples from RePORT South Africa

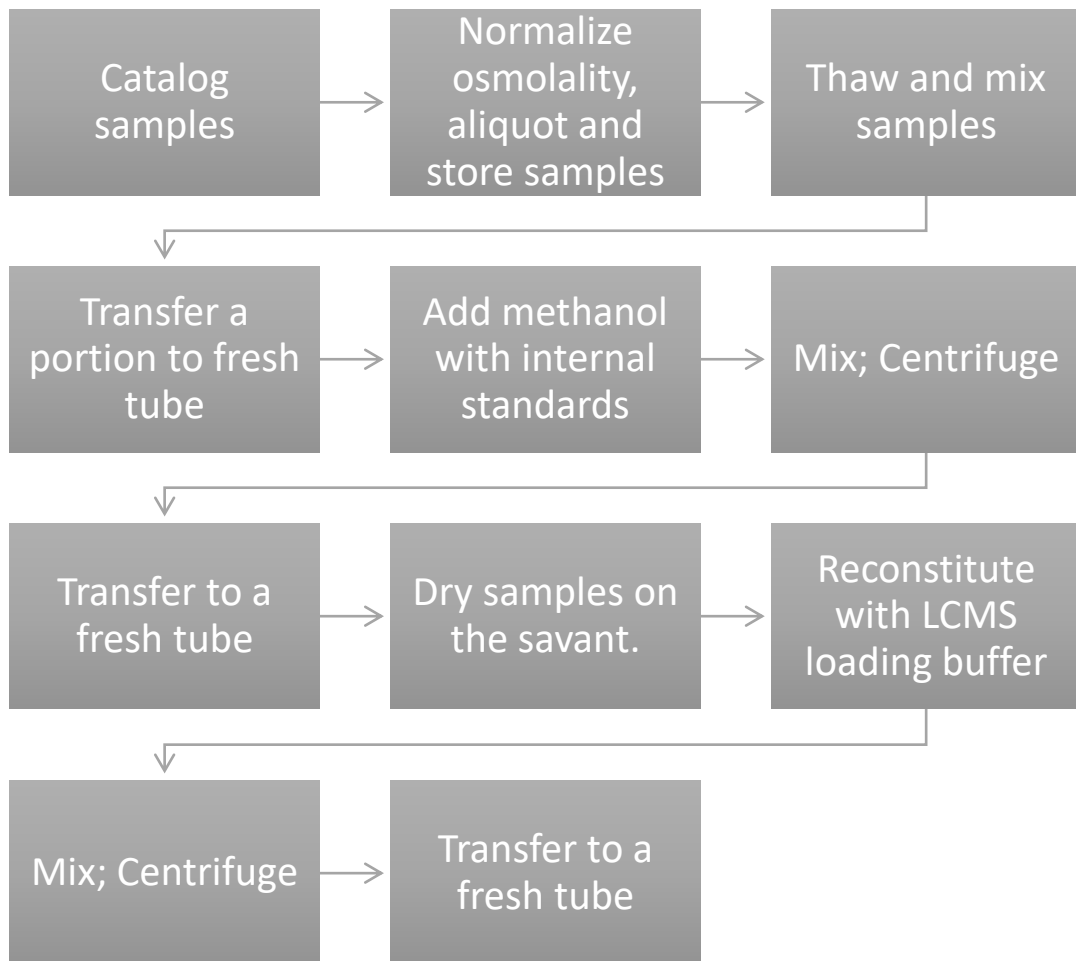
- *Colorado State University acknowledges, with respect, that the land we are on today is the traditional and ancestral homelands of the Arapaho, Cheyenne, and Ute Nations and peoples. This was also a site of trade, gathering, and healing for numerous other Native tribes. We recognize the Indigenous peoples as original stewards of this land and all the relatives within it. As these words of acknowledgment are spoken and heard, the ties Nations have to their traditional homelands are renewed and reaffirmed.*
- *CSU is founded as a land-grant institution, and we accept that our mission must encompass access to education and inclusion. And, significantly, that our founding came at a dire cost to Native Nations and peoples whose land this University was built upon. This acknowledgment is the education and inclusion we must practice in recognizing our institutional history, responsibility, and commitment.*



Analysis of Urine Metabolites as Biomarkers of Disease Progression



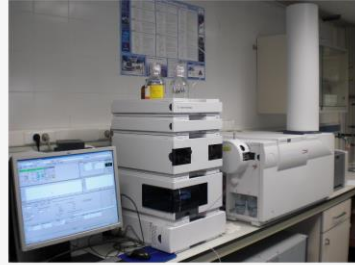
Analysis of Urine Metabolites as Biomarkers of Disease Progression



