MS Bioworks
Protein Mass Spectrometry Services

Immunopeptidomic Services: MHC

Introduction

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 typically involves extracting MHC complexes from tissue or cells, followed by enrichment via antibody-coated magnetic bead. The bound peptides are then eluted, analyzed, and characterized using mass spectrometry to map the immunopeptidome.

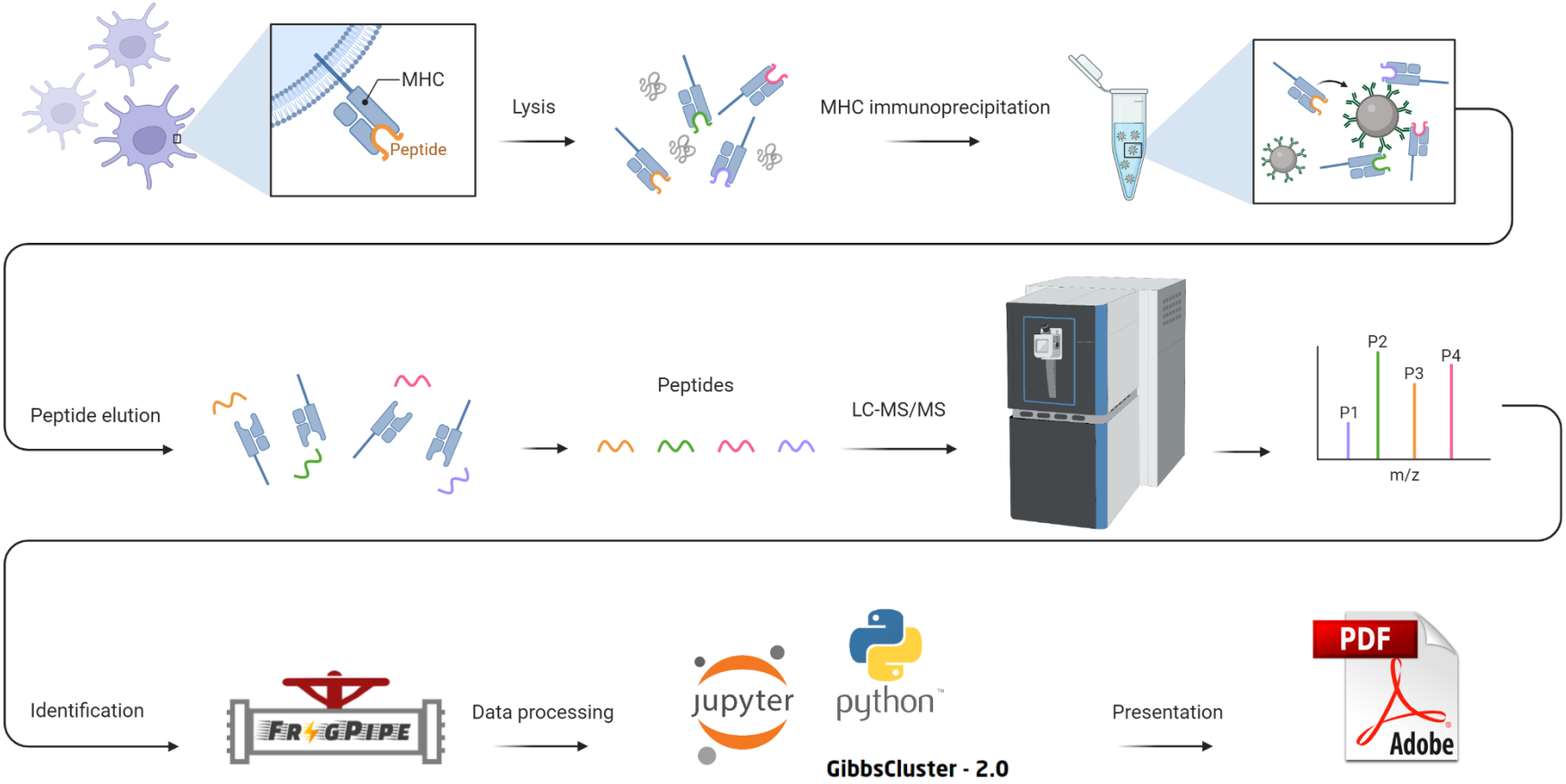

Figure 1. Diagram of the MS Bioworks MHC immunoaffinity assay workflow. Cells or tissue are lysed, and the HLA complex captured using bead-bound antibody. Peptides are eluted in acid and analyzed by nanoscale LC/MS/MS with an Orbitrap Astral. Data are searched using FragPipe and outputs further processed using custom in-house tools.

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.

