

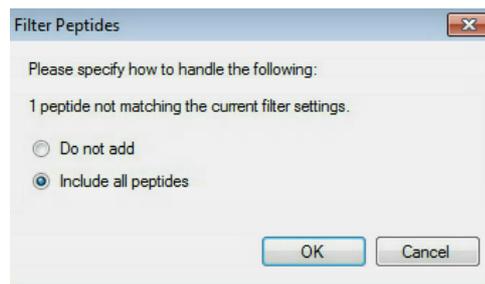
Tutorial 2: Prepare and export an SRM transition list

After setting up all peptide and transition settings in your Skyline document in *Tutorial 1 - Settings*, you can now rapidly generate transition lists for your SRM measurements. Depending on the level of available information you can insert already a transition list into Skyline (Edit → Insert → Transition list), or, if you just know your target proteins and suited representative peptides for those, you can insert peptide sequences (Edit → Insert → Peptides). And finally, if you only have a number of target proteins, you can simply insert a protein list (Edit → Insert → Proteins) and Skyline will automatically select peptides and transitions according to your settings.

In our case study we will monitor 10 target proteins, each represented by 3 proteotypic peptides. The optimal proteotypic peptides have been selected based on previously acquired shotgun and SRM data. In the following tutorial we use Skyline to automatically select the best transitions for each peptide from a spectral library.

- To insert the 30 target peptides into your Skyline document from *Tutorial 1 - Settings* called *TarProCourse_Tut1_Settings.sky*, first open the target peptide list in Excel (*target_peptides.xls* in folder *Tutorial-2_TransitionList*) and copy the column of peptide sequences with “control”+”c”. In Skyline go to “Edit” → “Insert” → “Peptides” → Paste the copied peptide sequences with “control”+”v”.
 - Skyline will search these peptides in the background proteome and automatically add the corresponding protein names.
 - In case you add a peptide sequence which is not unique for a single protein Skyline will ask you how to proceed in these cases.
 - To immediately insert peptides carrying modifications you can indicate these modifications directly in the peptide sequences using squared brackets containing the mass difference. For example, peptides carrying carbamidomethylated cysteines can be inserted as:
LPDGNGIELC[+57]R

As for one peptide no spectral library information is included in the activated “Mtb_proteome_library”, Skyline will give the following error message:



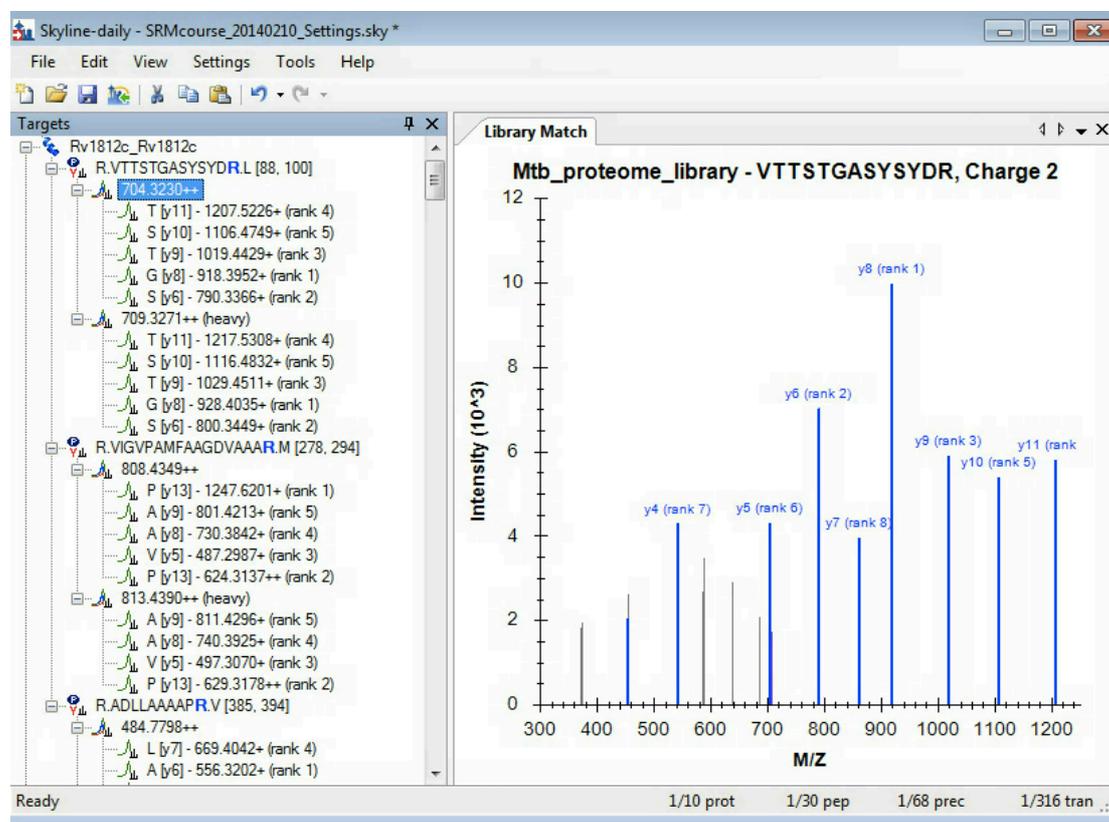
→ Select “include all peptides” → Click “OK” → Click the “Insert” button.

