Sample preparation for shotgun proteomics: comparison of stacking gel, tube-gel, FASP, S-TRAP, SPE and liquid methods

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Objective

- ✓ Sample preparation is a crucial step in high-throughput shotgun proteomics, challenged with detergent incompatibility that has a strong influence on the accuracy and robustness of MS analyses. Classical approaches using stacking-gel (SG), Solid Phase Extraction (SPE) or liquid digestion (LD) have been developed but show limitations due to the time-consuming and repetitive sample processing, their recovery efficiency and overall yield. In recent years, strategies by filtration such as filter-aided sample preparation (FASP) based on a molecular weight cut-off (MWCO), and its new alternative, the suspension traps (S-TRAP) confining particulate protein suspensions with the subsequent depletion of interfering substances, have tried to overcome these drawbacks.
- ✓ The objective of this work was to compare for the first time all these preparation methods, *i.e.* FASP, S-TRAP, SPE, SG, TG (tube-gel) and LD before subjecting the samples to a label-free semi-quantitative proteomic analysis (shotgun proteomics). A soluble fraction of muscle proteins (100 µg), spiked with 1.5 µg of casein, was used to assess sample preparation methods. Ten replicates were prepared for each method.

Materials & methods

Sample preparation

300 mg of pork muscle in 40 mM Tris-HCl, 2 mM EDTA pH 8 buffer

Homogenization - centrifugation 1000 *g* 10 min 4°C

Soluble fraction of muscle proteins (5 mg/ml) spiked with 1,5% casein as internal standard

A soluble fraction of pork muscle proteins was prepared as indicated above.
100 µg were used in 10 replicates for each preparation methods.
The quality of sample preparation was checked with a control 1D gel.

Control 1D Gel

20 µl proteins extract 5 µg/µl - - > 100 µg

In-gel methods

SG

Reference

Reduction with DTT 10 mM - 56°C - 30 mn

Alkylation with Iodoacétamide 55 mM

600 ng trypsin

TG

Balliau et al. 2018

In-solution methods

LD

Reduction with DTT 2,5 mM 56°C - 30 min

Alkylation with Iodoacétamide 25 mM

2,5 µg trypsin

SPE

Screenig sorbents : C18 (MN), OASIS HLB (Waters), EVOLUTE ABN (Biotage), ISOLUTE ENV+ (Biotage), HR-X (MN)

SPE ISOLUTE ENV+ (Biotage)

Sample dilution 1:3 (v/v) with 2% FA

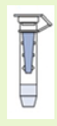
Equilibration 1 ml FA 2%

Elution 1 ml MeOH 0,1% TFA

Trypsin digestion

Filtration methods

FASP



Protein extract in 80 µl ammonium bicarbonate buffer 50 mM

Colgrave et al. 2013

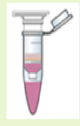
Load on a 3kDa millipore tube. Add 100 µL Urea 8 M

Spin 13 000 rpm - 15 mn

Reduction with DTT 10 mM

Alkylation with Iodoacétamide 25 mM

S-TRAP



Protein extract in 20 µl SDS buffer 10% 100 mM Ammonium bicarbonate pH 7,1

Reduction with DTT 20 mM

Alkylation with Iodoacétamide 40 mM

Phosphoric acid 12% - 1,2% final

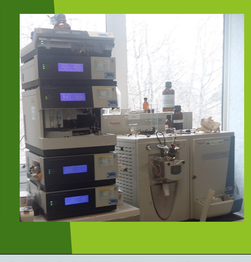
S-TRAP buffer (90% MeOH, 100 mM ABC pH 7,1

