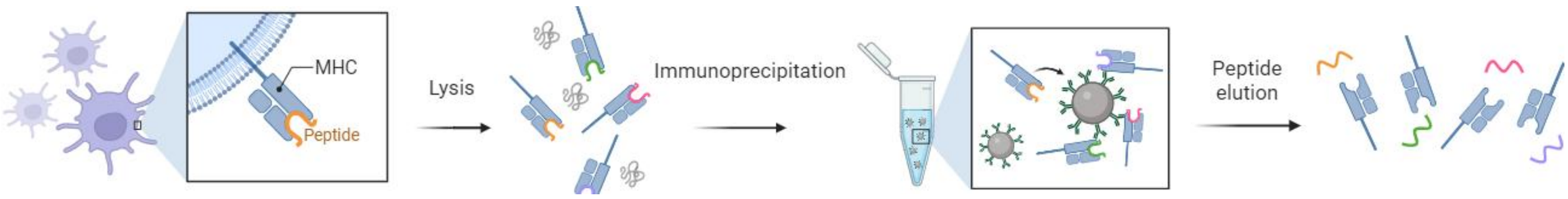
Immunopeptidome Services

Immunopeptidomics is the study of the set of peptides presented by major histocompatibility complex (MHC) proteins on the surface of antigen presenting cells (APC).

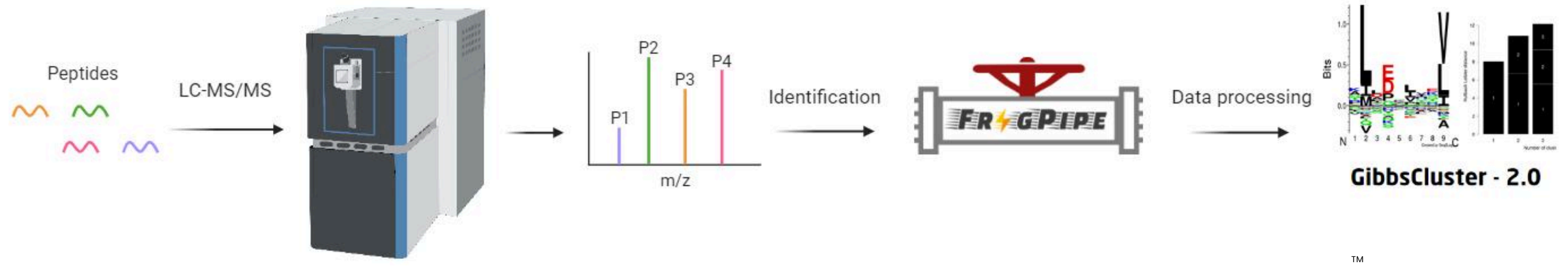
By investigating the repertoire of peptides that bind to MHC molecules and are displayed to T cells, we can further our insights on the power of adaptive immunity. This discipline provides insights into immune responses, including those related to infections, cancer, and autoimmune diseases.

An MHC immunoaffinity assay is an experimental technique developed to isolate and identify peptides that are bound to MHC proteins. The process involves extracting MHC complexes from cells or tissues, followed by MHC complex enrichment via antibody-coated magnetic bead. The bound peptides are then eluted, analyzed, and characterized using mass spectrometry to map the immunopeptidome.

Immunopeptidomics workflow



Cells are lysed and resulting preparations are subjected to immunoaffinity capture. Following multiple washes peptides are eluted from the MCH cleft with acid.



Purified peptides are analyzed by LC-MS/MS using nano-scale chromatography combined with an Orbitrap Astral mass spectrometer. FragPipe is used for peptide quantitation.

HCT116, A549, and HEPG2 Cell-lines as a Case Study

This study's purpose is to document our capability in immunopeptidomics. A secondary aim of this study is to showcase the number of MHC bound peptides identified using an Orbitrap Astral mass spectrometer. Using human cancer cell lines, HCT116, A549, and HEPG2, we confirm our ability in immunopeptidomics, and showed that the Orbitrap Astral performance meets expectations.

