Selected Reaction Monitoring Workflow

Additional tutorials:

Skyline targeted method development - https://skyline.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view?name=tutorial_method_edit

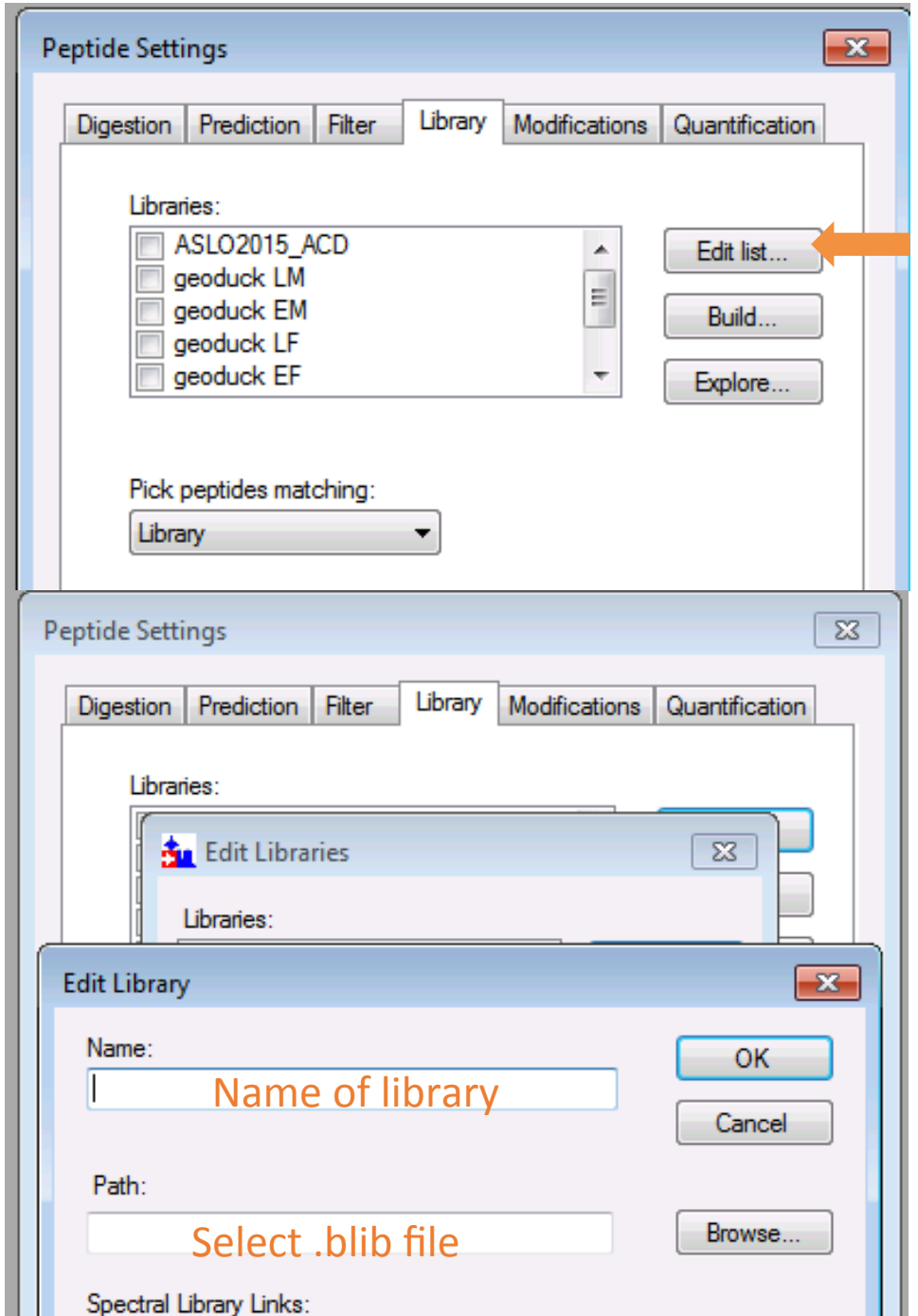
Pecan - https://www.evernote.com/shard/s347/sh/edcb06ab-d008-418f-b28f-52f6614f1c39/2984ab55f427fcfe

