



Oligo

User Guide

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Overview

Byos provides two different workflows for Oligonucleotide Analysis – the **Oligo** workflow, which incorporates much of the same functionality as the Intact workflow, and the **Digested Oligonucleotide** workflow, which uses the same general UI platform as the Peptide workflows.

Oligo Workflow

The Oligo module allows users to evaluate a variety of nucleotide macromolecules such as: DNA, RNA and custom assemblies, including DNA/RNA combinations, phosphorothioate backbones, and user-specified modifications. Common solvent- or LC column-related adducts are easily detected and highlighted.

Features include:

1. Support for all major mass spectrometry instruments and vendors
2. Automatic or manual integration of chromatographic time windows
3. Deconvolution of charge states to transform m/z spectra to neutral mass spectra
4. Automatic mass peak picking and intensity calculations
5. Sequence confirmation using MS2 fragmentation data
6. Side-by-side comparison of multiple samples
7. Reporting of summary data and figures

Tour of Oligonucleotide Analysis Dashboard Views

The Oligonucleotide Analysis user interface (UI), or dashboard, shown in the figure below has eight highly interactive plot and table views:

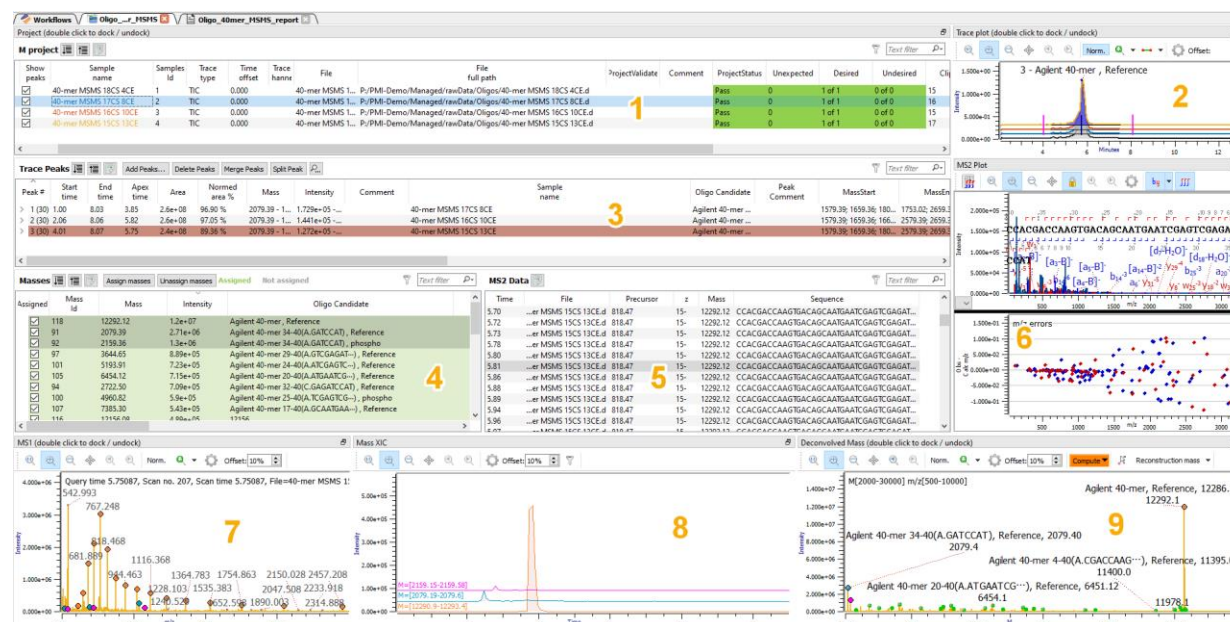


Figure 1: Oligonucleotide Analysis Dashboard

There are nine Oligonucleotide Analysis views: (1) Project table with input MS files, (2) Trace plot chromatogram with selected peak, (3) Trace Peaks table, (4) Masses table, (5) MS2 Data table, (6) MS2

and mass error plots, (7) MS1 spectrum (summed spectra), (8) Mass XIC plot, and (9) Deconvolved mass spectrum. Views can be rearranged, resized, docked, and undocked. Customized layouts can be saved and shared.

1. The **Project** table lists the data files in use. The paired check boxes for MS files and result files can be used to turn on or off the records displayed in the other views. The colors of the files correspond to colors used in the other views.
2. The **Trace plot** shows the total ion chromatogram (TIC), base peak intensity (BPI), ultraviolet (UV) trace, or electropherogram (icIEF).
3. The **Trace Peaks** table summarizes the Trace plot chromatogram or electropherogram. Selecting a row in the table selects a peak in the **Trace plot** (View 2). Trace peaks can be merged, split, added, or deleted using the buttons at the top. Time limits can be adjusted by editing **Start time**, **End time** and/or **Apex time** fields or dragging the magenta bars shown in the **Trace plot** (View 2).
4. The **Masses** table includes one row for each picked peak from **Deconvolved Mass spectrum** (View 9). The user can add, delete, or assign rows in the table. The name of a mass peak can be either its mass or an interpretation.
5. The **MS2 Data table** shows the sequence information, along with the charge state, acquisition time and other details of the oligonucleotide. This table gets populated when selecting the precursor mass (from one or more rows) in the **Masses** table (View 4). Note that the **MS2 Data table** gets populated only when the selected row in the **Masses** table (View 4) has MS2 data. Each row of the **MS2 Data table** represents a MS2 scan of a single charge state of the selected mass in the **Masses** table (View 4).
6. The **MS2 and mass error plots** show the MS2 spectrum of the oligonucleotide sequence selected in the **MS2 Data table** (View 5).
7. **MS1** shows the summed m/z plot for the chromatogram or electropherogram trace peak selected in the **Trace plot** (View 2) or **Trace Peaks table** (View 3).
8. The **Mass XIC** plot allows user to assess elution profile of deconvolved species.
9. The **Deconvolved Mass spectrum** is the primary output of the software. Neutral masses are plotted for the summed m/z plot (View 7) associated with the trace peak selected in the **Trace plot** (View 2) or **Trace Peaks table** (View 3). Hollow dots indicate picked peaks, the “candidate” masses that can be assigned for reporting. Once assigned, the dots become solid green and peaks become persistent. Outlined and colored dots interactively show selected peaks, connecting to the corresponding-colored dots in the m/z plot.

Creating a Project

Project Creation

If the user already has a saved workflow, the oligonucleotide analysis can be started by clicking **File > Launch Workflow**, selecting the workflow and clicking **Create Project** (shown in below figure).

The user has an option to edit an existing workflow by launching the workflow, making the edits to the workflow, and saving this as new workflow by clicking the **Save Workflow** (shown in below figure).

The user can also create a new workflow by clicking the Oligo icon. This will result in a Project Creation window (shown in below figure).

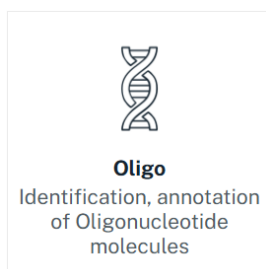


Figure 2: Oligo workflow icon

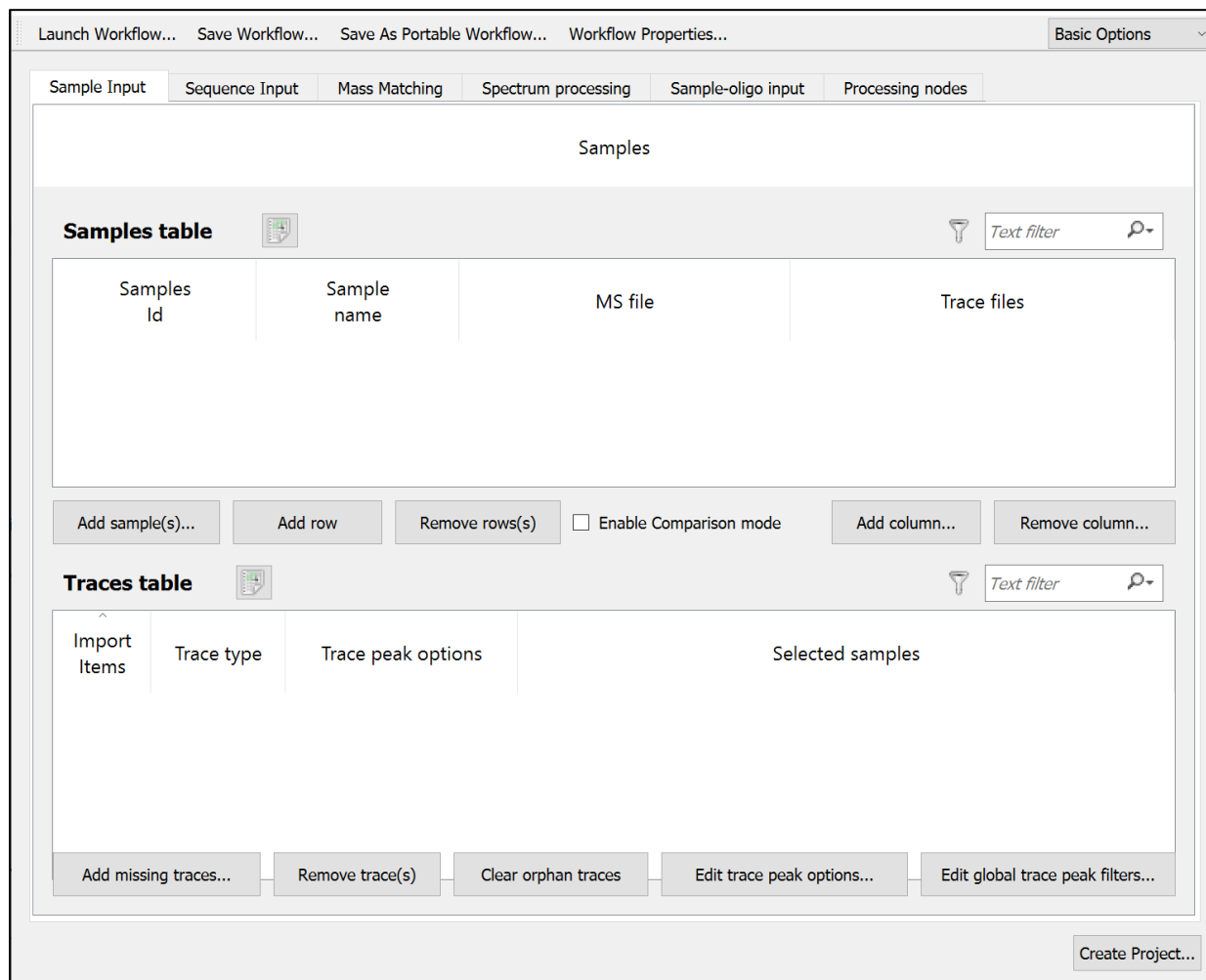
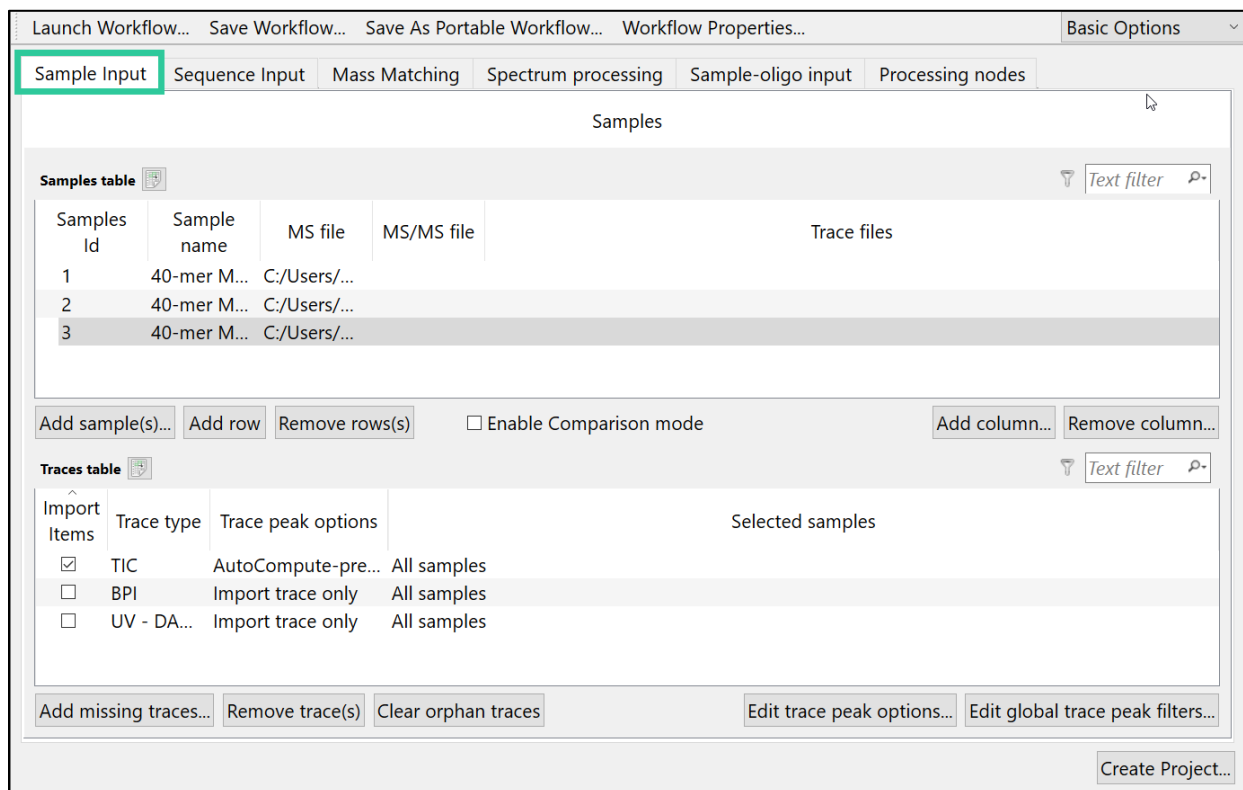


Figure 3: Oligonucleotide Analysis project creation window

This multi-tab window allows the user [1] to analyze one or multiple mass spectrometer files, [2] to add corresponding set of oligonucleotide sequences and [3] to set-up the parameters required for the oligonucleotide analysis. Below are the steps for creating a sample workflow.



Sample Input Tab



Launch Workflow... Save Workflow... Save As Portable Workflow... Workflow Properties... Basic Options



Sample Input Sequence Input Mass Matching Spectrum processing Sample-oligo input Processing nodes

Samples

Samples table  Text filter 

Samples Id	Sample name	MS file	MS/MS file	Trace files
1	40-mer M...	C:/Users/...		
2	40-mer M...	C:/Users/...		
3	40-mer M...	C:/Users/...		


Add sample(s)... Add row Remove rows(s) ☐ Enable Comparison mode Add column... Remove column...

Traces table  Text filter 


Import Items	Trace type	Trace peak options	Selected samples
<input checked="" type="checkbox"/>	TIC	AutoCompute-pre...	All samples
<input type="checkbox"/>	BPI	Import trace only	All samples
<input type="checkbox"/>	UV - DA...	Import trace only	All samples

Add missing traces... Remove trace(s) Clear orphan traces Edit trace peak options... Edit global trace peak filters... Create Project...

Figure 4: Sample Input tab

MS sample files are added in the **Sample Input** tab. Oligonucleotide Analysis accepts a variety of reference sample files (Bruker: *.d, Thermo: *.raw, Waters: *.raw, SCIEX: *.wiff, Agilent: *.d). To enter a file, drag and drop an MS raw file into the project window. Alternatively, click **Add sample**, double-click in the **MS file** column, click  and browse to the sample file. Click **Open**.

The sample is named for the file name; to change the sample name, click the default name twice and enter a new sample name. Click **Add sample** at left to add a new row to the **Samples table**. Then add the desired files as above. To remove an unwanted sample file row, select the row and click **Remove sample(s)**.

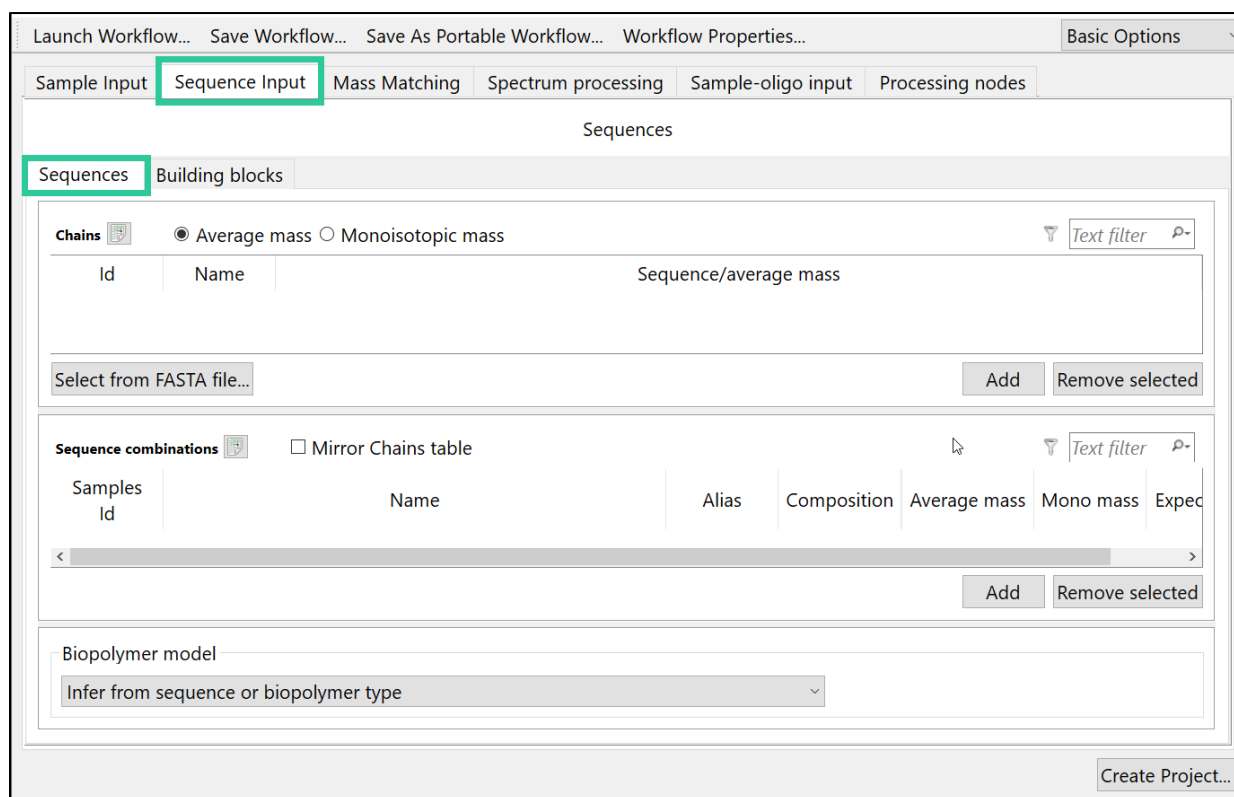
Individual traces can be imported from sample file types supported in the **MS file** cell, as well as *.csv, *.txt files. If there are traces associated with a raw MS file, the traces table would be populated automatically to reflect these traces with TIC being selected as default. To import a trace from a file, drag the file into the **Traces files** column in the Samples table. Alternatively, double-click in the Samples table row under the Traces file column, click  and browse to the sample file. Click **Open**. The trace file is added to the sample name and the trace is added to the Traces table.

Sequence Input Tab

Next in the project creation window is the **Sequence Input** tab, which is further divided between two sub-tabs: **Sequences** and **Building blocks**.

Sequences sub-tab

The **Sequences** sub-tab is further divided into three sections: **Chains**, **Sequence combinations**, and a section for Biopolymer model.






Launch Workflow... Save Workflow... Save As Portable Workflow... Workflow Properties... Basic Options

Sample Input **Sequence Input** Mass Matching Spectrum processing Sample-oligo input Processing nodes




Sequences

Sequences Building blocks

Chains  ☒ Average mass ☐ Monoisotopic mass  Text filter 

Id	Name	Sequence/average mass
Select from FASTA file...		


Add Remove selected

Sequence combinations  ☐ Mirror Chains table  Text filter 

Samples Id	Name	Alias	Composition	Average mass	Mono mass	Expected mass
< [Progress Bar] >						

Add Remove selected

Biopolymer model

Infer from sequence or biopolymer type 

Create Project...

Figure 5: Sequence Input Tab - Sequences sub-tab

To enter a nucleotide sequence, drag and drop a FASTA file into the **Chains** table. Alternatively, click **Select from FASTA file**, navigate to the file of interest, and click **Open**. Nucleotide sequences can also be typed or pasted in manually. To manually enter the sequence, click **Add** in the Chains section, and click in the **Name** and **Sequence/average mass** cells to enter text in those columns (See [Building Blocks sub-tab](#) section for Oligonucleotide naming convention). The chains can then be added as rows to the **Sequence combinations** table by selecting **Mirror Chains table** checkbox. The user can edit **Sequence combinations** table only after unselecting **Mirror Chains table** checkbox.

NOTE: **Sequence combinations** table must be populated with the expected/desired masses to reflect the annotations in the **Deconvolved Mass spectrum** and other related plots/tables.

The software automatically computes the average and monoisotopic mass for each selected FASTA entry, and these masses are then available for automatic peak assignment. Nucleotide sequences can be arbitrary if the user inputs the average or monoisotopic masses, overriding the computed masses. Average or monoisotopic masses give the software reference masses to assign mass peaks based on mass deltas (see [Delta masses sub-tab](#) section below).

Biopolymer model options include:

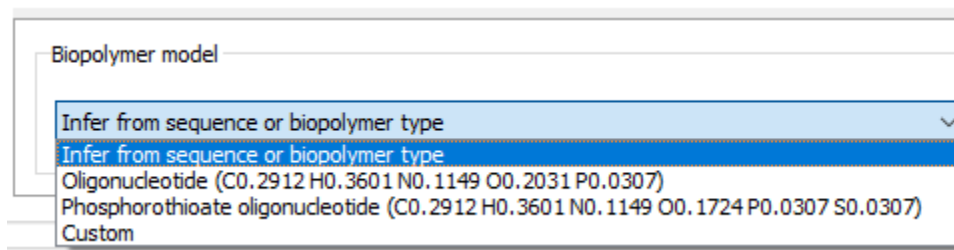


Figure 6: Biopolymer models

- **Infer from sequence or biopolymer type** is the default selection, which uses the standard model that Oligonucleotide Analysis uses to calculate mass. The standard model is defined by the **Chemical Elements** composition in the **Building blocks** sub-tab of the **Sequence-Input** tab (see [Building Blocks sub-tab](#) section below).
- **Oligonucleotide** uses the displayed averagine formula to calculate mass.
- **Phosphorothioate oligonucleotide** uses the displayed averagine formula to calculate mass.
- **Custom** opens a cell to the right of the selection to add a custom formula to calculate mass.

Note that the formula values refer to atom counts (molar ratios), not mass ratios.

Building Blocks sub-tab

The **Building blocks** sub-tab contains information on naming convention for building oligonucleotide sequences. The user has an option to add new building blocks or edit the existing building blocks to change chemical formula.

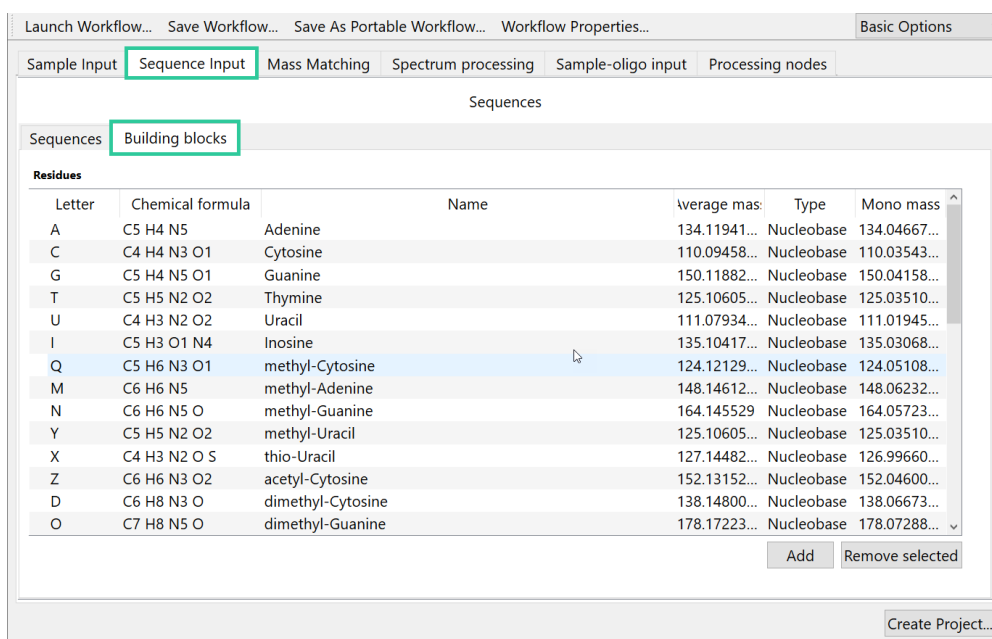


Figure 7: Sequence Input Tab - Building blocks sub-tab

Below is an example of naming convention for default residues shown in the **Residues** table (above figure). See Letter 'Q' in **Residues** table (above figure) as reference to build custom oligonucleotides.