In this experiment, each of the three cell lines, HCT116, A549, and HEPG2, were split into two cohorts of 100 million cells and 10 million cells. The samples were immunoprecipitated (IP) in triplicate. Peptides were eluted from the enriched MHC and loaded on a C18 plate, reduced, and alkylated on column, followed by elution. 25% of each IP was analyzed on a ThermoFisher Vanquish Neo system interfaced to the Astral mass spectrometer using a 1h gradient. All resulting data were processed using FragPipe.

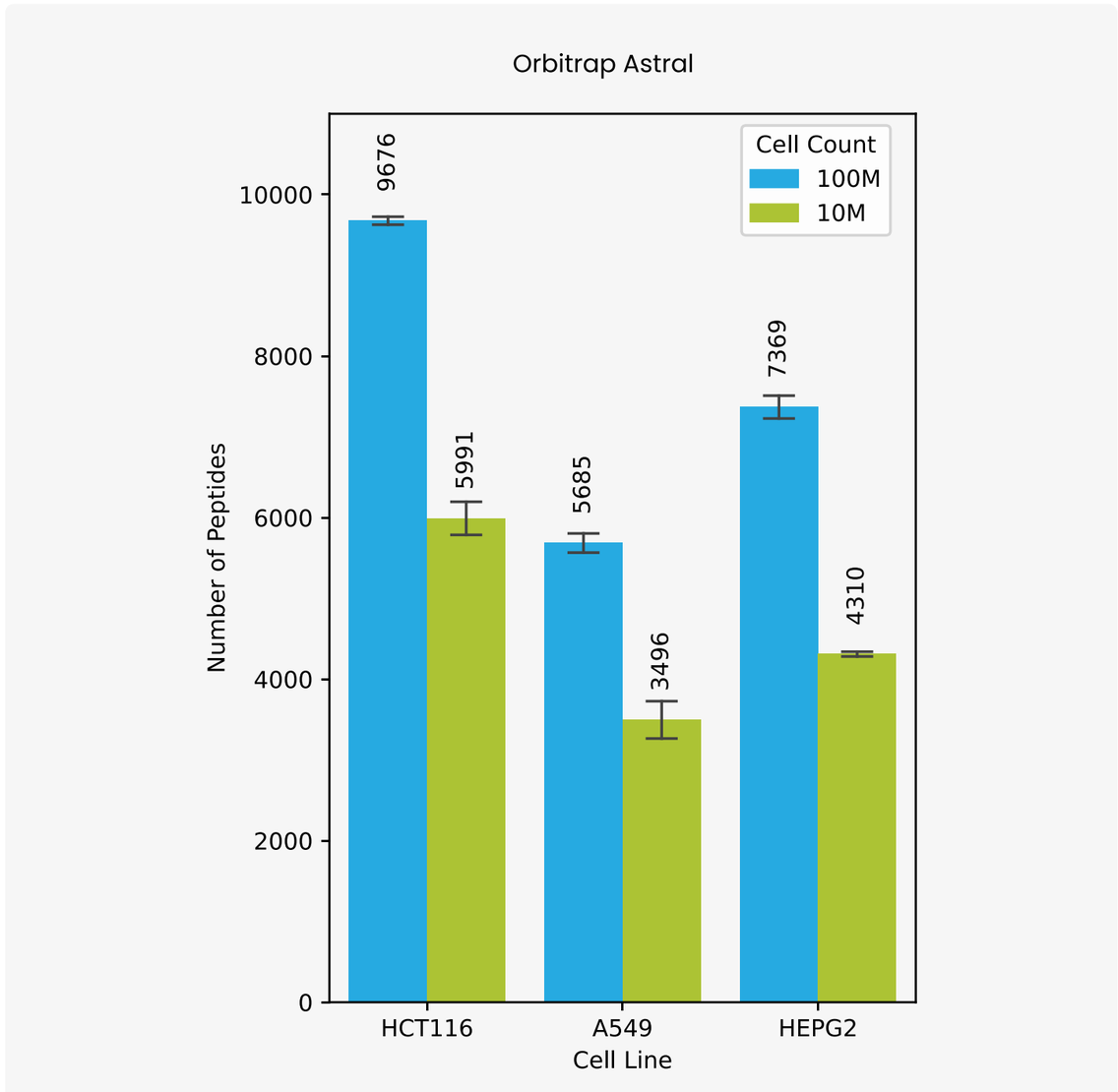


Figure 2. A bar chart depicting the number of sequences identified per cell line for two different cell equivalents.

