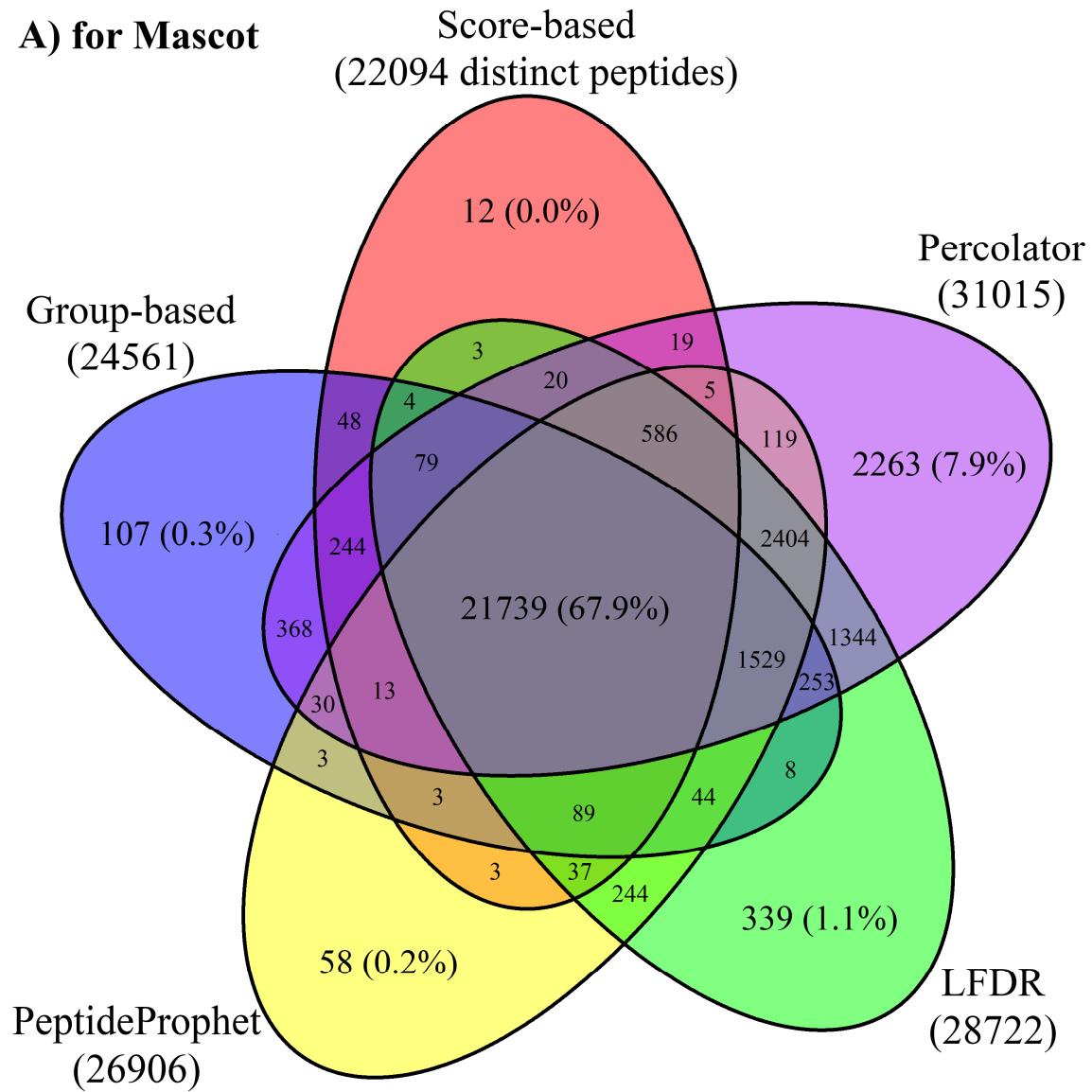
