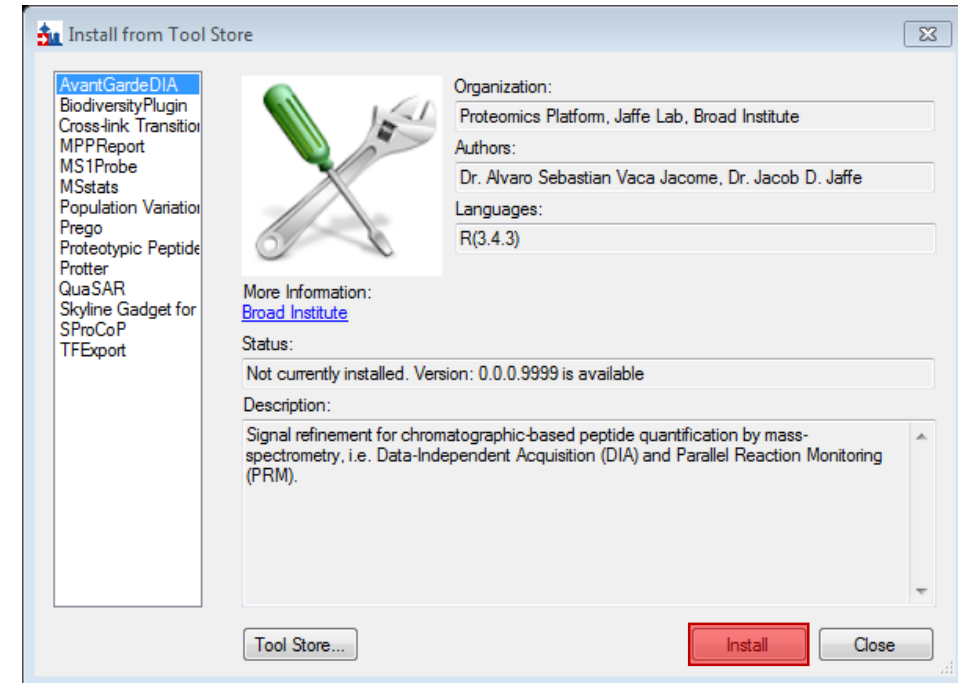
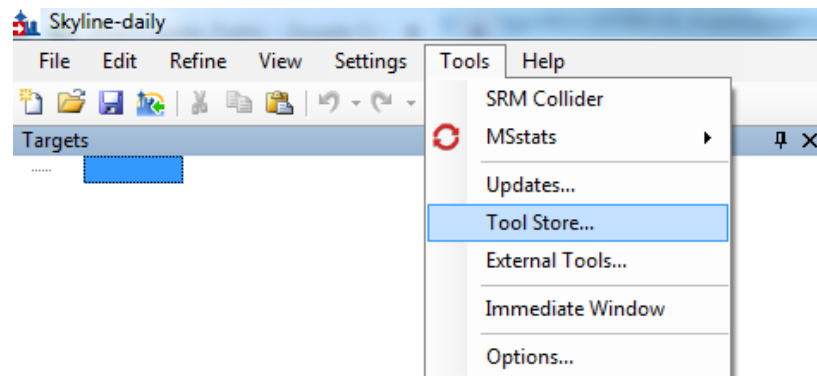


