ProMass for MassLynx
Overview and Preliminary User Guide

Novatia LLC

Overview

- General info about ProMass
- Features
- Basics of how ProMass works
- Example Spectra
- Manual Deconvolution with ProMass
- Deconvolution Parameters
- Automated Deconvolution with ProMass
- Examples

ProMass Features

- MassLynx layered application
- Uses Novatia's ZNova algorithm for "*artifact-free*" charge deconvolution of biomolecule ESI mass spectra
- Allows for automated deconvolution of spectra in LC/MS data from the MassLynx sample list
- Produces web-based results format including chromatograms, spectra, color-coded summary, and tabular results
- Works with oligonucleotides, proteins, peptides, etc.
- Applications include:
  - *High throughput (HT) oligonucleotide synthesis QC*
  - *Detailed LCMS oligonucleotide impurity/degradant profiling, and metabolite id*
  - *Detailed intact protein characterization – impurities, degs, PTM's*
  - *HT intact protein expression and bioprocess monitoring*

Why we need charge deconvolution

Unfortunately mass spectrometers measure m/z NOT Mass:

$$\text{Mass (M)} \neq m/z$$

But:

$$m/z = (M + zA)/z$$

Where:

A = mass of adduct providing charge

z = number of charges

Given 2 adjacent m/z peaks (mz_1 and mz_2) in a charge series of unknown charges (z_1 and z_2), you can use algebra:

$$z_1 = z_2 + 1$$

$$z_2 = (mz_1 – A)/(mz_2 – mz_1)$$

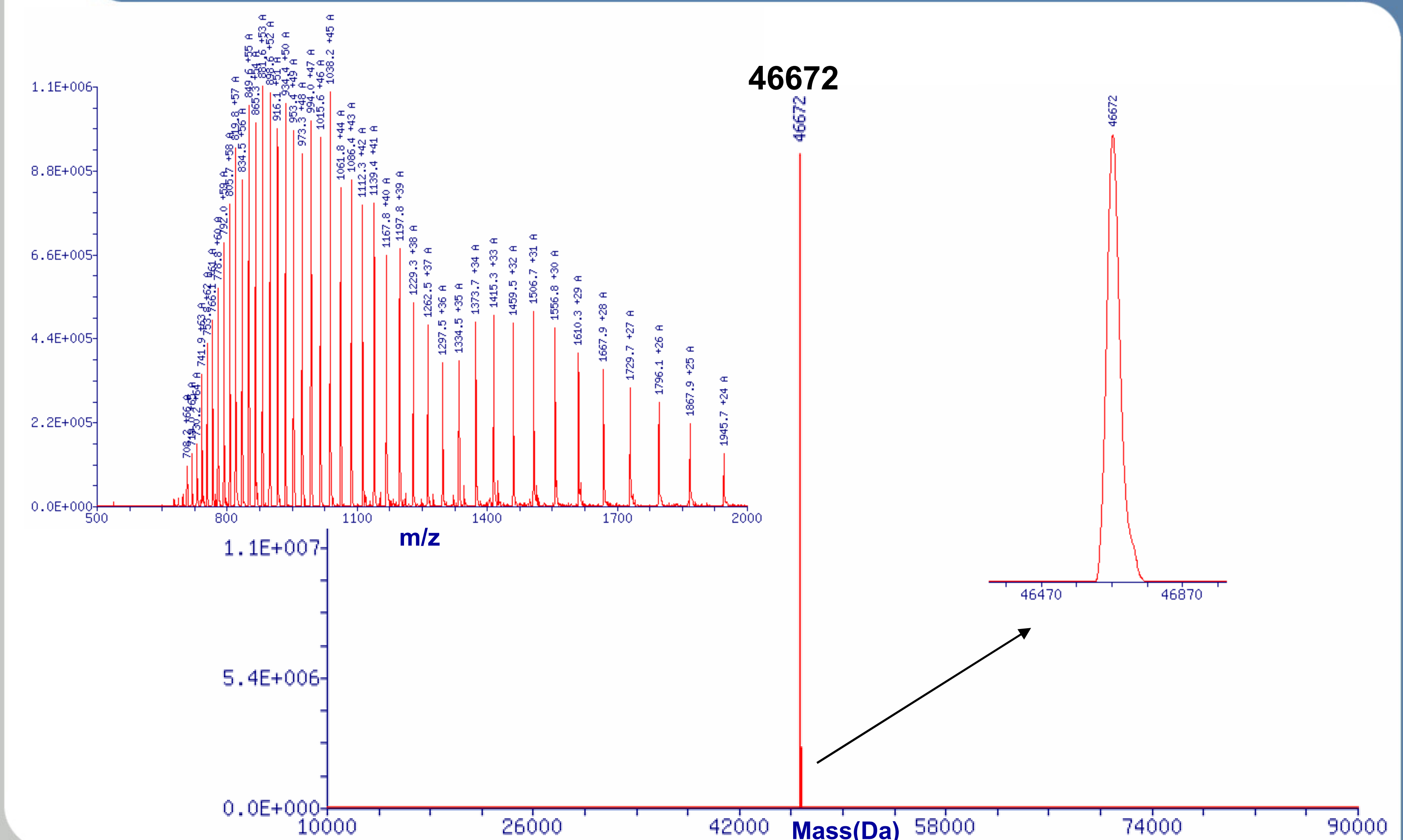
You would go insane if you had to do this for every spectrum!

