Creating parent mass lists with skyline

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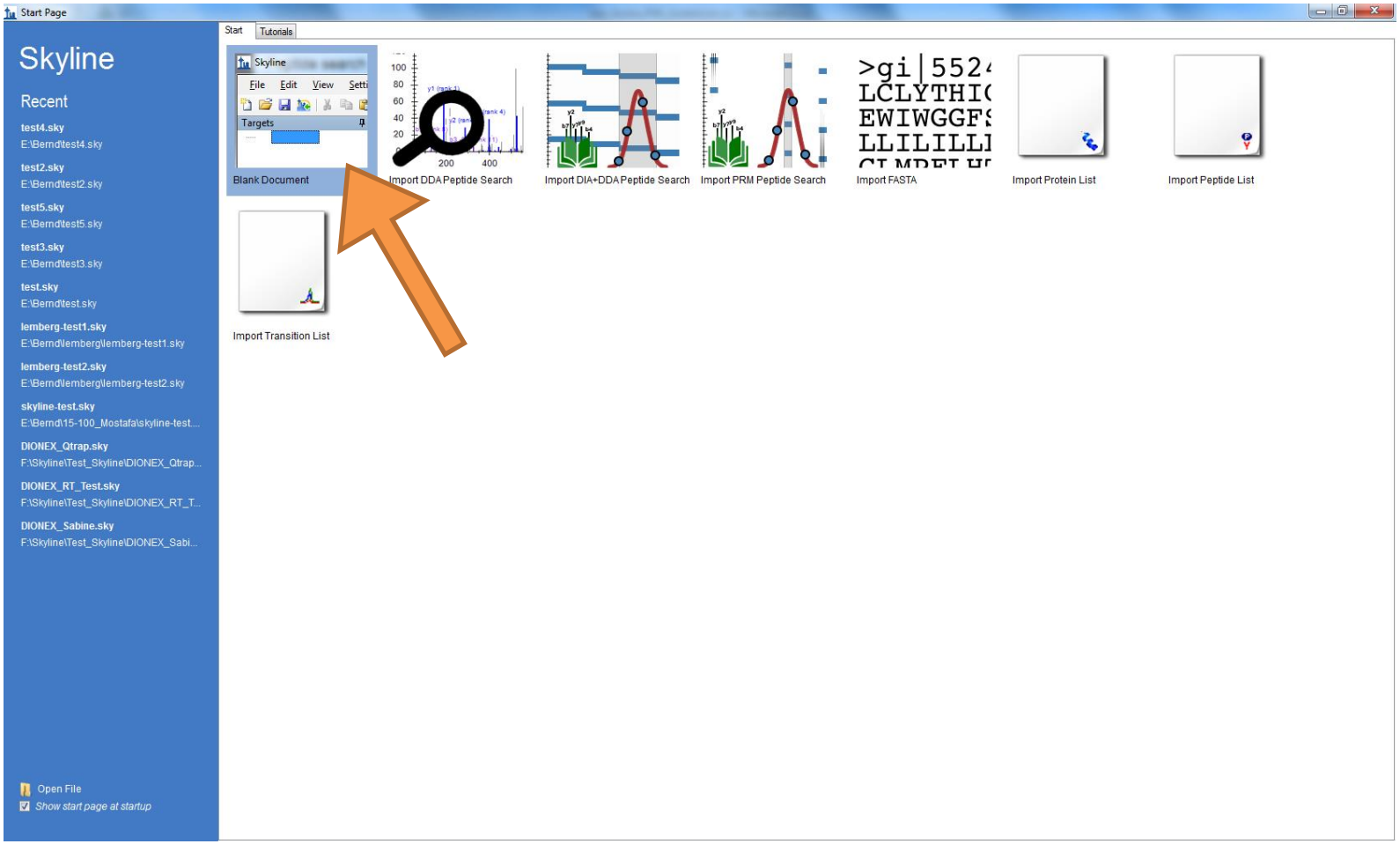