How to run *Avant-garde*

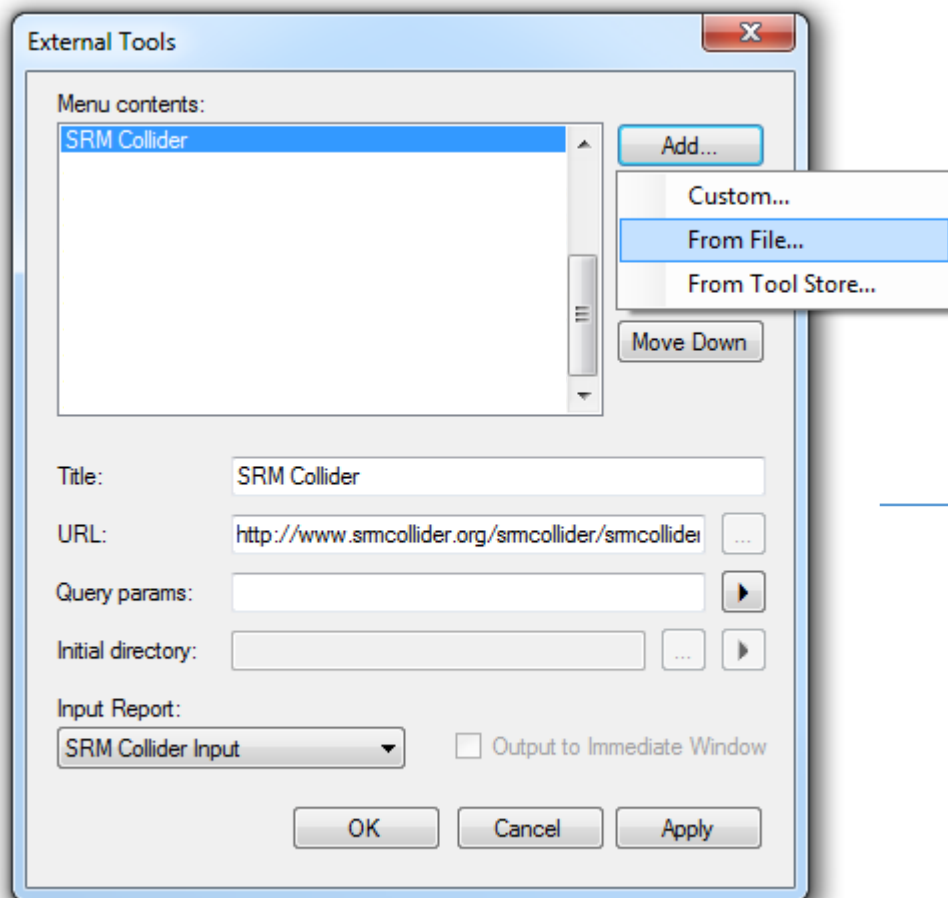


Install Avant-garde from the Skyline Tool Store

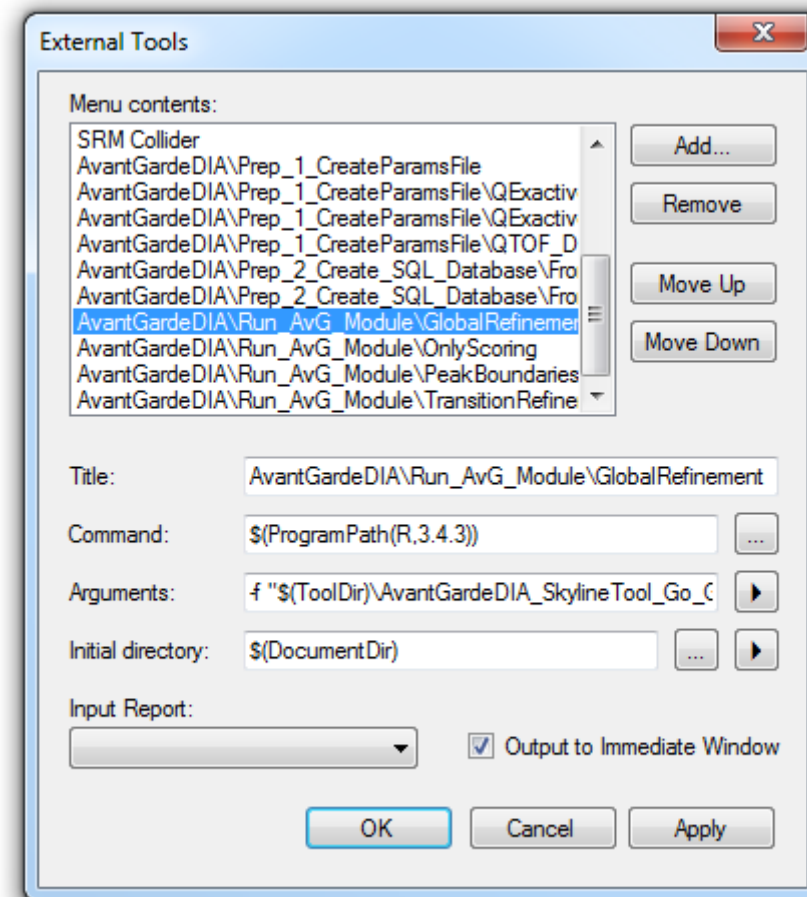


Install Avant-garde from a zip file

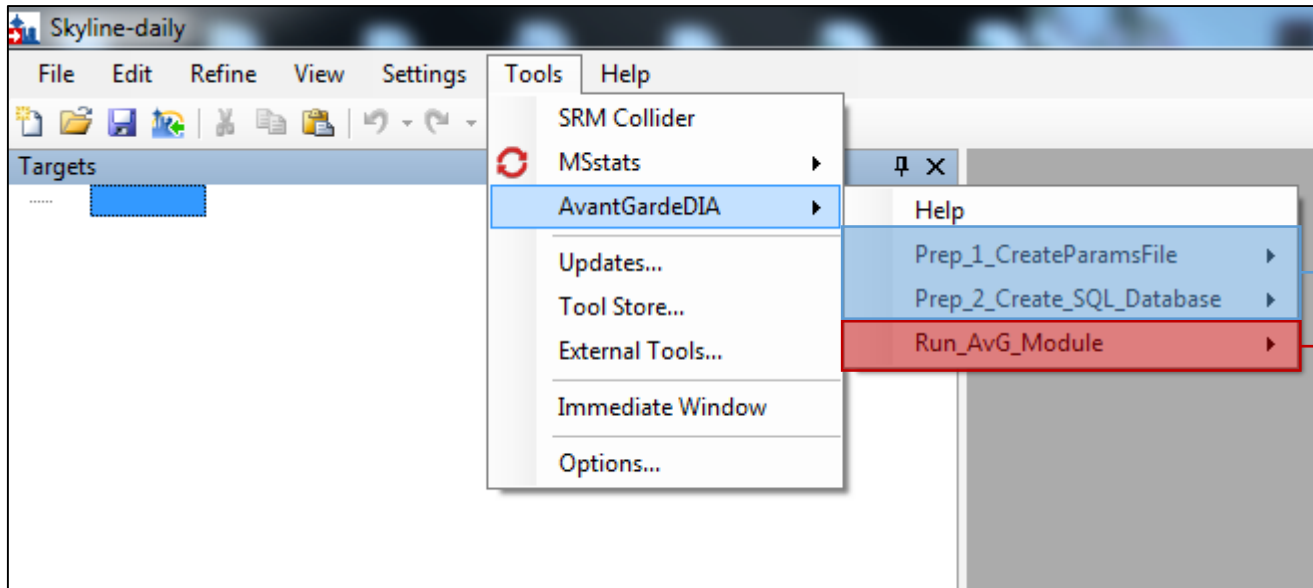
Go to: Tools → External tools



Select Zip file
Of Avant-garde DIA
Skyline External tool



Overview



Preparation scripts (Run only once):

- **Create Parameters File:**
 - Writes parameters file (.R file)
