

**Measurement of DNA concentration as a normalization strategy for metabolomic data from adherent cell lines**

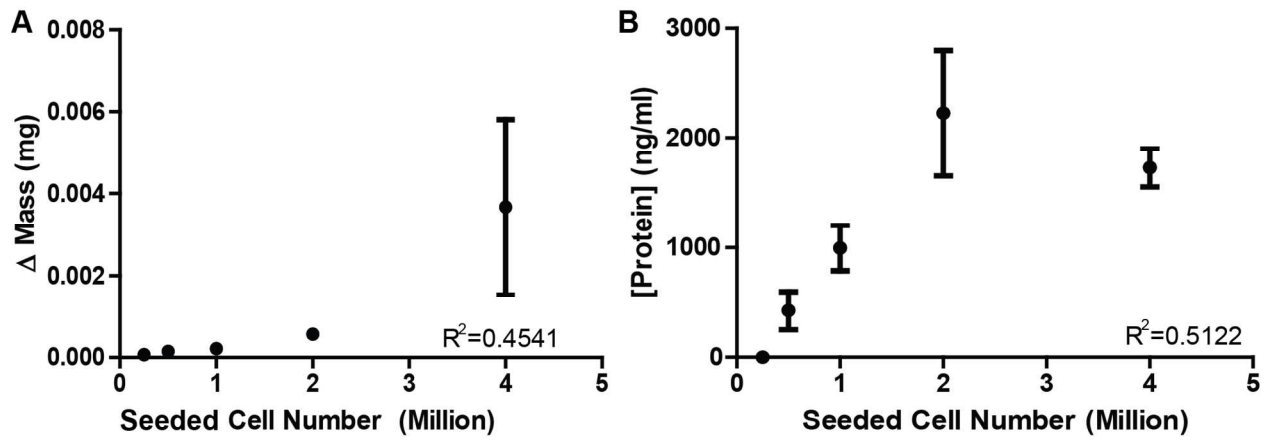
Leslie P. Silva<sup>†□</sup> Philip L. Lorenzi<sup>†□</sup>, Preeti Purwaha<sup>†</sup>, Valeda Yong<sup>†</sup>, David H. Hawke<sup>§</sup>, John N. Weinstein<sup>\*†</sup>

<sup>†</sup> Department of Bioinformatics & Computational Biology, University of Texas MD Anderson Cancer Center, 1400 Pressler St., Houston, TX, 77030, United States

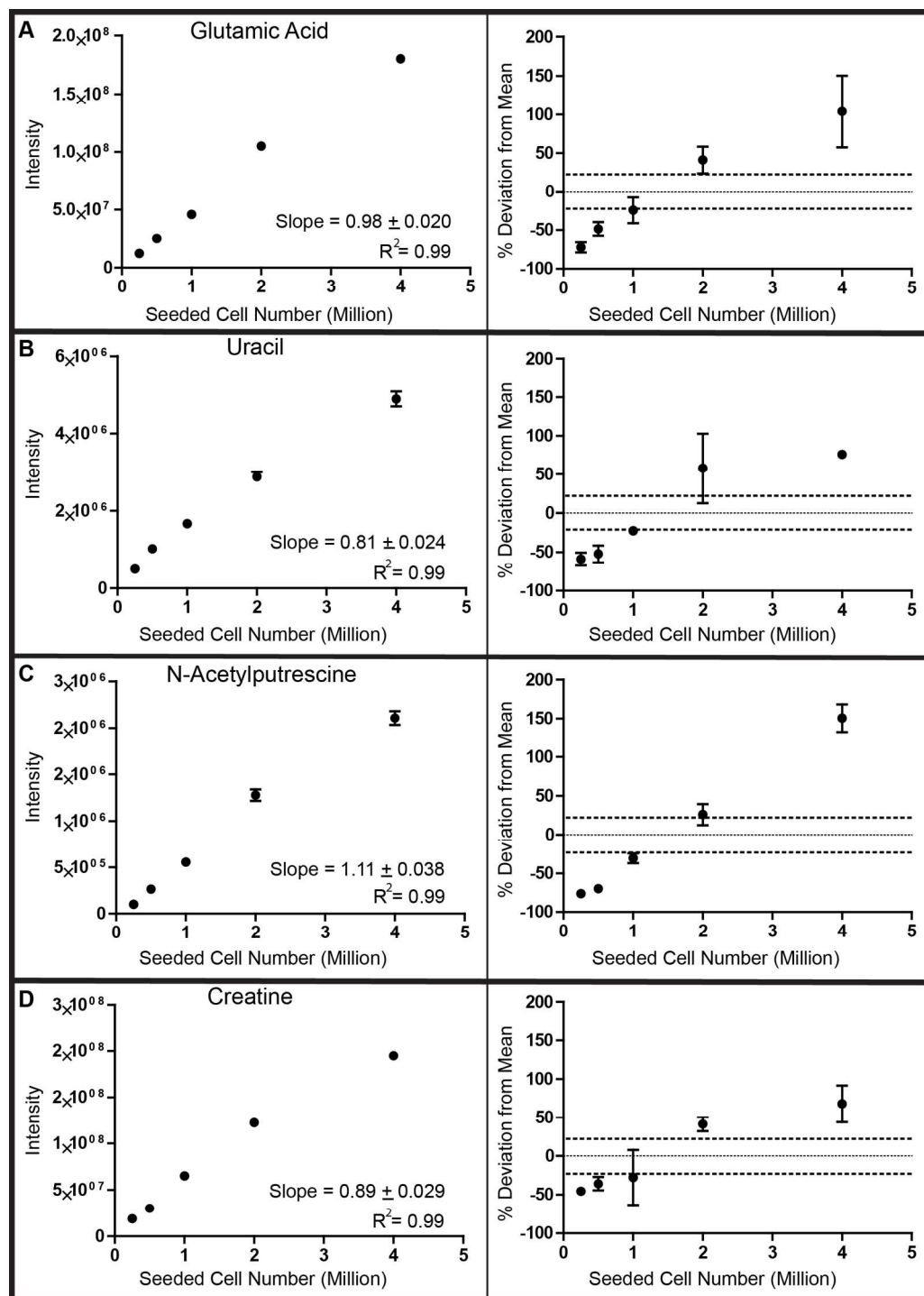
<sup>§</sup> Department of Pathology, University of Texas MD Anderson Cancer Center, 7435 Fannin St., Houston, TX, 77054, United States

□ Authors contributed equally to this manuscript

The supplementary information provided with this manuscript contains figures with less successful and unsuccessful metabolomic data normalization methods that were tested and mentioned in the manuscript. The following methods, metabolite normalization to cell debris mass, metabolite normalization to protein concentration from cell debris (Supplementary Figure S1 (A) and (B), respectively), and metabolite normalization to the median ion intensity (Supplementary Figure 2) showed too large of deviation to be considered as possible strategies for cell number normalization in adherent cells.



**Supplementary Figure S1.** Cell mass of metabolomic preparations. (A) Mass of air-dried cell pellets as a function of seeded cell number. (B) Total protein concentration determined from air-dried cell pellets as a function of seeded cell number. Large error bars, based on the standard error of the mean with triplicate sampling, were observed between replicate measurements in both instances.



**Supplementary Figure S2.** Metabolite intensity as a function of cell number. In the left-hand panels, values for four metabolite intensities are plotted against seeded cell number. In the right-hand panels, scatter plots display the percent deviation from the mean for metabolite intensity normalized by the median metabolite intensity for each sample. Dashed lines indicate 20% deviation from the mean.