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Comparison of two sampling and extraction protocols to perform metabolomic studies on human adherent cells

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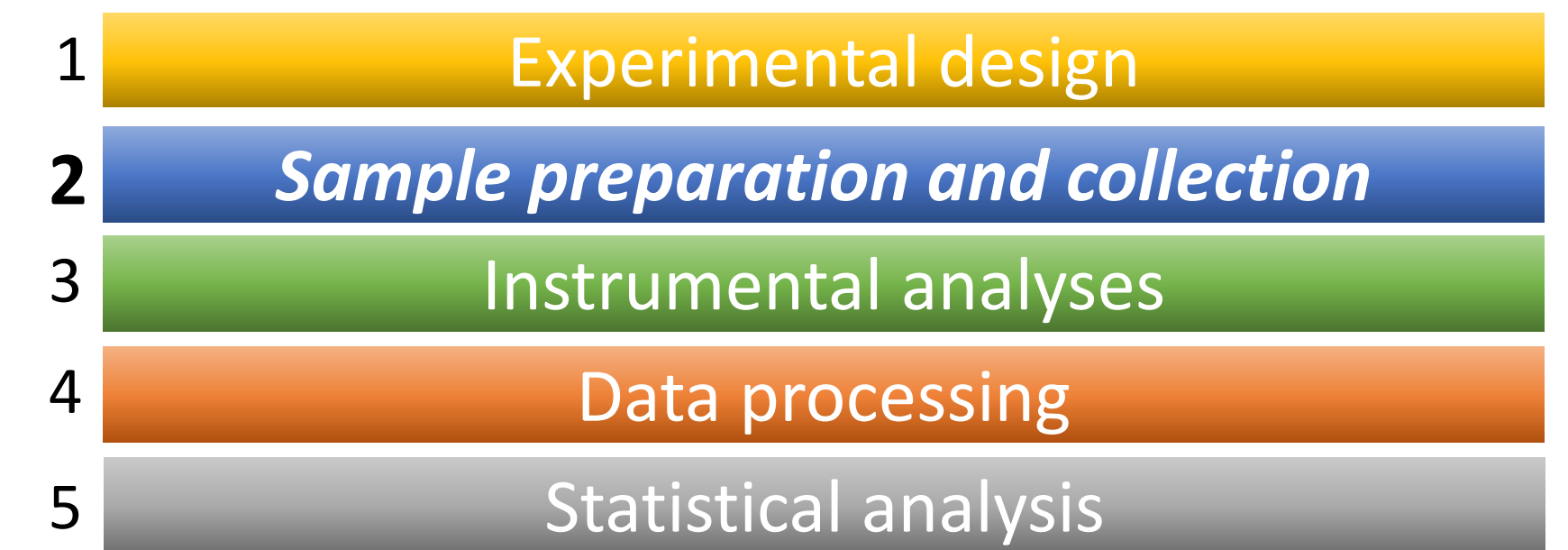
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Introduction

There are numerous ways to perform metabolomics on adherent cells [1]. The main steps for sample preparation and collection are :

- Cell Wash → must be efficient to avoid contamination by metabolites from media.
- Quenching → metabolism arrest has to be quick and reproducible to minimize unphysiological changes of metabolites (temperature change, cell stress ...)
- Cell detachment → appeared to be in favor of scrapping due to metabolites leakages with trypsination [2].
- Extraction solvent → should maximize metabolites recovery but impossible to observe the entire range of metabolites by a single extraction and analytical method [3].



Aim → Compare two metabolites sampling methods to perform targeted and untargeted metabolomics on adherent cells.

