



Introduction

- Glioblastoma multiforme (GBM) is a highly diverse tumour with metabolically distinct, heterogeneous regions.(1)
- Mapping the spatial distribution of analytes in GBM allows for the identification of molecularly homogeneous tumour regions.

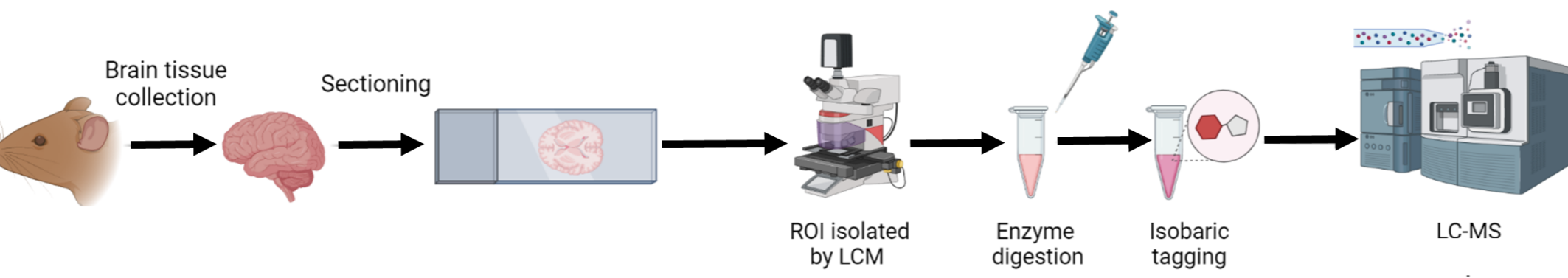
Research Focus:

- Identify proteomic markers associated with GBM heterogeneity and metabolic behaviour.
- Explore molecular signalling pathways, particularly in response to metabolic therapies.

Study Aims:

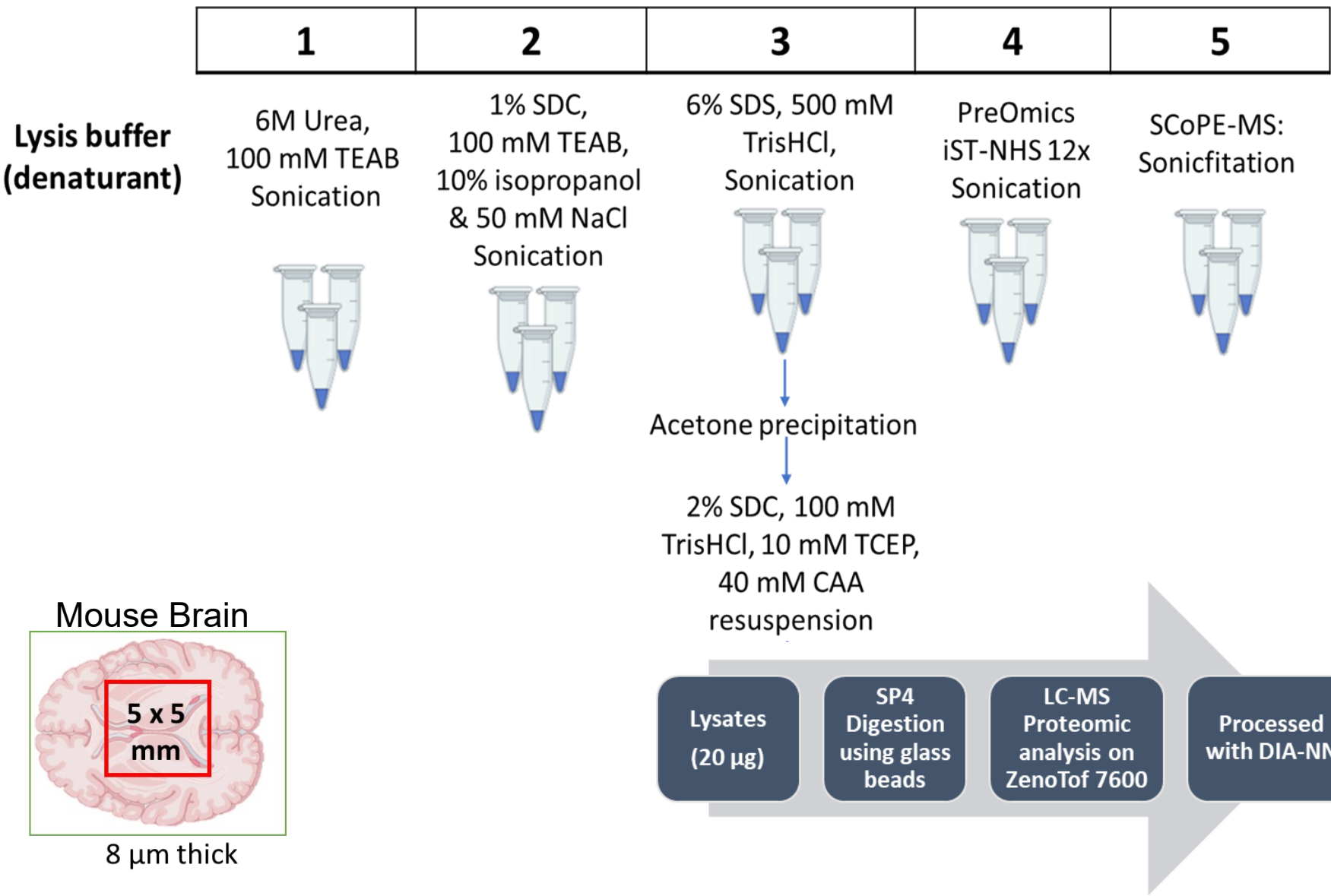
- Evaluate the efficiency of protein extraction across lysis methods
- Determine the minimum sample size for iTRAQ proteomic analysis
- Evaluate the destruction of proteins by DESI imaging
- Combine technologies to create a workflow incorporating DESI and LC-MS.

