

SpikeMix™ Isotope-Labeled Peptide Pools

Development & Characterization of SpikeMix™ ABRF (Cross-Species Standard) Consisting of 1,000 Stable Isotope Labeled Peptides

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The ABRF Proteomics Standards Research Group (sPRG) has spent the past two years (2012-2014) generating an interassay, interspecies, and interlaboratory peptide standard that can be used for assay development and relative quantitation of protein abundance measurements via mass spectrometry based quantitative proteomics analyses. The standard has been formulated as 1,000 stable isotope ¹³C/¹⁵N-labeled synthetic tryptic peptides whose sequences were derived from approximately 552 proteins, conserved across proteomes of commonly analyzed species: *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*. The 1,000 Peptide standard was then spiked into an HEK digest results and sent to 90 laboratories worldwide. Over half the participants (47 laboratories) returned datasets via Panorama Web Repository (<https://panoramaweb.org>). Processed data and results from the analysis scripts are available in the ABRF sPRG project on PanoramaWeb (<http://tinyurl.com/ABRF-sPRG>).

Introduction

The mission of the ABRF Proteomics Standards Research Group (sPRG) is to promote and support the development and use of standards in proteomics for the benefit of all research laboratories, resource facilities and individual scientists including ABRF members and member laboratories. In the spirit of this mission, the ABRF Proteomics Standards Research Group (sPRG) launched and completed a two-year study (2012-2014) resulting in the benchmarking of an ABRF cross species standard for mass spectrometry based quantitative proteomics analyses.

Results

First year efforts were focused on selection of appropriate protein and peptide candidates, peptide synthesis, quality assessment and LC-MS/MS evaluation conducted in laboratories of sPRG members. 1,000 isotope labeled peptides were synthesized by JPT Peptide Technologies and analyzed in the laboratories of sPRG members with various instruments and fragmentation methods. Greater than 99% of the peptides were identified during the validation runs. In LC-MS/MS peptide dilution experiments (ranging from 100-6.25 fmol), the majority of peptides behaved as expected with a linear response (**Figure 1**).

In the second year, the group launched the study to the entire proteomics community containing a lyophilized mixture of HEK 293 tryptic digest cell lysate spiked with the 1,000 stable isotope labeled (SIL) peptide standards was provided to each participant. The ABRF sPRG team provided a Skyline tutorial, tutorial datasets, three MS/MS spectral libraries generated from linear ion-trap (CID), Q-TOF/QQQ (CID), or Orbitrap (HCD) instrumentation, and a Panorama data repository interface. Participants were asked to analyze the sample in triplicate and calculate ratios of the spiked SIL to endogenous peptides and coefficients of variance for each peptide. The study received over 120 sample requests in 45 days for an average of 2.6 sample requests/day. The team had only generated 90 samples for the study, so the first 90 requested were honoured and all late requests were postponed to future studies. Over half the participants, 47 laboratories returned datasets via Panorama Web Repository. The results represent a wide range of LC-MS platforms and instrumentations from North America, Europe, and Asia.

