An Automated, Combined Workflow for Extracting Polar Metabolites and Lipids from Mammalian Cells

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Introduction

Cell Sample Preparation is Difficult, Time-Consuming, and Error-Prone

Challenges in Mammalian Cell Sample Preparation

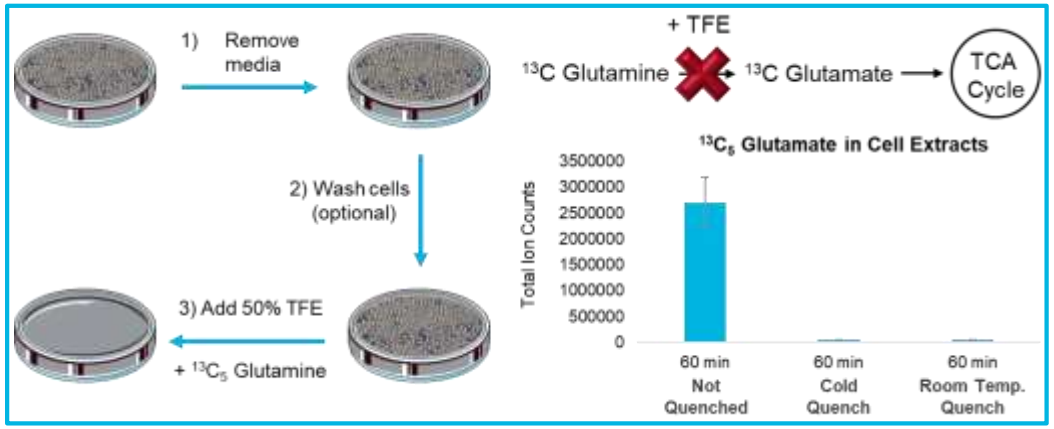
- Cold liquids (i.e. liquid nitrogen) used for fast metabolic quenching are difficult and, in some cases, dangerous to handle, especially in a time-sensitive workflow
- Liquid-liquid phase separations for metabolite and lipid separation require visualization of the liquid-liquid interface for accurate phase separation

Technological Solutions for Mammalian Cell Sample Preparation

- Room temperature 50% trifluoroethanol for cell lysis and metabolism quenching
- Solid phase extraction (SPE) for sequential metabolite and lipid collection from individual samples
- Automation of SPE workflow on Bravo Liquid Handler

Results and Discussion

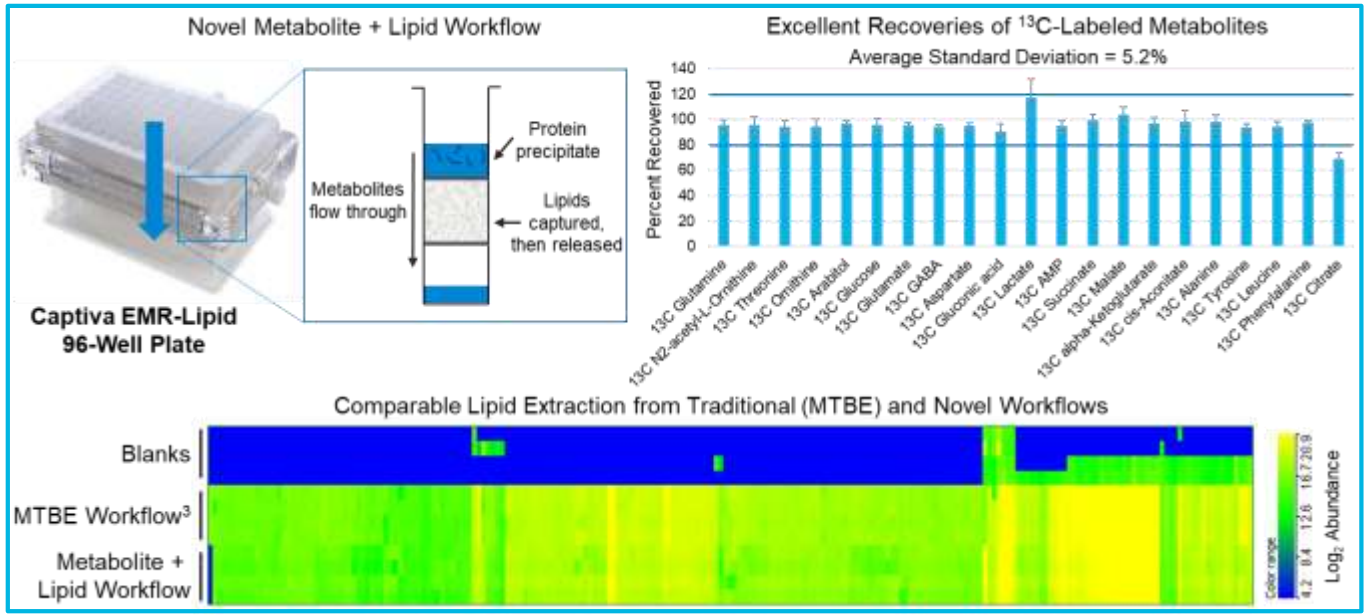
^{13}C Glutamine Stable Isotope Tracing Confirms Room Temperature Metabolism Quenching



No detectable conversion of ^{13}C glutamine into ^{13}C glutamate by K562 cellular enzymes during cold temperature metabolism quenching or during room temperature metabolism quenching with 50% trifluoroethanol (TFE, v/v). ATP is stable at room temperature in 50% TFE for at least 4 hours and the amount of ATP extracted is comparable to traditional cold quench workflows (not shown). Data analysis with MassHunter VistaFlux Software.

Results and Discussion

Novel Workflow Provides Good Polar Metabolite Recoveries and Lipid Extraction that is Comparable to a Traditional Workflow



Proteins precipitated from K562 cell lysate and soluble lipids are captured by the Captiva EMR-Lipid plate[2], providing a metabolite-containing flow-through. Lipids are sequentially eluted. Excellent metabolite recoveries for organic acid, amino acid, sugar, and nucleotide classes were measured from spiked-in ^{13}C-labeled metabolites using ion-pairing reversed-phase 6545 LC/Q-TOF and Quant 10.1 analysis. Representative lipid extractions analyzed by negative-ion mode, reversed-phase 6545 LC/Q-TOF, Lipid Annotator 1.0 and MPP 15.1 are presented in a heat map of relative peak areas for 225 lipid annotations representing 21 lipid classes across four extraction replicates from each workflow.

Conclusions

Improved, Semi-Automated Cell Sample Preparation

- Effective room temperature metabolism quenching improves safety and reduces complexity of workflow
- Unique Captiva EMR-Lipid SPE material enables sequential collection of polar metabolites and lipids for intra-sample multi-omics correlations
- SPE workflow is compatible with Bravo automation, which can reduce operator errors and improve reproducibility

References

[1]Hartman, T. E., et. al. (2017) *Agilent Technologies Application Note*.

[2]Zhao, L. et. al. (2017) *Agilent Technologies Application Note*.

[3]Matyash, V., et al., J Lipid Res 49, 1137-1146, (2008) doi: 10.1194/jlr.D700041-JLR200