

# Supporting Information

## **Evaluating False Transfer Rates from the Match-Between-Runs Algorithm with a Two-Proteome Model**

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**Supplementary Table 1:** Formatted tables of MaxQuant “peptide.txt” and “proteinGroups.txt” output files separated by species (yeast and human).

### Run Level Analysis - Peptides

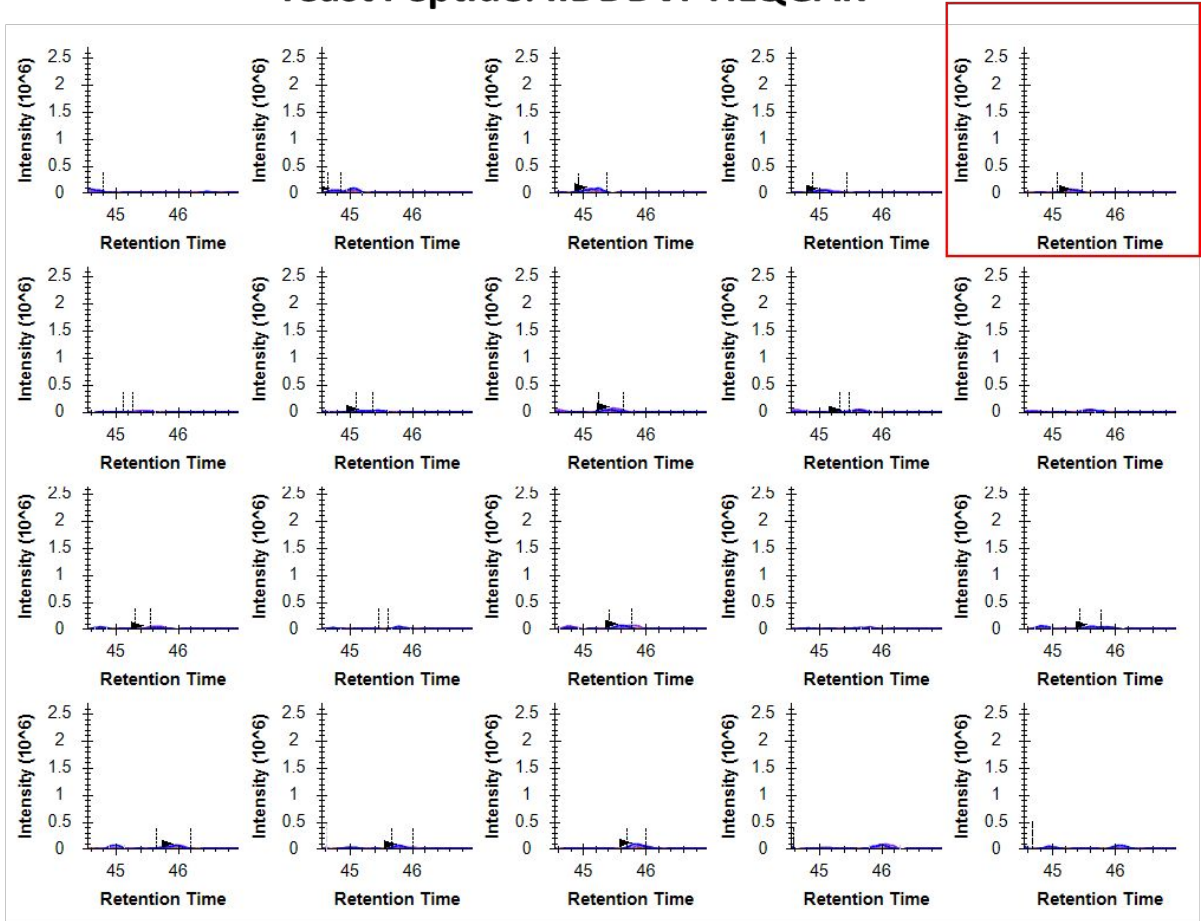
Total Unique Human Peptides	39,568 Unique Human Peptides Observed
Sample H, MBR -	22,529 ± 404 Human Peptides out of 39,568 (57%)
Sample HY, MBR -	22,241 ± 449 Human Peptides out of 39,568 (56%)
Sample H, MBR +	32,483 ± 243 Human Peptides out of 39,568 (82%)
Sample HY, MBR +	31,585 ± 380 Human Peptides out of 39,568 (80%)
Total Unique Yeast Peptides	3605 Unique Yeast Peptides Observed
Sample H, MBR -	11 ± 2 Yeast Peptides out of 3605 (0%)
Sample HY, MBR -	2127 ± 51 Yeast Peptides out of 3605 (59%)
Sample H, MBR +	79 ± 5 Yeast Peptides out of 3605 (2%)
Sample HY, MBR +	2974 ± 32 Yeast Peptides out of 3605 (83%)

**Figure S1:** Run level analysis of MBR false transfers as a percentage of total identifications at the peptide level. Total unique proteins and peptides reported are the combined unique protein list between all 40 LC-MS/MS runs performed in this study from the human (orange bar) and yeast (purple bar) proteomes. Yellow bars indicate percentage identified in analyses of Sample H while blue bars represent percentage identified in Sample HY.