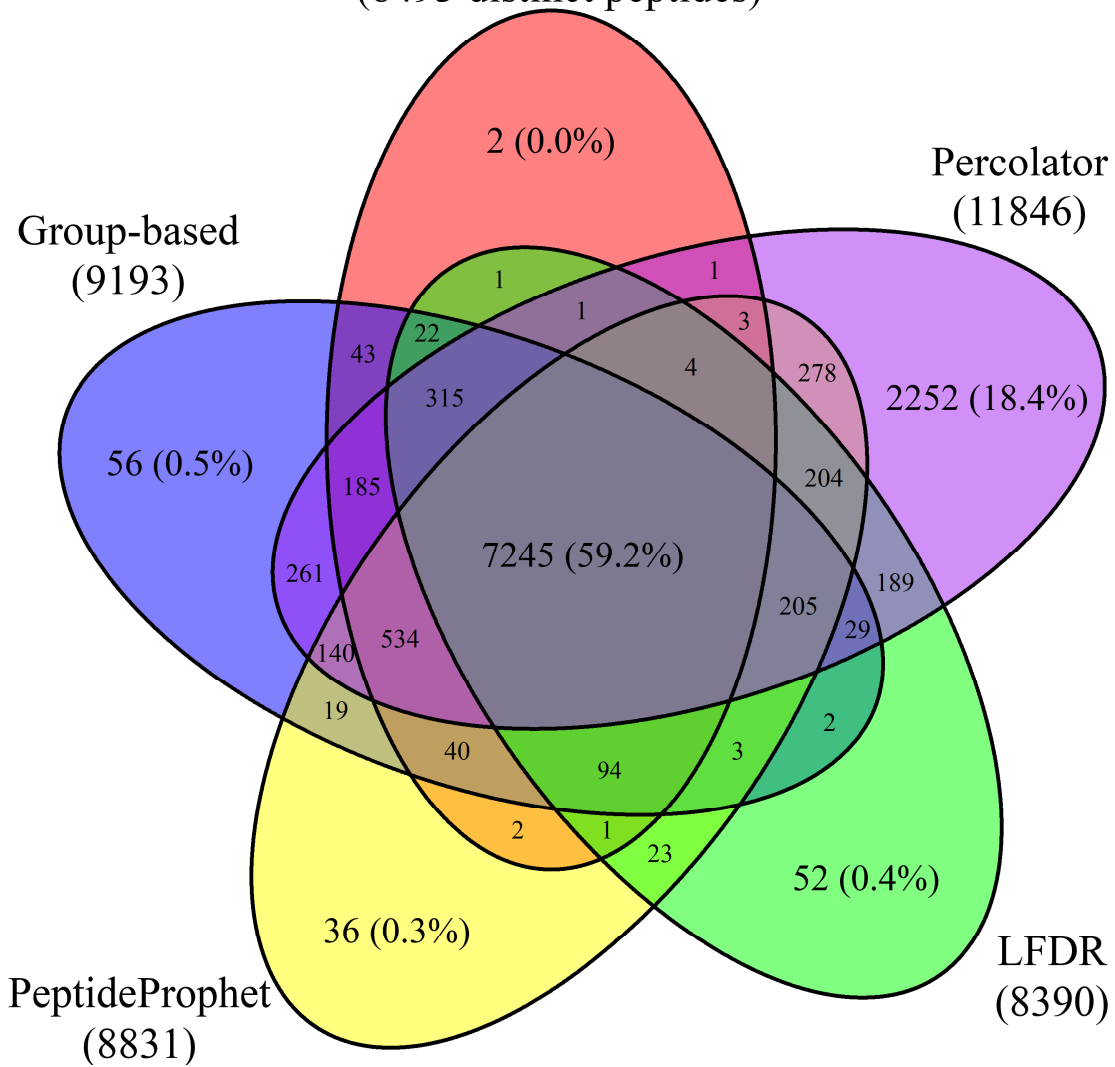