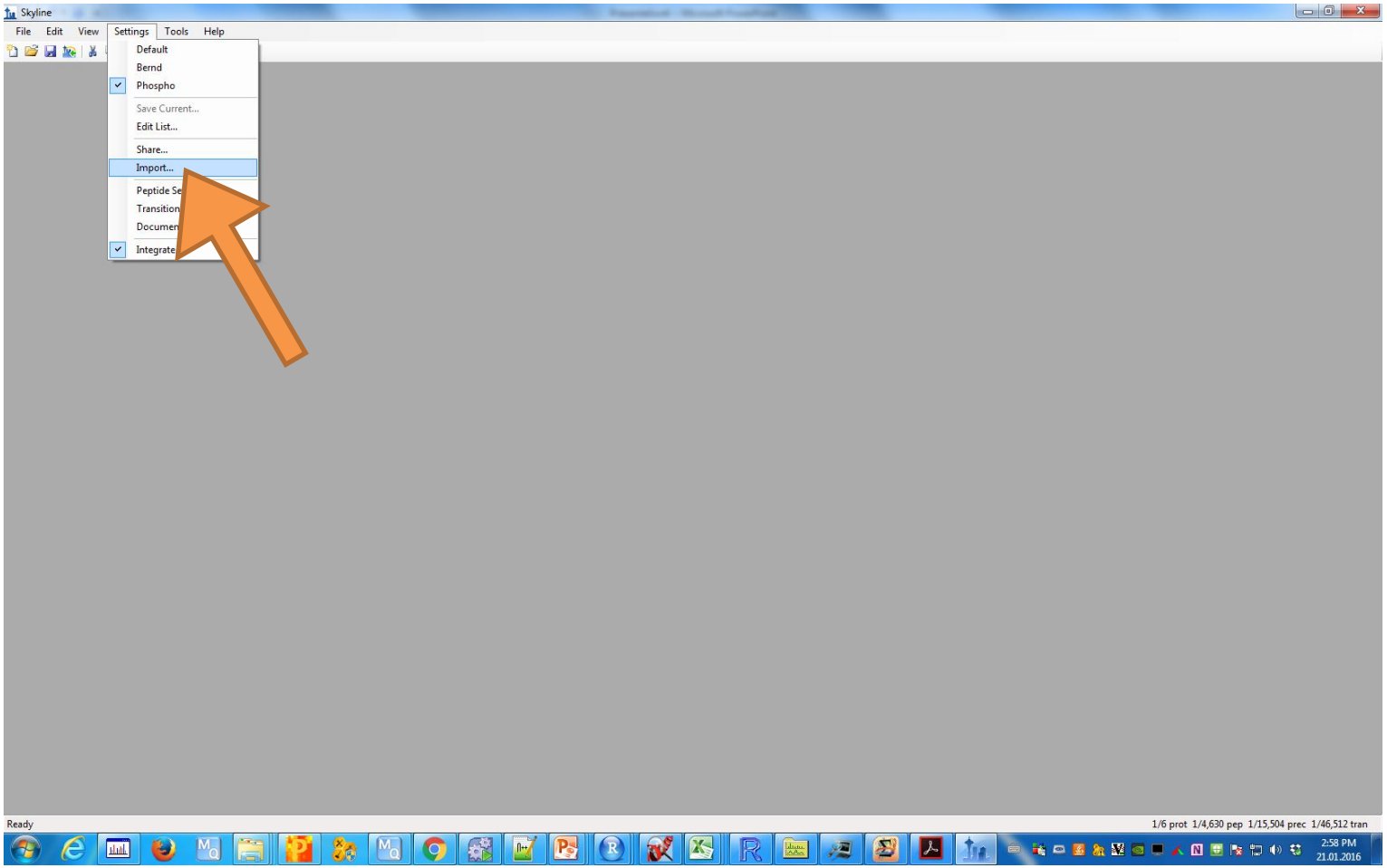
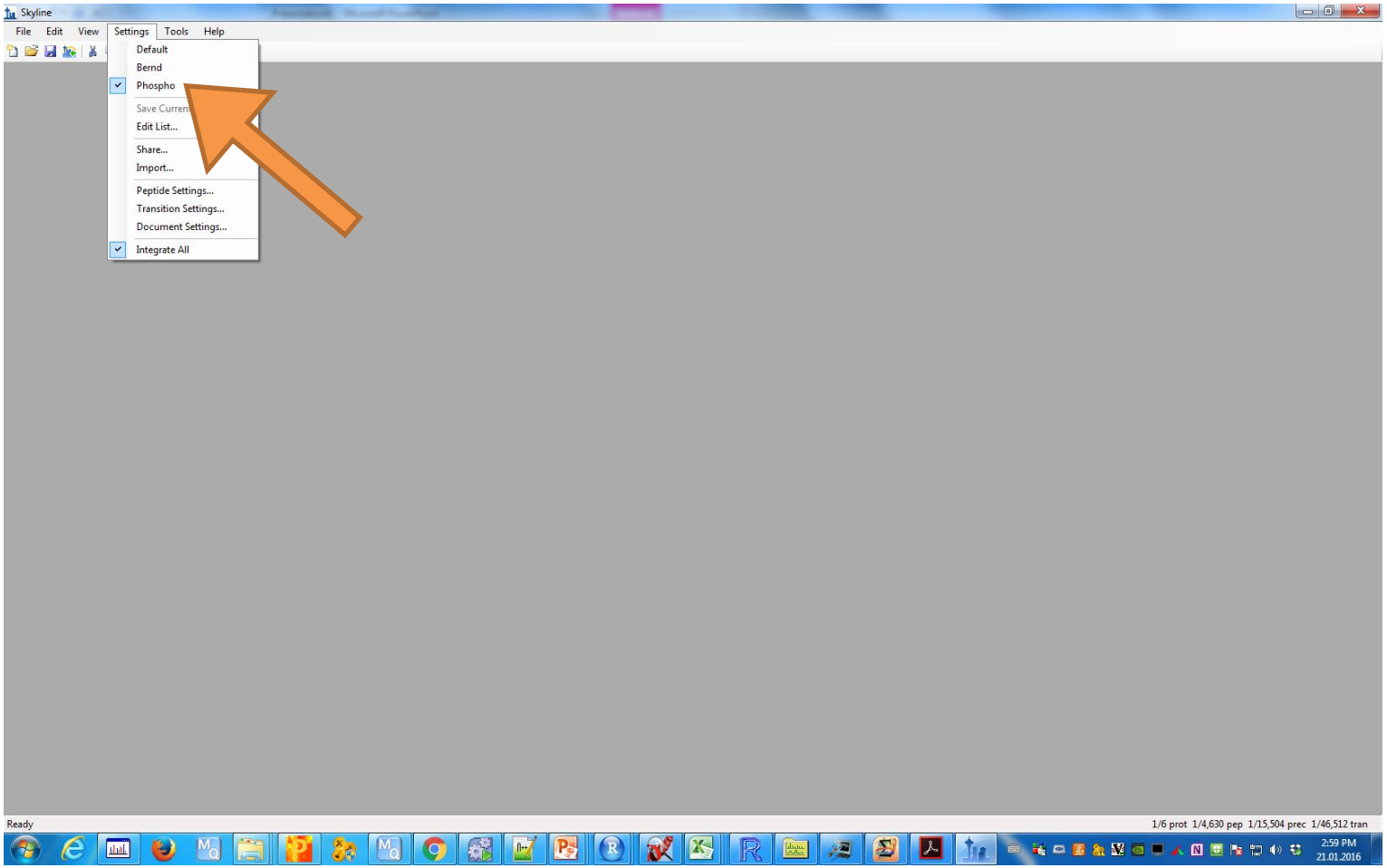
Open a fresh project in Skyline