If all settings are setup correctly, Skyline will automatically insert the 30 target peptides under the correct protein name with selected transitions according to the filter and library definition.

In total you should end up with a document containing (see lower right corner):

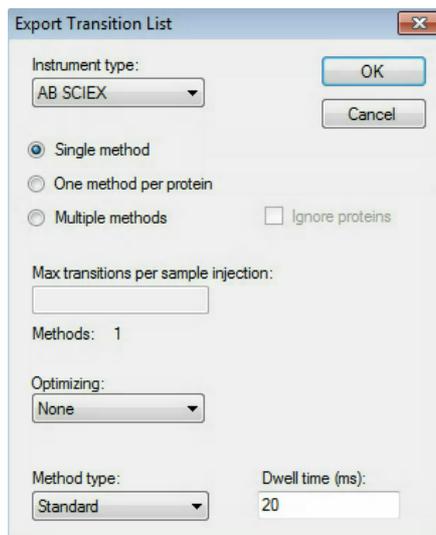
306 transitions, 68 precursors, 30 peptides and 10 proteins

- **Tip!** To see all selected transitions at once go to “Edit” → “Expand all” → “Precursors”. These “Collapse/Expand all” functions are very useful to quickly change views for all proteins/peptides/precursors.
- **Tip!** Hover with the cursor over the protein/peptide/precursor/transition to get specific information on the respective item.
- **Tip!** The numbers in the square brackets behind the peptide sequence indicate the position of the peptide in the protein.
- **Tip!** Right-click on protein/peptide/precursor to see several options for refining and/or modifying. If you select “Pick Children” on either level, you can add or remove selected peptides per protein, precursor charge states/isotypes per peptide, and transitions per precursor. Click on the funnel icon to see all options.
- **Tip!** For each target peptide you can view the corresponding MS2 spectrum of the library via the MS/MS Spectrum tab (usually by default visible, if not, go to “View” → “Library Match”). To view also other ion types than y-ions in the MS2 spectrum right-click on the spectrum and select any additional ion types you are interested in.
- Your Skyline document should now look like this:

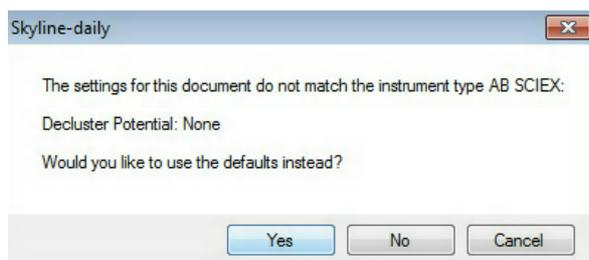


- Go through all target peptides and check the automatically selected transitions and the quality of the MS2 library spectra.
Note! The spectra in the Mtb_proteome_library contain profile data and therefore do not work so well with Skyline's ion ranking. This is because Skyline only considers a limited number of peaks per spectrum to optimise performance and because of this, for some spectra, the lower intense peaks are not added to the transition list.
- Save the Skyline file as *TarProCourse_Tut2_MtbProteomeLib.sky* in folder *Tutorial-2_TransitionList*.
- Export a transition list file:

→ “File” → “Export” → “Transition list” → Select: instrument type “AB SCIEX”, “Single method”, Method type “Standard”, dwell time (ms) of “20” → Click “OK”.



- Skyline will notify you that no declustering potential has been defined in your transition settings.



→ Select “No” to keep the settings, because we do not want to work with a declustering potential at the moment.

→ File Name saved: *TarProCourse_Tut2_MtbProteomeLib_TransList.csv*.

Note! Skyline can also export native instrument method files (File → Export → Method), though for this function Skyline must be running on a computer with the instrument software installed (vendor-dependent).

- Now open the exported .csv file in Excel and make sure you understand which information is given in each column. (To split the data by comma in Excel: Data → Text to Columns.)

Exercises

1. If a dwell time of 5 ms or 20 ms is used for each transition, what would be the resulting cycle times for the generated transition list in a non-scheduled SRM run? (Assuming that no additional time is needed to switch between transitions.) Are those two cycle times suitable and what is important to consider in this context?
2. Into how many methods do you need to split this transition list to end up with a dwell time of 15 ms and a maximal cycle time of 1.5 s? Generate those transition lists with Skyline using the “Multiple methods” option in the transition list export.
3. Why is for the peptide VPAARPDVAVLDVR only a single transition automatically selected? Is this suitable for an SRM measurement? What can you do?
4. For which proteins are two directly flanking peptides selected?

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