

ABRF PRG STUDY TO EVALUATE DATA-INDEPENDENT ACQUISITION FOR PROTEIN QUANTIFICATION IN CORE FACILITY SETTINGS

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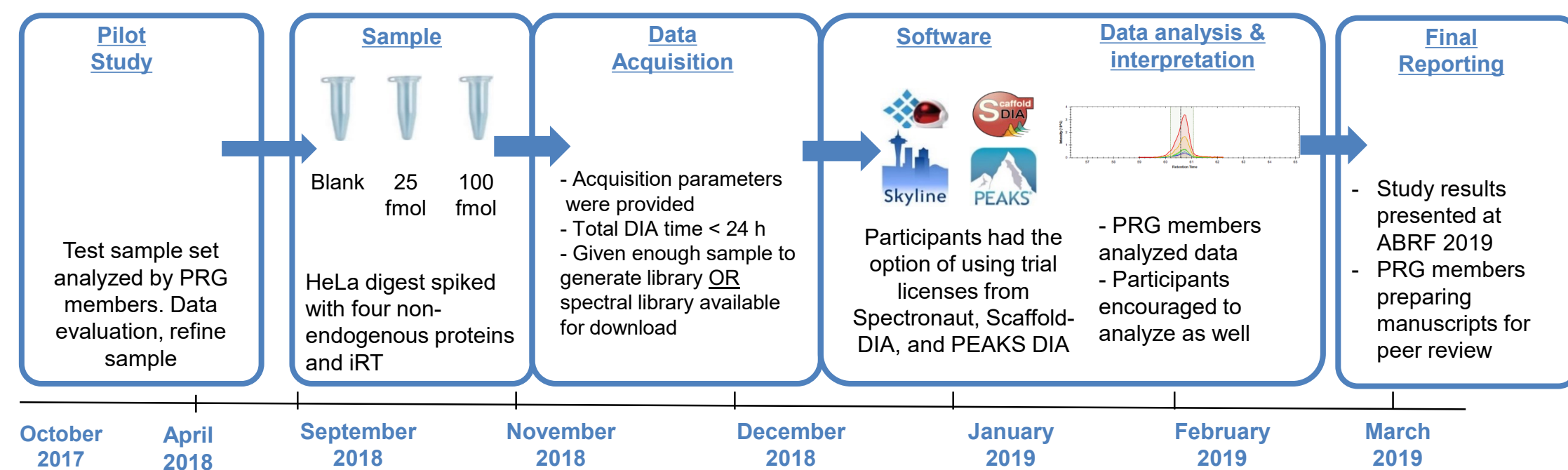
Study Highlights

- Study samples were sent to **63 labs** from **20 countries** around the globe
- **40 labs** uploaded data, **53 labs** filled out survey, **35 data sets** used for preliminary analysis
- Most participants were experienced LCMS users, but few had used DIA
- Data from most participants reflects expected fold change of spike-in proteins. Accuracy of quantitation was not correlated to previous DIA experience
- By providing prototype sample set and recommended methods for different instrument platforms, and extended trial licenses, the PRG provided an opportunity for labs to evaluate their readiness to carry out DIA analysis
- Samples were injection-ready, data was analyzed by PRG members, thus the results presented here reflects data quality

Study Design

- **Provided to participants**
 - Predigested, C18 cleaned samples (3 in each set), ready for analysis, 25 µg total peptide each with iRT spiked in
 - Recommended mass spec methods for certain instruments, guidelines for method generation
 - Trial software license for DIA data analysis
 - Chromatogram and/or Spectral library
- **Required from participants**
 - Maximum DIA acquisition time is 24 hours – DO NOT EXCEED
 - Online survey on data acquisition and analysis (if self analyzed)
 - Raw data is required
 - All submissions are anonymous
- **Optional for participants**
 - Generation of in-house spectral library – if enough sample left after DIA analysis
 - Self analyze data
 - trial licenses provided with extra time period
 - Spectral library available
- **Data analysis**
 - Carried out by experienced PRG members as “expected” data interpret for participants