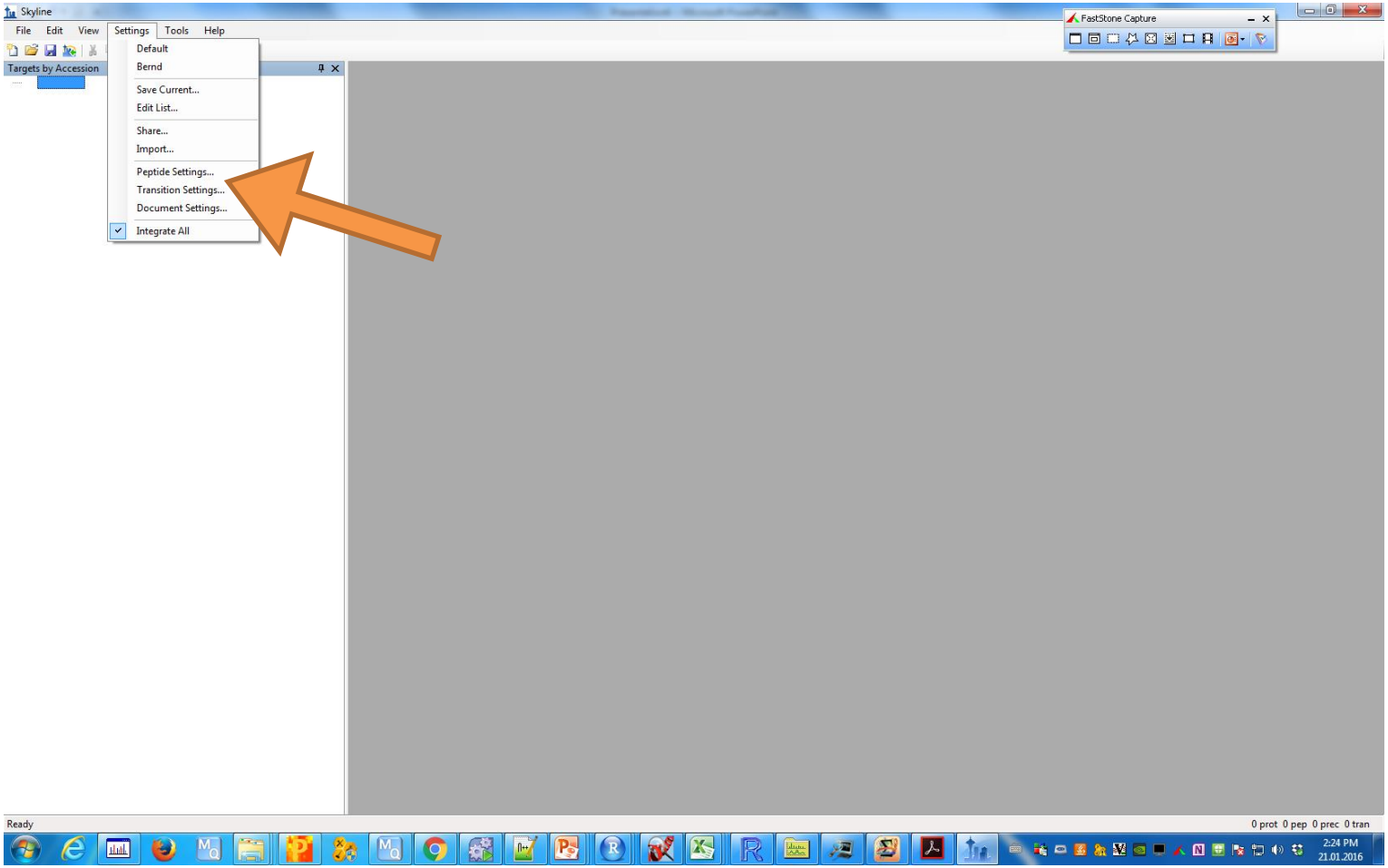
Import the email attached “Phospho_Skyline_Settings.skys”