Material and methods

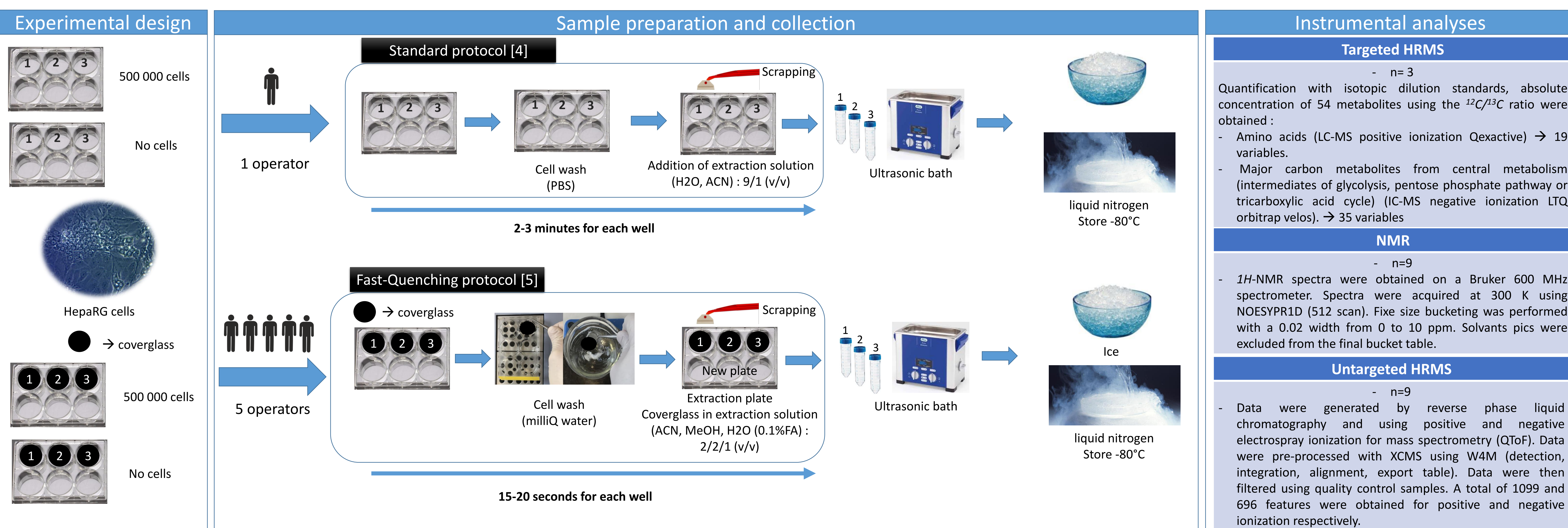


Fig. 1 Experimental design, sample collection and instrumental analyses used to compare both extraction protocols.