Study Timeline

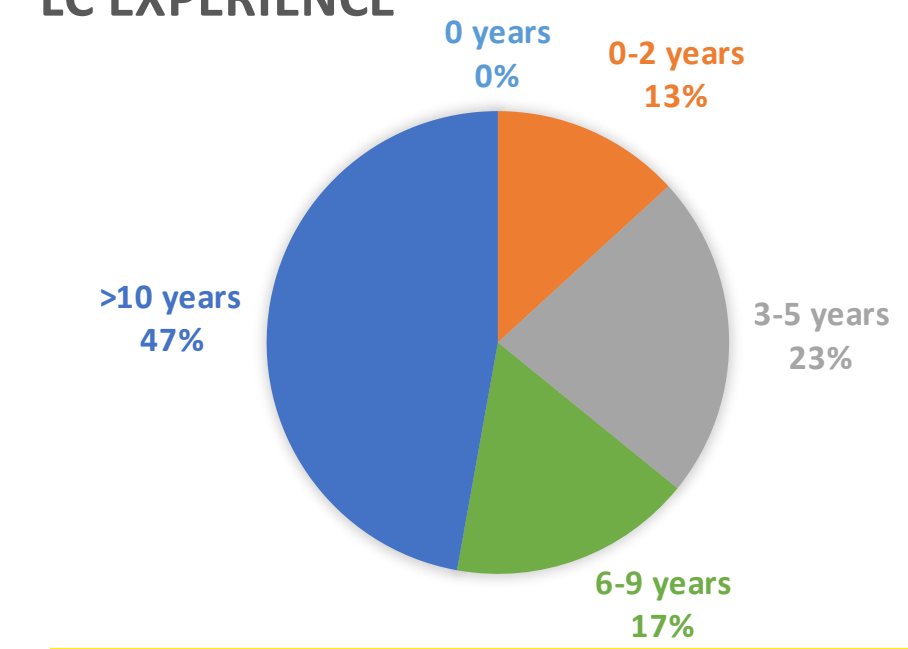


Participant Matrices

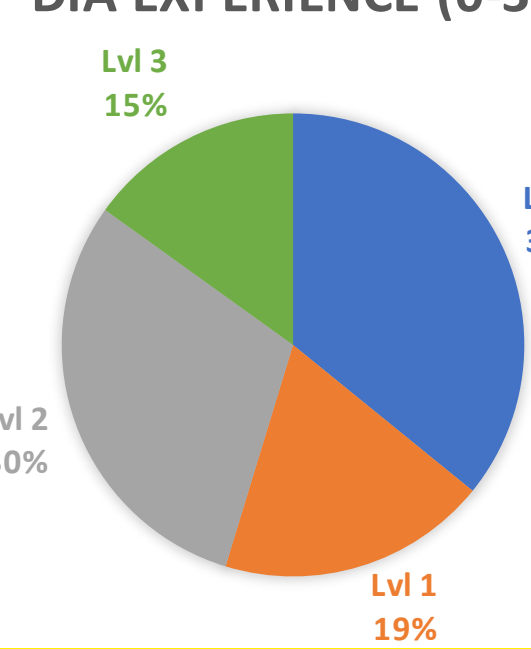


Study Participants: 63 labs, 