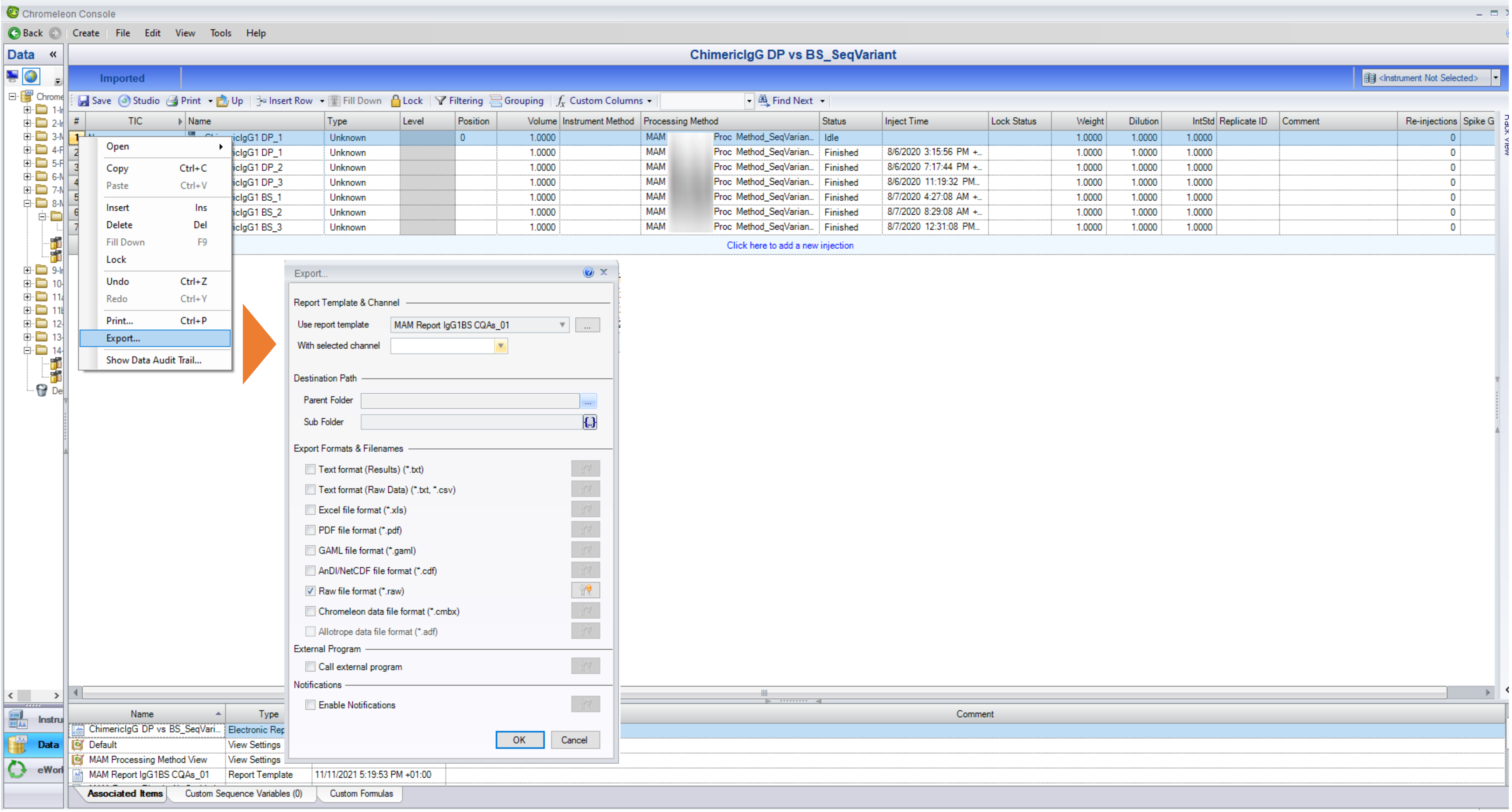
Stage 3: Data processing, analysis and reporting_**Step 8**



#	TIC	Name	Type	Level	Position	Volume	Instrument Method	Processing Method	Status	Inject Time	Lock Status	Weight	Dilution	IntStd	Replicate ID	Comment	Re-injections	Spike G
1		iclgG1 DP_1	Unknown		0	1.0000		MAM [redacted] Proc Method_SeqVarian..	Idle			1.0000	1.0000	1.0000			0	
2		iclgG1 DP_1	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian..	Finished	8/6/2020 3:15:56 PM +..		1.0000	1.0000	1.0000			0	
3		iclgG1 DP_2	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian..	Finished	8/6/2020 7:17:44 PM +..		1.0000	1.0000	1.0000			0	
4		iclgG1 DP_3	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian..	Finished	8/6/2020 11:19:32 PM..		1.0000	1.0000	1.0000			0	
5		iclgG1 BS_1	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian..	Finished	8/7/2020 4:27:08 AM +..		1.0000	1.0000	1.0000			0	
6		iclgG1 BS_2	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian..	Finished	8/7/2020 8:29:08 AM +..		1.0000	1.0000	1.0000			0	
7		iclgG1 BS_3	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian..	Finished	8/7/2020 12:31:08 PM..		1.0000	1.0000	1.0000			0	

Export...

Report Template & Channel

Use report template: MAM Report IgG1BS CQAs_01

With selected channel:

Destination Path

Parent Folder:

Sub Folder:

Export Formats & Filenames

- ☐ Text format (Results) (*.txt)
- ☐ Text format (Raw Data) (*.txt, *.csv)
- ☐ Excel file format (*.xls)
- ☐ PDF file format (*.pdf)
- ☐ GAML file format (*.gaml)
- ☐ AnDI/NetCDF file format (*.cdf)
- ☑ Raw file format (*.raw)
- ☐ Chromeleon data file format (*.cmbx)
- ☐ Allotrope data file format (*.adf)

External Program

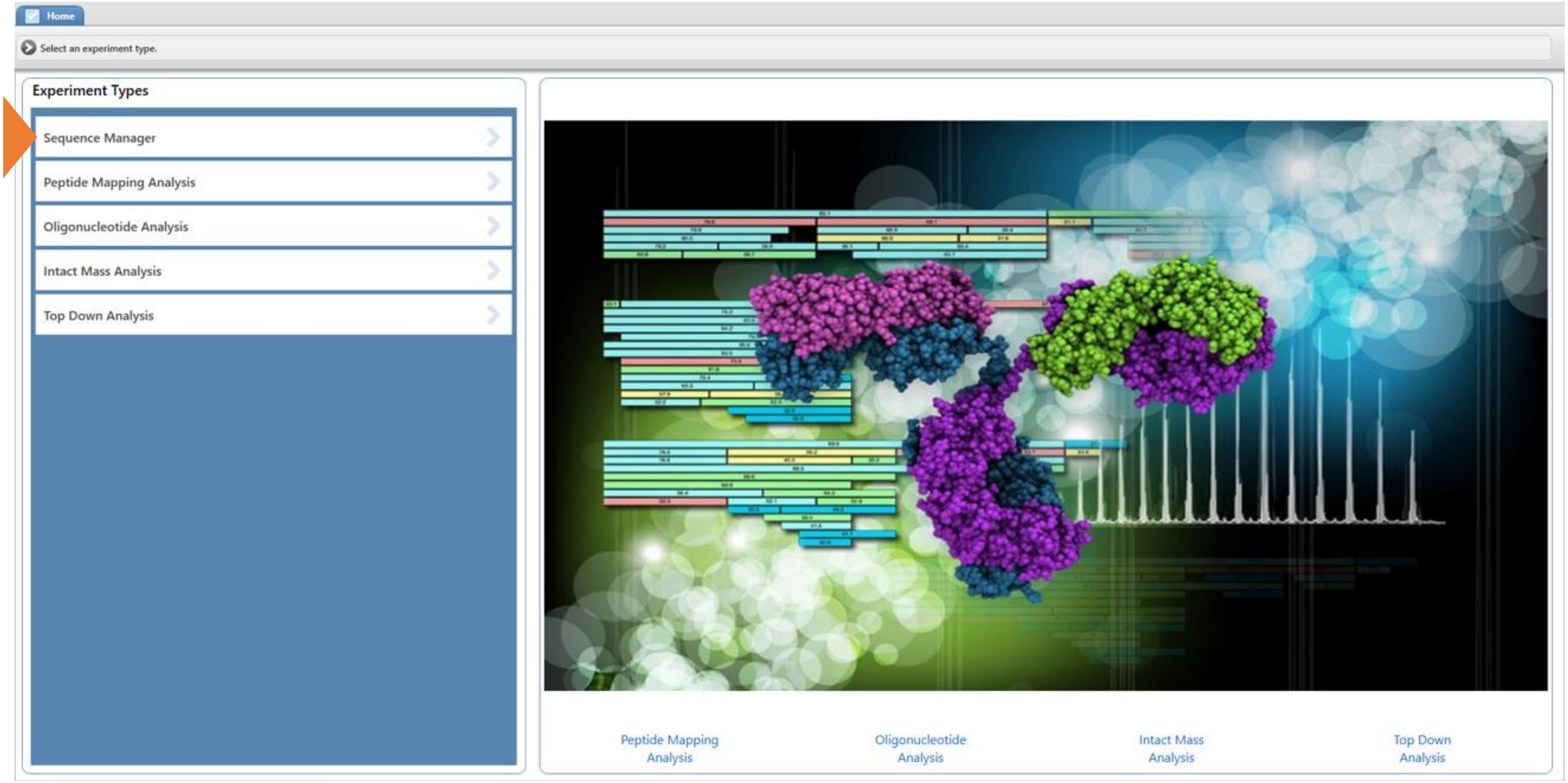
- ☐ Call external program

Notifications

- ☐ Enable Notifications

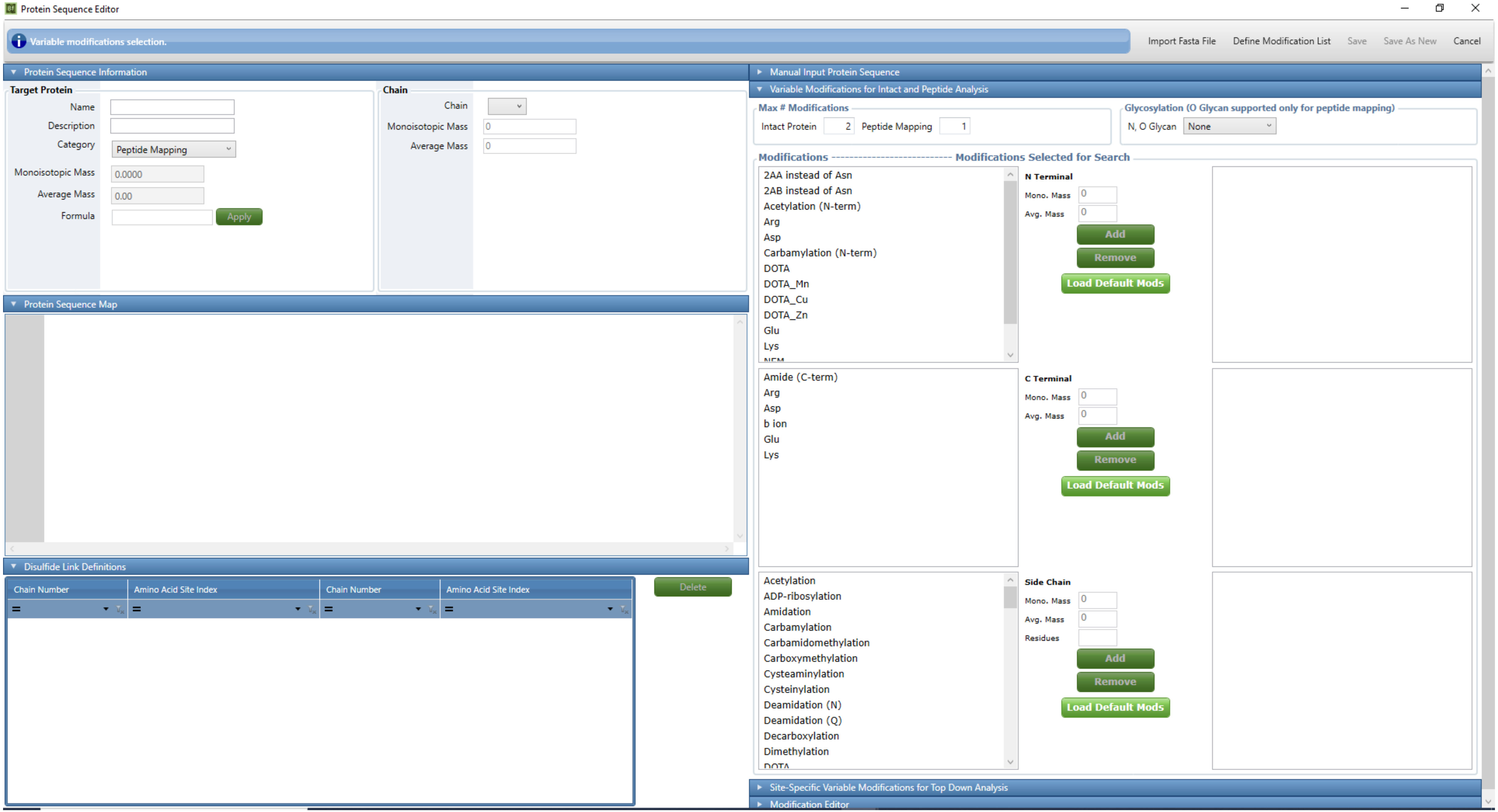
Name	Type		Comment
ChimericIgG DP vs BS_SeqVari...	Electronic Rep		
Default	View Settings		
MAM Processing Method View	View Settings		
MAM Report IgG1BS CQAs_01	Report Template	11/11/2021 5:19:53 PM +01:00	

Stage 3: Data processing, analysis and reporting_**Step 9**



Select an experiment type.

Stage 3: Data processing, analysis and reporting_**Step 9**



Protein Sequence Editor

Variable modifications selection.