Results

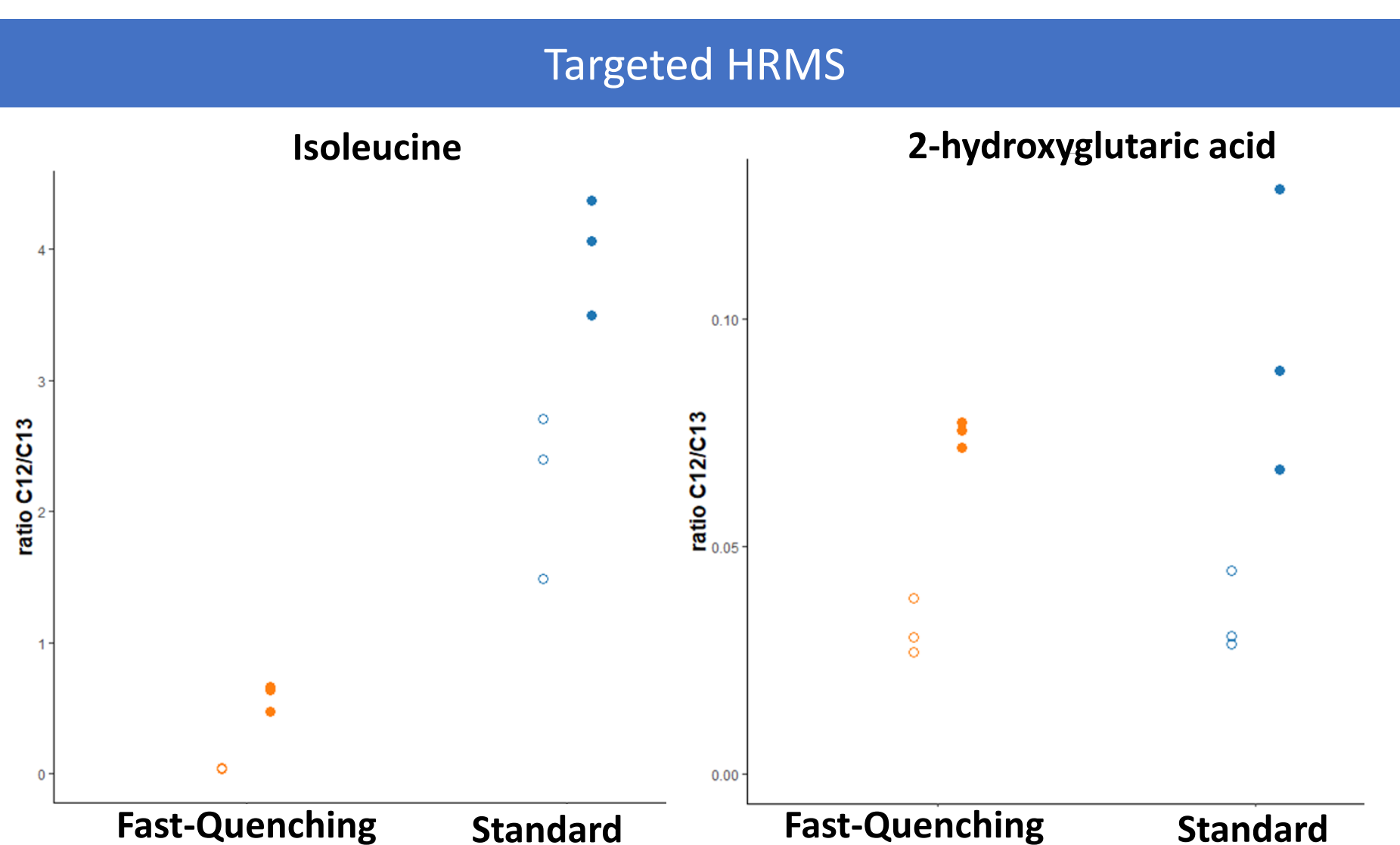


Fig. 2. Comparison of metabolites levels on blank (o) vs cell samples (•) in both protocols. ¹²C/¹³C ratio were used for these dotplots.

→ less pollution from cell culture media due to more efficient washing in fast-quenching protocol.