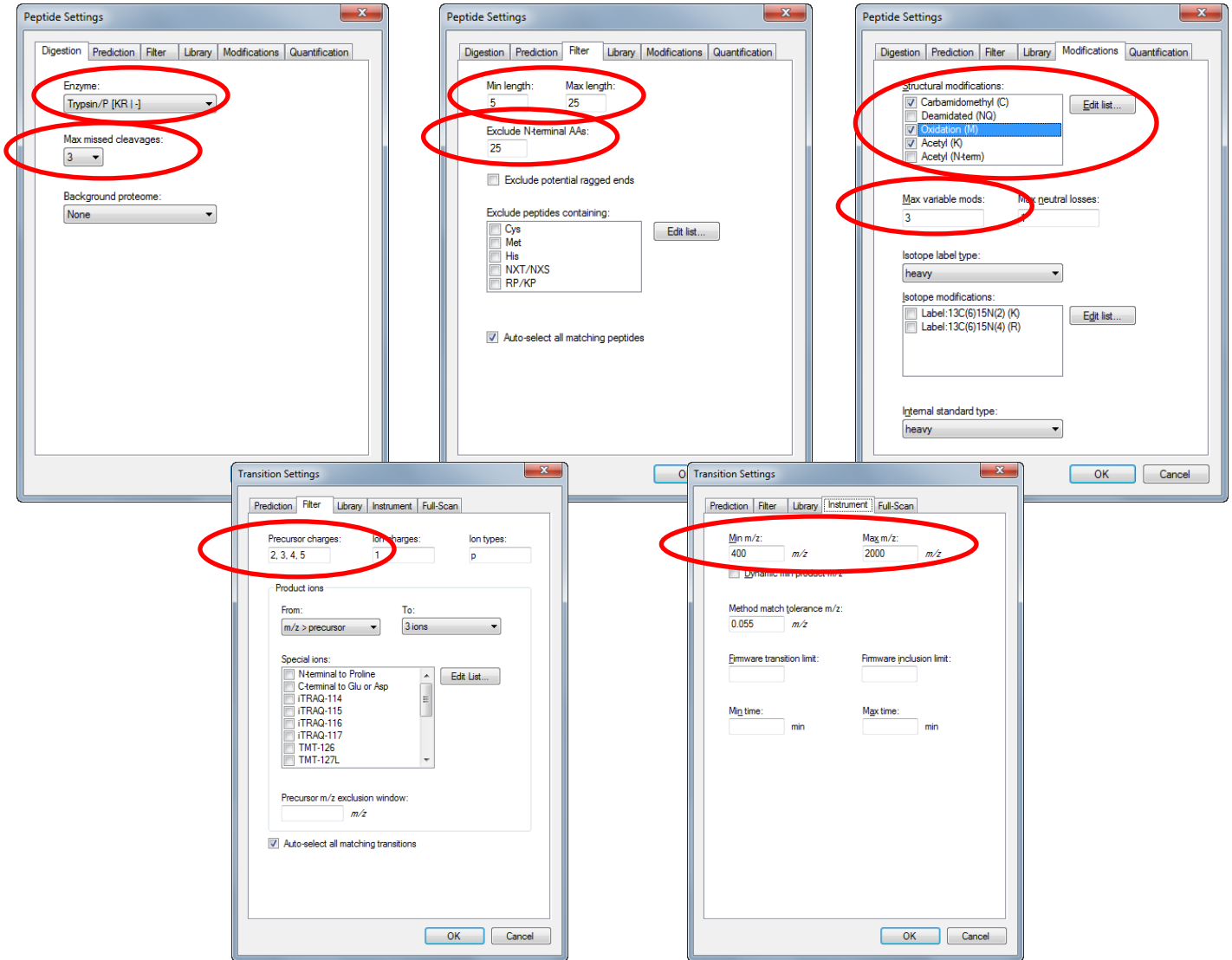
The default settings for creating Phospho-PML are loaded