Category: Peptide Mapping

Monoisotopic Mass: 0.0000

Average Mass: 0.00

Chain Monoisotopic Mass: 0

Chain Average Mass: 0

Max # Modifications: Intact Protein 2, Peptide Mapping 1

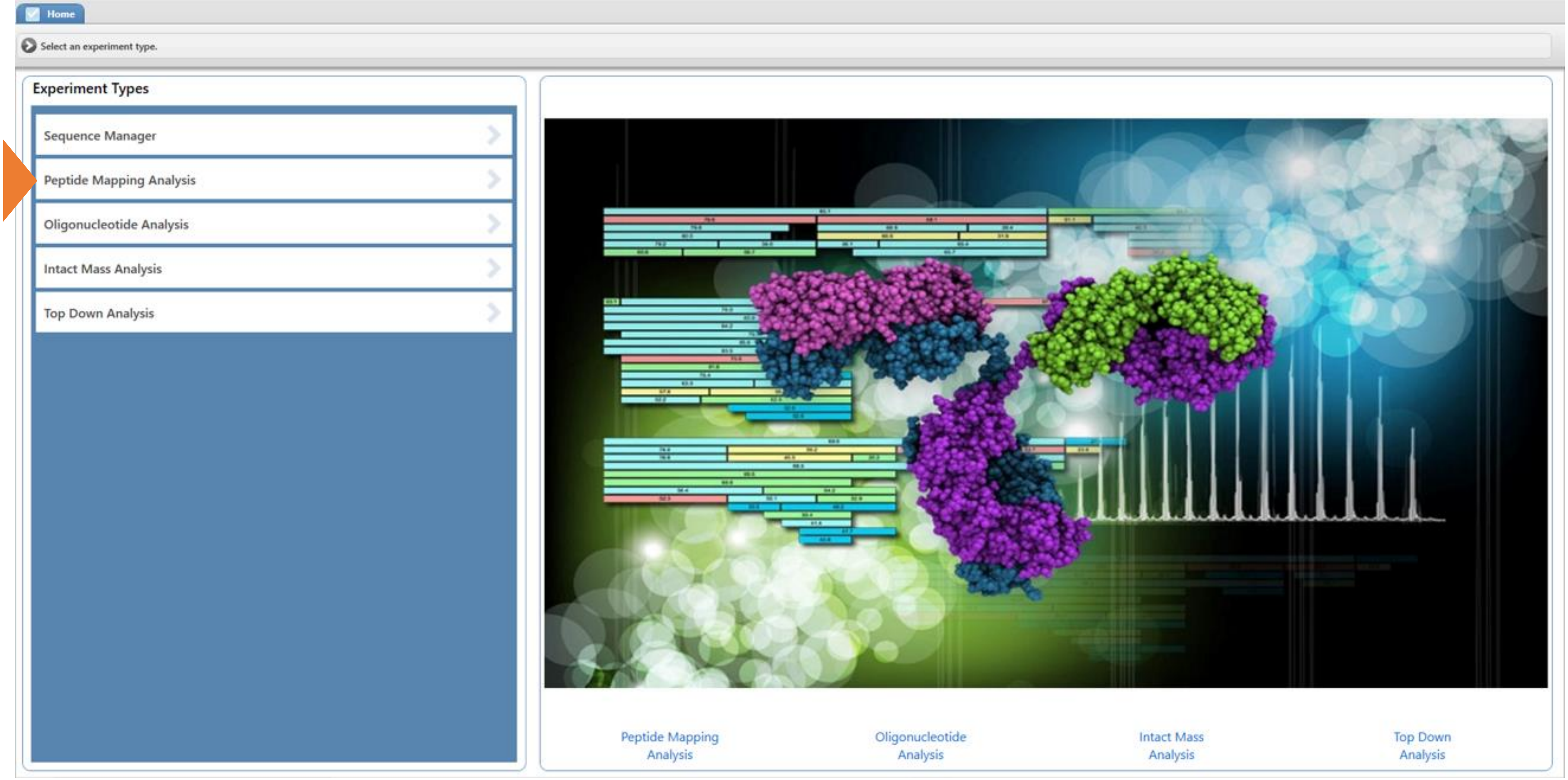
Glycosylation (O Glycan supported only for peptide mapping): N, O Glycan None

N Terminal Mono. Mass 0, Avg. Mass 0

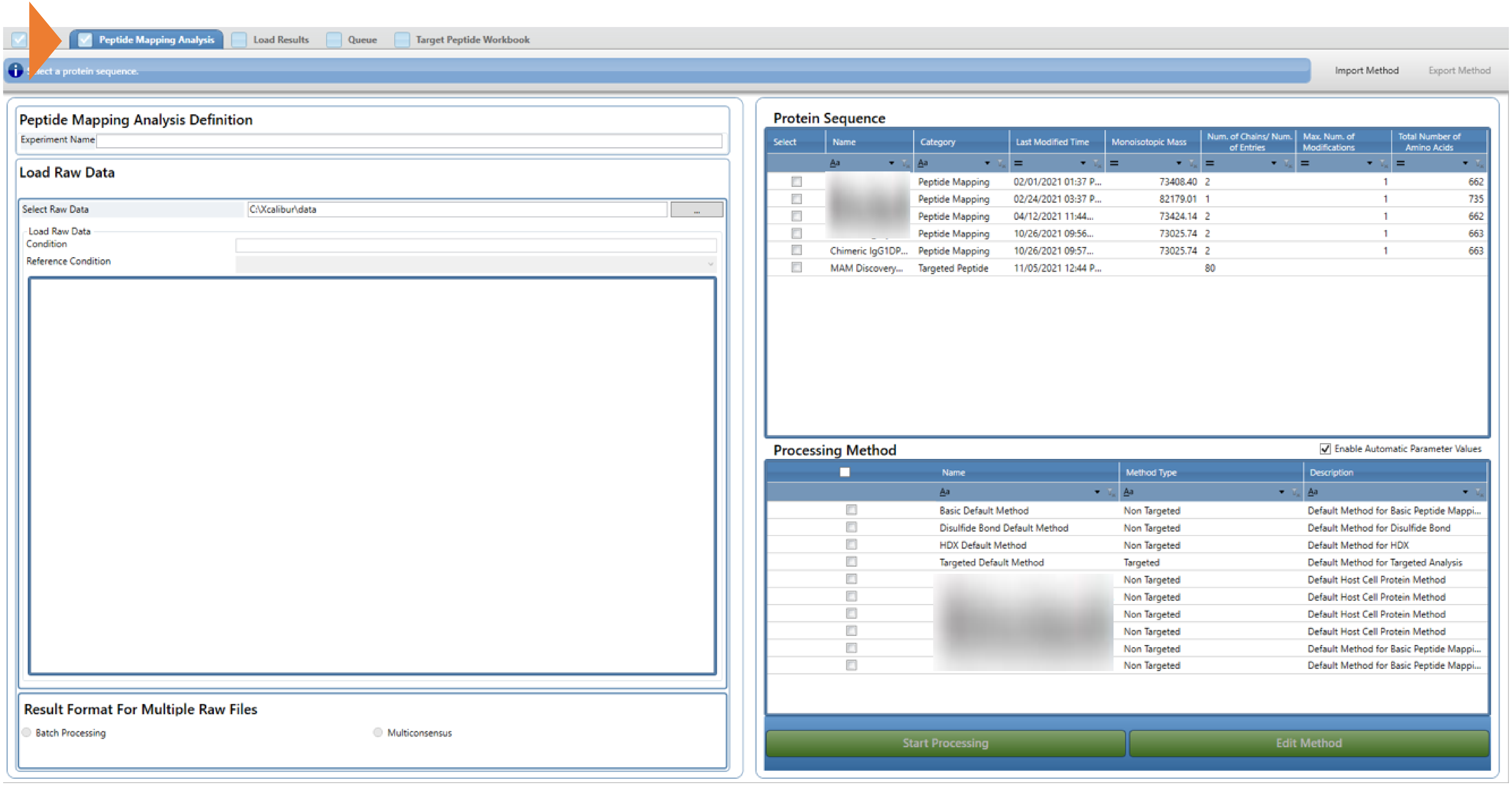
C Terminal Mono. Mass 0, Avg. Mass 0

Side Chain Mono. Mass 0, Avg. Mass 0

Stage 3: Data processing, analysis and reporting_**Step 10**



Stage 3: Data processing, analysis and reporting_**Step 10**



ect a protein sequence.

Peptide Mapping Analysis Definition

Experiment Name

Load Raw Data

Select Raw Data: C:\Xcalibur\data

Load Raw Data Condition

Reference Condition

Result Format For Multiple Raw Files

○ Batch Processing ○ Multiconsensus

Protein Sequence

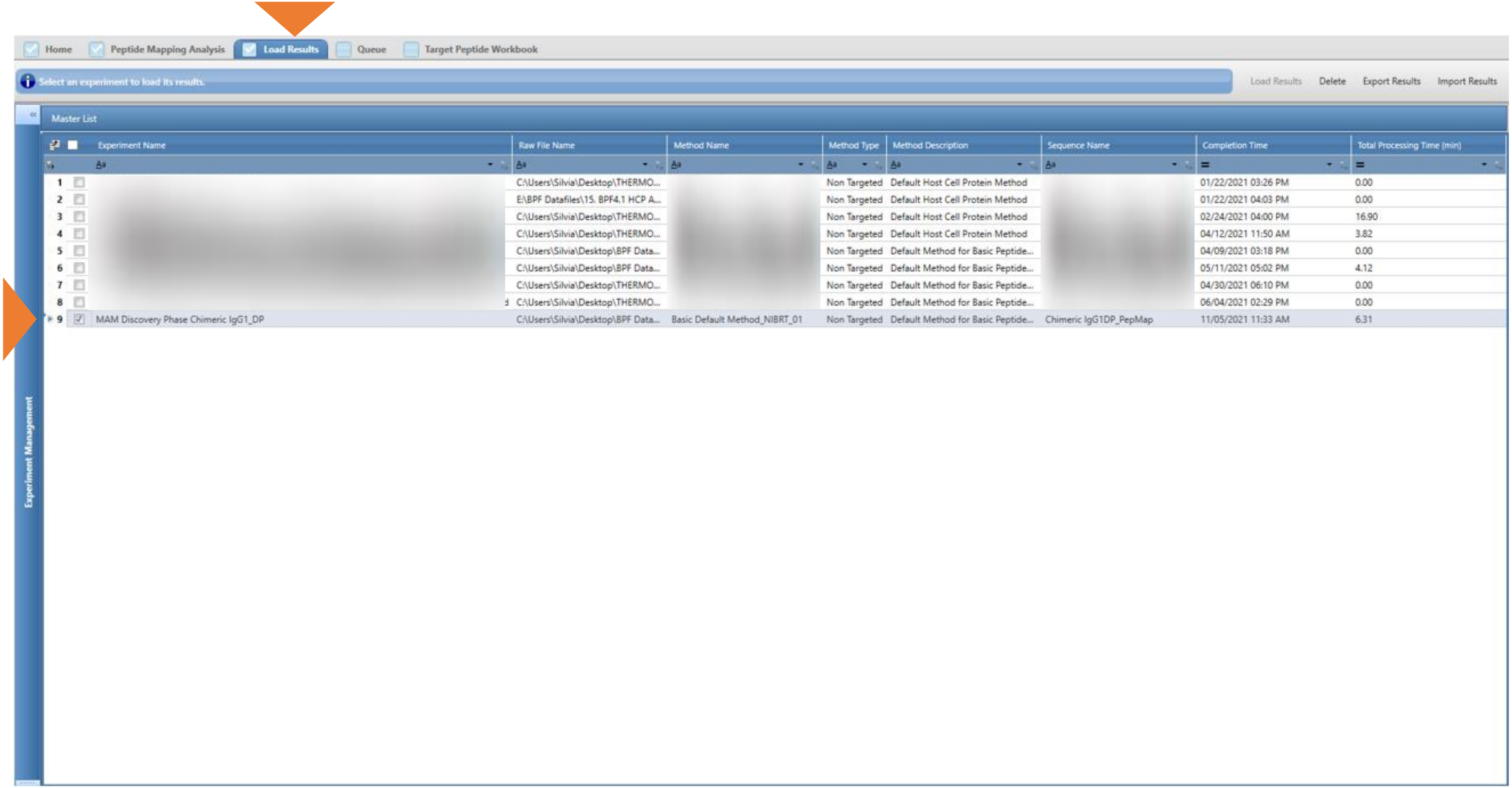
Select	Name	Category	Last Modified Time	Monoisotopic Mass	Num. of Chains/ Num. of Entries	Max. Num. of Modifications	Total Number of Amino Acids
☐	[redacted]	Peptide Mapping	02/01/2021 01:37 P...	73408.40	2	1	662
☐	[redacted]	Peptide Mapping	02/24/2021 03:37 P...	82179.01	1	1	735
☐	[redacted]	Peptide Mapping	04/12/2021 11:44...	73424.14	2	1	662
☐	[redacted]	Peptide Mapping	10/26/2021 09:56...	73025.74	2	1	663
☐	Chimeric IgG1DP...	Peptide Mapping	10/26/2021 09:57...	73025.74	2	1	663
☐	MAM Discovery...	Targeted Peptide	11/05/2021 12:44 P...		80		

Processing Method

☑ Enable Automatic Parameter Values

☐	Name	Method Type	Description
☐	Basic Default Method	Non Targeted	Default Method for Basic Peptide Mappi...
☐	Disulfide Bond Default Method	Non Targeted	Default Method for Disulfide Bond
☐	HDX Default Method	Non Targeted	Default Method for HDX
☐	Targeted Default Method	Targeted	Default Method for Targeted Analysis
☐	[redacted]	Non Targeted	Default Host Cell Protein Method
☐	[redacted]	Non Targeted	Default Host Cell Protein Method
☐	[redacted]	Non Targeted	Default Host Cell Protein Method
☐	[redacted]	Non Targeted	Default Host Cell Protein Method
☐	[redacted]	Non Targeted	Default Method for Basic Peptide Mappi...
☐	[redacted]	Non Targeted	Default Method for Basic Peptide Mappi...

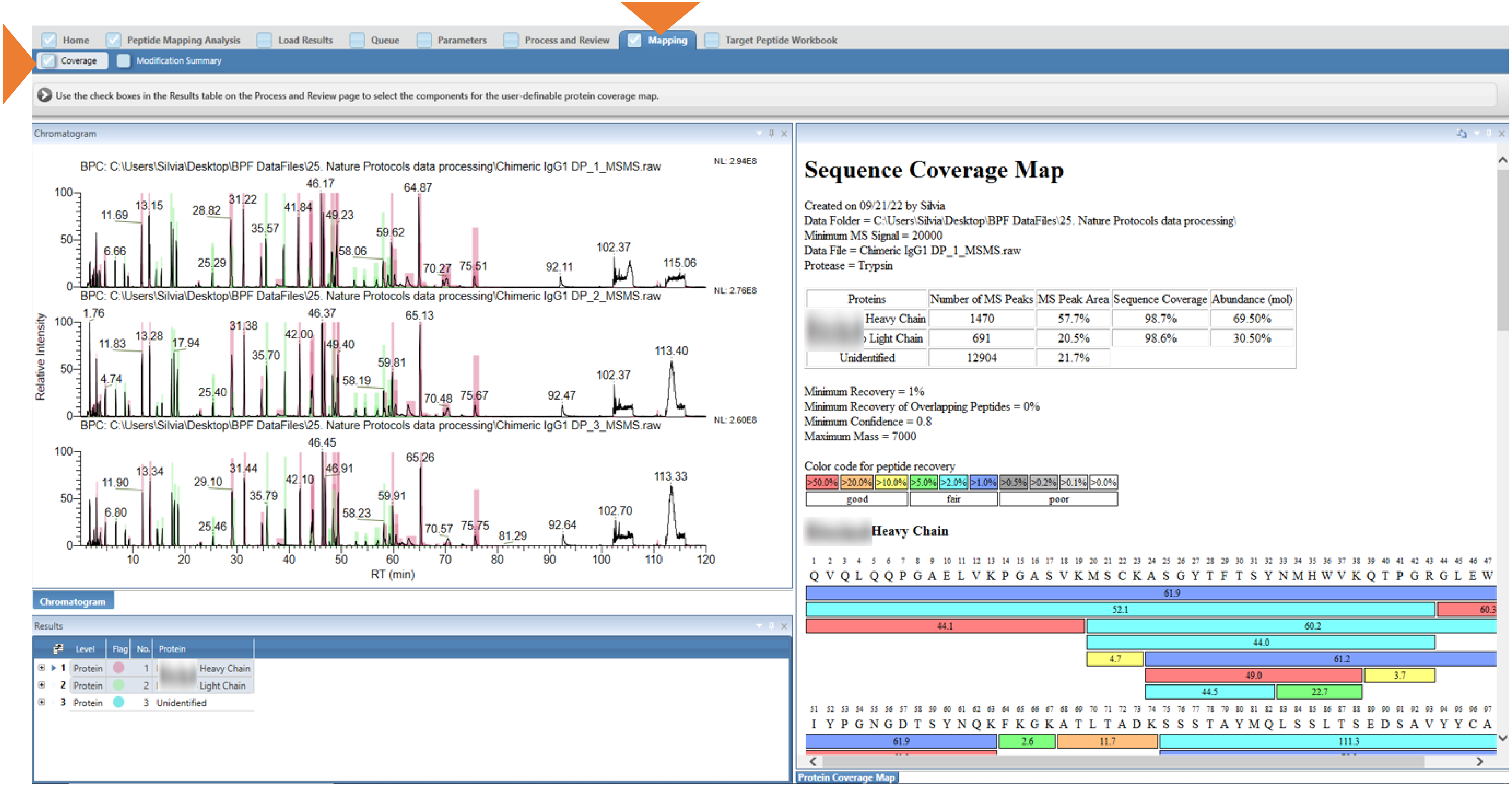
Stage 3: Data processing, analysis and reporting_**Step 11**



Select an experiment to load its results.