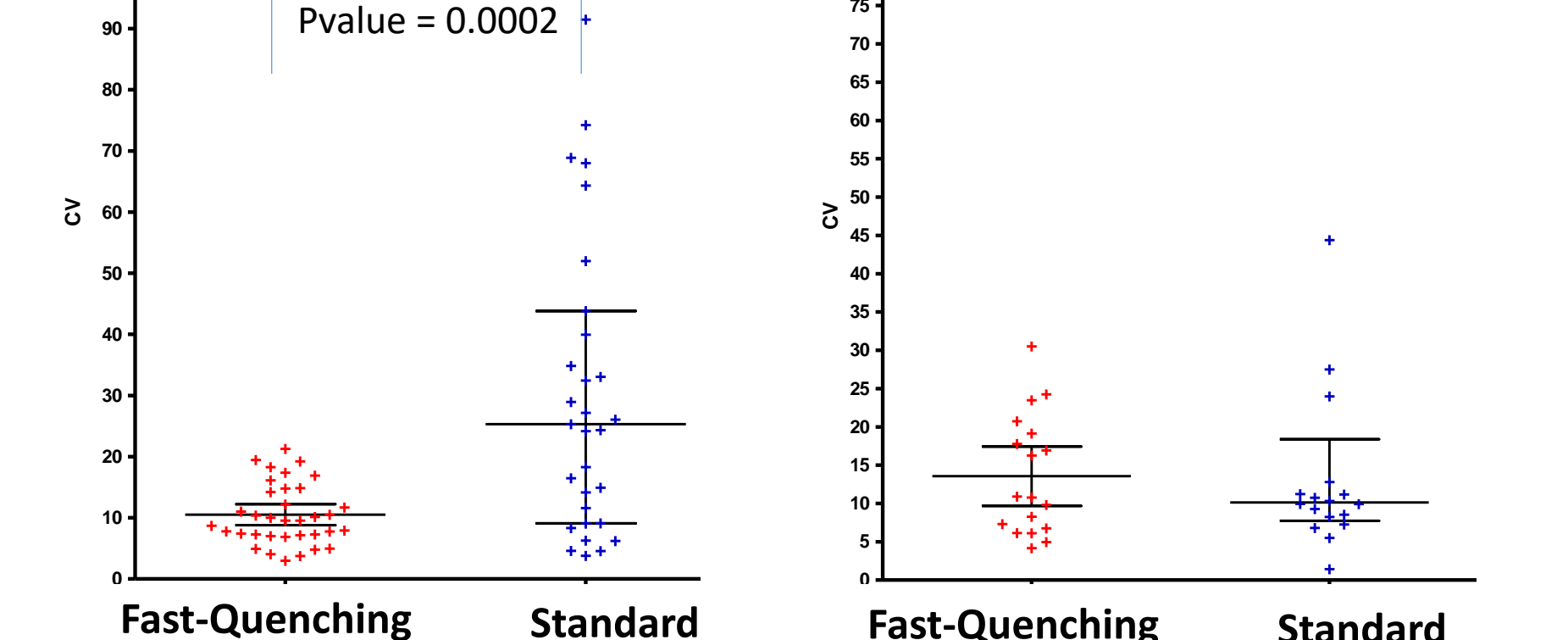


Fig. 3. Comparison of coefficients of variation obtained with absolute quantification between both protocols.

Coefficients of variation were obtained using the ¹²C/¹³C ratio for energetic metabolites (A) and for amino acids (B). Medians and interquartile range are represented for both protocols. Median were compared using a Mann & Whitney test → lower coefficient of variation for the fast-quenching protocol for energetic metabolites (pvalue=0.0002) (A). No significant differences for amino acids (B).

