Fast, High-Yield, and Universal Proteomics Sample Preparation Using the Reversible Protein Tag, ProMTag

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Abstract

High-quality protein extraction and cleanup during sample preparation are critical for achieving the coverage, yields, and reproducibility required for a successful proteomics experiment. Many current technologies for proteomics sample preparation rely on precipitation or filtration-based technologies, which suffer from sample loss, long processing times, incompatibility with certain lysis buffers, and limitations for automation.

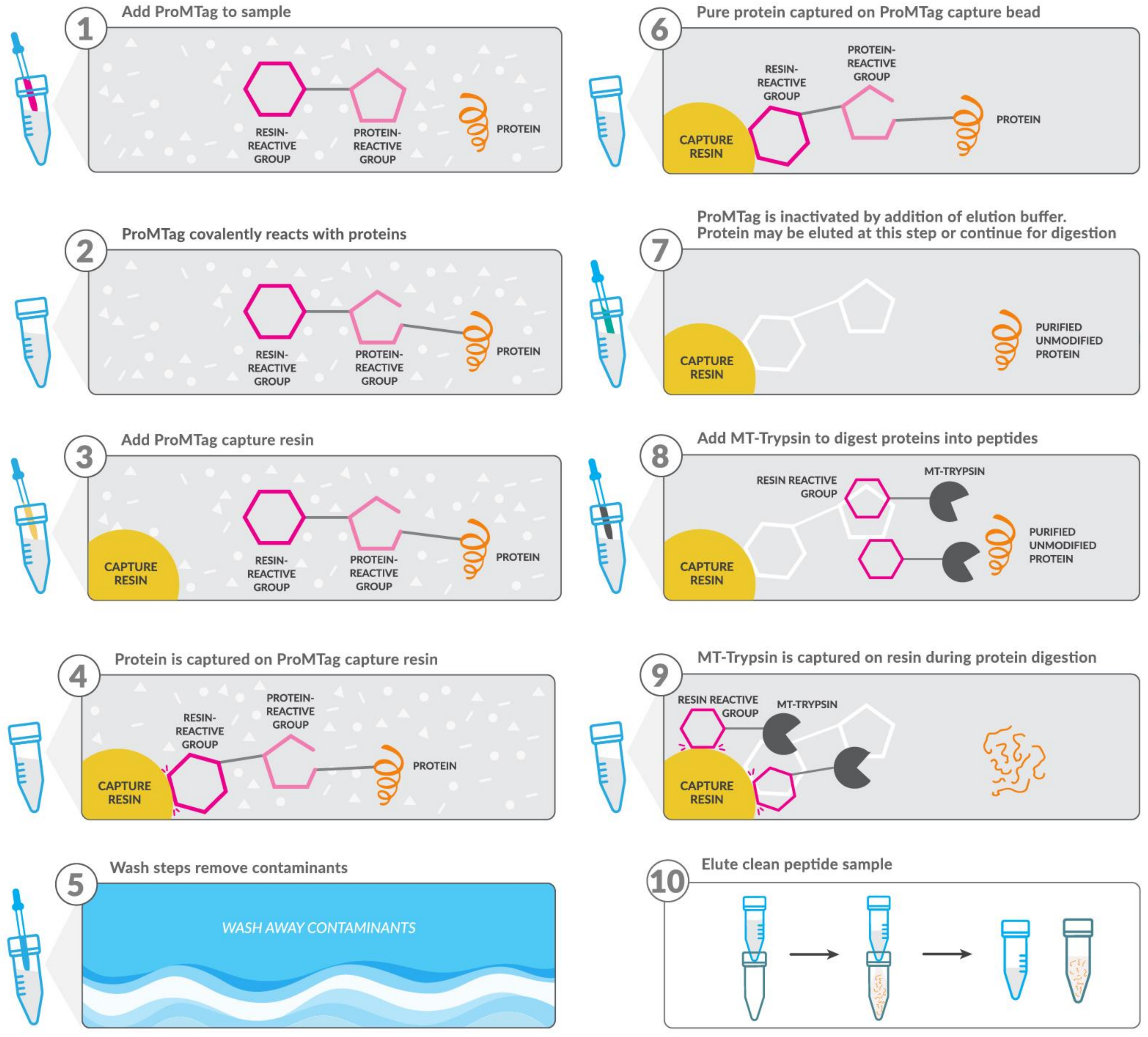
At Impact Proteomics, our Universal Protein Extraction and Cleanup Kits (UPECK) have overcome these limitations. Our novel protein sample preparation method utilizes a protein-specific tag (ProMTag) where one end of the tag forms a reversible, covalent link to proteins, and the other end irreversibly anchors the protein to a capture resin using a fast-acting, click chemistry reaction. The ProMTag reaction is not affected by high detergent, denaturant, or salt conditions accommodating harsh cell lysis conditions. Once ProMTagged proteins are covalently linked to the capture resin, contaminating salts, detergents, and other molecules that interfere with MS analysis are removed by rapid washing steps. Following washing, the ProMTag is reversed, releasing the protein in its original, unmodified form. To yield peptides ready for MS analysis, modified MT-Trypsin, which also binds to the capture resin, is added and digestion is complete in one hour.