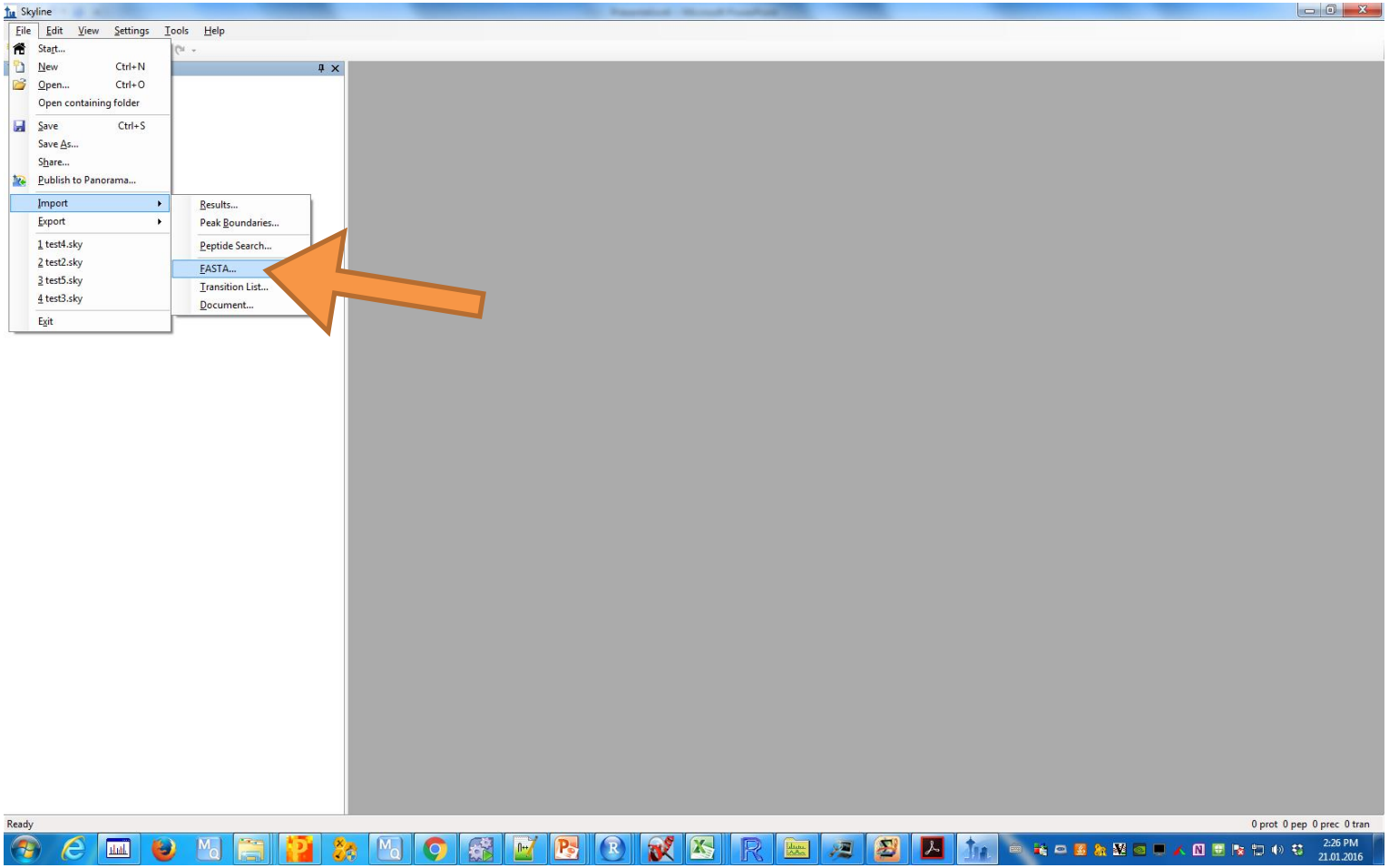
You can manually check and edit Peptide and transition settings



