



## Association of Biomolecular Resource Facilities

*Proteomics Standards Research Group (sPRG)*

9650 Rockville Pike, Bethesda MD 20814

Tel: 301-634-7306 Fax: 301-634-7455 Email: [abrf@abrf.org](mailto:abrf@abrf.org)

Thank you for participating in the Proteomics Standards Research Group 2012-2013 study.

The Proteomics Standards Research Group (sPRG) is pleased to provide you with our 2013 study sample.

This is the second part of a multi-year project that builds on the 2012 study started by the sPRG last year. The current study focuses on the development of a proteomic standard consisting of 1000 stable isotope label peptides conserved across *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*. Details about the information requested by the sPRG from each study participant can be found in this letter, on the [ABRF sPRG website](#), and [ABRF sPRG Panorama Website](#).

You have received one tube containing a lyophilized mixture of a tryptic digest of a HEK293 cell lysate spiked with 1000 stable isotope labeled (SIL) synthetic peptides. The peptides and lysate are present in sufficient quantities (5  $\mu$ g of HEK digested lysate + 325 fmol of each sPRG stable isotope-labeled peptide) to permit a minimum of triplicate analysis on the sample. We have provided a excel data reporting template, a skyline reporting template, two FASTA files, and three spectral libraries for helping with your analysis. All these items can be downloaded from either the [ABRF sPRG website](#) or [ABRF sPRG Panorama Website](#). The first FASTA file contains a copy of the human swissprot database and we ask that you use this database for all database searches. The second fasta file contains a concatenated peptide sequence from all 1000 SIL synthetic peptides, and this should be used for quantitation purposes only. Finally, using data collected among the ABRF sPRG team laboratories, we have generated three MS/MS spectral libraries using Ion-Trap (IT), Q-TOF/QQQ (CID), or Orbitrap (HCD) fragmentation. The libraries contain MS/MS spectra for all 1000 SIL peptides and are provided in [Skyline](#) format.

**PLEASE NOTE:** The sPRG is interested in collecting as many datasets as possible and will be committed to help any participants requesting assistance. If you have any questions or need help with any part of the analysis, please first carefully review this documentation as well as our Skyline and FTP tutorials. If you still have additional questions or concerns, please feel free to email [sPRG2013@gmail.com](mailto:sPRG2013@gmail.com) and your email will be anonymously forwarded to the sPRG team and they will respond ASAP.

### Recommendations for sample handling

1. Record the date when you received the sample.
2. Store the sample at  $-80^{\circ}\text{C}$  and minimize freeze-thaw cycles before analysis.
3. Use a sample reconstitution protocol that is compatible with your planned analytical strategy and effective for solubilizing hydrophobic peptides. The reconstitution protocol that was found effective by the sPRG is as follows:
  - a. Dissolve and vortex in 4  $\mu$ l of 70% formic acid
  - b. Add and vortex 21  $\mu$ l of 0.1% TFA in water to a total of 25  $\mu$ l
  - c. The resulting solution will contain 200 ng HEK + 15 fmol of each sPRG SIL Peptide in 1  $\mu$ l.
  - d. Inject 5  $\mu$ l (1  $\mu$ g HEK + 75 fmol of each sPRG SIL Peptide)
4. Run the sample in triplicate
5. Store any remaining sample at  $-80^{\circ}\text{C}$

## Directions for the study

1. Download the data reporting template and FASTA files from [www.abrf.org/sprg](http://www.abrf.org/sprg).
  2. Design the most effective, in your opinion, analytical strategy (strategies) for analysis of the provided standards.
  3. Reconstitute samples and analyze peptides by method(s) of choice.
  4. We strongly suggest you run your analysis for a minimum of three injections so you can calculate and report coefficient of variance for each peptide ratio between runs.
  5. Analyze the data. You can use a wide variety of tools to calculate the heavy/light ratios for each peptide in each run. As a way to encourage participation, we have provided a tutorial for analyzing label-free results from Skyline.  
([https://panoramaweb.org/labkey/wiki/ABRF%20sPRG/download.view?entityId=cde657ce-8b94-1030-bc9c-3013bb9bac3b&name=Skyline Tutorial ABRF sPRG.pdf](https://panoramaweb.org/labkey/wiki/ABRF%20sPRG/download.view?entityId=cde657ce-8b94-1030-bc9c-3013bb9bac3b&name=Skyline_Tutorial_ABRF_sPRG.pdf))
  6. **After processing your data, either upload your data results from Skyline to Panorama or complete the data reporting template downloaded in Step 1.**
  7. Rename your data results file with a unique anonymous identifier composed of six alphanumeric characters (e.g. "12345A"). If you are submitting several data reports corresponding to alternative analytical strategies, keep the first six characters the same and concatenate sequential numbers (e.g., "12345A-01," "12345A-02," and "12345A-03." Retain this identifier as this will be the only way for you to find your data in any presentation of our study results. Your data will only be referred to by this unique identifier in any presentation.
  8. **Upload your data results template and "raw" data files as a combined zip folder to the NIST dedicated secure file server** using the same identifier (e.g., "12345A.zip"). The raw files will be used by the sPRG for building the spectral library for this standard and for analysis of the peak area ratios by the sPRG.
- FTP login details are below
- FTP login server: chemdata.nist.gov
  - username: sprg2013
  - password: 2013SPRG\_100
9. Complete ABRF sPRG Study Survey at <http://www.surveymonkey.com/s/XLMP2WV>
  10. After completing all the steps above, send a quick email to [sPRG2013@gmail.com](mailto:sPRG2013@gmail.com) with subject header - "12345A data results completed"

Please check for possible updates and additional information on our web page [www.abrf.org/sprg](http://www.abrf.org/sprg) under sPRG2013 study. The completed reporting template and raw data needs to be returned by **October 11, 2013** so that sufficient time will be available for the sPRG to tabulate the results and present them at the 2014 ABRF Meeting (March 22-25; Albuquerque, NM). Confidentiality will be maintained at every stage, and all submitted data become the property of the ABRF sPRG, to be used accordingly for research, commercialization of standards, and publications.

We ask that you please consult the "[Research Group Study Participation Guidelines](#)" document accompanying this letter. Importantly, **an ABRF Research Group study is not a competition and under no circumstances should it be referred to as such.** Words and phrasing that imply a competition—such as "winner," "best of," etc. are strictly discouraged. The purpose of the study should be considered as "benchmarking." No one can claim to be "the winner," or to have obtained "the best results."

For problems with the samples, reporting, survey, or if you are interested in helping out the ABRF as a research group member or in any other capacity, please email us at [sPRG2013@gmail.com](mailto:sPRG2013@gmail.com).

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*