Overall Workflow:

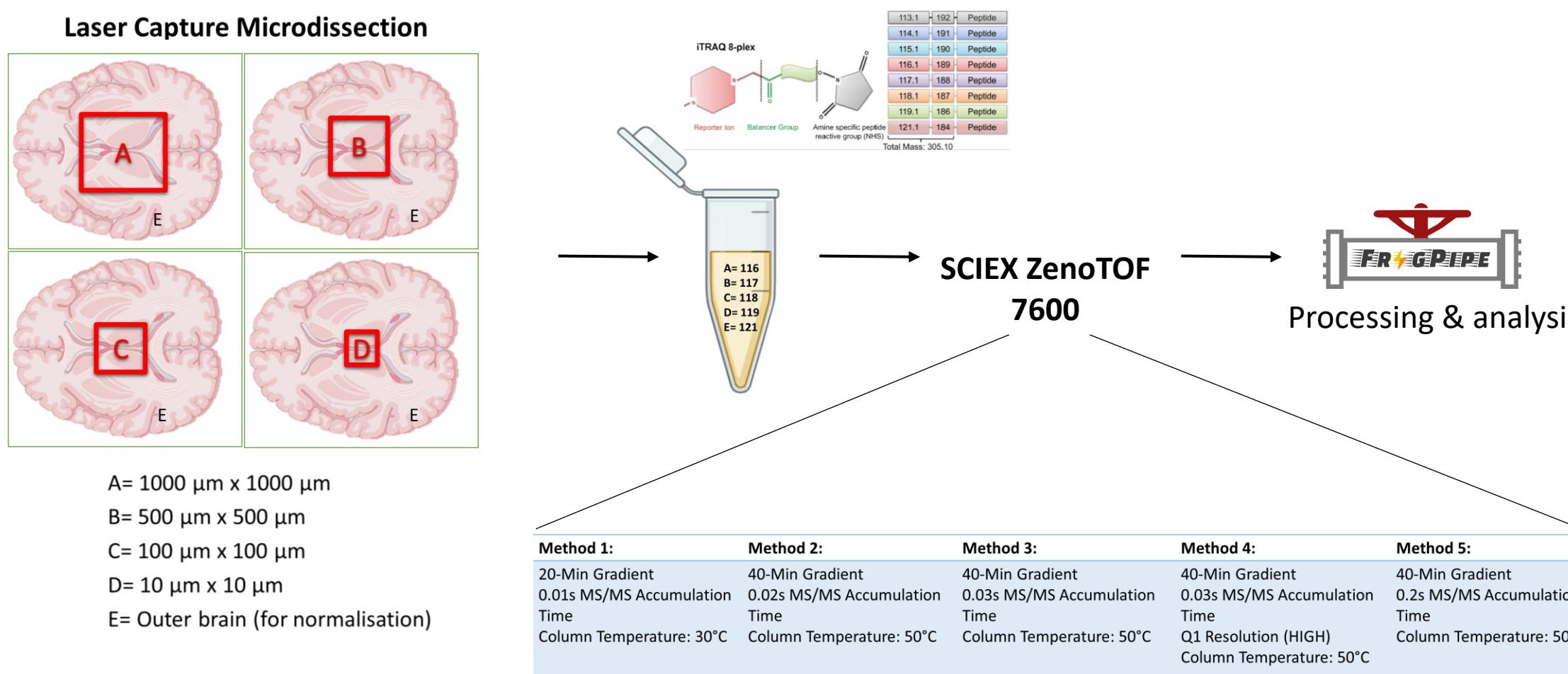


Methods

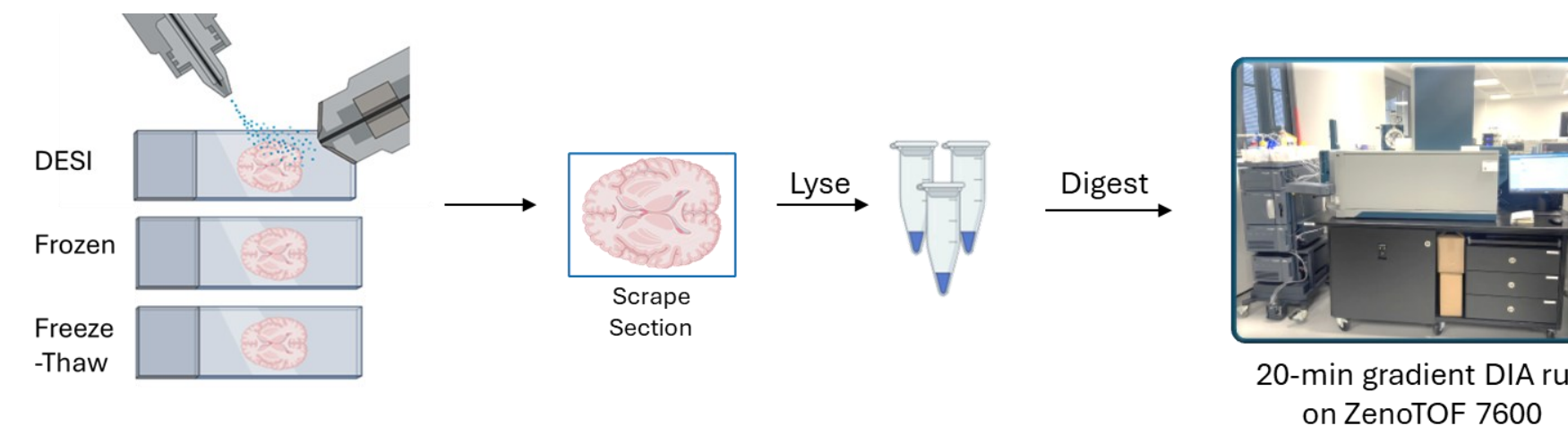
1. Protein Extraction Optimisation



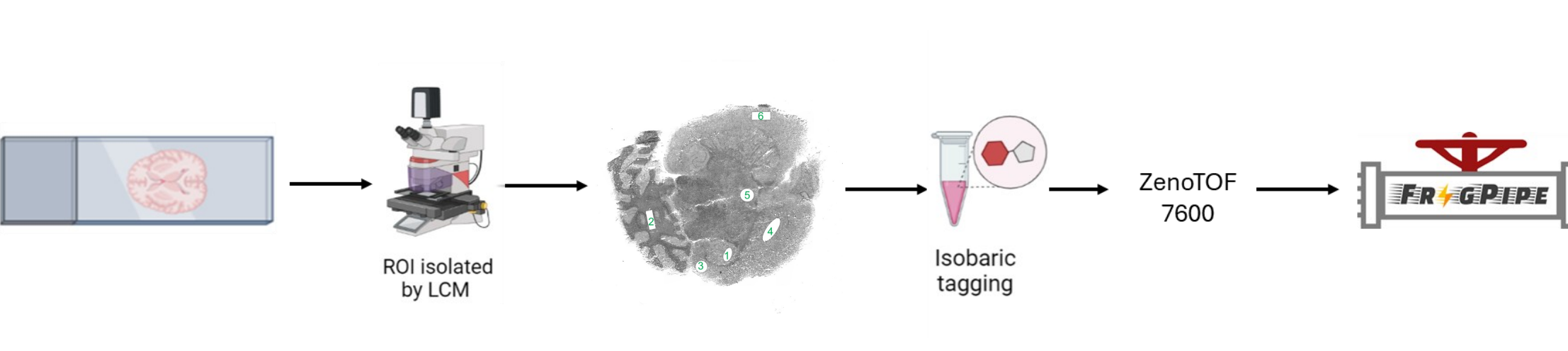
2. Minimum Tissue Size Optimisation



3. Effects of DESI on Proteomics Optimisation

