Parco Scientifico e Tecnologico della Sardegna

PortoConte Ricerche

Critical comparison of sample preparation strategies for shotgun proteomic analysis of formalin-fixed, paraffin-embedded samples

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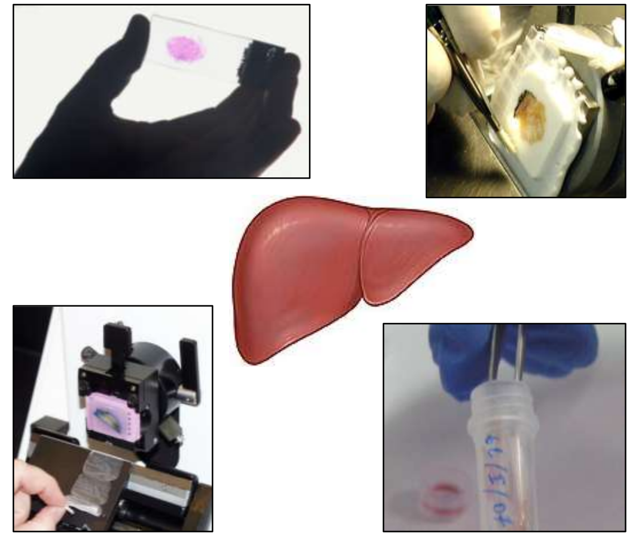
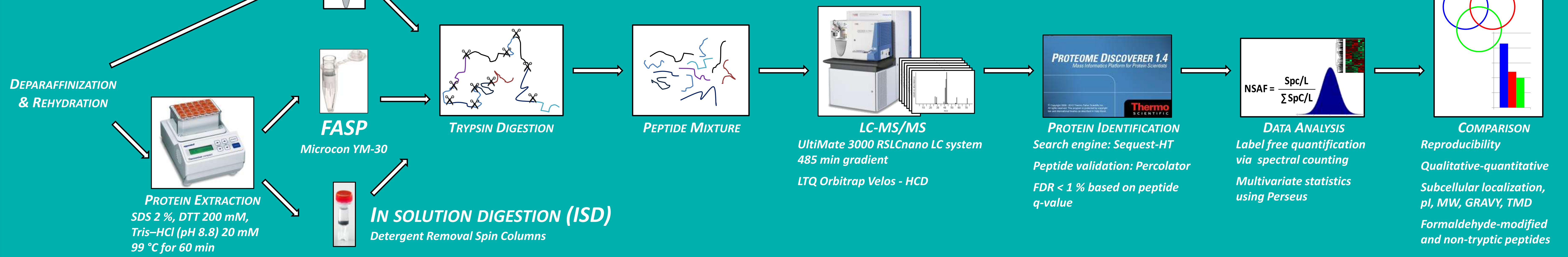


1. Introduction

The growing field of formalin-fixed paraffin-embedded (FFPE) tissue proteomics holds promise for improving translational research. Worldwide archival tissue banks hold a significant number and variety of tissue samples, as well as a wealth of retrospective information regarding diagnosis, prognosis, and response to therapy. This makes them an important resource for protein biomarker discovery and validation. Direct tissue trypsinization (DT) and protein extraction followed by in solution digestion (ISD) or filter-aided sample preparation (FASP) are the most common workflows for shotgun LC-MS/MS analysis of FFPE samples. However, there is currently no consensus on the optimal protocol, and no studies critically comparing the performance of the three different methods with FFPE specimens have been reported so far. Liver tissue was chosen as a model in consideration of its high proteome complexity in terms of expressed proteins and metabolic pathways.

