



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 study, “**N-terminal sequencing of standard proteins by dimethyl labeling and bottom-up mass spectrometry.**”

N-terminal sequencing is in the midst of a technology transition from classical Edman sequencing to mass spectrometry-based terminal identification. For core laboratories, the ultimate goal in the coming years will be to have a well-defined protocol for terminal sequence analysis by mass spectrometry that has the same level of maturity as Edman sequencing. Knowing the types of samples, sample preparation protocols and expected results are critical for core laboratories so that they can provide the most reliable data to their customers.

To help in development and establishment of such techniques, the PSRG is conducting a study using chemical derivitization to enhance N-terminal identification of proteins by mass spectrometry. As an extension of the 2014 study, the current 2015 study is focused on dimethyl labeling of protein N-termini and designed to test the limits of detection of this approach.

The goal for this year is to test the abilities of participating core laboratories to a) successfully derivitize the provided protein using dimethyl labeling, b) digest and identify the derivitized N-terminal peptide by mass spectrometry, and c) obtain MS/MS data to confirm N-terminal amino acid information. The protocol will be performed for one purified protein at 3 concentrations.

Participating laboratories will receive one known protein and protocols for dimethyl labeling.[1] The participants will follow the derivitization and sample cleanup with trypsin digestion and bottom-up mass spectrometry using the MS system and operating parameters of their choice. Recommendations for successful analytical and bioinformatics methods will be provided by the PSRG with requested samples. Participants will be asked to report the N-terminal peptide information from MS/MS data for each sample concentration analyzed.

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 2015 conference March 28-31, 2015, in St. Louis, MO, and posted on the ABRF website.

Study restrictions: Dimethyl labeling of protein N-termini is fast and inexpensive, but requires some reagents that the PSRG is not able to ship to participants as part of the study. Formaldehyde and sodium cyanoborohydride must be purchased by participants and can be obtained by vendors such as Sigma Aldrich for less than \$50 each. This study is not designed for Edman sequence analysis; participants must use bottom-up mass spectrometry. Analysis must include the use of bioinformatics tools to derive terminal sequences.

We encourage participation by all mass spectrometry, proteomics, and research laboratories interested in protein N-terminal identification methods. For inquiries about the study or to obtain a sample set, e-mail your request to: Dr. Henriette Remmer at hremmer@umich.edu. Please include in your request the address to which the samples should be sent. 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.

So that we may provide samples for as many participants as possible, 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 24, 2014**.
- Requested samples will be sent to participants starting **November 3, 2014**.
- Data and results should be submitted to PSRG by **February 6, 2015**.

Thank you for your interest in the PSRG 2015 study and we look forward to seeing you in St. Louis!

Sincerely,

The ABRF Protein Sequencing Research Group

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References:

[1] JL Hsu, SY Huang, NH Chos, SH Chen. **Stable-isotope dimethyl labeling for quantitative proteomics**. Anal Chem., 2003 Dec 15; 75(24):6843-52.