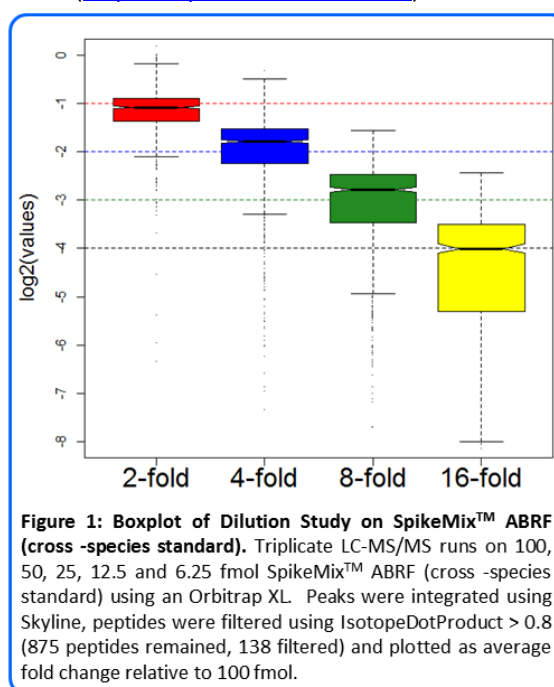


Figure 1: Boxplot of Dilution Study on SpikeMix™ ABRF (cross-species standard). Triplicate LC-MS/MS runs on 100, 50, 25, 12.5 and 6.25 fmol SpikeMix™ ABRF (cross-species standard) using an Orbitrap XL. Peaks were integrated using Skyline, peptides were filtered using IsotopeDotProduct > 0.8 (875 peptides remained, 138 filtered) and plotted as average fold change relative to 100 fmol.

Using the data in Panorama and LabKey Server interface for R, custom R scripts were integrated into Panorama data repository so users can visualize their results via web browser. Utilizing the 43 Skyline datasets in Panorama, we compared the Retention Time Correlation of each and generated a heatmap (**Figure 2**) were 26/43 labs had > 0.96 mean pairwise correlation and 30/43 had > 0.80 mean pairwise correlation. Filtering the data to the top 26 datasets, we then calculated a mean percentile rank and standard deviation for each peptide. The list was then filtered to the top 171 peptides which were found to have an Absolute Rank Deviation of less than 1% (**Supplemental Table 1**).

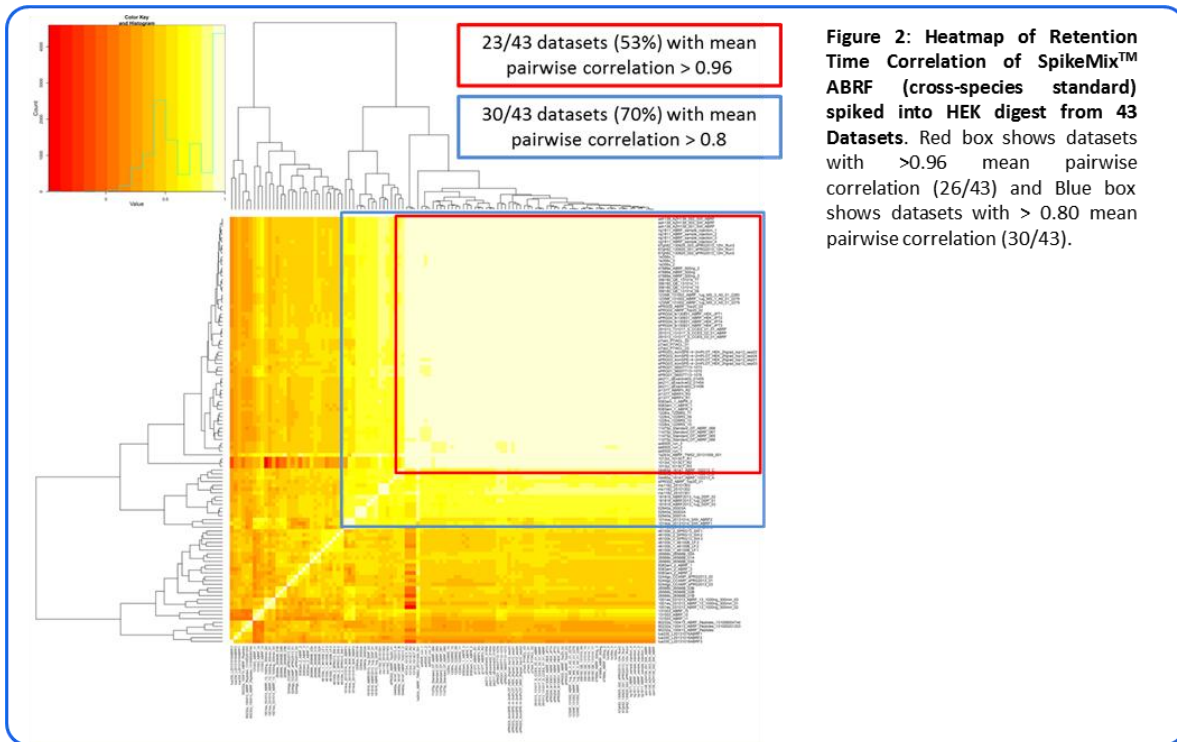


Figure 2: Heatmap of Retention Time Correlation of SpikeMix™ ABRF (cross-species standard) spiked into HEK digest from 43 Datasets. Red box shows datasets with >0.96 mean pairwise correlation (26/43) and Blue box shows datasets with > 0.80 mean pairwise correlation (30/43).

