Association of Biomolecular Resource Facilities
Protein Sequencing Research Group (PSRG)
9650 Rockville Pike, Bethesda, MD 20814
Tel: 301-634-7306 ♦ Fax: 301-634-7455 ♦ Email: abrf@abrf.org

Dear Colleagues:

The Protein Sequencing Research Group (PSRG) is pleased to announce the availability of test samples for the 2014 study, **"N-terminal sequencing of standard proteins by N-terminal labeling followed by bottom-up/top-down mass spectrometry"**

N-terminal sequencing is in the midst of a technology transition from classical Edman sequencing to mass spectrometry-based terminal sequencing. 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 sequencing of proteins by mass spectrometry. The current 2014 study entails **terminal sequencing and identification of 3 purified proteins. The goal for this year is to test the abilities of participating core laboratories to a) successfully derivitize the provided proteins, b) digest and identify the derivitized peptide fragments by mass spectrometry, and c) obtain terminal sequence information**.

This study is not designed for Edman sequence analysis; participants must use either top-down or bottom-up mass spectrometry. Analysis must include the use of bioinformatics tools to derive terminal sequences. Additional background information and specific instructions will be provided with the samples (experimental procedures have been modeled after a literature report by Gallien, et al[1]). We encourage participation by all mass spectrometry, proteomics, and research laboratories interested in protein N-terminal identification methods.

Participating laboratories will receive three known proteins provided separately, the labeling reagent (TMPP[2]), and protocols for TMPP labeling: **(A)** in-solution labeling with SDS-PAGE or cut-off filter cleanup, or **(B)** SDS-PAGE separation and in-gel labeling. Participants may choose which workflow to perform and will follow the sample preparation with trypsin digestion and bottom-up or top-down mass spectrometry using the MS system and operating parameters of their choice. Participants may also choose to analyze the provided protein standards with a mass spectrometry based N-terminal identification method of their choice such as dimethyl labeling by reductive amination[2]. If alternative methods are used, PSRG requests a brief description of the protocol used and literature references as appropriate. Recommendations for successful analytical and bioinformatics methods will be made by the PSRG. Participants will be asked to provide mass spectra containing the digested labeled fragments and report the N-terminal sequence as determined by bottom-up (or top-down) mass spectrometry to the PSRG.

Participating laboratories will submit their data electronically to the PSRG for tabulation of the results. Results will be presented at the ABRF 2014 meeting March 22-25, 2014 in Albuquerque, NM, and subsequently posted on the ABRF website. A brief methods survey will also be part of the data submission process.

For inquiries about the study and/or to obtain a sample set(s), e-mail your request to: hremmer@med.umich.edu. Please include in your request the address to which the samples should be sent and **which analysis technique(s) you would like to perform (A and/or B), so that you receive sufficient sample.**

(A) Workflow "in-solution labeling":

1) TMPP labeling of proteins at N-terminus
2) SDS-PAGE and in-gel tryptic digestion or on-membrane cut-off filter tryptic digestion
3) MS analysis including data analysis
4) Identification of N-termini

(B) Workflow "in-gel labeling and digest"

1) SDS-PAGE, excision of bands and in-gel TMPP labeling
2) In-gel tryptic digestion and cleanup
3) MS analysis including data analysis
4) Identification of N-termini

In order to serve our participants most efficiently, 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 **November 8, 2013.**
Requested samples will be sent to participants starting **November 18, 2013.**
Data and results should be submitted to PSRG by **February 7, 2014**.

As in the past, results will be stripped of all identifiers so as to maintain the anonymity of the participants. An identification number will be issued so you may compare your results to other participating laboratories at the meeting.

Thank you for your interest in the PSRG 2014 study and we look forward to seeing you in Albuquerque!

Sincerely,

The ABRF Protein Sequencing Research Group

Robert English (co-chair)
Sara McGrath (co-chair)
Greg Cavey
Mark Garfield
Pegah Jalili
Ejvind Mortz
Henriette Remmer (ad-hoc)
Bill Hendrickson (EB liaison)

References:

[1] S Gallien, E Perrodou, C Carapito, C Deshayes, JM Reyrat, A Van Dorsselaer, O Poch, C Schaeffer, O Lecompte. **Orthoproteogenomics: Multiple proteomes investigation through orthology and a new MS based protocol.** Genome, 2009; 19: 128-135; doi 10.1101/gr.081901.108.

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

[3] TMPP-Ac-OSu: N-succinimidyloxycarbonylmethyl-tris (2,4,6-trimethoxyphenyl)phosphoniumbromide