Semi-automated extraction of cell and tissue samples for multi-omic analysis using the Biomek i7 Hybrid Workstation

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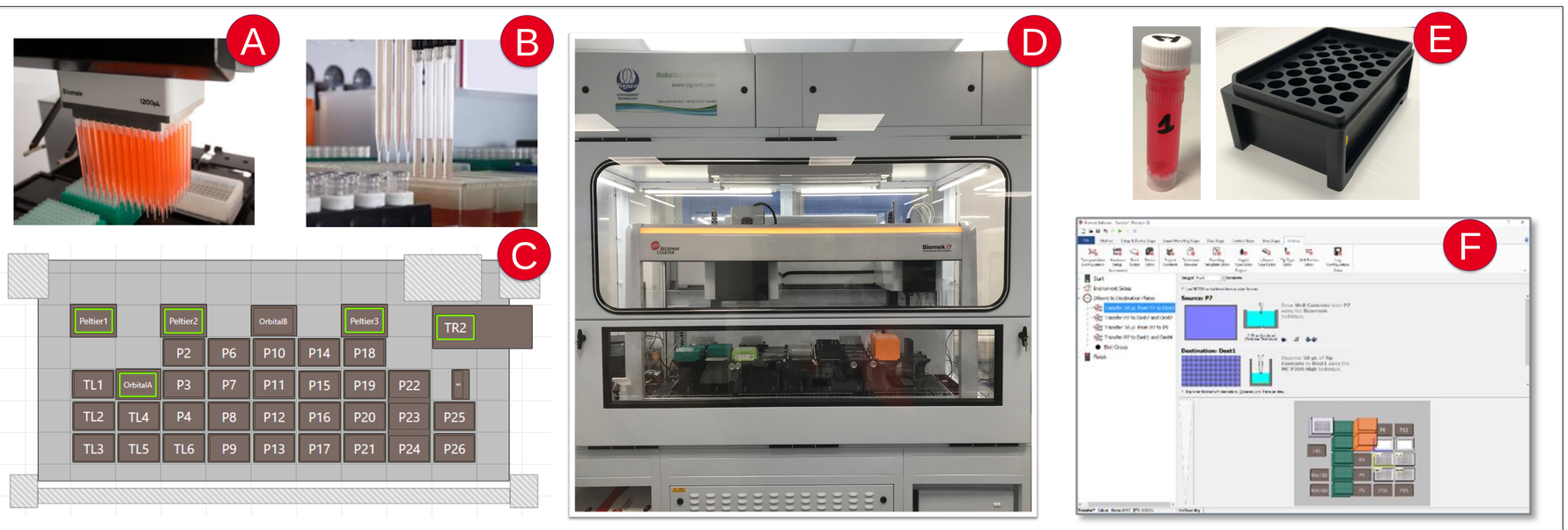
Introduction

‘PrecisionTox’, a €20M EU Horizon 2020-funded research project, aims to develop new methods for chemical hazard assessment based on a fusion of: 1) transcriptomics and metabolomics analyses, 2) machine learning, and 3) chemical exposure experiments in phylogenetically disparate model organisms (*Daphnia magna*, *Danio rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Xenopus laevis*) and the HepG2 human cell line. With >10,000 samples being generated across 6 different laboratories in Europe and the US, there is a considerable need to automate the extraction of polar and lipophilic compounds from cells and tissues. We report progress in the development of a high-throughput semi-automated extraction method using a Biomek i7 Hybrid automated workstation from Beckman Coulter Life Sciences.