20 countries, 16 US States

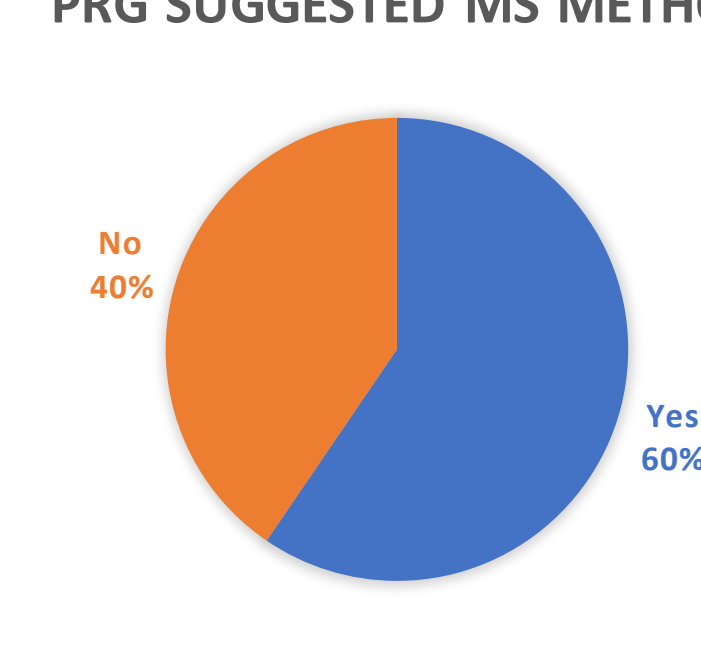
LC EXPERIENCE



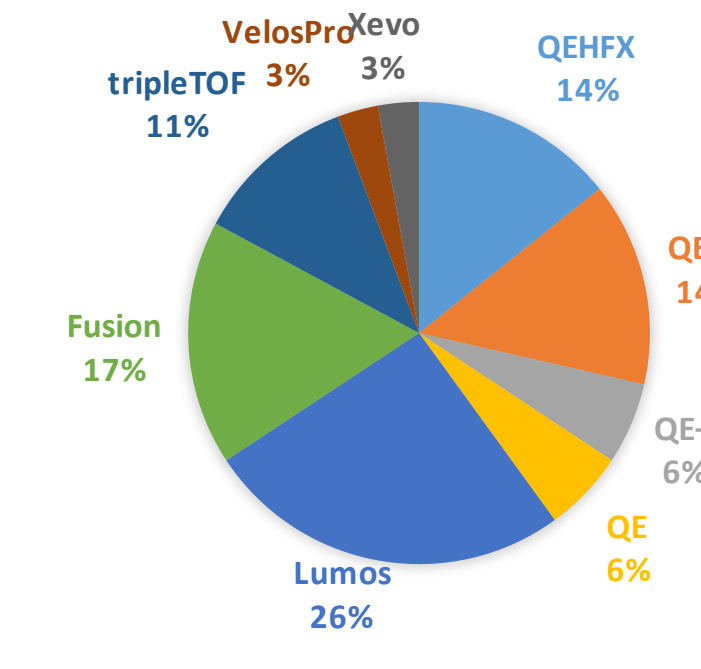
DIA EXPERIENCE (0-3)



