BECKMAN COULTER
Life Sciences

Sample preparation for metabolomics

"Having conducted research in metabolomics for almost 20 years, I've experienced firsthand the labor intensive nature of metabolite extractions as well as the importance of repeatable extractions to achieve high data quality. Establishing our extraction protocols on the Biomek i7 has opened a new horizon for high-throughput metabolomics, enabling the preparation of thousands of cell extracts for mass spectrometric analysis within just a few days"

Mark R. Viant, Ph.D.
Executive Director of Phenome Centre Birmingham
Team Leader of Metabolomics & Systems Toxicology Laboratory
University of Birmingham, UK

Metabolomics

Metabolomics is the science of measuring and statistically analyzing the low-molecular-weight metabolites within biological specimens. As the levels of metabolites change in response to external stimuli such as diet and exposure to chemicals, measuring metabolites over time can provide indications of physiological conditions, both healthy and diseased. Since metabolomics allows for the profiling of thousands of metabolites simultaneously, a much greater number than is currently possible with traditional laboratory techniques, it is widely used to understand complex physiological processes in animal and plant biology.

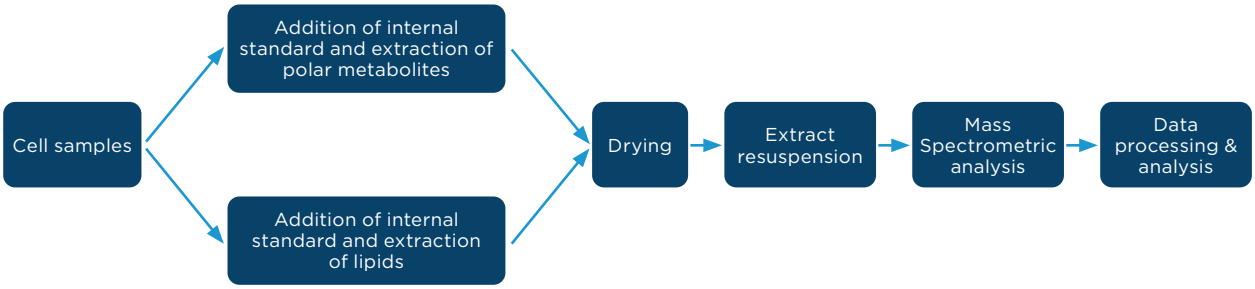

Figure 1. A workflow for high-throughput in *vitro* metabolomics.

Compared to genomics and proteomics workflows, metabolomics workflows are complicated because there is no single technology available to analyze the entire metabolome. Analyzing molecules of dissimilar physicochemical properties involves separate workflows for different classes of metabolites (e.g., polar vs. non-polar). In addition, metabolite levels will change during sample preparation - due to residual enzymatic activity - unless they are extracted according to strict protocols using low temperature and organic solvents. With the development of international quality assurance/quality control (QA/QC) practices in metabolomics, different types of QC samples need to be prepared during the extraction of study samples. Furthermore, large-scale metabolomics studies with thousands of samples should achieve the repeatability and processing requirements that are widely used during the manual preparation of small studies.

Challenges

- Increasing throughput without compromising repeatability
- Enabling the extraction of polar metabolites and lipids
- Maintaining sample integrity through strict processing requirements
- Ensuring appropriate use of quality control samples

Use of automation to overcome challenges

Increasing throughput with high repeatability via automation on the Biomek i7 automated workstation

As automation has advanced to culture cellular samples, and for mass spectrometric measurements, processing and statistical analysis, a significant bottleneck in metabolomics has formed around the labor-intensive manual extraction of samples. Until recently, a graduate student would typically prepare fewer than a thousand samples throughout their entire degree, requiring a week at the bench to extract each batch of 100-200 samples. Using the automation provided by the Biomek i7 Hybrid, Julia Malinowska - a student in the Metabolomics & Systems Toxicology Laboratory, University of Birmingham, UK - can prepare thousands of metabolite extracts for analysis each week, through the use of 96-well microplates.

Enabling the extraction of polar metabolites and lipids

Both the polar metabolome and lipidome can convey deep insights into normal and adverse physiological processes. To measure a wide range of metabolite classes of dissimilar physicochemical properties, different solvent systems need to be established to extract samples for analysis. The Biomek i7 automated workstation allows pipetting of a range of aqueous and organic solvents suitable for extracting low biomass samples, enabling protocols to be implemented and applied to study polar metabolites and lipophilic chemicals.