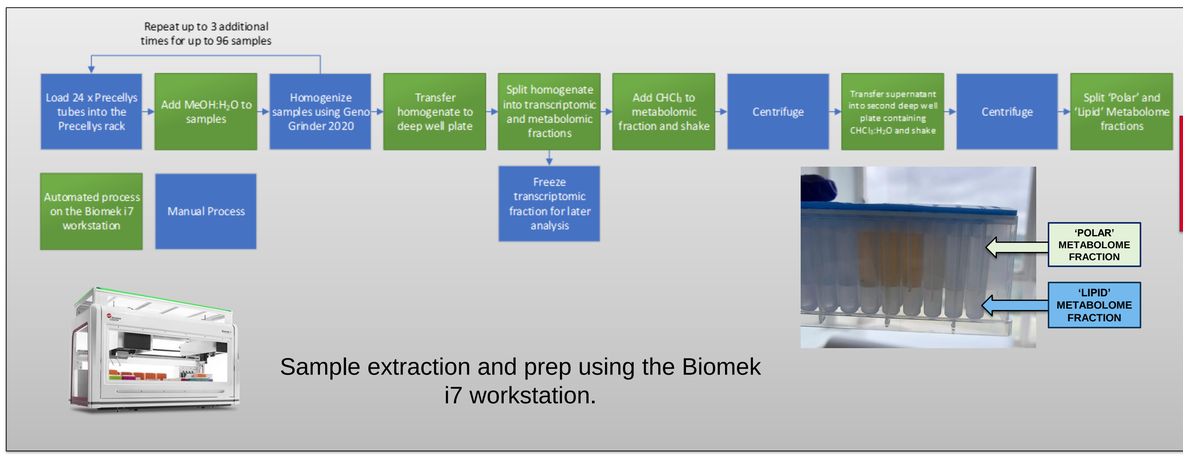
Methods

The Biomek i7 workstation used in this workflow includes the following features:

- Large-volume multichannel head capable of pipetting ranging between 5 µl and 1000 µl with select tip pipetting feature (**A**)
- Span-8 pipettor capable of independent volumes across all eight channels ranging from 1 µl to 1000 µl (**B**)
- Three static Peltier positions (temperature range 4 °C to 100 °C) (**C**)
- Eight cryogenically cooled positions (temperatures < -10 °C) (**C**)
- Two orbital shaker positions capable of shaking deep well plates up to 2000 rpm with a 3 mm orbit (**C**)
- BigNeat enclosure (**D**)
- Bespoke 24 position Precellys tube racks capable of interfacing with both the Geno Grinder tissue homogenization system and Biomek i7 workstation (**E**)
- Biomek i-Series software (**F**)



The overall workflow involves exposing various model systems to compounds of interest and then collecting samples in Precellys tubes, which provide a convenient way to support tissue homogenization in MeOH:H_2O using the Geno Grinder 2020 unit. Once homogenized, the samples are consolidated from the 24 tube Precellys racks into a standard 96 well format to allow for processing using the Biomek i7 workstation’s multichannel pipetting head. After splitting the homogenate and saving a fraction in a fresh plate for RNA-Seq analysis, the metabolomic fraction is then split into polar and lipid fractions using $CHCl_3$:H_2O. The separate fractions are then transferred to different plates with the polar fraction dried in a SpeedVac while the non-polar layer is dried under N_2 (g). Polar and non-polar fractions are then analyzed using multiplexed untargeted LC-HRMS(/MS) and RPLC-MS respectively in order to determine if the compound of interest has a biological effect on the model systems being studied.



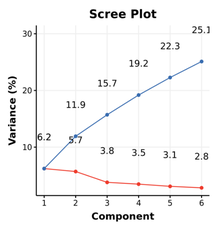
Results

Examining For Potential Batch Effect

To assess workflow reproducibility, technical replicate batches utilizing beef extract powder (Sigma B4888) and lamb kidney fractions were processed through the semi-automated workflow at Phenome Centre Birmingham. A total of 6 batches of 12 replicate samples each were conducted over the course of two days with beef extract powder samples. A single batch of 14 replicates of lamb kidney fractions was also processed to test the extraction workflow on tissue samples. Sample locations were randomized across the processing plate in each batch. Extraction blanks were included in each batch. Polar fractions were analyzed using HILIC-MS while non-polar fractions were analyzed using RPLC-MS. Both analyses were conducted on a Thermo Scientific Q Exactive Focus mass spectrometer with Thermo Scientific™ Dionex™ Ultimate™ RSLC 3000**.** Following signal correction (if required) and quality assessment, data was subjected to principal component analysis to examine batch effect

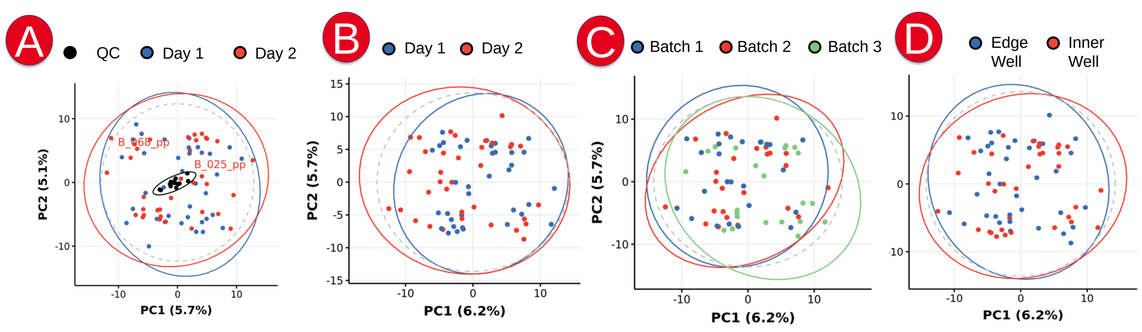
	01	02	03	04	05	06	07	08	09	10	11	12
A	1	6	4	2	5	3	1	6	4	2	5	3
B	3	6	4	2	3	EB	5	1	1	4	6	1
C	5	2	3	EB	4	4	6	LK	LK	3	2	6
D	2	EB	2	EB	EB	1	LK	LK	4	5	LK	4
E	4	4	3	EB	2	EB	3	LK	5	6	5	2
F	6	EB	6	EB	1	2	1	LK	LK	LK	LK	5
G	1	5	LK	6	5	3	EB	1	LK	LK	LK	3
H	3	5	2	4	6	1	3	5	2	4	6	1