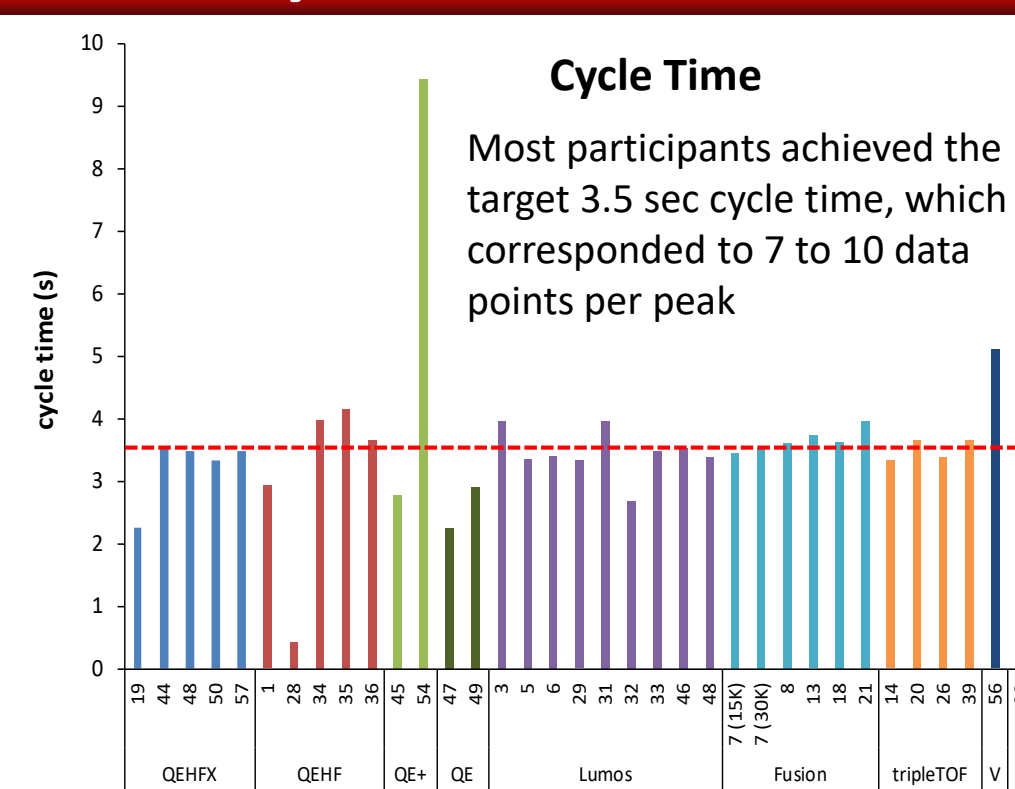