		Experiment Name	Raw File Name	Method Name	Method Type	Method Description	Sequence Name	Completion Time	Total Processing Time (min)
1	☐	[redacted]	C:\Users\Silvia\Desktop\THERMO...	[redacted]	Non Targeted	Default Host Cell Protein Method	[redacted]	01/22/2021 03:26 PM	0.00
2	☐	[redacted]	E:\BPF Datafiles\15. BPF4.1 HCP A...	[redacted]	Non Targeted	Default Host Cell Protein Method	[redacted]	01/22/2021 04:03 PM	0.00
3	☐	[redacted]	C:\Users\Silvia\Desktop\THERMO...	[redacted]	Non Targeted	Default Host Cell Protein Method	[redacted]	02/24/2021 04:00 PM	16.90
4	☐	[redacted]	C:\Users\Silvia\Desktop\THERMO...	[redacted]	Non Targeted	Default Host Cell Protein Method	[redacted]	04/12/2021 11:50 AM	3.82
5	☐	[redacted]	C:\Users\Silvia\Desktop\BPF Data...	[redacted]	Non Targeted	Default Method for Basic Peptide...	[redacted]	04/09/2021 03:18 PM	0.00
6	☐	[redacted]	C:\Users\Silvia\Desktop\BPF Data...	[redacted]	Non Targeted	Default Method for Basic Peptide...	[redacted]	05/11/2021 05:02 PM	4.12
7	☐	[redacted]	C:\Users\Silvia\Desktop\THERMO...	[redacted]	Non Targeted	Default Method for Basic Peptide...	[redacted]	04/30/2021 06:10 PM	0.00
8	☐	[redacted]	C:\Users\Silvia\Desktop\THERMO...	[redacted]	Non Targeted	Default Method for Basic Peptide...	[redacted]	06/04/2021 02:29 PM	0.00
9	☑	MAM Discovery Phase Chimeric IgG1_DP	C:\Users\Silvia\Desktop\BPF Data...	Basic Default Method_NIBRT_01	Non Targeted	Default Method for Basic Peptide...	Chimeric IgG1DP_PepMap	11/05/2021 11:33 AM	6.31

Stage 3: Data processing, analysis and reporting_**Step 11**



Use the check boxes in the Results table on the Process and Review page to select the components for the user-definable protein coverage map.

	Level	Flag	No.	Protein
1	Protein	●	1	[redacted] Heavy Chain
2	Protein	●	2	[redacted] Light Chain
3	Protein	●	3	Unidentified

Sequence Coverage Map

Created on 09/21/22 by Silvia
Data Folder = C:\Users\Silvia\Desktop\BPF DataFiles\25. Nature Protocols data processing\
Minimum MS Signal = 20000
Data File = Chimeric IgG1 DP_1_MSMS.raw
Protease = Trypsin

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
[redacted] Heavy Chain	1470	57.7%	98.7%	69.50%
[redacted] Light Chain	691	20.5%	98.6%	30.50%
Unidentified	12904	21.7%		

Minimum Recovery = 1%
Minimum Recovery of Overlapping Peptides = 0%
Minimum Confidence = 0.8
Maximum Mass = 7000

Color code for peptide recovery

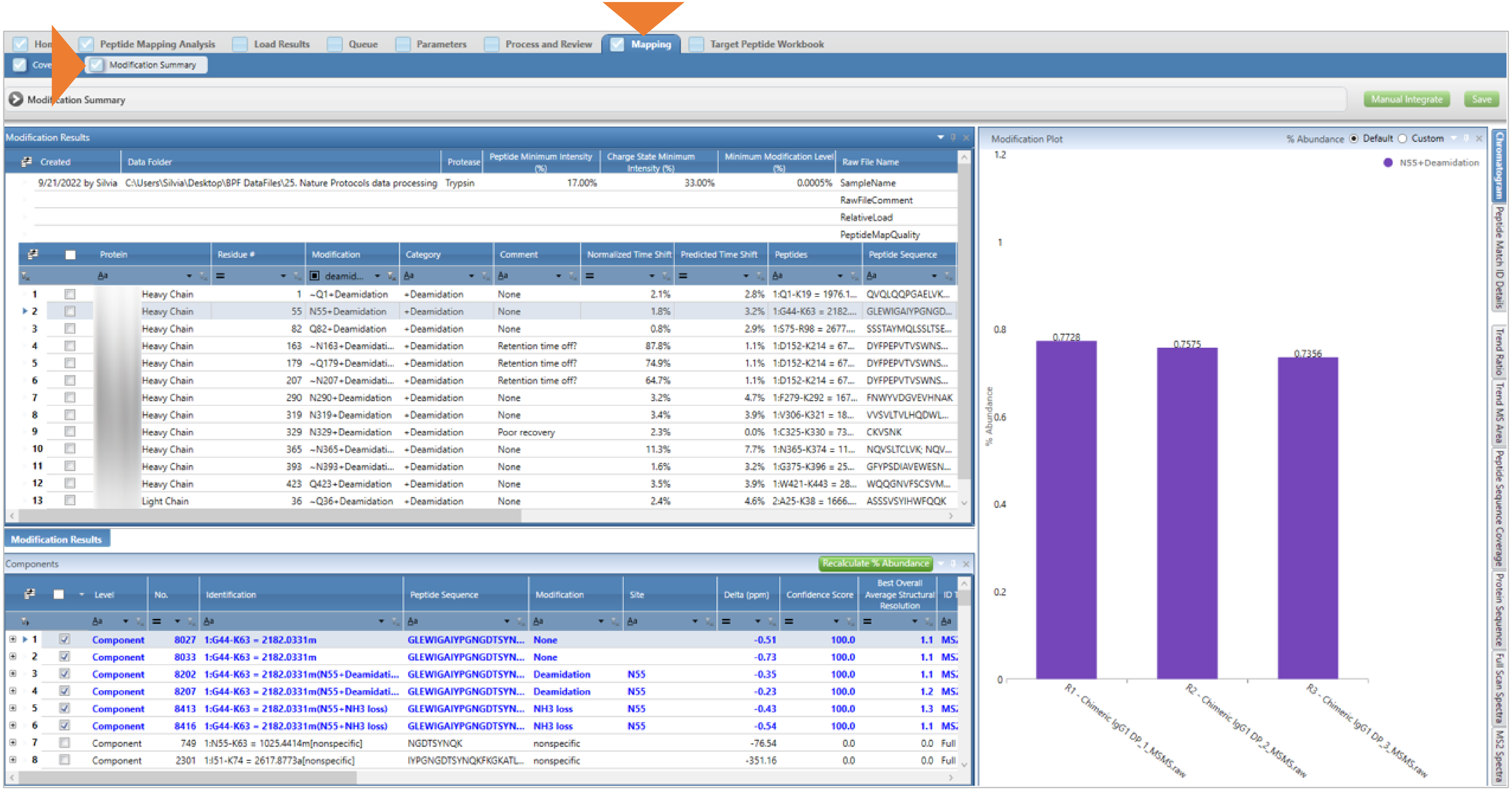
>50.0%	>20.0%	>10.0%	>5.0%	>2.0%	>1.0%	>0.5%	>0.2%	>0.1%	>0.0%
good			fair			poor			

[redacted] Heavy Chain

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47
Q V Q L Q Q P G A E L V K P G A S V K M S C K A S G Y T F T S Y N M H W V K Q T P G R G L E W

51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97
I Y P G N G D T S Y N Q K F K G K A T L T A D K S S S T A Y M Q L S S L T S E D S A V Y Y C A

Stage 3: Data processing, analysis and reporting_**Step 12**



Modification Results

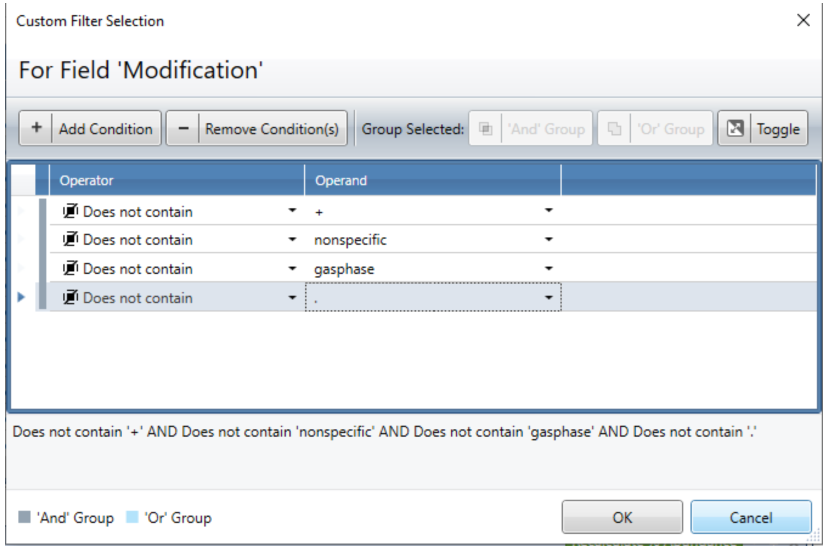
Created	Data Folder	Protease	Peptide Minimum Intensity (%)	Charge State Minimum Intensity (%)	Minimum Modification Level (%)	Raw File Name
9/21/2022 by Silvia	C:\Users\Silvia\Desktop\BPF DataFiles\25. Nature Protocols data processing	Trypsin	17.00%	33.00%	0.0005%	SampleName
						RawFileComment
						RelativeLoad
						PeptideMapQuality

	☐	Protein	Residue #	Modification	Category	Comment	Normalized Time Shift	Predicted Time Shift	Peptides	Peptide Sequence
			=	■ deamid...			=	=		
1	☐	[redacted] Heavy Chain	1	~Q1+Deamidation	+Deamidation	None	2.1%	2.8%	1:Q1-K19 = 1976.1...	QVQLQQPGAELVK...
2	☐	[redacted] Heavy Chain	55	N55+Deamidation	+Deamidation	None	1.8%	3.2%	1:G44-K63 = 2182....	GLEWIGAIYPGNGD...
3	☐	[redacted] Heavy Chain	82	Q82+Deamidation	+Deamidation	None	0.8%	2.9%	1:S75-R98 = 2677....	SSSTAYMQLSSLTSE...
4	☐	[redacted] Heavy Chain	163	~N163+Deamidati...	+Deamidation	Retention time off?	87.8%	1.1%	1:D152-K214 = 67...	DYFPEPVTVSWNS...
5	☐	[redacted] Heavy Chain	179	~Q179+Deamidati...	+Deamidation	Retention time off?	74.9%	1.1%	1:D152-K214 = 67...	DYFPEPVTVSWNS...
6	☐	[redacted] Heavy Chain	207	~N207+Deamidati...	+Deamidation	Retention time off?	64.7%	1.1%	1:D152-K214 = 67...	DYFPEPVTVSWNS...
7	☐	[redacted] Heavy Chain	290	N290+Deamidation	+Deamidation	None	3.2%	4.7%	1:F279-K292 = 167...	FNWYVDGVEVHNAK
8	☐	[redacted] Heavy Chain	319	N319+Deamidation	+Deamidation	None	3.4%	3.9%	1:V306-K321 = 18...	VVSVLTVLHQDWL...
9	☐	[redacted] Heavy Chain	329	N329+Deamidation	+Deamidation	Poor recovery	2.3%	0.0%	1:C325-K330 = 73...	CKVSNK
10	☐	[redacted] Heavy Chain	365	~N365+Deamidati...	+Deamidation	None	11.3%	7.7%	1:N365-K374 = 11...	NQVSLTCLVK; NQV...
11	☐	[redacted] Heavy Chain	393	~N393+Deamidati...	+Deamidation	None	1.6%	3.2%	1:G375-K396 = 25...	GFYPSDIAVEWESN...
12	☐	[redacted] Heavy Chain	423	Q423+Deamidation	+Deamidation	None	3.5%	3.9%	1:W421-K443 = 28...	WQQGNVFSCSVM...
13	☐	[redacted] Light Chain	36	~Q36+Deamidation	+Deamidation	None	2.4%	4.6%	2:A25-K38 = 1666....	ASSSVSYIHWFQQK

Components

	☐	Level	No.	Identification	Peptide Sequence	Modification	Site	Delta (ppm)	Confidence Score	Best Overall Average Structural Resolution	ID T
			=					=	=	=	
1	☑	Component	8027	1:G44-K63 = 2182.0331m	GLEWIGAIYPGNGDTSYN...	None		-0.51	100.0	1.1	MS2
2	☑	Component	8033	1:G44-K63 = 2182.0331m	GLEWIGAIYPGNGDTSYN...	None		-0.73	100.0	1.1	MS2
3	☑	Component	8202	1:G44-K63 = 2182.0331m(N55+Deamidati...	GLEWIGAIYPGNGDTSYN...	Deamidation	N55	-0.35	100.0	1.1	MS2
4	☑	Component	8207	1:G44-K63 = 2182.0331m(N55+Deamidati...	GLEWIGAIYPGNGDTSYN...	Deamidation	N55	-0.23	100.0	1.2	MS2
5	☑	Component	8413	1:G44-K63 = 2182.0331m(N55+NH3 loss)	GLEWIGAIYPGNGDTSYN...	NH3 loss	N55	-0.43	100.0	1.3	MS2
6	☑	Component	8416	1:G44-K63 = 2182.0331m(N55+NH3 loss)	GLEWIGAIYPGNGDTSYN...	NH3 loss	N55	-0.54	100.0	1.1	MS2
7	☐	Component	749	1:N55-K63 = 1025.4414m[nonspecific]	NGDTSYNQK	nonspecific		-76.54	0.0	0.0	Full
8	☐	Component	2301	1:I51-K74 = 2617.8773a[nonspecific]	IYPGNGDTSYNQKFKGKATL...	nonspecific		-351.16	0.0	0.0	Full

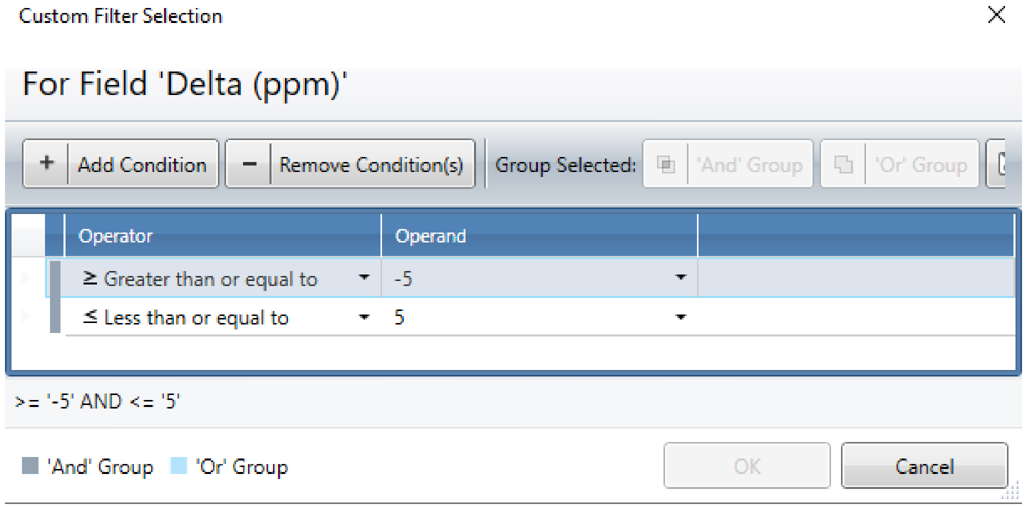
Stage 3: Data processing, analysis and reporting_**Step 13**



For Field 'Modification'

Operator	Operand
Does not contain	+
Does not contain	nonspecific
Does not contain	gasphase
Does not contain	.