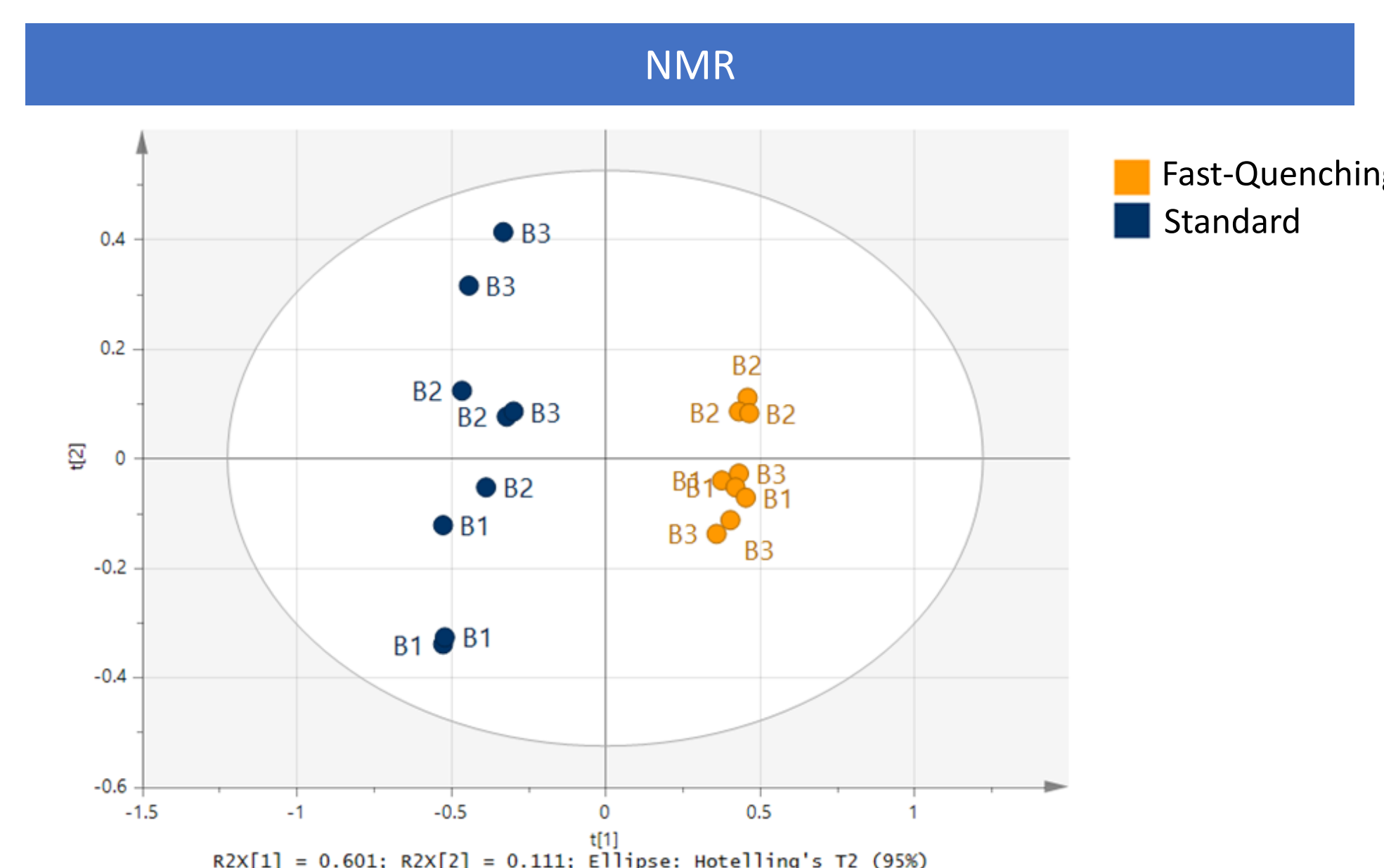


Fig. 4. PCA score plot of NMR spectra for fast-quenching and standard protocol. Score plots were obtained with a principle component analysis, each spectrum was normalized by the total integrated area and pareto scaling was performed for multivariate analyses. Biological batches are represented with the label B1/B2/B3.

→ less variability for the fast-quenching protocol.

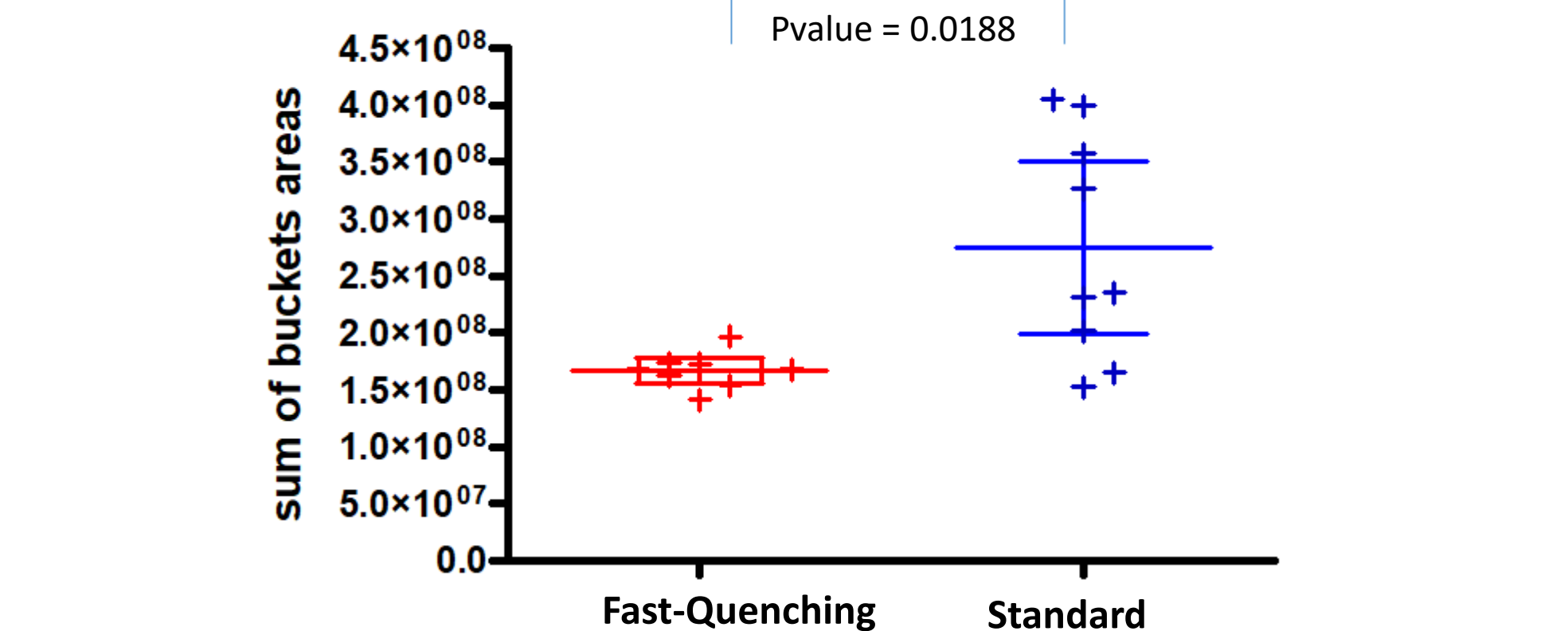


Fig. 5. Comparison of blank pollution between both protocols

Spectra were cut with a 0.02 ppm width without normalization. Bucket areas were summed for each spectra. Median were compared using a Mann & Whitney test). → less pollution in blank (pvalue = 0.0188) due to more efficient washing for the fast-quenching protocol.