 - User-defined parameters
- **DB:**
 - Creates SQLite database containing data and metadata

Avant-Garde DIA modules:

'GlobalRefinement' for

- 1) transition refinement
- 2) peak boundaries refinement
- 3) peak rescoring

'TransitionRefinement' for

- 1) transition refinement
- 2) peak rescoring

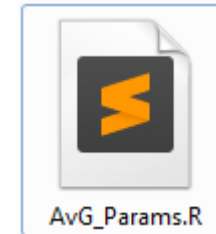
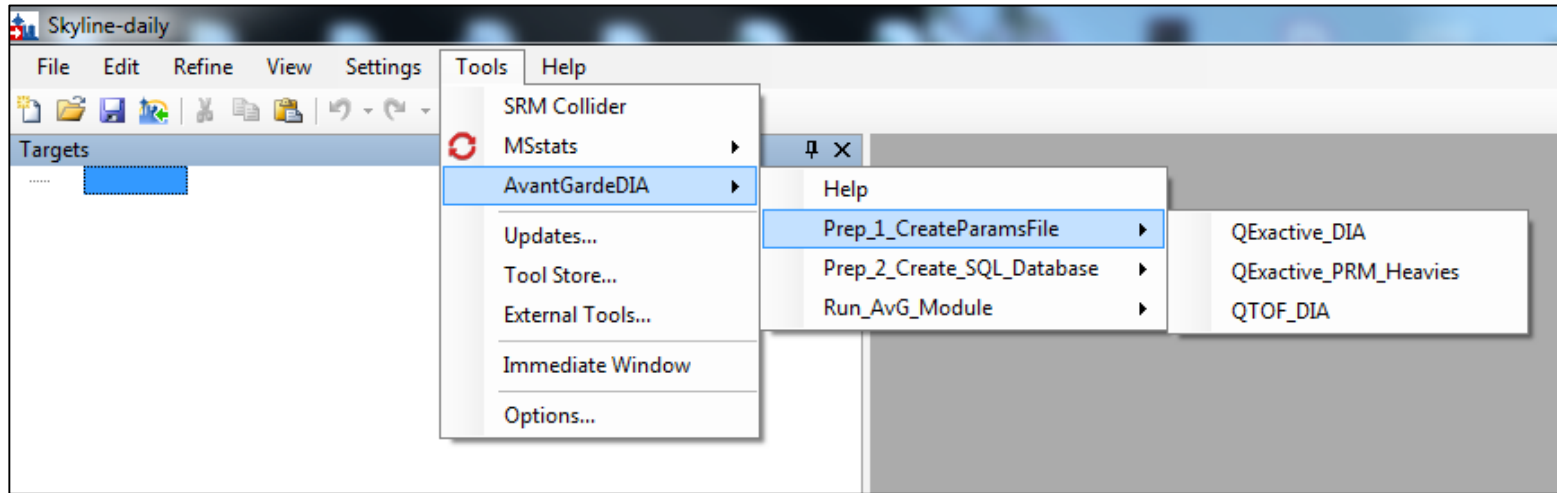
'PeakBoundariesRefinement' for