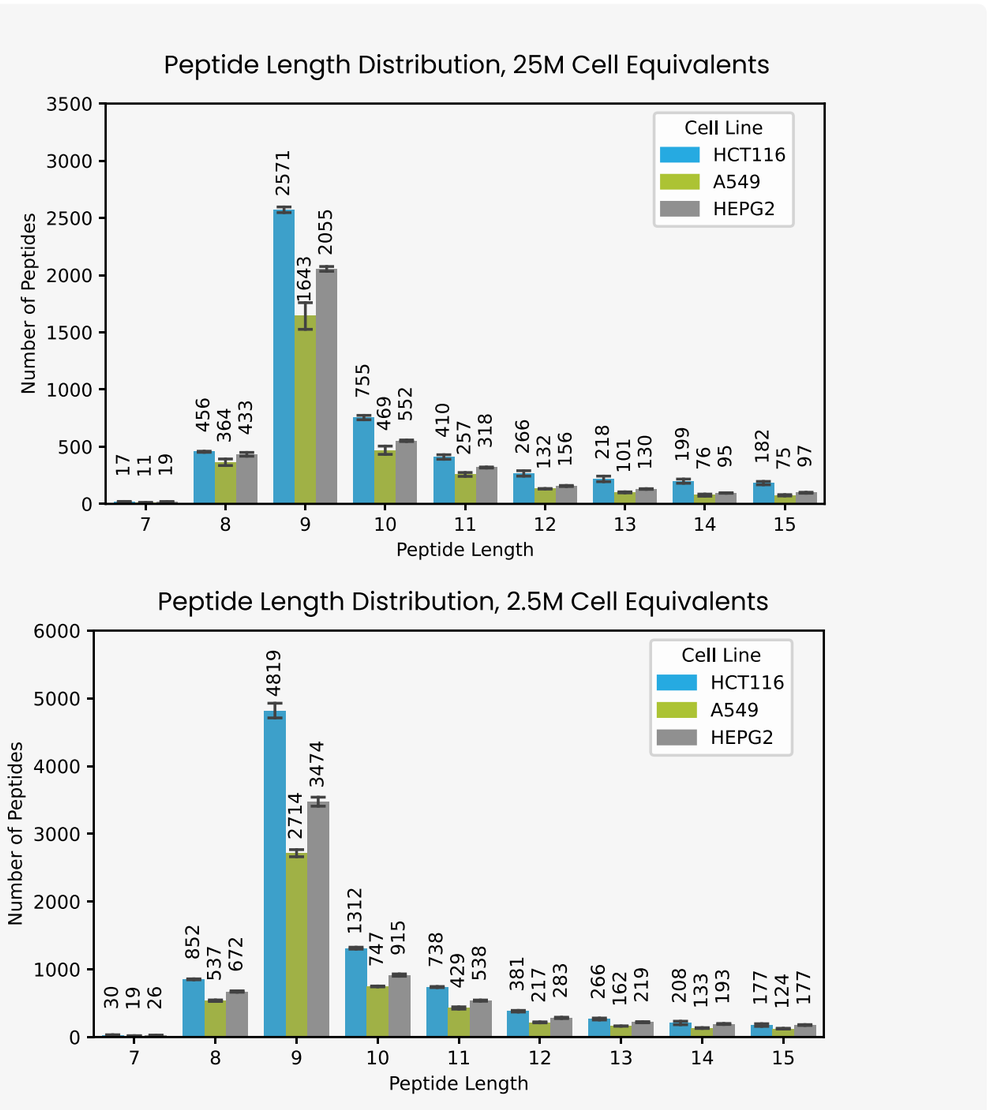


Figure 3. A histogram comparing the number of peptides of different lengths identified between (A) 25 million and (B) 2.5 million cell equivalents of each cell line.

This study showcases the effectiveness of MHC immunoaffinity assays in identifying peptides presented by MHC proteins. The ability to map the immunopeptidome using mass spectrometry highlights the potential of this technique to deepen our understanding of immune system function in the context of infections, cancer, and autoimmune diseases. This work demonstrates our capabilities and the power of the ThermoFisher Orbitrap Astral in advancing our knowledge for future immunological research.