

DIALib-QC v1.0 Tutorial

Here we present a spectral ion library (*K562_q3bad_PV*) referring from the Midha *et. al.* paper submitted in Nature communication (2020).

A) All the below mentioned steps works well with **PeakView, Spectronaut and OpenSWATH** library formats. Please download all the files to test running DIALib-QC.

Step 1: Objective: Assessment of the ion library (*K562_q3bad_PV.txt*)

```
perl assess_swathlib.pl --ion_library K562_q3bad_PV.txt --full_stats --rt_stats --assess_massdiff --coldefs --invert_output --swath_file SWATH_I00VW.txt --peptide_file Uniprot-Human.ppeps
```

DIALib-QC flagged fragment ions average mass error of .0064 in the *K562_q3bad_PV.txt.QC* report.

library	K562_q3bad_PV.txt	Name of library file being analyzed						
precursor_ok	36431	Number of assays where precursor is within 5 PPM (parts per million m/z) of theoretical						
precursor_bad	0	Number of assays where fragment is more than 5 PPM from theoretical						
fragment_ok	9837	Number of assays where fragment is within 1 PPM of theoretical						
fragment_bad	208749	Number of assays where precursor is more than 1 PPM from theoretical						
fragment_na	0	Number of assays where peak annotation not found in expected b/y series						
fragment_avg_mdifff	0.0064	Average m/z difference between reported and theoretical fragment						
swa_defined	36431	Number of peptide ions that fall into a defined SWATH bin						
swa_missing	0	Number of peptide ions that fail to fall into a defined SWATH bin (Pepions - swa_defined)						
swa_conflict	0	Number of fragment_ions that fall into same SWATH(s) as precursor						
swa_ok	255528	Number of fragment_ions that do not fall into same SWATH(s) as precursor						
swa_conflict_assay	0	Number of precursor that have at least one failing fragment						
swa_5	655	Number of fragment ions that fall within 5 Th of precursor ion						
swa_25	9397	Number of fragment ions that fall within 25 Th of precursor ion						
problem_assays	36431	Assays whose precursor or any fragment m/z values do not match SWATHs file or differ significantly from theoretical values						

Step 2: Objective: Correct fragment (q3) mass error in the ion library using theoretical masses.

```
perl assess_swathlib.pl --ion_library K562_q3bad_PV.txt --assess_massdiff --correct_mz --swath_file SWATH_I00VW.txt --peptide_file Uniprot-Human.ppeps
```

We assess the *K562_q3bad_PV.txt.mz_corrected* new library (output from this step)

```
perl assess_swathlib.pl --ion_library K562_q3bad_PV.txt.mz_corrected --full_stats --rt_stats --assess_massdiff --coldefs --invert_output --swath_file SWATH_I00VW.txt --peptide_file Uniprot-Human.ppeps
```

Now there is no fragment ion mass error but DIALib-QC flagged few conflict assays in the *K562_q3bad_PV.txt.mz_corrected.QC* report.

library	K562_q3bad_PV.txt.mz_corrected	Name of library file being analyzed						
precursor_ok	36431	Number of assays where precursor is within 5 PPM (parts per million m/z) of theoretical						
precursor_bad	0	Number of assays where fragment is more than 5 PPM from theoretical						
fragment_ok	218586	Number of assays where fragment is within 1 PPM of theoretical						
fragment_bad	0	Number of assays where precursor is more than 1 PPM from theoretical						
fragment_na	0	Number of assays where peak annotation not found in expected b/y series						
fragment_avg_mdifff	0	Average m/z difference between reported and theoretical fragment						
swa_defined	36431	Number of peptide ions that fall into a defined SWATH bin						
swa_missing	0	Number of peptide ions that fail to fall into a defined SWATH bin (Pepions - swa_defined)						
swa_conflict	5	Number of fragment_ions that fall into same SWATH(s) as precursor						
swa_ok	255523	Number of fragment_ions that do not fall into same SWATH(s) as precursor						
swa_conflict_assay	5	Number of precursor that have at least one failing fragment						
swa_5	652	Number of fragment ions that fall within 5 Th of precursor ion						
swa_25	9395	Number of fragment ions that fall within 25 Th of precursor ion						
problem_assays	5	Assays whose precursor or any fragment m/z values do not match SWATHs file or differ significantly from theoretical values						

