Dr. Alexandre Zougman[1], Dr. John P. Wilson[2]

[1]University of Leeds, Leeds, England

[2]ProtiFi LLC, Fairport, NY, United States

PROTIFI™ *Bringing precision omics to life!™*

Understanding all of biology with highest throughput: Si-Trap-based simultaneous multiomics sample preparation

Introduction

We present Si-Trap, representing Simultaneous Trapping, a new high-throughput solution to address all necessities for clinical implementation of omics analyses with application to clear cell renal carcinoma. A new development over the original S-Trap[2-5] sample processing platform, Si-Trap yields from the same sample both metabolomic and proteomic fractions with extraordinary inter- and intrarun reproducibility over time. It requires only minutes of processing time per sample and can be executed either in loose spin columns or in an automated 96-well format (4 - 96 samples simultaneously).

Motivation

Sample processing in omics analyses is obligatory and has traditionally been the largest source of variability in proteomics and metabolomics data (Fig. 1). Resultantly, many biomarker "discoveries" are subsequently traced to batch or run order effects, thus compelling for clinical deployment of omics technologies first serious improvements in reproducibility especially in sample processing. Second, for widespread use, sample throughput should be as high as possible, necessitating extension to an automated platform. Moreover, to enable eventual point of care (POC) deployment of omics techniques, sample processing time should be as rapid as possible. Third, as we do not necessarily have a priori knowledge of which biomolecule will prove to be useful as diagnostic, prognostic, therapeutic or predictive signals for a given biological condition, sample processing should ideally produce from one sample different fractions for multiple omics interrogations. Fourth, such a solution should be affordable enough to be accessible to the majority of labs.

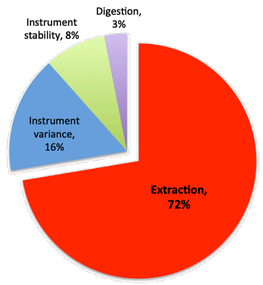

Figure 1 The vast majority of variability in omics data arises during protein extraction. Percent of total variability from [1].

Methods

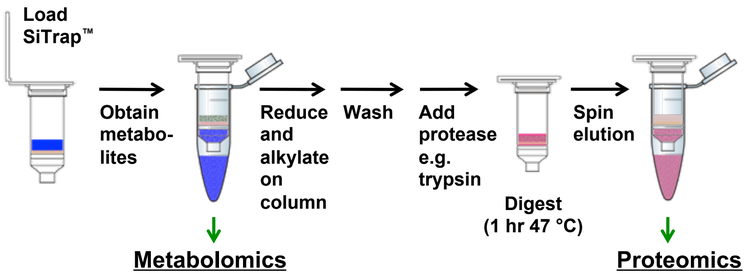

Fig. 2: The Si-Trap™ method. Biological samples are dissolved either in a native dissolution buffer or a denaturing buffer to which methanoloic binding buffer is added. The proteins are captured on a derivatized protein trap while metabolites flow thought. Reduction and alkylation is on column, as is digestion, and samples are ready for proteomics analysis after proteolytic treatment.

Results

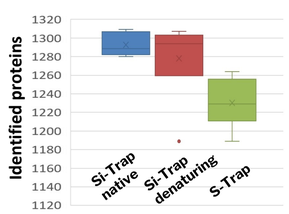

Fig. 3: Si-Trap and S-Trap methods achieved similar protein depth and coverage.

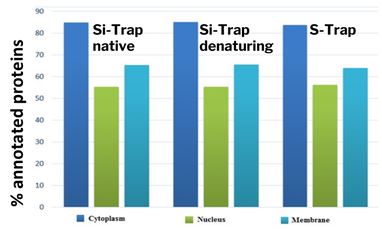

Fig. 4: Si-Trap and S-Trap methods reproducibly sampled all cellular components of the cell.

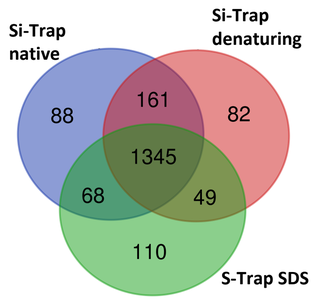

Fig. 5: Si-Trap in denaturing and native modes, and S-Trap methods, identified highly similar protein populations.

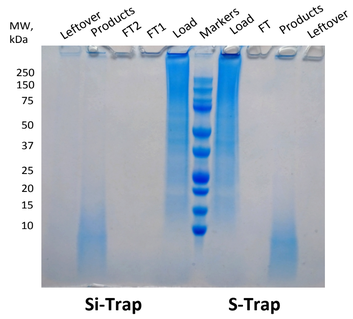

Fig. 6: Si-Trap was just as effective as S-Trap in the processing of FFPE tissues.