- 1) peak picking
- 2) peak rescoring

'OnlyScoring' for

- 1) peak rescoring

Preparation Step 1: Create parameters file



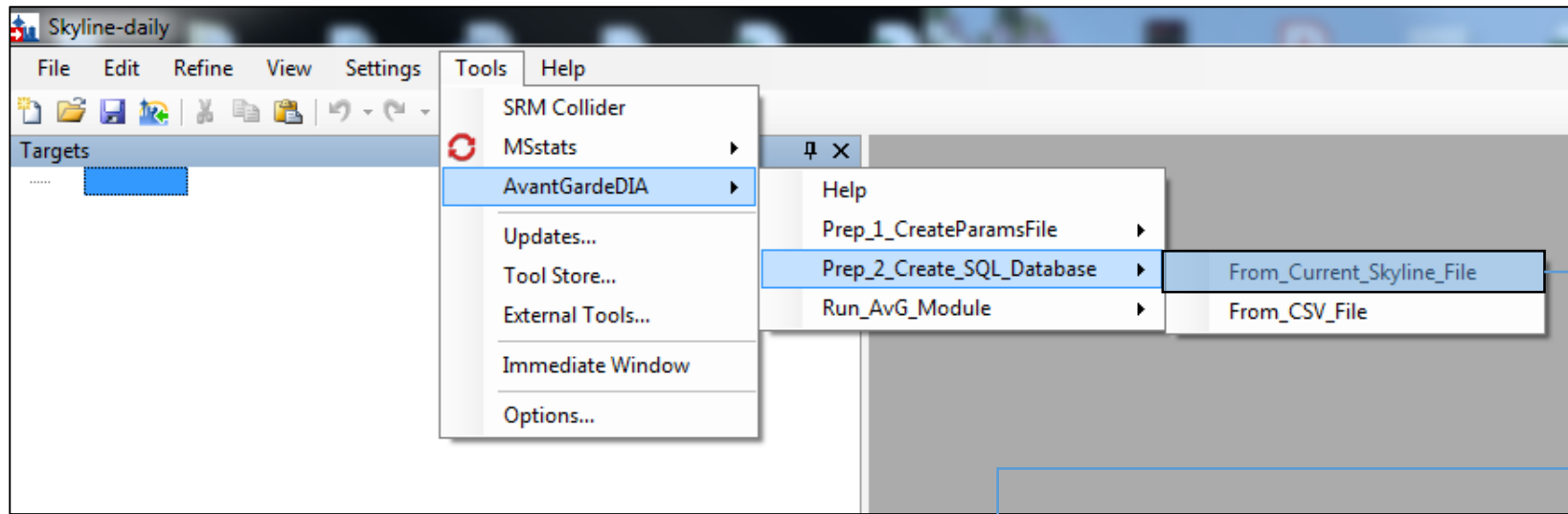
- This step creates:
 - Parameters file (.R file) with correct format
 - Folders for outputted intermediary data and results
- Default parameters files for three applications are given here
 - Qexactive_DIA: DIA dataset analyzed on a Q-Exactive series instrument
 - Qexactive_PRM_Heavies: PRM dataset analyzed on a Q-Exactive series instrument containing light and heavy peptides for all analytes.
 - QTOF_DIA: DIA dataset analyzed on a Q-TOF instrument (SWATH)
- All files will be written to the "AvantGardeDIA" folder in the same location as the Skyline File

Preparation Step 1: List of parameters

Parameter	Default	Comment
Name_Tag	'Name_Tag'	Short Name that will be added to all output files. (Must be in quotes)
MinimalInitialNumberOfTransitions_Filter	5	Peptides with less than N transitions will be removed and not included in the SQLite Database
RemoveTransitionBelowOrdinal_Filter	3	Transition ordinal below or equal to this value will be removed (for example if Max_TransitionIonOrdinal=3 transitions b1,b2,b3,y1,y2,y3 are removed)
RemoveSharedTransitionsBetweenLightAndHeavy_Filter	TRUE	Should be TRUE for DIA,FALSE for PRM.
ReadNumberOfLines	20000	The CSV file is read in chunks. This parameter sets the number of lines in each chunk.
NonZeroBaselineChromatogram	FALSE	TRUE for data having high noise baseline level (e.g. SWATH data on Q-TOF), FALSE (noise-reduced data, e.g demultiplexed Overlap DIA Data acquired on Q-Exactive series)
MinimalNumberOfTransitionsAfterOptimization	4	The optimization will find the best solution that will have at least this number of transitions.
KeepPeptidesWithLowerNumberOfTransitions	FALSE	Keep (or not) a peptide if it has a lower number of transitions than the limit above (recommended FALSE for DIA). The transition refinement will not be done on these peptides but the peak boundary refinement and scoring will be performed.
TopN_RankedbyIntensity	6	Number of most intense transitions in teh spectral library (N) among which at least n transitions need to be present.
MinimalNumberOfTransitionsAmongTopN	2	Number of transitions (n) that need to be present among the N most intense transitions in the spectral library.
alpha	0.005	AvG Fitness score: Intensity score coefficient
Beta	0.05	AvG Fitness score: Mass error score coefficient
SpectralLibraryDotProduct_limit	0.7	Library dot product cut off
MassError_Tolerance	10	Mass error tolerance (in PPM)
MassError_CutOff	20	Mass error cut-off (in PPM)
MinimalNumberOfConsecutivePoints	3	Minimal number of consecutive points above the limit of noise to consider a signal as a potential peptide.
MinimalIntensityPercentagePerTransition	3	Minimal percentage of the maximum intensity of each transition below which any point is no longer considered.
UseHeavyPeakBoundariesForLight	FALSE	Use the heavy peptide peak boundaries for the light peptides.

