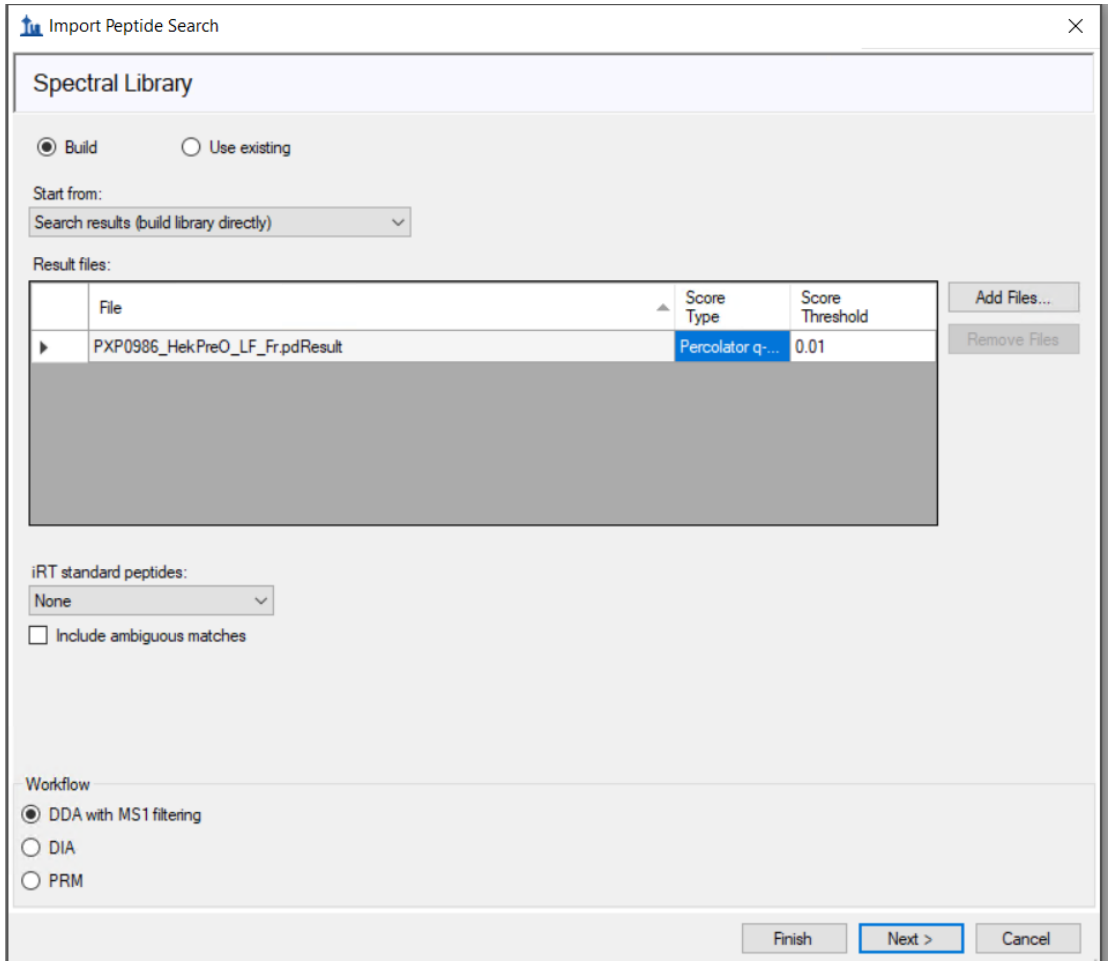
Import Peptide Search



Skyline claims to use ‘Percolator q-value’ as score type, which obviously is not the case (see next slide)