Supplemental Figure 1

**A) for Mascot**

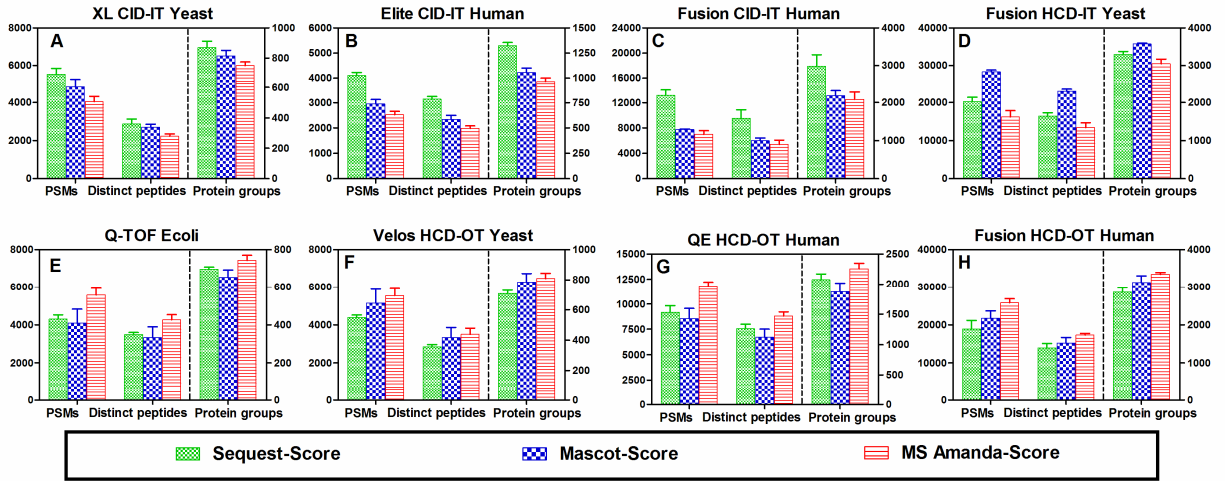


**B) for MS Amanda**      Score-based  
 (8493 distinct peptides)



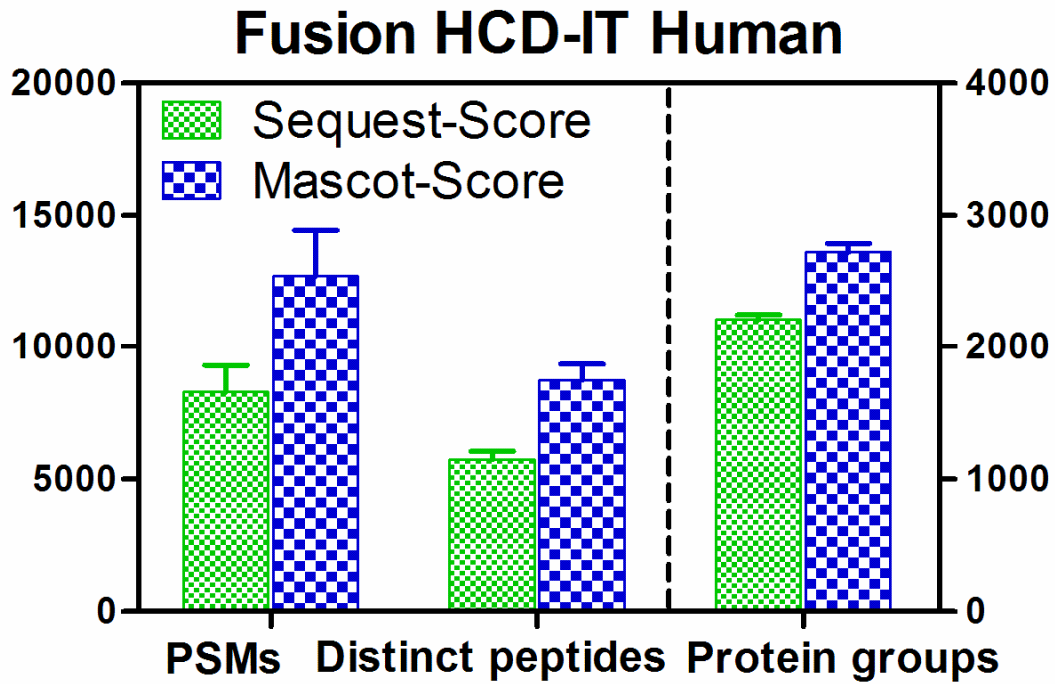
Supplemental Figure 1. Overlap of distinct peptides identified by different filtering approaches: score-based, group-based, LFDR, PeptideProphet, and Percolator coupled with (A) Mascot and (B) MS Amanda, respectively. One replicate of the Fusion HCD-IT Yeast dataset was used for Mascot database search and one replicate of the QE HCD-OT Human dataset was used for MS Amanda database search.