Default values for
“QExactive DIA”
example

Preparation Step 2



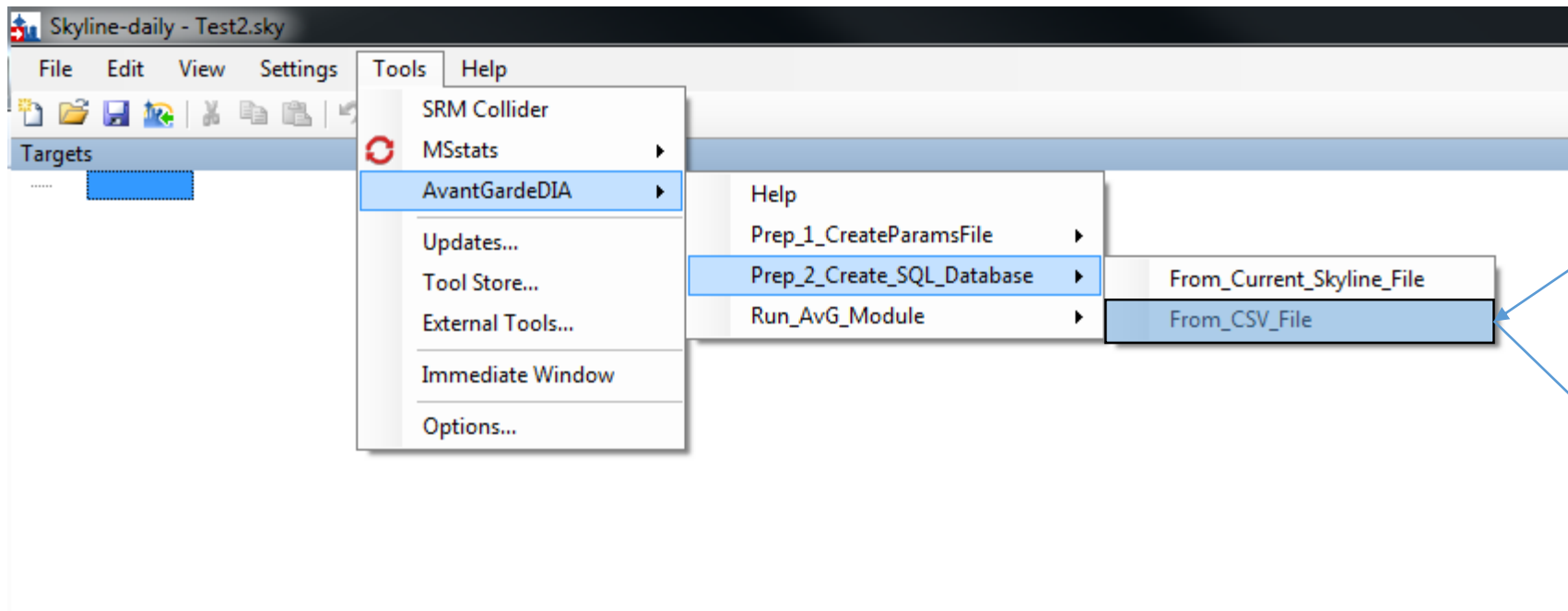
- Exports CSV file into a temporary folder
- Transforms CSV file into a SQLite file
- Works better for small files (PRM)

Preparation Step 2

For large dataset (DIA):

- Export → Report → AvantGardeDIA_Export.csv inside the “AvantGardeDIA” folder that was created in preparation step 1
- Launch AvantGardeDIA → Prep2_Create_SQL_Database → From_CSV_File
 - This transforms the CSV file into a SQLite file