The UPECK workflow ensures fast, high-yield, and streamlined sample preparation that yields highly reproducible results and requires about four hours to complete with less than an hour of hands-on time.

Here, we demonstrate the compatibility of the UPECK Peptide workflow with various sample types, including tissues from brain to muscle, and bodily fluids, all using a universal lysis and solubilization buffer.

ProMTag UPECK-Pep ensures streamlined sample preparation

- ❑ A reversible, covalent link is formed by one end of the ProMTag, and an irreversible anchor is formed by the other end via a fast-acting click chemistry reaction.
- ❑ Rapid washing steps are used to remove contaminating salts, detergents, and other molecules that would interfere with MS analysis.
- ❑ The ProMTag is reversed after washing, allowing the protein to return to its original state.
- ❑ A modified MT-Trypsin, which also binds to the capture resin, is added to yield peptides ready for MS analysis.



1 Add ProMTag to sample

2 ProMTag covalently reacts with proteins

3 Add ProMTag capture resin

4 Protein is captured on ProMTag capture resin

5 Wash steps remove contaminants

6 Pure protein captured on ProMTag capture bead

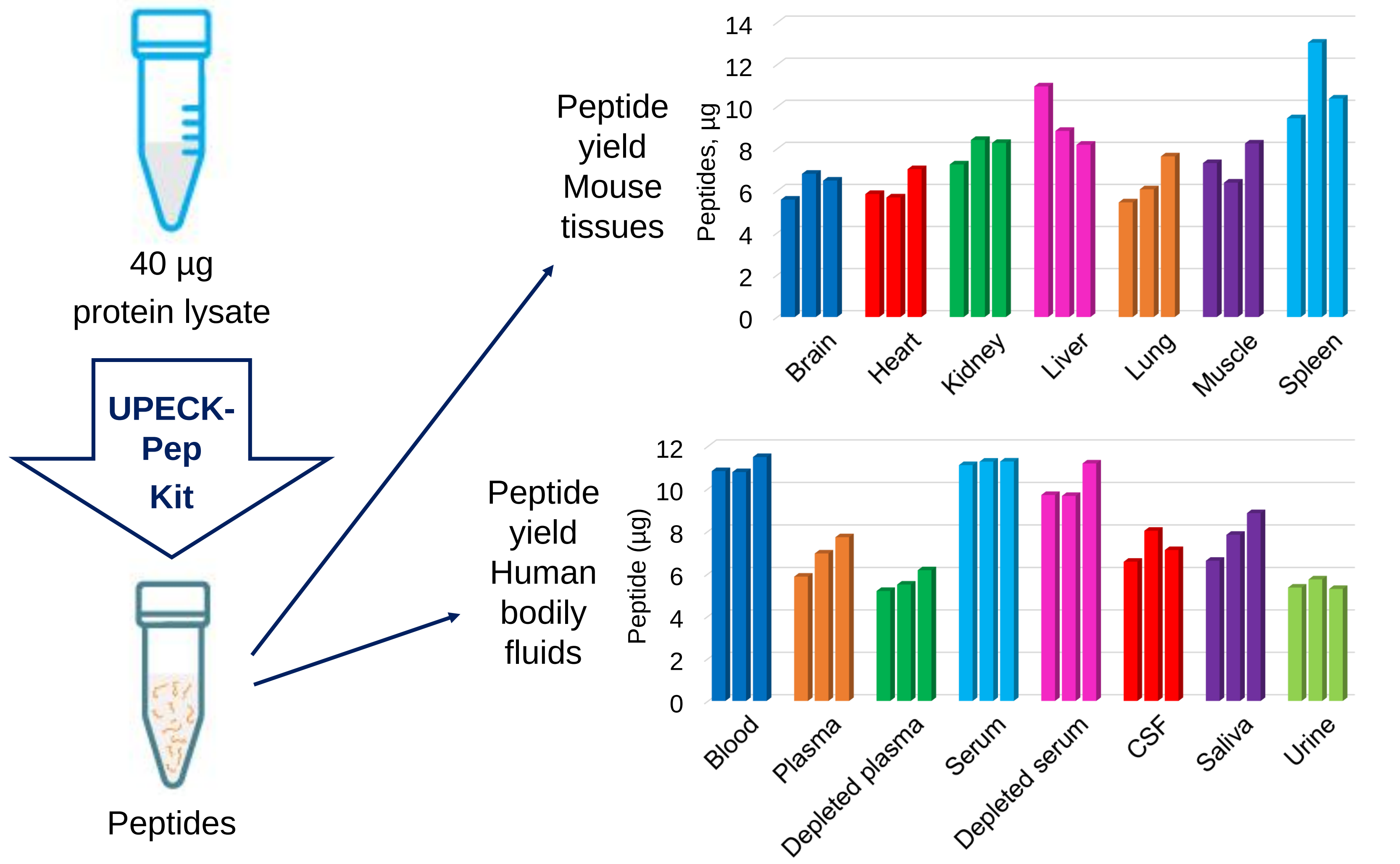
7 ProMTag is inactivated by addition of elution buffer. Protein may be eluted at this step or continue for digestion