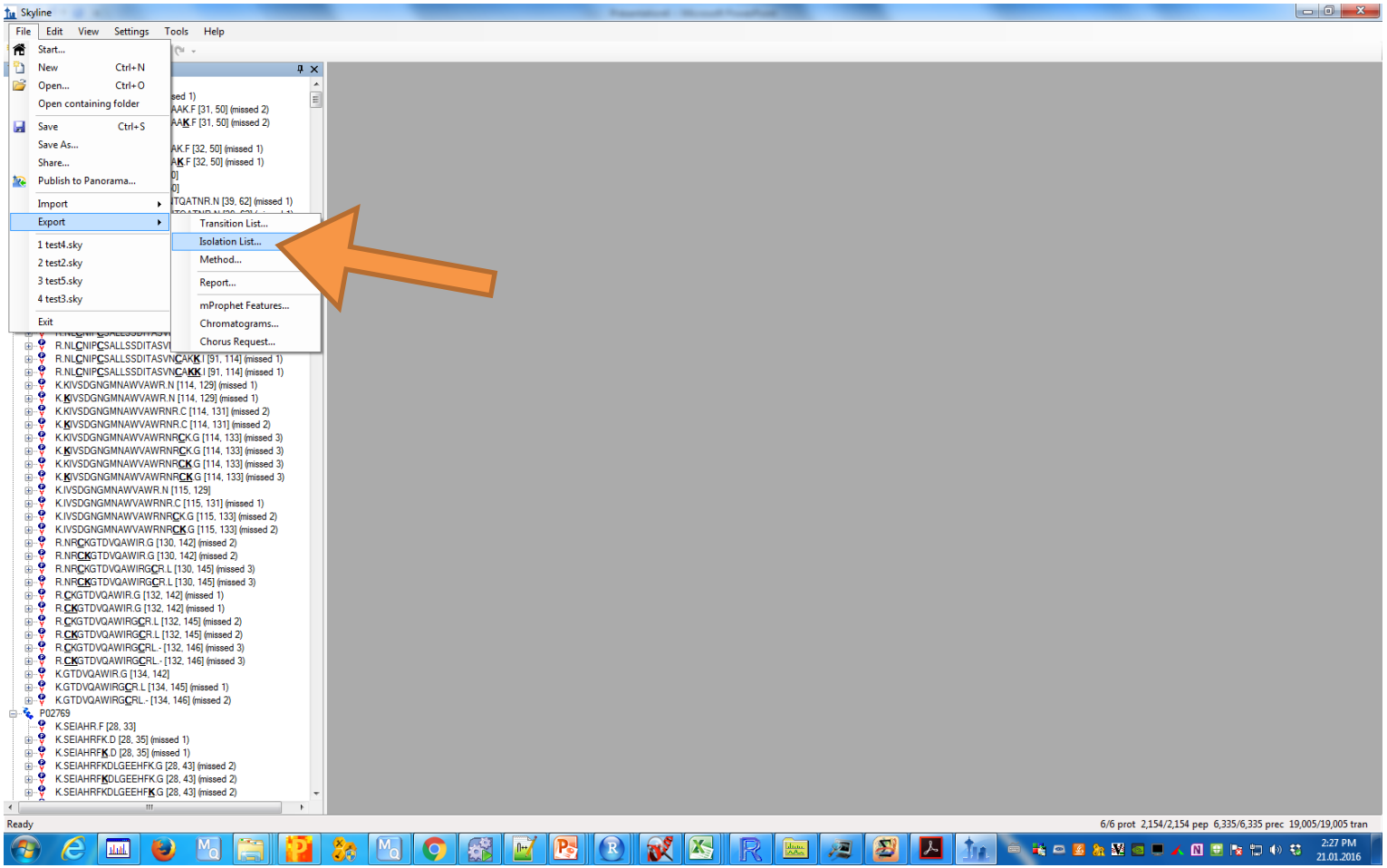
Here the most important parameter



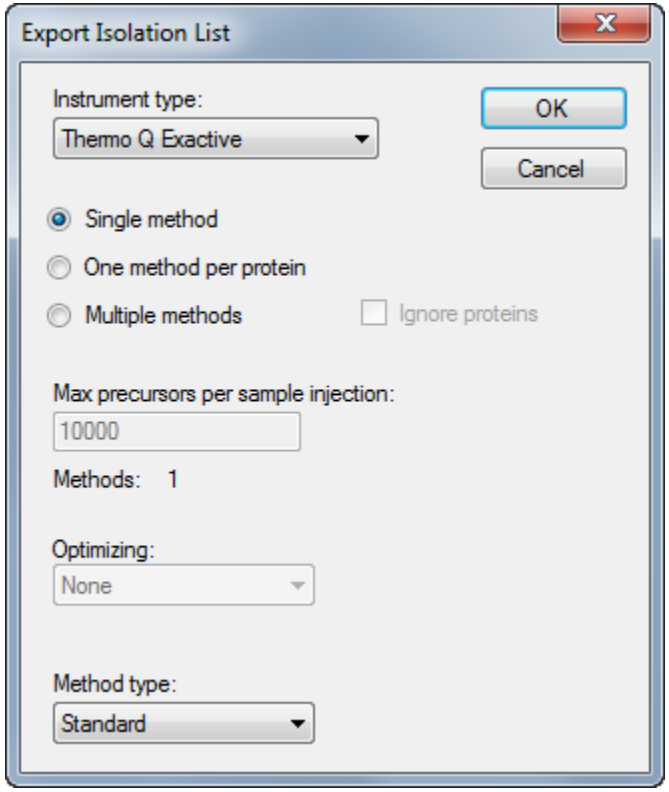