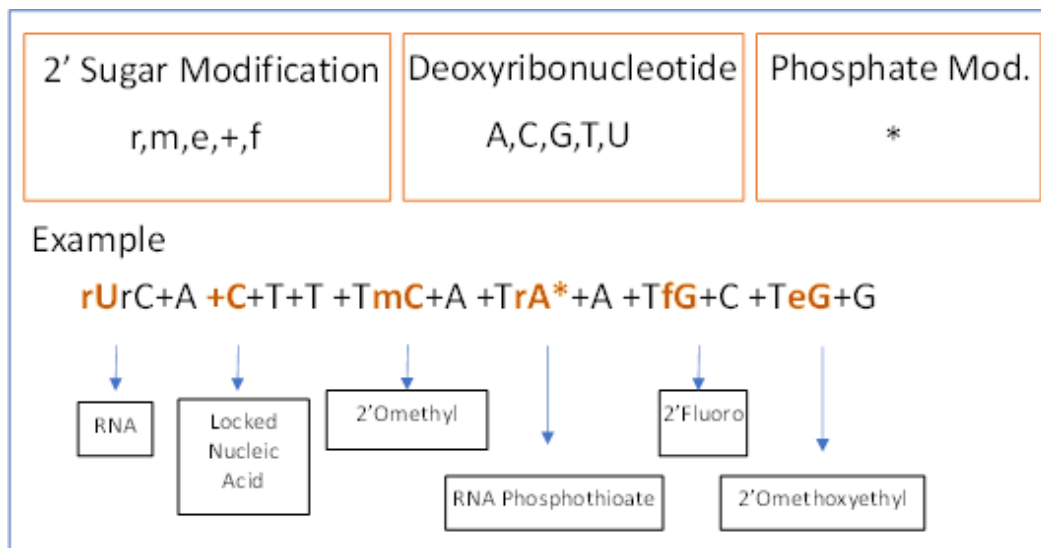


Figure 8: Example of oligonucleotide sequence naming convention

The Byos Oligo workflow allows for full flexibility in defining types of building blocks (e.g., nucleobase, ribose, phosphate).

Oligonucleotide molecules are specified by "building blocks", which are chemical formulas linked to letters or other characters. The 3' and 5' termini are single-character building blocks with the default formula -OH and -H respectively. Within the sequence, building blocks come in groups of three characters, representing nucleobase, ribose, and phosphate linker.

Any base can be combined with any sugar and any linker to form a nucleotide. Note that the order of building blocks within the sequence is important. The order is assumed to be:

```
[5' terminal][RiboseBasePhosphate_1 RiboseBasePhosphate_2 ...
RiboseBasePhosphate_n][3' terminal]
```

As an example (for notation, see the **Letter** and **Type** columns in [Figure 7](#)):

<rA* mC* fU>

specifies ribose adenine thiophospho for the first nucleotide, methylribose cytosine thiphospho for the second nucleotide, and fluororibose uracil for the third nucleotide. 5' terminates with an -H and 3' terminates with an -OH.

If a terminal is omitted, a default -OH terminal is assumed on 3' termini and -H on the 5' termini.

If a ribose symbol is omitted, a default C5O2H7 for ribose is assumed.

If a phosphate symbol is omitted, a default -HPO3 is assumed.

Note that these values have been updated as of release v5.8.

Mass Matching Tab

The **Mass Matching** tab has two sub-tabs: **Tolerances** and **Delta masses** sub-tabs.

Tolerances sub-tab

The **Tolerances** sub-tab allows for setting the precursor mass tolerance (**Tolerance** under MS1 section), fragment mass tolerance (**Tolerance** under MS2 section) and the MS2 **Fragmentation type**. In the figure below, the **MS1 Tolerance** is set to 2 Daltons, the **MS2 Tolerance** is set to 50 ppm.

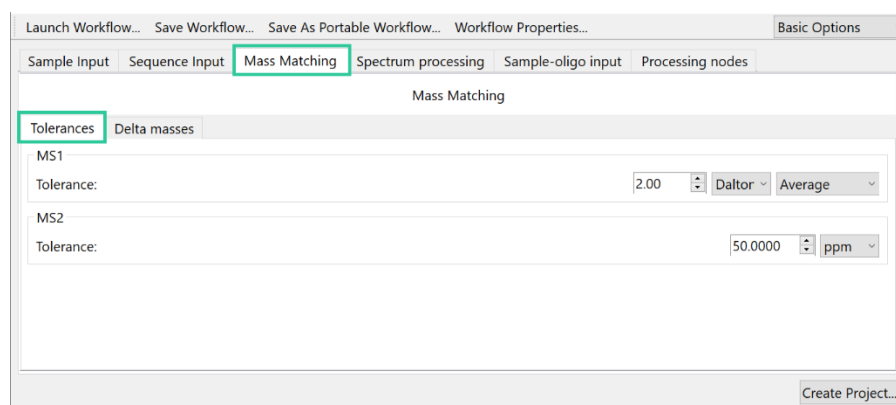


Figure 9: Mass Matching Tab – Tolerances sub-tab

Delta masses sub-tab

The **Delta masses** sub-tab gives a table of likely mass differences between observed peaks and the reference mass input by the user or computed from an oligonucleotide sequence. The table includes delta masses of common oligonucleotide impurities.

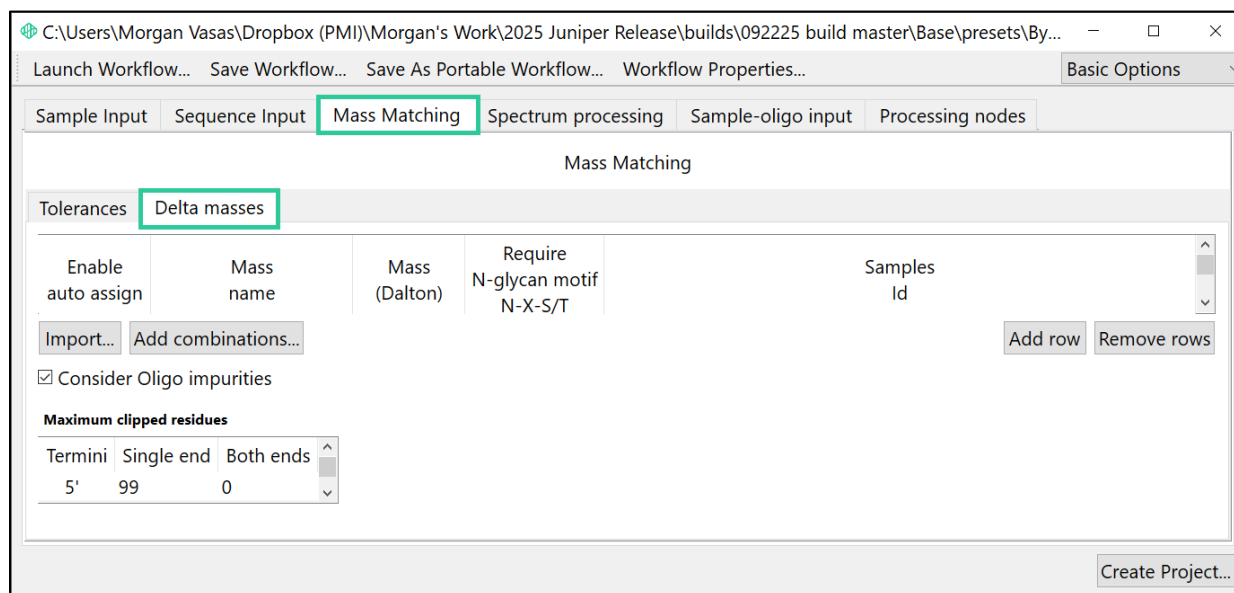


Figure 10: Mass Matching Tab – Delta masses sub-tab

Import allows the user to import a *.csv table of custom delta masses.

Add row and **Remove rows** allow the user to enter individual delta masses.

Add Combinations opens a dialog that allows the user to assign match tolerance modifications to a delta mass.

Clips can now be anticipated from either 5' or 3' termini or both. This feature can be turned on during project creation within the **Mass Matching** tab by checking **Consider Oligo impurities** and setting the parameters in the **Maximum clipped residues** box. The **Single end** column limits the maximum number of residues clipped from either the 5' or 3' terminal. The **Both ends** column limits the number of clipped residues from either the 5' or 3' terminal when the opposite terminal is also clipped. The users can then go to the **Masses** table within the project and check the **Oligo Candidate** column to see the annotation of the internal clips.

Spectrum Processing Tab

The **Spectrum Processing** tab includes two sub-tabs, **Basic** and **Advanced**.

Basic sub-tab

The **Basic** sub-tab contains the primary settings used in computing deconvolved masses.

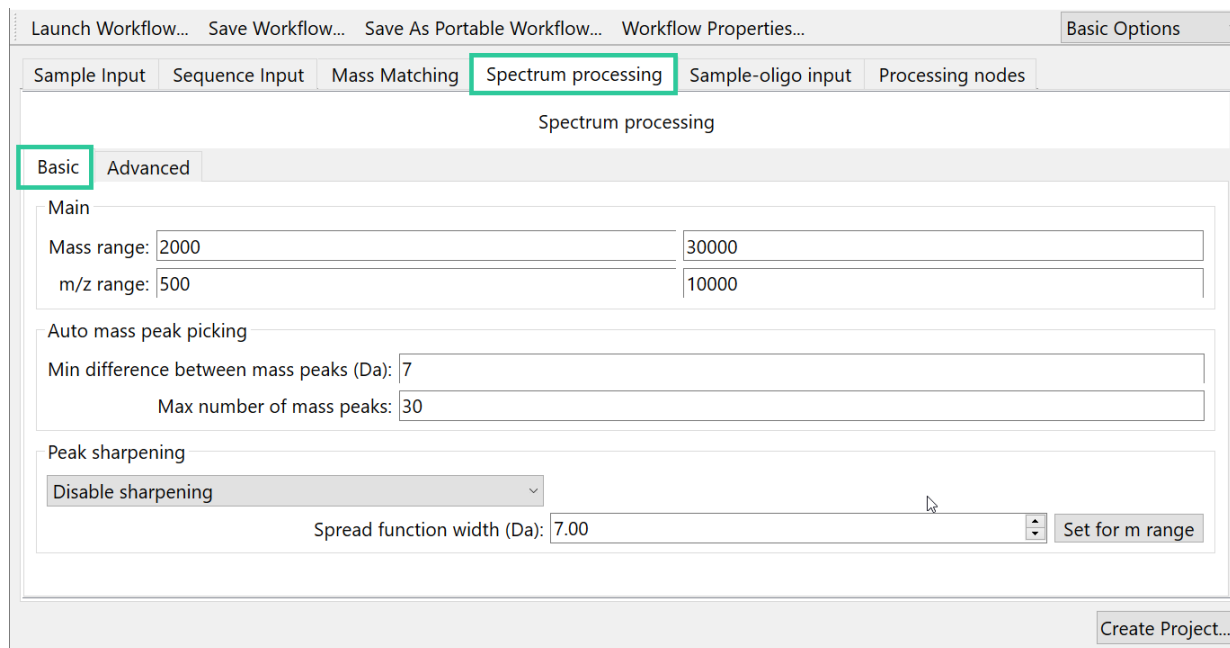


Figure 11: Spectrum Processing Tab - Basic sub-tab

Mass range and **m/z range** set ranges for neutral masses and m/z, respectively. Mass range defines the range of neutral masses displayed in the Deconvolved mass spectrum. m/z range defines the segment of the MS1 spectrum used to compute neutral masses.

The **Auto mass peak picking** parameters control peak picking. **Min difference between mass peaks (Da)** prevents the peak picker from picking multiple points on top of a ragged or isotope-resolved mass peak. **Max number of mass peaks** sets a limit on the number of picked peaks.

The Peak sharpening dropdown enables an optional “super-resolution step” for resolving larger masses beyond isotope resolution (if **Enable sharpening** from the drop-down menu is selected).

Advanced sub-tab

The **Advanced** sub-tab mainly concerns resolution. These parameters are only needed if the aim is to produce isotopically resolved neutral mass spectra.

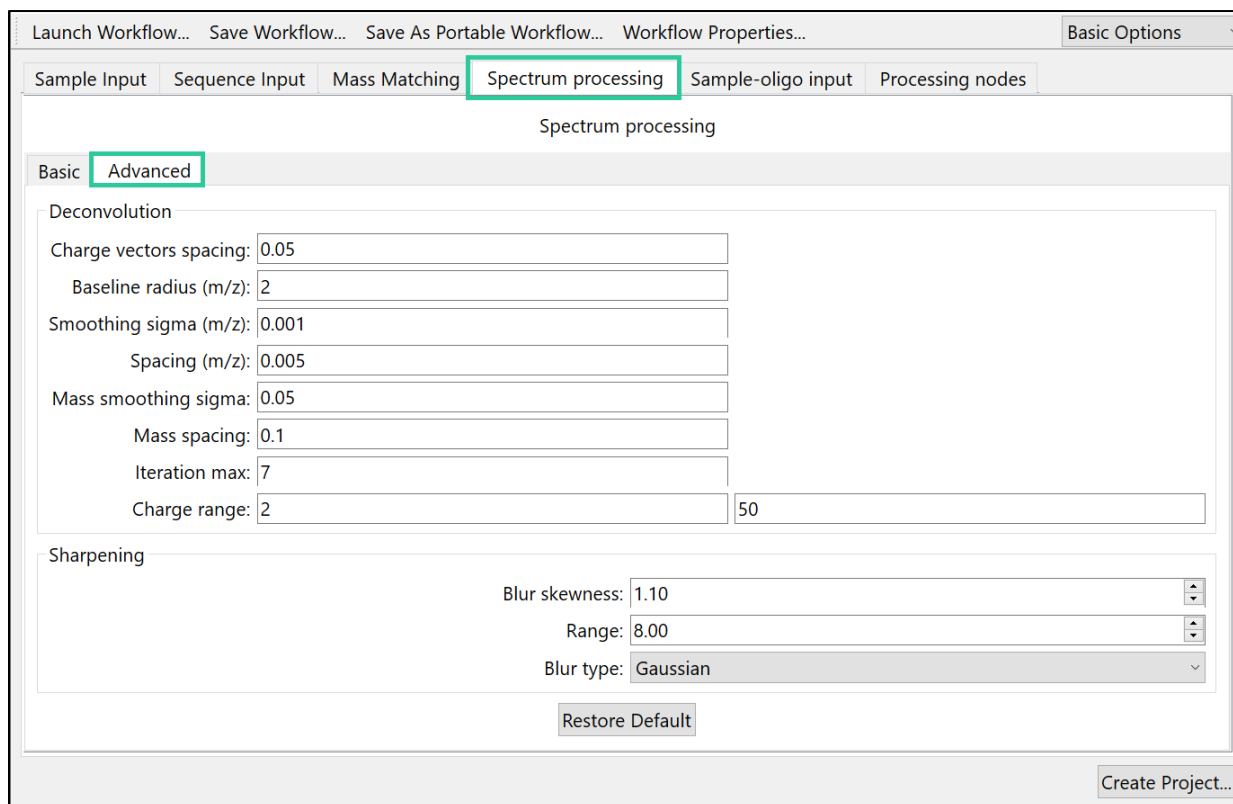


Figure 12: Spectrum Processing Tab - Advanced sub-tab

Charge vectors spacing assigns charge probabilities for each small interval of m/z points.

Baseline radius controls the stiffness of the baseline. A baseline radius of 2 gives a flexible baseline that will cut into m/z peaks broader than 2 Thomsons.

Spacing (m/z) controls the spacing of sample points in the m/z spectrum. The default value is 0.005.

Smoothing sigma can be helpful for producing an appropriately smoothed neutral mass spectrum with less smoothing at lower mass and more smoothing at higher mass, if set at a larger value than Spacing (m/z). The default value is 0.001.

Mass spacing controls the spacing of points in the neutral mass spectrum.

Iteration max set to 10 will work for most purposes. If speed is an issue, this value can be set to 5.

Charge range is best set to a wide range, in which case the charge range will be implied by the mass and m/z ranges. The default range of 1 – 30 covers most applications.

NOTE: **Spacing (m/z)** and **Mass spacing** will only be effective when the advanced parameter `SteppingMethod=constant` is added to the advanced configuration during the project creation (See [Oligo Analysis: Advanced Commands](#) for additional details).

Peak sharpening uses the **Spread function width** set in the Basic tab to deconvolve the data. The Advanced tab offers three infrequently used controls for peak sharpening. **Blur skewness** controls the asymmetry of the point spread function; the default value of 1.1 means that the right tail has sigma (standard deviation) 10% bigger than the left tail. 1.2 gives even more asymmetrical tailing; 1.0 gives a

symmetric point spread function. **Range** sets the length of the tails in standard deviations; a small value of 5 or 6 may work better in the case of Lorentzian point spread. The default is set to 8. **Blur type** has two options: Gaussian (skinny tails) and Lorentzian (fat tails).

Sample-oligo input Tab

Sample-oligo associations can be imported from *.csv files and from MS files. To create CSV files from other projects, use **File > Export > Generate MS path template CSV**. This capability is useful for making a single Oligo Analysis project with many samples.

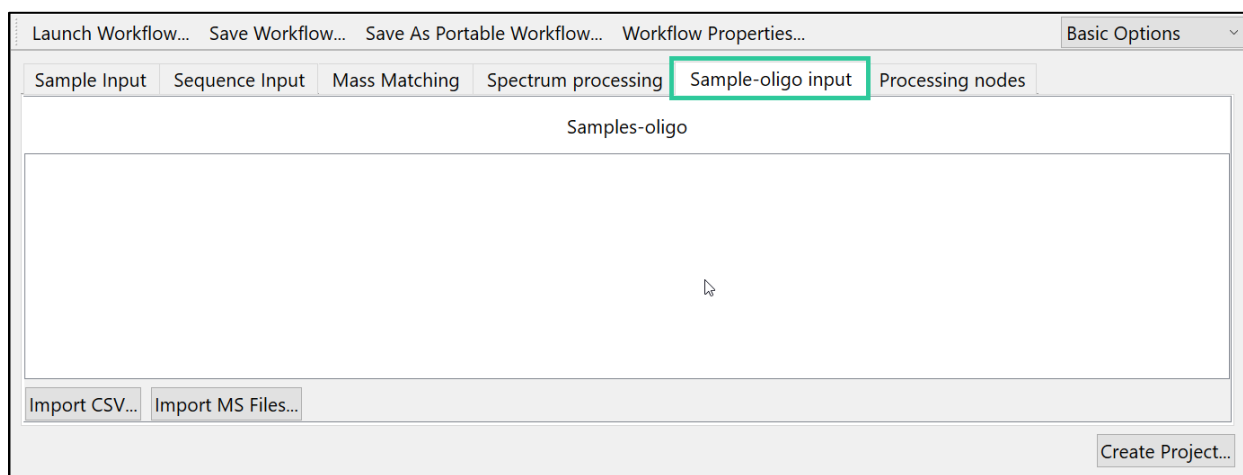


Figure 13: Sample-oligo input Tab

Processing nodes Tab

Processing nodes is where the user specifies the processing parameters to be applied during project creation and report generation. Each workflow is populated with default values, designed for the user to review once, and save for future projects. This empowers every member of a team, scientist within a lab, or colleagues across a project to complete the exact same analysis and generate identical reports regardless of Byos user-skill level, scientific expertise or experience, or location. Please note the next section will only have to be completed once for each analysis type and the workflow used for perpetuity.

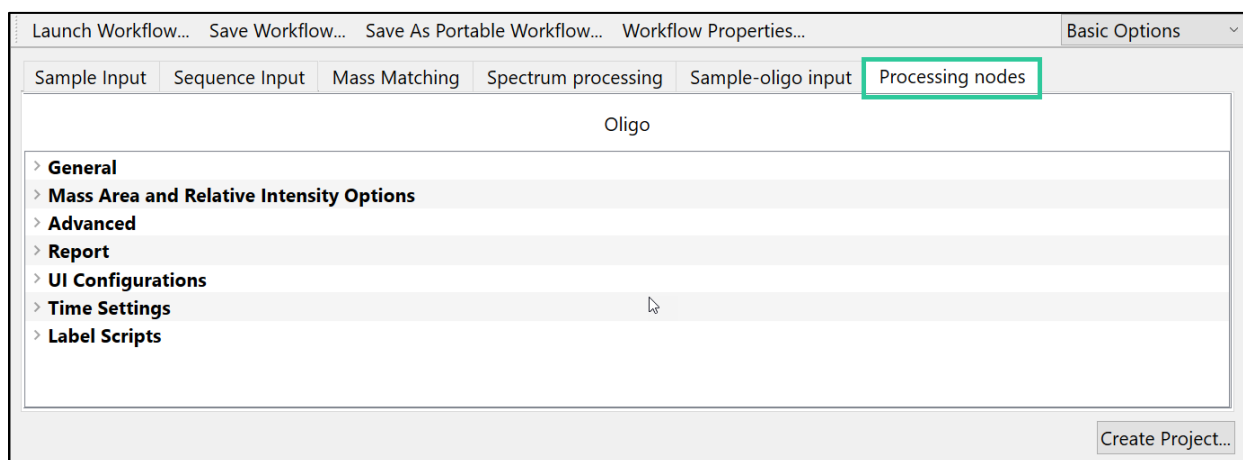


Figure 14: Processing nodes Tab

- **General**

▼ General	
Samples	*
Enable Lock-Mass Calibration	— No
Lock Mass (m/z)	556.2771
Mass assignments	Auto charge deconvolution and mass assignments

Figure 15: General parameters.

- **Samples** - The “*” asterisk character applies all parameters to all samples dragged and dropped into the Samples tab.
- **Enable Lock-Mass Calibration** can be set as Yes or No.
- **Lock Mass (m/z)** sets the calibrant m/z value. Several are available using the drop-down menu or the user can type in a numerical value. If empty, no calibration will be applied.
- **Mass assignments** allows the user to turn on/off charge deconvolution and automatic mass assignment.

- **Mass Area and Relative Intensity Options**

▼ Mass Area and Relative Intensity Options	
Compute Areas of Mass Peaks	✓ Yes
Mass Area Width	500
Report Intensities Relative to Local Base Peak	✓ Yes
Window for Local Base Peak (%)	20
Minimum % of Local Base Peak	10
Generate zoomed-in segments	None
Plot segment width	50.00

Figure 16: Mass Area and Relative Intensity Options

- **Compute Areas of Mass Peaks**
- **Mass Area Width** compute peak area within a band around each mass, defined by the mass area width value.
- **Report Intensities Relative to Local Base Peak** reports intensities based upon the two below parameters.
- **Window for Local Base Peak (%)** sets tolerance for local base peak (e.g. mass within +/- 20%).
- **Minimum % of Local Base Peak** filters out masses below a certain % of local base peak.
- **Generate zoomed-in segments**: The user has 3 options: None, Using reference masses, Using observed masses (per highest local base peak).
- **Plot segment width** can be set to automatically generate segments around reference/observed masses for deconvoluted mass spectrum and MS1 plots during project creation.

- **Advanced**

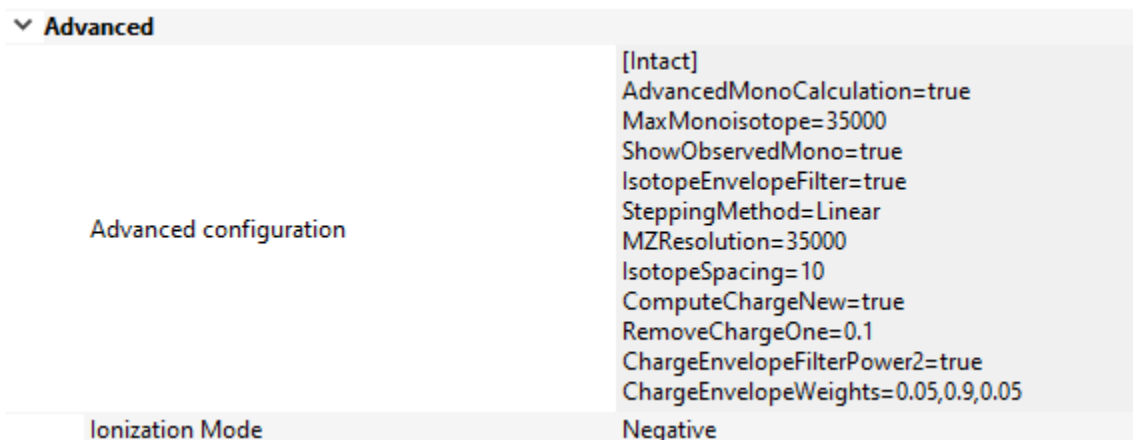


Figure 17: Advanced commands

Several advanced commands can be applied to Oligonucleotide Analysis processing by adding in the **Advanced configuration** text box. Please refer to the [Oligo Analysis: Advanced Commands](#) section of this user guide. For the **Ionization Mode**, the user has an option to select between **Positive** and **Negative** modes using the drop-down menu.