Maintaining sample integrity through strict processing requirements

Residual enzymatic activity is a significant problem for metabolomics researchers, changing the metabolite profile between the time of sampling and the analytical measurement, and potentially generating misleading results. A combination of low temperature, and organic solvents to denature proteins, are the keys to minimizing this problem. The Biomek i7 workstation deck can be configured to maintain cell samples at -15 ˚C throughout the extraction process using automated labware positioners (ALPs) on the deck, while solvents for the extraction are kept at +4 ˚C. A large deck with multiple types of ALPs allows for regulating the temperature of the samples depending on the step in the workflow, thereby minimizing enzymatic activity and ensuring sample integrity.

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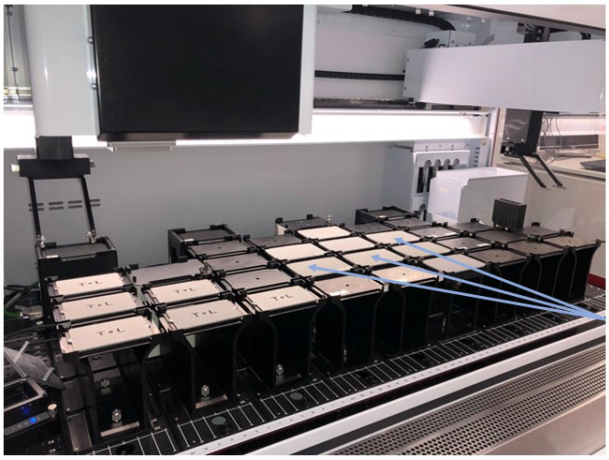

ALPs maintained at -15 ˚C

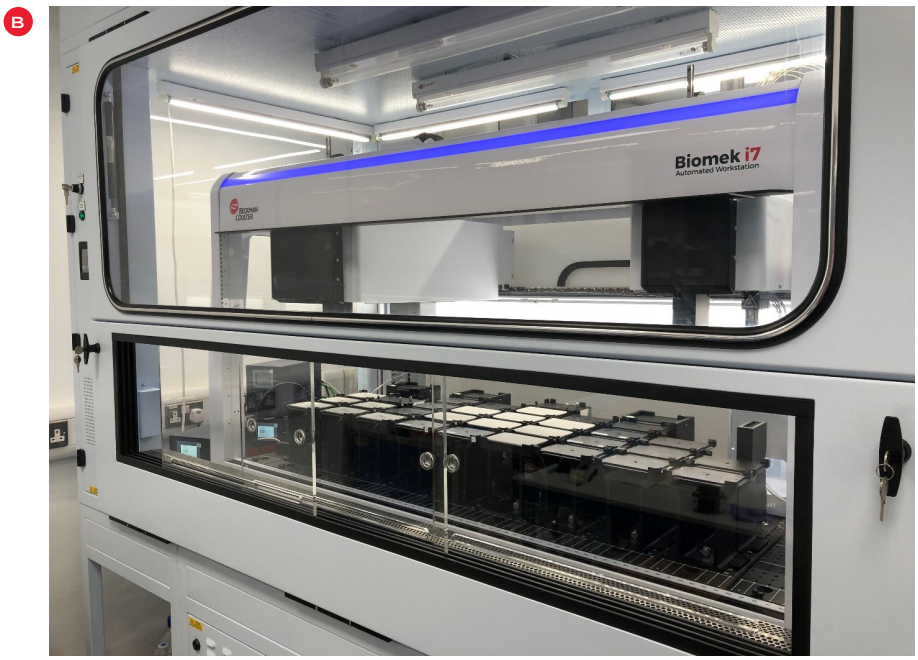

Figure 2. Beckman Coulter i7 hybrid workstation for high-throughput metabolomics. (a) Instrument deck. Arrows indicate examples of ALPs used to maintain cell samples at -15 ˚C. (b) Biomek i7 hybrid workstation with the enclosure.

Ensuring compatibility with quality control samples

Several quality control samples are now integral to a robust metabolomics study design. For example, an intrastudy QC is required for several data processing steps, including an assessment of any drift in signal sensitivity across and between analytical batches. The composition of this QC sample must be very similar to the study samples (i.e., a pool derived from an aliquot of each sample, or a pool of equivalent biological material from the same cell culture study), and hence the preparation of the intrastudy QC must be incorporated into the sample preparation protocol. Similarity, the extraction blank must be prepared using the same protocol. Using the 96-channel and Span-8 pods, the Biomek i7 workstation provides sufficient flexibility to pool, mix and re-aliquot metabolomics QC samples to ensure a robust study plan.

References

1. Clish C. B. (2015). Metabolomics: an emerging but powerful tool for precision medicine. Cold Spring Harbor Molecular Case Studies, 1(1), a000588. https://doi.org/10.1101/mcs.a000588

2. Viant M. R. et al. (2019) Use cases, best practice and reporting standards for metabolomics in regulatory toxicology. Nature Communications, 10, Article number: 3041. https://doi.org/10.1038/s41467-019-10900-y