Inside the pdResult file

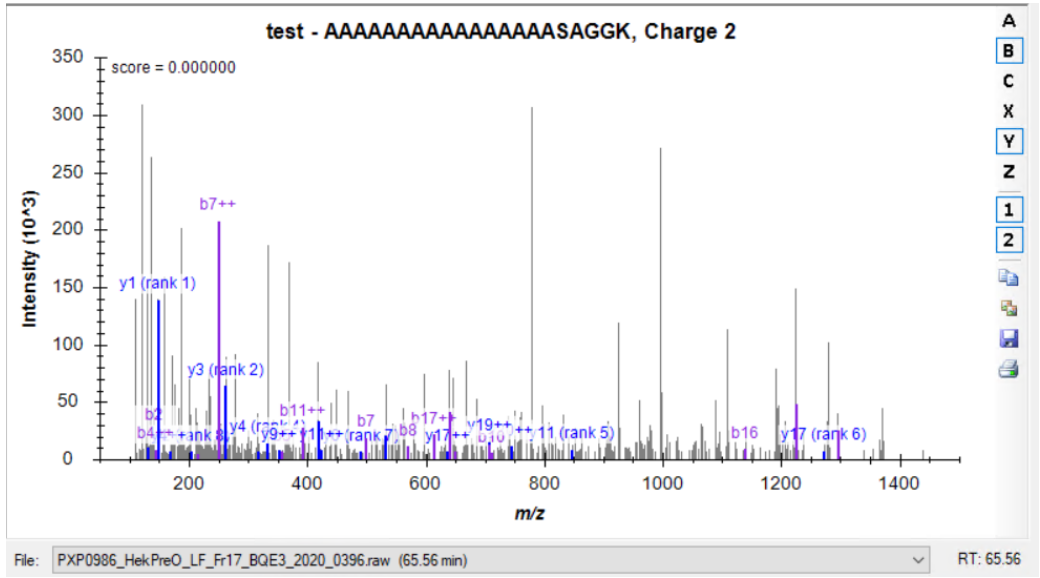
the first peptide: AAAAAAAAAAAAAAAASAGGK

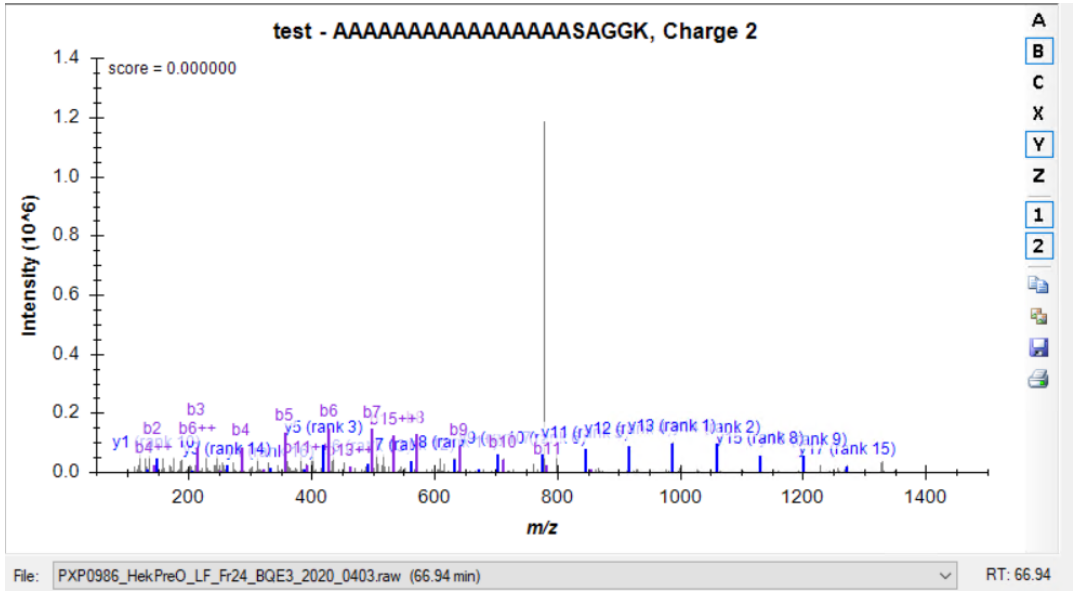
RetentionTime	55.102732	65.558789	66.93995
FirstScan	38413	41927	41923
LastScan	38413	41927	41923
SpectrumFileName	PXP0986_HekPreO_LF_Fr11_BQE3_2020_0389.raw	PXP0986_HekPreO_LF_Fr17_BQE3_2020_0396.raw	PXP0986_HekPreO_LF_Fr24_BQE3_2020_0403.raw
SpectrumFileId	614	620	627
StudyFileId	F1.11	F1.17	F1.24
ExcludedBy	3	3	-1
Checked	0	0	0
PeptidesMatched	223	224	211
XCorr	1.647716	2.299643	5.484662
PercolatorqValue	0.3913	0.3832	0.000006
PercolatorPEP	1.0	1.0	0.0

TargetPsms table opened in a Jupyter Notebook (Python) with sqlite and pandas

There are 3 PSMs for this peptide, two of which are clearly wrongly assigned and one that looks great, based on that same Percolator q-value which is called ‘PercolatorqValue’ in the table
So everything would work well if Skyline indeed would score by that value, only 1 spectrum would be left and it would very likely be the right one.