ZNova Deconvolution Algorithm

- *ZNova* is the charge deconvolution algorithm used by the ProMass software
- *ZNova* uses a “component deconvolution” approach which tests and determines the charge of every peak in the raw mass spectrum (Zhang & Marshall, JASMS 1998)
- *ZNova* determines charge by looking at the series of contiguous charge states (not isotope spacing)
- *ZNova* uses a simple intensity-based scoring algorithm to assign charge based on the highest scoring series of peaks
- *ZNova* has built-in signal processing techniques to improve reliability of deconvolution even on noisy data
  - *Automatic baseline removal*
  - *Decentroiding of centroid input data*
  - *Smoothing prior to decon*
  - *Normalization of scoring based on observed/predicted peaks*
- *ZNova* exhibits low incidence of deconvolution artifacts - unlike Mann algorithm
- Clean deconvolutions allow reliable confirmation of target components

ProMass Deconvolution of Yeast Enolase (MW 46670.9)

Example of Artifact-free Deconvolution



Novatia

Comparison of Deconvolution Algorithms

heavy / light chain IgG mixture

Mann deconvolution, with deconvolution artifacts

7909.0
11862.9
17181.6
20617.1
23725.8
31634
35587
39543
47454
51542
55366
59317
64422
68724
71175
77313

5000 10000 15000 20000 25000 30000 35000 40000 45000 50000 55000 60000 65000 70000 75000 80000

Mass

***ProMass* deconvolution**

1.6E+006
1.3E+006
9.8E+005
6.5E+005
3.3E+005
0.0E+000

5000 20000 35000 50000 65000 80000

23726.1

Light chain

51539.5

Heavy chain

How *ZNova* Deconvolution Works

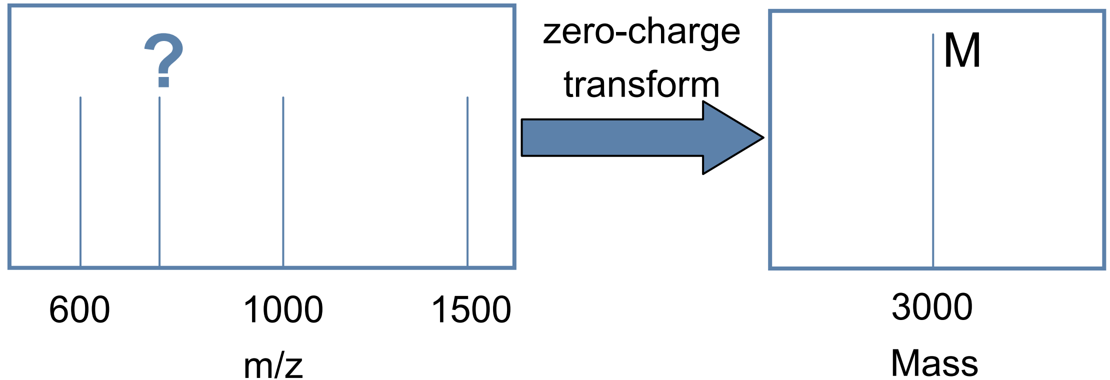
Example: Test all z values for m/z 749 in the spectrum below

