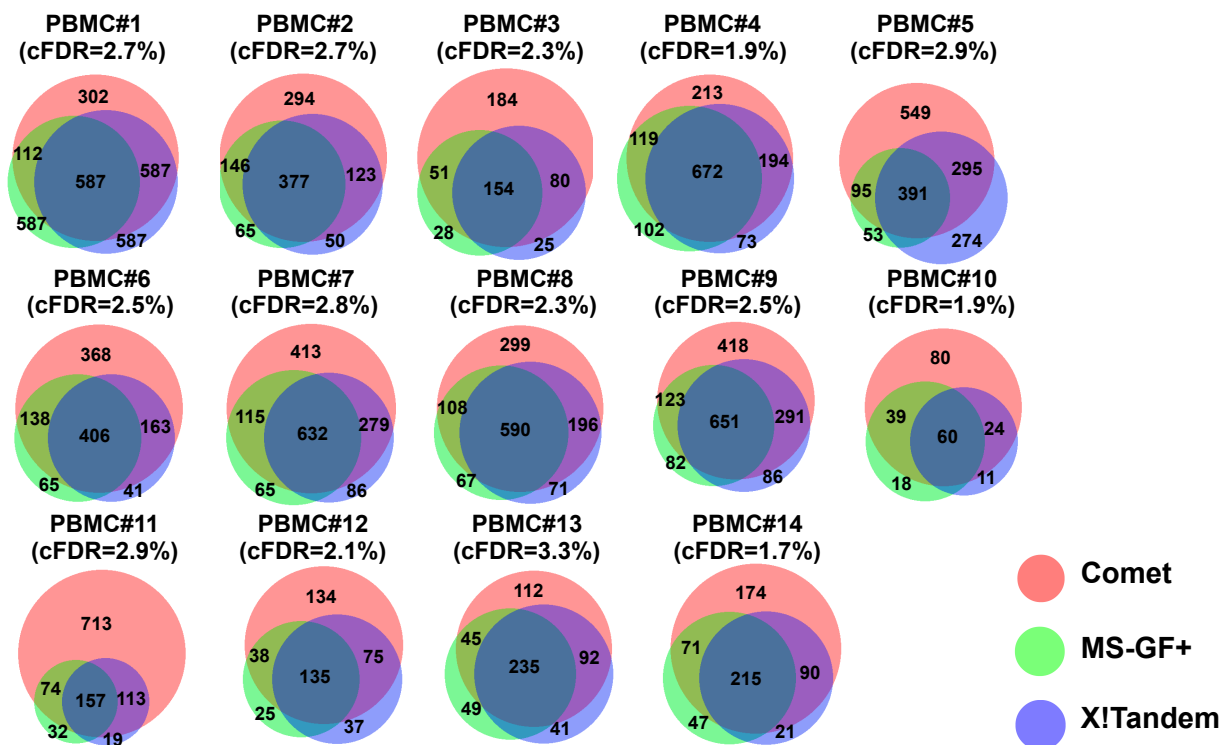


**A****B**

Sample	Union (venn diagram)	Intersection (venn diagram)	iProphet
PBMC#1	1405	587	1362
PBMC#2	1055	377	1203
PBMC#3	523	154	574
PBMC#4	1373	672	1220
PBMC#5	1657	391	1281
PBMC#6	1181	406	1121
PBMC#7	1590	632	1477
PBMC#8	1331	590	1178
PBMC#9	1651	651	1642
PBMC#10	232	60	260
PBMC#11	1108	157	1076
PBMC#12	444	135	456
PBMC#13	574	235	599
PBMC#14	618	215	697

**Figure 1—figure supplement 2. Combining results of three open-source database search engines in immunopeptidomics using iProphet.** (A) The HLA peptidome of fourteen PBMC samples were analyzed. Venn diagrams show the search results obtained from three database search engines (i.e., Comet, MS-GF+ and X!Tandem) at 5% peptide-level FDR. The search identifications were combined and statistically scored using PeptideProphet and iProphet within the Trans-Proteomic Pipeline (TPP). Following annotation of all identified peptides to their respective HLA allele, all non-annotated peptides were removed from the iProphet combined search result and a corrected false discovery rate (cFDR) was manually calculated based on the target-decoy approach. cFDR is indicated for each PBMC sample. At peptide-level FDR 1%, the cFDR was estimated on average at 0.5%. At peptide-level FDR 5%, the cFDR was estimated at 2.5%. (B) The table shows the number of HLA class I peptides identified from the iProphet combined search results that were used to build the spectral libraries. The sum of peptides identified by the three search engines (Union) as well as the number of overlapping peptides (Intersection) for each venn diagram/sample is also indicated.