additions and corrections Journal of proteome research

Network Analysis of Primary Hepatocyte Dedifferentiation Using a Shotgun Proteomics Approach

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The editors of this journal received the following comment from a reader.

"I would like to point out that the following recent article accepted to the *Journal or Proteome Research* does not anywhere indicate the instrument used for mass spectrometry. Whilst the other aspects of this study are otherwise good and well presented, the MS instrument detail is still an important difference in terms of what one might achieve. Other details of peak list creation etc., are also absent. Reference is made to another article that I cannot access. The article indicates they have adapted a micro-array data processing approach, which seems a useful alternative, but the readers would be benefit from knowing how."

The authors apologize to the reader for the omissions and would like to add the following information to address the points raised.

For LC−MS/MS analysis of iTRAQ-labeled samples, each cation exchange fraction was resuspended in 120 μL 5% ACN/0.05% trifluoroacetic acid (TFA) and 60 μL were loaded on column. Samples were analyzed on a QSTAR Pulsar i hybrid mass spectrometer (AB Sciex) and were delivered into the instrument by automated in-line liquid chromatography (integrated LCPackings System, 5 mm C18 nanoprecolumn and 75 μm $\times$ 15 cm C18 PepMap column; Dionex, Sunnyvale, CA) via a nanoelectrospray source head and 10 μm inner diameter PicoTip (New Objective, Woburn, MA). The precolumn was washed for 30 min at 30 μL/min with 5% ACN/0.05% TFA prior to initiation of the solvent gradient to reduce the level of salt in the sample. A gradient from 5% ACN/0.05% TFA (v/v) to 60% ACN/0.05% TFA (v/v) in 70 min was applied at a flow rate of 300 nL/min. The MS was operated in positive ion mode with survey scans of 1 s and with an MS/MS accumulation time of 1 s for the three most intense ions. Collision energies were calculated on the fly based on the m/z of the target ion and the formula, collision energy = (slope $\times$ m/z) + intercept. The intercepts were increased by 3−5 V compared to standard data acquisition to improve the reporter ion intensities/quantitative reproducibility.

Protein identification data analysis was performed using ProteinPilot software (Version 3, AB Sciex, Warrington, U.K.). The data were analyzed with a fixed modification of MMTS-labeled cysteine, biological modifications allowed, and with the confidence set to 10% to enable the False Discovery Rate to be calculated from screening the reversed Swiss-Prot database. Ratios for each iTRAQ label were obtained using freshly isolated cell protein as the denominator. The detected protein

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threshold ("unused protscore (conf)") in the software was set to 1.3 to achieve 95% confidence.

Differential expression statistical analysis was performed in the R computing environment using packages originally designed to analyze microarray data. These packages and instructions can be downloaded from Bioconductor (http://www.bioconductor.org/packages/release/bioc/). The *limma* package,[1] which generates linear models of microarray gene expression, was used for comparing multiple time points, and *marray* and *multtest* packages were used for comparison between culture types.

Reference

(1) Smyth, G. K. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* **2004**, *3*, Article3.

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