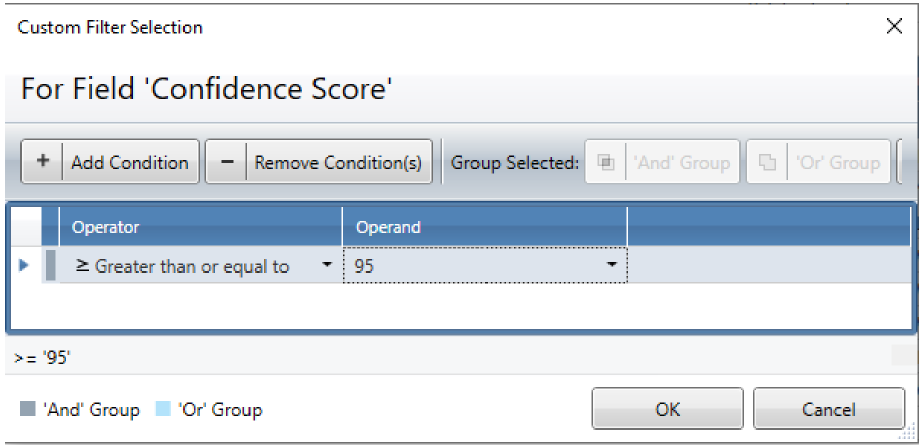
Does not contain '+' AND Does not contain 'nonspecific' AND Does not contain 'gasphase' AND Does not contain '.'



For Field 'Delta (ppm)'

Operator	Operand
≥ Greater than or equal to	-5
≤ Less than or equal to	5

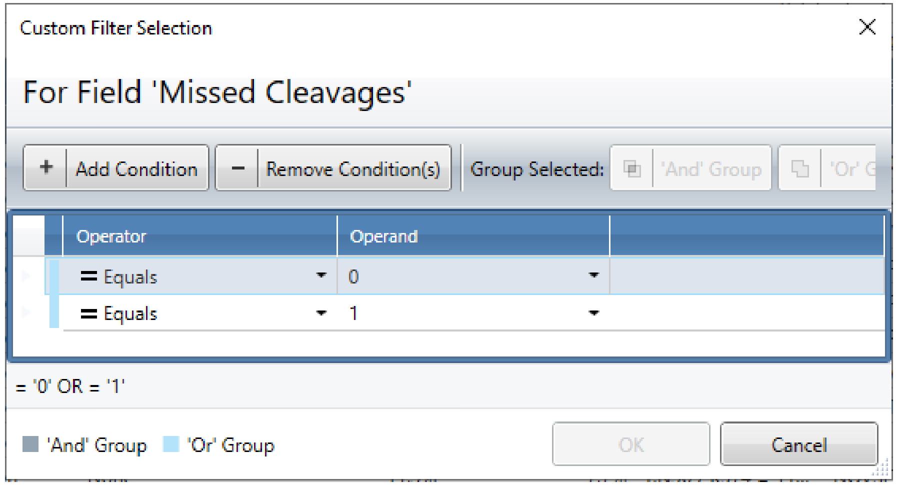
>= '-5' AND <= '5'



For Field 'Confidence Score'

Operator	Operand
≥ Greater than or equal to	95

>= '95'

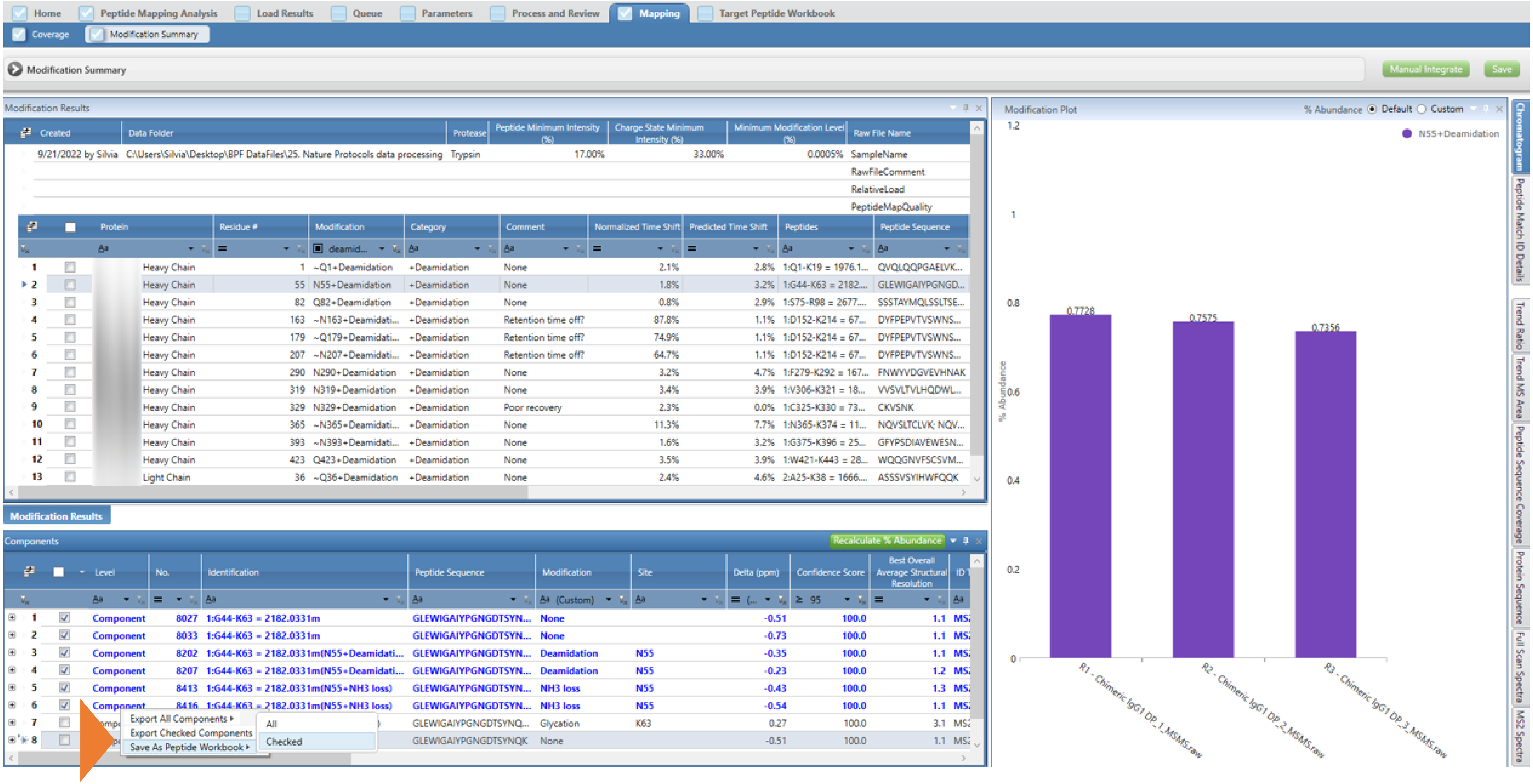


For Field 'Missed Cleavages'

Operator	Operand
= Equals	0
= Equals	1

= '0' OR = '1'

Stage 3: Data processing, analysis and reporting_**Steps 14 and 15**

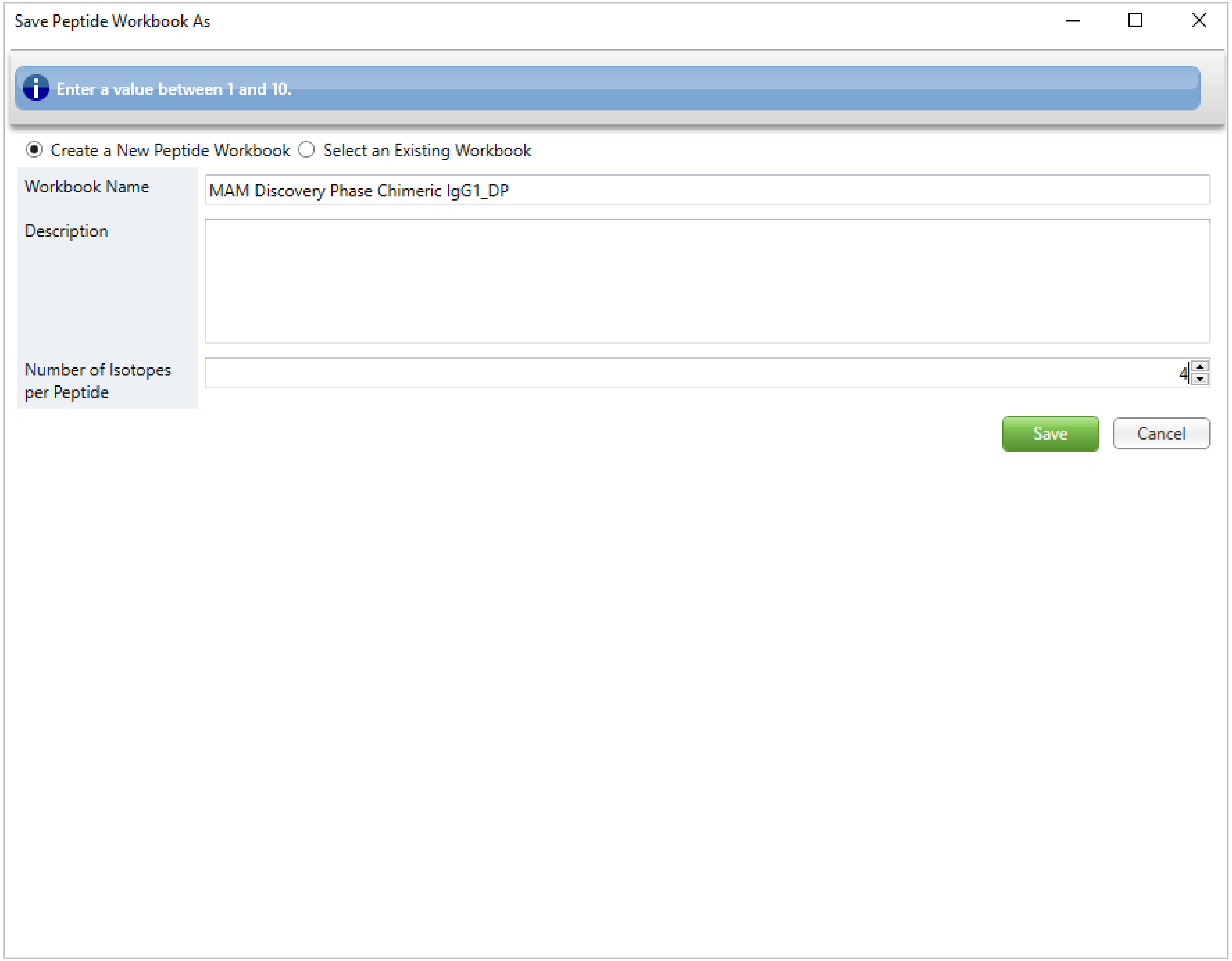


Created	Data Folder	Protease	Peptide Minimum Intensity (%)	Charge State Minimum Intensity (%)	Minimum Modification Level (%)	Raw File Name
9/21/2022 by Silvia	C:\Users\Silvia\Desktop\BPF DataFiles\25. Nature Protocols data processing	Trypsin	17.00%	33.00%	0.0005%	SampleName
						RawFileComment
						RelativeLoad
						PeptideMapQuality

	☐	Protein	Residue #	Modification	Category	Comment	Normalized Time Shift	Predicted Time Shift	Peptides	Peptide Sequence
				deamid...						
1	☐	[redacted] Heavy Chain	1	~Q1+Deamidation	+Deamidation	None	2.1%	2.8%	1:Q1-K19 = 1976.1...	QVQLQQPGAELVK...
2	☐	[redacted] Heavy Chain	55	N55+Deamidation	+Deamidation	None	1.8%	3.2%	1:G44-K63 = 2182....	GLEWIGAIYPGNGD...
3	☐	[redacted] Heavy Chain	82	Q82+Deamidation	+Deamidation	None	0.8%	2.9%	1:S75-R98 = 2677....	SSSTAYMQLSSLTSE...
4	☐	[redacted] Heavy Chain	163	~N163+Deamidati...	+Deamidation	Retention time off?	87.8%	1.1%	1:D152-K214 = 67...	DYFPEPVTVSWNS...
5	☐	[redacted] Heavy Chain	179	~Q179+Deamidati...	+Deamidation	Retention time off?	74.9%	1.1%	1:D152-K214 = 67...	DYFPEPVTVSWNS...
6	☐	[redacted] Heavy Chain	207	~N207+Deamidati...	+Deamidation	Retention time off?	64.7%	1.1%	1:D152-K214 = 67...	DYFPEPVTVSWNS...
7	☐	[redacted] Heavy Chain	290	N290+Deamidation	+Deamidation	None	3.2%	4.7%	1:F279-K292 = 167...	FNWYVDGVEVHNAK
8	☐	[redacted] Heavy Chain	319	N319+Deamidation	+Deamidation	None	3.4%	3.9%	1:V306-K321 = 18...	VVSVLTVLHQDWL...
9	☐	[redacted] Heavy Chain	329	N329+Deamidation	+Deamidation	Poor recovery	2.3%	0.0%	1:C325-K330 = 73...	CKVSNK
10	☐	[redacted] Heavy Chain	365	~N365+Deamidati...	+Deamidation	None	11.3%	7.7%	1:N365-K374 = 11...	NQVSLTCLVK; NQV...
11	☐	[redacted] Heavy Chain	393	~N393+Deamidati...	+Deamidation	None	1.6%	3.2%	1:G375-K396 = 25...	GFYPSDIAVEWESN...
12	☐	[redacted] Heavy Chain	423	Q423+Deamidation	+Deamidation	None	3.5%	3.9%	1:W421-K443 = 28...	WQQGNVFSCSVM...
13	☐	[redacted] Light Chain	36	~Q36+Deamidation	+Deamidation	None	2.4%	4.6%	2:A25-K38 = 1666....	ASSSVSYIHWFQQK

	☐	Level	No.	Identification	Peptide Sequence	Modification	Site	Delta (ppm)	Confidence Score	Best Overall Average Structural Resolution	ID T
						(Custom)		(...	≥ 95		
1	☑	Component	8027	1:G44-K63 = 2182.0331m	GLEWIGAIYPGNGDTSYN...	None		-0.51	100.0	1.1	MS
2	☑	Component	8033	1:G44-K63 = 2182.0331m	GLEWIGAIYPGNGDTSYN...	None		-0.73	100.0	1.1	MS
3	☑	Component	8202	1:G44-K63 = 2182.0331m(N55+Deamidati...	GLEWIGAIYPGNGDTSYN...	Deamidation	N55	-0.35	100.0	1.1	MS
4	☑	Component	8207	1:G44-K63 = 2182.0331m(N55+Deamidati...	GLEWIGAIYPGNGDTSYN...	Deamidation	N55	-0.23	100.0	1.2	MS
5	☑	Component	8413	1:G44-K63 = 2182.0331m(N55+NH3 loss)	GLEWIGAIYPGNGDTSYN...	NH3 loss	N55	-0.43	100.0	1.3	MS
6	☑	Component	8416	1:G44-K63 = 2182.0331m(N55+NH3 loss)	GLEWIGAIYPGNGDTSYN...	NH3 loss	N55	-0.54	100.0	1.1	MS
7	☐				GLEWIGAIYPGNGDTSYNQ...	Glycation	K63	0.27	100.0	3.1	MS
8	☐				GLEWIGAIYPGNGDTSYNQK	None		-0.51	100.0	1.1	MS

Stage 3: Data processing, analysis and reporting_**Step 16**



Save Peptide Workbook As

Enter a value between 1 and 10.