# Yeast Peptide: IIDDDVPTILQGAK

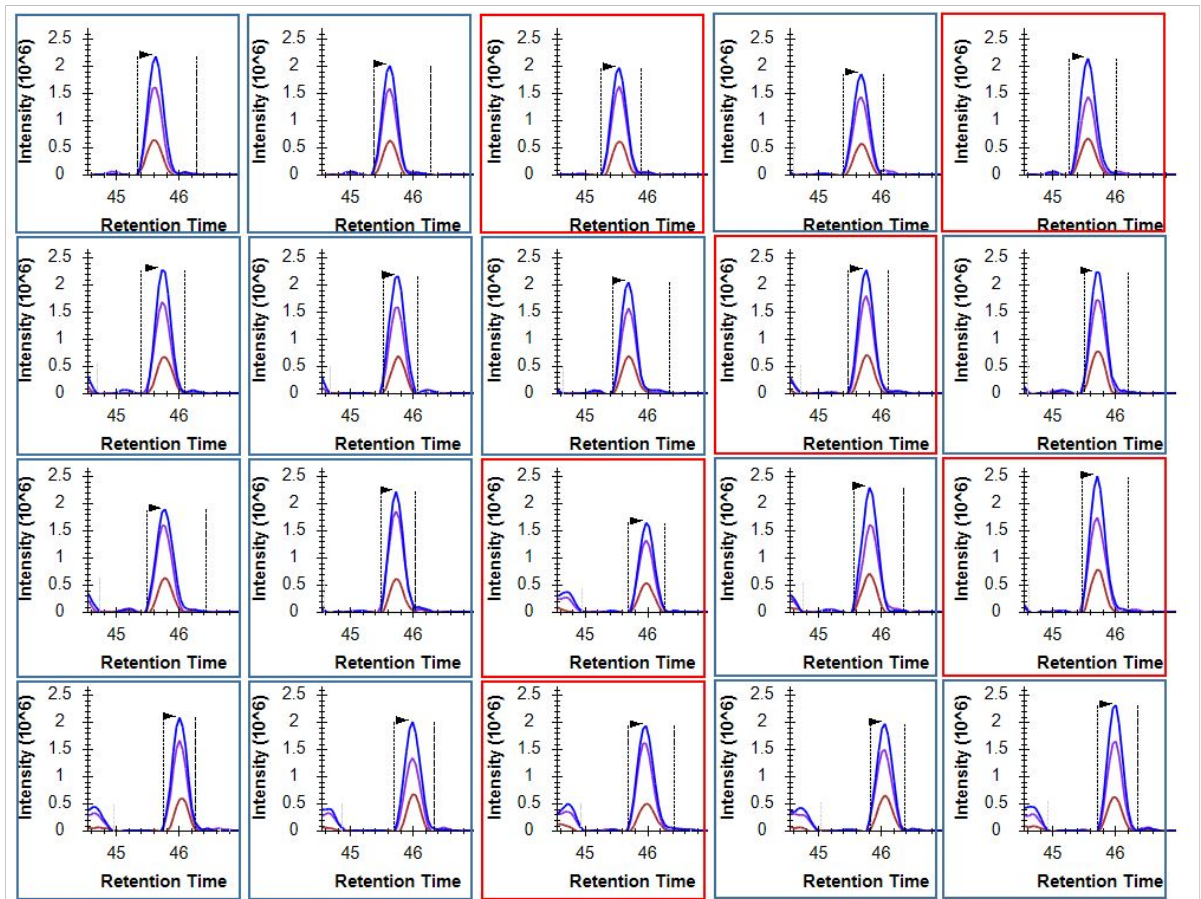
A

Extracted Ion Chromatograms for Sample H



B

Extracted Ion Chromatograms for Sample HY

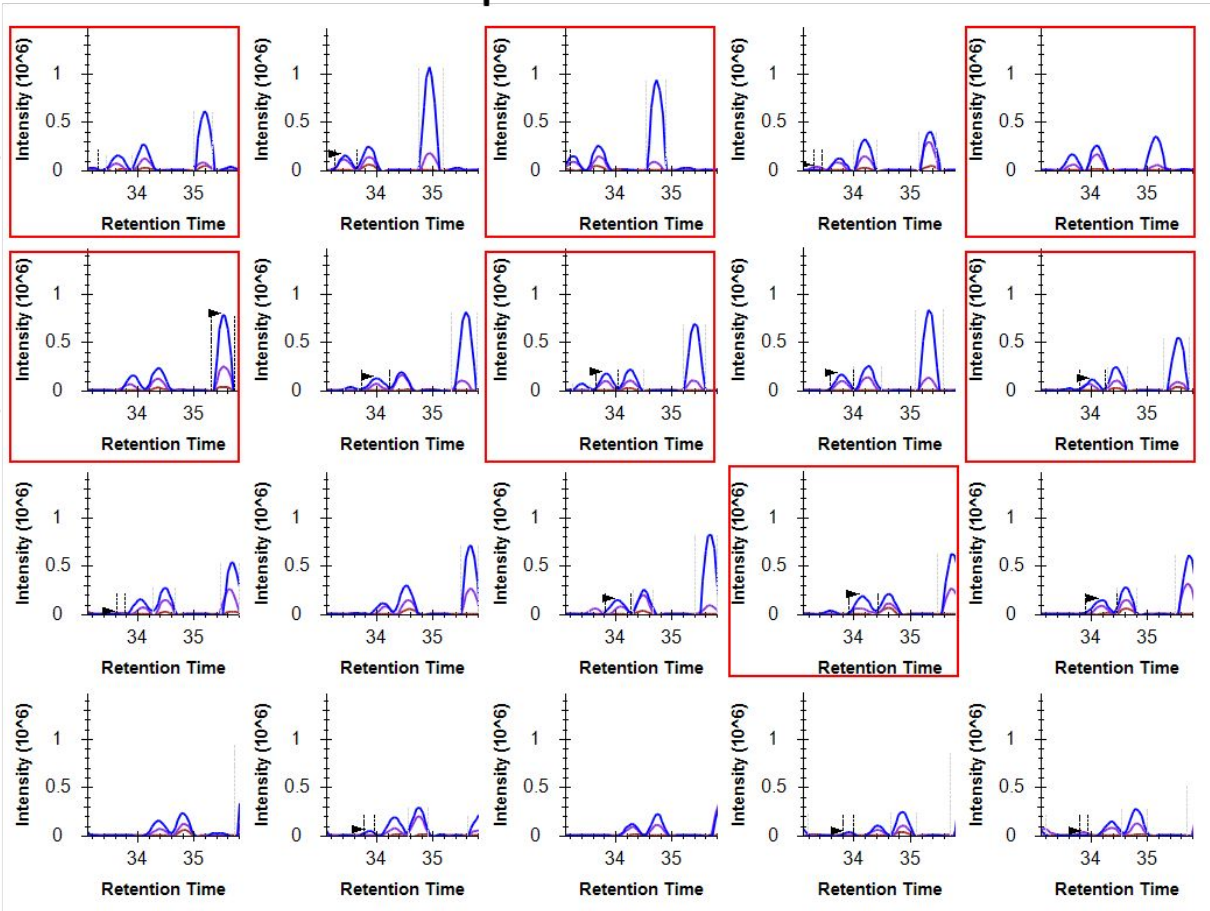


**Figure S2:** Extracted Ion Chromatograms for peptide IIDDDVPTILQGAK across 40 runs (previous page). **A)** Sample H and **B)** Sample HY. XICs of transferred peptide identifications are boxed in red while XICs of peptide identifications made by MS/MS are boxed in blue. Peptide IIDDDVPTILQGAK belongs to the yeast protein HS104 which was identified in Sample H with MBR but not quantified with LFQ. The MaxQuant msms.txt file with MBR and LFQ enabled was supplied to Skyline to generate XICs for this peptide ( $\pm 5$  PPM). Dashed lines represent the bounds of integration for a peak selected automatically by Skyline. Black arrows show the peak Skyline selected for integration. Monoisotopic precursor and the M+1 and M+2 isotopes are shown in blue, purple, and brown respectively in each Skyline plot.

