ABRF-sPRG 2013-2014 Study: Development of a Peptide Normalization Standard Consisting of 1,000 Stable Isotope Labeled Peptides

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ABRF-sPRG 2013-2014 Study and Results

50 Participants were shipped one kilo containing a lyophilized mixture of a tryptic digest of HEK293 cell lysate spiked with 1,000 stable isotope labeled (SIL) synthetic peptides. Participants had access to sPRG-generated spectral libraries, a Skyline tool, and ten FASTA files. Over half the participants (47 laboratories) returned data analysis results via Panorama Web Repository and RAVY data files to NIST FESRE.

Methods

Sequences of synthetic peptides were derived from approximately 552 proteins, conserved across proteomes of commonly analyzed species. Homo sapiens, Mus musculus and Flavus nonvegus. Peptides represent a wide range of hydrophobicity and ionization points typical of complex proteomic samples. The 1,000 isotope-labeled peptides were spotted-synthesized by JPT (www.jpt.com). Individual peptides were reconstituted, combined and desalted by solid-phase extraction. HEK293 cell lysates were prepared by RIPA lysis and sonication, and proteins were methanol-precipitated, neutralized and digested with Lysozyme-C and Trypsin. The HEK proteolytic peptide mixture was desalted by solid-phase extraction. For the combined formulation, 375 fmol of synthetic peptide was added to 5 pg of the HEK peptide mixture. This synthetic peptide mixture and the combined synthetic and HEK derived peptide mixture were characterized individually by sPRG members and study participants representing multiple LC-MS/MS instruments platform.

Characterization of Peptide Standards

1,000 isotopic labeled peptide were synthesized and analyzed in the laboratories of sPRG members with various instruments and fragmentation methods. Greater than 99% of the peptides were identified during the validation runs. For peptide dilution experiments, 100, 50, 25, 12.5 and 6.25 fmols were individually analyzed via LC-MS/MS on an Orbitrap XL. The majority of peptides behaved as expected with a linear response; however, a few peptides had a non-linear response (A, B). The ABRF SIL peptides were spiked into a HEK293 digest and showed minimal variation in retention time (DC) while enabling estimated abundance to be calculated (D).

Participant Information and Demographics

Over the past decade, proteomic research has been driven by a series of initiatives and events to comprehensively characterize proteomes, including quantitative proteomics. Technologies such as stable isotope labeling (SIL) synthetic tryptic peptides. For the current study, a lyophilized synthetic peptide mixture and the combined synthetic and HEK proteolytic peptide mixture was desalted by solid-phase extraction. HEK293 cell lysates were prepared by RIPA lysis and sonication, and proteins were methanol-precipitated, neutralized and digested with Lysozyme-C and Trypsin. The HEK proteolytic peptide mixture was desalted by solid-phase extraction. For the combined formulation, 375 fmol of synthetic peptide was added to 5 pg of the HEK peptide mixture. This synthetic peptide mixture and the combined synthetic and HEK derived peptide mixture were characterized individually by sPRG members and study participants representing multiple LC-MS/MS instruments platform.

Conclusion

The Proteomics Standards Research Group reports the progress for development of an innovative proteomics normalization standard, designed to represent proteins of various concentrations and spanning three orders of magnitude. The 1,000 Peptide standard was then shipped into an HEK digest baseline and relative peptide levels were analyzed and integrated directly into PanoramaWeb through an R script interface. Processed data and results are now available in the ABRF-sPRG project on PanoramaWeb (http://tinyurl.com/ABRF-sPRG).

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Thank you for participating in the sPRG2013-2014 Study!!! Visit www.abrf.org/sprg

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Panorama and Integrated R scripts

PanoramaWeb (https://panoramaweb.nist.gov) was chosen as the repository for normalized data submitted by participants. PanoramaWeb is a public server hosted at the University of Washington for storing, selecting and analyzing results contained in Skyline documents. Of the 38 datasets processed with Skyline, 36 were published as PanoramaWeb lists directly from Skyline. Results from the remaining 11 datasets, including those processed with other software, were added to PanoramaWeb as Excel documents. Analysis scripts designed to assess data quality were developed in the R statistical language and integrated directly into PanoramaWeb through an R script interface. Processed data and results are now available in the ABRF-sPRG project on PanoramaWeb (http://tinyurl.com/ABRF-sPRG)