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

Thank you for participating in the Proteomics Standards Research Group 2018 study.

The Proteomics Standards Research Group (sPRG) is pleased to provide you with our 2018 study sample. The study focuses on the development of a phosphoproteomic standard consisting of 150 stable isotope label phosphopeptides from Homo sapiens. Details about the information requested by the sPRG from each study participant can be found in this letter and on the ABRF sPRG website.

You have received one tube containing a lyophilized mixture of 1mg tryptic digest of a stimulated HELA cell lysate spiked with 2pmol each of 150 stable isotope labeled (SIL) synthetic phosphopeptides. Also included is a vial with 5pmol of each of the 150 stable isotope labeled phosphopeptides that was spiked into the HELA tryptic lysate to use as you see fit (eg. creating a spectral library). We have provided an excel data reporting template with all sequences of the phosphopeptides as well as a fasta file with the sequences (containing two sequences, one containing all tryptic peptides and one containing the half-tryptic peptides). All these items can be downloaded from the ABRF sPRG website: https://abrf.org/research-group/proteomics-standards-research-group-sprg

PLEASE NOTE: The sPRG is interested in collecting as many datasets as possible and will be committed to help any participants requesting assistance. If you have any questions or need help with any part of the analysis, please first carefully review this. If you still have additional questions or concerns, please feel free to email abrfsPRG2018@gmail.com and your email will be anonymously forwarded to the sPRG team and they will respond ASAP.

Recommendations for sample handling:

1. Record the date when you received the sample.

2. Store the sample at -80°C and minimize freeze-thaw cycles before analysis.

3. Use a sample reconstitution protocol that is compatible with your planned analytical strategy and effective for solubilizing hydrophobic peptides.

Please use a phosphopeptide enrichment strategy that you feel comfortable with, either a kit from a vendor or your in-house protocol for the tryptic HELA lysate containing the heavy phosphopeptide.

4. We strongly suggest you run your analysis for a minimum of three injections so you can calculate and report coefficient of variance for each peptide ratio between runs. But this is not a requirement.

5. Analyze the data. You can use a wide variety of tools to calculate the heavy/light ratios for each peptide in each run.

6. After processing your data, fill out the excel data reporting form (on the sPRG website) and upload the raw files.

7. Rename your data results file with a unique anonymous identifier composed of six alphanumeric characters (e.g. “12345A”). If you are submitting several data reports corresponding to alternative analytical strategies, keep the first six characters the same and concatenate sequential numbers (e.g., “12345A-01,” “12345A-02,” and “12345A-03.” Retain this identifier as this will be the only way for you to find your data in any presentation of our study results. Your data will only be referred to by this unique identifier in any presentation.

8. Upload your data results template and “raw” data files as a combined zip folder to the UCDavis Bioshare FTP dedicated secure file server using the same identifier (e.g.,“12345A.zip”). The raw files will be used by the sPRG for building the spectral library for this standard and for analysis of the peak area ratios by the sPRG.

sFTP login details, use e.g. ‘FileZilla’:

sFTP login server: bioshare.bioinformatics.ucdavis.edu (make sure you use sFTP)
Port: 2200
User: abrfsPRG2018@gmail.com
Password: PVZBZVgxTs

And upload files into the SPRG2018 folder.

Or use a webbrowser to:

https://bioshare.bioinformatics.ucdavis.edu/bioshare/view/SPRG2018/

with the same login credentials as above.

9. Complete ABRF sPRG Study Survey at https://goo.gl/QRYG5F

10. After completing all the steps above, send a quick email to abrfsPRG2018@gmail.com with subject header - “12345A data results completed”.

Sincerely,

The ABRF Proteomics Standards Research Group

https://abrf.org/research-group/proteomics-standards-research-group-sprg