Conclusion

The ABRF Proteomics Standards Research Group (sPRG) is hopeful that the designed formulation of SpikeMix™ ABRF (cross-species standard) will become a valuable resource in various mass spectrometry-based proteomic applications, including quantitative and differential protein profiling, as well as general benchmarking for LC-MS performance (e.g., chromatographic retention time) and inter-laboratory assays.

The Authors



<http://www.abrf.org>

<https://panoramaweb.org/labkey/project/ABRF%20sPRG/begin.view?>

The sPRG is committed to identifying and implementing technical standards that reflect the ABRF's commitment to accuracy, clarity, and consistency in the field of proteomics. Examples of technical standards include, but are not limited to, reference materials, data sets, conditions and procedures that give proteomics researchers and analysts independent criteria to evaluate their abilities to produce predictable, consistent results. The sPRG strongly supports ongoing efforts for standardization of the recording and reporting of proteomics experiments.

The Company

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Supplemental Table 1.			
Top 171 Peptides with Mean Retention Time Deviation Less than 1%			
Peptide	Absolute Rank Deviation	Average Retention Time Rank	Standard Deviation
TATPQQAQEVHEK	0.002	0.013	0.007
AANEVSSADVK	0.001	0.023	0.010
IVQAEGEAEAAK	0.005	0.042	0.016
AVAHHTDCTFIR	0.007	0.046	0.023
GLSATVTGGQK	0.003	0.059	0.017
LVEPGSPA EK	0.009	0.063	0.017
HSGPNSADSANDGFVR	0.008	0.073	0.030
HSVGVVIGR	0.010	0.076	0.037
HSAILASPNPDEK	0.006	0.102	0.031
LSVEAPPK	0.005	0.111	0.035
SPDCTHDNPLETR	0.004	0.111	0.037
GTPQQIDYAR	0.007	0.119	0.032
VDNDENEHQLSLR	0.006	0.122	0.043
TAIQAAGYPDK	0.008	0.123	0.037
YVECSALTQK	0.008	0.131	0.040
VVGCSCVVVK	0.005	0.135	0.042
NSNPALNDNLEK	0.005	0.145	0.040
LVEVNGENVEK	0.007	0.156	0.042
FESPEVAER	0.008	0.163	0.048
YLAEVATGEK	0.006	0.164	0.041
LSPDAIPGK	0.008	0.166	0.041
SEALGVGDVK	0.006	0.178	0.043
AVENSSTAIGIR	0.007	0.186	0.050
FVVQNVSAQK	0.007	0.187	0.047
LQSEVAELK	0.007	0.190	0.052
LTVTAYDCGK	0.010	0.194	0.042
TQIQSVEPYTK	0.009	0.223	0.042
GASGSFVVVQK	0.008	0.226	0.049
EAALGAGFSDK	0.008	0.233	0.043
EAIEGTYIDK	0.008	0.239	0.046
VTQSNFAVGYK	0.009	0.264	0.051
INCPVYITK	0.007	0.281	0.043
VETFSGVYK	0.010	0.287	0.046
LVASAYSIAQK	0.006	0.291	0.051
IPEQILGK	0.009	0.297	0.051
LNEQASEEILK	0.008	0.315	0.051
FGISSVPTK	0.007	0.320	0.053
GTEELYAIK	0.008	0.329	0.044
TGTTIAGVVYK	0.008	0.331	0.048
SLVQGELVTASK	0.008	0.354	0.046
LVQAFQYTDK	0.009	0.364	0.043
IPVGPETLGR	0.008	0.366	0.053
LYDIDVAK	0.009	0.370	0.046
DAHNALLDIQSSGR	0.007	0.405	0.041
GFVEIQTPK	0.008	0.418	0.050
VGAPLVCCEIK	0.008	0.425	0.050
NTGIICTIGPASR	0.006	0.438	0.046
VLSIGDIAR	0.007	0.442	0.054
NFGIGQDIQPK	0.005	0.443	0.047
ILYLTPEQEK	0.008	0.452	0.039
LQQLPADFGR	0.009	0.461	0.042
LAEVALAYAK	0.009	0.466	0.043
GVSCSEVTASSLIK	0.010	0.466	0.042
IEAACFATIK	0.006	0.467	0.044
VCGSNLLSICK	0.007	0.481	0.075
FVALENISCK	0.007	0.485	0.044
EGYSGVGLLSR	0.008	0.486	0.037
ISELGAGNGGVVFK	0.010	0.529	0.035
LALASLGYEK	0.009	0.544	0.045
FAEIVNTLDK	0.007	0.558	0.037
ATLYVTAIEDR	0.010	0.561	0.037