1. Use Pecan to create a library for targeted method development in Skyline.

- Input files for Pecan
  - Raw data files converted to mzML files and a file with a list of paths to these files
  - Bacground proteome, if not already included in Pecan
  - List of peptides of interest and a path to this list
  - List of isolation windows
- Run pecanpie to prepare files for Pecan
  - Pecanpie –o [name of directory to be created] –b [name of background proteome] –n [name of blib file to be created] --isolationSchemeType BOARDER --pecanMemRequest 10 [list of mzML paths] [list of peptide list paths] [isolation scheme list]
- Run Pecan
  - Navigate to directory created by pecanpie
  - Submit Pecan jobs: ./run_search.sh
- Output from Pecan is used directly in Skyline

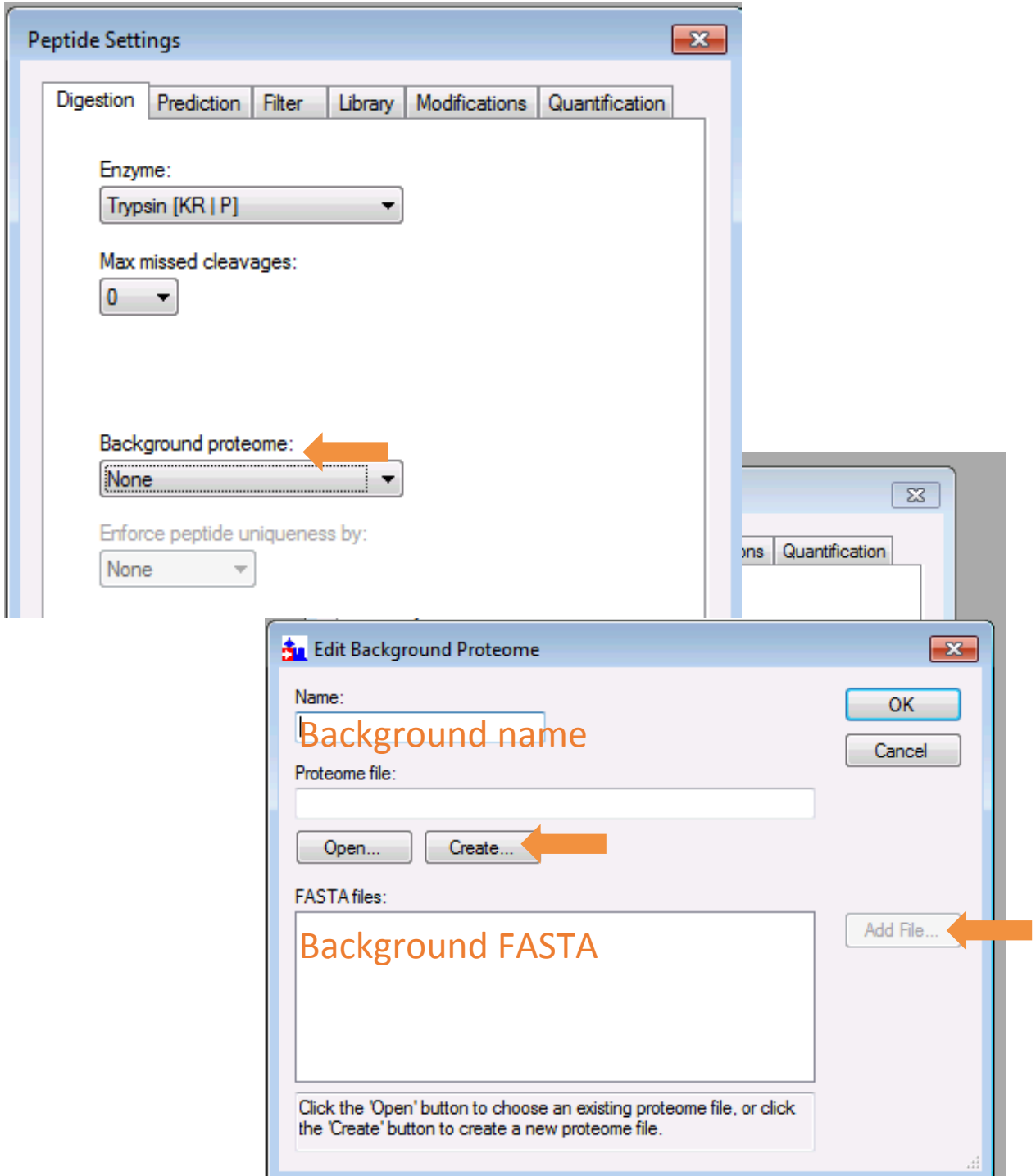
2. Import spectral library into Skyline.

