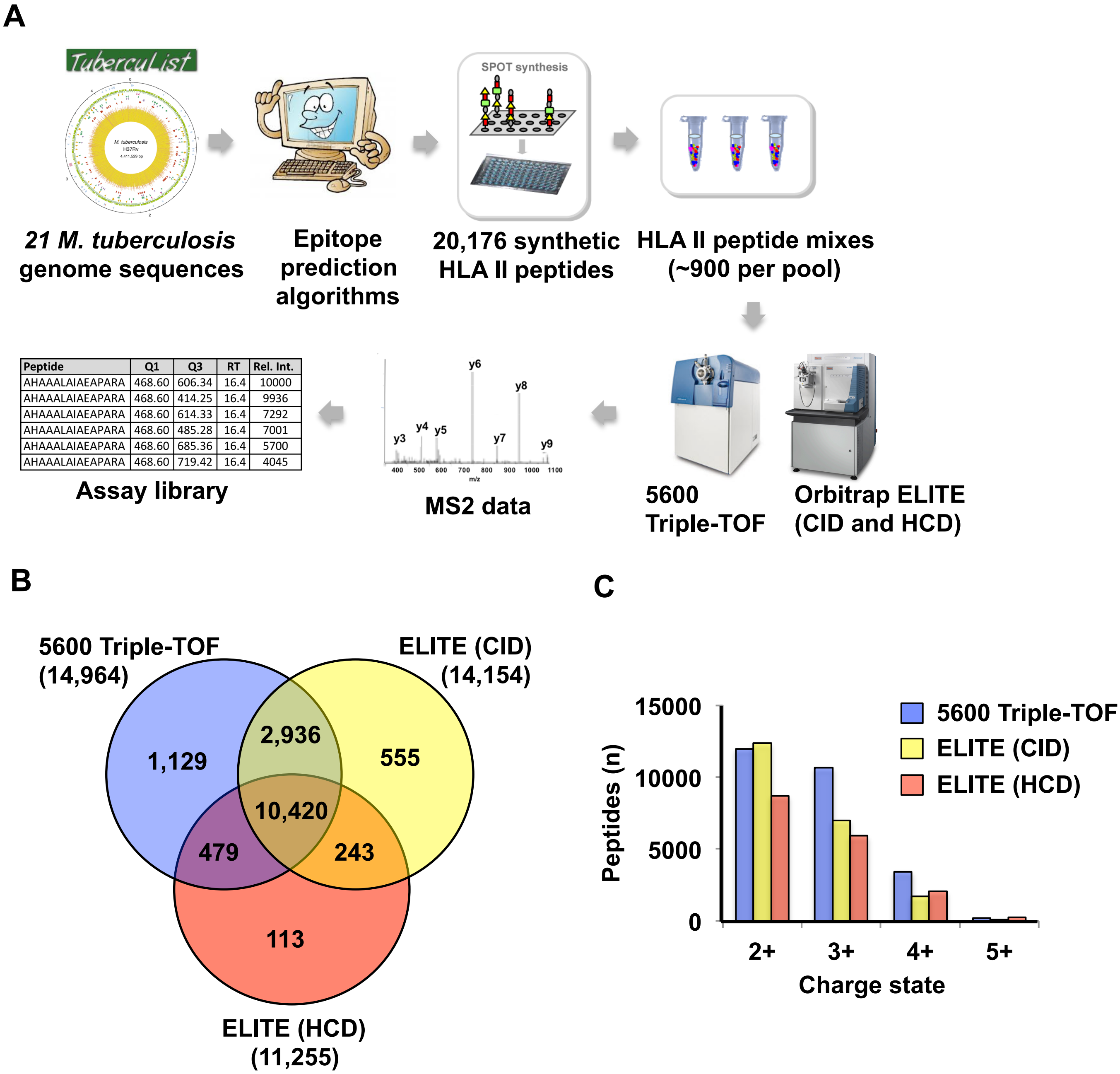

Figure 2—figure supplement 3. Generation of assay libraries from a large collection of synthetic HLA class II peptides. (**A**) Workflow to generate an assay library from synthetic peptides. A total of 20,176 predicted peptides (with a range of 2 to 10 per ORF, and an average of 5), were synthesized and arranged into 23 peptide pools of ~900 peptides (Lindestam Arlehamn et al., PLoS Pathog, 2013). Spiked-in reference iRT peptides were used and the pools of synthetic peptides were analyzed in DDA mode using a 5600 Triple-TOF and an Orbitrap ELITE (CID and HCD fragmentation). The identified peptides were then processed through our computational pipeline to generate the assay library. (**B**) Venn diagram showing the overlap between peptides identified by the 5600 Triple-TOF and by the ELITE (CID and HCD fragmentation methods). Number of peptides identified is indicated in parenthesis. (**C**) Histogram showing the distribution of the precursor charge state.