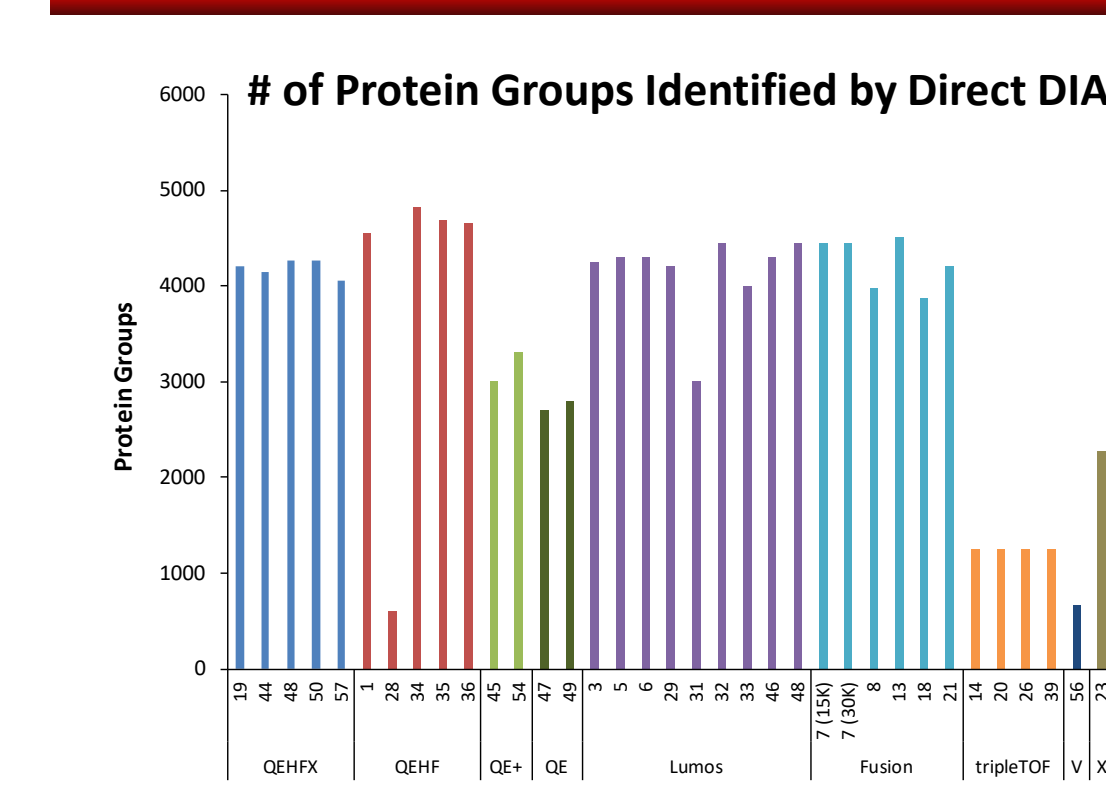
PRG SUGGESTED MS METHOD