- **Report**

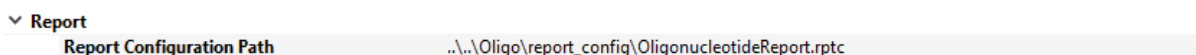


Figure 18: Report Configuration Path

Each Byos default workflow includes a report template created by our Customer Success team that is optimized for the specific type of analysis. If the user prefers a customized report template, they can direct Byos to this file using the light blue “...” button (double-click on the row). They will be prompted to select a file.

- **UI Configurations**

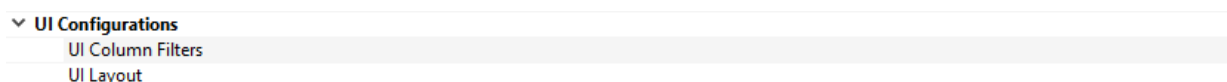


Figure 19: UI Configurations – UI Column Filters and UI Layout.

The user can import column filters as well as layout files. This is designed to standardize analyses across all users, labs, and sites. The user can direct Byos to the preferred file for each using the light blue “...” button (double-click on the row). The user will be prompted to select a file.

- **Time Settings**



Figure 20: Time Settings

- **Alignment max time** sets the maximum alignment value between plots (for example, UV and TIC). This value will limit the allowed alignment time between the two signals. The default value is set to 1.00.

- **Label Scripts**

This feature allows users to customize peak labels for Trace plot and Deconvolved Mass Spectrum.

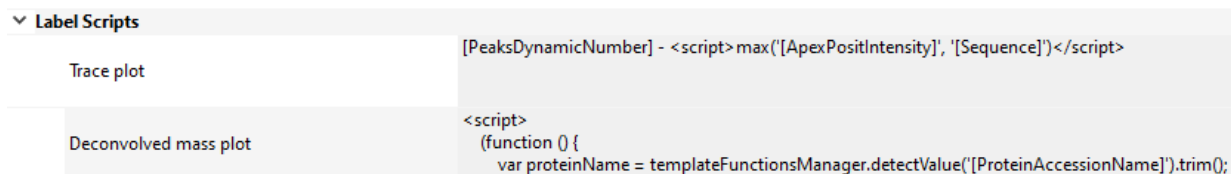


Figure 21: Label Scripts

Scripts related to trace plot are in C:\Program Files\ProteinMetrics\PMI-Suite\Base\labelscripts\traceplot folder, while the scripts related to deconvolved mass plot are in C:\Program Files\ProteinMetrics\PMI-Suite\Base\labelscripts\dmsplot folder. To load a script during project creation, select **Processing nodes**, expand **Label Scripts**, click on ... for Trace plot (or Deconvolved mass plot), then click **Load** to select a script, then click **OK** and **Create Project**. This will result in creation of the project with custom labels as specified in the script.

The user has an option to load scripts after project creation as well. To customize peak labels after project creation, select **Rendering options** icon, click **Edit Annotations**, then click **Load** to select a script, then click **Open** and click **OK** to display new custom labels.

Oligonucleotide Analysis

Finally, save the workflow and start the oligonucleotide analysis by clicking **Create Project**. This will result in the generation of *.olms project file and an Oligo specific report being attached to the project.

Below is the example project file generated that is discussed in detail in the [Project Inspection section](#):

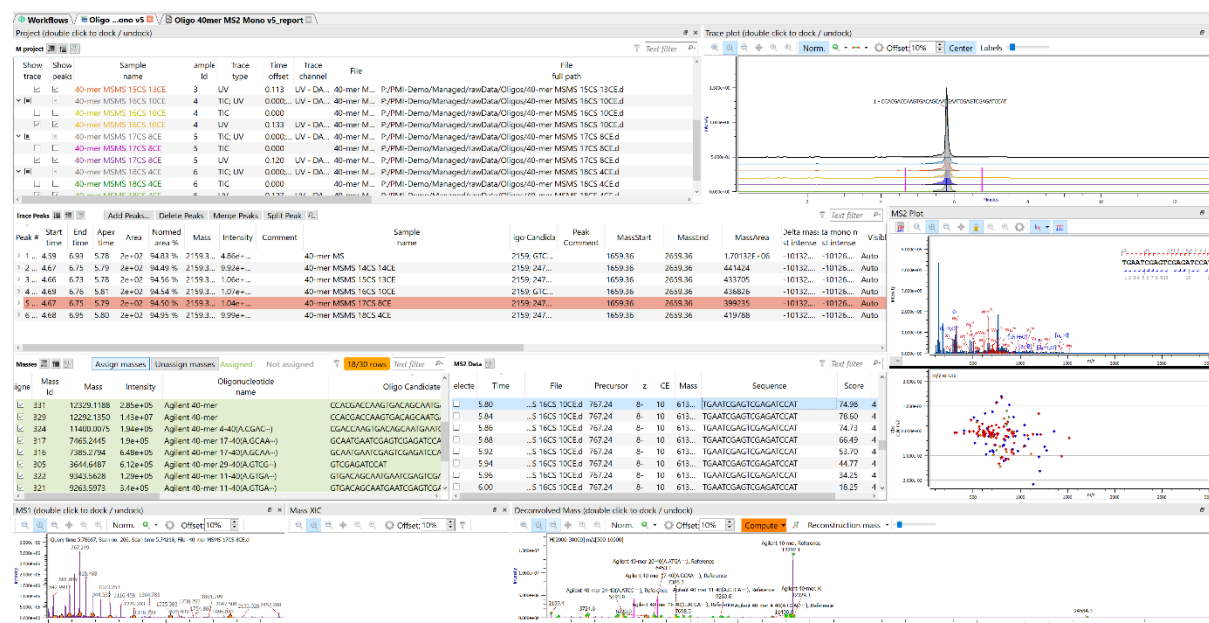


Figure 22: Oligonucleotide project

Below is the example report generated that is discussed in detail in the [Reporting section](#):

Workflows / Oligo ... v5.11 / Oligo 40mer MS2 Mono v5.1 report

File Edit Tabs

Show configuration Copy pivot table to clipboard Find: Enter text

Summary Expected Mass - Intensity Expected Mass - Relative Intensity (%) All Mass - Intensity All Mass - Relative Intensity (%) Expected Mass - Bar Chart Expected And Observed Mass - ppm error PLOTS Project options

Protein Metrics

Export of: C:\Users\Meagan\Downloads\Oligo 40mer MS2 Mono v5.1.xlsx
Creation time: 2025.09.25 16:09:41
Created by: Meagan Vaisa

Project table:

Show trace	Sample name	Sample Id	Trace type	Time offset	Trace channel	File	File full path	Project/Validate	Comment	ProjectStatus	Unexpected	Desired	Undesired	Clipped	Digestion MS file Type	MS file count
<input checked="" type="checkbox"/>	40-mer MSMS 14CS 14CE	1	TIC; UV	0.000; 0.127	UV - DAD 200nm	40-mer MSMS.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS.d			Review	24	1 of 1	0 of 0	14	1	1
<input checked="" type="checkbox"/>	40-mer MSMS 14CS 14CE	2	TIC; UV	0.000; 0.127	UV - DAD 200nm	40-mer MSMS 14CS 14CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 14CS 14CE.d			Review	8	1 of 1	0 of 0	14	1	1
<input checked="" type="checkbox"/>	40-mer MSMS 16CS 10CE	3	TIC; UV	0.000; 0.113	UV - DAD 200nm	40-mer MSMS 16CS 10CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 16CS 10CE.d			Review	8	1 of 1	0 of 0	14	1	1
<input checked="" type="checkbox"/>	40-mer MSMS 16CS 10CE	4	TIC; UV	0.000; 0.133	UV - DAD 200nm	40-mer MSMS 16CS 10CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 16CS 10CE.d			Review	8	1 of 1	0 of 0	13	1	1
<input checked="" type="checkbox"/>	40-mer MSMS 17CS 8CE	5	TIC; UV	0.000; 0.130	UV - DAD 200nm	40-mer MSMS 17CS 8CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 17CS 8CE.d			Review	9	1 of 1	0 of 0	13	1	1
<input checked="" type="checkbox"/>	40-mer MSMS 18CS 8CE	6	TIC; UV	0.000; 0.127	UV - DAD 200nm	40-mer MSMS 18CS 8CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 18CS 8CE.d			Review	9	1 of 1	0 of 0	13	1	1







Figure 23: Oligonucleotide report

Project Inspection

Project Table

The **Project** Table contains information about the MS samples:

Project (double click to dock / undock)

M project      Text filter 

Show trace	Show peaks	Sample name	Sample Id	Trace type	Time offset	Trace channel	File	File full path	Project/Validate	Comment	ProjectStatus	Desired
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	40-mer MSMS 18CS 4CE	1	TIC	0.000		40-mer MSMS 1...	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 18CS 4CE.d			Pass	1 of 1
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	40-mer MSMS 17CS 8CE	2	TIC	0.000		40-mer MSMS 1...	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 17CS 8CE.d			Pass	1 of 1
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	40-mer MSMS 16CS 10CE	3	TIC	0.000		40-mer MSMS 1...	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 16CS 10CE.d			Pass	1 of 1
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	40-mer MSMS 15CS 13CE	4	TIC	0.000		40-mer MSMS 1...	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 15CS 13CE.d			Pass	1 of 1

Figure 24: Project View

Check or uncheck **Show trace** or **Show peaks** to show or hide these features for sub-set of samples. To edit a MS sample, right-click the entry to edit under the **Sample Name** header, click **Edit selected row(s)**, edit the name, and then click **OK**.

Trace Plot

The **Trace plot** shows the total ion chromatogram (TIC), base peak intensity (BPI), ultraviolet (UV) trace, or electropherogram (icIEF).

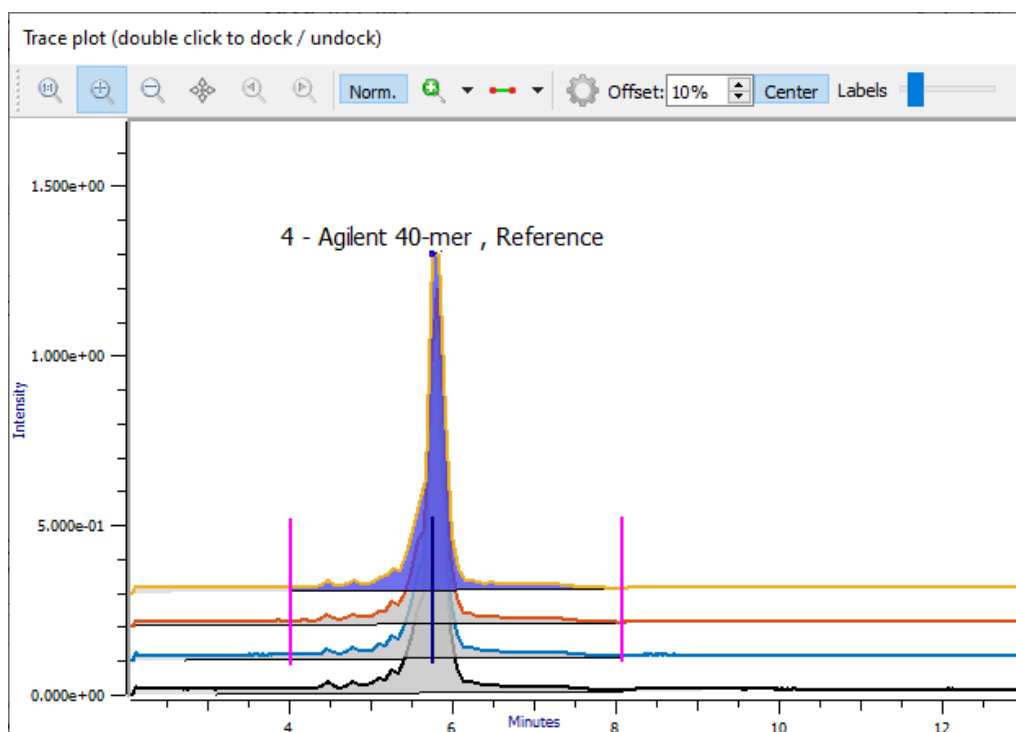













Figure 25: Trace Plot

The menu bars at the top of the three plot views, **Trace plot**, **MS1**, and **Deconvolved mass spectrum**, share icons that support generic operations on plots.

- The  icon resets the plot to default zoom level. Shortcut = double left-click.
- The  icon enables zooming in. After clicking, the cursor changes to . Drag across the range of the plot to display to zoom to that x-range.
- By default, the plot's y-range scales according to the maximum y-value within the x-range, but the software also supports freeform zooming.
- The  icon enables zooming out. Click anywhere in the plot to zoom out.
- The  icon enables moving (panning) across the plot. Click the plot and drag up or down, right or left to view a part of the plot that is off-screen.
- The  icon performs an undo of the last zoom step. Shortcut = Shift-left arrow key.
- The  icon performs a redo of the last zoom step. Shortcut = Shift-right arrow key.
- The **Norm.** button scales the y-coordinates so that the tallest peak has intensity 1.
- The  icon manages plot segments or zoom states. The number displayed in the icon shows how many plot segments are stored (named by the date and time of the save). To save the current zoom state as a plot segment, simply click the  icon. To switch to a previously stored plot segment, click the drop-down arrow and select the segment.
- The  icon manages how plots are displayed (render options), as well as zoom modes.

The Trace plot menu bar includes the following specialized control:

- The  icon shows the options for managing baseline anchors. *The Trace plot baseline has no effect on the neutral mass spectrum, only the areas of the trace peaks.*

Trace Peaks Table

The **Trace Peaks** table contains details about identified peaks, organized by sample:

Trace Peaks										Add Peaks...		Delete Peaks		Merge Peaks		Split Peak				Text filter	
Peak #	Start time	End time	Apex time	Area	Normed area %	Mass	Intensity	Comment	Sample name	Oligo Candidate	Peak Comment	MassStart									
> 1 (30)	3.09	8.08	5.82	2.5e+08	92.78 %	2079.39 - 1...	1.080e+05 ...	40-mer MSMS 18CS 4CE		Agilent 40-mer ...		1579.39; 1659.36;									
> 2 (30)	2.72	8.03	5.82	2.5e+08	94.25 %	2079.39 - 1...	1.377e+05 ...	40-mer MSMS 17CS 8CE		Agilent 40-mer ...		1579.39; 1659.36;									
> 3 (30)	2.06	8.06	5.82	2.6e+08	97.05 %	2079.39 - 1...	1.441e+05 ...	40-mer MSMS 16CS 10CE		Agilent 40-mer ...		1579.39; 1659.36;									
> 4 (30)	4.01	8.07	5.75	2.4e+08	89.36 %	2079.39 - 1...	1.272e+05 ...	40-mer MSMS 15CS 13CE		Agilent 40-mer ...		1579.39; 1659.36;									


Figure 26: Trace Peaks

To view specific peak data for a sample, click the > symbol to the right of that sample:

Trace Peaks										Text filter		
Peak #	Start time	End time	Apex time	Area	Normed area %	Mass	Intensity	Comment	Sample name	Oligo Candidate	Peak Comment	MassStart
> 1 (30)	3.09	8.08	5.82	2.5e+08	92.78 %	2079.39 - 1...	1.080e+05 ...	40-mer MSMS 18CS 4CE		Agilent 40-mer ...		1579.39; 1659.36;
> 2 (30)	2.72	8.03	5.82	2.5e+08	94.25 %	2079.39 - 1...	1.377e+05 ...	40-mer MSMS 17CS 8CE		Agilent 40-mer ...		1579.39; 1659.36;
> 3 (30)	2.06	8.06	5.82	2.6e+08	97.05 %	2079.39 - 1...	1.441e+05 ...	40-mer MSMS 16CS 10CE		Agilent 40-mer ...		1579.39; 1659.36;
> 4 (30)	4.01	8.07	5.75	2.4e+08	89.36 %	2079.39 - 1...	1.272e+05 ...	40-mer MSMS 15CS 13CE		Agilent 40-mer ...		1579.39; 1659.36;
> 4 (30)	4.01	8.07	5.75	2.4e+08	89.36 %	2079.3934	2.71e+06	40-mer MSMS 15CS 13CE		Agilent 40-mer ...		1579.39; 1659.36;
> 4 (30)	4.01	8.07	5.75	2.4e+08	89.36 %	2159.3617	1.3e+06	40-mer MSMS 15CS 13CE		Agilent 40-mer ...		1659.36
> 4 (30)	4.01	8.07	5.75	2.4e+08	89.36 %	2307.5576	2.15e+05	40-mer MSMS 15CS 13CE		2308		1807.56
> 4 (30)	4.01	8.07	5.75	2.4e+08	89.36 %	2722.4993	7.09e+05	40-mer MSMS 15CS 13CE		Agilent 40-mer ...		2222.5
> 4 (30)	4.01	8.07	5.75	2.4e+08	89.36 %	3011.6574	4.76e+05	40-mer MSMS 15CS 13CE		Agilent 40-mer ...		2611.66

Figure 27: Trace Peaks - Expanded sub-table

The Trace Peaks table includes the following specialized buttons:

- **Add Peaks** opens a dialog for the input of start and end times for a new peak.
- **Delete Peaks** deletes the selected peak.
- **Merge Peaks** merges a contiguous set of selected peaks into a single peak. Use left-click and shift-click to select a set.
- **Split Peak** splits a peak into two peaks. The time values for the two peaks can then be adjusted separately.
- **Filter options:** The magnifying glass  opens a dialog that allows the user to adjust the trace peak filter by overall trace time or minimum peak area (percent of TIC) to determine which trace peaks will be processed.

The Trace Peaks table includes the specialized right-click menu **Edit selected row(s)**. This menu is available from certain numeric fields such as Start time, End time and Apex time, and for annotation fields, such as Mass Comment, Peak Comment and Visible. This allows the user to overwrite the computed values for those fields for the given sample, as well as mark or comment on samples peak records.

Masses Table

The **Masses** table reports the masses of the most intense peaks that are selected in the Trace Peaks table.






Masses    							Text filter 	
Assign	Mass Id	Mass	Intensity	Oligonucleotide name	Oligo Candidate	Comment		
<input checked="" type="checkbox"/>	10	2687.4406	4.68e+06	Spinraza LNA 11-18(T.+A+A+T+G--)	+A+A+T+G+Q+T+G+G		2	
<input checked="" type="checkbox"/>	11	2688.4390	2.92e+06	Spinraza LNA 11-18(T.+A+A+T+G--)	+A+A+T+G+Q+T+G+G		2	
<input checked="" type="checkbox"/>	12	2689.4415	1.44e+06	Spinraza LNA 11-18(T.+A+A+T+G--)	+A+A+T+G+Q+T+G+G		2	
<input checked="" type="checkbox"/>	9	2686.4410	4.21e+06	Spinraza LNA 11-18(T.+A+A+T+G--)	+A+A+T+G+Q+T+G+G		2	
<input checked="" type="checkbox"/>	5	2345.3811	7.82e+06	Spinraza LNA 12-18(A.+A+T+G+Q--)	+A+T+G+Q+T+G+G		1	
<input checked="" type="checkbox"/>	7	2347.3891	4.37e+06	Spinraza LNA 12-18(A.+A+T+G+Q--)	+A+T+G+Q+T+G+G		1	
<input checked="" type="checkbox"/>	6	2346.3860	6.91e+06	Spinraza LNA 12-18(A.+A+T+G+Q--)	+A+T+G+Q+T+G+G		1	
<input checked="" type="checkbox"/>	8	2348.3875	2.03e+06	Spinraza LNA 12-18(A.+A+T+G+Q--)	+A+T+G+Q+T+G+G		1	
<input checked="" type="checkbox"/>	14	3019.4844	2.03e+06	Capped 9-17(A.+T+A+A+T--)	+T+A+A+T+G+Q+T+G+G		2	
<input checked="" type="checkbox"/>	15	3020.4861	1.46e+06	Capped 9-17(A.+T+A+A+T--)	+T+A+A+T+G+Q+T+G+G		2	
<input checked="" type="checkbox"/>	13	2619.4896	1.57e+06	Capped 9-17(A.+T+A+A+T--)	+T+A+A+T+G+Q+T+G+G		2	

Figure 28: Masses Table

The Masses table includes the following specialized buttons:

- **Assign Masses** assigns selected rows, an alternative to checking the Assigned boxes. If there is no selected row, **Assign Masses** opens a dialog to define a new mass by name, neutral mass, start and end times, intensity, and comment.
- **Unassign Masses** can be used to unassign selected rows.

The Masses table includes the specialized right-click menu **Edit selected row(s)**. This menu is available from certain numeric fields such as Mass Start, Mass End and Mass area, and along with other fields, such as Mass Comment and Name. This allows the user to overwrite the computed values for those fields for the given sample, as well as mark or comment on mass records.

MS2 Data Table

For a row highlighted in **Masses** table, the **MS2 Data** table provides information of the sequences, along with other details such as the mass, charge state, acquisition time, and score (a value which reflects the probability of randomly matching observed peaks to theoretical fragments). Each row of the **MS2 Data** table represents a single MS2 scan.



MS2 Data  114/114 rows 							
Selected	Time	File	Precursor	z	Mass	Sequence	Score
<input type="checkbox"/>	4.33	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	1.58
<input type="checkbox"/>	4.34	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.07
<input type="checkbox"/>	4.36	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.00
<input type="checkbox"/>	4.41	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.17
<input type="checkbox"/>	4.42	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.05
<input type="checkbox"/>	4.44	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.11
<input type="checkbox"/>	4.49	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.21
<input type="checkbox"/>	4.50	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.01
<input type="checkbox"/>	4.52	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.00
<input type="checkbox"/>	4.57	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.06
<input type="checkbox"/>	4.58	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.14
<input type="checkbox"/>	4.60	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.14
<input type="checkbox"/>	4.65	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	1.87
<input type="checkbox"/>	4.66	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.01
<input type="checkbox"/>	4.68	...0-mer MSMS 14CS 14CE.d	876.93	14-	12291.98	CCACGACCAAGTGACAGCAATGAATCGAG...	0.00

Figure 29: MS2 Data Table

Oligo scoring is based on a difference between the theoretical isotope pattern and the observed isotope pattern, and reflects the degree of correlation, with 100+ corresponding to nearly identical theoretical and observed patterns.

MS2 and Mass Error Plots

The **MS2 and mass error plots** display plots of the MS2 spectra with the corresponding oligonucleotide fragments and a plot of their m/z mass errors:

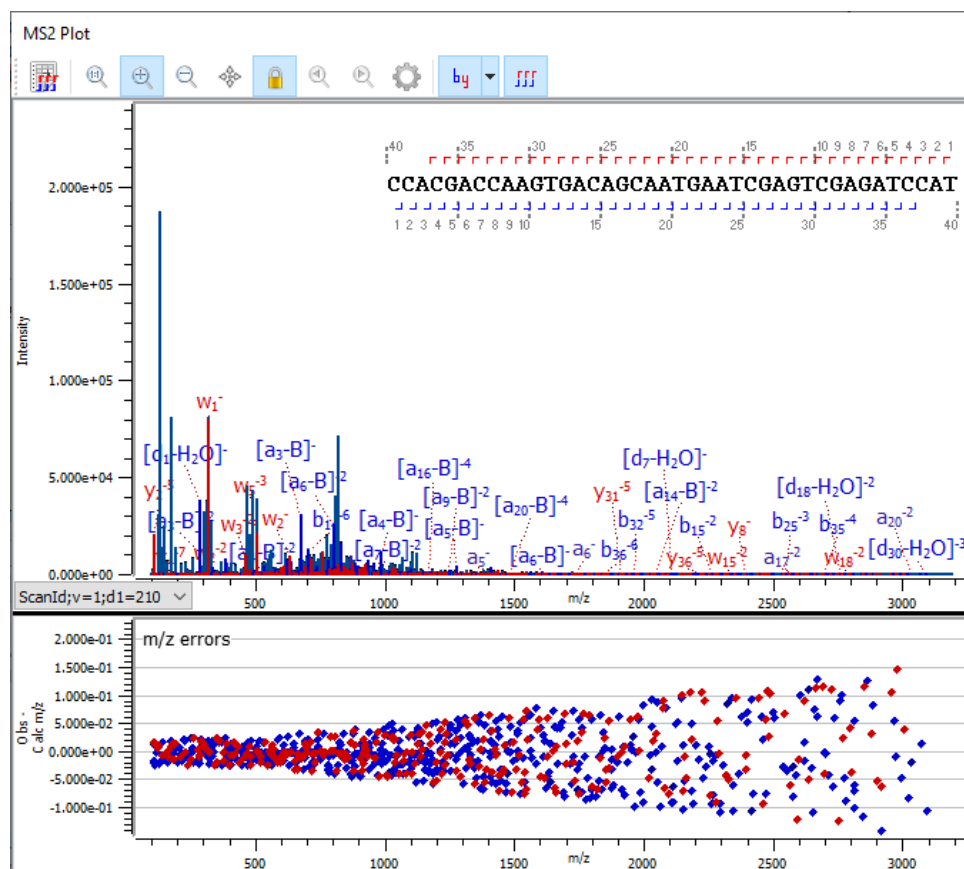


Figure 30: MS2 and mass error plots

Depending on the selection of a scan in **MS2 Data table**, the MS2 spectra and the corresponding mass error plots are populated.