- Settings > Peptide settings > Library
  - Click “Edit list”
    - Click “Add” if your library is not already in the list
- Name your library and select .blib file.
- After clicking ok, select correct library from list.
- Select “Library” under “Pick peptides matching:”



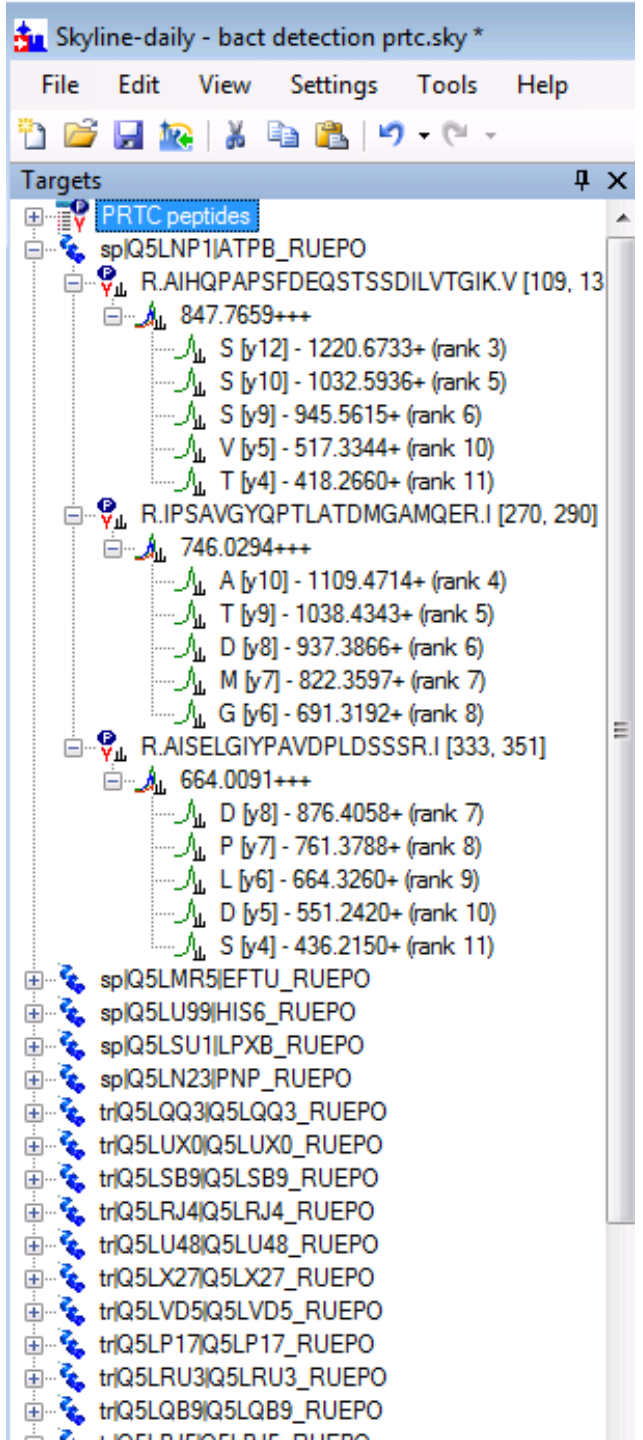
3. Add background proteome to Skyline.

- The background is the fasta version of the background proteome from Pecan (since we analyzed a species not included in Pecan's known list).
- Settings > Peptide settings > Digestion
  - Select "Add" under "Background proteome"
- Name background, click "Create" under "Proteome file" to choose where you will save the background.
- Click "Add file" under "FASTA files" and select your background proteome fasta file.
- Under Prediction tab, make sure "none" is selected for retention time predictor.