Load the FASTA file containing your protein of interest



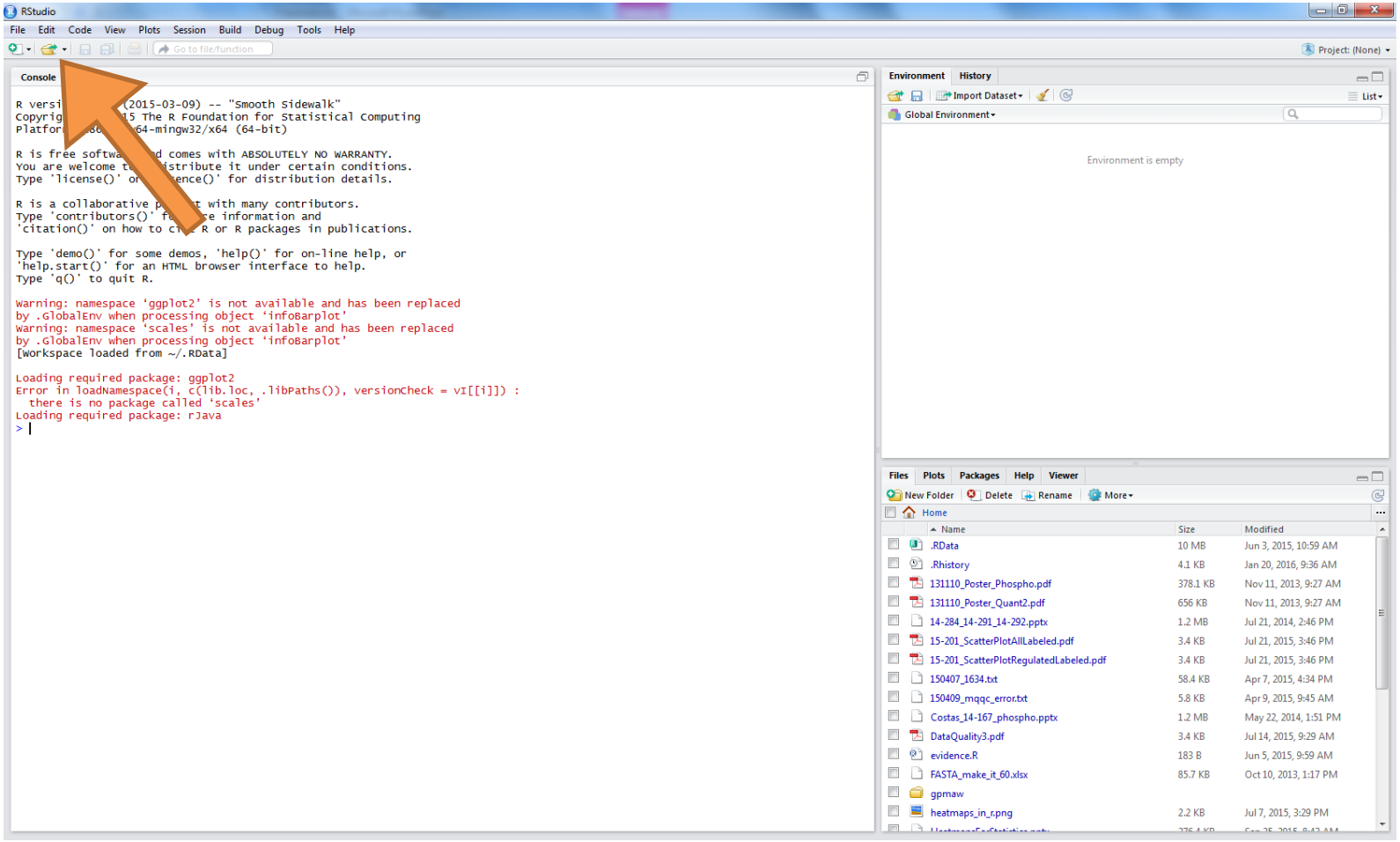
Export your PML as “Isolation List”...