8 Add MT-Trypsin to digest proteins into peptides

9 MT-Trypsin is captured on resin during protein digestion

10 Elute clean peptide sample

Peptides were prepared in triplicate via ProMTag UPECK-Pep from a variety of samples



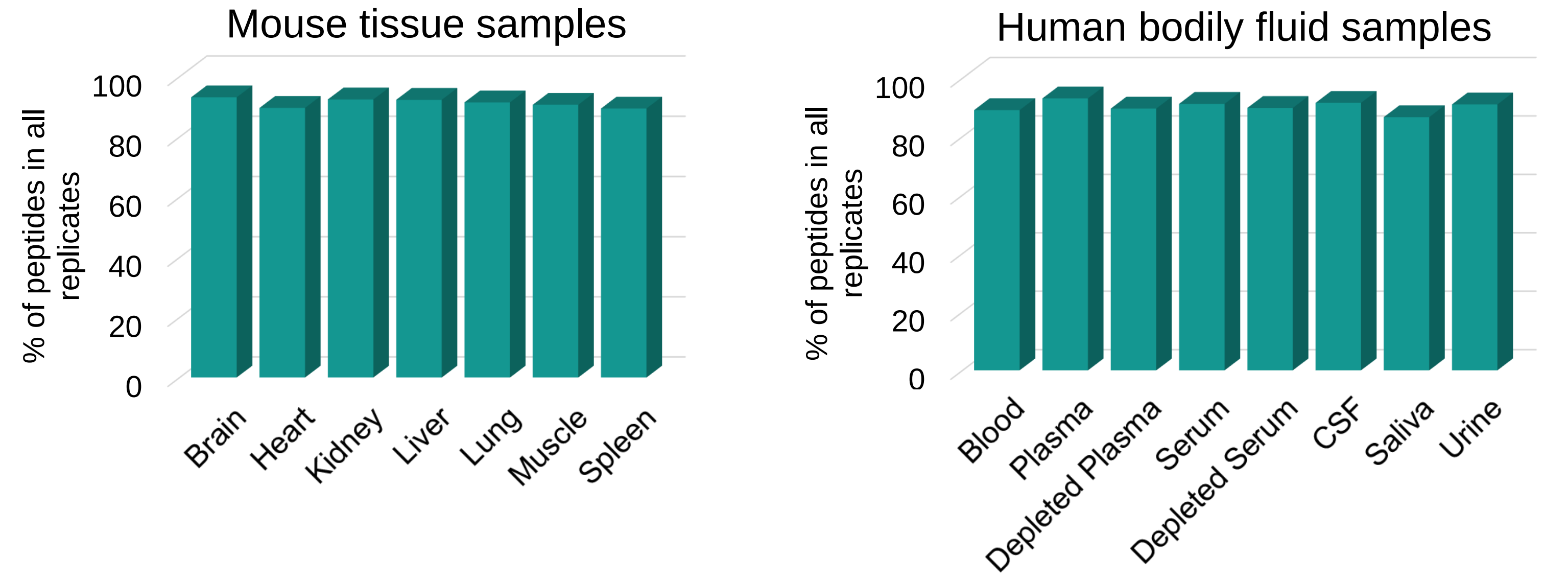
40 µg protein lysate

UPECK-Pep Kit

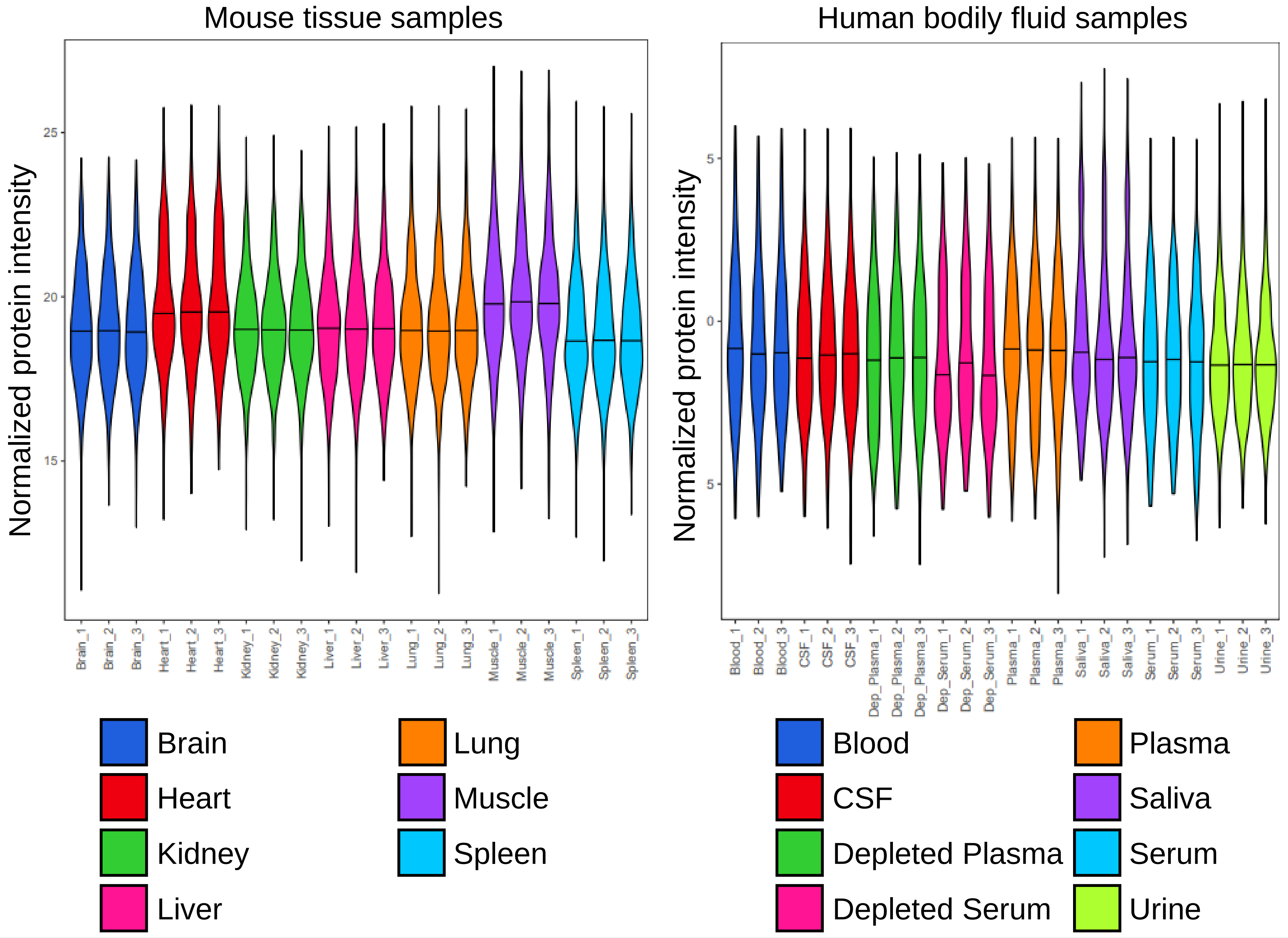
Peptides

Triplicate samples prepared with ProMTag UPECK-Pep showed high reproducibility

Technical replicates share a high percentage of peptide IDs