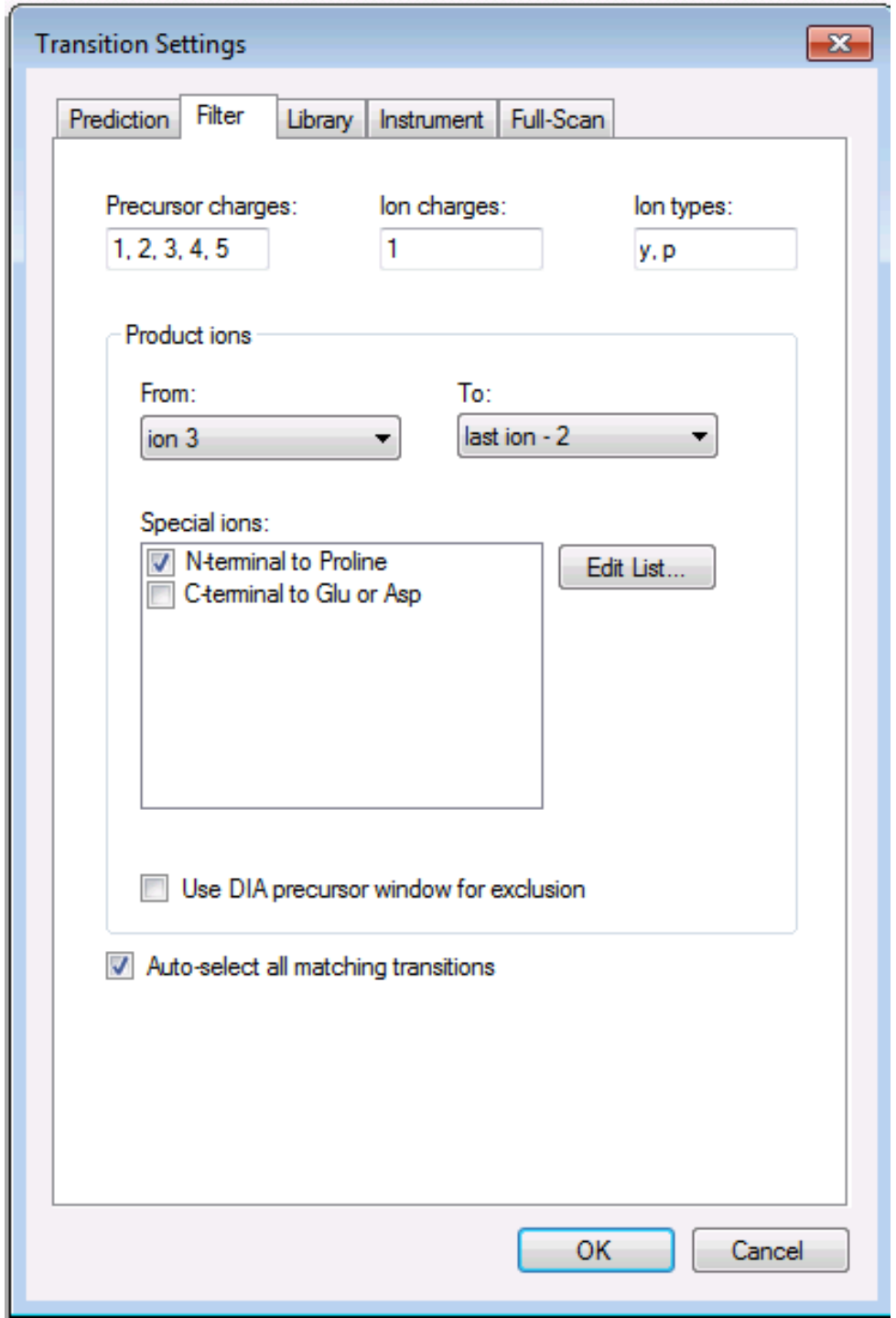
4. Populate the target analyte tree.

- Open the background proteome fasta file and copy all protein sequences.
- Paste the sequences into the lefthand, long window in the main Skyline view.
- Skyline will keep the proteins, peptides, and transitions that match what it finds in the library you gave it.