Step 3: Objective: Remove conflict assays from the library

```
perl assess_swathlib.pl --ion_library K562_q3bad_PV.txt.mz_corrected --assess_massdiff --filter_assays --swath_file SWATH_100VW.txt --peptide_file Uniprot-Human.ppeps
```

We assess the *K562_q3bad_PV.txt.mz_corrected.clean* new library (output from this step)

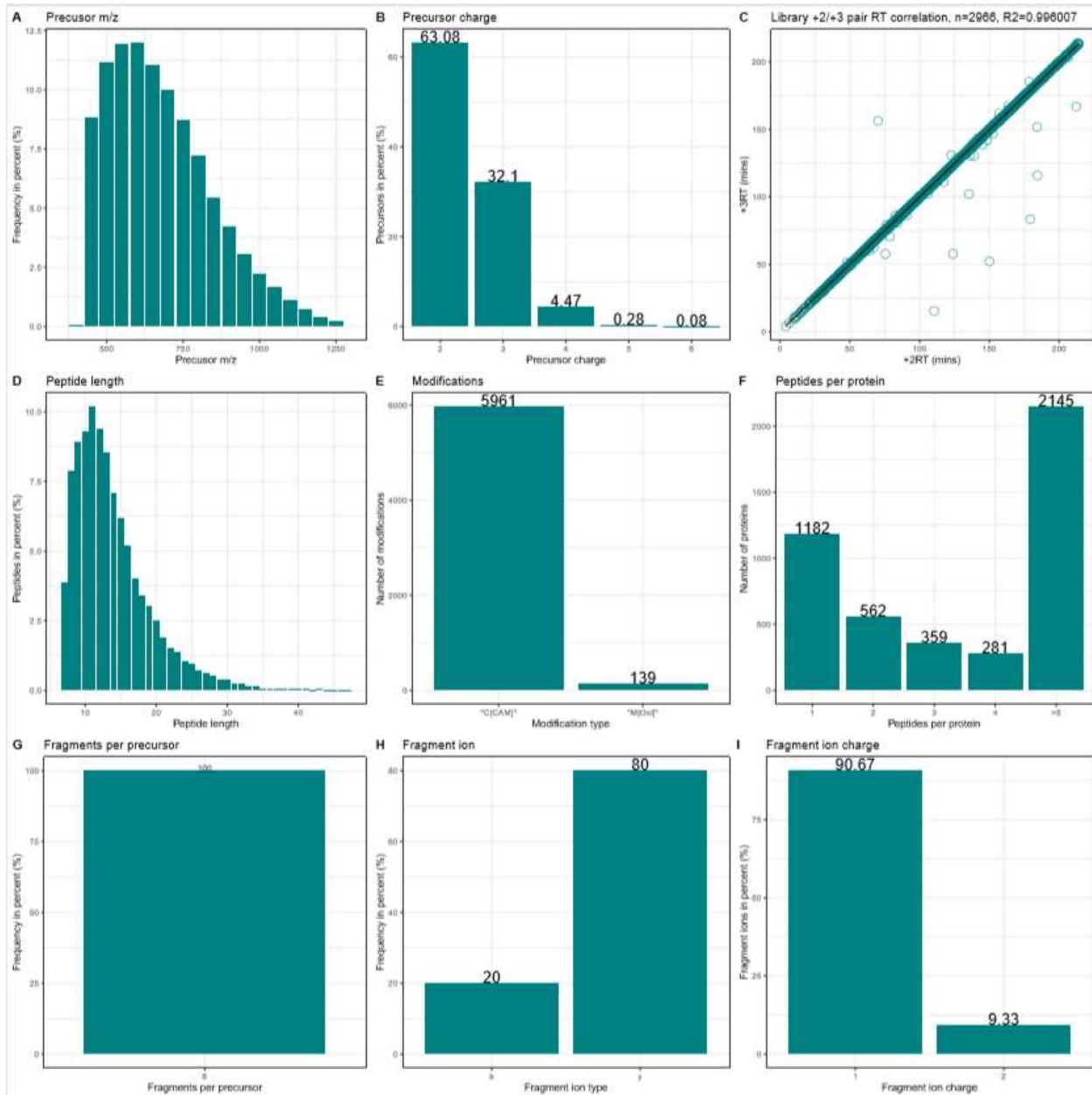
```
perl assess_swathlib.pl --ion_library K562_q3bad_PV.txt.mz_corrected.clean --full_stats --rt_stats --assess_massdiff --coldefs --invert_output --swath_file SWATH_100VW.txt --peptide_file Uniprot-Human.ppeps
```

Now there is no fragment ion mass error or conflict assays in the *K562_q3bad_PV.txt.mz_corrected.QC* report.

