



**Association of Biomolecular Resource Facilities**  
***Protein Sequencing Research Group (PSRG)***

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Dear Colleagues:

The ABRF Protein Sequencing Research Group (PSRG) is pleased to announce the availability of test samples for the 2015-2016 study, **“C-terminal identification of standard proteins by O18 labeling and bottom-up mass spectrometry.”**

C-terminal identification of proteins is a desired technology, especially when coupled with N-terminal identification experiments. For purposes of structural studies of large proteins, it provides identity of fragments generated by limited proteolysis. For Core laboratories, C-terminal identification technology should be reproducible, easy to perform, sensitive, and robust. Julka, et al, (2008) demonstrated that O18 water and bottom-up mass spectrometry can be used to determine the C-terminal sequence of a protein, provided that the C-terminal peptide of the protein does not end with Lysine and Arginine and is recovered during the steps leading to mass spectrometry. This approach is simple and can easily be adapted to a Core Laboratory operation since it does not require complicated chemistries and additional new steps during routine bottom-up mass spectrometry analyses used to identify proteins. The PSRG therefore feels that acquired experience performing C-terminal identification will be beneficial to Core laboratories.

The PSRG will provide two soluble, known proteins and O18 water to participating laboratories to carry out (i) individual analysis of each protein and (ii) analysis of a mixture of two proteins; PSRG members will carry out pilot studies to determine how many and in what stoichiometry the mixture of the two proteins will be. Participants will be encouraged to carry out protein in-gel or solution digestion, peptide clean-up, LCMS/MS or MALDI-TOF MS/MS and database searches for peptide identification using in-house optimized protocols; PSRG will also provide recommendations for all of the steps above as well as a detailed outline of O18 digestion and C-terminal peptide identification. At the end of the study, the participants will be asked to report the C-terminal sequence as determined by bottom-up mass spectrometry, to the PSRG. Mass spectrometry is required for participation in this proposed study.

**The goal for this year is to test the abilities of participating core laboratories to a) successfully digest the provided protein in presence of 50% (v/v) O18 labeled water, b) identify the pairs of internal peptides with and without O18 tag, c) identify C-terminal most peptide with no O18 tag d) obtain MS/MS data to confirm C-terminal amino acid information.**

Participating laboratories will submit their data electronically to the PSRG for tabulation of the results. A brief methods survey will be part of the data submission process. Results will be presented at the ABRF 2016 conference February 20-23, 2016, in Ft.Lauderdale, Florida, and posted on the ABRF website.

**We encourage participation by all mass spectrometry, proteomics, and research laboratories interested in protein C-terminal identification methods.** For inquiries about the study or to obtain a sample set, e-mail your request to: Dr. Sara McGrath at [Sara.Mcgrath@fda.hhs.gov](mailto:Sara.Mcgrath@fda.hhs.gov). Please include in your request the address to which the samples should be sent, contact email address, and phone number. As in the past, results will be stripped of all identifiers to maintain the anonymity of the participants. An identification number will be issued so that you may compare your results to other participating laboratories at the meeting.

**The O18 water used in this study is in limited supply. We ask that you please request samples only if you definitely can run the experiments and return your results. Results need not be completely successful; negative results and complications are just as valuable to us as positive ones, as they will help us refine our protocols for the future.**

- Final sample requests should be made by **October 15th, 2015**
- Requested samples will be sent to participants starting **October 19th, 2015**
- Data and results should be submitted to PSRG by **December 31st, 2015**

Thank you for your interest in the PSRG 2016 study and we look forward to seeing you in Ft. Lauderdale.

*Sincerely,*

The ABRF Protein Sequencing Research Group

Greg Cavey  
Robert English (*ad-hoc*)  
Hediye Erdjument-Bromage (*chair*)  
Brian Feild  
Xuemei Luo  
Sara McGrath (*ad-hoc*)  
David Wood  
Frances Weiss-Garcia (*EB liason*)

References:

[4] Julka S, Dielman D, Young SA. Detection of C-terminal peptide of proteins using isotope coding strategies. 2008, Journal of Chromatography B, 874:101-110.