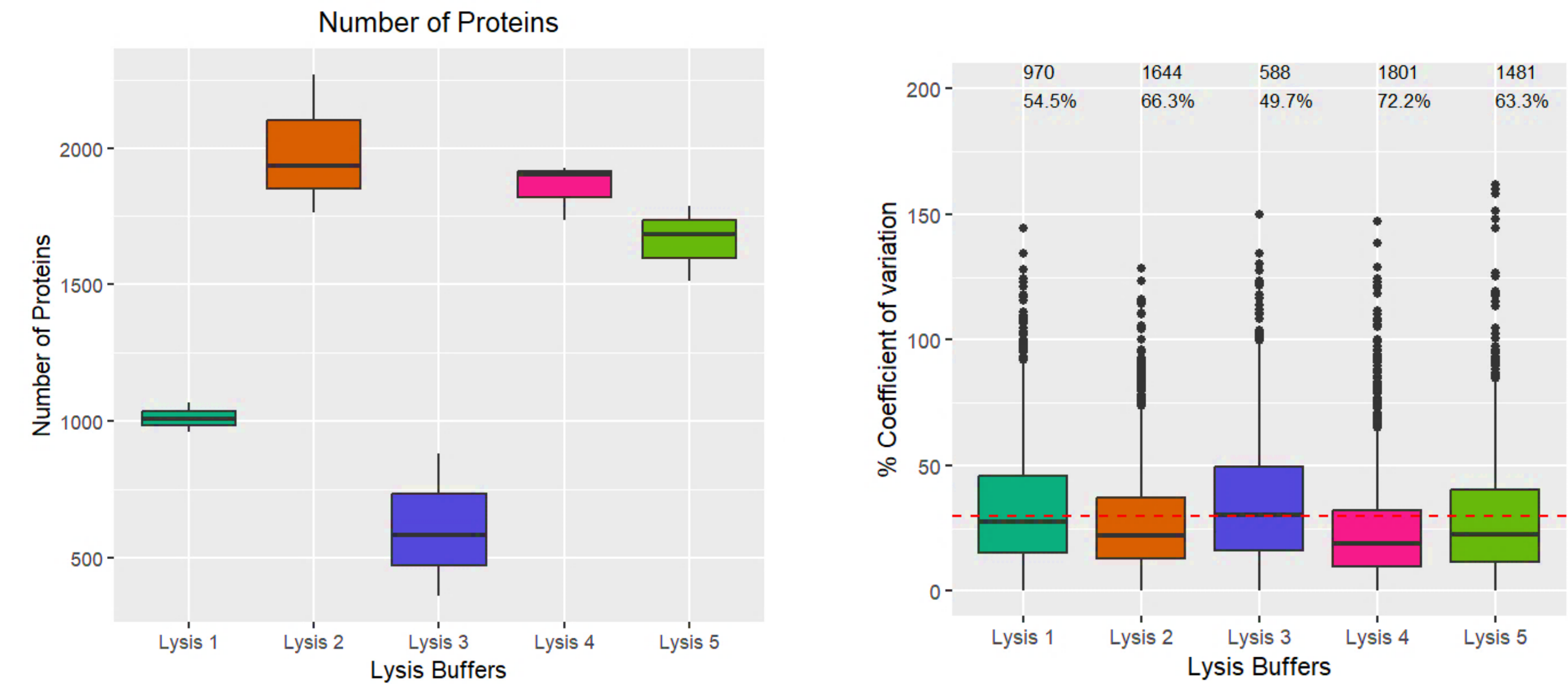


4. Regions of Interest Proteomics Optimisation

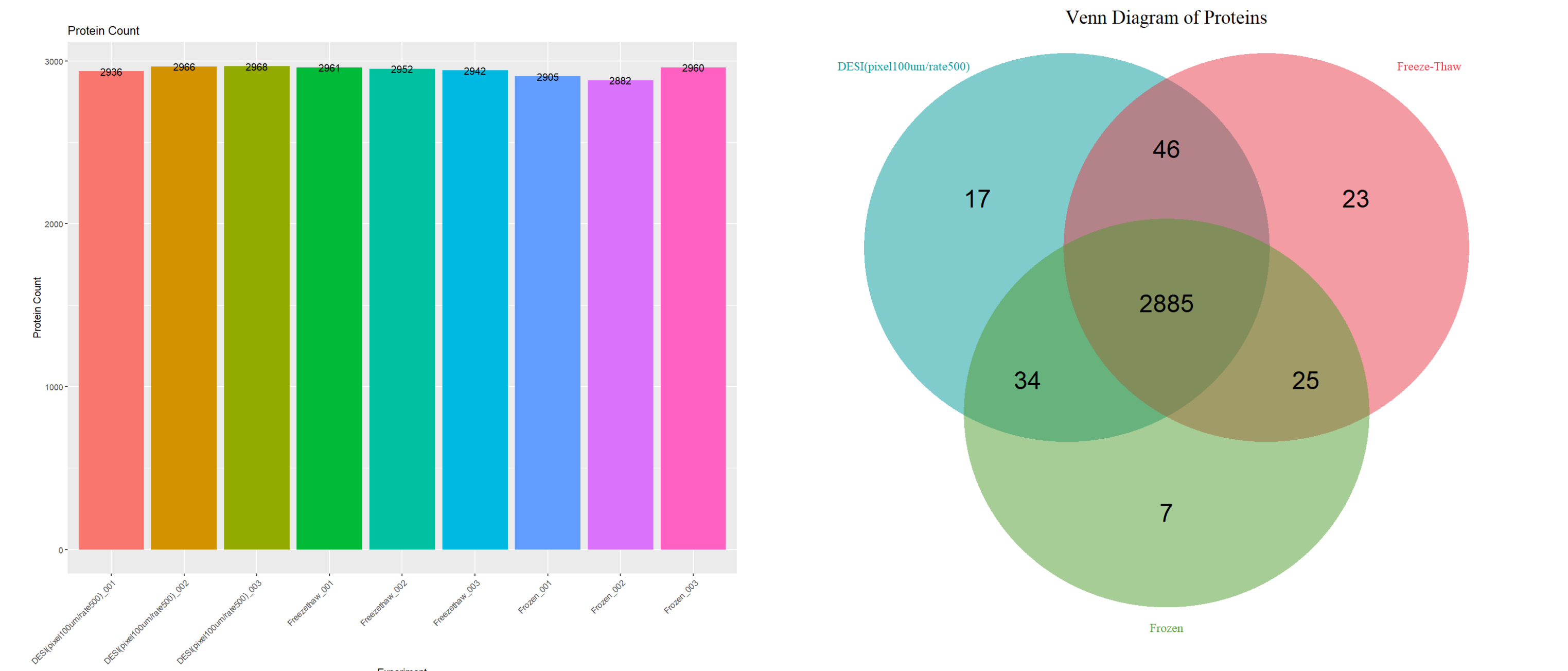


Results

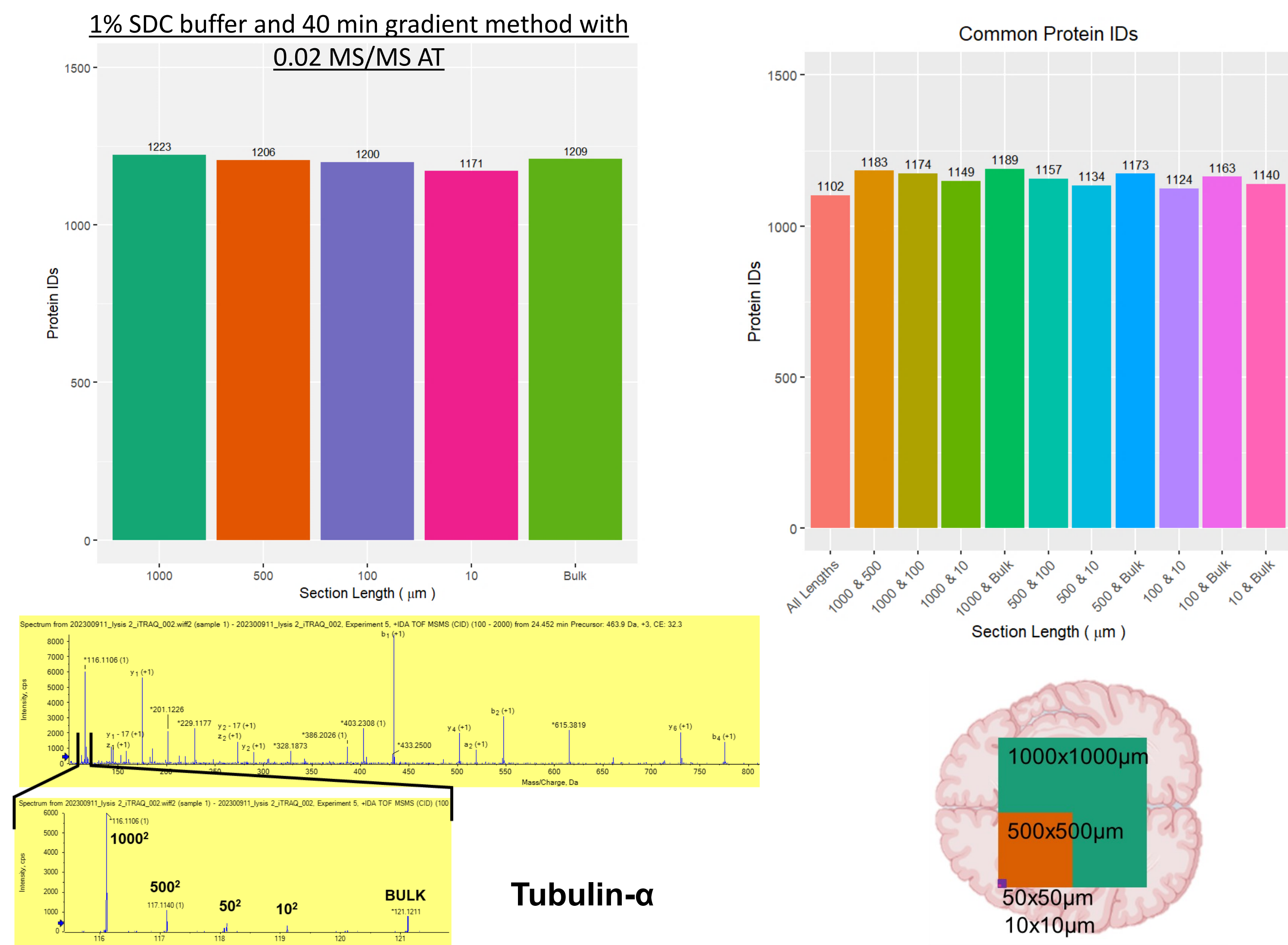
1. Protein Extraction



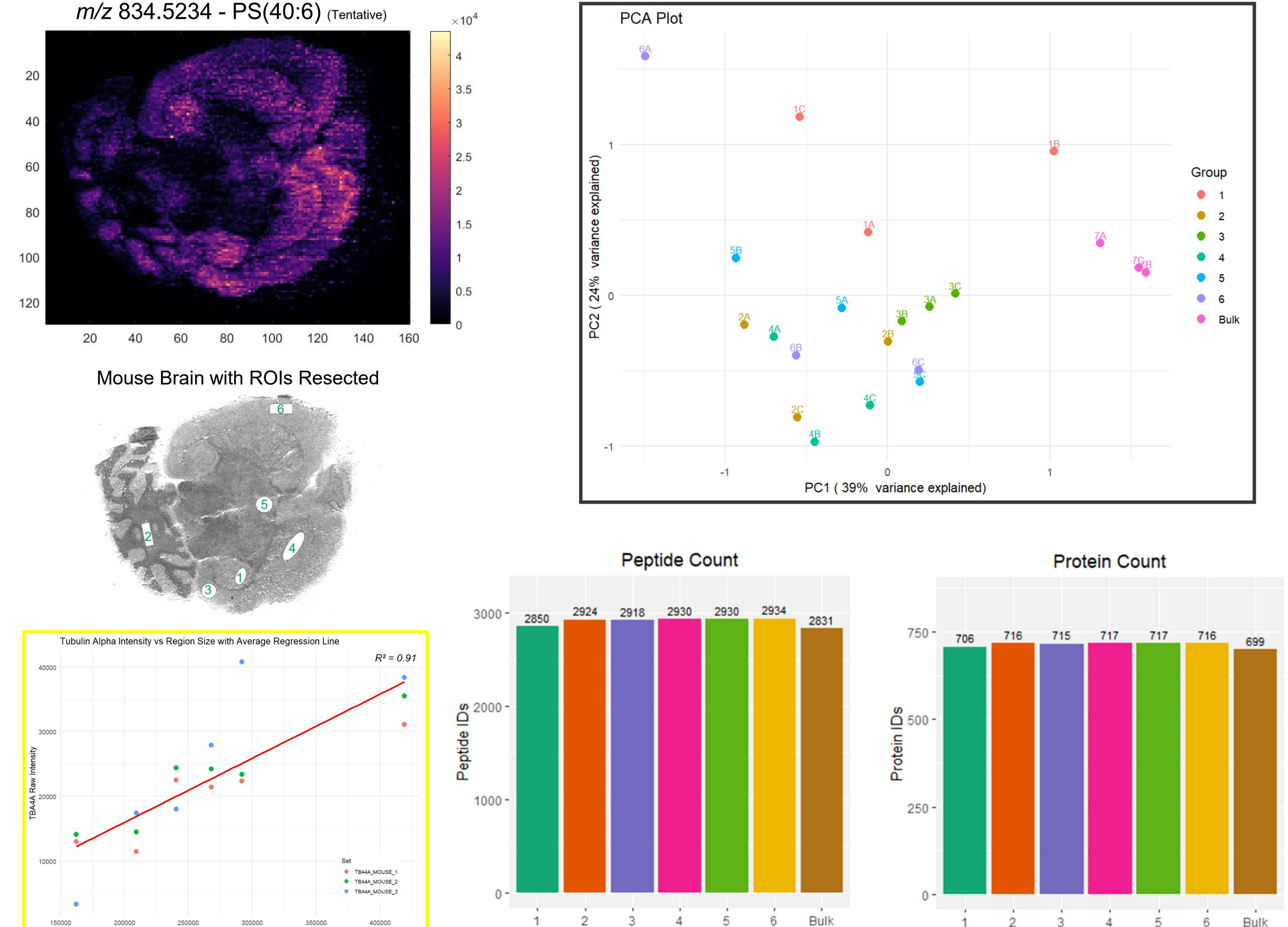
3. DESI Spray Effects on Proteins



2. Minimum Tissue Needed for Protein Tagging and LC-MS/MS



4. ROIs From DESI for Proteomics



Conclusion

- 1% sodium deoxycholate (SDC) was the optimal lysis buffer for efficient protein extraction in moles brain
- Peptide identification was maximised with a 40-minute gradient, 0.02s MS/MS AT, and 50°C column temperature.
- This combination gives the best results for analysing 10 µm x 10 µm (single cell) areas in mouse brain, helping to facilitate the identification of molecular signalling pathways associated with GBM in future work.
- DESI-MS is a soft ionisation technique used for MS1 analysis, with minimal impact on protein extraction.
- DESI-MS can be used to guide ROI selection, and regions can be precisely cut using LCM for targeted proteomics using iTRAQ

Future work

- Implement the optimised methods on GBM models.
- Enhance protein identification by leveraging nanoflow LC-MS to further increase sensitivity.

Reference

- Randall EC, Lopez BGC, Peng S, Regan MS, Abdelmoula WM, Basu SS, et al. Localized Metabolomic Gradients in Patient-Derived Xenograft Models of Glioblastoma. Cancer research. 2020;80(6):1258-67.