K562_q3bad_PV.txt.mz						
library	_corrected.clean	Name of library file being analyzed				
precursor_ok	36426	Number of assays where precursor is within 5 PPM (parts per million m/z) of theoretical				
precursor_bad	0	Number of assays where fragment is more than 5 PPM from theoretical				
fragment_ok	218556	Number of assays where fragment is within 1 PPM of theoretical				
fragment_bad	0	Number of assays where precursor is more than 1 PPM from theoretical				
fragment_na	0	Number of assays where peak annotation not found in expected b/y series				
fragment_avg_mdifff	0	Average m/z difference between reported and theoretical fragment				
swa_defined	36426	Number of peptide ions that fall into a defined SWATH bin				
swa_missing	0	Number of peptide ions that fail to fall into a defined SWATH bin (Pepions - swa_defined)				
swa_conflict	0	Number of fragment_ions that fall into same SWATH(s) as precursor				
swa_ok	255498	Number of fragment_ions that do not fall into same SWATH(s) as precursor				
swa_conflict_assay	0	Number of precursor that have at least one failing fragment				
swa_5	649	Number of fragment ions that fall within 5 Th of precursor ion				
swa_25	9393	Number of fragment ions that fall within 25 Th of precursor ion				
problem_assays	0	Assays whose precursor or any fragment m/z values do not match SWATHs file or differ significantly from theoretical values				

Step 4: Objective: To generate graphical plots (*K562_q3bad_PV.txt.mz_corrected.clean.pdf*) summarizing library characteristics

```
perl DIALib-QC_RPlot.pl K562_q3bad_PV.txt.mz_corrected.clean.fullstats K562_q3bad_PV.txt.mz_corrected.clean.RT
```



B) In-silico predicted library in Prosit (generic text: Spectronaut compatible)

We used a larger spectral ion library *HeLa_ce28.prosit.library.csv* submitted in iProx database (Chen *et. al.* 2019).

Step 1: Objective: Assessment of the ion library (*HeLa_ce28.prosit.library.csv*)

```
perl assess_swathlib.pl --assess_massdiff --full_stats --rt_stats --coldefs --invert_output --ion_library
HeLa_ce28.prosit.library.csv
```

DIALib-QC flagged few problem assays in the *HeLa_ce28.prosit.library.csv.QC* report because of difference with theoretical masses.

library	HeLa_ce28.prosit.library
precursor_ok	83976
precursor_bad	0
fragment_ok	5038047
fragment_bad	8243
fragment_na	0
fragment_avg_mdif	0
problem_assays	7742

Step 2: Objective: Correct to theoretical masses.

```
perl assess_swathlib.pl --ion_library HeLa_ce28.prosit.library.csv --assess_massdiff --correct_mz
```

We assess the new mass corrected *HeLa_ce28.prosit.library.csv.mz_corrected* library (output from this step)

```
perl assess_swathlib.pl --ion_library HeLa_ce28.prosit.library.csv.mz_corrected --full_stats --rt_stats --assess_massdiff --coldefs --invert_output
```

Now there is no fragment ion mass error and no problem assays in the mass corrected *HeLa_ce28.prosit.library.csv.mz_corrected.QC* report.

library	HeLa_ce28.prosit.library.csv.mz_corrected			
precursor_ok	83976			
precursor_bad	0			
fragment_ok	5046290			
fragment_bad	0			
fragment_na	0			
fragment_avg_mdif	0			
problem_assays	0			

Step 3: Objective: To generate graphical plots (*HeLa_ce28.prosit.library.csv.mz_corrected.pdf*) summarizing *in-silico* predicted ion library (free from any error) characteristics

```
perl DIALib-QC_RPlot.pl HeLa_ce28.prosit.library.csv.mz_corrected.fullstats HeLa_ce28.prosit.library.csv.mz_corrected.RT
```