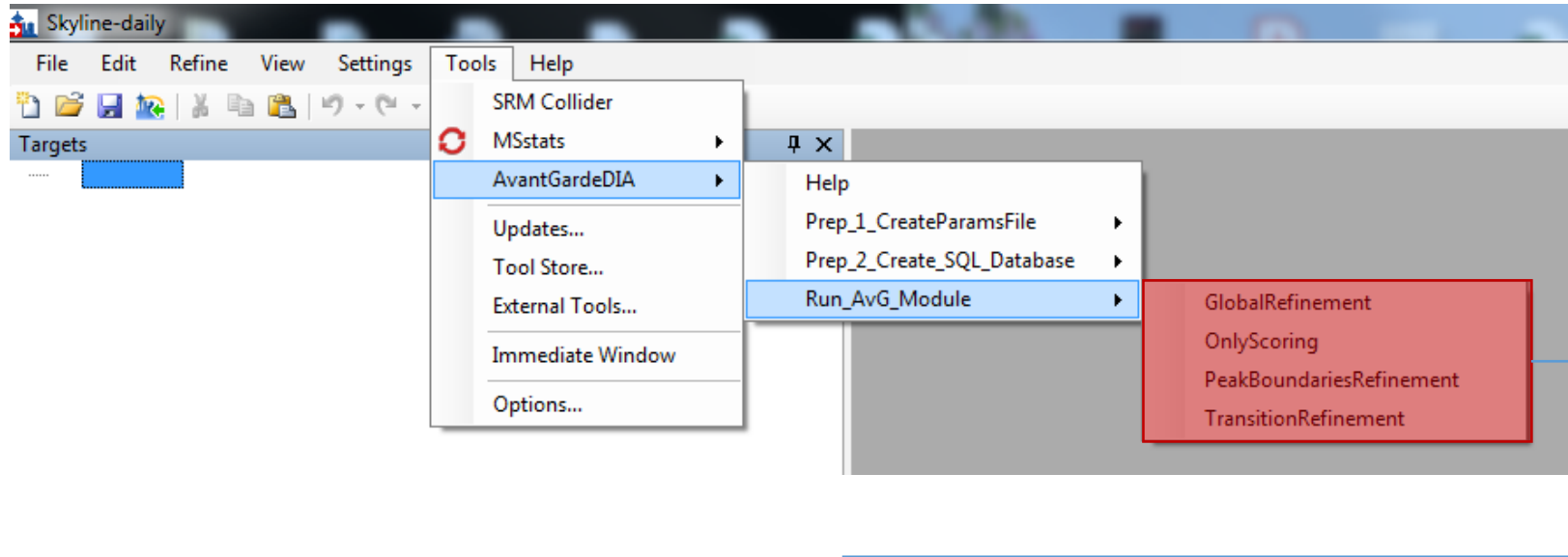
For files larger than 2Gb



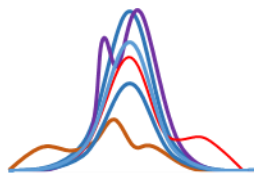
AvantGardeDIA_Export.csv

DB_*Name_Tag*.sqlite

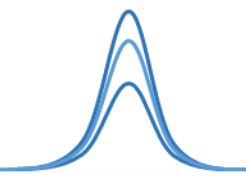
Run *Avant-garde* DIA



- Choose the module that you would like to run
- The preparation steps should be run only once. They should not be launched again every time a new module is used.
- Parallelized analysis: AvG uses N-1 cores (where N is total number of available cores)



Import Results



Import results into Skyline:

- Import → Peak boundaries → “GR_NewPeakBoundaries_*Name_TAG*_Formatted_Filtered.csv”
- Import → Annotations → “GR_Transitions_Annotations_*Name_TAG*.csv”

Prefix indicating the results of each module

GR_ : Global refinement

TR_ : Transition refinement

PB_ : Peak Boundaries

NO_ : Ony Scoring (No Optimization)

Name of the project that is indicated in the Parameters file

- The Transitions_Annotations will complete the “quantitative” annotation for each transition in Skyline.
 - Quantitative= TRUE means that the results of the transition will be used.
 - Quantitative= FALSE means that the results of the transition can be observed but its results will not be considered.

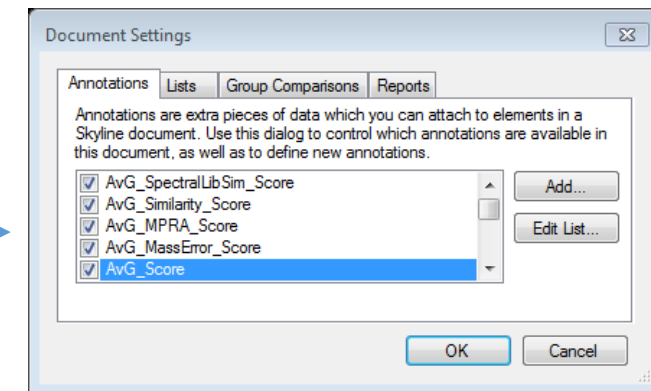
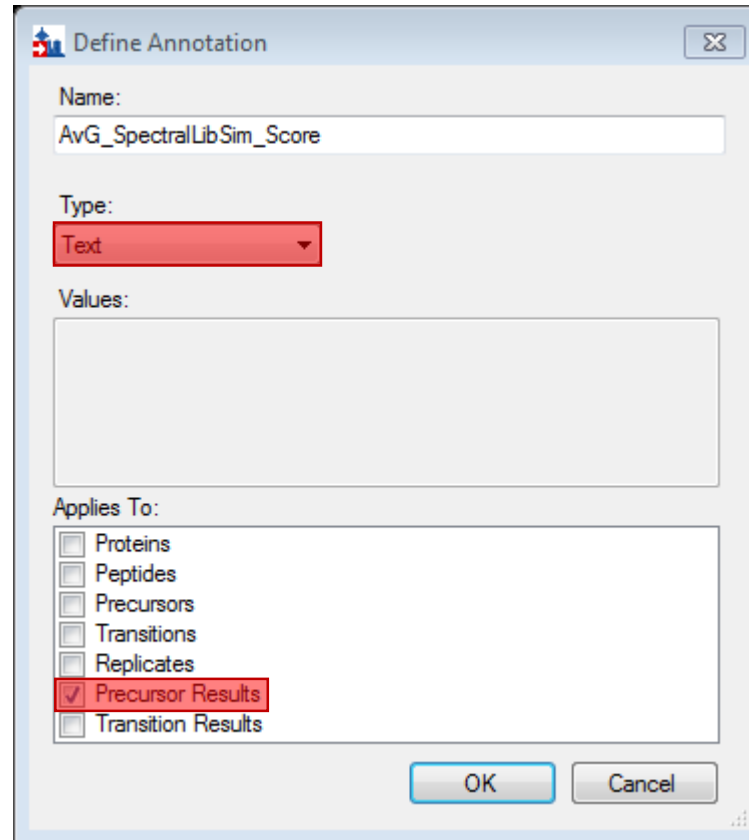
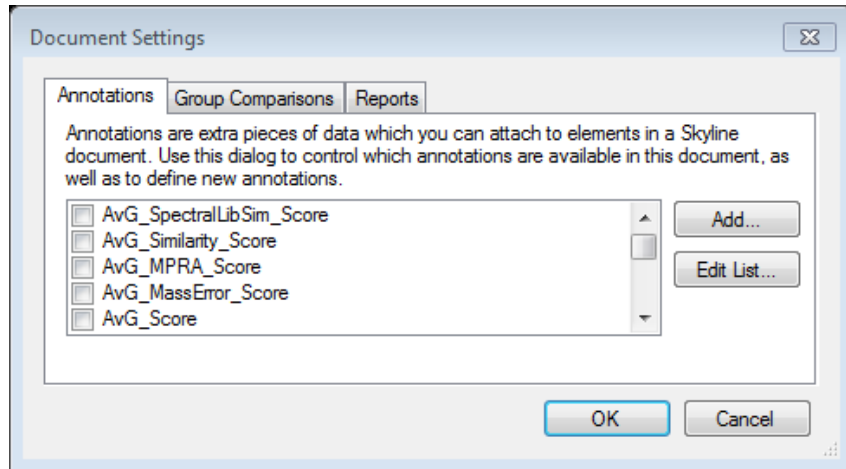
Import and view Scores directly in Skyline