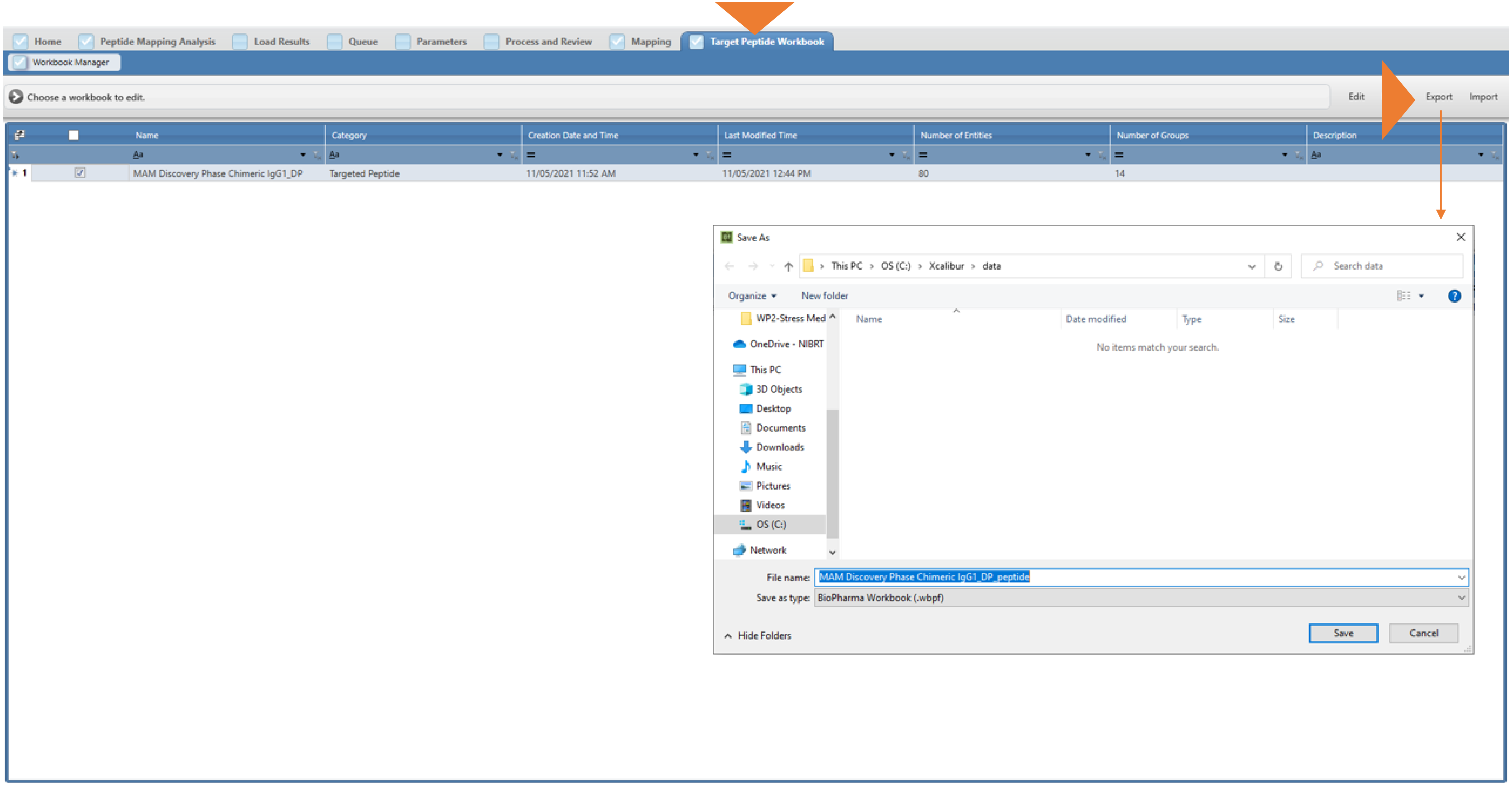
● Create a New Peptide Workbook ○ Select an Existing Workbook

Workbook Name: MAM Discovery Phase Chimeric IgG1_DP

Description:

Number of Isotopes per Peptide: 4

Stage 3: Data processing, analysis and reporting_**Step 17**



Choose a workbook to edit.

	☐	Name	Category	Creation Date and Time	Last Modified Time	Number of Entities	Number of Groups	Description
1	☑	MAM Discovery Phase Chimeric IgG1_DP	Targeted Peptide	11/05/2021 11:52 AM	11/05/2021 12:44 PM	80	14	

This PC › OS (C:) › Xcalibur › data

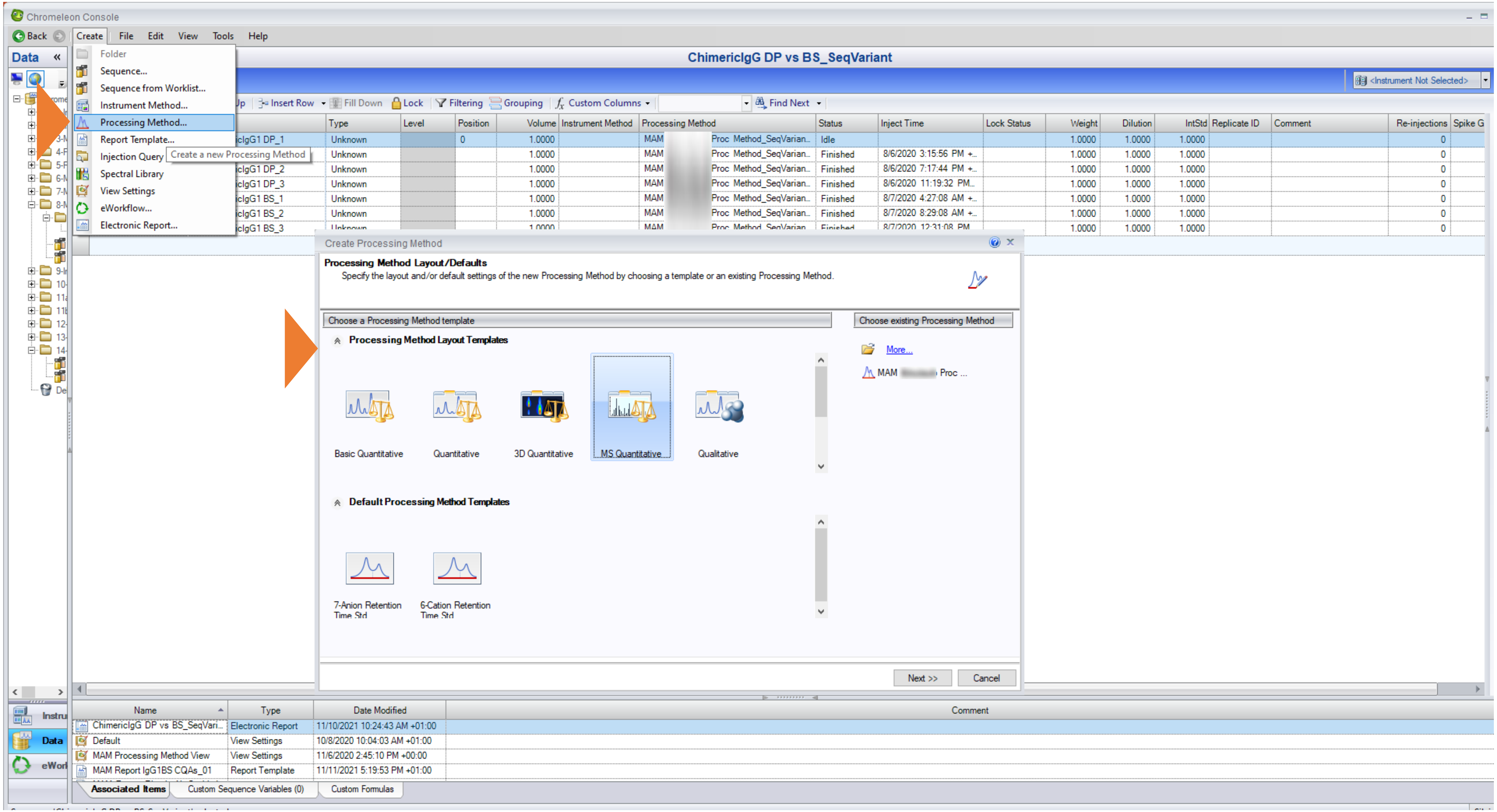
Name	Date modified	Type	Size

No items match your search.

File name: MAM Discovery Phase Chimeric IgG1_DP_peptide

Save as type: BioPharma Workbook (.wbpf)

Stage 3: Data processing, analysis and reporting_**Step 18**



ChimericIgG DP vs BS_SeqVariant

	Type	Level	Position	Volume	Instrument Method	Processing Method	Status	Inject Time	Lock Status	Weight	Dilution	IntStd	Replicate ID	Comment	Re-injections	Spike G
iclgG1 DP_1	Unknown		0	1.0000		MAM [redacted] Proc Method_SeqVarian...	Idle			1.0000	1.0000	1.0000			0	
	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 3:15:56 PM +...		1.0000	1.0000	1.0000			0	
iclgG1 DP_2	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 7:17:44 PM +...		1.0000	1.0000	1.0000			0	
iclgG1 DP_3	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 11:19:32 PM...		1.0000	1.0000	1.0000			0	
iclgG1 BS_1	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/7/2020 4:27:08 AM +...		1.0000	1.0000	1.0000			0	
iclgG1 BS_2	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/7/2020 8:29:08 AM +...		1.0000	1.0000	1.0000			0	
iclgG1 BS_3	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/7/2020 12:31:08 PM...		1.0000	1.0000	1.0000			0	

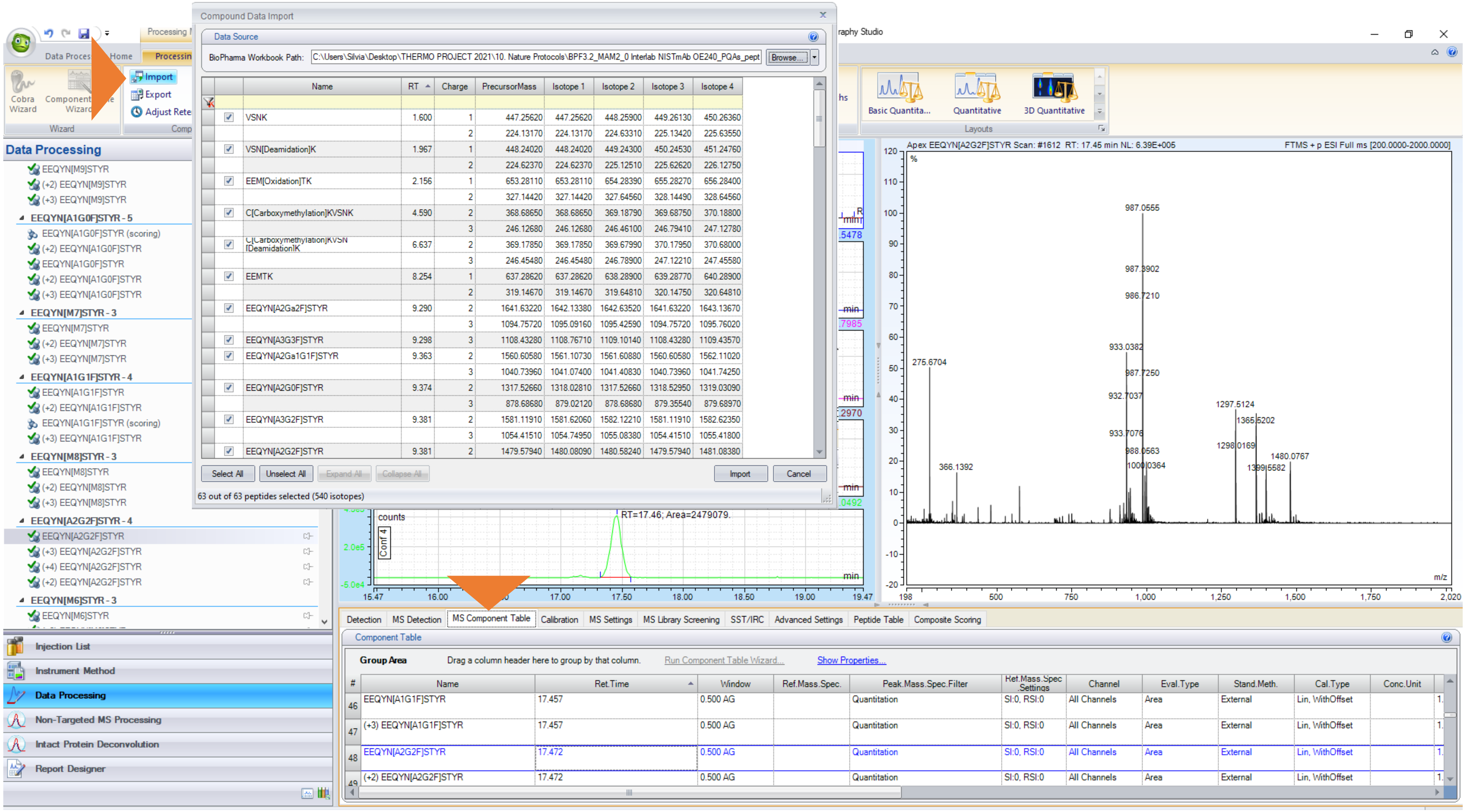
Create Processing Method

Processing Method Layout/Defaults

Specify the layout and/or default settings of the new Processing Method by choosing a template or an existing Processing Method.