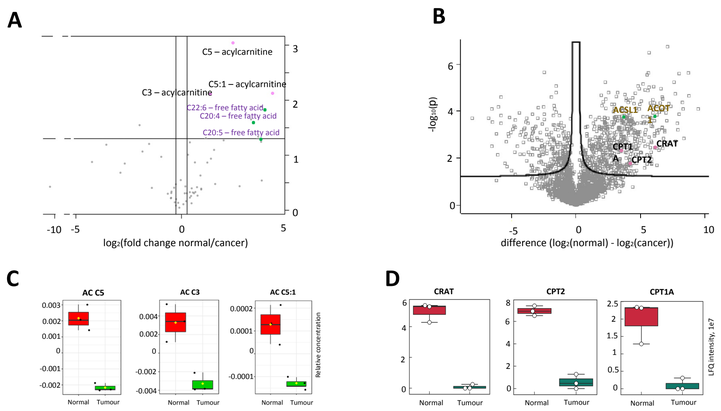

Fig. 7: Volcano plots of metabolomics and proteomics analyses for normal vs tumor renal sections (5% false discovery rate, FDR). **(A, C)** Metabolomics results indicate a decrease in both short chain acylcarnitines (C5, C5:1 and C3) and in polyunsaturated free fatty acids (C20:5, C20:4, C22:6) in the tumor samples. **(B, D).** Results of the proteomics analysis indicate downregulation of enzymes in the carnitine pathway, Carnitine O-acetyltransferase (CRAT), Carnitine O-palmitoyltransferase 2 (CPT2) and Carnitine O-palmitoyltransferase 1 (CPT1A) in the tumor samples. Further, a downregulation of enzymes in the polyunsaturated fatty acid pathway, Acyl-CoA Thioesterase 1 (ACOT1) and long chain Fatty acid-CoA ligase (ACSL1) is observed.

Results

Si-TRAP

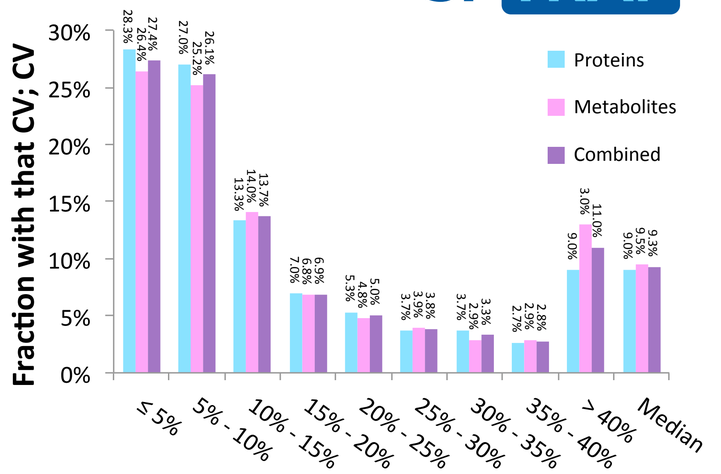

Fig. 8: >50% of CVs for all quantified protein and metabolite analytes fell below 10%. >67% of CVs fell below 15%.

Conclusion

Si-Trap yields both metabolomic and proteomic fractions from the same sample with extraordinary inter- and intrarun reproducibility over time. It requires only minutes of processing time per sample and can be executed either in loose spin columns or in an automated 96-well format (4 - 96 samples simultaneously). Automation and its commensurate high reproducibility can be achieved on an inexpensive Tecan A200 positive pressure workstation, a general automation platform accessible – due to its low cost – to the majority of research and clinical labs. We anticipate that Si-Trap will become an essential omics tool in laboratory and clinical settings and will enable novel discoveries, thereby helping to usher in a new era of clinical proteomics.

Fig. 9: The Tecan A200 is a low cost automation solution which can process 4 – 96 S-Trap or Si-Trap samples simultaneously.

References

1. Sources of Technical Variability in Quantitative LC-MS Proteomics. Piehowski et al. J. Proteome Res. 12(5) 2013.
2. Zougman A, Selby PJ, Banks RE. Suspension trapping (STrap) sample preparation method for bottom-up proteomics analysis.
3. Proteomics. 2014 May;14(9):1006-0. 2. Ludwig KR, Schroll MM, Hummon AB. Comparison of in-solution, FASP, and S-Trap based digestion methods for bottomup proteomic studies. Journal of proteome research. 2018 May 13;17(7):2480-90.
4. HaileMariam M, Eguez RV, Singh H, Bekele S, Ameni G, Pieper R, Yu Y. S-Trap, an Ultrafast Sample-Preparation Approach for Shotgun Proteomics. Journal of proteome research. 2018 Aug 16;17(9):2917-24.
5. Elinger D, Gabashvili A, Levin Y. Suspension trapping (S-trap) is compatible with typical protein extraction buffers and detergents for bottom up proteomics. Journal of proteome research. 2019 Mar 1.
6. Van De Meent MH, De Jong GJ. Improvement of the liquid-chromatographic analysis of protein tryptic digests by the use of long-capillary monolithic columns with UV and MS detection. Analytical and bioanalytical chemistry. 2007 May 1;388(1):195-200.

UNIVERSITY OF LEEDS

PROTIFI™ *Bringing precision omics to life!™*