MS INSTRUMENT



Overall Data Quality



Acknowledgements

Software support

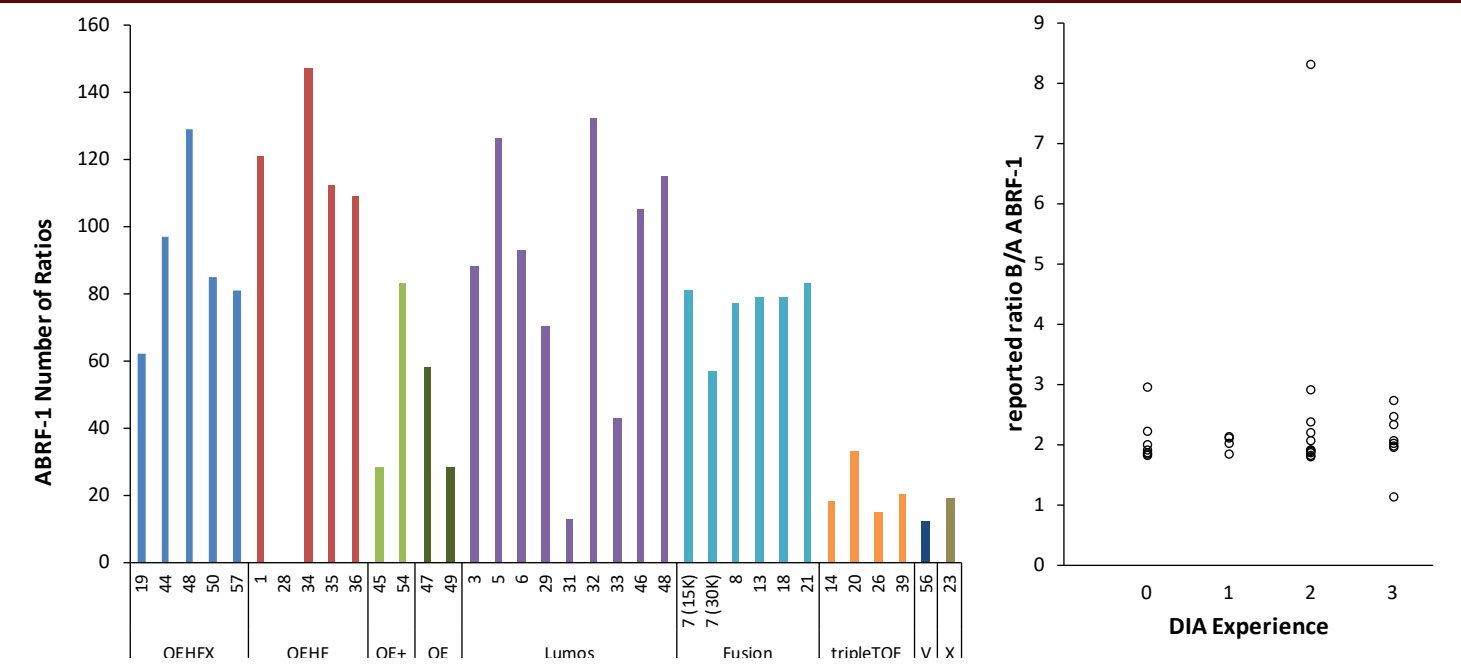
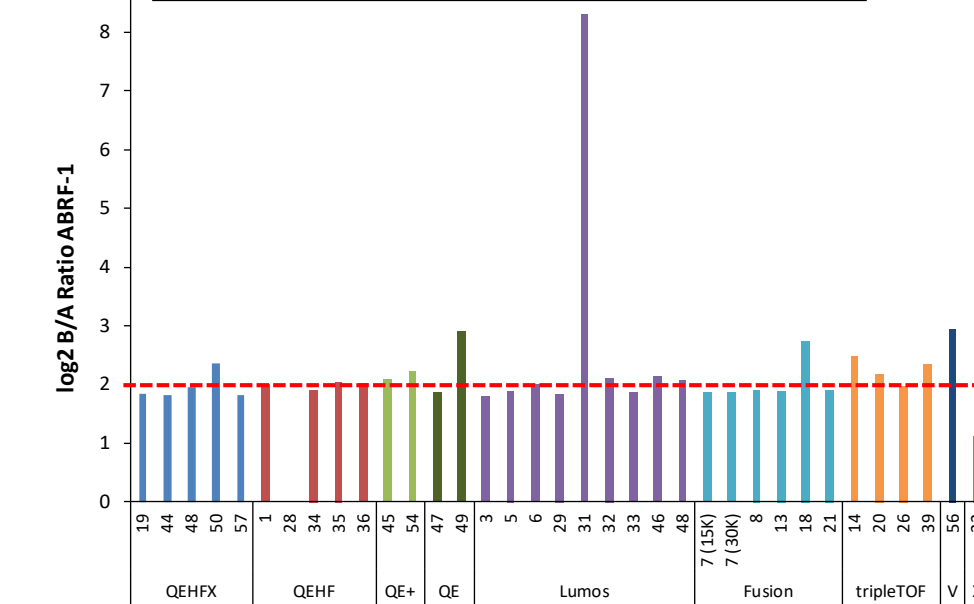
- Biognosys:
- Bioinformatic Solutions
- Proteome Software