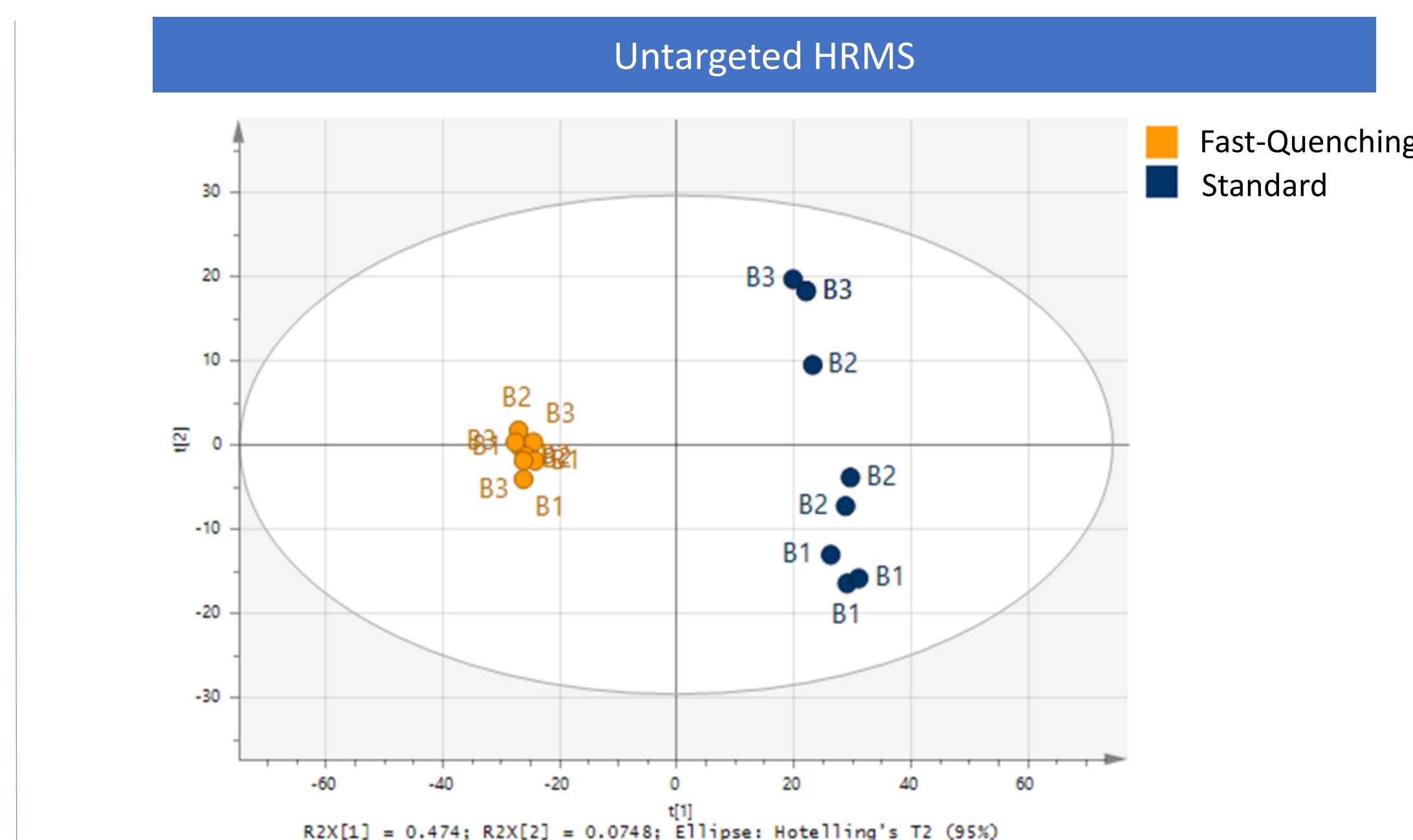


Fig. 6. PCA score plot of mass spectrometry spectra for fast-quenching and standard protocol. Score plots were obtained using a principle component analysis in negative mode of ionization. Log-pareto scaling was performed for multivariate analyses. Biological batches are represented with the label B1/B2/B3.

→ less variability for the fast-quenching protocol.