- The  icon switches between the default spectrum view and the mass table view:






MS2 Plot						
  <input checked="" type="checkbox"/> Calculated <input type="checkbox"/> Observed <input type="checkbox"/> Delta						
PID=0: CCACGACCAAGTGACAGCAATGAATCGAGTCGAGATCCAT						
#	a ⁺ calc.	a ² calc.	[a-B] ⁺ calc.	[a-B] ² calc.	[a-B] ³ calc.	[a-B] ⁴ calc.
1	208.0728	103.5327				
2	497.1191	248.0559	386.0759	192.5343	128.0204	
3	810.1767	404.5847	675.1222	337.0575	224.3692	168.0251
4	1099.2231	549.1079	988.1799	493.5863	328.7218	246.2895
5	1428.2756	713.6342	1277.2262	638.1095	425.0706	318.5511
6	1741.3332	870.1630	1606.2787	802.6357	534.7547	400.8142
7	2030.3796	1014.6862	1919.3364	959.1645	639.1073	479.0786
8	2319.4260	1159.2094	2208.3827	1103.6877	735.4561	551.3402
9	2632.4836	1315.7382	2497.4291	1248.2109	831.8048	623.6018
10	2945.5412	1472.2670	2810.4867	1404.7397	936.1574	701.8662

Figure 31: Fragment Masses table

The reported fragment data that was confirmed in the analysis is displayed in red bold font. Unconfirmed fragment data is displayed in black font. In this view, the  icon switches the view back to MS2 plots. The  icon copies the content of this table to the clipboard.

- The  icon switches between showing and hiding the fragment labels associated with oligo fragmentation.

MS1 Plot

MS1 shows the summed m/z plot for the chromatogram or electropherogram peak selected in **Trace peaks** table.

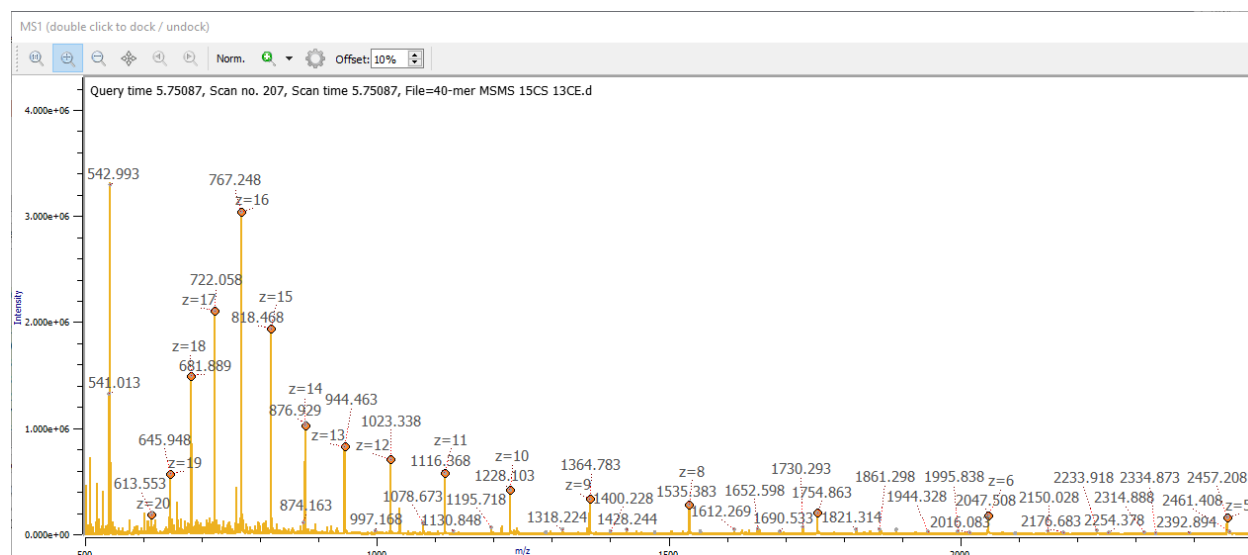


Figure 32: MS1 plot

Mass XIC Plot

The Mass XIC feature allows the user to quickly assess the elution profile of deconvolved species.

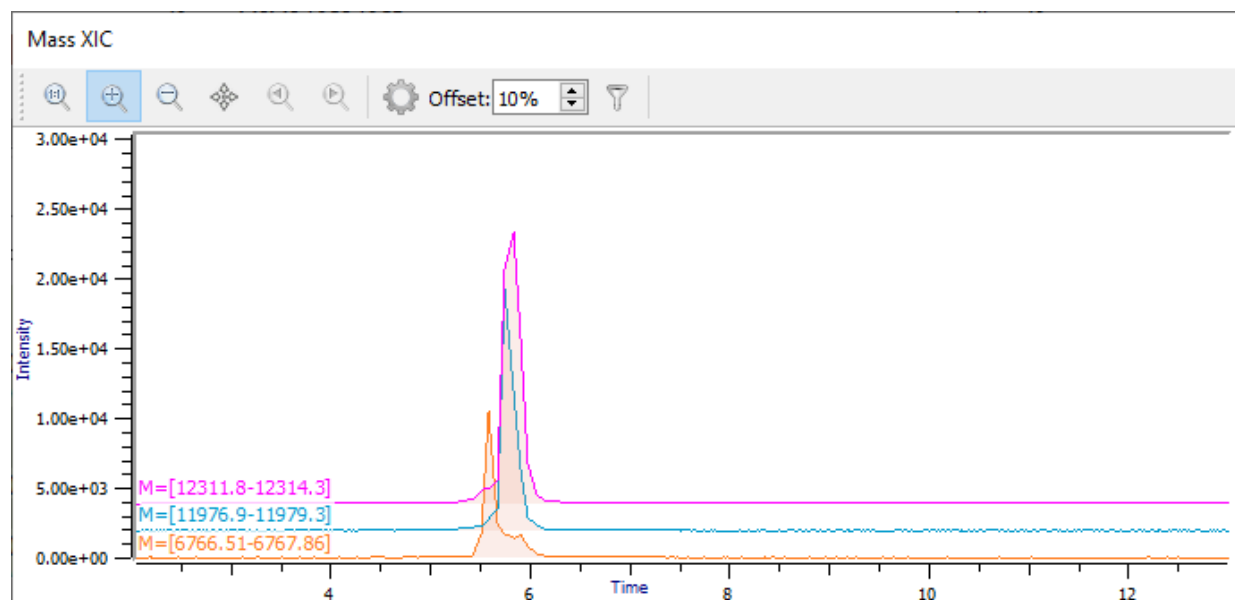


Figure 33: Mass XIC Plot

The Oligo module uses a sophisticated algorithm to improve signal to noise of XIC profile by optimally assembling information from multiple charge states of the same molecular compound. Mass XIC calculation, depending on LC peak width, can be computationally intensive and may need a runtime caching step. To provide XIC caching progress feedback, a progress bar **Constructing Mass XICs** tracks

the progress if the user selects non-cached data. Multiple peak selection in the Deconvolved Mass plot would result in showing Mass XICs for each of these peaks.

If the user wishes to disable Mass XIC generation and remove it from the Project layout, the option “Enable Mass XIC” can be unchecked in the **Window** tab.

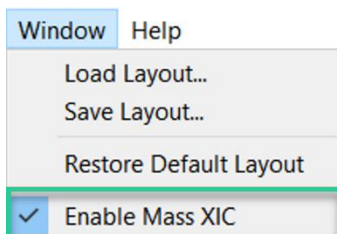


Figure 34: Enable Mass XIC

Mass XIC plot labeling can be customized within the **Template Editor**, which can be accessed by opening **Plot Settings > Labels > Edit Annotations**. From there, the user can enter an HTML script to customize labels as desired.

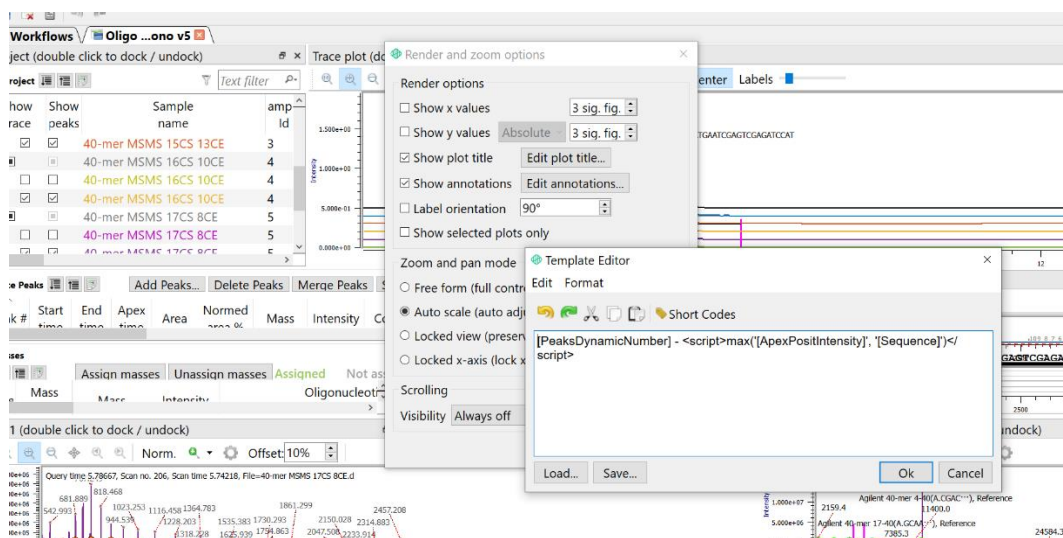


Figure 35: Labeling Mass XIC plot in Template Editor

Deconvolved Mass Spectrum

Below is a **Deconvolved mass** spectrum from the m/z plots. The neutral mass peaks in a deconvolved mass spectrum represent oligo forms present in the sample.

The **Deconvolved Mass** spectrum menu includes the **single most important** specialized control: the orange **Compute** button, which sets the parameters for the deconvolution. This Compute button functionality is like the [Spectrum Processing Tab](#) mentioned above. The user has the option to edit the parameters after project creation and re-run the analysis with new parameters and instantly get updated results.

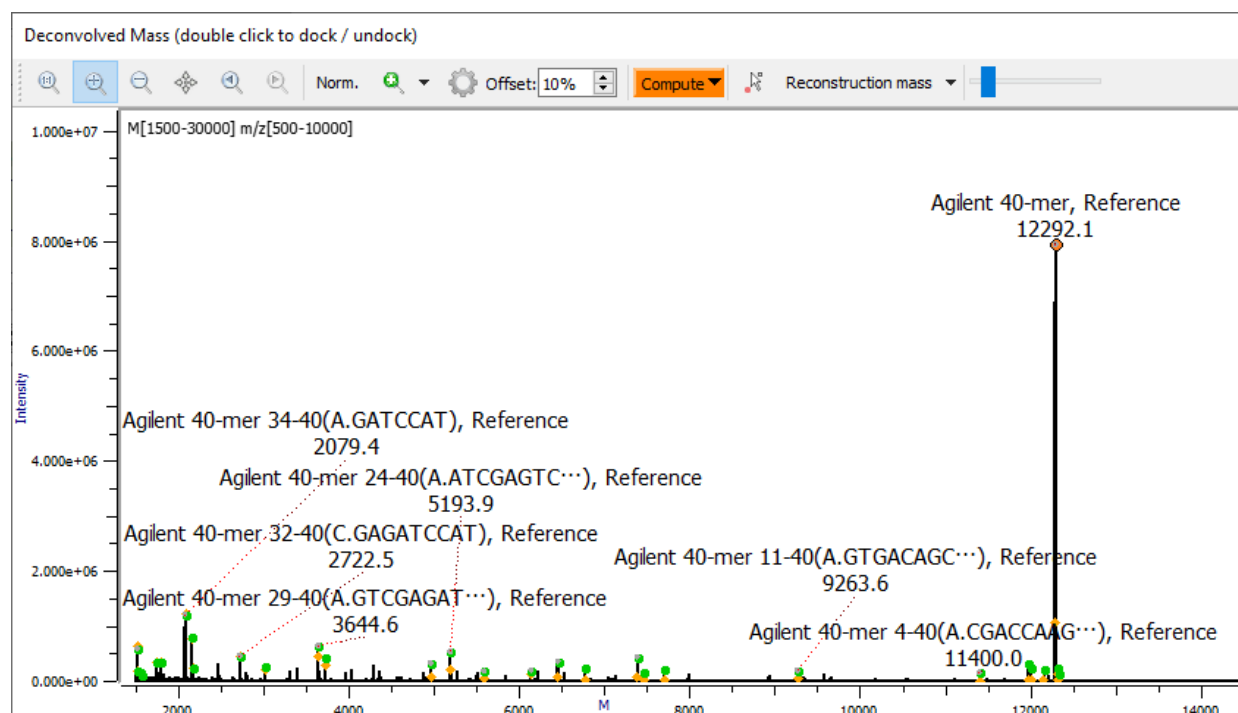



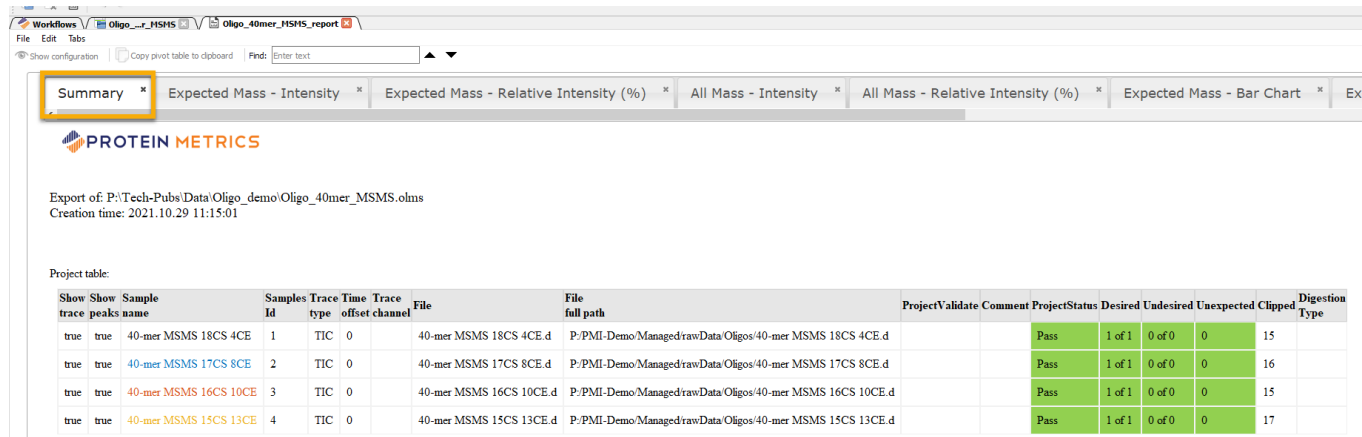
Figure 36: Deconvolved Mass spectrum

Reporting

To create a report, select **File > Export > Report** or simply click the report icon  below the Help menu. The report allows a user to visualize the results of their analyses more simply in table or variable graph formats. There are multiple tabs that are populated within the Oligo reporting tab defined by the default report template. However, each tab can be customized to meet the individual research needs.

Summary Tab

The Summary Tab provides information on the samples processed along with high-level summary of deconvolved mass spectrum output. The Traffic Light view displays the status of Desired, Undesired and Unexpected counts. If the project fails to report any desired masses, this will result in **ProjectStatus** column being reported as "Fail".

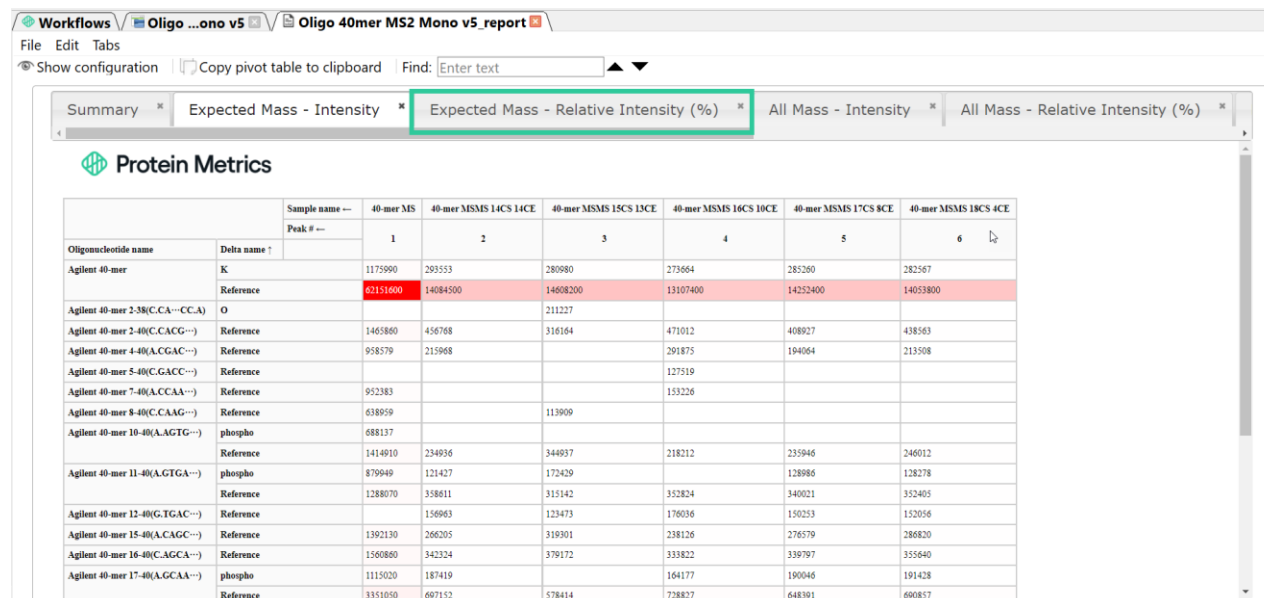


Show	Show	Sample	Samples	Trace	Time	Trace	File	File	Project	Validate	Comment	ProjectStatus	Desired	Undesired	Unexpected	Clipped	Digestion
trace	peaks	name	Id	type	offset	channel	full path	full path									Type
true	true	40-mer MSMS 18CS 4CE	1	TIC	0		40-mer MSMS 18CS 4CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 18CS 4CE.d				Pass	1 of 1	0 of 0	0	15	
true	true	40-mer MSMS 17CS 8CE	2	TIC	0		40-mer MSMS 17CS 8CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 17CS 8CE.d				Pass	1 of 1	0 of 0	0	16	
true	true	40-mer MSMS 16CS 10CE	3	TIC	0		40-mer MSMS 16CS 10CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 16CS 10CE.d				Pass	1 of 1	0 of 0	0	15	
true	true	40-mer MSMS 15CS 13CE	4	TIC	0		40-mer MSMS 15CS 13CE.d	P:\PMI-Demo\Managed\rawData\Oligos\40-mer MSMS 15CS 13CE.d				Pass	1 of 1	0 of 0	0	17	

Figure 37: Reporting – Summary Tab

Expected Mass - Relative Intensity % Tab

This tab allows the user to tabulate the relative intensities of expected oligonucleotides, i.e., those masses that were combinations found in reference masses and delta masses table (see [Delta masses sub-tab](#) section). Each vertical column will add up to 100% and allow the user to better understand which oligo fragments predominate each sample (column name represent sample name).

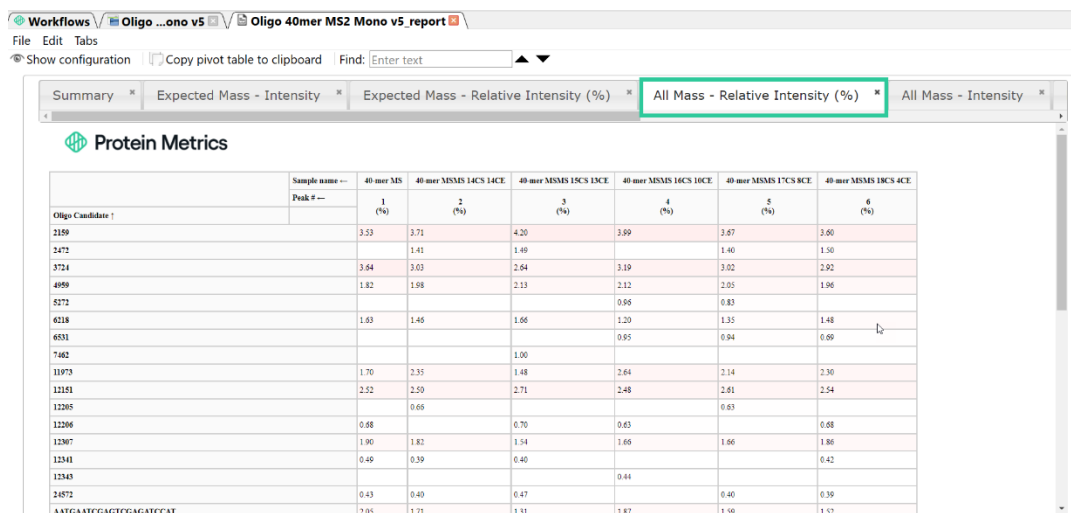


		Sample name -- Peak # --	40-mer MS	40-mer MSMS 14CS 14CE	40-mer MSMS 15CS 13CE	40-mer MSMS 16CS 10CE	40-mer MSMS 17CS 8CE	40-mer MSMS 18CS 4CE
Oligonucleotide name	Delta name ?		1	2	3	4	5	6
Agilent 40-mer	K	Reference	1175990	293553	280980	273664	285260	282567
	Reference		62151600	14084500	14608200	13107400	14252400	14053800
Agilent 40-mer 2-38(C.CA---CC.A)	O				211227			
Agilent 40-mer 2-40(C.CACG---)	Reference		1465860	456768	316164	471012	408927	438563
Agilent 40-mer 4-40(A.CGAC---)	Reference		958579	215968		291875	194064	213508
Agilent 40-mer 5-40(C.GACC---)	Reference					127519		
Agilent 40-mer 7-40(A.CCAA---)	Reference		952383			153226		
Agilent 40-mer 8-40(C.CAAG---)	Reference		638959		113909			
Agilent 40-mer 10-40(A.AGTG---)	phospho		688137					
	Reference		1414910	234936	344937	218212	235946	246012
Agilent 40-mer 11-40(A.GTGA---)	phospho		879949	121427	172429		128986	128278
	Reference		1288070	358611	315142	352824	340021	352405
Agilent 40-mer 12-40(G.TGAC---)	Reference			156963	123473	176036	150253	152056
Agilent 40-mer 15-40(A.CAGC---)	Reference		1362130	266205	319301	238126	276579	286820
Agilent 40-mer 16-40(C.AGCA---)	Reference		1560860	342324	379172	333822	339797	355640
Agilent 40-mer 17-40(A.GCAA---)	phospho		1115020	187419		164177	190046	191428
	Reference		3351050	697152	578414	728827	648391	690857

Figure 38: Relative Intensity of Expected Masses

All Mass - Relative Intensity % Tab

This tab allows the user to tabulate the relative intensities of all peaks identified in the Deconvolved Mass spectrum.



		Sample name -- Peak # --	40-mer MS	40-mer MSMS 14CS 14CE	40-mer MSMS 15CS 13CE	40-mer MSMS 16CS 10CE	40-mer MSMS 17CS 8CE	40-mer MSMS 18CS 4CE
Oligo Candidate ?			1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
2159			3.53	3.71	4.20	3.99	3.67	3.60
2472				1.41	1.49		1.40	1.50
3724			3.64	3.03	2.64	3.19	3.02	2.92
4959			1.82	1.98	2.13	2.12	2.05	1.96
5272							0.95	0.83
6218			1.63	1.48	1.66	1.20	1.35	1.48
6531						0.95	0.94	0.89
7462					1.00			
11973			1.70	2.35	1.48	2.64	2.14	2.30
12151			2.52	2.50	2.71	2.48	2.61	2.54
12285				0.66			0.63	
12286			0.68		0.70	0.63		0.68
12367			1.90	1.82	1.54	1.66	1.66	1.88
12341			0.49	0.39	0.40			0.42
12343						0.44		
24672			0.43	0.40	0.47		0.40	0.39
AATGAATCGAGTCGAGATCCAT			2.05	1.71	1.31	1.87	1.59	1.52

Figure 39: Relative Intensities of All Masses

Bar chart of Intensities

This interactive tab allows the user to visualize the relative intensity of selected oligo fragments from the project tab as a bar chart. Individual bars in the graph can be shown or hidden to gain a better understanding of their relative abundance by checking/unchecking the legend box at the bottom of the plot.

