Protocol Methods for

Trace: Machine Learning of Signal Images for Trace-Sensitive Mass Spectrometry – A Case Study from Single-Cell Metabolomics

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Project Summary. The goal of this study was to validate the performance of a custom-written software tool, called Trace, for finding molecular features from ultrasensitive metabolomics experiments using high-resolution mass spectrometry. The software uses a trained neural network model to extract molecular features. As model for validation, we performed MS profiling of single identified cells from early developing embryos of the South African clawed frog (*Xenopus laevis*) using a custom-built capillary electrophoresis electrospray ionization platform coupled to a quadrupole time-of-flight mass spectrometer. The MS dataset from these measurements was manually curated for molecular features, and the resulting list of molecular features were used to test the robustness and accuracy of Trace at predicting molecular features that were detected from the single cells.

Treatments of Animals. Embryos were obtained by natural mating of adult *Xenopus laevis*, approved by the Institutional Animal Care and Use Committee of the George Washington University (IACUC #A311) and University of Maryland, College Park (R-DEC-17-57). Embryos were cultured to the 16-cell stage, and the midline dorsal animal cell (D11) was identified following established cell fate maps. The protocols that were used to handle animals and embryos in this study are identical to those published in reference: Sieve, H. L.; Grainger, R. M.; Harland, R. M. Early development of Xenopus laevis: A laboratory manual; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2000

Collection of Samples. An about 10 nL portion of each identified D11 cell were aspirated using a microfabricated capillary under a stereomicroscope. Protocols of sample preparation, sample measurement, and data analysis were identical to those in reference: R. M. Onjiko, E. P. Portero, S. A. Moody, and P. Nemes, *In situ* microprobe single-cell capillary electrophoresis mass spectrometry: Metabolic reorganization in single differentiating cells in the live vertebrate (*X. laevis*) embryo, Anal. Chem. 2017, 89, 7069–7076, DOI: 10.1021/acs.analchem.7b00880.

Sample Preparation. Metabolites from the aspirated cell content were extracted in 4 μL of solvent containing 40% acetonitrile and 40% methanol. A 10 nL portion of each extract was

analyzed in a custom-built capillary electrophoresis (CE) electrospray ionization (ESI) interface coupled to a high-resolution time-of-flight mass spectrometer (MS, Impact HD, Bruker Daltonics, Billerica, MA). For further details, please refer to protocols published in reference: R. M. Onjiko, E. P. Portero, S. A. Moody, and P. Nemes, *In situ* microprobe single-cell capillary electrophoresis mass spectrometry: Metabolic reorganization in single differentiating cells in the live vertebrate (*X. laevis*) embryo, Anal. Chem. 2017, 89, 7069–7076, DOI: 10.1021/acs.analchem.7b00880.

CE-ESI-MS. The following experimental settings were used: CE separation voltage, 19–21 kV; CE capillary dimensions, 40/110 µm inner/outer diameter; coaxial sheath flow rate, 1 µL/min; electrospray potential, –1,700 V (positive ion mode, grounded emitter); MS^1 spectral acquisition rate, 2 Hz; mass range, 50–550 Da. For further technical details, please refer to protocols published in reference: R. M. Onjiko, E. P. Portero, S. A. Moody, and P. Nemes, *In situ* microprobe single-cell capillary electrophoresis mass spectrometry: Metabolic reorganization in single differentiating cells in the live vertebrate (*X. laevis*) embryo, Anal. Chem. 2017, 89, 7069–7076, DOI: 10.1021/acs.analchem.7b00880.

Data Analysis. Raw files were converted to mzML format using CompassXport version 3.0.9.2. The mzML files were further analyzed using Trace that we report in this work. Results of Trace analysis are provided as text files (.txt) in this submission.