2. Methods

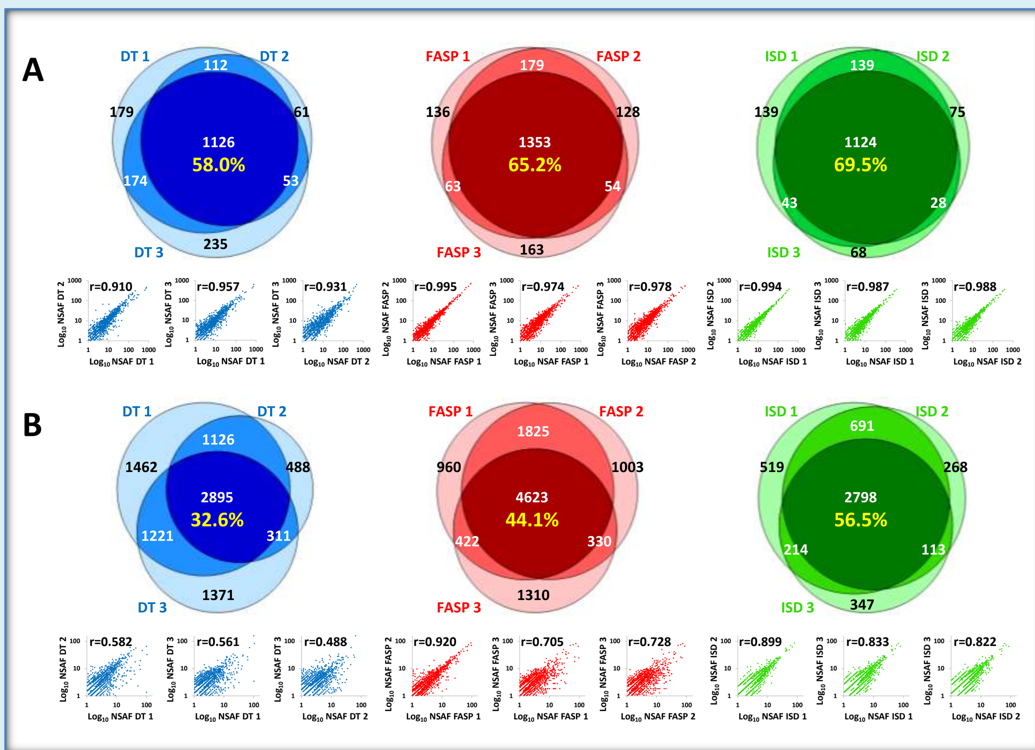
Human liver tissue
3 independent replicates per method
5 5-µm-thick slices per replicate