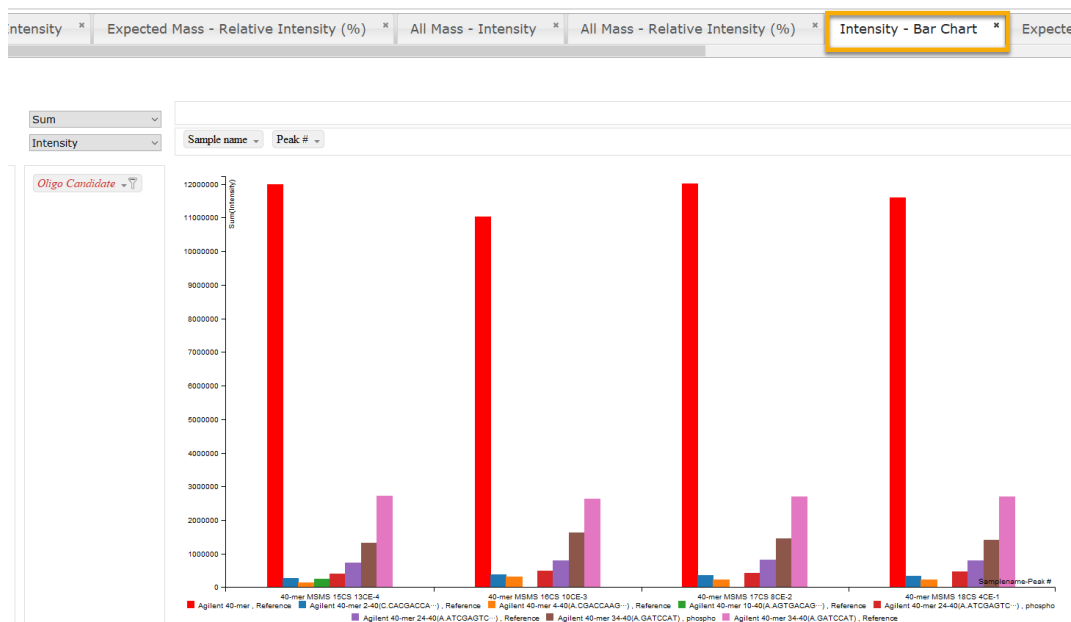


Figure 40: Reporting – Intensities of few oligo candidates

Expected and Observed Mass errors

This tab provides information on the mass errors between expected and observed masses.

Workflows | Oligo ...ono v5 | Oligo 40mer MS2 Mono v5_report

File Edit Tabs

Show configuration | Copy pivot table to clipboard | Find: Enter text

Mass - Relative Intensity (%) | All Mass - Intensity | Expected Mass - Bar Chart | **Expected And Observed Mass - ppm error** | PLOTS | Proje

Protein Metrics

Oligonucleotide name	Delta name	Expected mono mass	Sample name Peak #	40-mer MS 1	40-mer MSMS 14CS 14CE 2	40-mer MSMS 15CS 15CE 3	40-mer MSMS 16CS 16CE 4	40-mer MSMS 17CS 17CE 5	40-mer MSMS 18CS 18CE 6
Agilent 40-mer	K	12324.1897		-2.4293	-2.7131	-2.8677	-3.1573	-3.1974	-2.3593
Reference		12286.1897		0.4822	0.8497	0.3965	0.3547	0.6971	1.2550
Agilent 40-mer 2-38(C,CAC...CC-A)	O	11365.9597				0.4405			
Reference		11997.0633		0.4802	0.8443	0.6543	0.6191	1.2071	1.2152
Agilent 40-mer 4-40(A,CAGC...)	Reference	11394.9594		1.1297	-1.0394		-1.9182	-1.1803	1.2353
Agilent 40-mer 5-40(C,GACC...)	Reference	11105.9130					-0.5427		
Agilent 40-mer 7-40(A,CCAA...)	Reference	10463.8029		0.4578			0.8326		
Agilent 40-mer 8-40(CCAAG...)	Reference	10174.7565		0.2032		-0.1892			
Agilent 40-mer 10-40(A,AGTG...)	phospho	9652.6525		-3.7205					
Reference		9573.6525		0.4402	-0.5066	0.1468	0.7506	0.0123	1.5555
Agilent 40-mer 11-40(A,GTGA...)	phospho	9339.5949		-4.3518	-4.6832	-4.0473		-3.9763	-3.8040
Reference		9259.5949		-0.4504	0.0467	-0.1323	-0.0577	0.0261	0.0292
Agilent 40-mer 12-40(G,TGAC...)	Reference	8950.5424			-0.8937	0.0598	-0.7316	-0.6423	0.0998
Agilent 40-mer 15-40(A,CAGC...)	Reference	7984.3862		-0.3804	-0.0472	-1.1920	-2.5025	-0.1602	1.3370
Agilent 40-mer 16-40(C,AGCA...)	Reference	7695.3399		0.4986	0.8177	0.5371	0.2344	0.1768	1.2385
Agilent 40-mer 17-40(A,GCAA...)	phospho	7462.2822		-4.0700	-4.1061		-4.6863	-4.3759	-4.0876
Reference		7582.2822		-0.4648	-0.6747	-0.8854	-0.7366	-0.8335	0.0942

Figure 41: Expected and Observed mass differences across all samples

For custom report generation, please contact support@proteinmetrics.com.

Digested Oligonucleotides Workflow

The **Digested Oligonucleotides** workflow is intended for use with samples that have enzymatically digested oligonucleotides like mRNA and MSMS acquisition with (high resolution) accurate mass instruments. It will match sequences and provide fragmentation displays in a viewer.



Figure 42: Digested Oligonucleotides workflow

This workflow includes the following actions:

- Sequence Database Oligo Spectrum Matching (Byonic Oligo)
- Confident isomer assignment
- Detection and localization of modifications
- Wildcard searching for modification discovery
- XIC Plotting and MS1 Quantitation
- Interactive data review tools and visualizations
- Automated and customizable reporting

Digested Oligonucleotide Analysis Dashboard

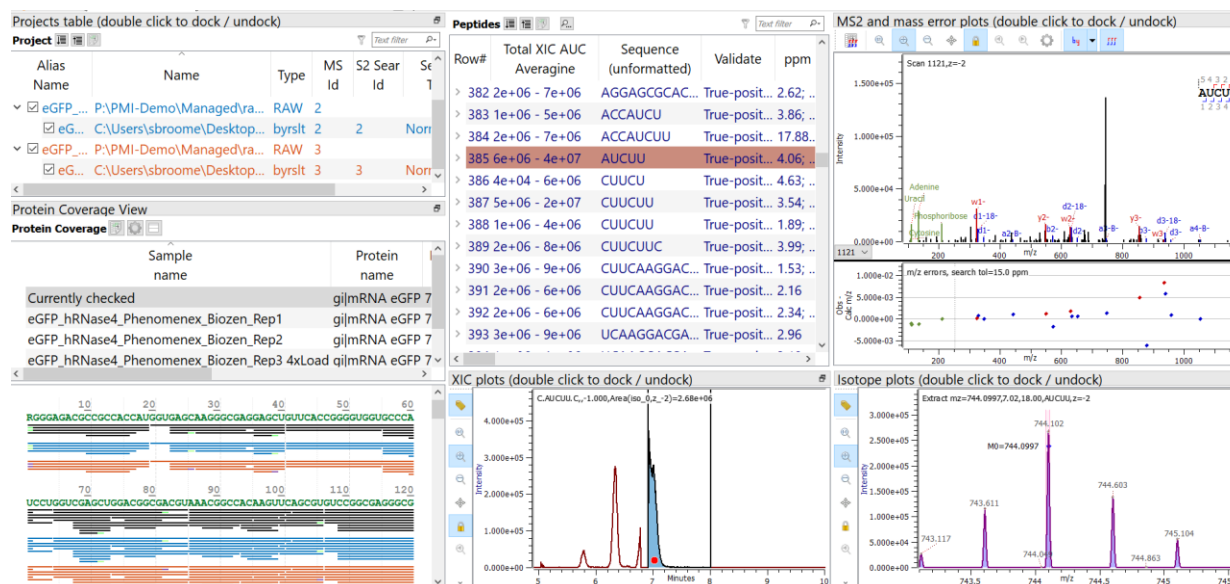


Figure 43: Digested Oligonucleotide project dashboard

Creating a Project

The Digested Oligonucleotides uses the same general UI platform as the Peptide workflows.

The user has an option to edit an existing workflow by launching the workflow, making the edits to the workflow, and saving this as new workflow by clicking the **Save Workflow** (shown in below figure).




The user can also create a new workflow by clicking the Oligo icon. This will result in a Project Creation window (shown in below figure).

Samples

Launch Workflow... Save Workflow... Save As Portable Workflow... Workflow Properties... Basic Options ▾

Samples Sequences Processing nodes


Samples (drag and drop MS files)

Samples table   Text filter 

Sample name	Sample type	MS file	MS/MS search	Digestion

Add sample(s)... Add row Remove rows(s) Add column... Remove column... Create Project...

Figure 44: Sample input

MS sample files are added in the **Samples** tab. Oligonucleotide Analysis accepts a variety of reference sample files (Bruker: *.d, Thermo: *.raw, Waters: *.raw, SCIEX: *.wiff, Agilent: *.d). To enter a file, drag and drop an MS raw file into the project window. Alternatively, click **Add sample**, double-click in the **MS file** column, click  and browse to the sample file. Click **Open**. Users can also drag and drop samples into the dialog to populate the samples row.

The sample is named for the file name; to change the sample name, click the default name twice and enter a new sample name. Click **Add sample** at left to add a new row to the **Samples table**. Then add the desired files as above. To remove an unwanted sample file row, select the row and click **Remove sample(s)**.

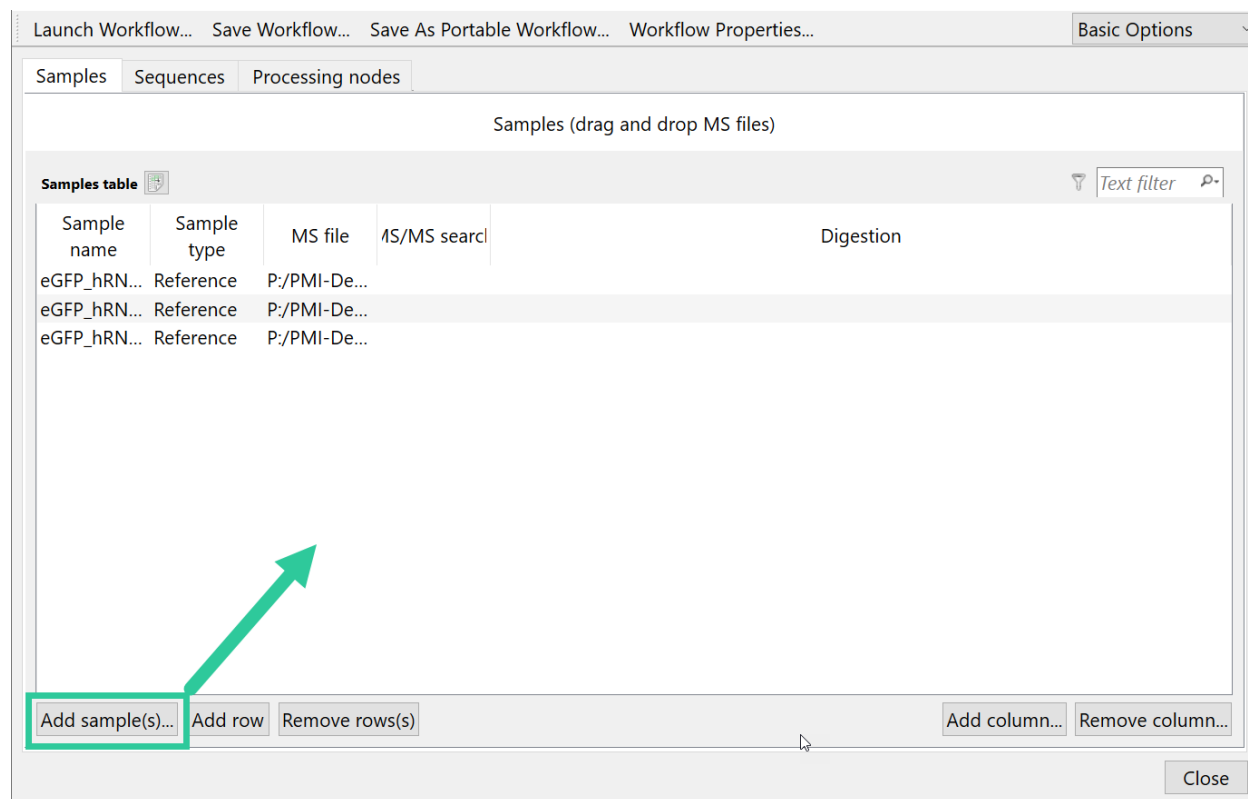


Figure 45: Add Samples

Sequence tab

In the **Sequences** tab, the user should add the oligo nucleobase sequence. Currently we support a 1-character code. Byos Digested Oligo assumes a phosphodiester backbone, ribose or deoxyribose, and unmodified A, G, C, U, and T. Modifications can be used to change the default sugar, nucleobase, and phosphate linker.

Launch Workflow... Save Workflow... Save As Portable Workflow... Workflow Properties... Basic Options ▾

Samples Sequences Processing nodes

Oligonucleotides (drag and drop FASTA files)

Oligo Name	Oligo Alias Name	Oligo sequence
gijmRNA e...		RGGGAGACGCCGCCACCAUGGUGAGCAAGGGCGAGGAGCUGUUCACCGGGGUGGUGCCCAUCCUGGUCGA...

Browse for FASTA file... Add row... Remove selected

Oligonucleotide Annotations 🔍 Text filter ⓘ

Oligo Name	Start Residue	Length	Annotation value
------------	---------------	--------	------------------

Import CSV... Export CSV... Add row Remove row(s)

Close

Figure 46: Add sequence via FASTA

Users can browse to add the sequence or drag and drop a FASTA file into the dialog to populate the Protein Sequence.

Processing nodes

Instrument Parameters	
Precursor Mass Tolerance	10.00 ppm
Molecule Type	RNA ▾
Fragment Mass Tolerance 1	RNA
Fragment Mass Tolerance 2	DNA
Recalibration (lock mass)	20.00 ppm
	None

Figure 46: Digested Oligonucleotide workflow parameters

Under the **Digestion** tab, the user should define digestion to meet RNase digest rules. Digestion specificity allows for full/semi specific digestion search and a set of # of Missed Cleavages. A nonspecific search is unlikely to be useful for oligonucleotides.

▼ Digestion		
Cleavage Site(s)	G	
Cleavage Side	C-terminal	
Digestion Specificity	Fully specific (fastest)	
Missed Cleavages	3	

Figure 47: Digestion tab

Under the **Modifications** tab, the user should set Total Common and Rare Max as needed. Custom modifications can be updated by clicking on the ellipses in the Modifications row.

> Digestion		
▼ Modifications		
Modifications	Cyclic Phosphate / +61.955 @ CTerm rare1	...
Total Common Max	1	
Total Rare Max	1	

Figure 48: Modifications tab

By default, the Digested Oligo workflow assumes 5' (NTerm) PO₄H₂ and 3' (CTerm) OH termini for each digested oligo. NTerm and CTerm fixed mods are used to change the 5' and 3' termini to match the specific RNase used. Here, fixed NTerm and CTerm mods are used to set the termini for RNase T1 (5' OH and 3' PO₄H₂).

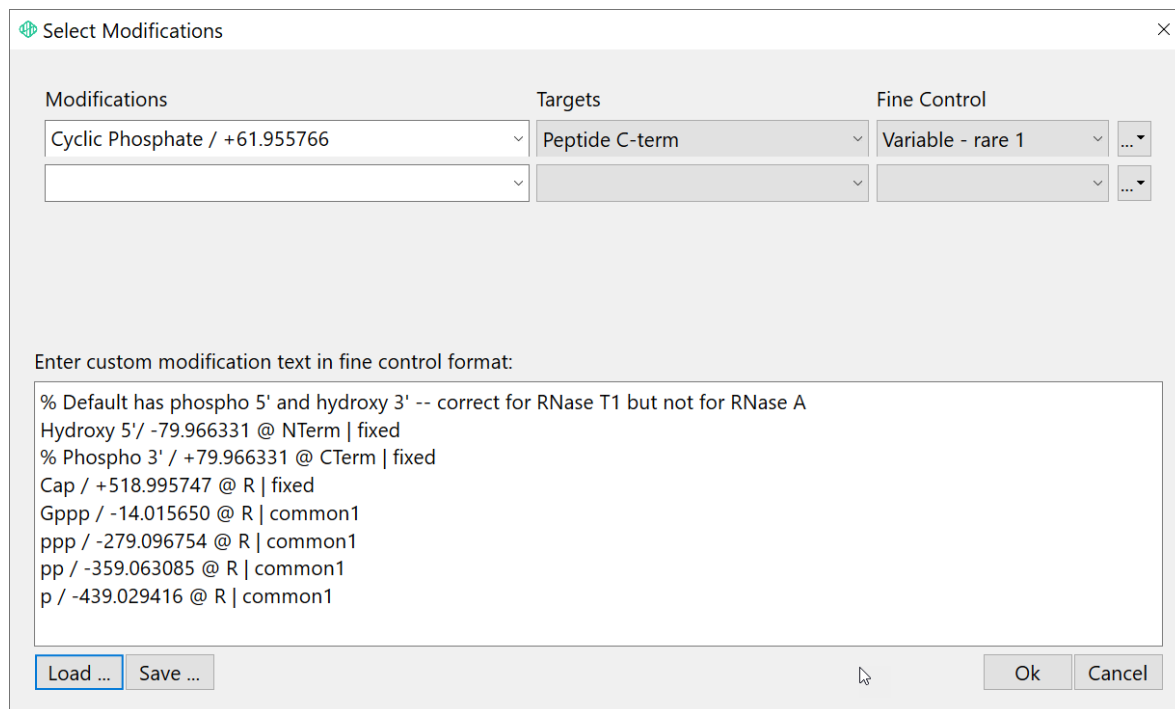


Figure 49: Select Modifications

If no terminal modifications are applied, Byos Digested Oligonucleotides expects 5' PO₄H₂ and 3' OH.
 5' **PO₄H₂**-ACCCAUUG-**OH** 3'

The Fixed Mods below adjust the Default 3' and 5' termini masses for an RNase T1 digested sample.
 5' **OH**-ACCCAUUG-**PO₄H₂** 3'

Hydroxy 5' / -79.966331 @ NTerm | fixed

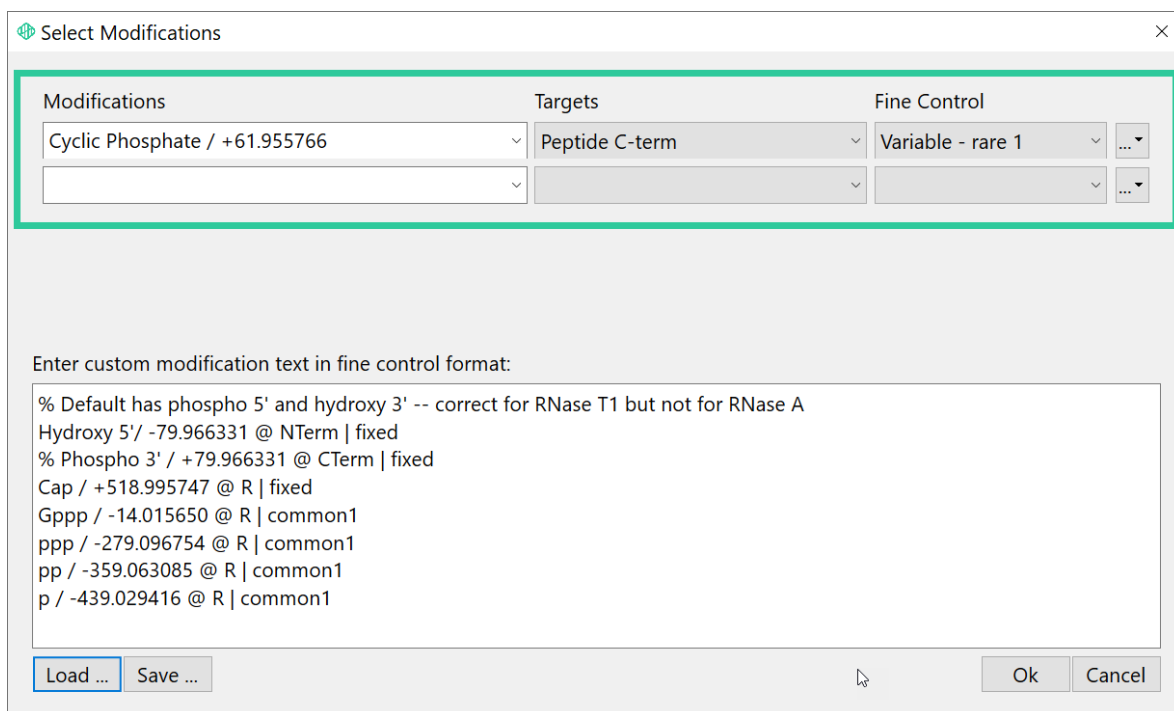
Phospho 3' / +79.966331 @ CTerm | fixed

Adding % "comments out" the terminal modification.

5' OH-ACCCAUUG-OH 3'

Hydroxy 5' / -79.966331 @ NTerm | fixed

% Phospho 3' / +79.966331 @ CTerm | fixed



Select Modifications

Modifications	Targets	Fine Control
Cyclic Phosphate / +61.955766	Peptide C-term	Variable - rare 1

Enter custom modification text in fine control format:

```
% Default has phospho 5' and hydroxy 3' -- correct for RNase T1 but not for RNase A
Hydroxy 5' / -79.966331 @ NTerm | fixed
% Phospho 3' / +79.966331 @ CTerm | fixed
Cap / +518.995747 @ R | fixed
Gppp / -14.015650 @ R | common1
ppp / -279.096754 @ R | common1
pp / -359.063085 @ R | common1
p / -439.029416 @ R | common1
```

Load ... Save ... Ok Cancel

Figure 50: Modifications




Set fixed or variable modifications as needed. Modifications can be applied generally or to localized spots with fixed mods on “unused” characters.

Digested Oligonucleotide Analysis

Project Inspection

Project Table

The **Project** table contains information about the MS samples and results files:

Project   Text filter 

Alias Name	Name	Type	MS Id	S2 Sear Id	Search Type	Sample type
✓ <input checked="" type="checkbox"/> eGFP_...	C:\Users\Morgan Vasas\Dr...	RAW	1			Reference
<input checked="" type="checkbox"/> test...	C:\Users\Morgan Vasas\Dr...	byrsIt	1	1	Normal	
✓ <input checked="" type="checkbox"/> eGFP_...	C:\Users\Morgan Vasas\Dr...	RAW	2			Reference
<input checked="" type="checkbox"/> test...	C:\Users\Morgan Vasas\Dr...	byrsIt	2	2	Normal	
✓ <input checked="" type="checkbox"/> eGFP_...	C:\Users\Morgan Vasas\Dr...	RAW	3			Reference
<input checked="" type="checkbox"/> test...	C:\Users\Morgan Vasas\Dr...	byrsIt	3	3	Normal	

Figure 51: Project table

To display or remove the MS sample and result combination, check or uncheck the box at left of either a sample or result record. To edit a MS sample or result alias name, right-click the entry to edit under the **Alias Name** header, click **Edit selected row(s)**, edit the name, and then click **OK**.

Oligonucleotide Coverage Table and Menu


The **Oligonucleotide Coverage** table displays **sequences** and corresponding oligonucleotide coverage. The accompanying display maps the sequences of detected oligonucleotides to colored lines below each sequence representing colors assigned to different MS samples:



Figure 52: Oligo coverage table

The different colors within the colored bars indicate the presence of modifications at those positions. (The colors used are chosen for contrast and do not reflect the colors assigned to the MS sample files.)