Technical support

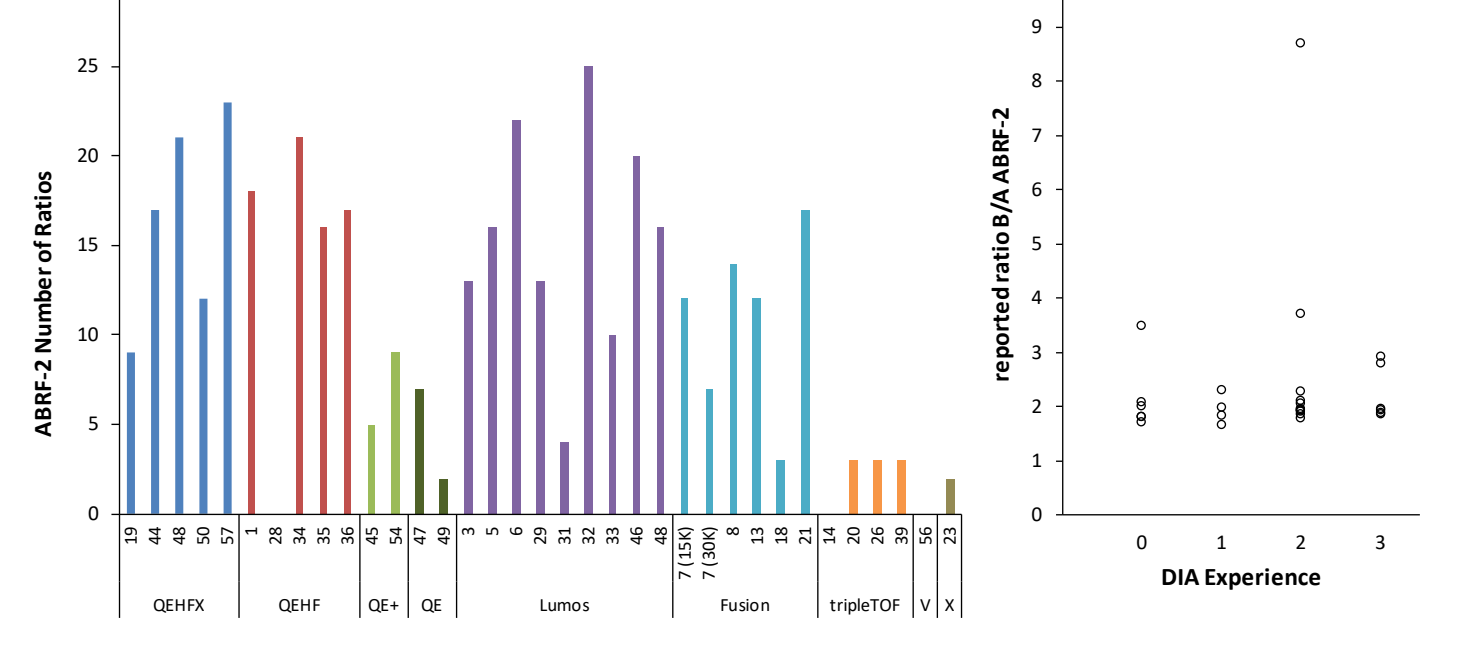
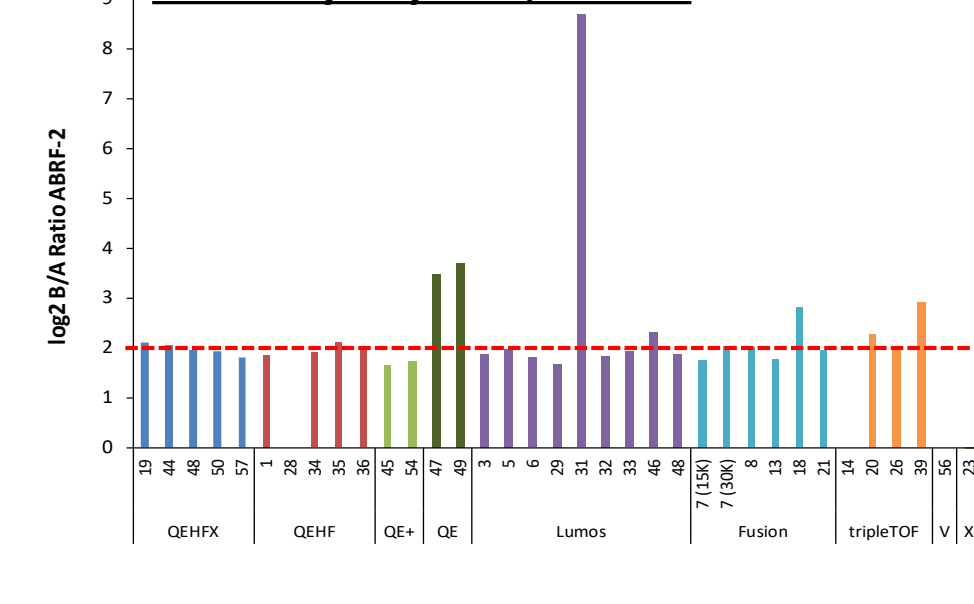
- Waters Corp
- Thermo Scientific
- Sciex