3. Results and Discussion

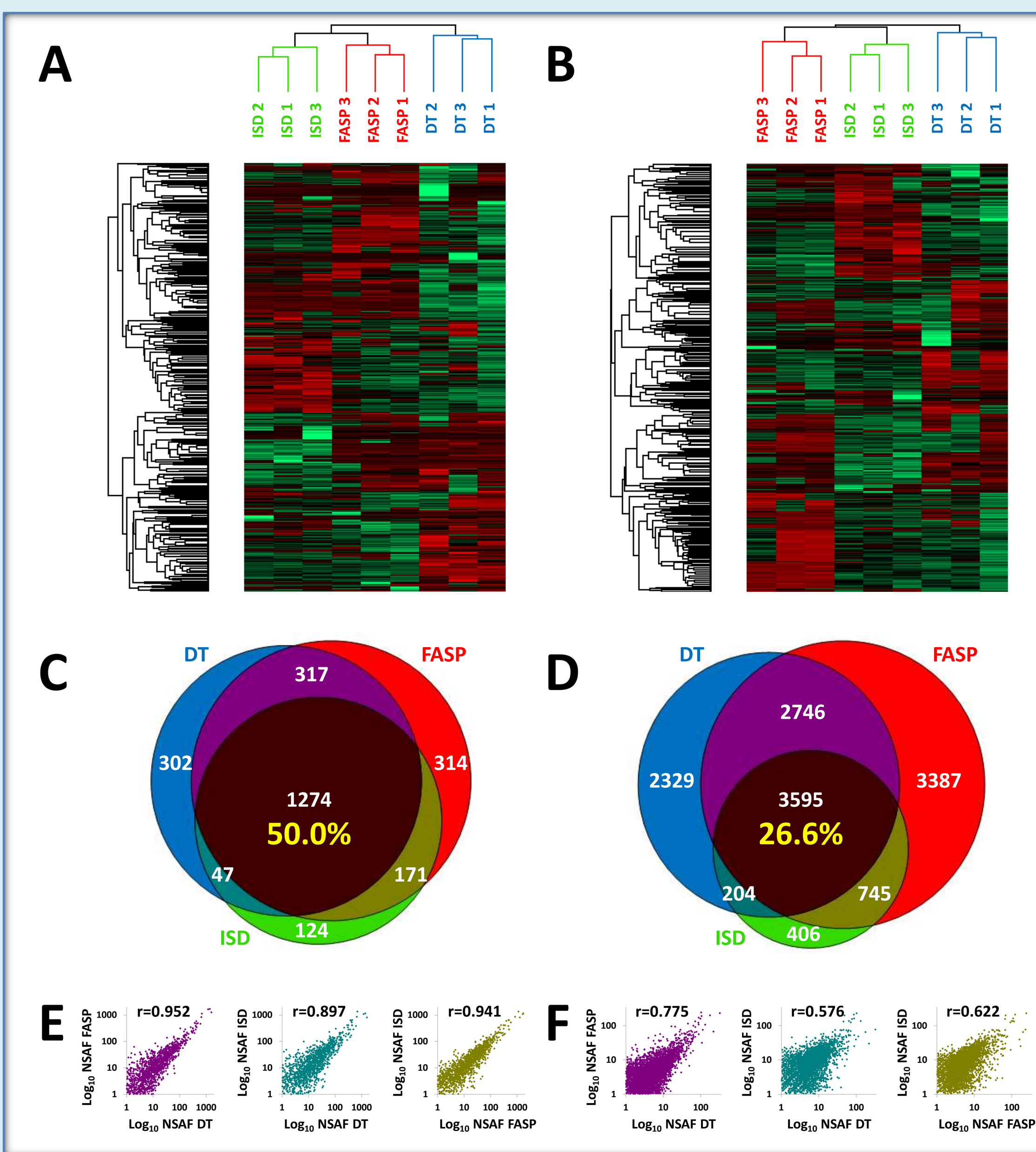
DT	• lower reproducibility • good preservation of high-MW proteins • much lower keratin contamination • higher abundance of non tryptic peptides
FASP and ISD	• depletion of high-MW proteins • enrichment in hydrophobic and membrane proteins
FASP	• higher identification yields
ISD	• higher reproducibility