The Oligonucleotide Coverage table also includes the following specialized menu icon buttons:

- The  icon opens the rendering options dialog:

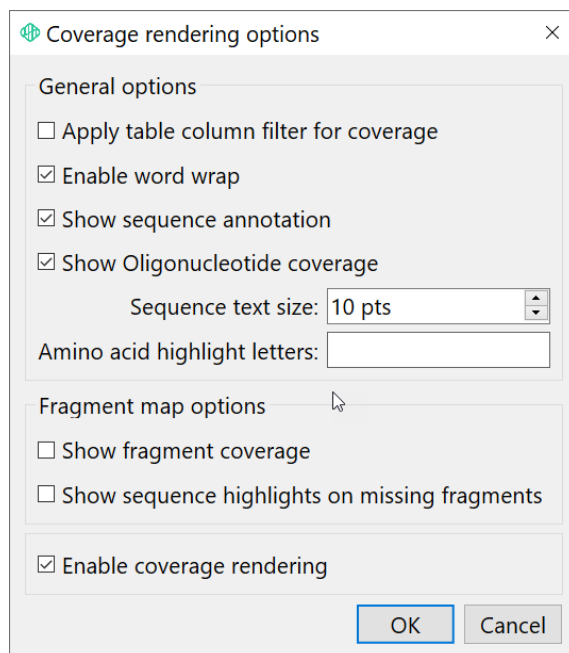


Figure 53: Protein coverage rendering options

- The  icon turns on and off the display of the tabular protein coverage data.

The Oligonucleotide Coverage view has context menu items revealed by a right-click on the graphical portion of the display:

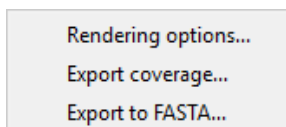


Figure 54: Protein coverage right click menu

- **Rendering options** also opens the Protein coverage rendering options dialog shown above.
- **Export coverage** creates a *.png file of the graphical protein coverage display.
- **Export to FASTA** creates a *.fasta file of the proteins found in the Protein Coverage view. The *.fasta file is then available to be imported into new projects.

Oligonucleotides Table and Menu


The **Oligonucleotides** table contains detailed information about all identified oligos:

Oligonucleotides															Unique Repeat sequence Non-unique isoform															Text filter	
Row#	Sequence unformatted	Validate	ppm	z	XIC area summed	XIC AUC	Calc. m/z	Obs. m/z	Oligo Name	Oligo mass	3' cap	5' cap	Start Residue	End Residue	PID																
> 1	ACCU	True-positive	1.99; 1....	-2	7.43e+06	1.57e+06 - 3.39...	621....	621.574...	gilmRNA eG...				618	621	59; 58...																
> 2	GAGC		1.71; 2....	-2	1.37e+06	301 - 9.46e+05	661....	661.088...	gilmRNA eG...				23	26	34; 58...																
> 3	ACCCC		2.28; 2....	-2	7.04e+06	1.26e+06 - 4.5e...	742....	742.625...	gilmRNA eG...				241	245	36; 11...																
> 4	ACCCU		2.52; 2....	-2	3.55e+07	6.27e+06 - 2.25...	743....	743.117...	gilmRNA eG...				372	376	39; 41...																
> 5	CAUCU		2.43; 3....	-2	1.87e+07	2.24e+06 - 1.38...	743....	743.609...	gilmRNA eG...				158	162	26; 29...																
> 6	AUCCU		4.40; 4....	-2	1.63e+07	6.05e+05 - 8.16...	743....	743.611...	gilmRNA eG...				426	430	45; 11...																
> 7	GCCCU	True-positive	5.22; 5....	-2	1.16e+07	5.49e+04 - 1.03...	751....	751.117...	gilmRNA eG...				185	189	1; 108...																
> 8	CACAU		4.01; 2....	-2	1.72e+07	3.04e+06 - 1.09...	755....	755.124...	gilmRNA eG...				668	672	42; 44...																
> 9	ACCAU		2.72; 3....	-2	7.04e+06	3.71e+04 - 3.56...	755....	755.123...	gilmRNA eG...				15	19	60; 11...																
> ...	GCACC		2.83; 3....	-2	1.27e+07	2.08e+06 - 5.4e...	762....	762.628...	gilmRNA eG...				163	167	21; 2; ...																
> ...	GACCU		3.14; 3....	-2; -1	1.65e+08	2.37e+04 - 9.74...	763....	763.121...	gilmRNA eG...				212	216	30; 14...																
> ...	ACAAC		2.68; 2....	-2	3.74e+07	7.03e+06 - 2.32...	766....	766.637...	gilmRNA eG...				448	452	72; 69...																
> ...	CAAGU		2.70; 3....	-2	1.48e+08	4.02e+06 - 1.11...	775....	775.126...	gilmRNA eG...				730	734	66; 37...																

Figure 55: Oligonucleotides table

The **Peptide** table organizes records in the following hierarchy: Peptide > Sample > Charge state (if combined) > MS2 scan. Records in the **Peptide** table can be modified directly by the user for three fields: Validate, Comment, and Labels. In the Validate field, the user selects between “True-positive”, “False-positive”, and “Uncertain”. Comments are input directly. Values for Labels are selected from a list. To create new labels to use in the Labels field, see **Edit > Label Manager**.

Note: The MS1 correlation score compares the measured MS1 isotope distribution to the theoretical distribution calculated using cosine similarity. A score of 1 is a perfect match.

In addition to the usual table menu buttons, the **Peptides** table contains the  icon button which opens the same Search Filter dialog that is opened by the **Edit > Search Filter** menu. The *text filter* entry box allows the records to be filtered based on the occurrence of the given text string searched across all fields.

The **Peptides** table has context menu items applicable to specific peptides. The menu is revealed by a right-click on a peptide cell in the table:

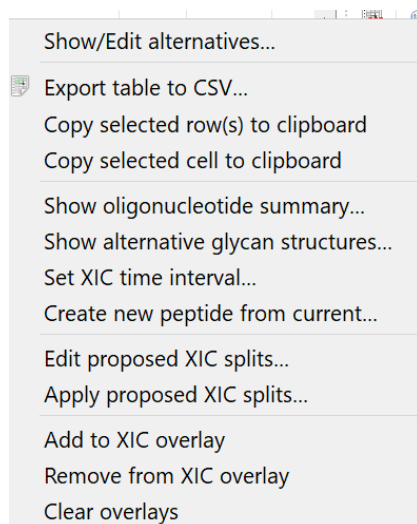
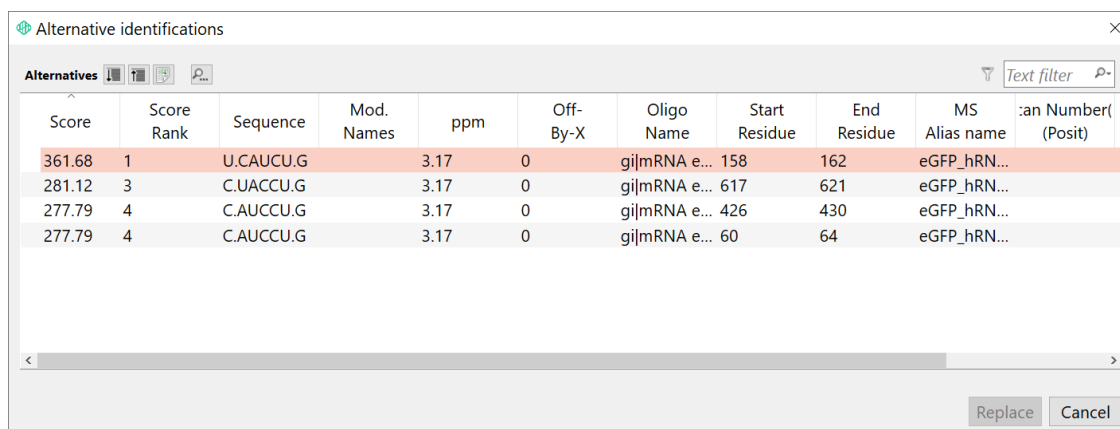



Figure 56: Peptides table right click menu

- **Show/Edit alternatives** displays a table of alternative MS2 identifications (with different sequences and/or modifications) for the selected peptide, along with their scores:



Alternative identifications

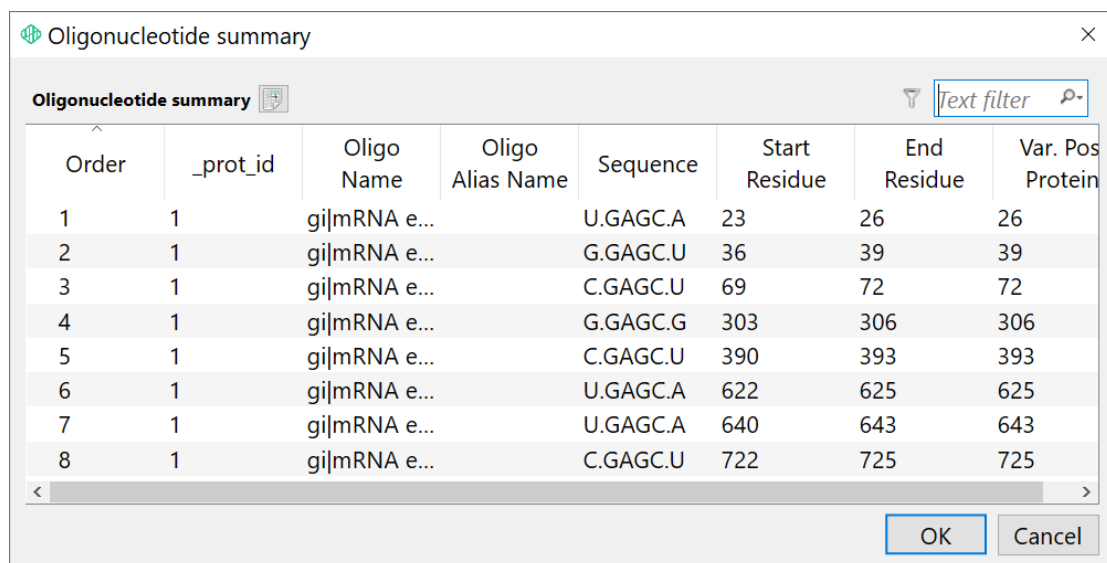
Alternatives 


Score	Score Rank	Sequence	Mod. Names	ppm	Off-By-X	Oligo Name	Start Residue	End Residue	MS Alias name	Ion Number (Posit)
361.68	1	U.CAUCU.G		3.17	0	gilmRNA e...	158	162	eGFP_hRN...	
281.12	3	C.UACCU.G		3.17	0	gilmRNA e...	617	621	eGFP_hRN...	
277.79	4	C.AUCCU.G		3.17	0	gilmRNA e...	426	430	eGFP_hRN...	
277.79	4	C.AUCCU.G		3.17	0	gilmRNA e...	60	64	eGFP_hRN...	

Figure 57: Peptide alternatives table

Select an alternative peptide and click **Replace** to replace the selected peptide in the **Peptides** table. Protein coverage, observed and calculated masses and *m/z* ratios all are updated.

- **Show Oligonucleotide summary** opens a table with protein data for that oligo:



Oligonucleotide summary 

Order	_prot_id	Oligo Name	Oligo Alias Name	Sequence	Start Residue	End Residue	Var. Pos Protein
1	1	gilmRNA e...		U.GAGC.A	23	26	26
2	1	gilmRNA e...		G.GAGC.U	36	39	39
3	1	gilmRNA e...		C.GAGC.U	69	72	72
4	1	gilmRNA e...		G.GAGC.G	303	306	306
5	1	gilmRNA e...		C.GAGC.U	390	393	393
6	1	gilmRNA e...		U.GAGC.A	622	625	625
7	1	gilmRNA e...		U.GAGC.A	640	643	643
8	1	gilmRNA e...		C.GAGC.U	722	725	725

Figure 58: Oligonucleotide summary

The values in the Protein summary can be captured in a report using the template **Blgc_PRM_wProteinDuplicates.rptc**.

- **Show alternative glycan structures...** allows the user to access alternative glycan structures rendered for glycopeptide modifications in generated reports featuring glycan cartoons. The table provides associated structures currently in the backend library. Users can also select **Add new structure from IUPAC...** to enter a new glycan structure to add to the library. See the **PMI Chromatogram Analysis Manual** for more information about building alternative glycan structures from IUPAC.

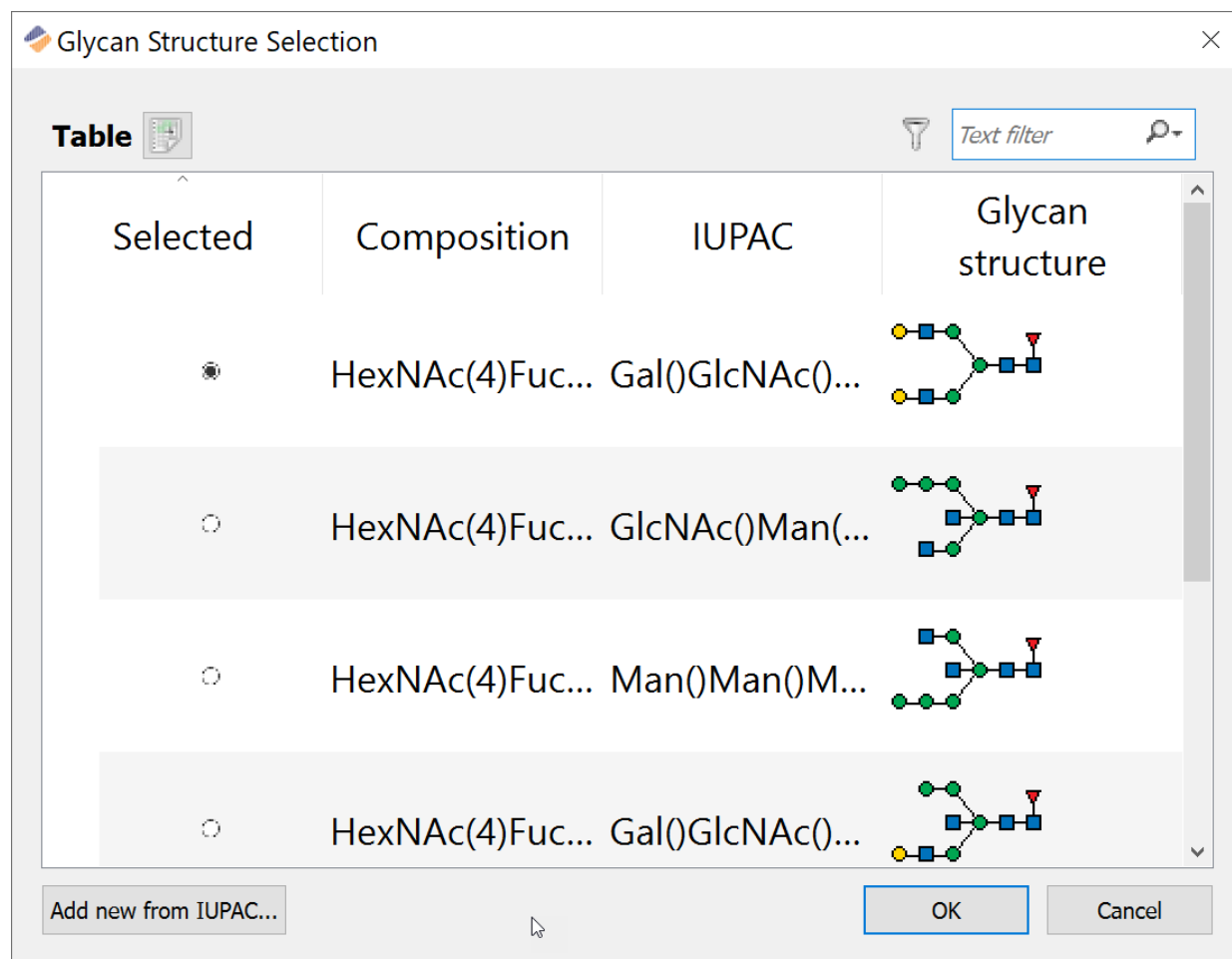


Figure 59: Glycan structure selection

- **Set XIC time interval** allows the user to edit the XIC start and end times for that peptide and to set the time tolerance (time warp) to improve performance:

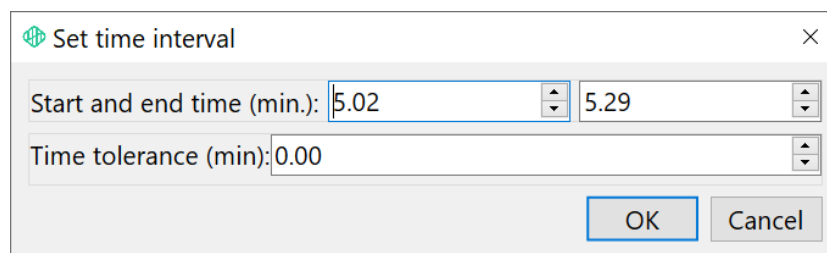
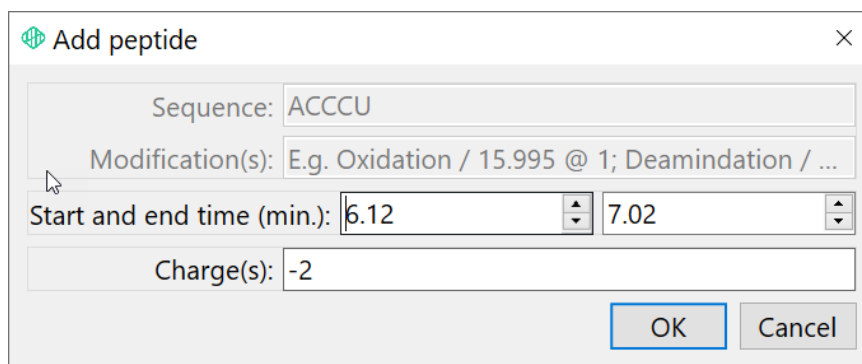


Figure 60: Set time interval

- **Create new peptide from current** makes a copy of that peptide. This allows XIC plots to be split according to separate integrations. The user edits the start and end times and the charge of the peptide before the copy is created:



Add peptide

Sequence: ACCCU

Modification(s): E.g. Oxidation / 15.995 @ 1; Deamidation / ...

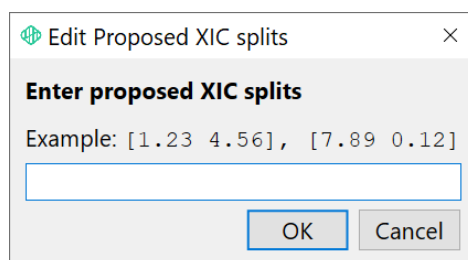
Start and end time (min.): 6.12 7.02

Charge(s): -2

OK Cancel

Figure 61: Create new peptide from current

- **Edit proposed XIC splits** opens a dialog to enter pairs of time ranges to split XIC plots for that peptide record into two or more chromatograms:



Edit Proposed XIC splits

Enter proposed XIC splits

Example: [1.23 4.56], [7.89 0.12]

OK Cancel

Figure 62: Edit proposed XIC splits

After a click on the **OK** button, the **Peptides** table record field **XIC proposals** is updated with the time range entries. The split must be applied to take effect.

- **Apply proposed XIC splits** separates a record in the **Peptides** table into two or more records using the time ranges entered using the Edit proposed XIC splits function.
- **Add to XIC overlay** adds the selected **Peptides** table record (subject to the right-click) or collection of records as an overlay to the XIC plots view mapped to the same time axis:

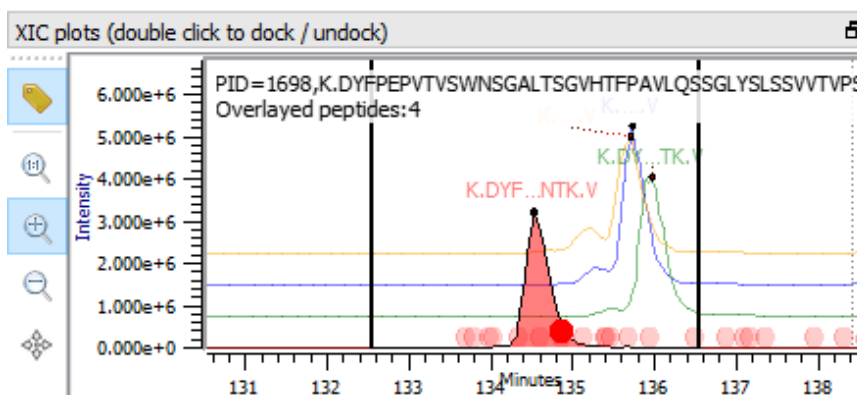


Figure 63: Add to XIC overlay

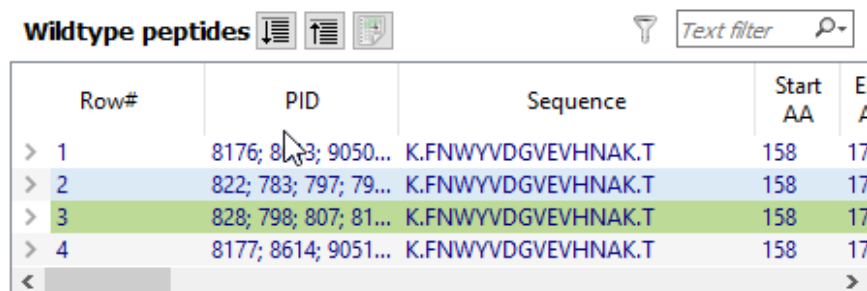
When this function is applied to a collection of records in the table, all of the records below it are added to the XIC plots view.

- **Remove from XIC overlay** removes the overlay from the selected Peptides table record (subject to the right-click) from the XIC plots view.

- **Clear overlays** removes all sample overlays from the XIC plots view.

Wildtype Peptides Table and Menu

The **Wildtype peptides** table contains detailed information about naturally occurring or wildtype peptides associated with the peptide selected in the Peptides table:



Row#	PID	Sequence	Start AA	End AA
> 1	8176; 8173; 9050...	K.FNWWYVDGVEVHNAK.T	158	17
> 2	822; 783; 797; 79...	K.FNWWYVDGVEVHNAK.T	158	17
> 3	828; 798; 807; 81...	K.FNWWYVDGVEVHNAK.T	158	17
> 4	8177; 8614; 9051...	K.FNWWYVDGVEVHNAK.T	158	17

Figure 64: Wildtype peptides table

The Wildtype peptides table has one context menu item specific to wildtype peptides. The menu is revealed by a right-click on a wildtype peptide cell in the table. **Set primary wildtype** marks that record as the primary wildtype peptide for the selected record in the Peptides table.

XIC Plots and Menu

The **XIC plots** view displays chromatograms for specific regions of the peptides and wildtype peptides associated with the record selected in the Peptides table:

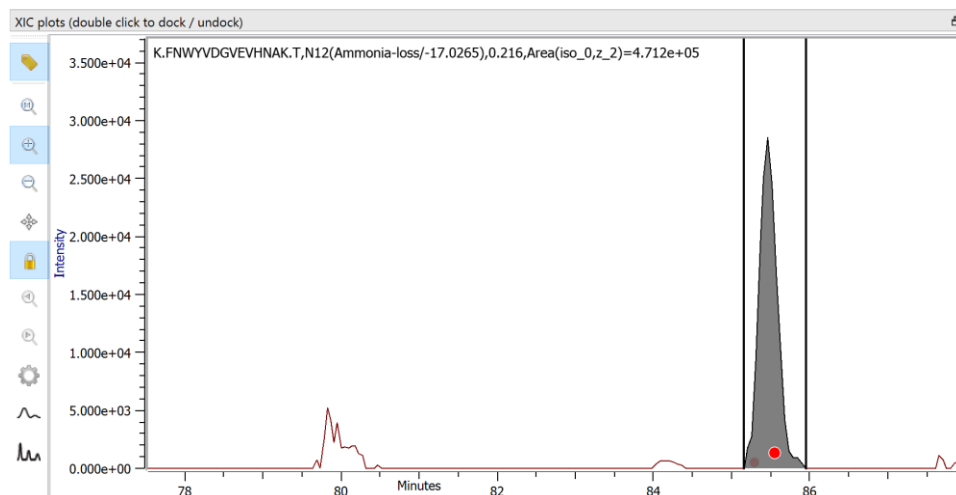







Figure 65: XIC plots

In addition to the menu icon functions shared by all the plot views, there are some functions specific to XIC plots:

- The  icon turns plot annotations on or off. In the XIC plots, annotations are displayed as circles at the base of the chromatograms. Dark red circles represent the position of currently displayed MS2 scans. Light red circles represent the position of other MS2 scans with the same identification. Light gray circles represent the position of MS2 scans with the same precursor mass as the selected peptide, but with different attribution. Hover over the circles and the associated sequence ID and value are displayed. Note that the cursor changes to a hand. Click

the circle and the corresponding Peptides table row is selected and the views are updated to display for that record.

- The  icon is used to compute the XIC plot integration for a specified time range. A dialog opens to enter start and end times.
- The  icon enables MS scan extraction. This displays isotope values derived from the XIC plot. A blue vertical line appears in the XIC plots view. As the line is dragged across the XIC trace, the Isotope plots view is updated to show the MS scan for that XIC time value. Two new icons appear on the Isotope plots menu bar in this mode: The  icon clears the extracted MS scan and restores the original isotope plots. The  icon saves the extracted MS scan to the project document.

If **Window > Enabled Stacked Plots** is selected, XIC plots for multiple samples can be overlaid and their explicit XIC integration limits defined.

