

Promega 6x5 and the SUMS Proteomics Workflow

Mass Spectrometry

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Quality control procedures consume a significant portion of instrument capacity in biomolecular facilities, reducing equipment availability for experimental work. Here, we demonstrate a comprehensive strategy for integrating the Promega 6x5 System Suitability standard into our core facility proteomics workflow to address these concerns. If successful, we hope that the implementation of the 6x5 will significantly reduce the amount of instrumentation time spent on quality control checks. We investigate several chromatography and mass spectrometry parameters, including retention time reproducibility, sensitivity, and linear dynamic range across various backgrounds including: 1) A blank sample matrix, consisting of our reconstitution buffer (97.9% water, 2% acetonitrile, 0.1% formic acid) 2) A complex, standardized sample matrix, specifically our yeast LC-MS quality control standard (0.1 ng/uL of digested yeast peptides in our reconstitution buffer) 3) Real-world sample matrices at different preparation timepoints (during sample reconstitution versus before sample desalting, for example) to identify critical points of sample loss. These experiments will be run using our in-house LC-MS methods in parallel with those described in the original 2015 Analytical Chemistry article detailing the application of the 6x5 standard, for comparison. We might also run further experiments incorporating the 6x5 standard even earlier in the sample preparation workflow (for example, before digestion) to assess our protocols' efficiency.