# Yeast Peptide: FGPIVSASLEK

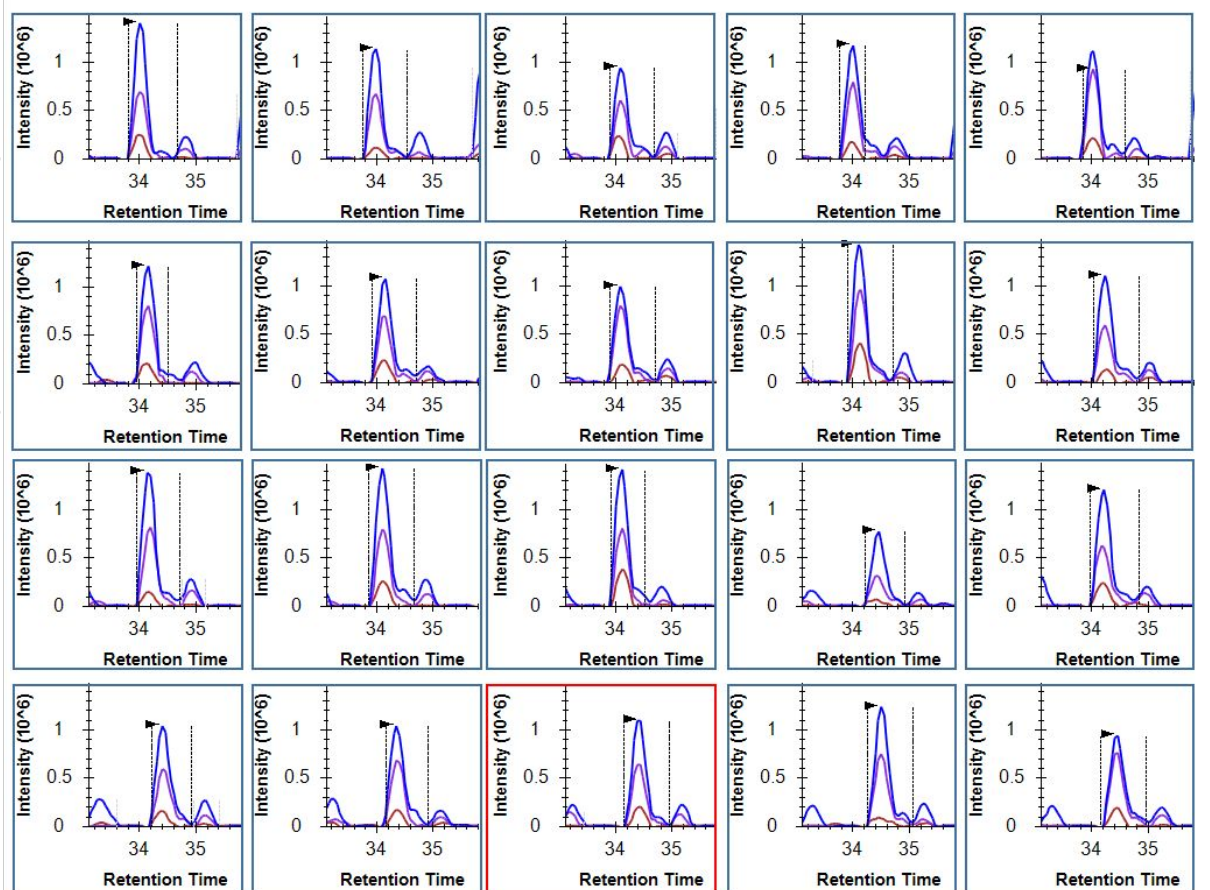
A

Extracted Ion Chromatograms for Sample H



B

Extracted Ion Chromatograms for Sample HY



**Figure S3:** Extracted Ion Chromatograms for yeast peptide FGPIVSASLEK across 40 runs (previous page). **A)** Sample H and **B)** Sample HY. XICs of transferred identifications are boxed in red. Peptide FGPIVSASLEK belongs to the yeast protein PABP which was identified in Sample H with MBR and quantified with LFQ in 6 of the 20 Sample H replicates. Even though Sample H contains no yeast proteins, peaks sharing the same precursor M/z value can be found near the calibrate retention time for FGPIVSASLEK. This highlights the potential difficulty the MBR algorithm faces when attempting to assign an identification transfer. The MaxQuant msms.txt file with MBR and LFQ enabled was supplied to Skyline to generate XICs for this peptide ( $\pm 5$  PPM). Dashed lines represent the bounds of integration for a peak selected automatically by Skyline. Black arrows show the peak Skyline selected for integration. Monoisotopic precursor and the M+1 and M+2 isotopes are shown in blue, purple, and brown respectively in each Skyline plot.