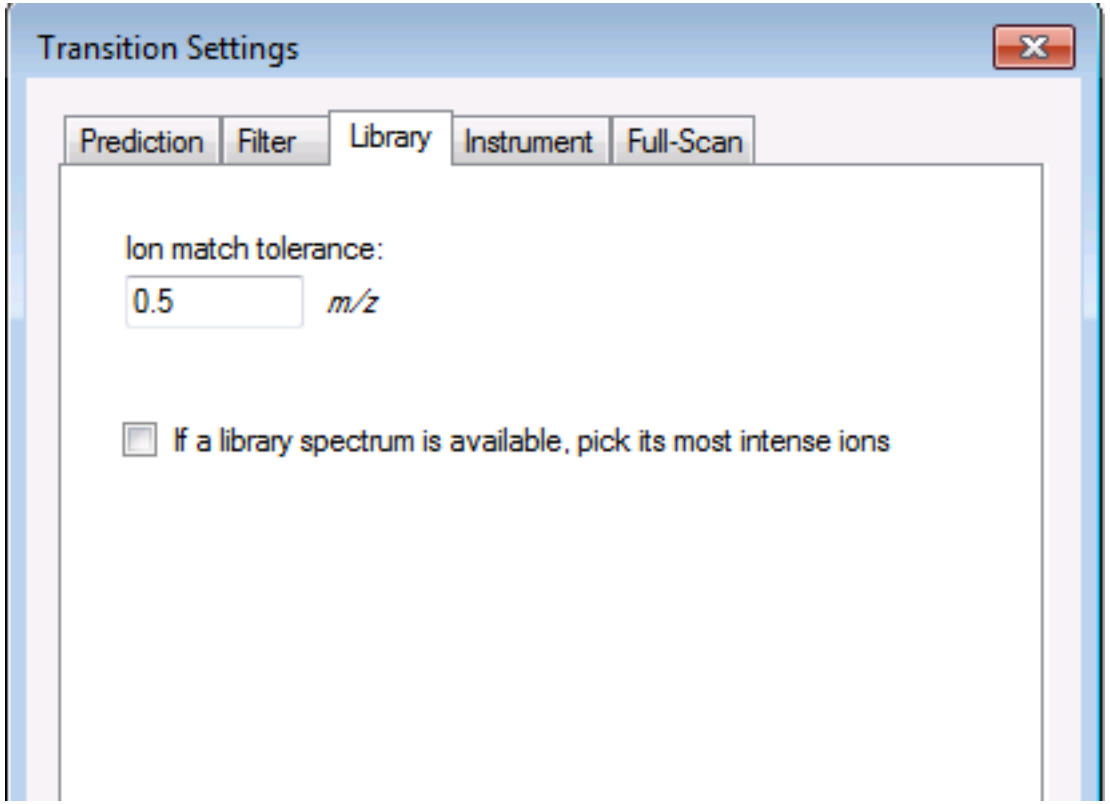
5. Adjust transition settings in Skyline.

- Settings > transition settings > Filter
  - Precursor charges: 1,2,3,4,5
  - Ion charges: 1 (this is the prevalent fragment, additional charges will increase interference)
  - Ion types: y,p (including b ions for Q-Exactive data will increase noise)
  - Product ions: from ion 3 to last ion -2