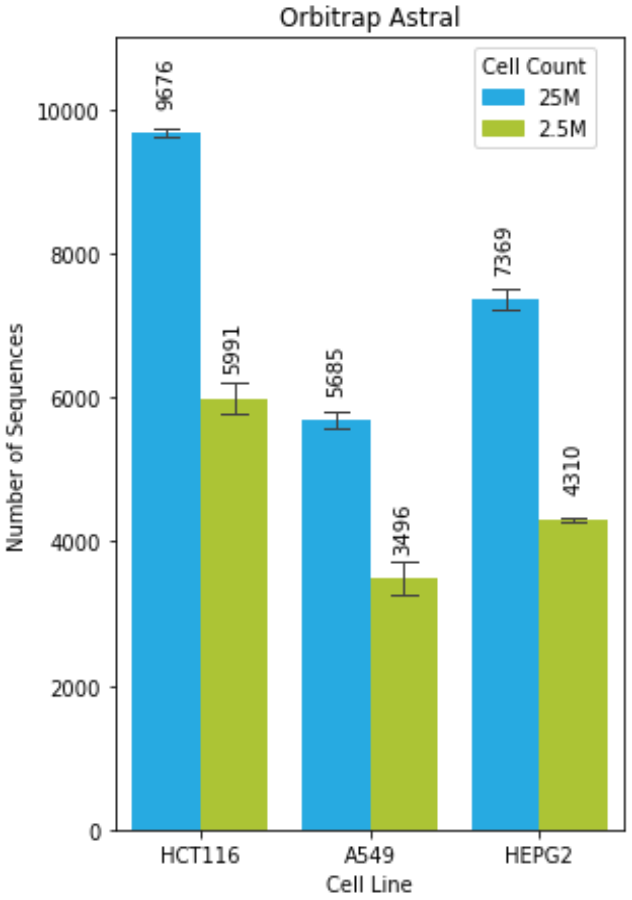

Figure 2. A bar chart depicting the number of sequences identified per cell line for two different cell equivalents.

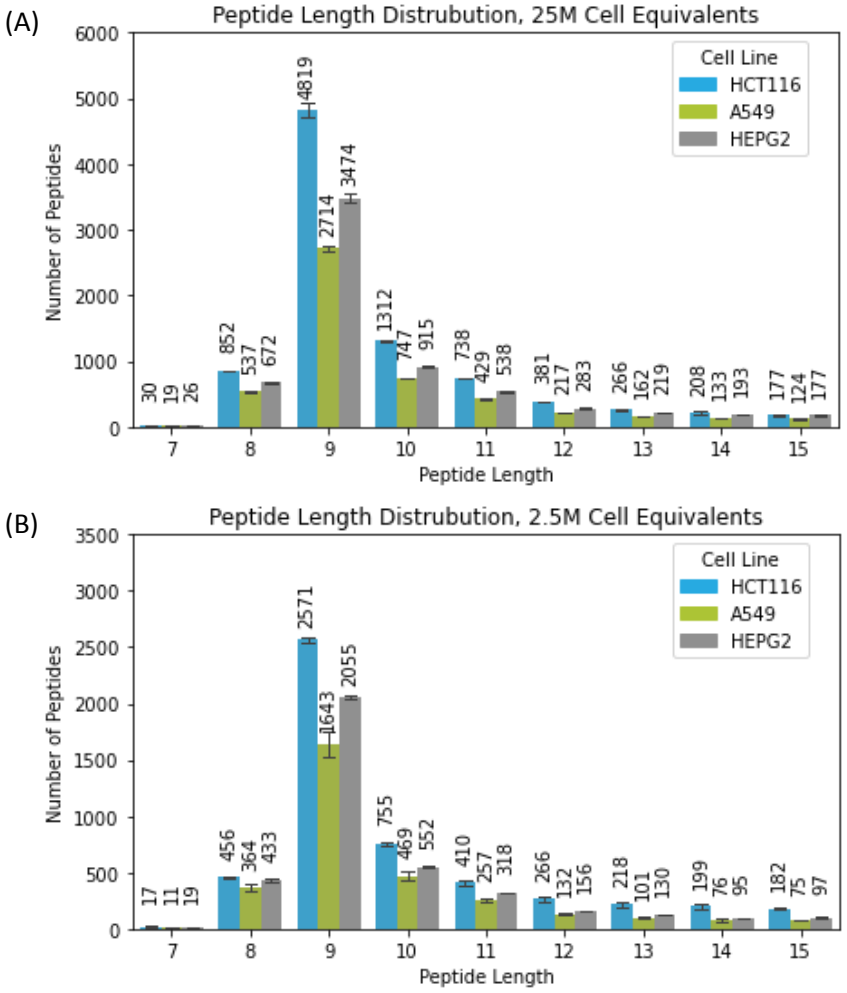

Figure 3. A histogram comparing the number of peptides of different lengths identified between (A) 25 million and (B) 2.5 million cell equivalents of each cell line.