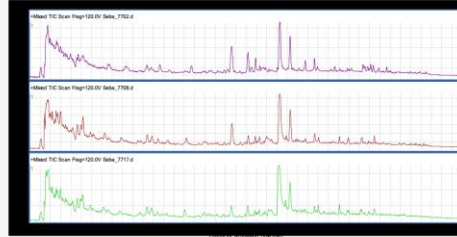
Process samples



Process samples



Analyze samples by liquid chromatography-mass spectrometry (LC-MS)

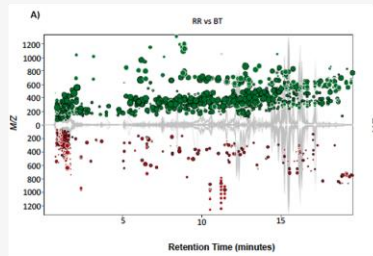


Spectra of patient sera (thousands of molecular features)

Combine data of patient groups



Computational analyses

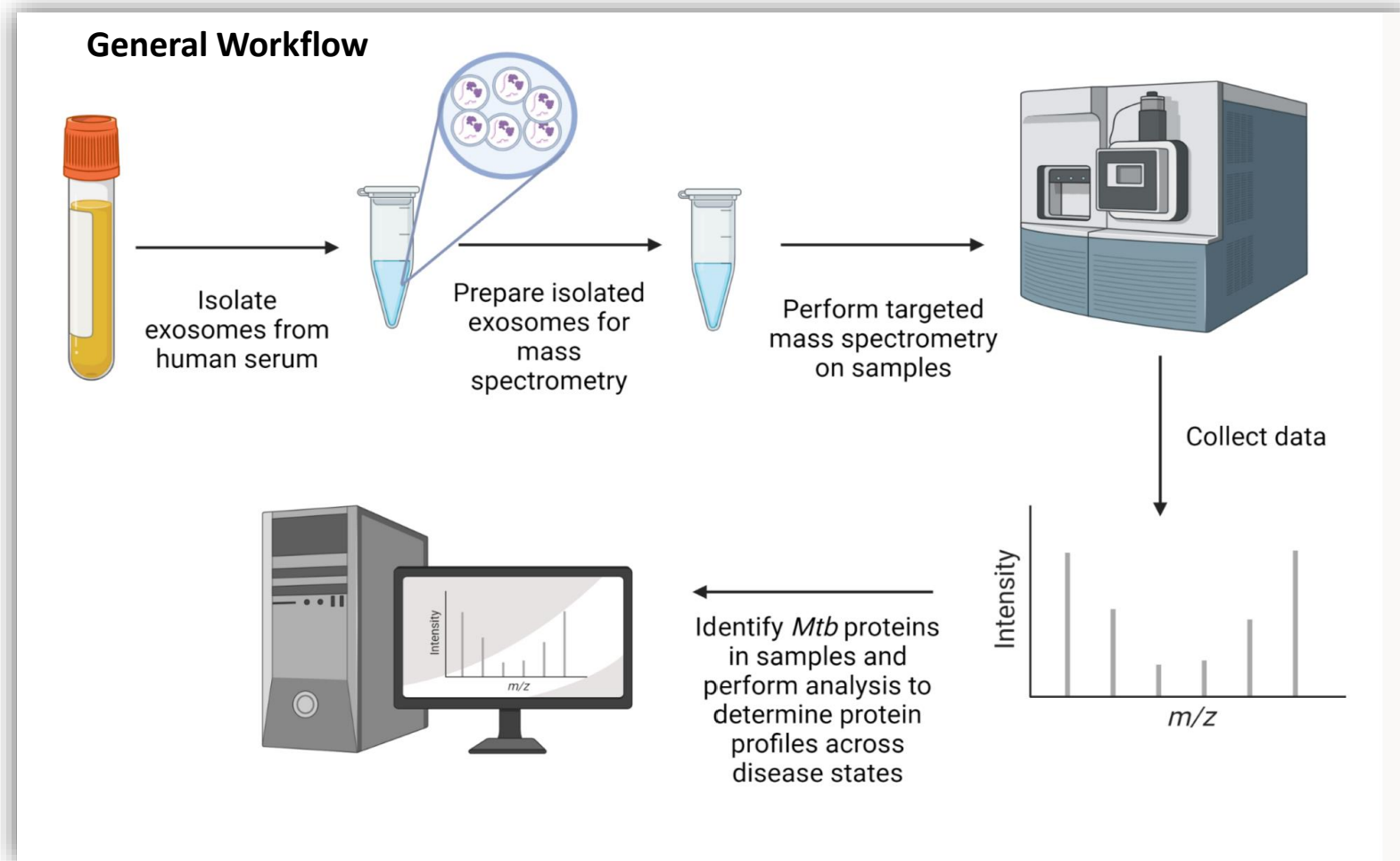


Disease or treatment specific metabolic profile

Analysis of Urine Metabolites as Biomarkers of Disease Progression

- Samples cataloged and aliquoted
- Collected LC-MS data on all 261 CORTIS samples
- Extracted LC-MS data for 41 targeted metabolites used for TB treatment response (75% completed)
- Extracting LC-MS data for all untargeted molecular features
- Data analyses to evaluate group separation to be started mid September