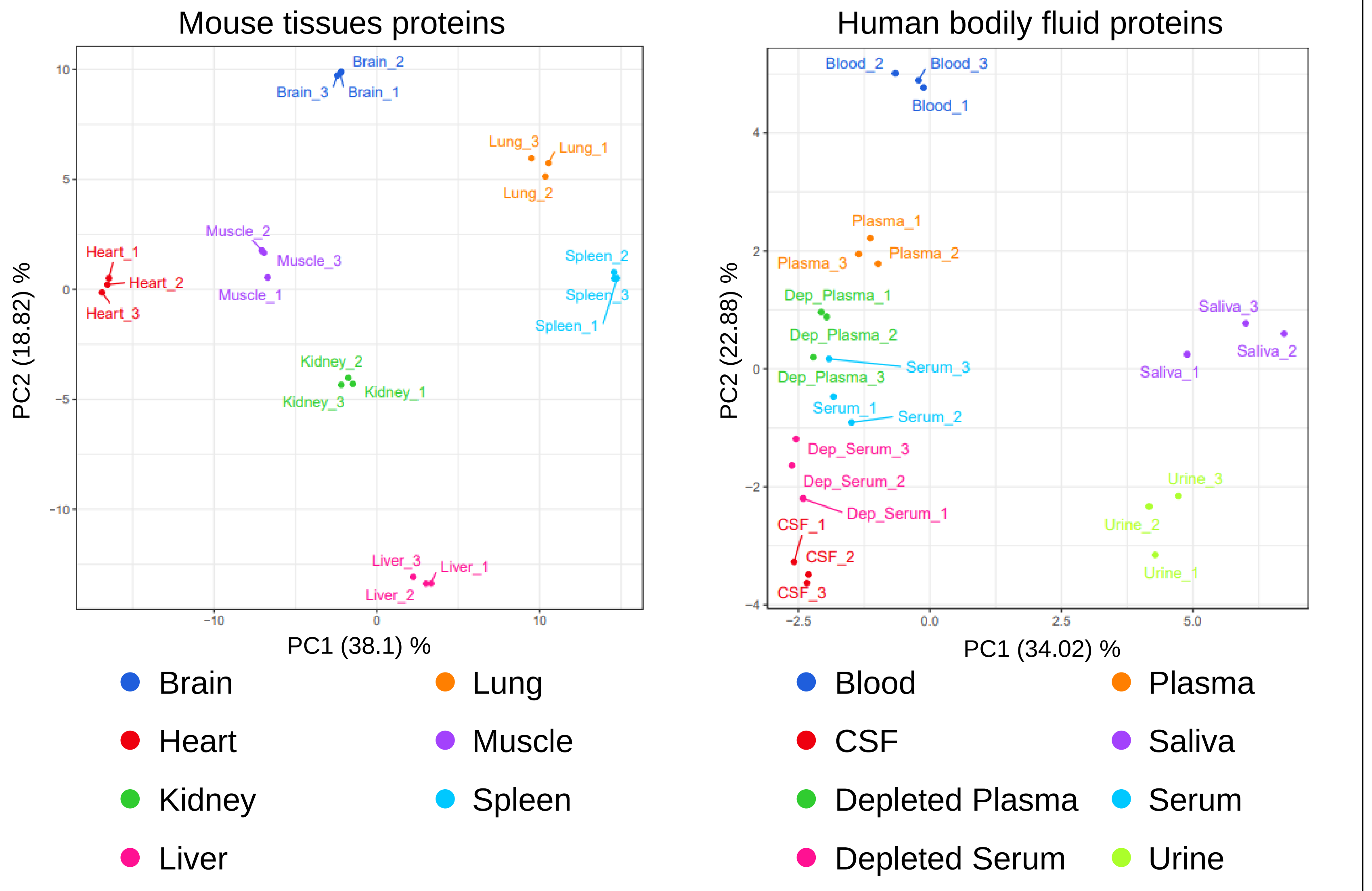


Analysis confirms high consistency of sample replicates

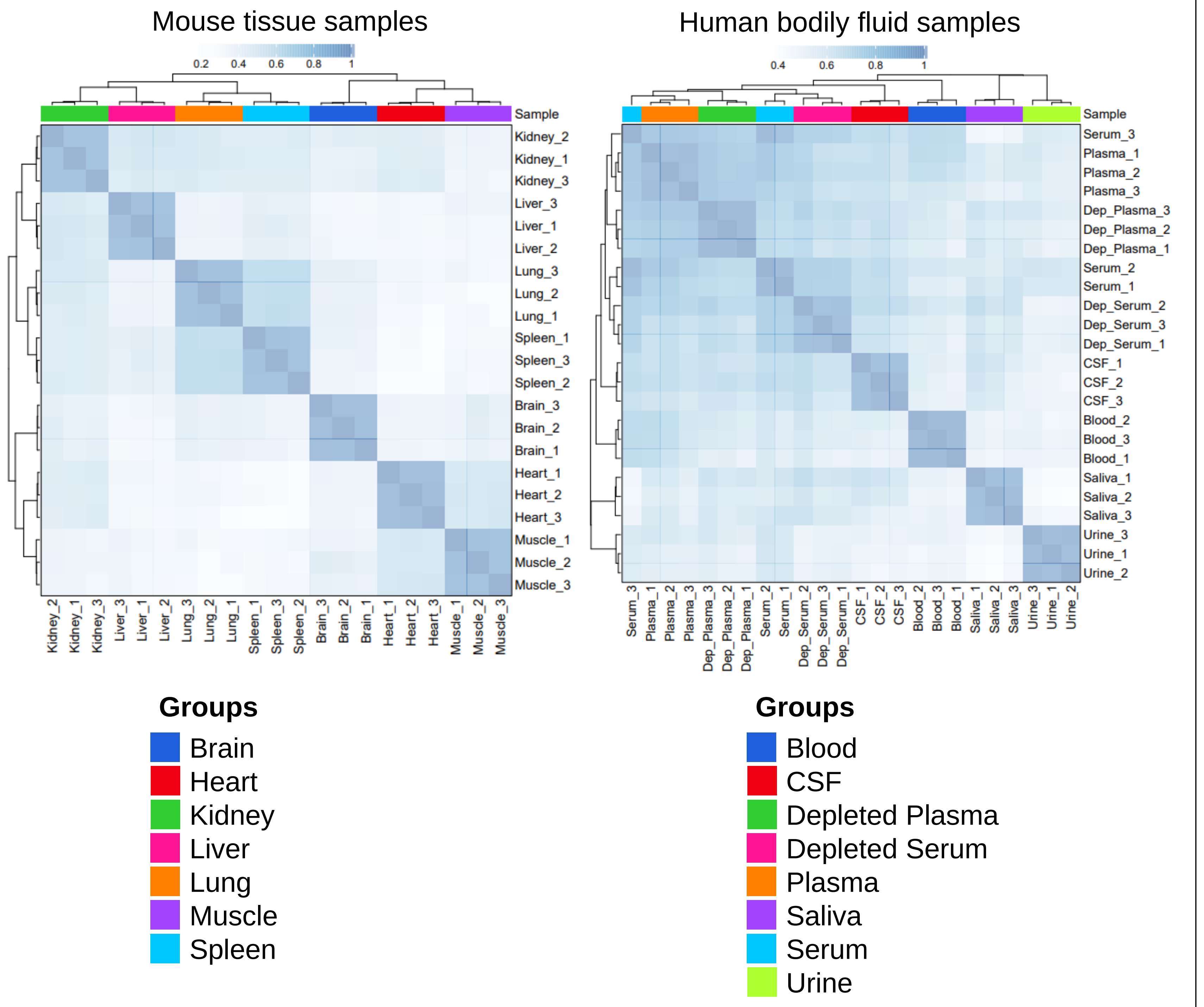


The ProMTag UPECK-Pep workflow is compatible with a wide variety of sample types

PCA of MS data shows high reproducibility and similarity between samples



Pearson's correlation analysis confirms a high correlation between sample replicates and tissue origins



Acknowledgements

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