Name	Type	Date Modified	Comment
ChimericIgG DP vs BS_SeqVari...	Electronic Report	11/10/2021 10:24:43 AM +01:00	
Default	View Settings	10/8/2020 10:04:03 AM +01:00	
MAM Processing Method View	View Settings	11/6/2020 2:45:10 PM +00:00	
MAM Report IgG1BS CQAs_01	Report Template	11/11/2021 5:19:53 PM +01:00	

Stage 3: Data processing, analysis and reporting_**Step 18**



Compound Data Import

Data Source

BioPharma Workbook Path: C:\Users\Silvia\Desktop\THERMO PROJECT 2021\10. Nature Protocols\BPF3.2_MAM2_0 Interlab NISTmAb OE240_PQAs_pept

	Name	RT	Charge	PrecursorMass	Isotope 1	Isotope 2	Isotope 3	Isotope 4
☑	VSNK	1.600	1	447.25620	447.25620	448.25900	449.26130	450.26360
			2	224.13170	224.13170	224.63310	225.13420	225.63550
☑	VSN[Deamidation]K	1.967	1	448.24020	448.24020	449.24300	450.24530	451.24760
			2	224.62370	224.62370	225.12510	225.62620	226.12750
☑	EEM[Oxidation]TK	2.156	1	653.28110	653.28110	654.28390	655.28270	656.28400
			2	327.14420	327.14420	327.64560	328.14490	328.64560
☑	C[Carboxymethylation]KVSNK	4.590	2	368.68650	368.68650	369.18790	369.68750	370.18800
			3	246.12680	246.12680	246.46100	246.79410	247.12780
☑	C[Carboxymethylation]KVSN [Deamidation]K	6.637	2	369.17850	369.17850	369.67990	370.17950	370.68000
			3	246.45480	246.45480	246.78900	247.12210	247.45580
☑	EEMTK	8.254	1	637.28620	637.28620	638.28900	639.28770	640.28900
			2	319.14670	319.14670	319.64810	320.14750	320.64810
☑	EEQYN[A2Ga2F]STYR	9.290	2	1641.63220	1642.13380	1642.63520	1641.63220	1643.13670
			3	1094.75720	1095.09160	1095.42590	1094.75720	1095.76020
☑	EEQYN[A3G3F]STYR	9.298	3	1108.43280	1108.76710	1109.10140	1108.43280	1109.43570
☑	EEQYN[A2Ga1G1F]STYR	9.363	2	1560.60580	1561.10730	1561.60880	1560.60580	1562.11020
			3	1040.73960	1041.07400	1041.40830	1040.73960	1041.74250
☑	EEQYN[A2G0F]STYR	9.374	2	1317.52660	1318.02810	1317.52660	1318.52950	1319.03090
			3	878.68680	879.02120	878.68680	879.35540	879.68970
☑	EEQYN[A3G2F]STYR	9.381	2	1581.11910	1581.62060	1582.12210	1581.11910	1582.62350
			3	1054.41510	1054.74950	1055.08380	1054.41510	1055.41800
☑	EEQYN[A2G2F]STYR	9.381	2	1479.57940	1480.08090	1480.58240	1479.57940	1481.08380

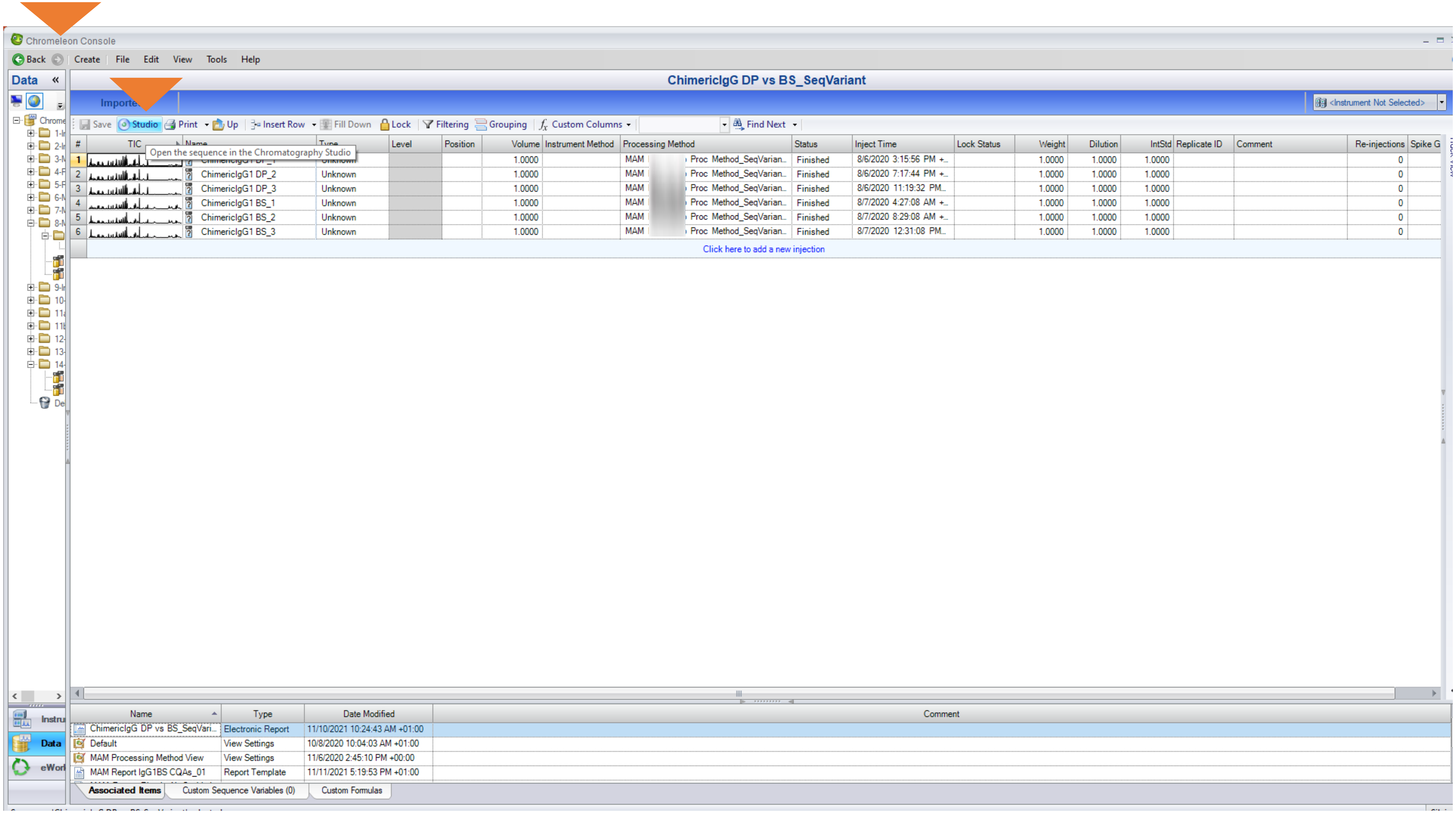
63 out of 63 peptides selected (540 isotopes)

Component Table

Group Area Drag a column header here to group by that column. Run Component Table Wizard... Show Properties...

#	Name	Ret.Time	Window	Ref.Mass.Spec.	Peak.Mass.Spec.Filter	Ref.Mass.Spec.Settings	Channel	Eval.Type	Stand.Meth.	Cal.Type	Conc.Unit
46	EEQYN[A1G1F]STYR	17.457	0.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
47	(+3) EEQYN[A1G1F]STYR	17.457	0.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
48	EEQYN[A2G2F]STYR	17.472	0.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
49	(+2) EEQYN[A2G2F]STYR	17.472	0.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	

Stage 3: Data processing, analysis and reporting_**Step 19**



ChimericIgG DP vs BS_SeqVariant

#	TIC	Name	Type	Level	Position	Volume	Instrument Method	Processing Method	Status	Inject Time	Lock Status	Weight	Dilution	IntStd	Replicate ID	Comment	Re-injections	Spike G
1		ChimericIgG1 DP_1	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 3:15:56 PM +...		1.0000	1.0000	1.0000			0	
2		ChimericIgG1 DP_2	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 7:17:44 PM +...		1.0000	1.0000	1.0000			0	
3		ChimericIgG1 DP_3	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 11:19:32 PM...		1.0000	1.0000	1.0000			0	
4		ChimericIgG1 BS_1	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/7/2020 4:27:08 AM +...		1.0000	1.0000	1.0000			0	
5		ChimericIgG1 BS_2	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/7/2020 8:29:08 AM +...		1.0000	1.0000	1.0000			0	
6		ChimericIgG1 BS_3	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/7/2020 12:31:08 PM...		1.0000	1.0000	1.0000			0	

Name	Type	Date Modified	Comment
ChimericIgG DP vs BS_SeqVari...	Electronic Report	11/10/2021 10:24:43 AM +01:00	
Default	View Settings	10/8/2020 10:04:03 AM +01:00	
MAM Processing Method View	View Settings	11/6/2020 2:45:10 PM +00:00	
MAM Report IgG1BS CQAs_01	Report Template	11/11/2021 5:19:53 PM +01:00	

Stage 3: Data processing, analysis and reporting_**Step 19**

Injections:

1 ChimericIgG1 DP_1
2 ChimericIgG1 DP_2
3 ChimericIgG1 DP_3
4 ChimericIgG1 BS_1
5 ChimericIgG1 BS_2
6 ChimericIgG1 BS_3

Channels:

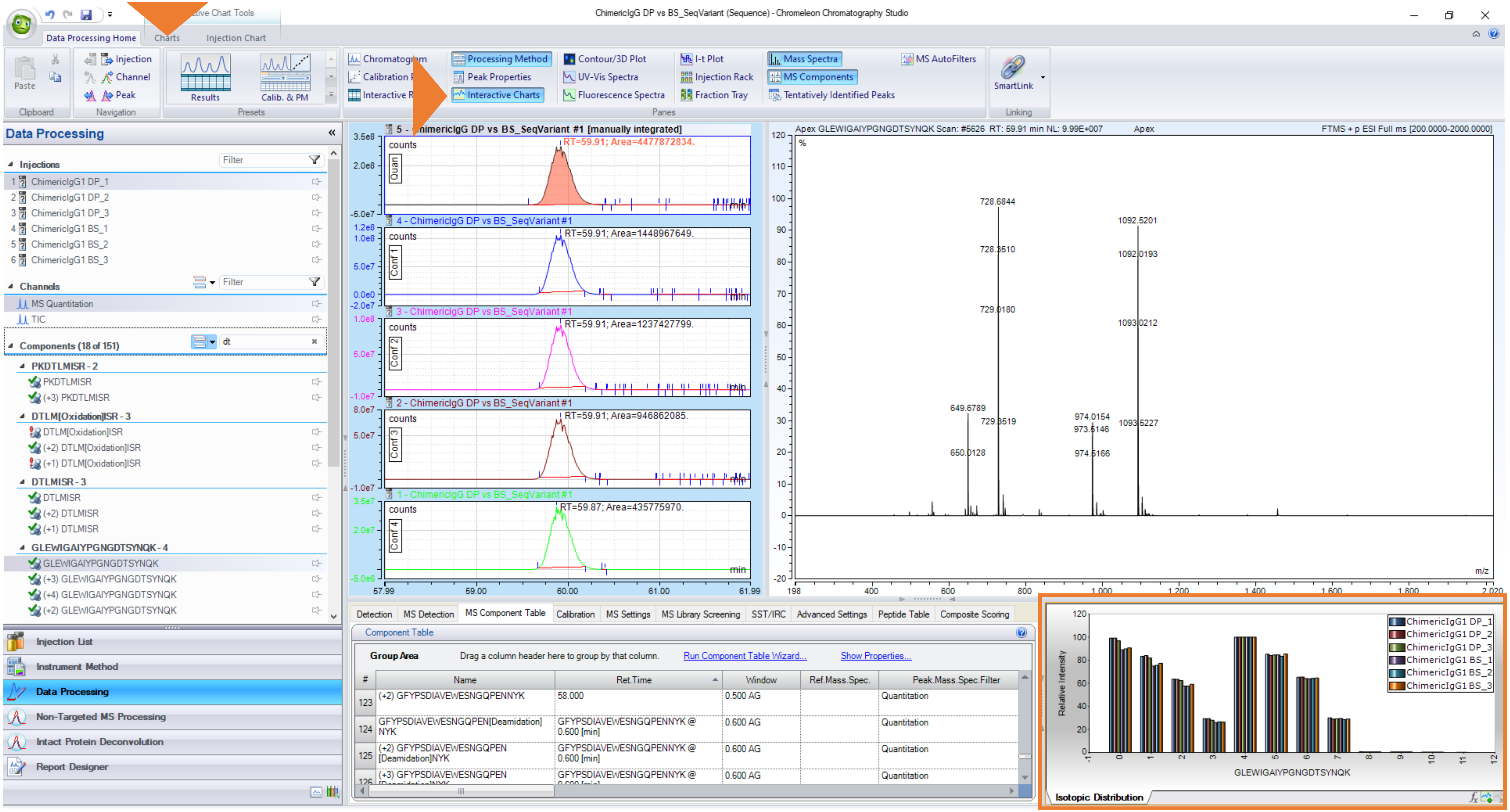
- MS Quantitation
- TIC

Components (18 of 151):

- PKDTLMISR
- (+3) PKDTLMISR
- DTLM[Oxidation]ISR
- (+2) DTLM[Oxidation]ISR
- (+1) DTLM[Oxidation]ISR
- DTLMISR
- (+2) DTLMISR
- (+1) DTLMISR
- GLEWIGAIYPGNGDTSYNQK
- (+3) GLEWIGAIYPGNGDTSYNQK
- (+4) GLEWIGAIYPGNGDTSYNQK
- (+2) GLEWIGAIYPGNGDTSYNQK
- GLEWIGAIYPGN[Deamidation]GDTSYNQK
- (+2) GLEWIGAIYPGN[Deamidation]GDTSYNQK
- (+3) GLEWIGAIYPGN[Deamidation]GDTSYNQK
- GLEWIGAIYPGN[NH3 loss]GDTSYNQK
- (+2) GLEWIGAIYPGN[NH3 loss]GDTSYNQK