Loading 3 x 150 µl - Spin

Elution 50 µl ABC buffer 50 mM 0,2%FA 80 µl 50% ACN 0,2% FA twice

2,5 µg trypsin in 150 µl

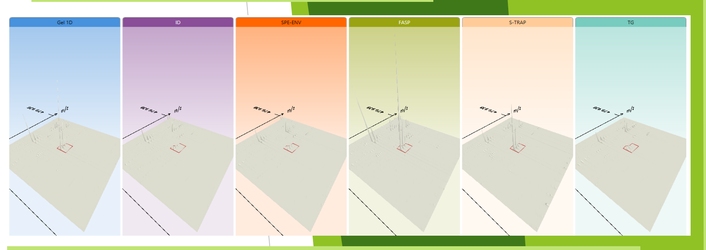
Quantitative analysis



Label-free shotgun by LC-MSMS and MASCOT identification

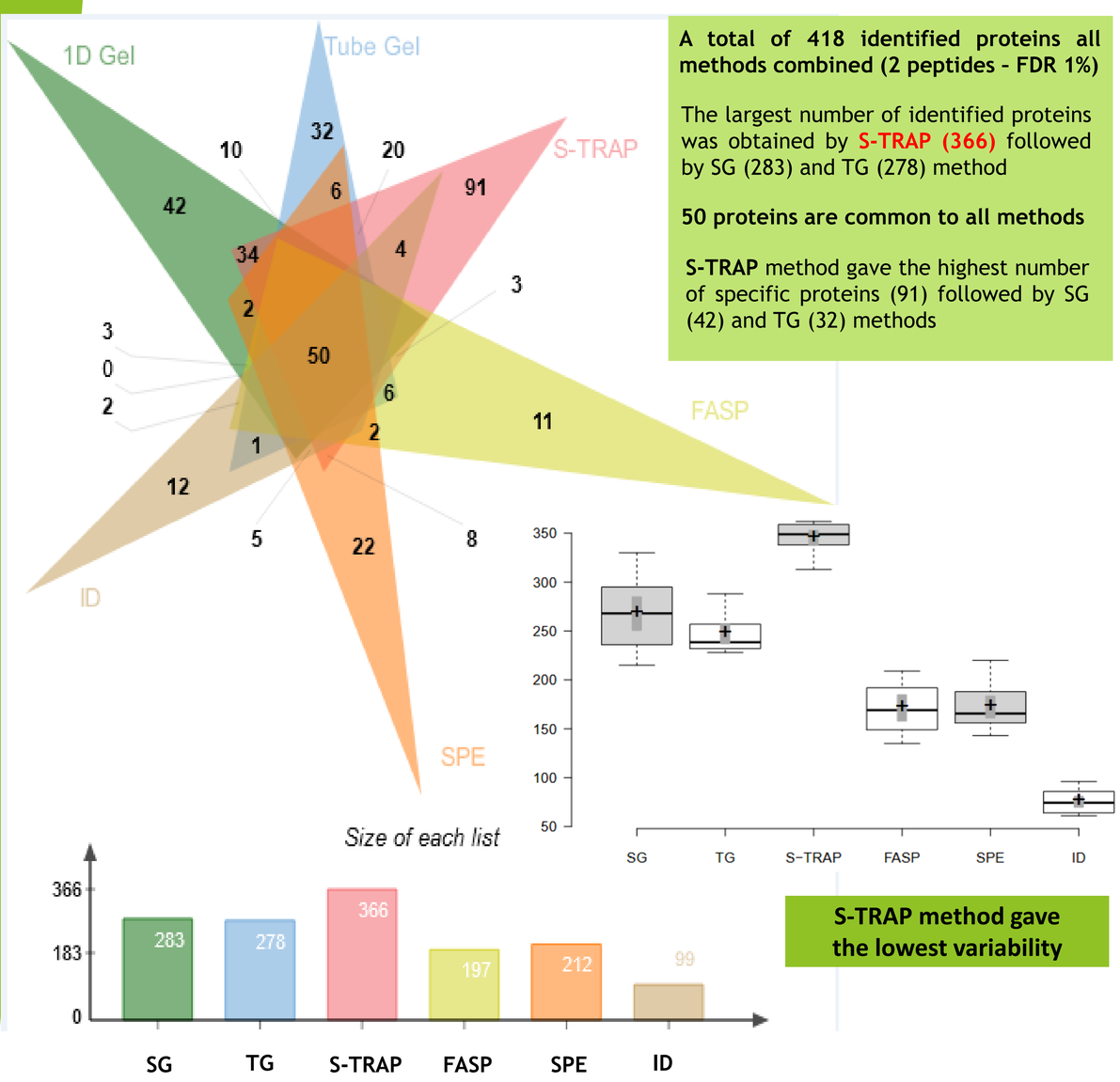
Alignment of Ionic maps

Progenesis QI for proteomics



Results

Identifications



A total of 418 identified proteins all methods combined (2 peptides - FDR 1%)

The largest number of identified proteins was obtained by **S-TRAP (366)** followed by SG (283) and TG (278) method

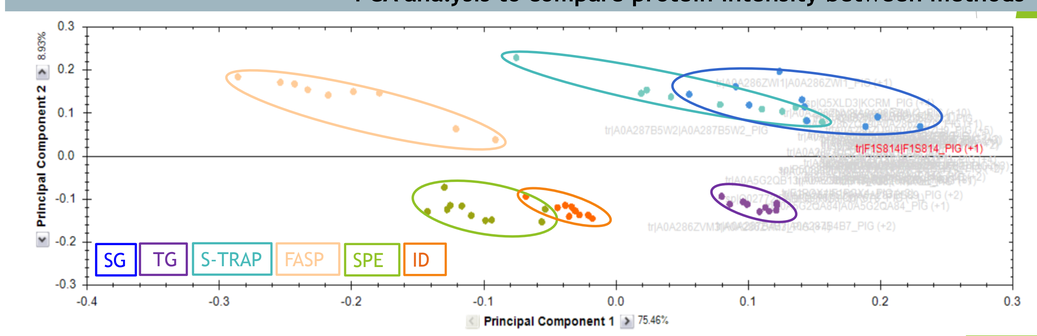
50 proteins are common to all methods

S-TRAP method gave the highest number of specific proteins (91) followed by SG (42) and TG (32) methods