… and save it for “Instrument type” “Thermo Q Exactive”



Open RStudio and load the attached script: “Skyline_ParentMassList_filtering.R”



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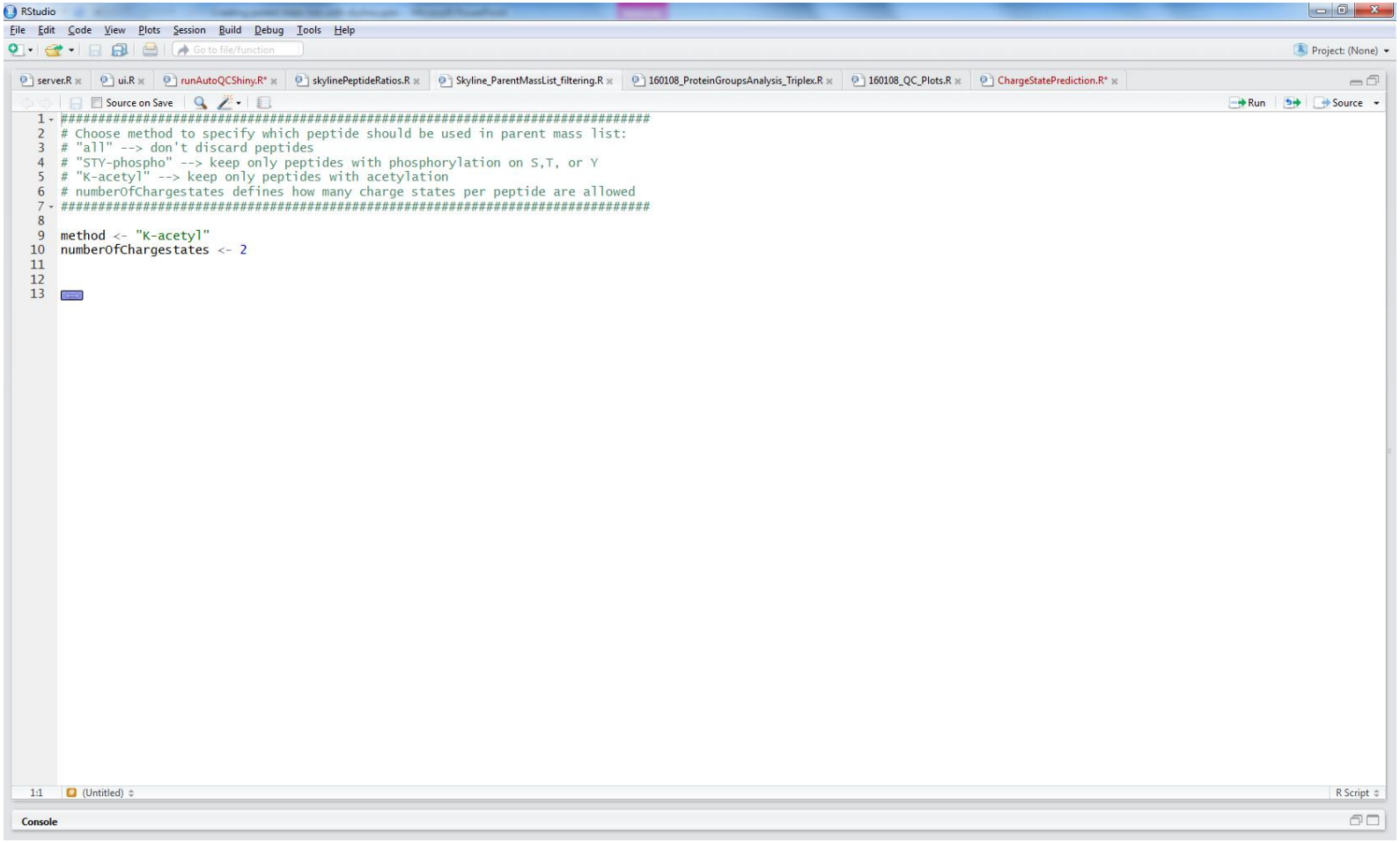
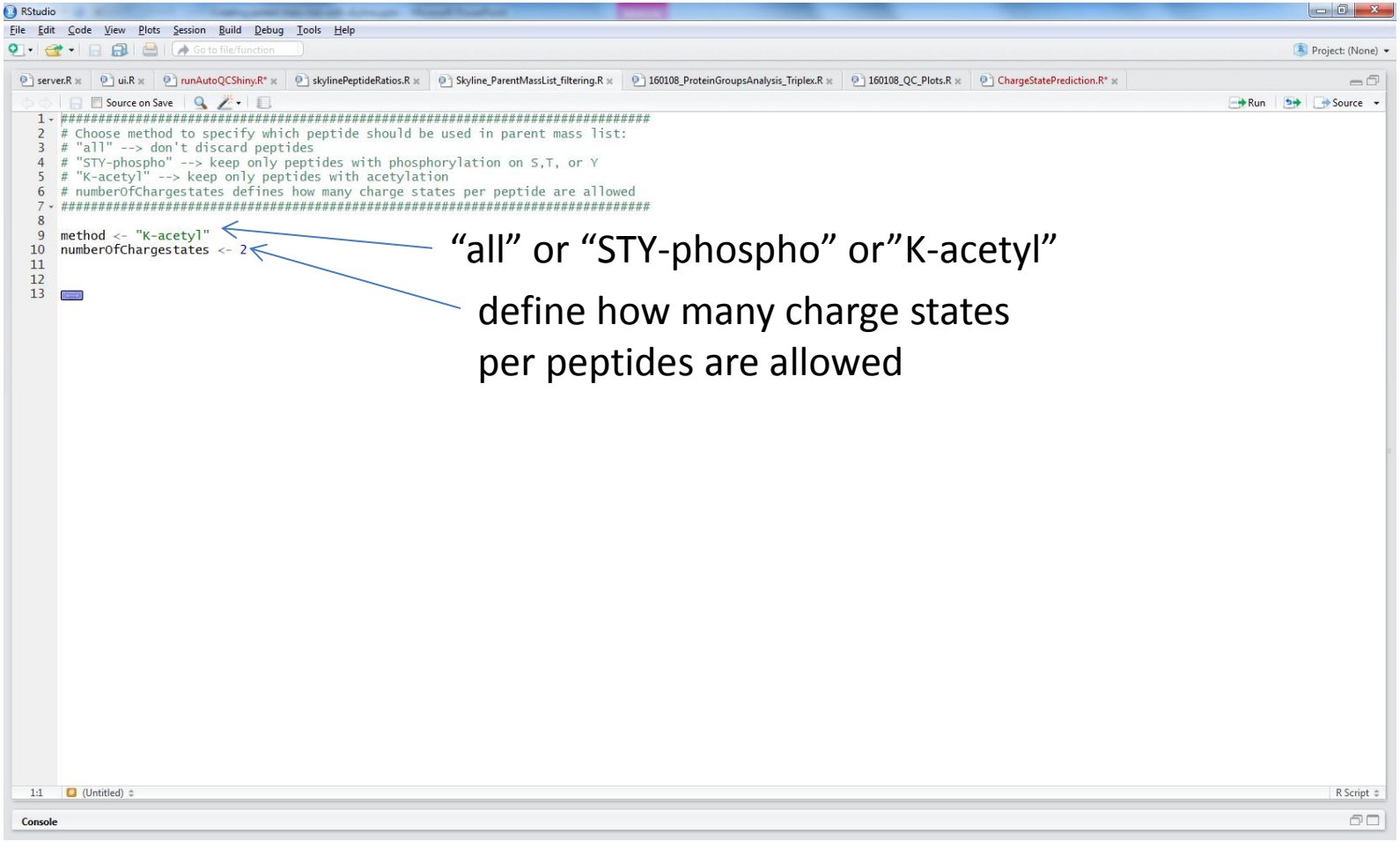


Choose the parameters that should be applied to the dataset:



The Script applies following filtering of data:

- calculate the two most likely charge states for each peptide by sum of H,K,R and only keep these charge states
- delete duplicate m/z entries
- according to specified method:
  - “all” = no further filtering
  - “STY-phospho” = keep only peptides bearing a phosphorylation
  - “K-acetylation” = keep only peptides bearing an acetylation not located at c-terminal lysines
- numberOfChargeStates defines how many chargestates per peptide are allowed:
  - 2: the maximum charge state of a peptide and maximum charge state minus 1
  - 3: the maximum charge state and maximum minus 1 and 2
  - ...

Output file

- output file is stored in same location as input file
- can be directly imported into Q-Exactive
- for other instruments m/z vales need to be copied manually