Assume z = -2 (MW 1500) : -1 = 1499, score = 2

Assume z = -3 (MW 2250) : -4 = 561.5, -2 = 1124, -1 = 2249, score = 1

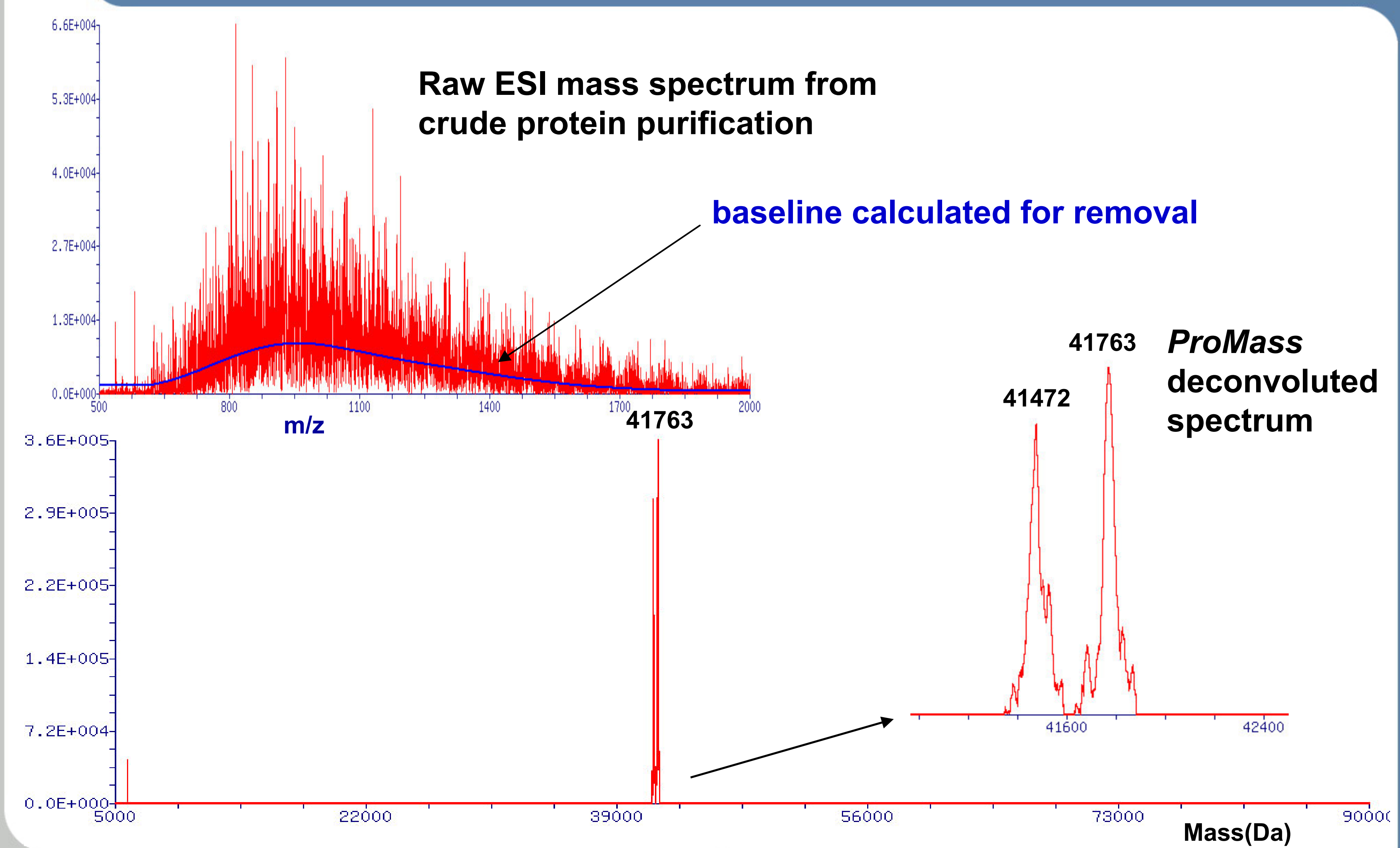
Assume z = -4 (MW 3000) : -5 = 599, -3 = 999, -2 = 1499, score = 4

Assume z = -5 (MW 3750) : -6 = 624, -4 = 936.5, -3 = 1249, score = 1



- Scoring based on accumulated log(S/N) of all peaks in a charge series.
- Highest scoring test charge state represents the correct charge state (z = -4, above).
- Once correct charges are determined, only those signals are transformed to the zero-charge (deconvoluted) spectrum.
- The process is repeated until all m/z values are processed.