S-TRAP method gave the lowest variability

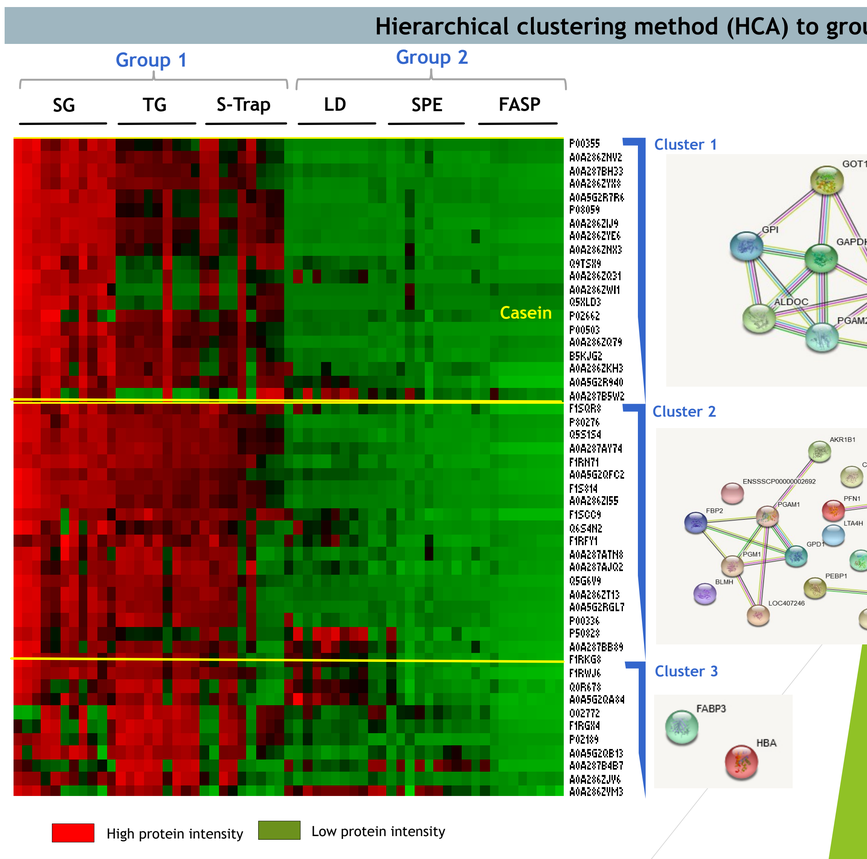
Statistical analysis : multivariate analysis of the 50 common proteins

PCA analysis to compare protein intensity between methods



Based on protein intensities considering each protein as a variable, PCA analysis revealed that all groups can be distinguished from each other. It shows similarities between SG and S-TRAP, and between LD and SPE

Hierarchical clustering method (HCA) to group methods



SG, TG and S-TRAP methods are the closest.
They allow to purify the proteins with the highest intensities.
Cluster 1 contains Glycolytic proteins

TG and S-TRAP methods are complementary to SG method
Cluster 2 includes small molecules metabolic process

Conclusion

- ➢ The originality of this study lay in the comparison of proteins identified by LC-MS/MS from the same sample by implementing several preparation methods based on different principles: gel, liquid and filtration.
- ➢ The analysis of the results by Venn diagram, principal-component analysis, hierarchical clustering and the abundance ranking of quantitative proteins highlights significant differences in identified proteins, according to the sample preparation method. Moreover, there is a specificity in the nature of extracted proteins according to the method.
- ➢ A total of 418 proteins were identified combining all the methods and the largest number of identified proteins was obtained by S-TRAP (366), followed by SG (283) ant TG (278) methods.
- ➢ Statistical results and the qualitative analyses of significant proteins indicate that S-TRAP method outperforms SG method.
- ➢ S-TRAP would purify the majority of the proteins in a sample rapidly and with the greatest intensity.
- ➢ The faster and easier S-TRAP method turns out to be the best alternative to replace classical in-gel and in-solution methods, resulting in an ultrafast sample-preparation approach for shotgun proteomics.

References : Balliau T., Blein-Nicolas M., Zivy M. - Evaluation of Optimized Tube-Gel Methods of Sample Preparation for Large-Scale Plant Proteomics - Proteomes 2018, 6, 6; doi:10.3390/proteomes6010006
Colgrave ML*,Stockwell S., Grace A., McMillan M., Davey R., Lehnert S., Schmoelzl S. Global proteomic profiling of the membrane compartment of bovine testis cell populations - JIOMICS, 3, 2, 2013, 99-111

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