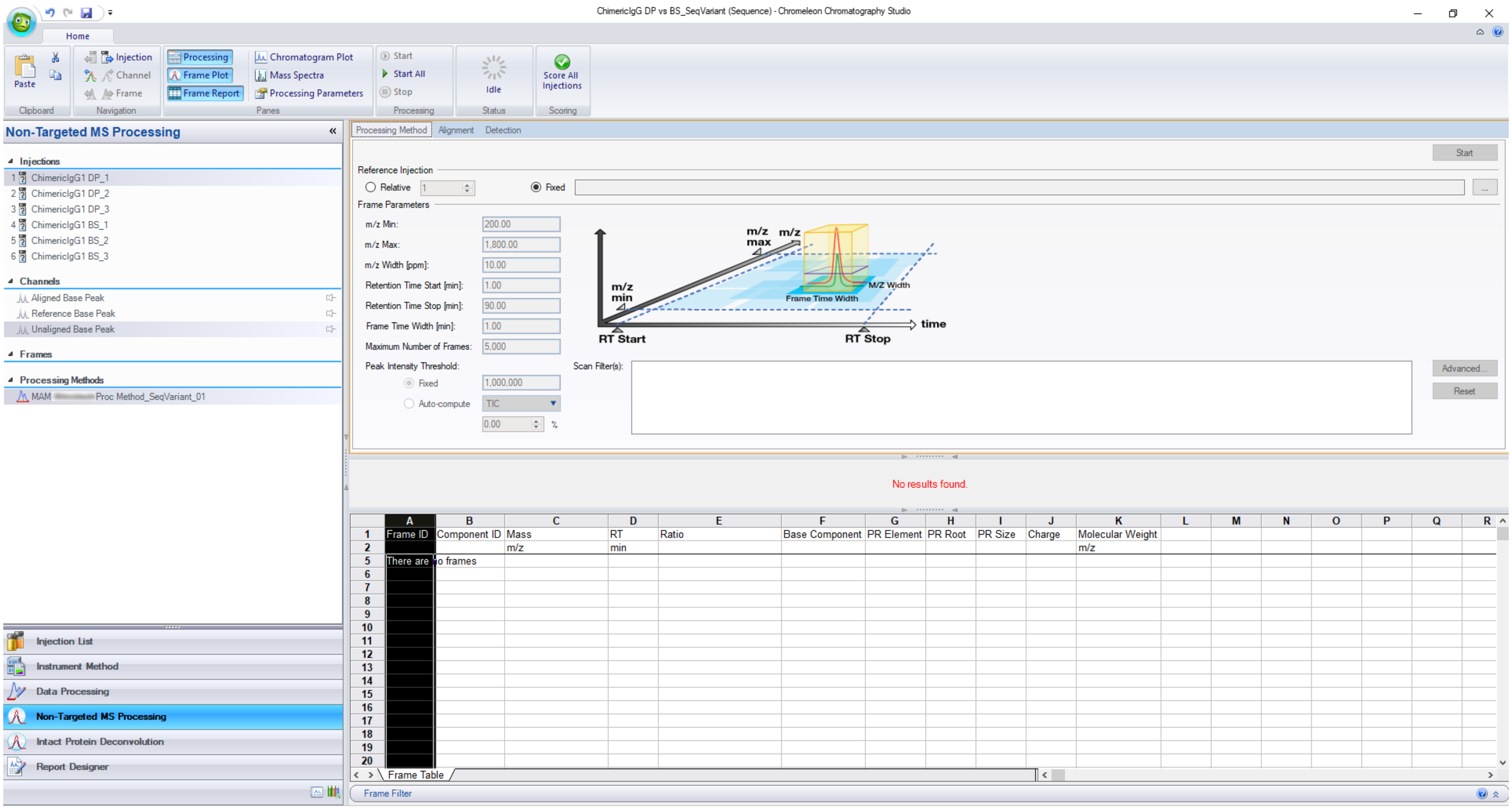
#	Name	Ret.Time	Window	Ref.Mass.Spec.	Peak.Mass.Spec.Filter	Ref.Mass.Spec .Settings	Channel	Eval.Type	Stand.Meth.	Cal.Type	Conc.Unit
123	(+2) GFYPSDIAVEWESNGQPENNYK	58.000	0.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
124	GFYPSDIAVEWESNGQPEN[Deamidation] NYK	GFYPSDIAVEWESNGQPENNYK @ 0.600 [min]	0.600 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
125	(+2) GFYPSDIAVEWESNGQPEN [Deamidation]NYK	GFYPSDIAVEWESNGQPENNYK @ 0.600 [min]	0.600 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
126	(+3) GFYPSDIAVEWESNGQPEN [Deamidation]NYK	GFYPSDIAVEWESNGQPENNYK @ 0.600 [min]	0.600 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
127	GFYPSDIAVEWESN[NH3 loss] GQPENNYK	GFYPSDIAVEWESNGQPENNYK @ 1.600 [min]	0.600 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
128	(+2) GFYPSDIAVEWESN[NH3 loss] GQPENNYK	GFYPSDIAVEWESNGQPENNYK @ 1.600 [min]	0.600 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
129	(+3) GFYPSDIAVEWESN[NH3 loss] GQPENNYK	GFYPSDIAVEWESNGQPENNYK @ 1.600 [min]	0.600 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	
130	GLEWIGAIYPGNGDTSYNQK	59.990	0.500 AG		Quantitation	SI:0, RSI:0	All Channels	Area	External	Lin, WithOffset	

Stage 3: Data processing, analysis and reporting_**Step 20**



#	Name	Ret.Time	Window	Ref.Mass.Spec.	Peak.Mass.Spec.Filter
123	(+2) GFYPSDIAVEWESNGQPENNYK	58.000	0.500 AG		Quantitation
124	GFYPSDIAVEWESNGQPEN[Deamidation] NYK	GFYPSDIAVEWESNGQPENNYK @ 0.600 [min]	0.600 AG		Quantitation
125	(+2) GFYPSDIAVEWESNGQPEN [Deamidation]NYK	GFYPSDIAVEWESNGQPENNYK @ 0.600 [min]	0.600 AG		Quantitation
126	(+3) GFYPSDIAVEWESNGQPEN	GFYPSDIAVEWESNGQPENNYK @	0.600 AG		Quantitation

Stage 3: Data processing, analysis and reporting_**Step 21-24**



Injections

1 ChimericIgG1 DP_1
2 ChimericIgG1 DP_2
3 ChimericIgG1 DP_3
4 ChimericIgG1 BS_1
5 ChimericIgG1 BS_2
6 ChimericIgG1 BS_3

Channels

- Aligned Base Peak
- Reference Base Peak
- Unaligned Base Peak

Frames

Processing Methods

- MAM [redacted] Proc Method_SeqVariant_01

Reference Injection

○ Relative 1 ● Fixed

Frame Parameters

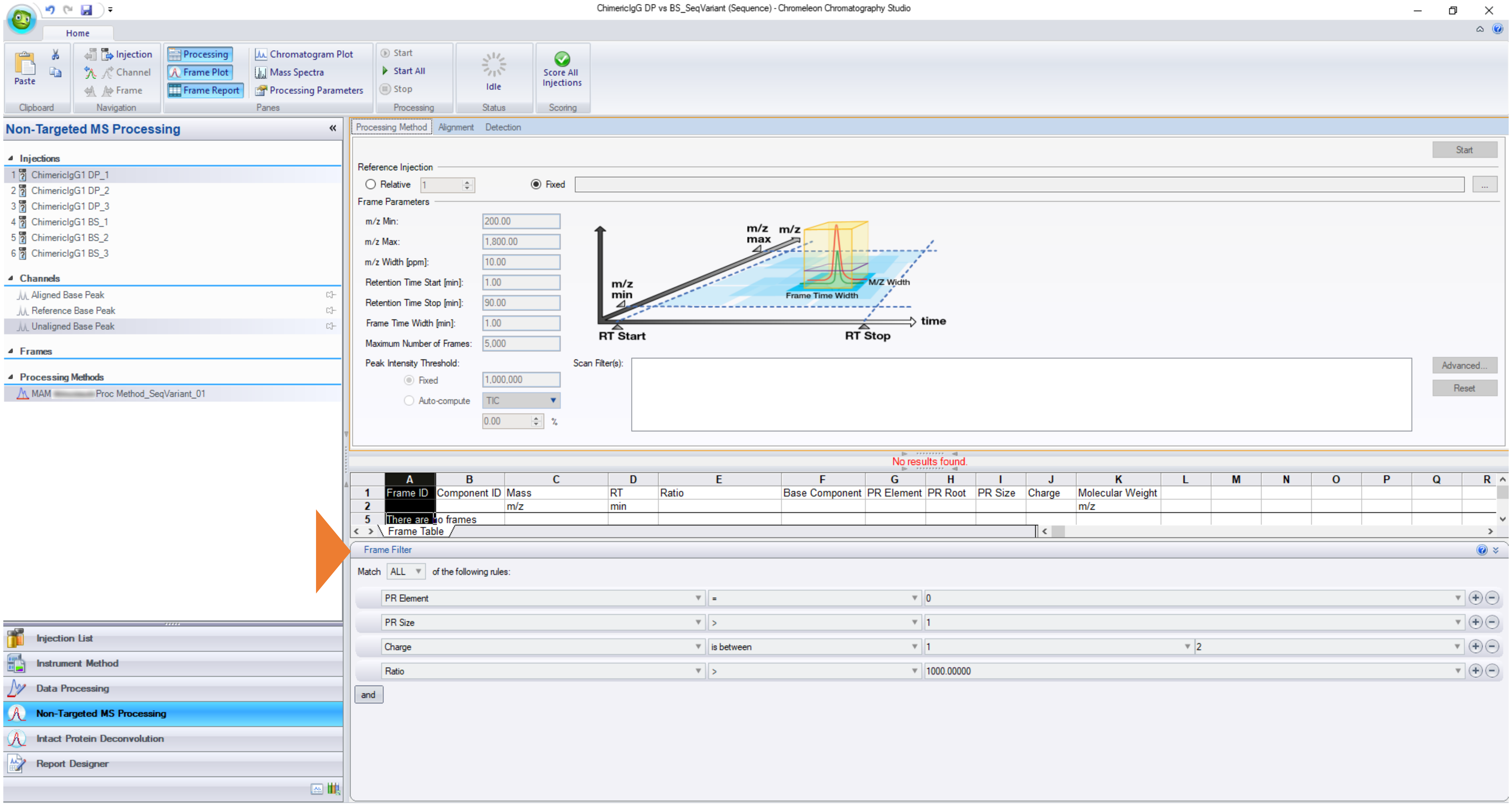
m/z Min: 200.00
m/z Max: 1,800.00
m/z Width [ppm]: 10.00
Retention Time Start [min]: 1.00
Retention Time Stop [min]: 90.00
Frame Time Width [min]: 1.00
Maximum Number of Frames: 5,000
Peak Intensity Threshold:
● Fixed 1,000,000
○ Auto-compute TIC
0.00 %

Scan Filter(s):

No results found.

	A	B	C	D	E	F	G	H	I	J	K
1	Frame ID	Component ID	Mass	RT	Ratio	Base Component	PR Element	PR Root	PR Size	Charge	Molecular Weight
2			m/z	min							m/z
5	There are no frames										

Stage 3: Data processing, analysis and reporting_**Step 25-26**



Non-Targeted MS Processing

Injections

1 ChimericIgG1 DP_1
2 ChimericIgG1 DP_2
3 ChimericIgG1 DP_3
4 ChimericIgG1 BS_1
5 ChimericIgG1 BS_2
6 ChimericIgG1 BS_3

Channels

Aligned Base Peak
Reference Base Peak
Unaligned Base Peak

Frames

Processing Methods

MAM [redacted] Proc Method_SeqVariant_01

Reference Injection

○ Relative 1 ● Fixed

Frame Parameters

m/z Min: 200.00
m/z Max: 1,800.00
m/z Width [ppm]: 10.00
Retention Time Start [min]: 1.00
Retention Time Stop [min]: 90.00
Frame Time Width [min]: 1.00
Maximum Number of Frames: 5,000
Peak Intensity Threshold:
● Fixed 1,000,000
○ Auto-compute TIC 0.00 %

Scan Filter(s):

No results found.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Frame ID	Component ID	Mass	RT	Ratio	Base Component	PR Element	PR Root	PR Size	Charge	Molecular Weight							
2			m/z	min							m/z							
5	There are no frames																	

Frame Table

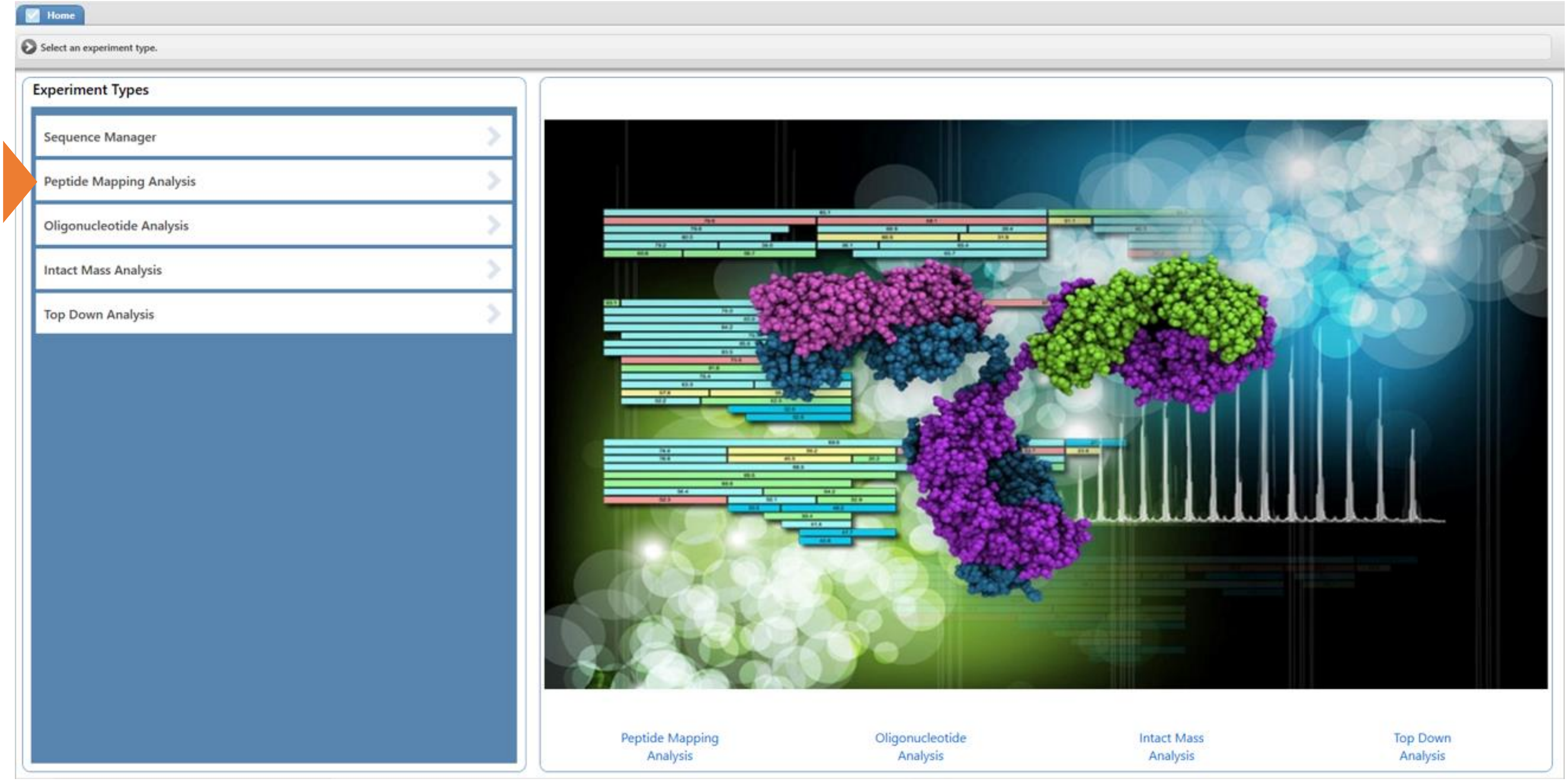
Frame Filter

Match ALL of the following rules:

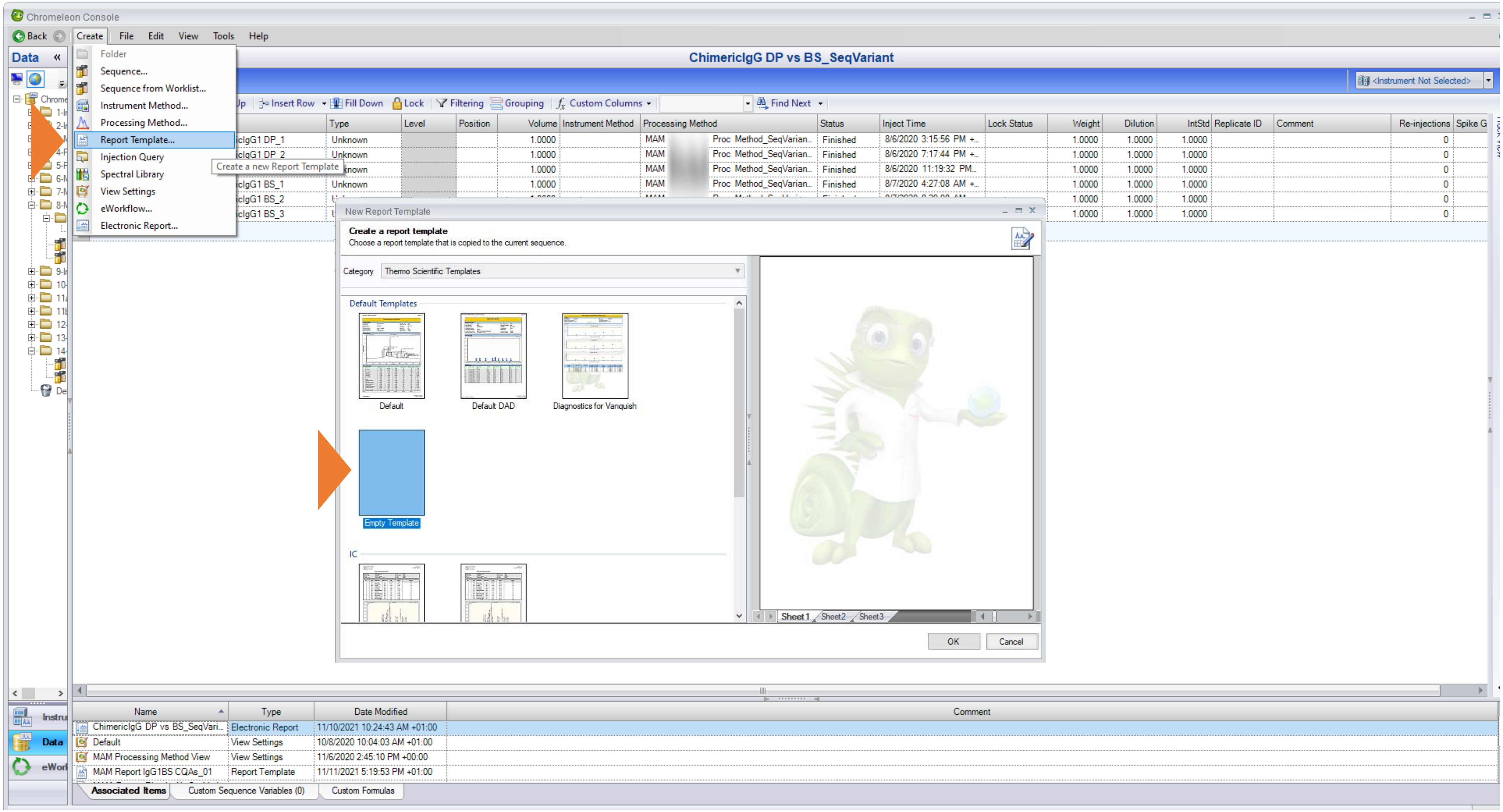
PR Element = 0
PR Size > 1
Charge is between 1 2
Ratio > 1000.00000

and

Stage 3: Data processing, analysis and reporting_**Step 27**



Stage 3: Data processing, analysis and reporting_**Step 28**



ChimericlgG DP vs BS_SeqVariant

	Type	Level	Position	Volume	Instrument Method	Processing Method	Status	Inject Time	Lock Status	Weight	Dilution	IntStd	Replicate ID	Comment	Re-injections	Spike G
iclgG1 DP_1	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 3:15:56 PM +...		1.0000	1.0000	1.0000			0	
iclgG1 DP_2	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 7:17:44 PM +...		1.0000	1.0000	1.0000			0	
	known			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/6/2020 11:19:32 PM...		1.0000	1.0000	1.0000			0	
iclgG1 BS_1	Unknown			1.0000		MAM [redacted] Proc Method_SeqVarian...	Finished	8/7/2020 4:27:08 AM +...		1.0000	1.0000	1.0000			0	
iclgG1 BS_2										1.0000	1.0000	1.0000			0	
iclgG1 BS_3										1.0000	1.0000	1.0000			0	

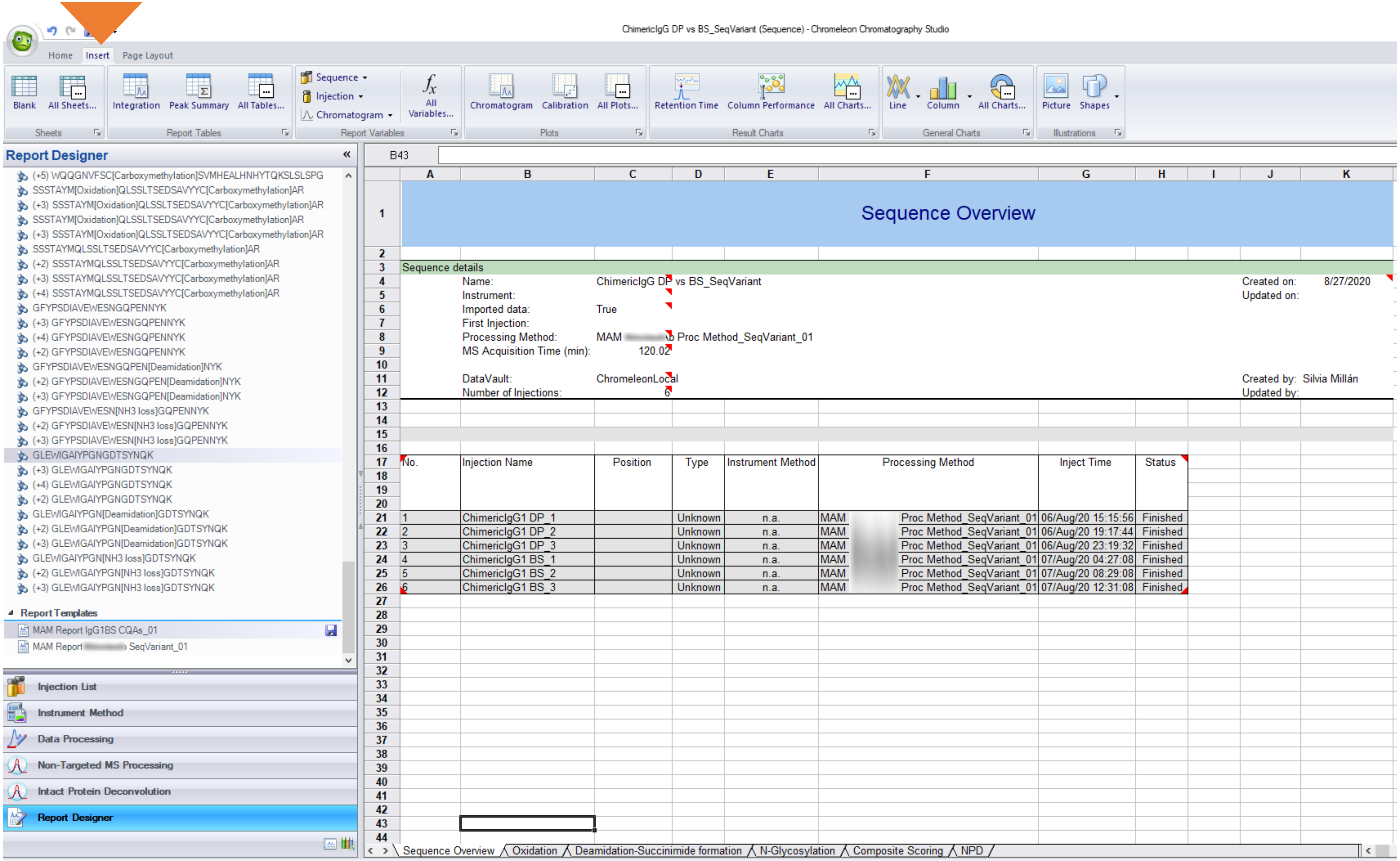
New Report Template

Create a report template
Choose a report template that is copied to the current sequence.

Category: Thermo Scientific Templates

Name	Type	Date Modified	Comment
ChimericlgG DP vs BS_SeqVari...	Electronic Report	11/10/2021 10:24:43 AM +01:00	
Default	View Settings	10/8/2020 10:04:03 AM +01:00	
MAM Processing Method View	View Settings	11/6/2020 2:45:10 PM +00:00	
MAM Report IgG1BS CQAs_01	Report Template	11/11/2021 5:19:53 PM +01:00	

Stage 3: Data processing, analysis and reporting_**Step 29-32**



Sequence Overview

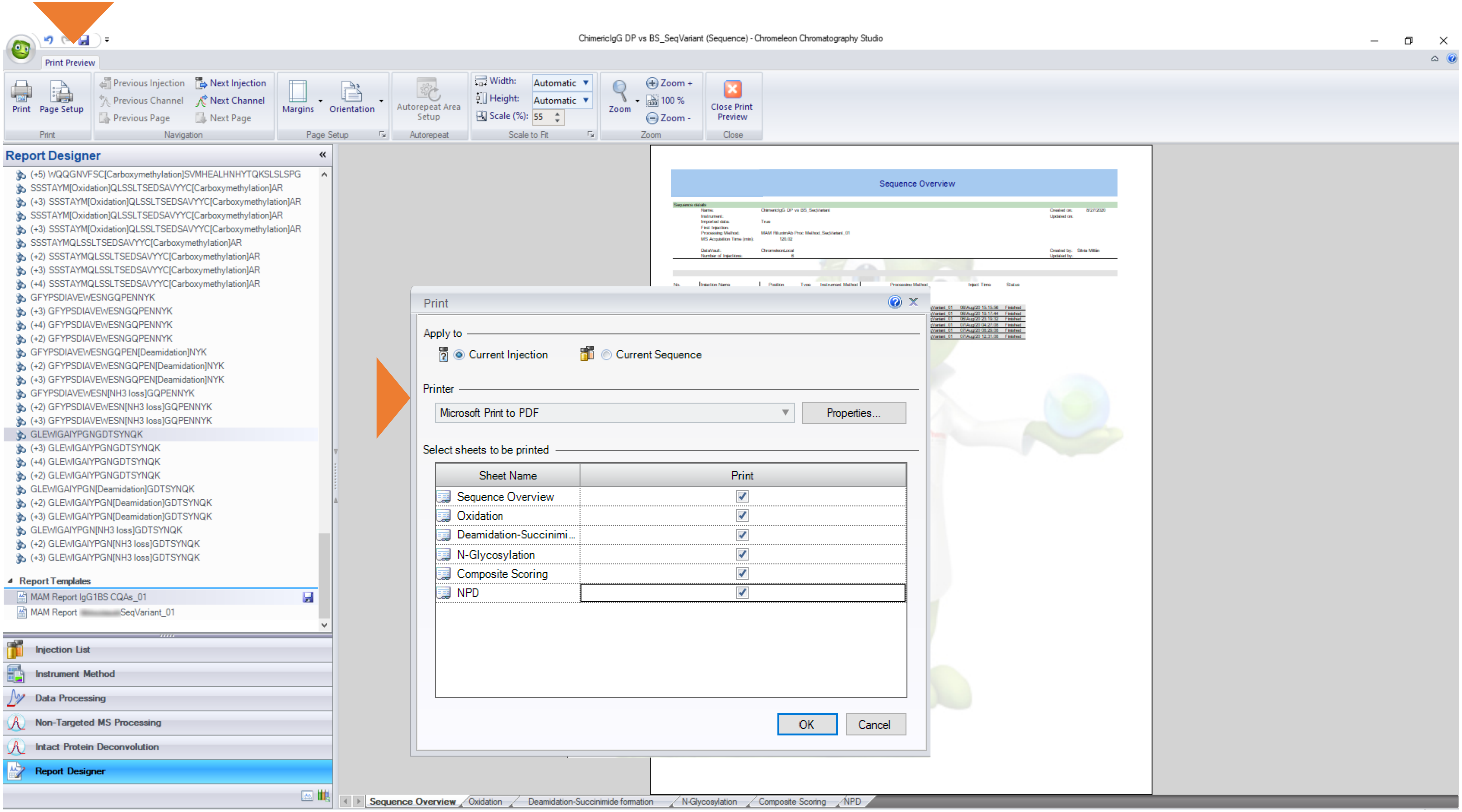
Sequence details

Name: ChimericIgG DP vs BS_SeqVariant
Instrument:
Imported data: True
First Injection:
Processing Method: MAM [redacted] Proc Method_SeqVariant_01
MS Acquisition Time (min): 120.02
DataVault: ChromeleonLocal
Number of Injections: 6

Created on: 8/27/2020
Updated on:
Created by: Silvia Millán
Updated by:

No.	Injection Name	Position	Type	Instrument Method	Processing Method	Inject Time	Status
1	ChimericIgG1 DP_1		Unknown	n.a.	MAM [redacted] Proc Method_SeqVariant_01	06/Aug/20 15:15:56	Finished
2	ChimericIgG1 DP_2		Unknown	n.a.	MAM [redacted] Proc Method_SeqVariant_01	06/Aug/20 19:17:44	Finished
3	ChimericIgG1 DP_3		Unknown	n.a.	MAM [redacted] Proc Method_SeqVariant_01	06/Aug/20 23:19:32	Finished
4	ChimericIgG1 BS_1		Unknown	n.a.	MAM [redacted] Proc Method_SeqVariant_01	07/Aug/20 04:27:08	Finished
5	ChimericIgG1 BS_2		Unknown	n.a.	MAM [redacted] Proc Method_SeqVariant_01	07/Aug/20 08:29:08	Finished
6	ChimericIgG1 BS_3		Unknown	n.a.	MAM [redacted] Proc Method_SeqVariant_01	07/Aug/20 12:31:08	Finished

Stage 3: Data processing, analysis and reporting_**Step 33**



Width: Automatic
Height: Automatic
Scale (%): 55

Report Designer

- (+5) WQQGNVFSC[Carboxymethylation]SVMHEALHNHYTQKSLSLSPG
- SSSTAYM[Oxidation]QLSSLTSEDSAVYYC[Carboxymethylation]AR
- (+3) SSSTAYM[Oxidation]QLSSLTSEDSAVYYC[Carboxymethylation]AR
- SSSTAYM[Oxidation]QLSSLTSEDSAVYYC[Carboxymethylation]AR
- (+3) SSSTAYM[Oxidation]QLSSLTSEDSAVYYC[Carboxymethylation]AR
- SSSTAYMQLSSLTSEDSAVYYC[Carboxymethylation]AR
- (+2) SSSTAYMQLSSLTSEDSAVYYC[Carboxymethylation]AR
- (+3) SSSTAYMQLSSLTSEDSAVYYC[Carboxymethylation]AR
- (+4) SSSTAYMQLSSLTSEDSAVYYC[Carboxymethylation]AR
- GFYPSDIAVEWESNGQPENNYK
- (+3) GFYPSDIAVEWESNGQPENNYK
- (+4) GFYPSDIAVEWESNGQPENNYK
- (+2) GFYPSDIAVEWESNGQPENNYK
- GFYPSDIAVEWESNGQPEN[Deamidation]NYK
- (+2) GFYPSDIAVEWESNGQPEN[Deamidation]NYK
- (+3) GFYPSDIAVEWESNGQPEN[Deamidation]NYK
- GFYPSDIAVEWESN[NH3 loss]GQPENNYK
- (+2) GFYPSDIAVEWESN[NH3 loss]GQPENNYK
- (+3) GFYPSDIAVEWESN[NH3 loss]GQPENNYK
- GLEWIGAIYPGNGDTSYNQK
- (+3) GLEWIGAIYPGNGDTSYNQK
- (+4) GLEWIGAIYPGNGDTSYNQK
- (+2) GLEWIGAIYPGNGDTSYNQK
- GLEWIGAIYPGN[Deamidation]GDTSYNQK
- (+2) GLEWIGAIYPGN[Deamidation]GDTSYNQK
- (+3) GLEWIGAIYPGN[Deamidation]GDTSYNQK
- GLEWIGAIYPGN[NH3 loss]GDTSYNQK
- (+2) GLEWIGAIYPGN[NH3 loss]GDTSYNQK
- (+3) GLEWIGAIYPGN[NH3 loss]GDTSYNQK

Report Templates

- MAM Report IgG1BS CQAs_01
- MAM Report [redacted]SeqVariant_01

Sequence Overview

Print

Apply to

● Current Injection ○ Current Sequence

Printer

Microsoft Print to PDF | Properties...

Select sheets to be printed

Sheet Name	Print
Sequence Overview	☑
Oxidation	☑
Deamidation-Succinimi...	☑
N-Glycosylation	☑
Composite Scoring	☑
NPD	☑

OK | Cancel