SpikeMix™ Isotope-Labeled Peptide Pools

VQQTVQDLFGR	0.009	0.589	0.041
STLNEIFYGK	0.010	0.589	0.036
SNFGYNIPLK	0.007	0.617	0.037
FFLCQVAGDAK	0.007	0.666	0.031
LGDGLFLQCCR	0.008	0.668	0.028
IDSLSAQLSQLQK	0.008	0.674	0.039
TILPAAAQDVYYR	0.010	0.675	0.031
GPATVEDLPSAFEEK	0.007	0.691	0.027
VADIGLAAWGR	0.005	0.707	0.027
IEELQLIVNDK	0.006	0.712	0.035
EFSPTGTTITSAK	0.007	0.715	0.029
EFIEGVSQFSVK	0.009	0.720	0.031
LDYILGLK	0.009	0.728	0.033
STFVLDEFK	0.008	0.733	0.031
EVSFDELPEK	0.006	0.735	0.031
VPQDGFDFLQK	0.009	0.751	0.020
LAYINPDLALEEK	0.009	0.751	0.031
IYGISFPDPK	0.004	0.754	0.025
VLTPELYAELR	0.007	0.756	0.027
SVILEAFSSPSEEVK	0.003	0.762	0.027
DLLNHAFFQEETGVR	0.008	0.782	0.025
LSVLGAIQSVQQR	0.009	0.796	0.031
ETEEILADVLEK	0.008	0.804	0.041
IINDLLQSLR	0.005	0.810	0.035
IPDWFLNR	0.009	0.810	0.020
YAVLYQPLFDK	0.006	0.816	0.020
VPPAINQFTQALDR	0.009	0.820	0.029
LTTVPTQAFEYLSK	0.010	0.828	0.020
EADDIVNWLEK	0.008	0.831	0.022
ATNDEIFSIK	0.008	0.833	0.028
SDFEVEFDALQK	0.008	0.834	0.016
FCIWTESAFLR	0.006	0.838	0.019
TVLELQYVLDK	0.006	0.839	0.028
FYGPAGPYGIFAGR	0.009	0.840	0.022
ALLSAPWYLNLR	0.006	0.847	0.017
FGFPAFSGISR	0.003	0.851	0.018
EGDVLTLLESER	0.007	0.853	0.024
FIATLQYIVGR	0.005	0.854	0.023
WLLLTGISAQQNR	0.005	0.860	0.017
DFNHINVELSLLGK	0.006	0.862	0.021
SPNELVDLDFK	0.006	0.863	0.021
LFTAESLIGLQK	0.005	0.866	0.022
EFVEAVLELR	0.008	0.869	0.024
GAFGQVIEADAFGIDK	0.007	0.870	0.015
IFGVTTLDIVR	0.005	0.870	0.021
VAVFFGGLSIK	0.005	0.876	0.018
FYFENLLAK	0.004	0.878	0.016
TLSQFTDALVTIR	0.007	0.881	0.062
FDEISFVNFAR	0.006	0.883	0.016
ILEFFGLK	0.007	0.886	0.016
LIFQVLDAVK	0.010	0.886	0.028
VVLAYEPVWAIGTGK	0.008	0.893	0.016
AFSVFLFNTENK	0.006	0.893	0.015
GLETFSQLVWVK	0.006	0.894	0.015
ALTVPELTQQVFDALQK	0.006	0.898	0.018
IVEPYIAWGYPNLQK	0.009	0.899	0.020
FTDEYQLFEELGK	0.010	0.899	0.015
LFIFETFCR	0.009	0.909	0.090
EVEPALELLEPIDQK	0.007	0.910	0.017
LDQLIYIPLPDEK	0.010	0.913	0.018
TIAIIAEGIPEALTR	0.008	0.914	0.020
IILDLISESPIK	0.008	0.914	0.023
VFSATLGLVDIVK	0.006	0.915	0.020
FTQISPVWLQK	0.006	0.916	0.013
EFIWGVFGK	0.010	0.921	0.013
LGIYTVLFLER	0.009	0.921	0.016
ASSSLGLQDFDLLR	0.005	0.921	0.015