3.1. Reproducibility



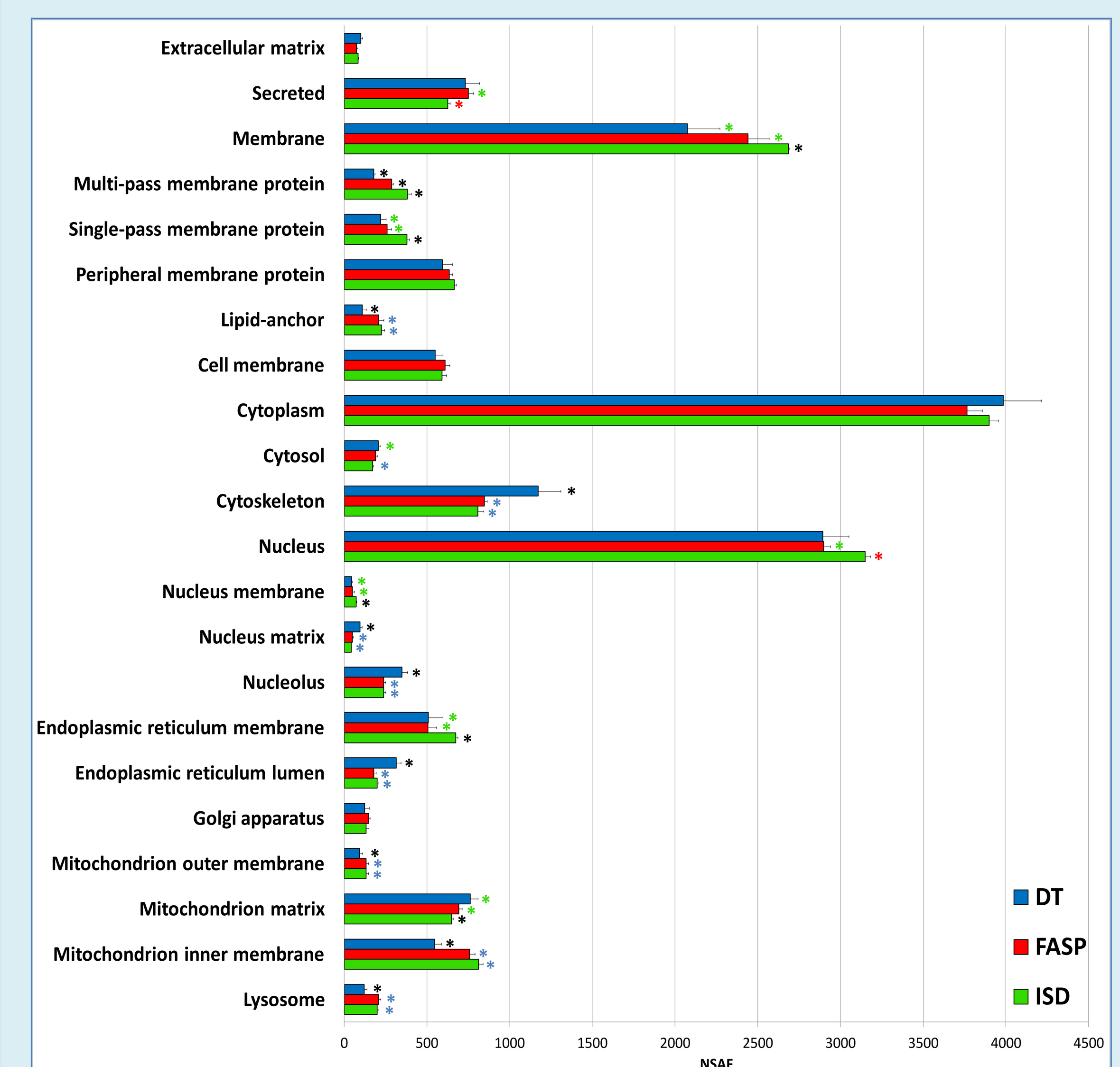
Qualitative and quantitative reproducibility of DT, FASP and ISD.
A) Top: distribution of identified proteins among replicates. Percentage of common proteins are indicated in yellow.
Bottom: correlation of protein abundance between all replicates combinations for every method. Pearson correlation coefficients are also reported.
B) Same as Panel A but at peptide level.

3.2. Qualitative and quantitative comparison



Top: Unsupervised hierarchical cluster analysis based on protein (**A**) and peptide (**B**) label-free quantitative data, respectively.
Middle: Venn diagrams illustrating distribution of all identified proteins (**C**) and peptides (**D**). Percentage of common proteins and peptides are indicated in yellow.
Bottom: Dot plots describing correlation of protein (**E**) and peptide (**F**) abundance between **DT and FASP**, **DT and ISD**, **FASP and ISD**. Pearson correlation coefficients are also reported.