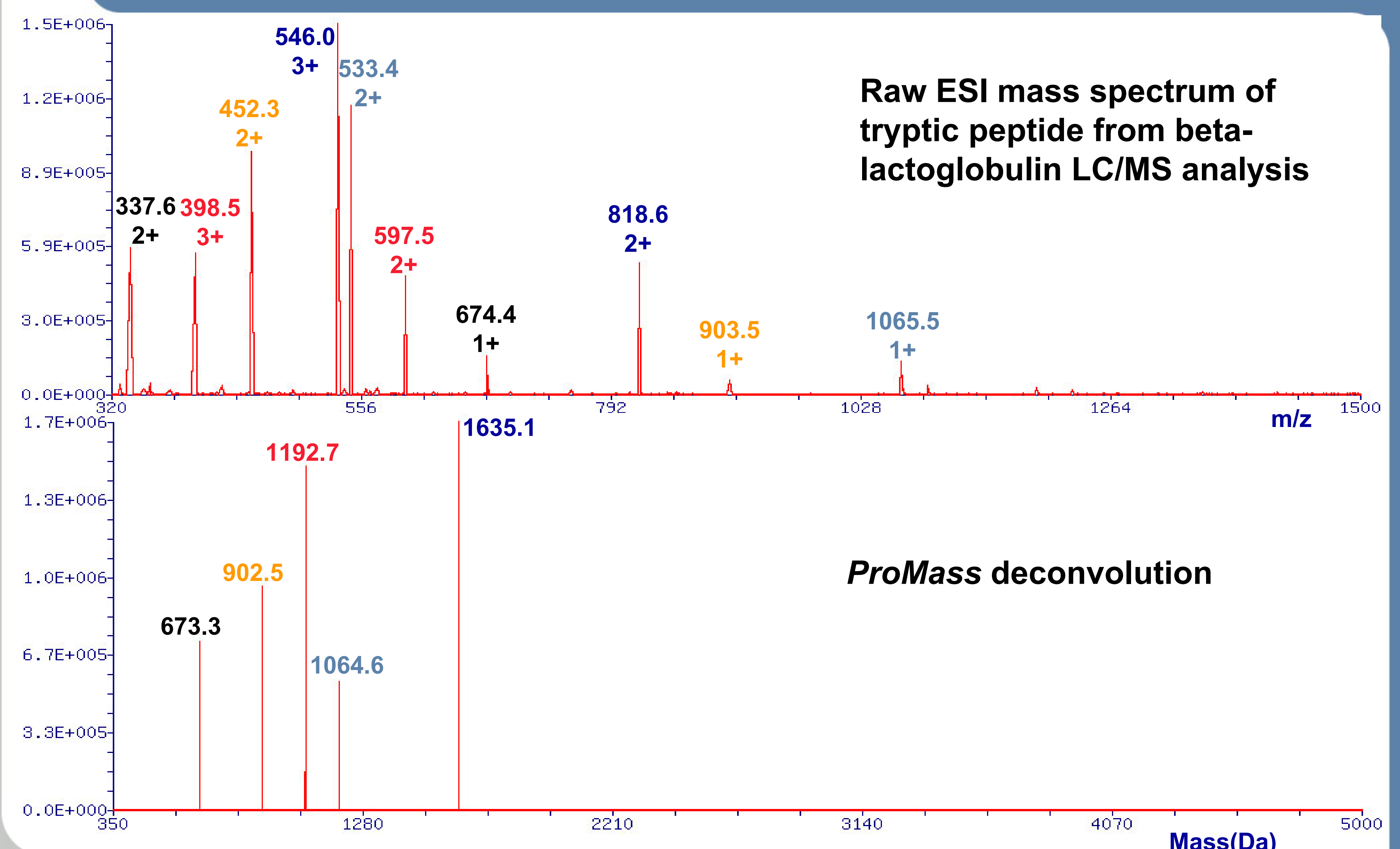
ProMass Deconvolution of Low S/N ESI Data