Supplemental Figure 2



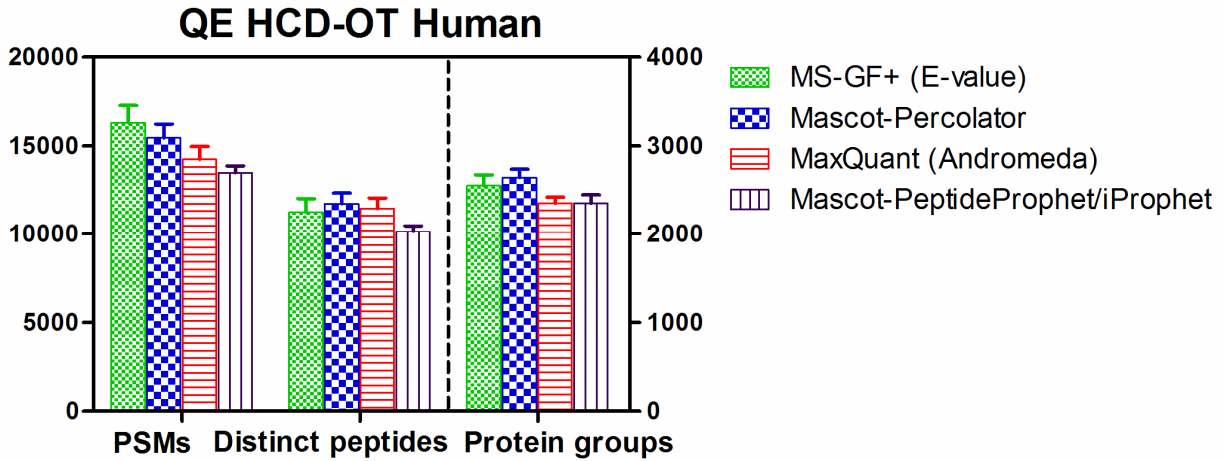
Supplemental Figure 2. Comparison of PSMs, distinct peptides, and protein groups at 1% protein FDR with respective SEQUEST, Mascot, and MS Amanda search engines coupled with score-based filtering approach.

Supplemental Figure 3



Supplemental Figure 3. Comparison of PSMs, distinct peptides, and protein groups at 1% protein FDR with respective SEQUEST-Score and Mascot-Score combinations. Two replicates from the HeLa cell sample by Oribtrap Fusion in our lab with the HCD-IT mode were analyzed. A significant increase of 53%, 52%, and 23% respectively at PSM, distinct peptide and protein group level were achieved by Mascot-Score than SEQUEST-Score.

Supplemental Figure 4



Supplemental Figure 4. Comparison of PSMs, distinct peptides, and protein groups at 1% protein FDR with respective MS-GF+ (E-value), Mascot-Percolator, Mascot-PeptideProphet/iProphet, and MaxQuant (Andromeda) approaches. Three replicates from the QE HCD-OT human dataset were analyzed. MS-GF+ (E-value) identified more PSMs than others, while Mascot-Percolator identified the largest number of distinct peptides and protein groups.