



Association of Biomolecular Resource Facilities

Business Office:

2019 Galisteo Street, Bldg. I, Santa Fe, NM 87505

Tel: 505-983-8102 ♦ Fax: 505-989-1073 ♦ Email: abrf@abrf.org

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Dear Colleagues,

Thank you for participating in the ABRF 2004 Proteomics Research Group (PRG) study. Please find enclosed the sample (ABRF-PRG04) that you requested. The results of your analyses should be transmitted by completing the online “Data Analysis Form” and supporting evidence **ONLY** for the results should be faxed using the cover sheet that can be downloaded after completing the online Data Analysis Form (see below). All results are submitted anonymously, and are identified only by a **5-digit identification number** that you select. There are several primary goals of this study:

- To provide a mechanism so that participating labs can evaluate their capabilities in identifying unknown proteins and discriminating between closely related protein isoforms
- To evaluate methods for preparing intact proteins for downstream identification analysis (i.e. solution or in-gel digestion, choice of enzymes, chromatographic or electrophoretic separation)
- To obtain data that would allow a comparison of the strategies used and aid in optimization of these techniques.
- To increase awareness of the subtleties and importance of good database search techniques

Sample Information: ABRF-PRG04 contains 3 pmol each of three closely related intact proteins. The sample is supplied as a dried pellet, and can be dissolved in most common aqueous solutions. As with any real life sample, there may also be contaminant(s).

Analysis:

- Identify the three most abundant protein isoforms, from up to two different species, in the sample.
- Identify one posttranslational modification common to all three isoforms excluding those that may happen during sample handling such as oxidation and deamidation.
- Provide discriminatory evidence for the specific identifications (e.g. ms/ms spectrum or amino acid sequence for a diagnostic peptide, exact protein mass, etc.) for each isoform and the modification.
- Submit your results online by filling out the online form and by faxing up to 4 pieces of supporting data for the identifications (tandem mass spectra with key diagnostic fragment ions labeled, etc.) according to the instructions given below.

Discriminating between closely related proteins can be considerably more difficult than simply identifying a single protein. We would like to emphasize that we are just as interested in information about unsuccessful analyses as we are in successful analyses. All results are

compiled in a completely anonymous manner, so there is absolutely no need to feel shy about submitting negative results.

- **Please fill out the online Data Analysis Form regardless of your results. This study is not a contest!**

Returning Data: Please return your results no later than **November 14, 2003** so that the PRG can complete the data analysis prior to the ABRF 2004 meeting, *Integrating Technologies in Proteomics and Genomics*, February 28-March 2 in Portland, Oregon. The results should be transmitted by completing the online Data Analysis Form at <http://www.surveymonkey.com/s.asp?u=96715250169>. **Please note that if your computer accepts cookies, you can return to the form and make changes later as long as you enter the data from the same computer. Otherwise you can fill out the entire survey again using the same identification code so that we can recognize the duplication and ignore your previous entry.** A link to the fax form cover sheet is provided at the end of the survey. Only supporting data (4 pieces maximum) should be faxed to the impartial third party below who will remove all marks from the data that would trace the analysis back to your laboratory. **Most importantly**, in order for you to obtain feedback about your analyses you need to choose a unique **5-digit identification number** that only you will know, and write it in the box of the FAX cover sheet that was provided to you as well as in the upper right hand corner of the supporting data sheets. The data analysis results will be identified by the number that you pick. **Use the same 5-digit number** for the faxed supporting data as you do for the online survey so that all data can be correlated properly.

After completing the online Data Analysis Form, please fax or mail ONLY your supporting evidence for the identification of the protein isoforms along with the common modification (maximum 4 pieces of data) to:

Lora Goodridge
Protein Core Facility
630 W. 168th Street
Box 54
New York, NY 10032

The fax number is 212-305-1599 (attn. Lora Goodridge)

Faxing is preferred and please use the link at the end of the survey for the Fax Cover sheet.

If you have any questions about filling out the online Data Analysis Form, please e-mail David Arnott at arnott@gene.com or Kaye Speicher at kspeicher@wistar.upenn.edu. Thank you for your participation in this study!

The ABRF Proteomics Research Group

Kaye Speicher (chair)	Wistar Institute
Dave Arnott	Genentech
Mary Ann Gawinowicz	Columbia University
Jeff Kowalak	National Institutes of Health
Bill Lane (EB Liaison)	Harvard University
Tom Neubert	New York University
Christoph Turck	Max Planck Institute of Psychiatry
Karen West	Cleveland Clinic Foundation - Cole Eye Institute