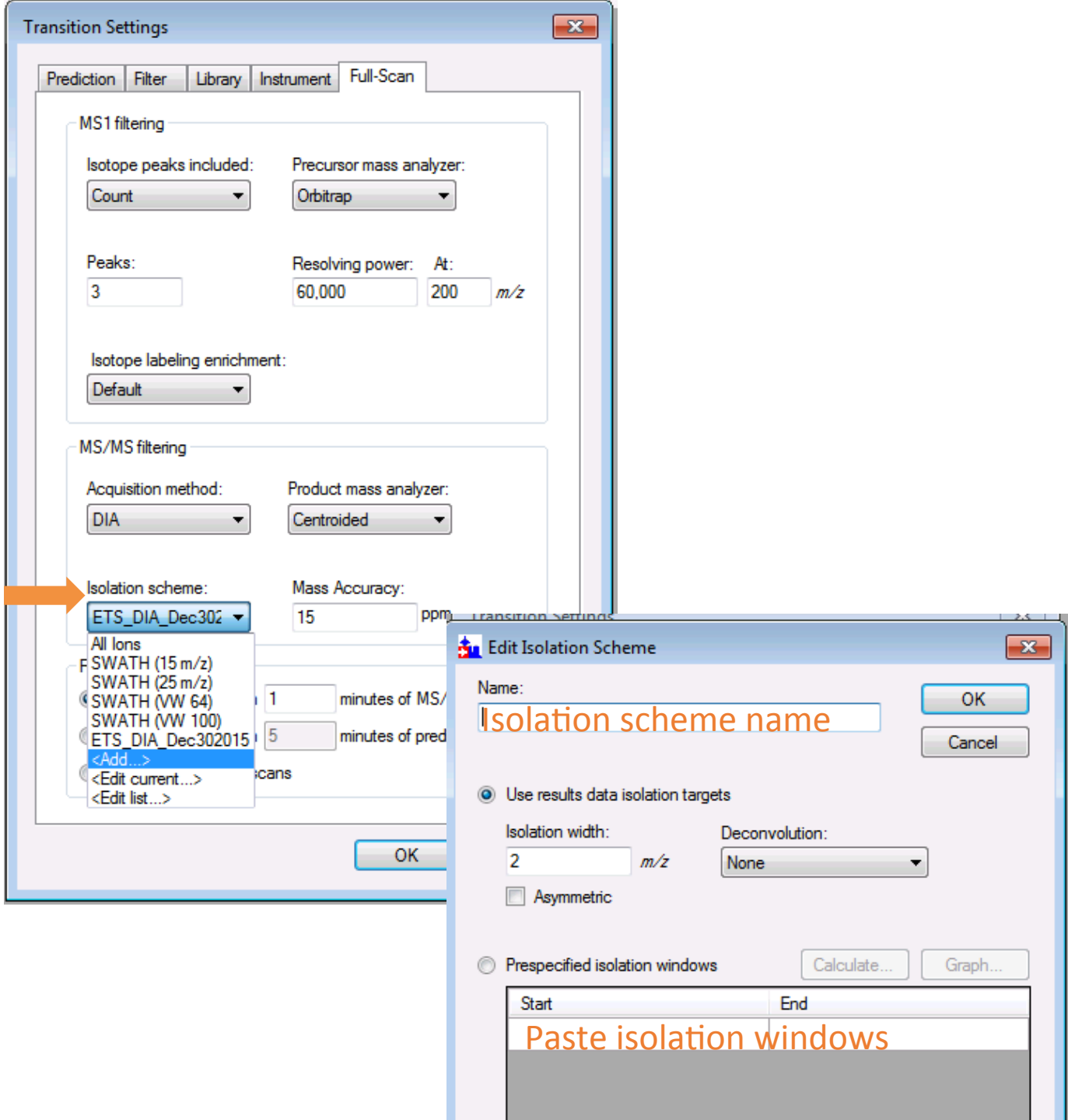
5. Adjust transition settings in Skyline.

- Settings > transition settings > Library
  - Ion match tolerance: 0.5 m/z
  - Unselect “if a library spectrum is available, pick its most intense ion”



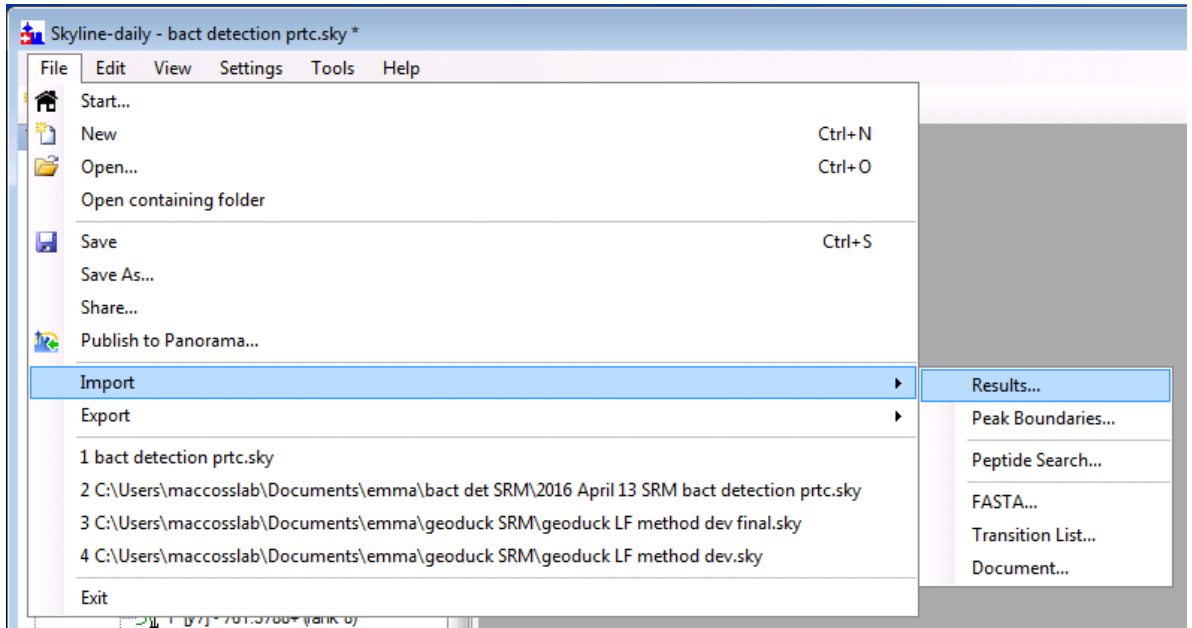
5. Adjust transition settings in Skyline.