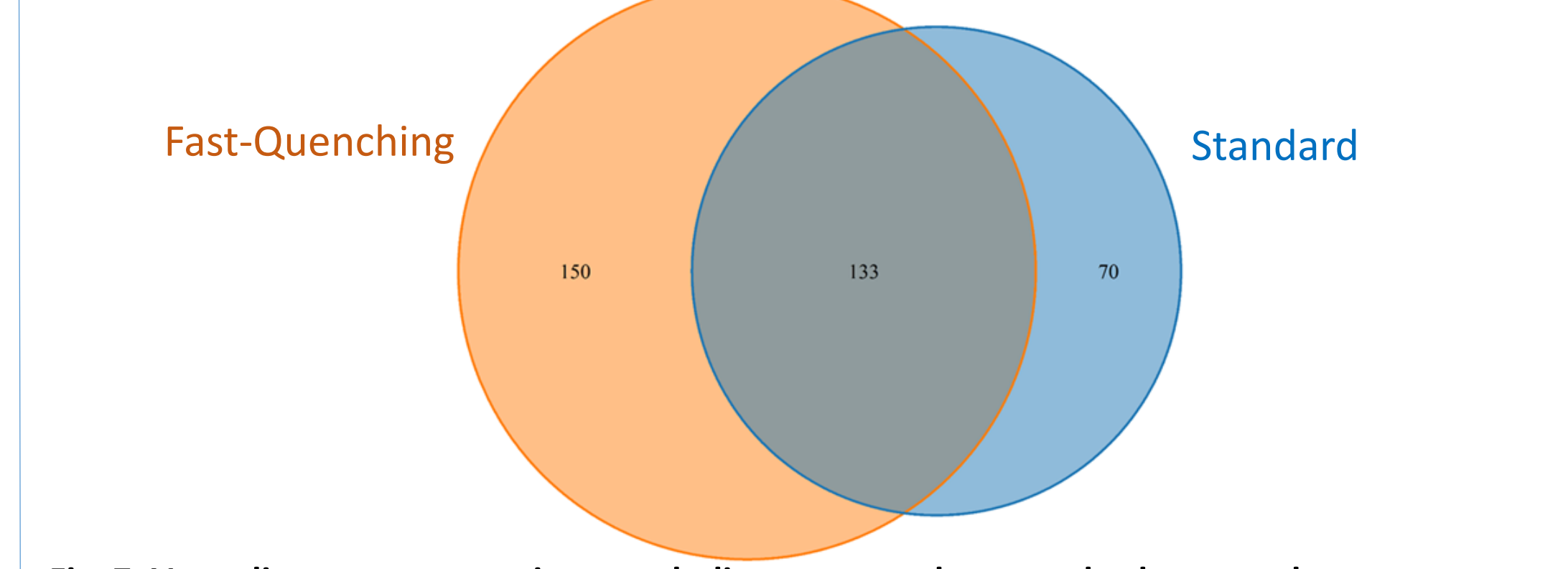


Fig. 7. Venn diagram representing metabolites recovery between both protocols

Data were obtained in negative mode of ionization. CAMERA was used to associate variables to specific metabolites adducts. → differences in metabolites extraction due to the use of different solvent between both protocols. More metabolites were obtained with the fast-quenching protocol.

Discussion and Conclusion

- Samples obtained with the fast-quenching method showed less variability than samples from the standard protocol (fig. 3, fig.4 and fig. 6). Regarding the score plots, Biological batches appear to be less important in the fast-quenching protocol. This is due to the fast stop of metabolism which limits cell stress and prevents the unphysiological transformation of metabolites. Cells' metabolites can be observed in more physiological state.
- Samples without cells appear to be less polluted with residual metabolites from media with the fast-quenching method (fig 2. and fig. 5.). This observation is very important for targeted metabolomics to prevent overestimation of metabolite concentrations and also for untargeted metabolomics during the blank filtration in order to preserve a maximum of variables. Cell culture on coverglass allows the extraction of metabolites in a new plate with a minimum of residual metabolites from medium and more efficient washing.
- Strong separation observed in PCA score plots between both protocols (fig. 4 and fig. 6) is explained by major differences in the metabolites extraction (fig. 7). These differences of metabolites recovery could be explained by the different extraction solutions used in this study. As already demonstrated in the literature, each extraction solution is linked with specific advantages and disadvantages as regards metabolites characterization, due to their different physicochemical properties [6]. However, the extraction solution can easily be adapted depending of the targeted metabolites.

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