A High-Throughput, Semi-Automated, Sample Handling Platform for Quantitative Proteomics: A Test Case Study of Gene Regulation in Mouse Hypothalamus

Paul D. Piehowski, Vladislav A. Petyuk, Arshad Khan, Anil K. Shukla, Desmond Smith and Richard D. Smith

Biology Sciences Division, Pacific Northwest National Laboratory, Richland, WA *Department of Molecular and Medical Pharmacology, University of California Los Angeles, Los Angeles, CA

Introduction

• The technical variability of our platform was 22%, while the variability of the HDMP was 28%. Thus, we estimate that 38% of the variance observed is due to biological variability.

Methods

1. Frozen, dissected tissue was loaded into a homogenization plate leaving the outermost wells empty to reduce variation from edge effects.

2. Samples were homogenized in a denaturing buffer using a Tissuelyser (Qiagen).

3. Samples yield was measured by Coomassie assay and protein concentration was normalized using a custom script and an EpMotion liquid handler.

4. Samples were digested, alkylation and digestion were carried out on the EpMotion with an external plate incubator customized to uniformly distribute temperature in well plates.

5. SPE was achieved using a positive pressure manifold (SPEware) and SPE tips (Agilent).

6. Peptide concentrations were measured using the BCA assay and normalized using a custom script and an EpMotion liquid handler.

7. Samples were separated with a 100-minute, reverse-phase gradient on a Waters nanoACQUITY UPLC and analyzed using a Velos Orbitrap MS (ThermoScientific).

Analysis of Single Nucleotide Polymorphisms (SNPs)

Figure 3. Test LMM was used to map genetic variation that potentially affects expression of the 4732 unique peptides. Allele frequency >5% (ui>0.05/SNP) were informative and used in the analysis. This resulted in > 150 SNPs (ui>0.05) with a significant correlation. A) Manhattan plot showing best LMM results for two different correlations of the protein gene detected by the blue dot. B) Peptide with many strongly correlated SNPs. B) Peptide with many strongly correlated SNPs in the same gene region as the SNP.

Results

Alignment and Peak Matching

Figure 1. Peak matching to an AARP tag database resulted in 4732 unique peptides, mapping to 1123 protein groups, which includes a minimum of 55 datasets. A) Mass error histogram for peptides across 109 samples. B) Normalized retention time (RT) error histogram across all 109 datasets. The narrow width and normal distribution of these measurements demonstrate the robust performance of the LC-MS analysis, performed on a nanoACQUITY (Waters) and Orbitrap Velos (ThermoScientific).

Measurement Variability

Figure 2. Peptide abundances were calculated as the area under the curve. Labeling errors deriving from planting and assay variance were corrected for by adjusting the median log2(plight/healthy) to 0. Alanine differ in significant statistical test of coefficient of variance (CV) expressed as a percentage. Controls are displayed in red and individual strains (samples) in purple. B) Peptide CV’s in Control vs. CV’s in the sample set. In all, 2013 peptides showed higher variance across strains than the within strain control.

Conclusions

• 109 samples are processed from start to finish in 1.5 working days.

• Samples were processed in a single batch to minimize batch effects.

• Using biological replicates of an inbred mouse strain as quality control allows the assessment of sample handling variability.

• Technical variability for the entire sample handling process from dissection through to analysis was limited to 22%.

• Minor variations in the genome can be detected at the proteome level using this platform.

Acknowledgements

This work was funded by grant DE-SC0004262 from the DOE. Samples were acquired using capabilities developed under the support provided under DOE grant DE-AC05-76RL01830. This work was performed in the Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility on the PNNL campus. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under contract DE-AC05-76RL01830. For more information about career opportunities at PNNL please visit http://omics.pnl.gov/careers.

References


CONTACT: Paul Piehowski, Ph.D.
Biology Sciences Division
Pacific Northwest National Laboratory
E-mail: paul.piehowski@pnnl.gov

Career Opportunities: For potential openings in the Omics Separations and Mass Spectrometry Department at PNNL please visit http://omics.pnl.gov/careers.