October 2009

Dear colleagues,

Please find enclosed the ABRF-2010 PSRG samples that you requested from the ABRF Protein Sequence Research Group. This is the 22nd study in an annual series designed to aid laboratories in evaluating their abilities to obtain and interpret amino acid sequence data. The purpose of this year's study is to investigate both traditional and alternative methods for obtaining terminal sequence information on an antibody molecule. A monoclonal antibody is being provided to participants with the goal of obtaining as much terminal sequence of both the light and heavy chains as possible. Participants may use any available Edman, mass spectrometric, biochemical method or technology, or a combination of techniques. Quantities sufficient for multiple analyses of the antibody have been provided. We would like to compare the extent of sequence obtained by a variety of methods, and to that end, we encourage all mass spectrometry and proteomic laboratories as well as Edman sequencing facilities to participate.

For this year's study, two vials (PSRG2010) of an intact monoclonal antibody are provided. Each vial contains approximately 50 ug of protein. The two vials are identical. You may use one or both of these vials for your analysis. The PSRG committee suggests that the antibody be solubilized with any of the following:

-0.1 %TFA
-25mM ammonium bicarbonate
-0.1% TFA / 20% acetonitrile
-250 mM sodium phosphate buffer (pH7.0) containing 50 mM DTT and 5 mM EDTA
-100 mM Tris

Please use any sample treatment or sample fractionation method prior to sequencing that you feel will enhance the chances of obtaining sequence data for your particular method.

Data submission:

The PSRG is asking each participant to log on to the PSRG 2010 Google Docs survey to report the results of their analysis. This survey is completely anonymous. You will be receiving an identification number via email from Robert English. Please save this number and record the number in the appropriate field in the survey. For those facilities submitting more than one data set, please add the letter "A", "B", "C" *etc*. to your assigned identification number. Robert is the only person who will know the relation between your email and your identification number.

The survey can be accessed from the following link:

http://spreadsheets.google.com/viewform?formkey=dDd5cl9lc3YxWXZMZ1pzVVRtQTBnaVE6MA

The link can also be found on the ABRF web site. Go to the Research Groups tab and select the Protein Sequencing Research Group bullet point. Under "Studies"

select the “PSRG 2010 Data Survey”. If you cannot open the link, contact Robert English (rdenglis@utmb.edu) and he will fax you a paper copy.

For those performing MS analysis, please complete the PSRG 2010 Google Docs survey and email power point files with relevant spectra supporting your sequence call directly to rdenglis@utmb.edu. **Please include your laboratory identification number in the title of the supporting document.** In order to ensure anonymity, Robert English will remove all identifying marks prior to forwarding the data to the sequence committee for analysis. The sequencing results will be presented at ABRF 2010, March 20-23, 2010 in Sacramento, CA., and will also help guide future potential studies and tutorial sessions.

If your sample arrived damaged, or if you have questions about the study, please contact Steve Smith (jssmith@utmb.edu). Equipment failures and “no data obtained” analyses are as important to us as data from “successful” runs. Please send us your results whatever happens.

The deadline for receiving data for inclusion in the study is December 4th, 2009.

Thank you for your valuable participation in this year’s study!

The Protein Sequencing Research Group:

Steve Smith – University of Texas Medical Branch
Wendy Sandoval – Genentech
Peter Hunziker – Functional Genomics Center Zurich
Jim Walters – Sigma-Aldrich
Bosong Xiang – Monsanto Company
Kwasi Mawuenyega – Washington University School of Medicine
Jack Simpson - EB liaison