This step is not necessary but it can be helpful to have the scores imported into Skyline.

To import the scores into skyline:

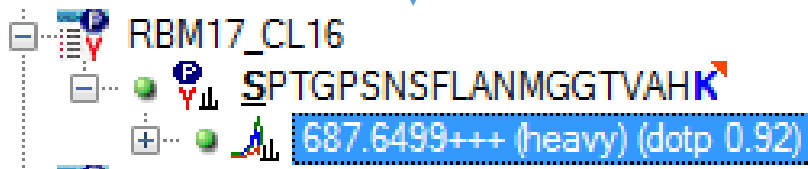
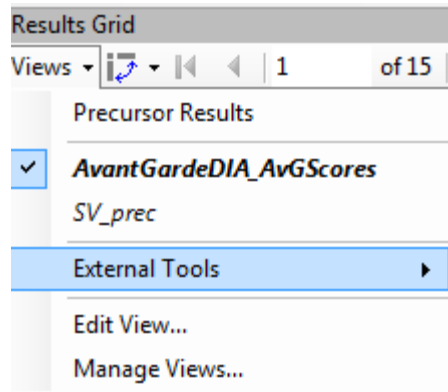
Settings → Document Settings

- Create the following 5 annotation fields
- The names should be the same as below
- All of them choose:
 - Type: Text
 - Applies to: Precursor Results



Import and view Scores directly in Skyline

- Open Results_Grid
- Select AvantGardeDIA_AvGScores
- When a precursor is selected the AvG subcores and the AvG Score are shown.
- Import → Annotations → “GR_AnnotationsPrecursorResults_Name_TAG.csv”
- The scores are now imported into Skyline. They can be added to any report or view using the results grid (see below).

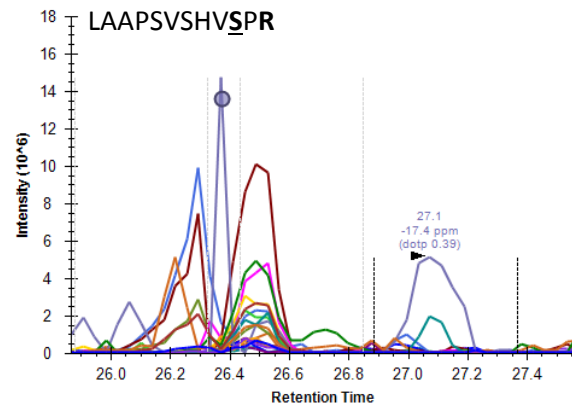


Select a precursor on the targeted peptide tree to see the calculated scores.

