



Association of Biomolecular Resource Facilities

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PRG2009 Relative Protein Quantification in a Clinical Matrix Study

November 15st , 2008

Dear PRG2009 Study Participant:

Thank you for agreeing to participate in the "**Relative Protein Quantification in a Clinical Matrix Study**" conducted by the ABRF Proteomics Research Group (PRG). Accompanying this letter are six sample vials. If for some reason the samples did not arrive in good condition, please notify the PRG by sending an e-mail to **PRG2009anonymous@gmail.com**. Results returned by **January 05, 2009** will be included in the PRG presentation at the upcoming ABRF Meeting, which will take place February 7 – 10, 2009 in Memphis, TN, and will be published on the ABRF website so that other researchers can compare results and adopt best practices.

PRG2009: An increasingly common request for proteomics laboratories today is determining quantitative differences among samples in clinical matrices such as urine, plasma or CSF. The experiment often begins with a small set of pilot samples (case versus control) where putatively differentially expressed protein species are identified. Subsequently, further sample sets are provided for a more detailed examination of these proteins to determine if the initial observation of quantitative differences holds within a larger patient cohort. The major challenges associated with this type of analysis are detection and accurate quantification in these very complex matrices.

The PRG has designed a study that explores the use of different approaches for determining quantitative differences for several target proteins in six samples of human plasma.

The PRG anticipates that the samples can be successfully characterized by scientists with different levels of experience using a wide variety of approaches and platforms. The primary goals of this study are to document the breadth of approaches used by the ABRF community and to highlight the type of information obtained.

Study samples: You will find enclosed with this letter 3 duplicate samples, which contain 40µl undepleted, freeze dried human plasma (**please see enclosed safety sheet**). Each duplicate set of vials is spiked with different amounts of the following two human proteins and two rabbit proteins:

- Prostate specific antigen (human)
- Beta human chorionic gonadotropin (human)
- Glycogen phosphorylase A (rabbit)
- Glycogen phosphorylase B (rabbit)

These proteins are spiked into plasma at a range of 2.5 fmol/µl to 1.25 pmol/µl

Participants will be asked to provide the following:

- i. Relative quantification of the four specified proteins in human plasma.
- ii. Information about methods used to analyze the samples.
- iii. Information about the experimental design used for relative quantification.

The sequences of the target proteins and typical tryptic peptide MS/MS derived fragment spectra from the four proteins can be found at **the PRG website** (<http://www.abrf.org/prg>) under 'Studies'.

Returning Results: When submitting their results, participants are asked to report the relative quantification for the four target proteins in the plasma samples by completing the sample survey. Information on how to access the survey will be posted on the PRG website: www.abrf.org/PRG.

When submitting your results, you will be asked to supply a 5-digit identification number so that all entries remain anonymous. It is requested that you use UniProt accession numbers when reporting results. In addition to the online survey, please prepare a brief written summary of the experimental methods used and the corresponding results obtained from your analysis. If you used more than one independent experimental strategy to obtain your results, please enter each one separately in the on-line survey. It will be necessary to use a separate 5-digit identification number for each entry. This will allow us to associate the results obtained with each analytical approach. The PRG will compile descriptions of the experimental methods that were used and highlight methods that successfully determined known differences in the sample sets.

Please note that if your browser is set to accept cookies, you will be able to revisit the survey form and make changes in your entries until the submission deadline, as long as you are using the same computer. If you need to make changes in your entry but are using a different computer, it will be necessary for you to fill out the complete survey again using the same 5-digit identification code you used previously. Before compilation of the results, we will retain only the most recent submission and remove prior, duplicate entries. Remember that your results will be listed only by their 5-digit identification number. So, please select a unique number that you will recognize. We would like to emphasize that information about all analyses is vital to this study, whether successful or not. All results will be compiled in a completely anonymous manner (even to members of the PRG), so there is absolutely no need to feel shy about submitting negative results.

Please fill out the online data analysis form regardless of your confidence in your results. This study is not a contest!

Any updates about the study will be posted on the ABRF PRG website: www.abrf.org/PRG

If you have any questions about filling out the online data analysis form, please e-mail the PRG at **PRG2009anonymous@gmail.com**.

This year's study is again open to both ABRF members and non-members. However, the total number of samples is limited, and priority has been given to ABRF members. Non-members are encouraged to join the ABRF (For more information, go to <http://www.abrf.org> and membership application form included with your samples).

We thank you for your support of the ABRF and look forward to your participation in this study.

Sincerely,

The ABRF Proteomics Research Group

Michael MacCoss (Chair) - University of Washington

Allis S. Chien - Stanford University

David B. Friedman - Vanderbilt University

David Hawke – MD Anderson Cancer Center

Jeroen Krijgsveld - Utrecht University

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Nicholas E. Sherman - University of Virginia

Chris W. Turck (EB Liaison) - Max Planck Institute of Psychiatry