Mycobacterium tuberculosis biomarkers sequestered in exosomes isolated from serum – current progress

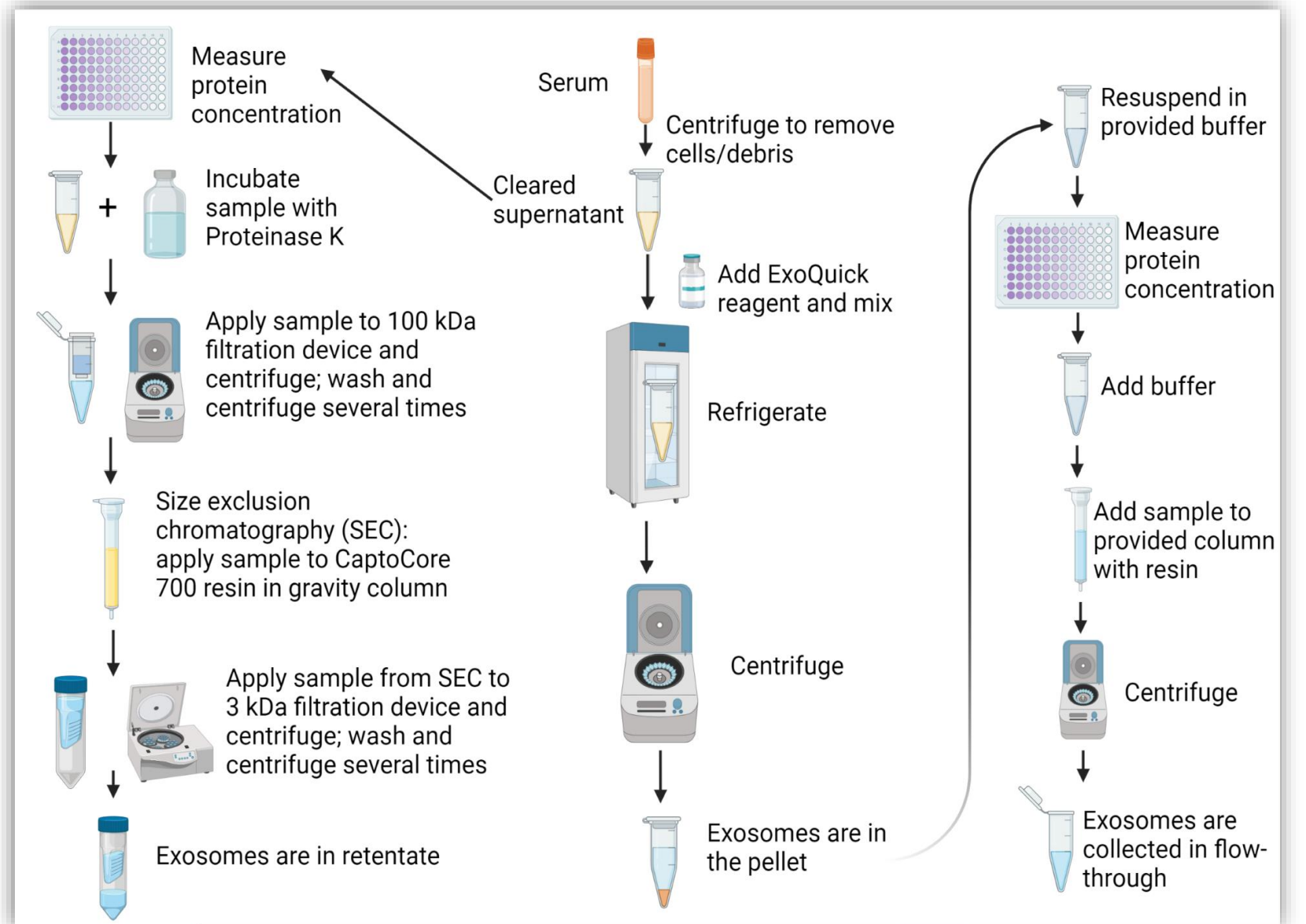


Optimizing sample processing:

- Captopcore SEC
- Exoquick ppt
- Exoquick ultra ppt

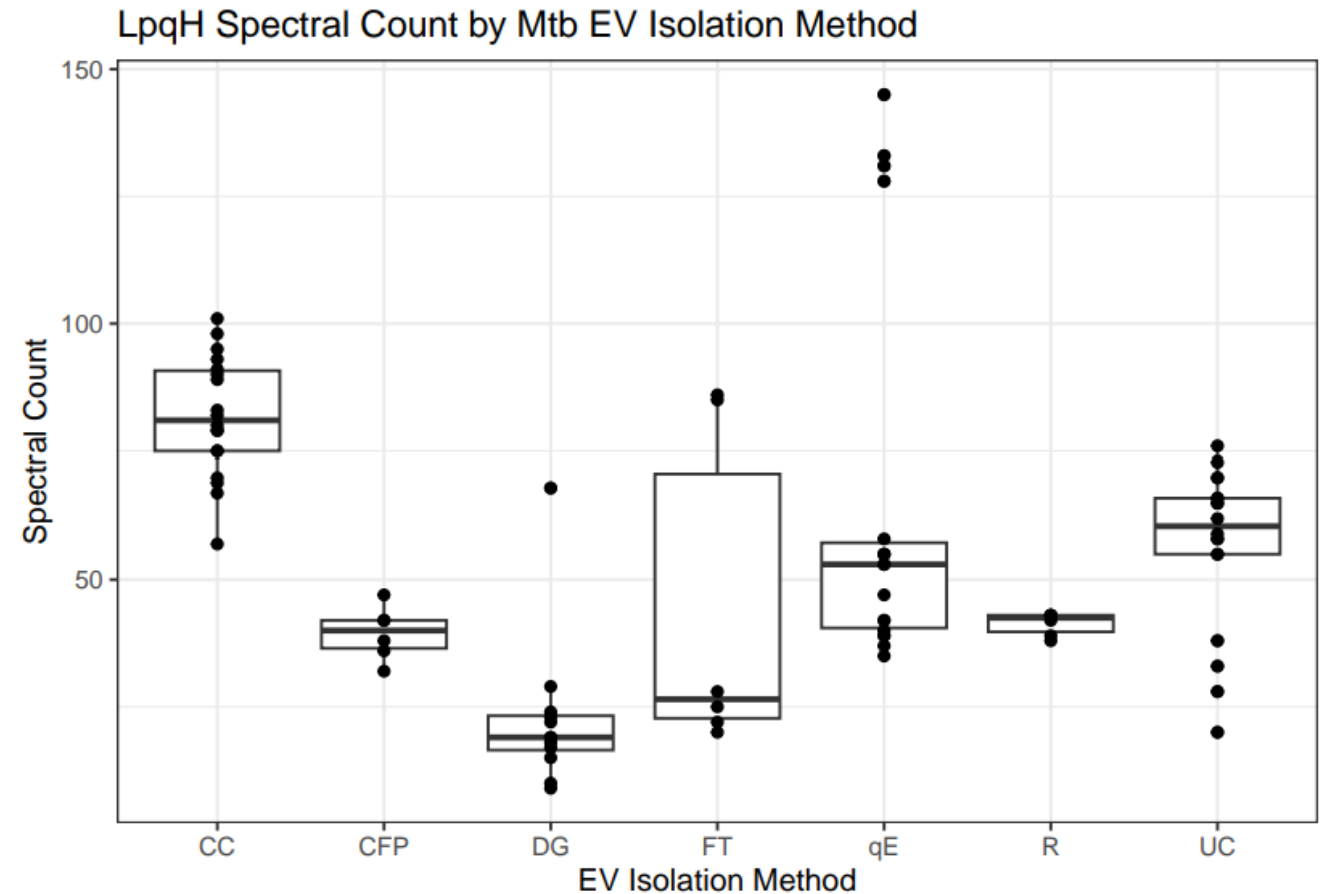
Then peptide access:

- In solution digestion
- In gel digestion



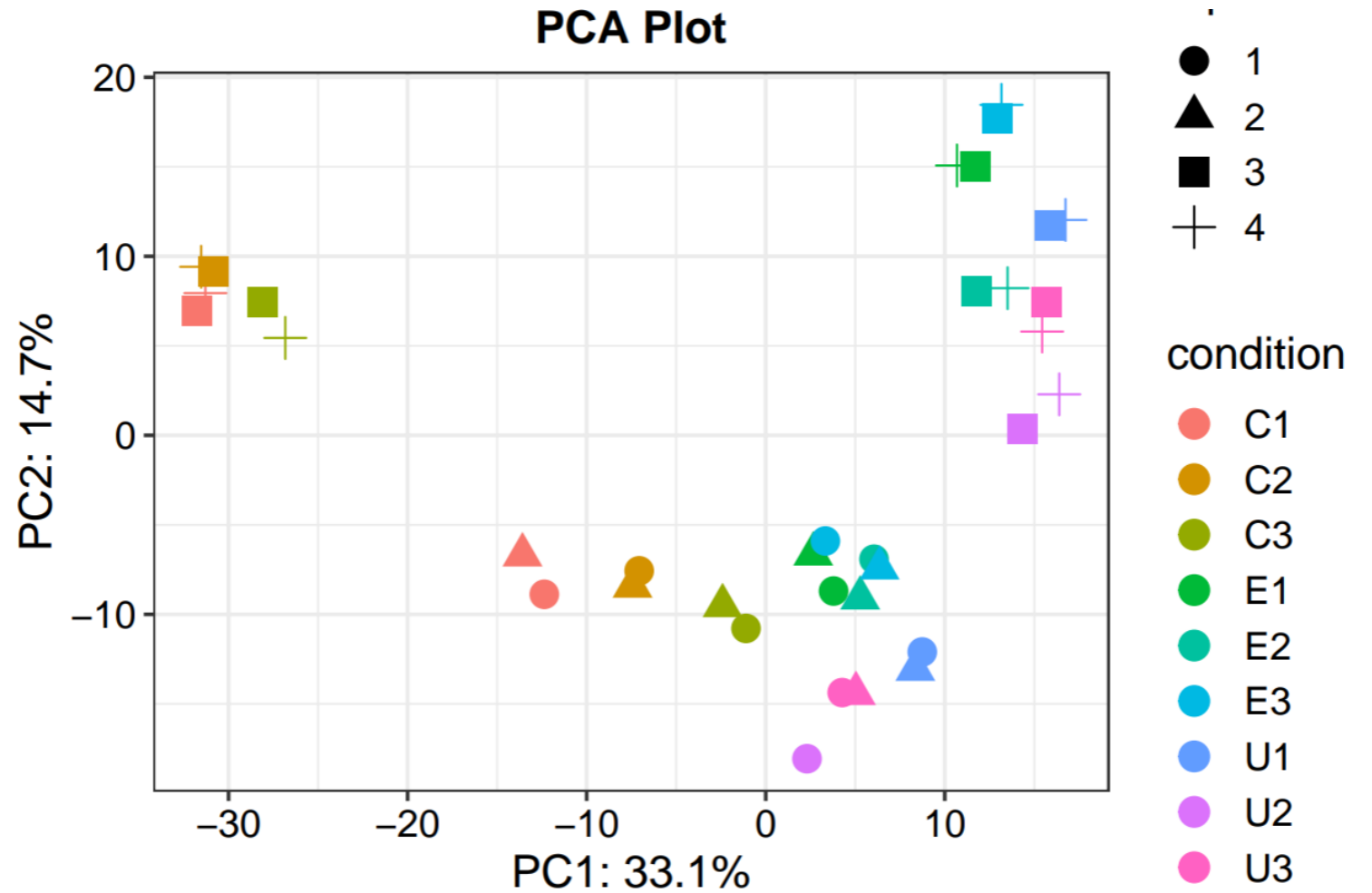
Sample processing rationale: Why?

- Processing matters (even with simple vesicles!) and especially with complex samples!
(Mtb proteins in CFP as example)
- Transfer processing method to robotics (build from John's efforts; serum processing is next level)



Protein feature relationships based on EV processing and protein digestion

- Samples processed:
 - C = captocore
 - E = exoquick
 - U = ultra
-
- Then:
 - 1, 2 = in gel digest
 - 3, 4 = in solution digest



Protein feature relationships based on EV processing and protein digestion

Samples processed:

C = captocore

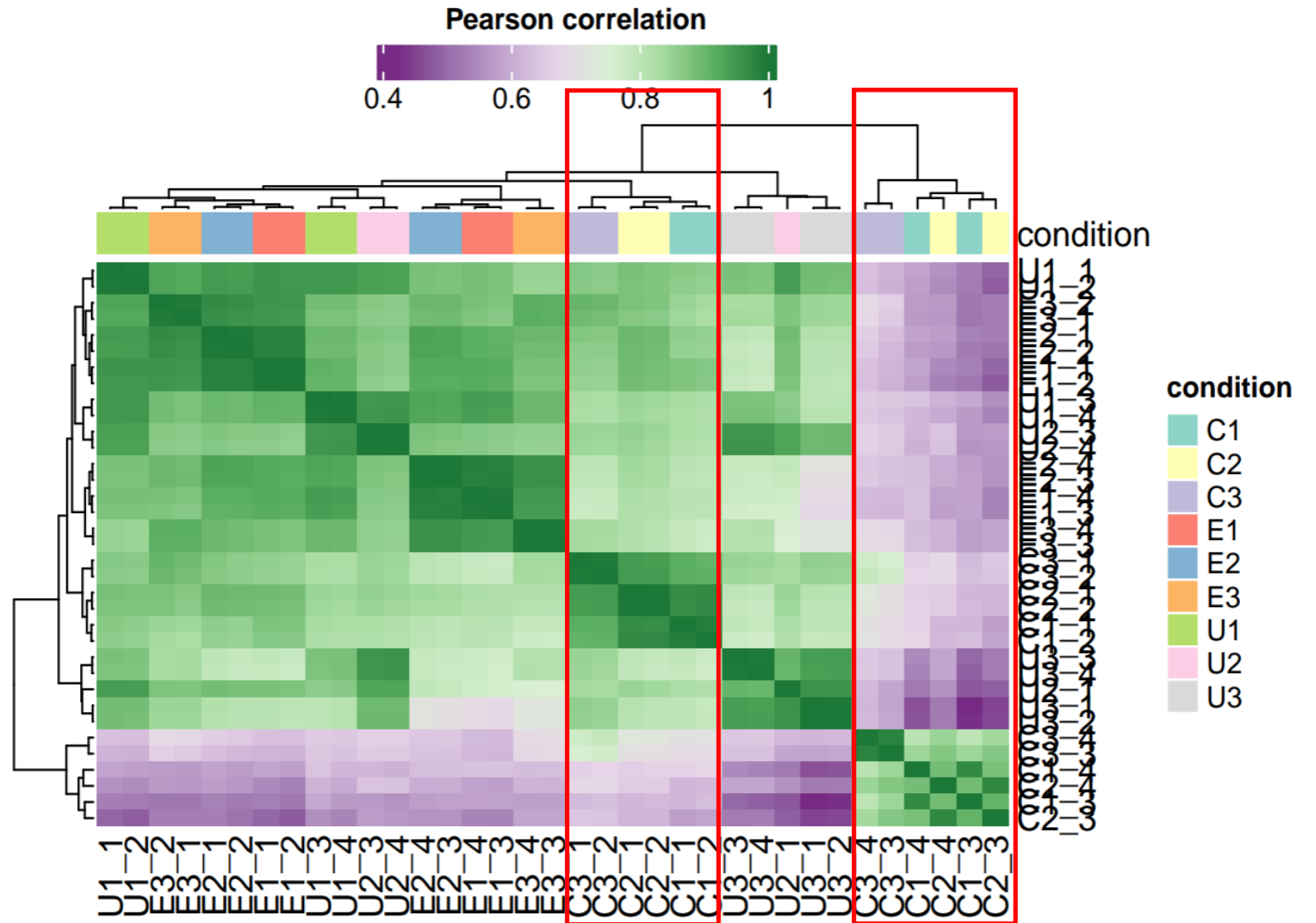
E = exoquick

U = ultra

Then:

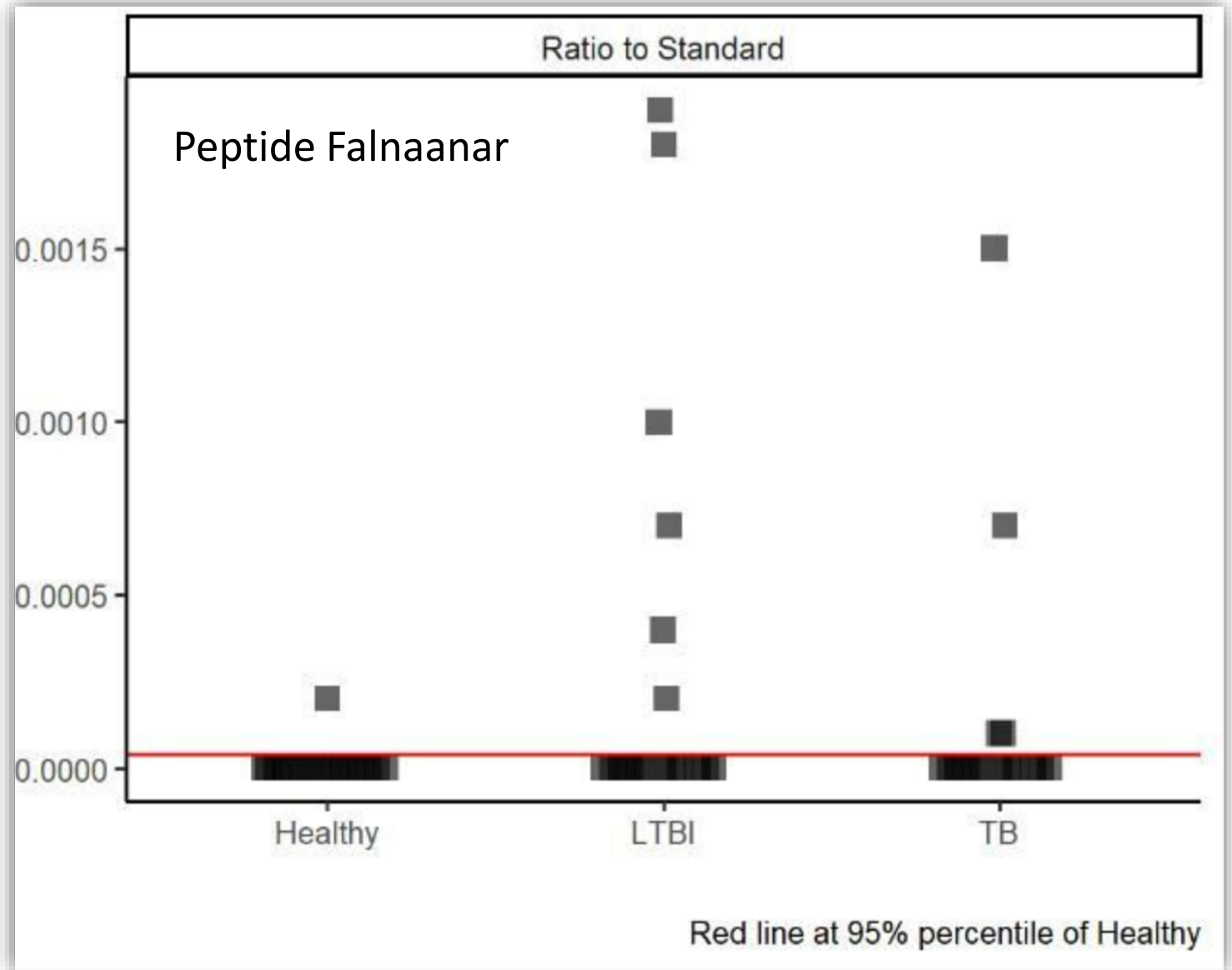
1, 2 = in gel digest

3, 4 = in solution digest



Segue....A quick analysis with banked samples from FIND –

- Captocore purified
- In gel digested
- Some promise – instrument errors

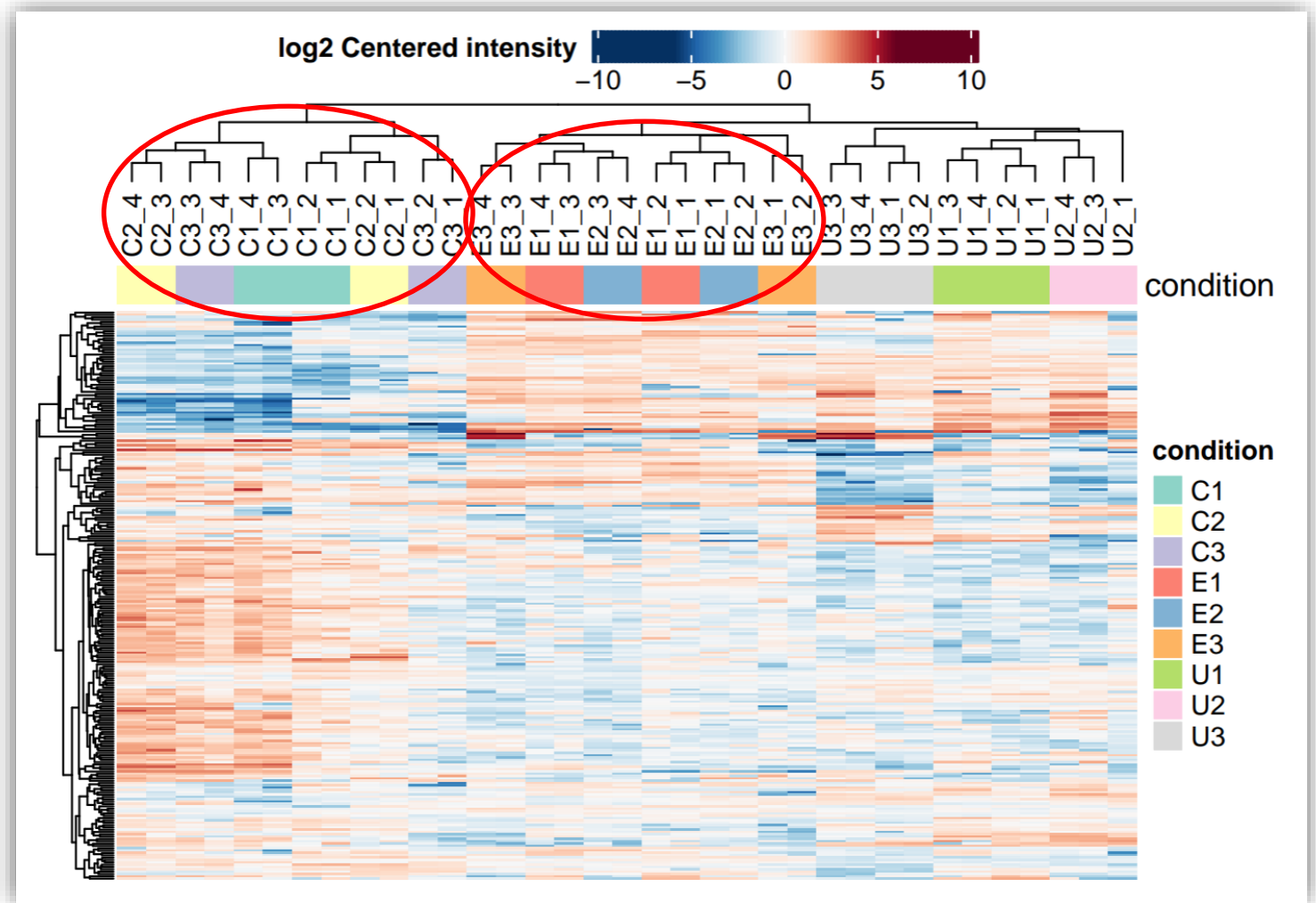


Final Method? Two horse race

- Samples processed:
- C = captocore
- E = exoquick
- U = ultra

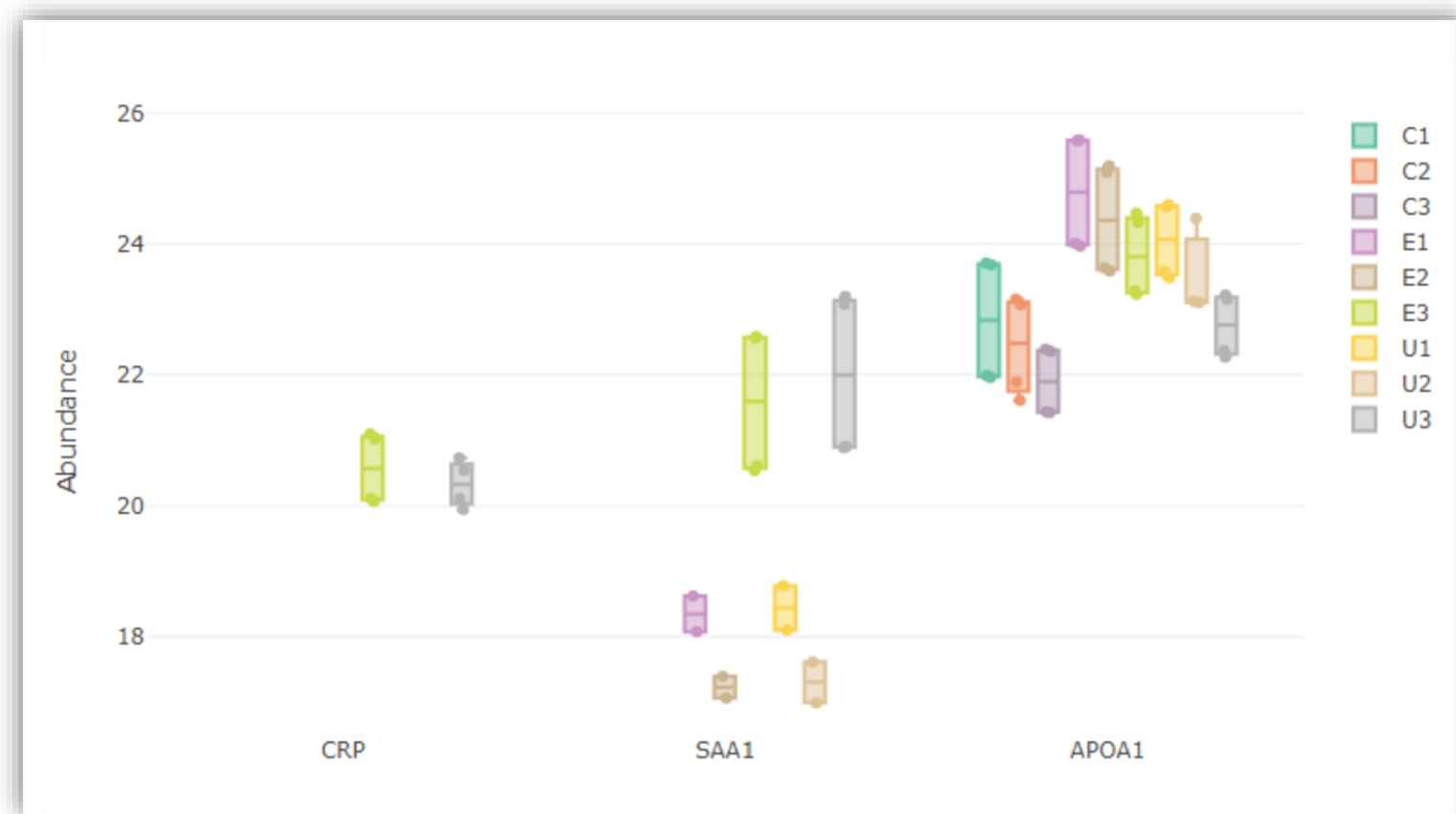
- Then:
- 1, 2 = in gel digest
- 3, 4 = in solution digest

**To Do: Final QC Assay with EV
specific tetraspanins!!**



Possible future directions for
Mtb exosome biomarker studies

Multiplex with host marker to drive ultimate sensitivity and
specificity for early detection / risk of developing disease?



Dobos and Belisle Laboratories @ Colorado State University

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Nicole Kruh-Garcia, Joanie Ryan and Dobos Lab (past and present)

The MRL and RMRBL @ CSU

RePORT-SA

CDRF

NIAID/NIH

CCTSI (CU Anschutz)