Results Grid							
Views	14	of 15	Export...	Find:			
Precursor Result Locator	Replicate	AvG_Score	AvG_SpectralLibSir	AvG_Similarity_Sco	AvG_MPRA_Score	AvG_MassE	Average Mass Error PPM
PrecursorResult/...	CS20170831 SV HEK SpikeP100_108ng_Overlap22_01	0.973826588400735	0.999124920628...	0.997627282185...	0.999970305275...	1	5.5
PrecursorResult/...	CS20170831 SV HEK SpikeP100_108ng_Overlap22_02	0.92286180614668	0.999041486277...	0.992070941463...	0.999331980342...	1	4.9
PrecursorResult/...	CS20170831 SV HEK SpikeP100_108ng_Overlap22_03	0.9475340615793	0.999021205110...	0.994805563255...	0.999980200567...	1	4.6
PrecursorResult/...	CS20170831 SV HEK SpikeP100_13point5ng_Overlap22_01	0.635265691601597	0.998903048758...	0.989650638335...	0.999898763349...	0.8693961...	5.7
PrecursorResult/...	CS20170831 SV HEK SpikeP100_13point5ng_Overlap22_02	0.710025154389462	0.997922415141...	0.974696695527...	0.998547401055...	0.9650670...	10.3
PrecursorResult/...	CS20170831 SV HEK SpikeP100_13point5ng_Overlap22_03	0.441896240319349	0.998508140442...	0.950892411610...	0.999210440439...	0.8759202...	9.9
PrecursorResult/...	CS20170831 SV HEK SpikeP100_27ng_Overlap22_01	0.768268029797405	0.998149505228...	0.973509190064...	0.999549029703...	1	8.1
PrecursorResult/...	CS20170831 SV HEK SpikeP100_27ng_Overlap22_02	0.946890250746204	0.998739686489...	0.994889194866...	0.999560193472...	1	6.1
PrecursorResult/...	CS20170831 SV HEK SpikeP100_27ng_Overlap22_03	0.920693907755081	0.998979258159...	0.991829284277...	0.999817394096...	1	7.1
PrecursorResult/...	CS20170831 SV HEK SpikeP100_54ng_Overlap22_01	0.901455990426699	0.998740295351...	0.989754548869...	0.999524134098...	1	6.2
PrecursorResult/...	CS20170831 SV HEK SpikeP100_54ng_Overlap22_02	0.952769298085154	0.998941664307...	0.995420274480...	0.999979743973...	1	5.6
PrecursorResult/...	CS20170831 SV HEK SpikeP100_54ng_Overlap22_03	0.854204204480794	0.999180224809...	0.983947789882...	0.999677463215...	1	6
PrecursorResult/...	CS20170831 SV HEK SpikeP100_6point75ng_Overlap22_01	0.498291014697295	0.998094022166...	0.965195936983...	0.998308098648...	0.8691462...	5.8
PrecursorResult/...	CS20170831 SV HEK SpikeP100_6point75ng_Overlap22_02	0.205466314558495	0.998638012573...	0.960342348452...	0.997584941695...	0.6210872...	#N/A
PrecursorResult/...	CS20170831 SV HEK SpikeP100_6point75ng_Overlap22_03	0.30636326486527	0.997885638078...	0.963523636604...	0.995986285301...	0.7208113...	9.2

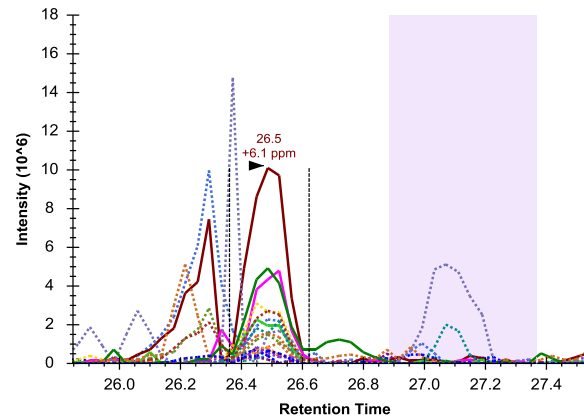
Example

Before Avant-garde

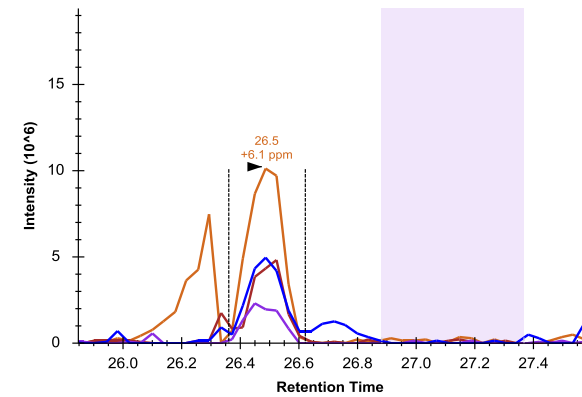


Avant-garde

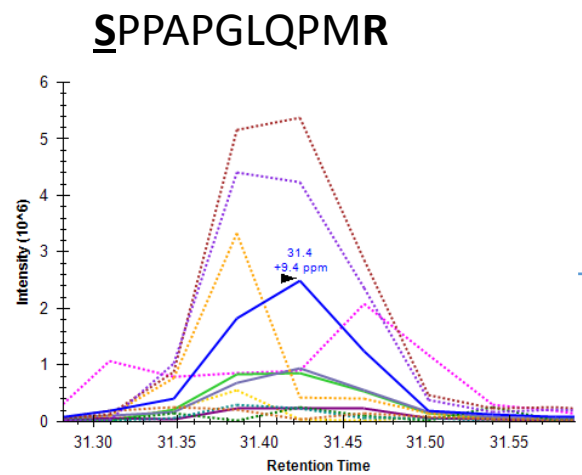
Peak boundary adjustment



Observing only quantitative transition



How to see only quantitative transitions



--- Non quantitative transitions
— Quantitative transitions