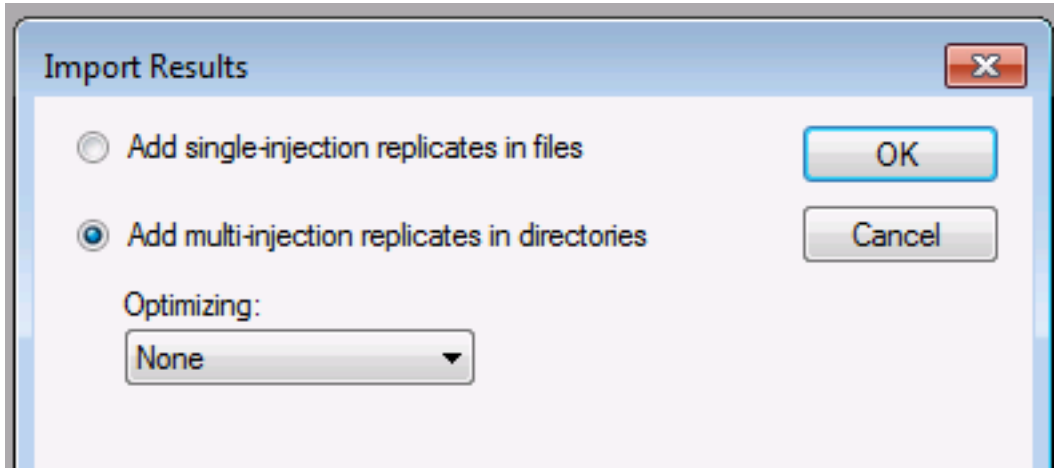
- Settings > transition settings > Full Scan
  - MS1 filtering
    - Isotope peaks included: count
    - Precursor mass analyzer: orbitrap (instrument specific)
    - Resolving power: 60,000 at 200 m/z (experiment specific)
  - MS/MS filtering
    - Acquisition method: DIA (experiment specific)
    - Product mass analyzer: centroided (use with automated peak detection)
    - Mass accuracy: 15 ppm (experiment specific)
    - Isolation scheme: copy and paste list of isolation targets (experiment specific)
  - Retention time filtering
    - Use scans within 1 minute of MS/MS IDs



6. Import raw DIA data into Skyline.

- Organize your raw files in a directory under a main directory called “raw” with sub-directories for each sample/organism that contains all files for that sample.
- In Skyline, File > Import > Results. Select “Add multi-injection replicates in directories”
- Select the “raw” directory and Skyline will recognize subfolders.





7. Refine selection of transitions for targeted method.

- This will depend on the goal for your project. Here are the parameters that I used, with a goal of creating a list of <400 transitions.
- Remove all precursor ions.
- Remove a protein if:
- Remove a peptide if:
  - It has <4 transitions
  - There are >3 peptides per protein and it is one of the lower quality peptides
- Remove a transition if:
  - There is not a clear, single peak in both technical replicates collected in DIA
  - There are >5 transitions per peptide and it is one of the lower quality transitions

8. Export transition list from Skyline to paste into an instrument method.

- File > export > transition list
  - Thermo instrument type
  - Single method
  - Paste transition list into triple quadrupole method