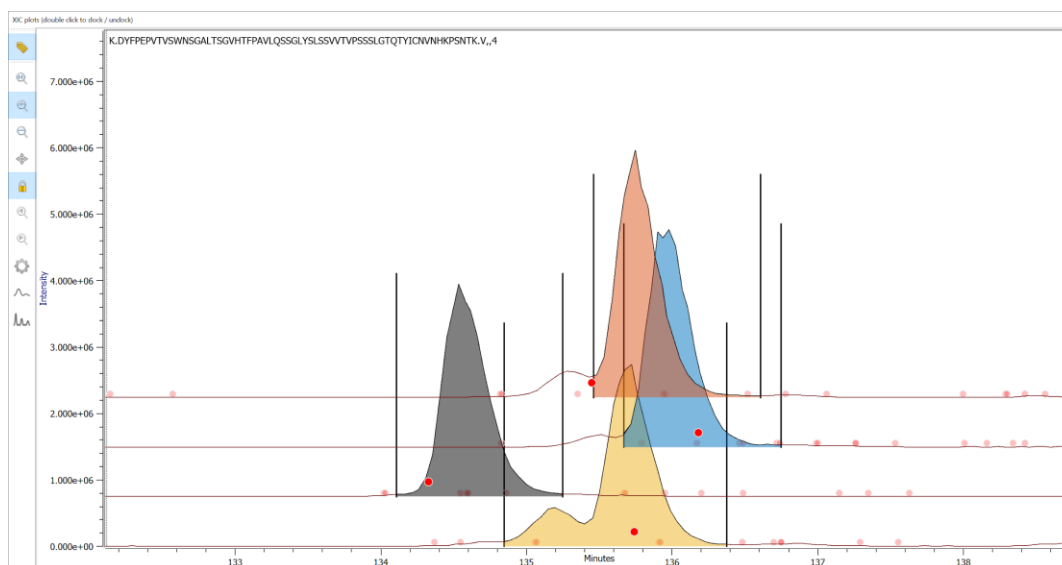


Figure 66: Stack XIC plots

With stacked plots enabled (via **Window> Enable Stacked Plots**), users can click and drag integration boundaries within XIC plots to simultaneously update start and end times for all samples and charges of a peptide. Subsequent changes will be reflected in the Peptides table.

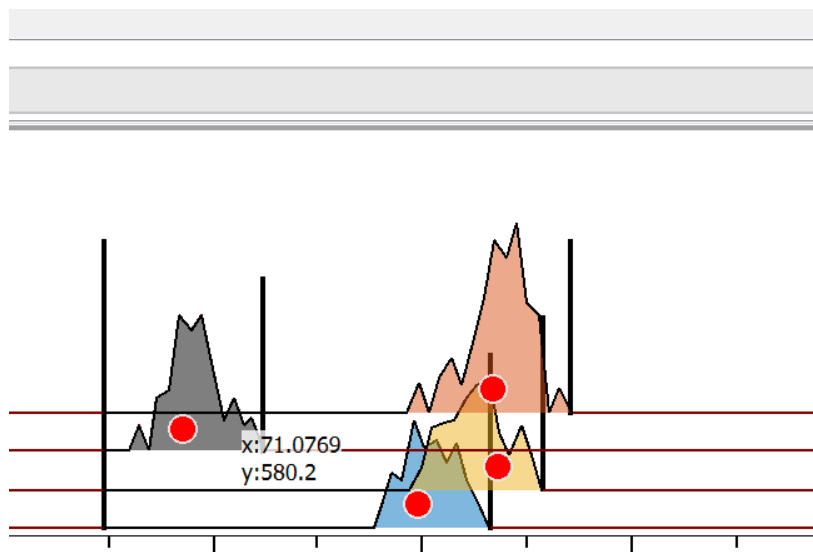


Figure 67: Selection of multiple plots

The option to edit multiple integration boundaries displayed in a set of stacked XICs at once is available with 'Shift+Click' and 'Ctrl+Click' actions. To move all start or end boundaries to the same retention time, hold down shift when clicking and dragging a boundary marker.

Alternatively, holding ctrl when clicking and dragging will result in boundary markers moving by the same amount.

If the user selects **Combine Charge States** under **Edit>Search Filter>Peptide Grouping Method** the application will display XIC traces for **multiple charge states** of the same molecule form in a single plot. This may provide additional evidence for the identity of the molecule.

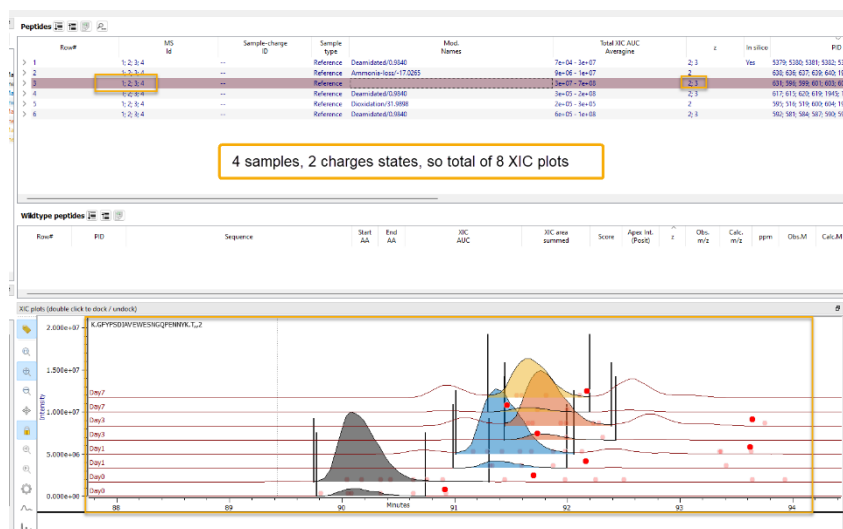


Figure 68: XIC traces for multiple charge states on one plot

Isotope Plots and Menu

The **Isotopes plots** display the peptide isotope (m/z scan) for the selected Peptides table record:

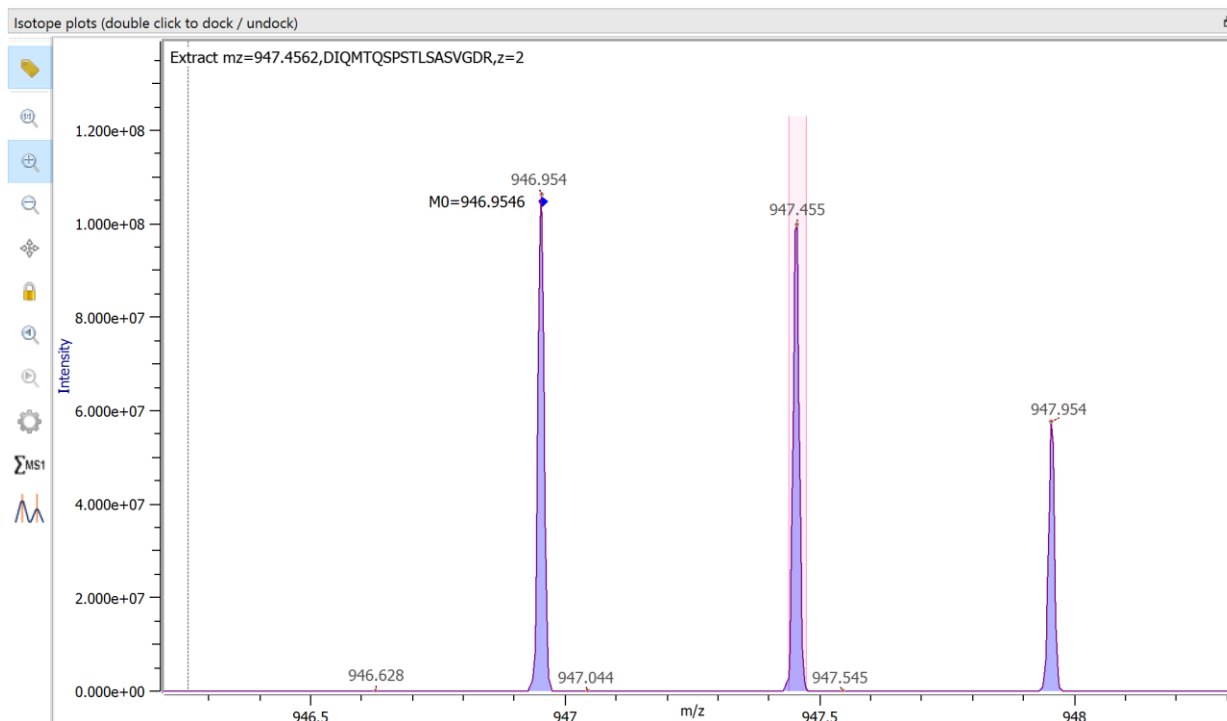





Figure 69: Isotope plots

In addition to the menu icon functions shared by all the plot views, there are some functions specific to Isotope plots:

- The  Show/hide annotations icon turns on and off the display of the molecular weight for the primary peptide beside a blue dot that identifies the peak with that calculated value.
- The  Sum MS1 icon is a local per-peptide summing option that sums MS1 scans for the selected peptide. If the XIC interval is adjusted by the user, the isotope plot will update accordingly. **Note:** Scan summing only works on projects created using v4.6 or later of Byos.
- The  Theoretical isotope distribution icon renders the theoretical averagine distribution. The theoretical isotope distribution is calculated using the averagine model rather than the exact chemical composition. Theoretical isotope distribution is rendered using green sticks surrounded by green shaded transparent rectangular boxes.

MS2 and Mass Error Plots and Menu

The **MS2 and mass error plots** display plots of the MS2 peptide fragments with the corresponding m/z mass error table:

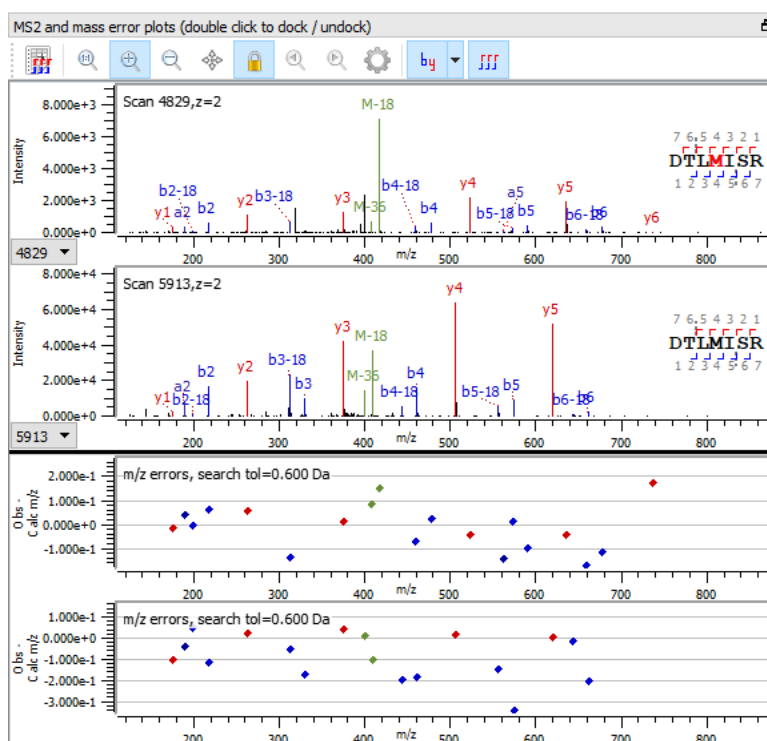





Figure 70: MS2 and Mass Error Plots

The MS2 plots are identified by Scan ID. The plots are colored blue for N-terminus b ions and red for C-terminus y ions. Other fragments, including c and z ions are colored green

In addition to the menu icon functions shared by all the plot views, there are some functions specific to MS2 and mass error plots:

- The  icon switches between the default plot view and the mass table view:



MS2 and mass error plots (double click to dock / undock)




☒ Calculated ☐ Observed ☐ Delta

PID=239: DTLMISR PID=337: DTLMISR

#	a calc.	b calc.	b-18 calc.	b-HexN calc.	Seq.	y calc.	y++ calc.	#
1	88.0393	116.0342	98.0237		D			7
2	189.0870	217.0819	199.0713		T	736.4022	368.7047	6
3	302.1710	330.1660	312.1554		L	635.3545	318.1809	5
4	449.2064	477.2014	459.1908		M	522.2704	261.6389	4
5	562.2905	590.2854	572.2748		I	375.2350	188.1212	3
6	649.3225	677.3174	659.3069		S	262.1510	131.5791	2
7					R	175.1190	88.0631	1

Figure 71: MS2 and mass error plots mass table view

The reported fragment data that was confirmed in the analysis is displayed in red bold font. Unconfirmed fragment data is displayed in black font. In this view, the  icon switches the view back to MS2 plots. The  icon copies the content of this table to the clipboard.

- The  icon switches between showing and hiding the b and y ion labels. The icon is accompanied by a drop-down arrow that reveals three sub-menus:
 - **Open annotation options** opens a dialog that allows the user to set options for determining the assignment of the MS2 annotations:

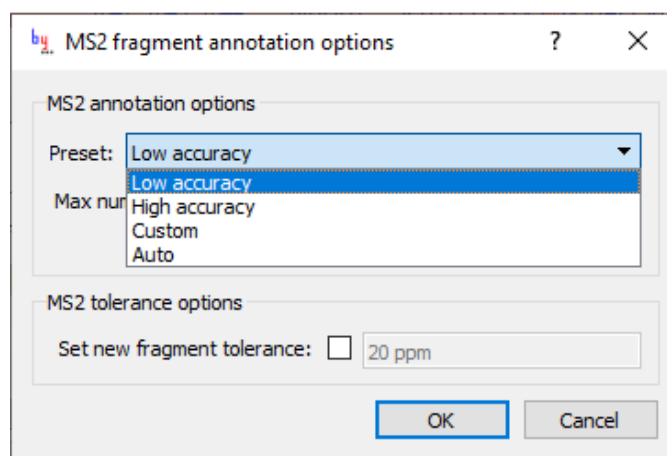



Figure 72: MS2 fragment annotation options

The maximum number of peaks per 100 m/z and MS2 mass-accuracy tolerance options can use presets for Low accuracy, High accuracy, or Custom, in which the user sets these values. The dialog defaults to the Auto preset, in which the software determines the MS2 fragment annotation settings.

- **Remove fragment coverage cache** clears the existing cache of detected fragments used for the protein fragment coverage rendering.

- **Export fragment to CSV**, exports the fragment sequence to a *.csv file.
- The  icon turns on or off the fragment sequences displayed to the right of the plots.

The MS2 and mass error plots view has two specific context menu items revealed by a right-click in the plot:

- **Apply peak filtering** opens a dialog that allows the filtering of peaks by an entered signal-to-noise ratio value.

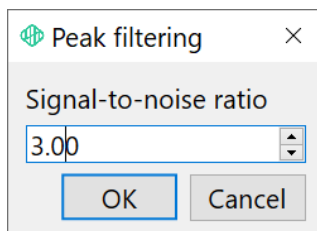


Figure 73: Peak filtering by signal to noise ratio

- **Combine MS2 spectra** (MS2 and mass error plots only) sums the MS2 Plots for all the peptides for a selected row in the Peptides table. The title of the plot updates to show the counts of the peptide scans in the combined MS2 plot:

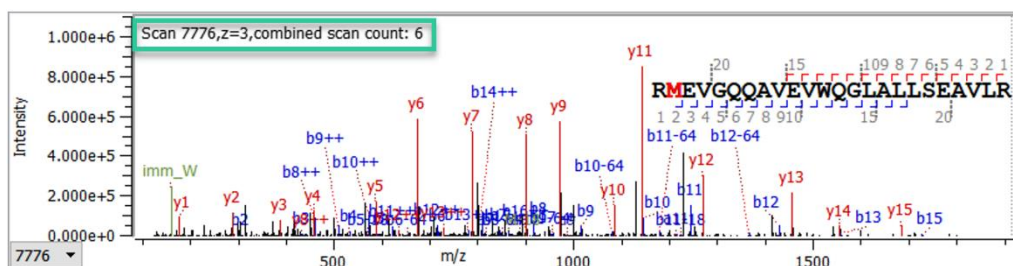


Figure 74: MS2 plots after Combine MS2 spectra is applied

Chromatogram XIC Plot and Menu

The **Chromatogram XIC plot** displays the [1] Total Ion Current (**TIC**), [2] Extracted Ion Chromatogram (**XIC**) of a specific peptide, [3] Summed Extracted Ion Chromatograms (**XIC summed**) of one or multiple peptides eluting at a given time, and the [4] Total Ion Current difference (**TIC difference**). **XIC Summed** calculation features the core step of the chromatogram XIC plot. This feature helps the user visualize how much of the total signal (represented by the TIC) is accounted for by the collection of XICs. TIC difference is defined as TIC – XIC Summed. To open the Chromatogram XIC plot, choose **Window > Show Chromatogram XIC Plot**.

The default view for the Chromatogram XIC plot shows the **TIC** curve and the **XIC** curve of all the peptides. The two peaks shown for the selected peptide (peaks between magenta lines) are the Total Ion Current (no-fill top peak) and Extracted Ion Chromatogram (red-filled bottom peak).

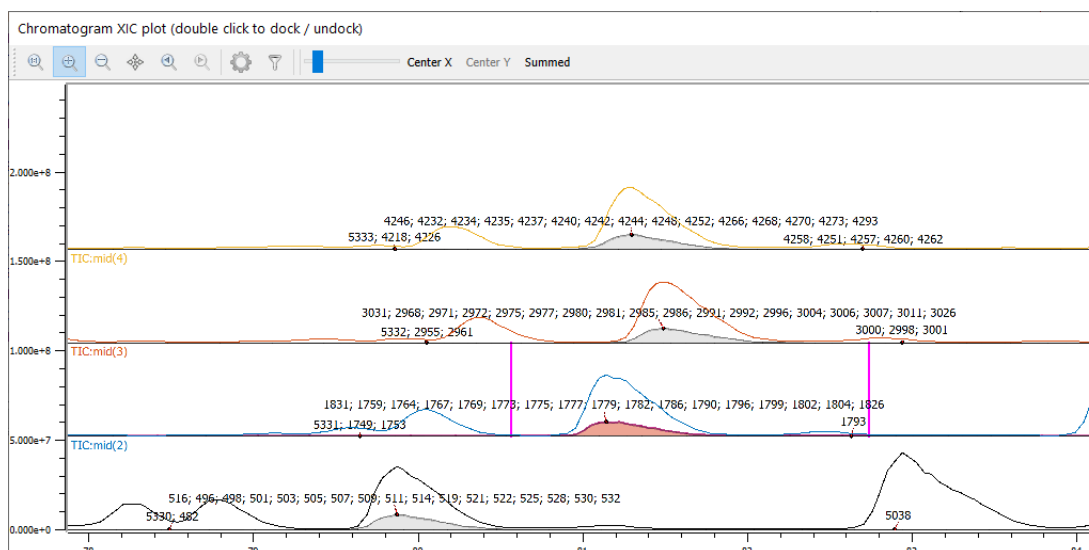



Figure 75: Default view of Chromatogram XIC plot

In addition, **XIC Summed** and **TIC Difference** traces can also be overlaid. **XIC summed** is calculated by summing the **XICs** of isotopes of peptides, including different charge states. **TIC difference** can then be calculated using the formula, **TIC – XIC summed**.

In addition to the menu icon functions shared by the other Peptide Analysis plot views, there are some functions specific to the Chromatogram XIC plot:

- The  **Settings** icon opens the **Plot Settings** dialog. The dialog includes navigation to four areas [a] General, [b] Labels, [c] Summed mode, [d] Zoom options.
 - **General** – contains options to show and edit the plot title and limit the number of displayed peptides:

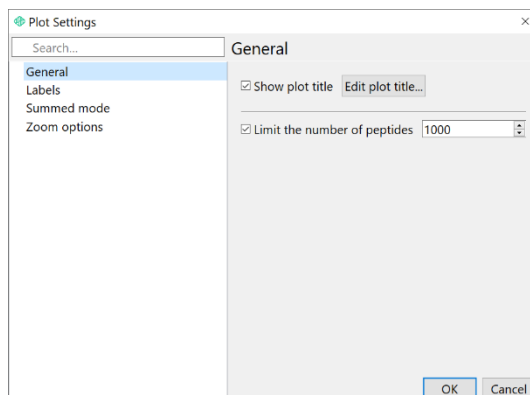


Figure 76: General tab

Edit plot title opens the **Template Editor** dialog:

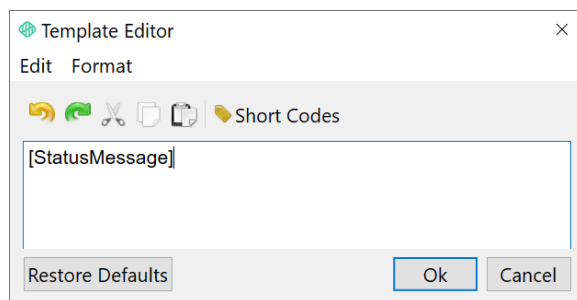


Figure 77: Template Editor within General tab

The Template Editor allows free entry of a plot title or the use of Short Codes.

- **Labels** – contains options to show and edit annotations, limit the total number of labels and orient (rotate) labels:

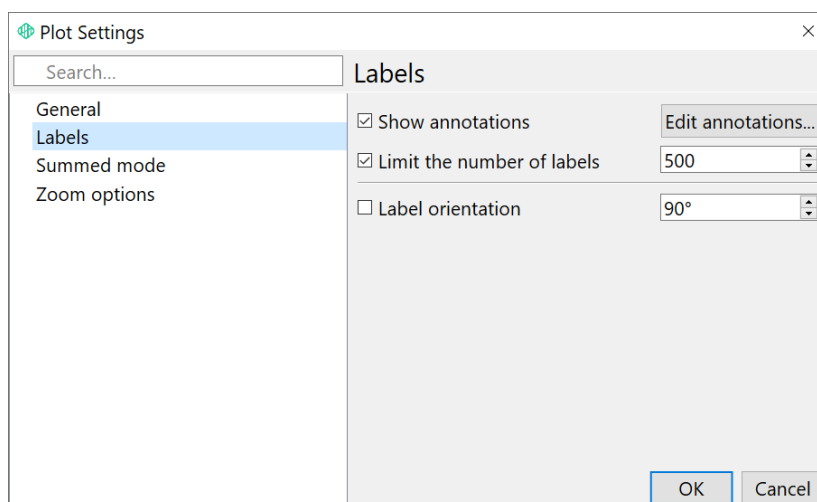


Figure 78: Labels tab

Edit annotations also opens the **Template Editor** dialog. The default [PeptidesId] can be replaced with other annotations available in the “Short Codes” tab on the Template Editor menu (**Note:** a Chromatogram Analysis license is required to see the Labels section for Chromatogram XIC plots).

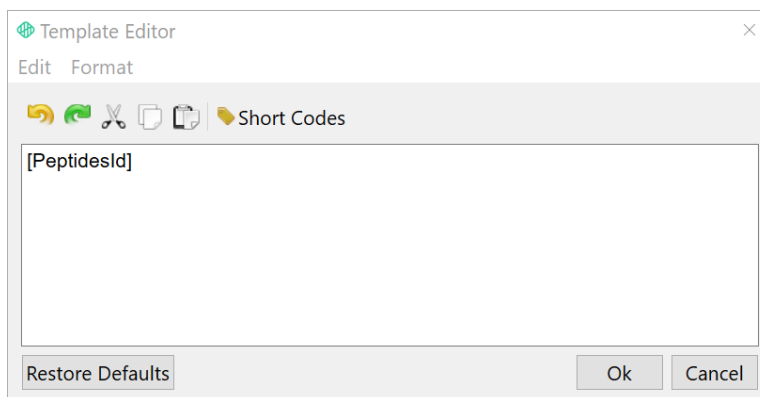


Figure 79: Edit annotations

Below are some of the options for the **Short Code** (1st column).

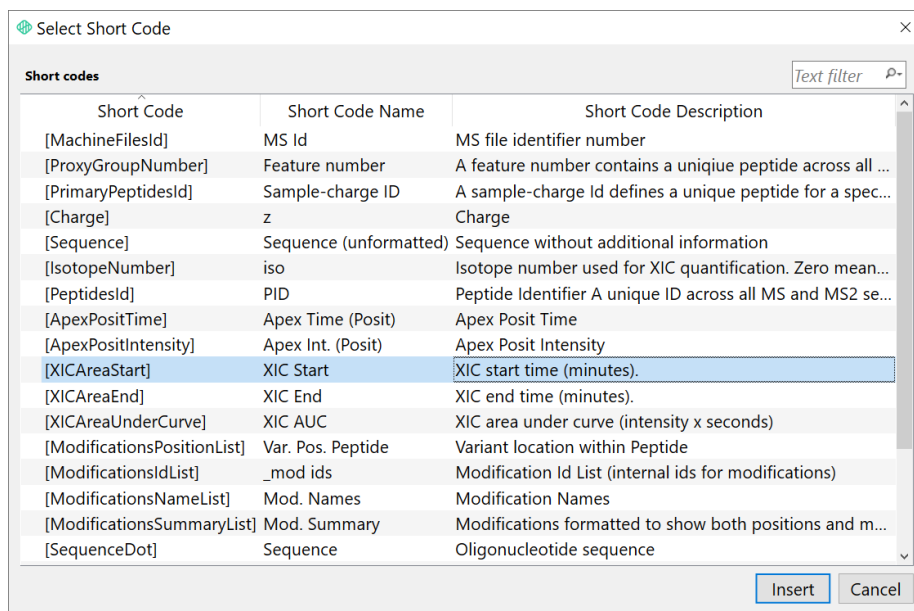


Figure 80: Short code options

Selecting the **[Sequence]** Short Code would result in the below annotation of the peaks.



