

## Yeast-based reference materials for quantitative metabolomics

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<b>Abstract</b>	<p>We introduce a new concept of yeast-derived biological matrix reference material for metabolomics research relying on in vivo synthesis of a defined biomass, standardized extraction followed by absolute quantification with isotope dilution. The yeast <i>Pichia pastoris</i> was grown using full control- and online monitoring fed-batch fermentations followed by fast cold methanol quenching and boiling ethanol extraction. Dried extracts served for the quantification campaign. A metabolite panel of the evolutionarily conserved primary metabolome (amino acids, nucleotides, organic acids, and metabolites of the central carbon metabolism) was absolutely quantified by isotope dilution utilizing uniformly labeled C-13-yeast-based internal standards. The study involved two independent laboratories employing complementary mass spectrometry platforms, namely hydrophilic interaction liquid chromatography-high resolution mass spectrometry (HILIC-HRMS) and gas chromatography-tandem mass spectrometry (GC-MS/MS). Homogeneity, stability tests (on a panel of &gt;70 metabolites over a period of 6 months), and excellent biological repeatability of independent fermentations over a period of 2 years showed the feasibility of producing biological reference materials on demand. The obtained control ranges proved to be fit for purpose as they were either superior or comparable to the established reference materials in the field.</p>
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