Batch layout. 1-6 denotes beef extract powder batch, EB denotes extraction blank, LK indicates lamb kidney fraction (data not shown).



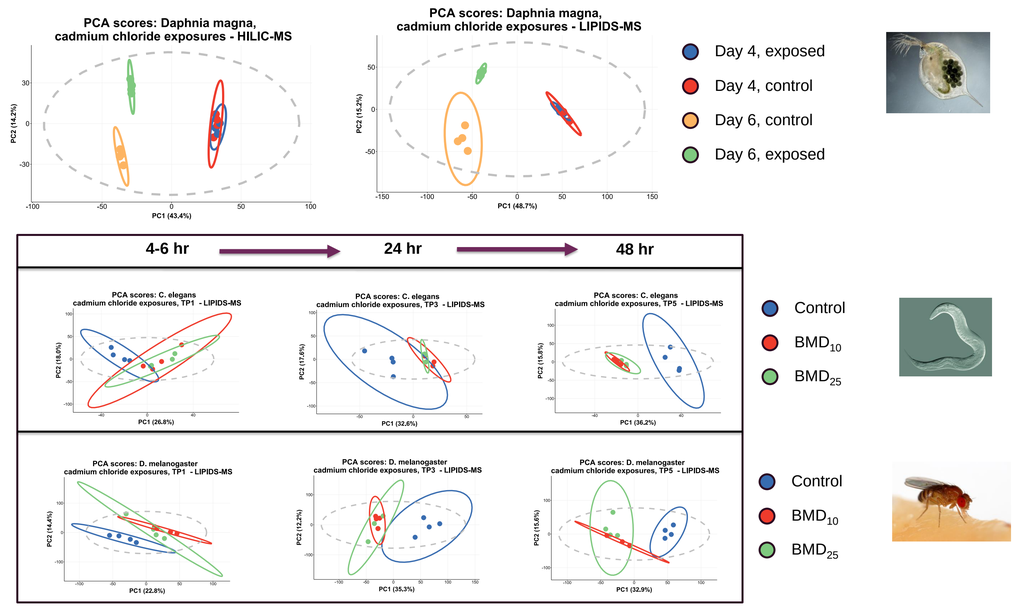
Beef extract powder batches scree plot.

The first six principal components explain only 25.1% of total variance. QCs cluster centrally (Graph A), while no discernable pattern could be determined between principal components 1 and 2 when assessing the day the batch was processed (Graph B), the batch on each day (Graph C), or location of the samples in the processing plate (Graph D).



Exploring chemical-induced metabolic changes

To explore if chemical-induced adverse health effects can be detected within the metabolic profiles of relevant model systems, samples from the water flea *Daphnia magna,* the nematode worm *Caenorhabditis elegans*, and the fruit fly *Drosophila melanogaster* were processed using the semi-automated workflow following a 48-hour chemical exposure to the toxicant cadmium chloride at BMD_{10} (eliciting immobilization/death in 10% of organisms treated) in the case of *Daphnia magna* or BMD_{10} and BMD_{25} for *Caenorhabditis elegans and Drosophila melanogaster.*



PCA scores plots for lipidomics analysis *of Caenorhabditis elegans* (top) *and Drosophila melanogaster* (bottom) control samples and samples exposed to cadmium chloride at BMD_{10} and BMD_{25} levels.

Conclusions and Future Work

In conclusion, we have shown that the semi-automated workflow for the extraction of tissue samples for multi-omic workflows using the Biomek i7 Hybrid Workstation allows for the processing of samples from a wide variety of sample types and is consistent across batches. In our ongoing work, we plan to integrate the Geno Grinder 2020 unit and centrifuge with the Biomek i7 Hybrid Workstation to reduce manual interventions still further. In addition, we plan to expand the workflow’s application domain to include cultured human cell lines in 24- and 96-well format.