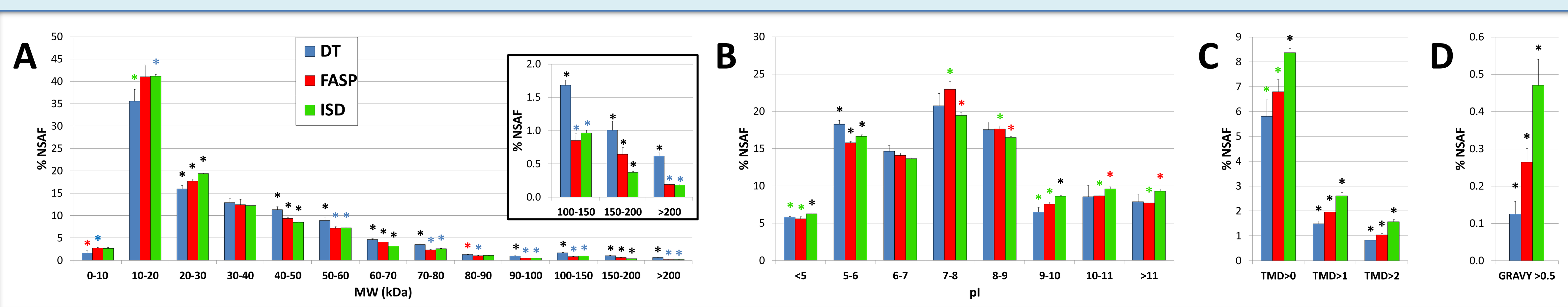
3.3. Quantitative protein distribution: Subcellular localization



Mean and SD value of NSAF percentage for three independent experimental replicates are shown. NSAF values were expressed as percentage of the annotated proteins.

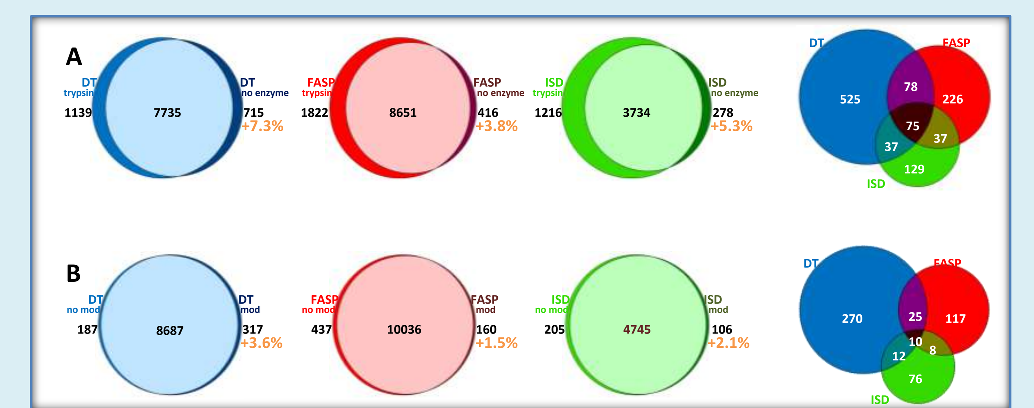
Asterisks indicate statistical significance according to Student's t-test (p value < 0.05):
* statistically significant difference versus DT
* versus FASP
* versus ISD
* versus all other methods

3.4. Quantitative protein distribution: physicochemical features



Quantitative protein distribution according to MW (**A**), pI (**B**), number of transmembrane domains (TMD, **C**) and hydrophobicity (GRAVY score, **D**). Mean and SD value of NSAF percentage for three independent experimental replicates are shown. NSAF values were expressed as percentage of all proteins.
Asterisks indicate statistical significance according to Student's t-test (p value < 0.05): * statistically significant difference versus DT, * versus FASP, * versus ISD and * versus all other methods.

3.5. Non-tryptic and formaldehyde-modified peptides



A) Left: distribution of peptides identified with 'trypsin' and 'no enzyme' searches in **DT**, **FASP** and **ISD** samples. **Right**: distribution of non-tryptic peptides among all methods.
B) Left: distribution of peptides identified with standard search ('no mod') and search comprising formaldehyde-induced modifications ('mod') in **DT**, **FASP** and **ISD** samples. **Right**: distribution of formaldehyde-modified peptides among all methods.

4. Conclusions

These results highlight that diverse sample preparation strategies provide qualitatively and quantitatively different proteomic information, and present typical biases that should be taken into account when planning a shotgun proteomic investigation dealing with FFPE samples. In view of the considerable portion of unique identifications provided by each method (particularly by DT and FASP), when a sufficient amount of tissue is available, a complementary, parallel use of different sample preparation strategies is suggested to increase proteome coverage, width and depth.

5. References

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