Figure 81: Selecting [Sequence] Short Code to show peptide sequences as annotations

- **Summed mode** – contains the option to select TIC difference calculation, along with XIC summed:

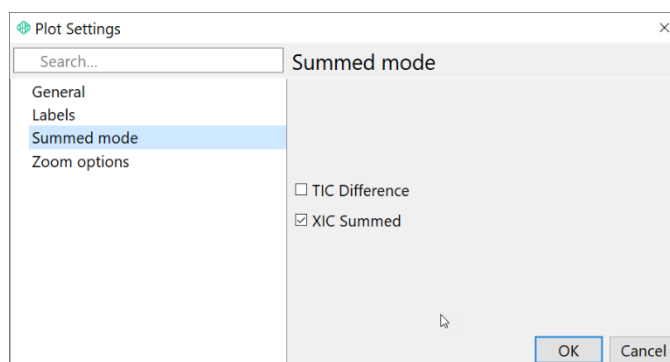


Figure 82: Summed mode tab

- **Zoom options** – contains the standard zoom options, along with scrolling visibility:

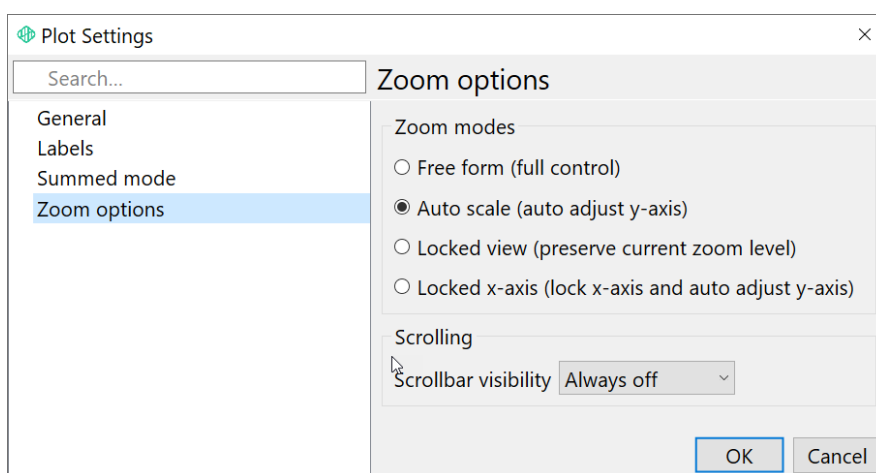


Figure 83: Zoom options tab

- The **filter**  icon opens the Column Filters Editor dialog. The dialog enables filtering based on combinations of column values:

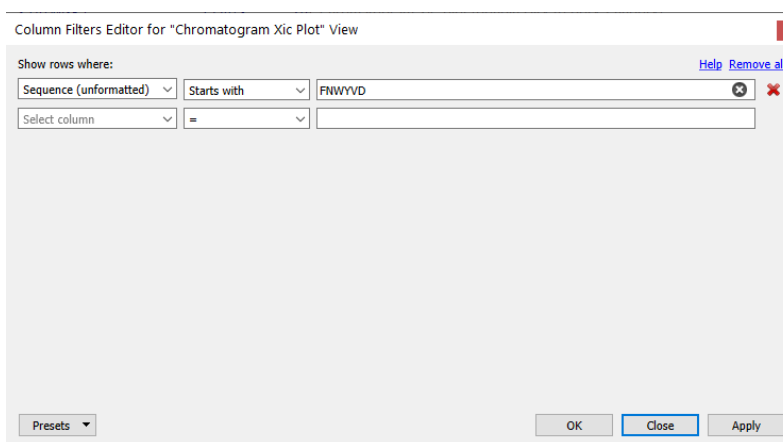


Figure 84: Column Filters Editor for filtering data to plot

- **Center X** zooms the segment and centers it in the frame:

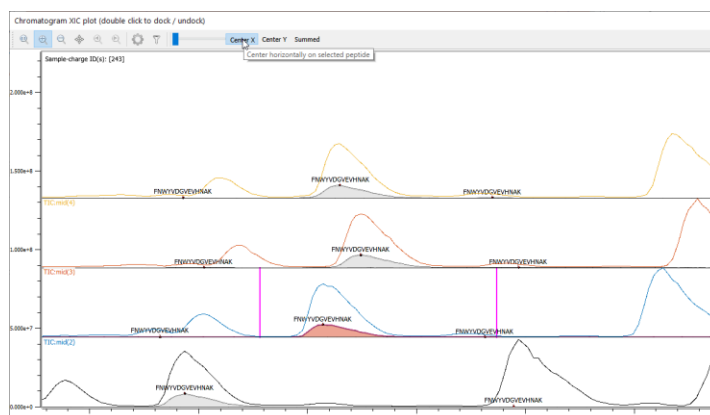


Figure 85: Center X allows the user to center the plot on X-axis

- **Center Y**, available when Center X is selected, zooms the segment and centers the height of the designated peak in the segment in the frame:

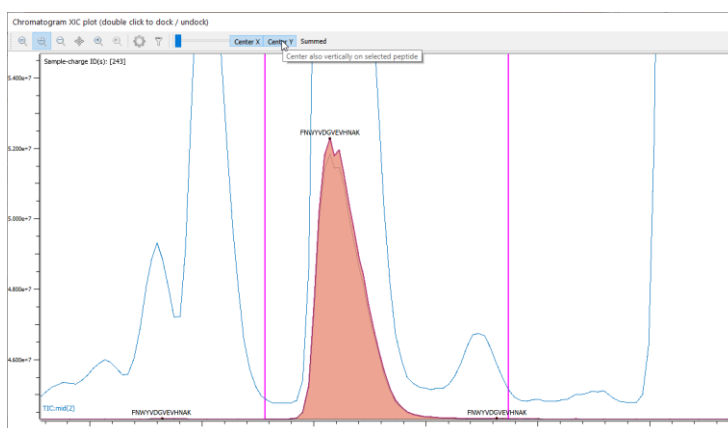


Figure 86: Center Y allows to center the plot vertically

- **Summed** button features the core step of the chromatogram XIC plot. This feature helps the user visualize how much of the total signal (represented by the TIC) is accounted for by the collection of XICs. In addition to the default TIC and XIC layers, additional layer **XIC summed** is generated when **Summed** icon is clicked. **XIC Summed** is the sum of all XICs of all the isotopes and at different charge states of the peptide.

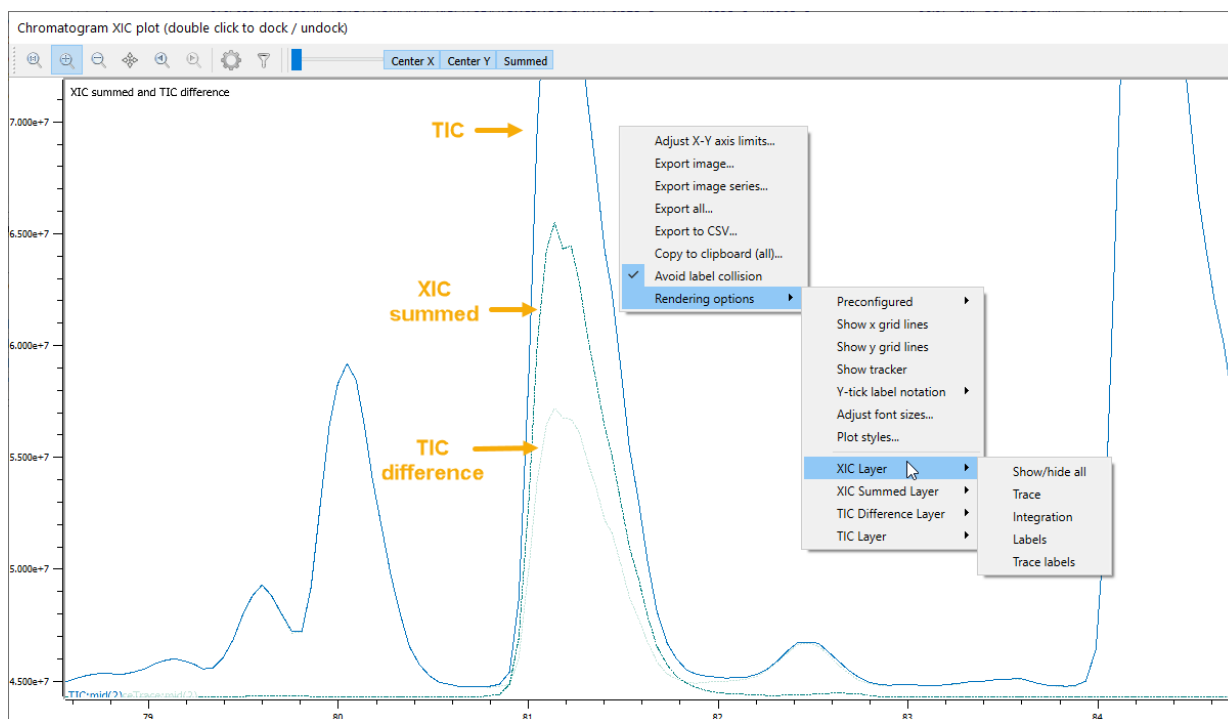


Figure 87: XIC Summed, TIC Difference and TIC curves

Note that the calculation of the Summed XIC (and TIC difference, if checkbox is enabled in the **Settings icon > Summed mode > TIC Difference** shown in Figure 137) might take a while depending on the size of the raw MS files and the RAM of the desktop computer.

The traces are plotted as layers (shown in the figure above) and user has an option to pick the set of traces to visualize. In the above figure, XIC layer rendering is hidden. This means, except the XIC layer, the rest of all of the layers can be seen.

Reporting

Oligo Summary Tab

The **Oligo Summary Tab** provides information on the Project Creation options applied to the project as well as information concerning the samples processed including the Oligonucleotide coverage table.

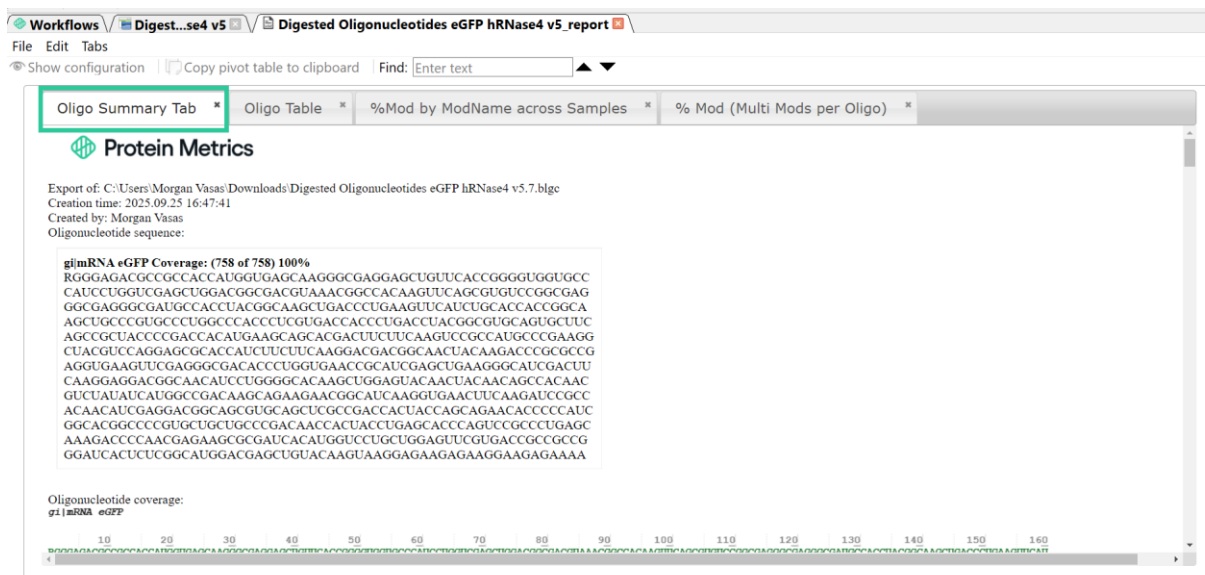


Figure 88: Oligo Summary Tab

Oligo Table

The **Oligo Table** is a pivot table that provides XIC area summed isoX normalized per sample.

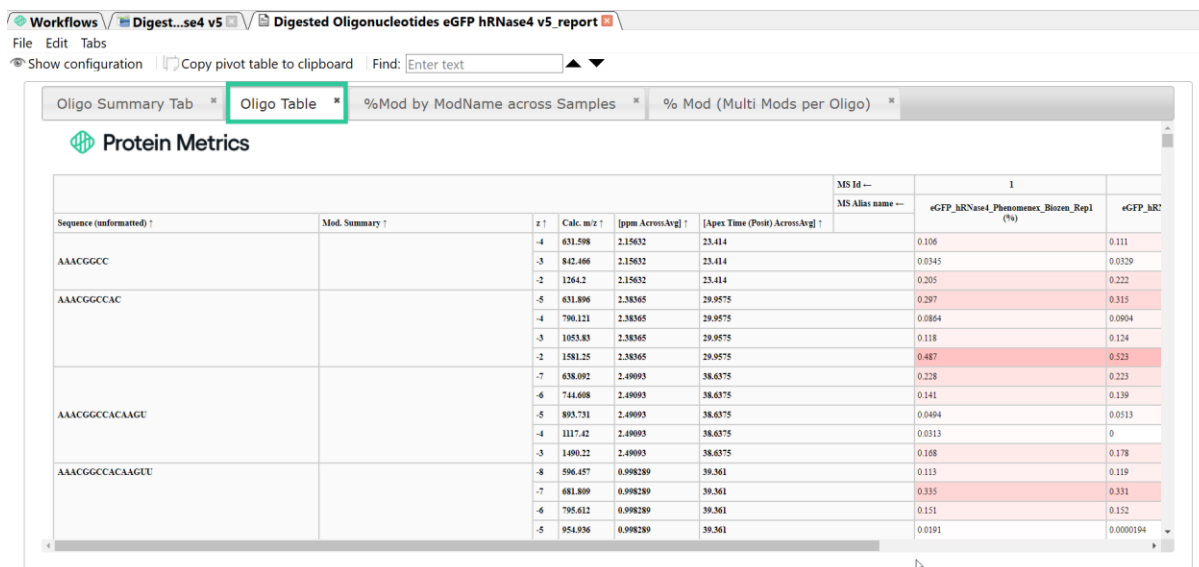


Figure 89: Oligo table

%Mod by ModName Across Samples

The **%Mod by ModName Across Samples** tab is a pivot table that provides XIC ratio % of named modifications per sample.

Workflows | Digest...se4 v5 | Digested Oligonucleotides eGFP hRNase4 v5_report

File Edit Tabs

Show configuration | Copy pivot table to clipboard Find: Enter text

Oligo Summary Tab | Oligo Table | %Mod by ModName across Samples | % Mod (Multi Mods per Oligo)

Mod. Names	Mod. AAs	Var. Pos. Protein	Labels	MS Id -- MS Alias name --	1 eGFP_hRNase4_Phenomenex_Biozen_Rep1	2 eGFP_hRNase4_Phenomenex_Biozen_Rep2	3 eGFP_hRNase4_Phenomenex_Biozen_Rep3 4sLoad
		26		No WT	No WT	No WT	No WT
		40		No WT	No WT	No WT	No WT
		55		No WT	No WT	No WT	No WT
		57		No WT	No WT	No WT	No WT
		73		No WT	No WT	No WT	No WT
		79		No WT	No WT	No WT	No WT
		81		No WT	No WT	No WT	No WT
		82		No WT	No WT	No WT	No WT
		83		No WT	No WT	No WT	No WT
		91		No WT	No WT	No WT	No WT
		93		No WT	No WT	No WT	No WT
		106		No WT	No WT	No WT	No WT
		113		No WT	No WT	No WT	No WT
		125		No WT	No WT	No WT	No WT
		127		No WT	No WT	No WT	No WT
		135			4.72	4.06	4.66
		141		No WT	No WT	No WT	No WT
		162		No WT	No WT	No WT	No WT

Figure 90: %Mod by ModName across Samples


%Mod (Multi Mods Per Oligo)

Workflows | Digest...se4 v5 | Digested Oligonucleotides eGFP hRNase4 v5_report

File Edit Tabs

Show configuration | Copy pivot table to clipboard Find: Enter text

Oligo Summary Tab | Oligo Table | %Mod by ModName across Samples | % Mod (Multi Mods per Oligo)

 Protein Metrics

Sequence (unformatted)	Mod. Names	Mod. AAs	Var. Pos. Protein	Labels	MS Id -- MS Alias name --	1 eGFP_hRNase4_Phenomenex_Biozen_Rep1 (%)	2 eGFP_hRNase4_Phenomenex_Biozen_Rep2 (%)	eG
AAGGAGAAGAGAAGAGAAGAAA	Cyclic Phosphate-61.9558	C-Term	758			100	100	100
ACAACAGCCACAAAGCUCUAUACAUAGCCGACAGCAG	Cyclic Phosphate-61.9558	C-Term	491			100	100	100
ACAACATCGAGGACGGCAGCGUGCAGCTCGCCGACACCT	Cyclic Phosphate-61.9558	C-Term	564			100	100	100
ACAAGACCCCGCCGCGAGGUGAAGUCGAG	Cyclic Phosphate-61.9558	C-Term	365			100	100	100
ACAAGACCCCGCCGCGAGGUGAAGUCGAGGCGGACAC	Cyclic Phosphate-61.9558	C-Term	373			100	100	100
ACCAGCAGAACACCCCT	Cyclic Phosphate-61.9558	C-Term	581			3.38	0.0508	1.62
ACCU	Cyclic Phosphate-61.9558	C-Term	621			100	100	100
ACGGCGGCGAGUGCTUCAGCGGCUACCCCG	Cyclic Phosphate-61.9558	C-Term	246			100	100	100
ACGTCGAGGAGCGCACATCTTCTCAGGAGACGGCAA CTACAGACCGCGCGGAGGT	Cyclic Phosphate-61.9558	C-Term	355			100	100	100
AGACCCCAACGAGAGCGGCAUCACATGGUCCGCGGAG UTCGT	Cyclic Phosphate-61.9558	C-Term	690			100	100	100
CAGAACACCCCAUCGGACGGCCCGU	Cyclic Phosphate-61.9558	C-Term	597			100	100	100
CCGACCCCTCGGACACCCU	Cyclic Phosphate-61.9558	C-Term	211			100	0	100
CGACACCTCGGUGAACCGCACTCGAGCT	Cyclic Phosphate-61.9558	C-Term	394			100	100	100
CGCCCTGAGCAAGACCCCAACGAGAAGCGGCAUCACATG GCCCC	Cyclic Phosphate-61.9558	C-Term	678			100	100	100

Figure 91: %Mod (Multi Mods per Oligo)

Oligo Analysis: Advanced Commands

- **Non-uniform stepping-based Deconvolution**

The user has an option to use nonuniform point spacing for both the m/z and m spectra using the advanced command below.

```
[Intact]
SteppingMethod=Linear
MZResolution=35000
```

SteppingMethod has 3 different settings: Linear, SquareRoot or Constant.

- **Increased Resolution for Isotopically Resolved Data**

IsotopeEnvelopeFilter reduces the spurious peaks in the m spectrum. Peaks are considered spurious if they do not exhibit the expected “average” isotope envelope, either isotope resolved or almost isotope resolved. For most spectra, **IsotopeEnvelopeFilter**, especially used in conjunction with **ChargeEnvelopeFilterPower2**, will remove artifact peaks.

```
[Intact]
IsotopeEnvelopeFilter=true
```

- **Increased Sensitivity and Dynamic Range**

A more sensitive deconvoluted m spectra for low signal-to-noise oligo forms with many charge states can be obtained using **ChargeEnvelopeFilterPower2** advanced command. The new algorithm can improve Limit of Detection (LoD) more than 10-fold.

```
[Intact]
ChargeEnvelopeFilterPower2=true
```

For smaller masses (less than 20KDa) which is typical for Oligo workflow, additional parameter **ChargeEnvelopeWeights** can be used:

```
[Intact]
ChargeEnvelopeWeights=0.05, 0.9, 0.05
```

The default charge envelope weights (i.e., **ChargeEnvelopeWeights**=0.07, 0.11, 0.2, 0.24, 0.2, 0.11, 0.07) will work well for masses with at least 5 or 6 charge states.

- **Improved Monoisotopic Mass Calculation**

Deconvolved isotope series may get slightly distorted by one or more signal interference events at specific charge states, especially for larger masses. This might result in monoisotopic mass calling errors. Advanced command **AdvancedMonoCalculation** improves the monoisotopic mass calculation by using both the deconvolved mass spectrum and the original MS1 spectrum to make the monoisotopic mass calls. Using MS1 spectrum improves the monoisotopic mass calls, given that there are many isotope envelopes (corresponding to different charge states) from which a reliable statistic can be derived.

```
[Intact]
AdvancedMonoCalculation=true
```

- **Remove Singly Charged Peaks** allows the user to remove singly charged peaks with m/z (maximum 2500). For example, a value of 0.02 directs the software to remove singly charged peaks down to 2% of base peak.

```
[Intact]
RemoveChargeOne=0.02
```

- **Monoisotopic Mass Calculation** feature allows the users to compute monoisotopic mass using an oligonucleotide averagine model for mass peaks up to the set **MaxMonoisotope** value. This command should be used in conjunction with convolution parameters that support isotopic resolution (for example, m/z spacing of 0.01 Thomsons and mass spacing 0.1 Da, with similarly small smoothing sigmas). However, the software will fit the averagine model whether or not the mass peak has isotopic resolution.

```
[Intact]
MaxMonoisotope=30000
```

- **Show Observed Mono** shows orange diamonds at the observed monoisotopic mass on the Deconvolved Mass Spectrum plot

```
[Intact]
ShowObservedMono=true
```

- **IsotopeSpacing** helps the deconvolution whenever peaks are isotope resolved and do not appear in 3 or more charge states.

```
[Intact]
IsotopeSpacing=5
```

- **ExtraSmoothingforCentroid** can be used to better resolve peaks with overlapping isotopic envelopes. The value for ExtraSmoothingforCentroid is false by default.

```
[Intact]
ExtraSmoothingforCentroid=false
```

- **Improvement in performance for large projects**

The advanced command **MS2TableMaxNumberSequences=10** adds a time filter to MS2 data when there are too many sequences (>10) to load. This command modifies how many sequences are displayed in the MS2 data table. A lower value = better performance upon opening the project. **Note:** This applies only when no candidates have been selected (immediately after project open)

```
[Intact]
MS2TableMaxNumberSequences=10
```

- **Adjustment of annotation rendering within the MS2 plot**

The advanced commands **Alpha** and **AnnotationStickMinWidth** adjust the rendering of the annotation bars within the MS2 plot. **Alpha** represents the transparency of the annotation bars on a 0-255 RBY color intensity scale and **AnnotationStickMidWidth** represents the width of the annotation bar.

```
[MS2Plot]
Alpha=50
```

```
AnnotationStickMinWidth=3
```

- **Fragment ion intensity** is now taken into account for MS2 fragment scoring
As of Byos v5.6, when MSMS fragments are available, the software is now able to use the intensities of the fragments to add validity to the scoring.

The scoring can be evaluated during MS2 annotation process by checking “Score fragments” option in MS2 annotation options and setting a desired score threshold for ion annotations.

The default score cutoff is 10. A different score cutoff can be set under the Advanced settings during Project Creation, affecting all MS2 scores in the table.

```
[MS2]
```

```
FragmentScoreThreshold=10
```

When applied, the scores will be displayed and the cutoff applied during scoring and MS2 annotation. Users can change this within the MS2 annotation options.

Intensity plays significant role in scores if otherwise desirable isotope patterns are observed in conjunction with low scores due to a lower intensity advanced parameter. The following advanced command can be used to turn off intensity consideration:

```
[MS2]
```

```
IntegrateSNIntoFragmentScore=false
```

To switch to the original method of fragment isotope scoring, the following advanced parameter can be used:

```
[MS2]
```

```
FragmentMatchScoringMethod=KL
```