SpikeMix™ Isotope-Labeled Peptide Pools
PROTEOMICS

LDCPFFGSPITLR	0.008	0.925	0.013
LLVPTQYVGAIGK	0.006	0.929	0.020
EIVLADVIDNDSWR	0.006	0.929	0.011
ISQGLGLQDFDLIR	0.004	0.930	0.012
FGLEGCEVLIPALK	0.004	0.932	0.012
HENVIGLLDVFTPAR	0.009	0.935	0.015
ATLWYVPLSLK	0.003	0.939	0.008
LICCDILDVLDK	0.004	0.939	0.012
AVFVDLEPTVIDEIR	0.005	0.942	0.026
ALTLGALTLPLAR	0.005	0.943	0.020
TVYSVFGFSFK	0.007	0.944	0.010
TGEAIVDAALSALR	0.008	0.945	0.018
EVCQLLPFLVR	0.004	0.946	0.011
LLYPETGLFLVR	0.003	0.947	0.008
LGTLSALDILIK	0.005	0.951	0.017
VNFLPEIITLSK	0.003	0.952	0.009
NPILWNVADVVIK	0.004	0.955	0.009
GSLTFEPLTLVPIQTK	0.005	0.956	0.009
ESYPVFYFR	0.006	0.958	0.009
FILNLPTFSVR	0.003	0.962	0.008
ASVDELFAEIVR	0.004	0.963	0.009
LNWLSVDFNNWK	0.005	0.963	0.008
ELYLFDVLR	0.003	0.963	0.008
DALSDLALHFLNK	0.003	0.967	0.008
DLYSGLIGPLIVCR	0.005	0.971	0.009
FQDNFEFVQWFK	0.003	0.973	0.007
TAFDEAIAELDTLNEESYK	0.004	0.974	0.007
EFSITDVVPYPISLR	0.004	0.974	0.007
AAVENLPTFLVELSR	0.004	0.976	0.007
ITPENLPQILLQLK	0.005	0.978	0.008
DICNDVLSLEK	0.006	0.979	0.008
LLLAGYDDFNCNVWDALK	0.004	0.980	0.006
FFADLLDIK	0.002	0.982	0.005
AYPFYWAWLPQAK	0.003	0.982	0.005
YEISSVPTLFFK	0.002	0.982	0.005
STLINSFLTDLYPER	0.002	0.984	0.005
ECLPLIIFLR	0.002	0.986	0.005
IGLVEALCGFQFTFK	0.001	0.989	0.004
IDIPSFWDWPIAPFPR	0.001	0.990	0.004
GIPEFWLTVFK	0.000	0.991	0.003
EVFGTFGIPFLLR	0.000	0.993	0.003
IEQLSPFPDLLLLK	0.000	0.995	0.002
DFLPLYFGWFLTK	0.000	0.999	0.001