Quantification of Spike-in Proteins

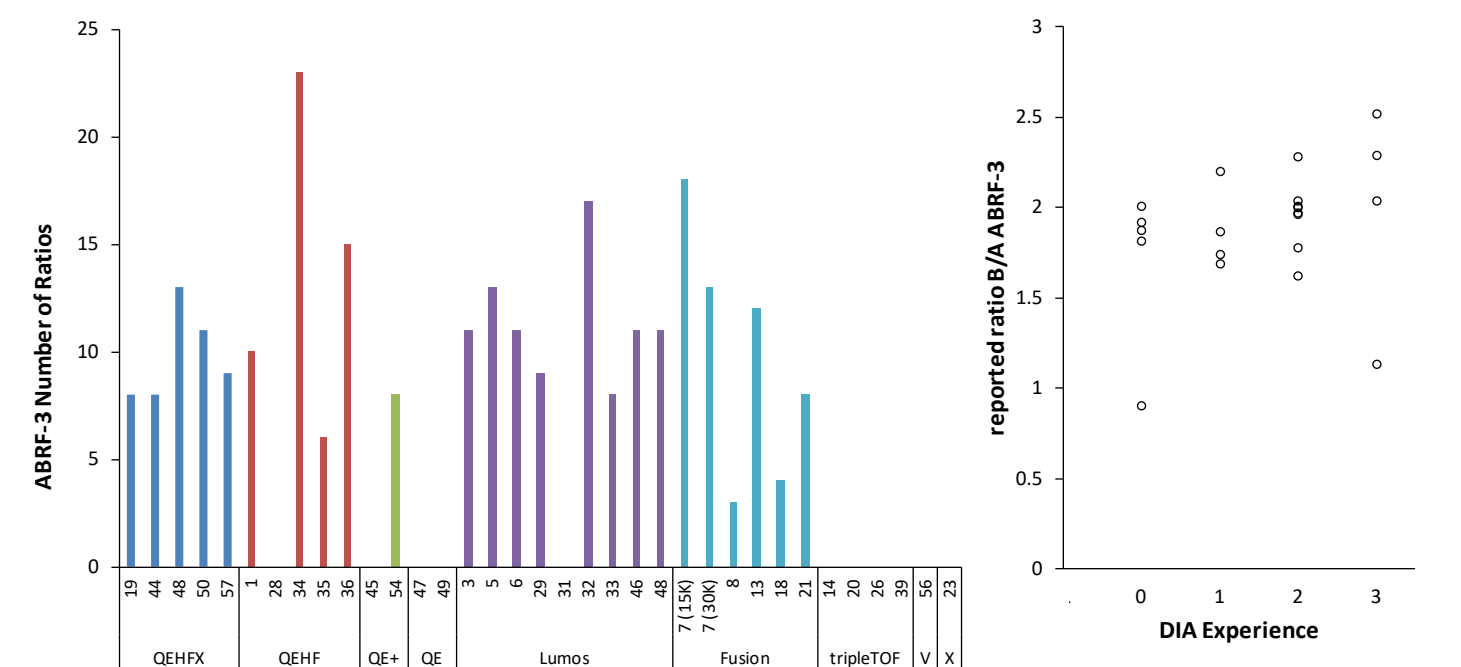
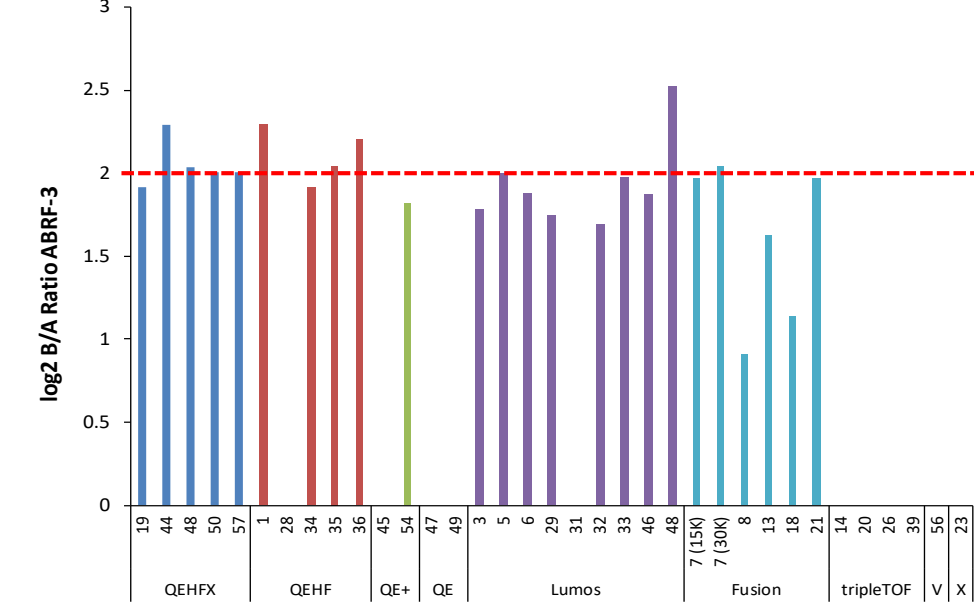
ABRF-1 beta-galactosidase, 1024 AA



ABRF-2 lysozyme C, 147 AA



ABRF-3 glucoamylaseC, 640 AA



ABRF-4 protein G, 185 AA