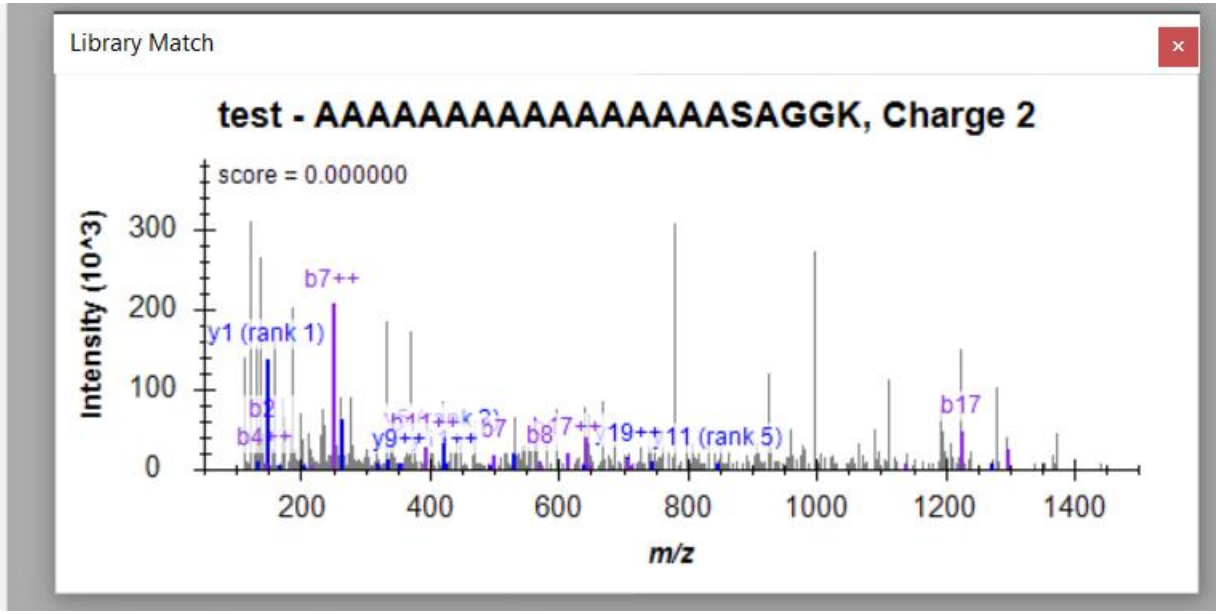
Skyline import result: all three spectra are imported - here just 2 of the 3 as example





Not surprisingly the spectrum at 65.56 min is useless and the one at 66.94 min looks great (ok, could have fragmented a bit harder...). Both have score ‘0.00000..’ even though we know we provided the q-values

Skyline library match



Not a good idea to pick this spectrum (the first in the list..?) as the library match

Skyline library (blib)

[10]:

	150949
id	400569
peptideSeq	AAAAAAAAAAAAAAAASAGGK
precursorMZ	778.922278
precursorCharge	2
peptideModSeq	AAAAAAAAAAAAAAAASAGGK
prevAA	-
nextAA	-
copies	3
numPeaks	645
ionMobility	0.0
collisionalCrossSectionSqA	0.0
ionMobilityHighEnergyOffset	0.0
ionMobilityType	0
retentionTime	65.558789
startTime	None
endTime	None
totalIonCurrent	13232063.447754
moleculeName	
chemicalFormula	
precursorAdduct	
inchiKey	
otherKeys	
fileID	17
SpecIDinFile	147.1208431
score	0.0
scoreType	1

And sure enough the same crappy spectrum is the only one that ends up in the (non-redundant) blib

Opening the blib in Python with sqlite and pandas