

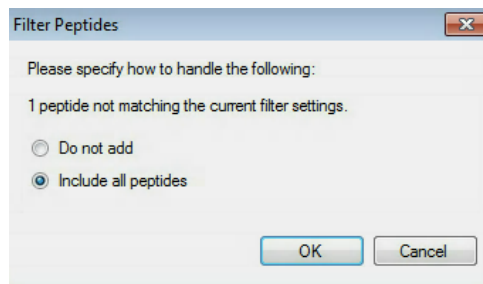
## Tutorial 2: Prepare and export an SRM transition list

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 simply 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 by SRM 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.

- Insert the 30 target peptides into Skyline. First open the target peptide list in Excel (*target\_peptides.xls* in folder *Tutorial-2\_TransitionList*) and copy the peptide sequences with “control”+”c”. In Skyline go to: Edit → Insert → Peptides → paste the copied peptides with “control”+”v”.
  - Skyline will search these peptides in the background proteome and automatically fill in 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 further 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

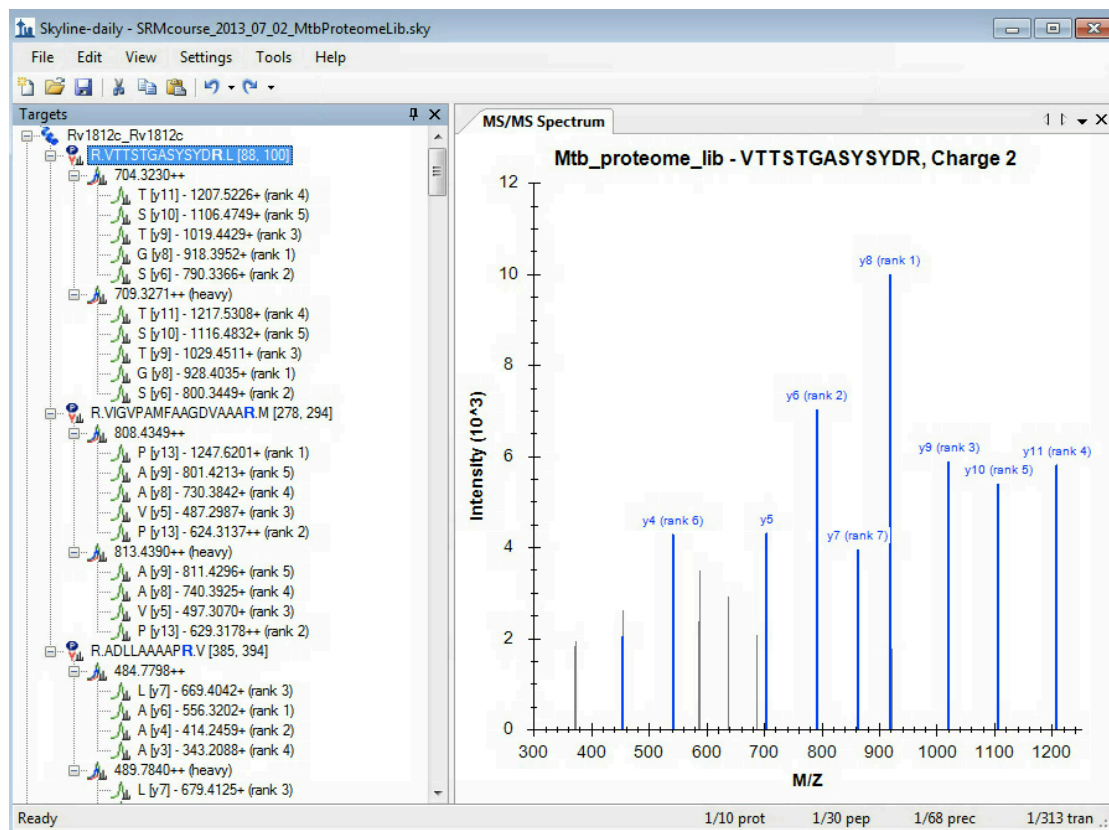
As for a single peptide no spectral library information is included in the activated Mtb proteome library Skyline will give the following error message:



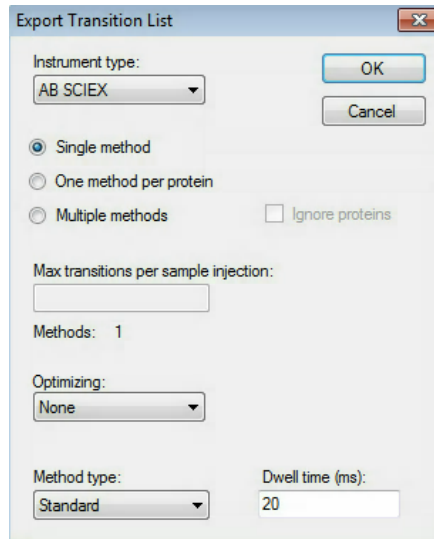
→ Select “include all peptides”.

- Select → Insert
  - 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 313 transitions, 68 precursors, 30 peptides and 10 proteins (see lower right corner).
    - **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 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 → MS/MS Spectra). To view also other ion types than y-ions in the MS2 spectrum right-click on the spectrum and select additional ion types.
- 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!** Some of the spectra in the Mtb\_proteome\_library contain profile data, and, therefore, do not work so well with Skyline's ion ranking. There is a 50-peak cut-off imposed for performance reasons and this causes that in some spectra the peak picking is not optimal.
- Save the Skyline file as *SRMcourse\_20130715\_MtbProteomeLib.sky* in folder *Tutorial-2\_TransitionList*.
- Export a transition list file:  
→ File → Export → Transition list → select “AB Sciex”, “Single method”, “Dwell time” = “20s” → OK → Name: *SRMcourse\_20130715\_MtbProteomeLib\_Qtrap.csv*.



- Note: Skyline can also export native instrument method files (File → Export → Method), though 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? 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?

We would like to thank Prime-XS and SystemsX for supporting the SRM Course 2013.

