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

Thank you for participating in the Proteomics Standards Research Group 2011-2012 Study.

The Proteomics Standards Research Group (sPRG) is pleased to provide you with our study sample — a comprehensive standard for the analysis of post-translational modifications (PTMs). This is the second part of a multi-year project that builds on the phosphorylation standard study completed by the sPRG last year. The current study focuses on development of a standard that can be used for both assessment of capabilities for detection and identification of an array of PTMs in a complex proteomic sample and development of new approaches for characterization of post-translationally modified proteins.

You have received two tubes: one containing a lyophilized mixture of the synthetic modified peptides, and the second containing the same mixture combined with a tryptic digest of the six proteins from which the synthetic peptides were derived. The peptides and digests are present in sufficient quantities (approximately 10 pmole of each) to permit multiple analyses and utilization of a variety of strategies. A FASTA file containing the protein sequences and a reporting template can be downloaded from **www.abrf.org/sprg** (see the section corresponding to the sPRG Study 2011-2012).

Recommendations for sample handling

1. Record the date when you received the sample.
2. Store the samples at -80^{o}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: add 5 µL of a solution containing 2% formic acid / 0.1% trifluoroacetic acid / 30% aqueous acetonitrile to the bottom of the sample tube; sonicate for five minutes and vortex for two minutes. Shake or spin the sample solution down to the bottom of the tube and add 45 µL of 0.1% formic acid in water; sonicate for another five minutes and vortex for two minutes. The resulting solution will contain approximately 200 fmol/µL of each constituent.
4. Consider aliquoting the reconstituted sample for storage and minimization of freeze-thaw cycles.

Directions for the study

1. Download the data reporting template and FASTA file from **www.abrf.org/sprg**.
2. Details about the information requested by the sPRG from each study participant can be found in this letter and all spreadsheets of the data reporting template.
3. Design the most effective, in your opinion, analytical strategy (strategies) for analysis of the provided standards. Consider the presence of various modifications, isobaric positional isomers, and peptides with very similar molecular weights and sequences while designing your experimental and data analysis strategy (strategies).
4. Consider using technical replicates for each experimental strategy.
5. Reconstitute samples and analyze peptides by method(s) of choice.
6. Analyze the data.
7. For each peptide analyzed, fill in the corresponding row in the template according to the instructions found in the template. Report your best match for each peptide and provide the filename and scan number for the identification. Please use the "Synthetic Peptide Mix" worksheet for Sample 1 and "Peptide Mix + Protein Digests" worksheet for Sample 2. The theoretical m/z values for synthetic peptides are provided on a separate spreadsheet for your reference.

8. Complete the "Sample Analysis" worksheet describing each analytical strategy for which you are reporting a best match. Complete the "% Coverage of target proteins" worksheet in the template. If SRM type analysis was used, please add the transitions to the report template, ranked by their intensities.
9. Complete the survey questions in the template.
10. Please name the template 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 for future reference, 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 its unique identifier in any presentation).
11. Email the completed template to **sPRG2011Standard@gmail.com**. Enter "ABRF sPRG 2011-2012 Study Data Report" followed by the unique anonymous identifier in the subject of the email message (e.g., "ABRF sPRG 2011-2012 Study Data Report 12345A").
12. Upload your "raw" data files to a dedicated secure server. Please see www.abrf.org/sprg for information on uploading your "raw" data. The raw files will be used by the sPRG for building the spectral library for this standard and for analysis of the reported matches by the sPRG.
13. Please check for possible updates and additional information at our web page www.abrf.org/sprg under sPRG2011-2012 study.

The completed report template needs to be returned by **September 15, 2011** so that sufficient time is available for the sPRG to tabulate the results and present them at the 2012 ABRF Meeting (March 17 – 20; Orlando, Florida). 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.

Please consult the "Research Group Study Participation Guidelines" document that accompanies this letter (and is also available at http://www.abrf.org/index.cfm/page/resources/RG_Com_guidelines.htm). 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 **sPRG2011Standard@gmail.com**.

Sincerely,

The ABRF Proteomics Standards Research Group:

Alexander R. Ivanov (Chair), Harvard School of Public Health
Christopher Colangelo, Yale University
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