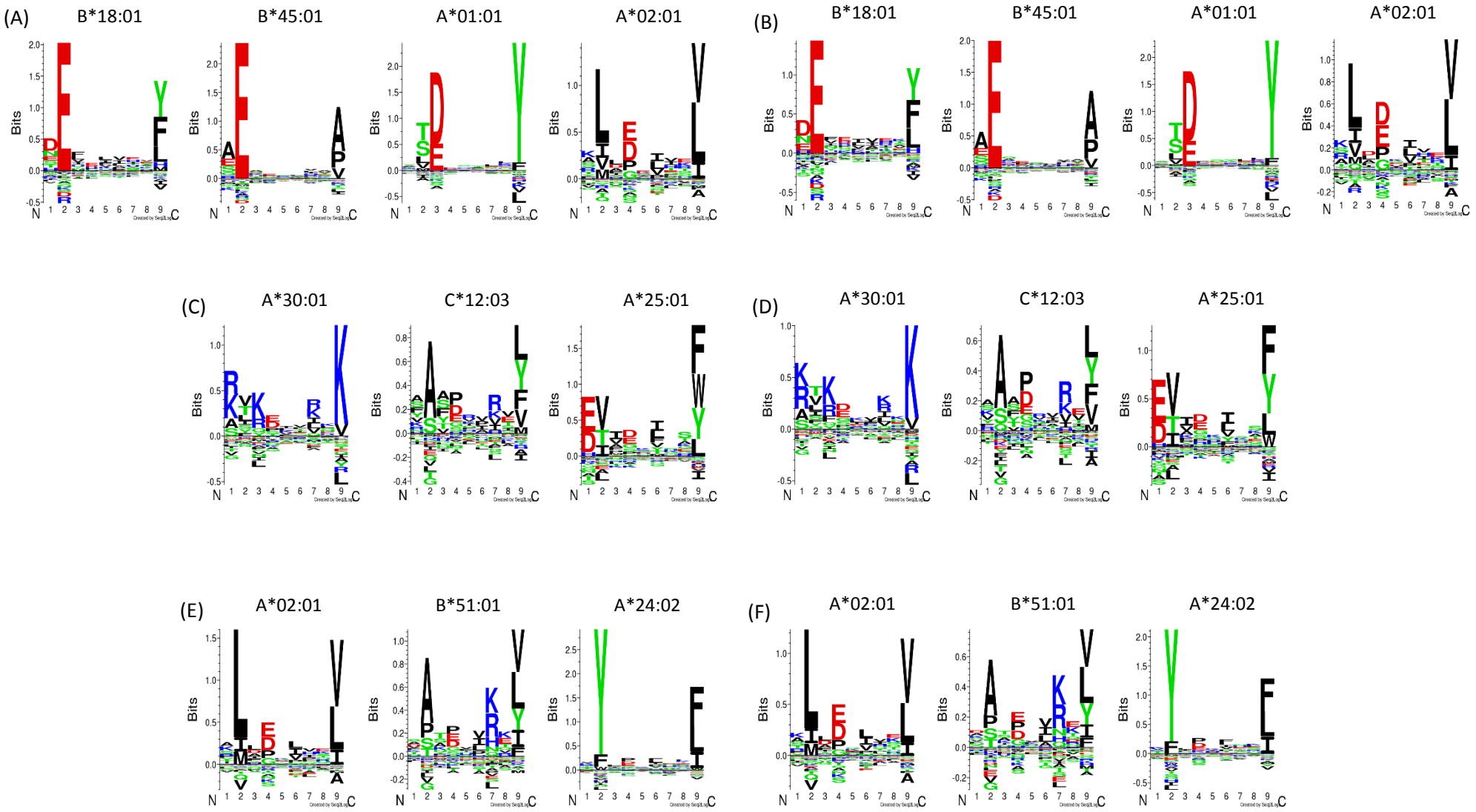

Figure 4. Detailed motif analysis of peptides 8-13 amino acids long. This data shows the conservation of motifs across cell lines at different concentrations: (A) 25 M HCT116 cell equivalents, (B) 2.5 M HCT116 cell equivalents, (C) 25 M A549 cell equivalents, (D) 2.5 M A549 cell equivalents, (E) 25 M HEPG2 cell equivalents, and (F) 2.5 M HEPG2 cell equivalents.

Conclusion

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.