

Association of Biomolecular Resource Facilities

Proteomics Standards Research Group (sPRG): 9650 Rockville Pike, Bethesda, MD 20814

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Re: sPRG2013 Study Sample Request

August 12, 2013

Proteomics technologies are an integral part of biological and clinical research. The ABRF Proteomics Standards Research Group (sPRG) functions to design and develop performance standards and resources for mass spectrometry-based proteomics applications. The sPRG is developing a new proteomic standard, derived from a series of stable isotope-labeled ("heavy") peptides as internal standards conserved across *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*. We invite you to participate in a collaborative study that focuses on the ability of core facilities to determine relative quantitation of up to 1,000 heavy/light peptide pairs in a single sample.

The primary goals of this study are the following:

- To allow labs to evaluate their ability to measure heavy/light peptide ratios for a large number of peptides in a complex biological background
- To provide a learning opportunity for labs unfamiliar with experimental techniques and data analysis strategies for measuring heavy/light peptide ratios
- To benchmark the standard across a large number of labs

Laboratories requesting samples will receive a single vial containing a lyophilized mixture of stable isotope labeled (SIL) synthetic peptides combined with a tryptic digest of HEK293 cells. The peptides and digests are present in sufficient quantities (5 µg digested lysate and 375 fmol of each heavy peptide) to permit at least three technical replicates. We have also provided Skyline files containing fragmentation-specific spectral libraries (HCD, Ion Trap resonance excitation, and QTOF) and a two FASTA files.Participants are asked to analyze the sample in **triplicate** using the LC-MS instrument platform of their choice and report **peak area ratios and coefficients of variation**. After analysis participants are asked to complete an on-line survey, publish any Skyline results to Panorama, and submit both your raw data and data reporting template to the sPRG NIST server.

This year's study is once again open to both ABRF members and non-members. However, the total number of samples is limited, and priority will be given to ABRF members. Non-members are encouraged to join the ABRF (for more information, go to http://www.abrf.org). Vendors/sponsors are welcome to participate. Each laboratory interested in participating should only request one sample per lab.

The sPRG anticipates beginning distribution of the samples on August 12, 2013 and requests the resulting data to be returned by **October 11, 2013** so that sufficient time will be available to tabulate the results and present them at the 2014 ABRF Meeting (March 22-25; Albuquerque, NM).

Requests for samples must be submitted by e-mail to spreadings-green Please include the words "Sample request" in the subject line of the message and provide a contact name, affiliation, and complete mailing address in the body of the message. To reiterate, because of limited availability and the significant effort involved in preparation of the samples by the sPRG, the Research Group asks that samples only be requested if there is a reasonable probability that you will be able to return data by the deadline. As a bonus, each lab completing the entire study will receive an aliquot of the 1000 SIL peptides for their own use. As in the past, result submissions will be coded to insure anonymity of the participating

laboratories. A summary of the results of this study will be presented orally and as a poster at the ABRF 2014 meeting; the results will be subsequently posted on the ABRF website and will ultimately be published.

We thank you for your support of the ABRF. We look forward to your participation in this study and your help developing this standard.

Sincerely,

The ABRF Proteomics Standards Research Group

Dr. Christopher Colangelo (Chair) - Yale University

Dr. Craig P. Dufresne - Thermo Fisher Scientific

Dr. David Hawke - UT MD Anderson Cancer Center

Dr. Alexander R. Ivanov - Northeastern University

Dr. Antonius Koller - Stony Brook University

Brendan MacLean - University of Washington

Dr. Kristie L. Rose - Vanderbilt University Medical Center

Dr. Paul A Rudnick - NIST

Brian C. Searle - Proteome Software Inc.

Dr. Scott A. Shaffer - University of Massachusetts Medical School

Dr. Brett S Phinney (EB Liaison) - Proteomics Core UC Davis Genome Center