Novatia

ProMass Deconvolution of Peptide Mixtures

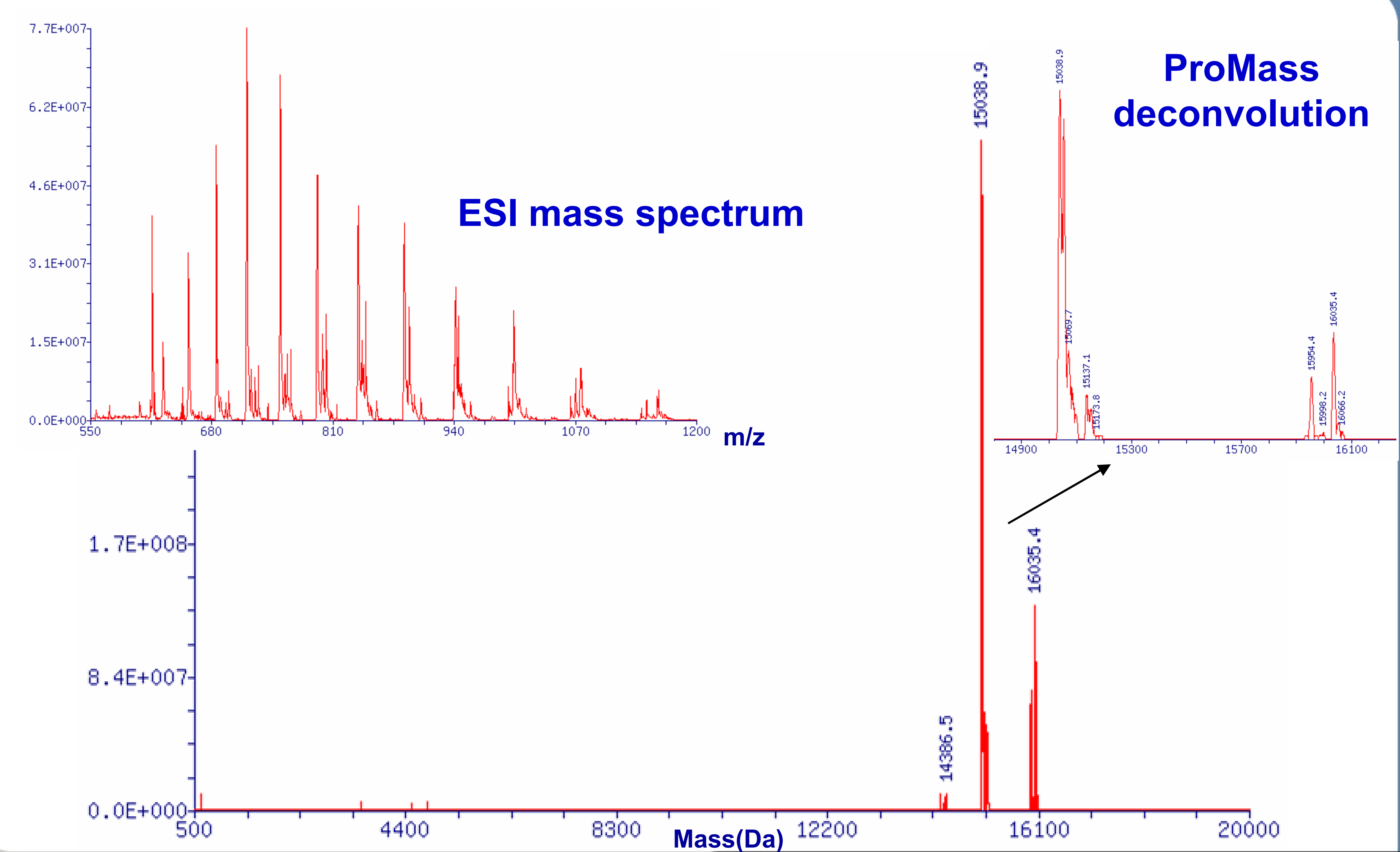
ProMass works on low charge state spectra too



ProMass Deconvolution of Hemoglobin Mixture

Example of overlapping, closely-related mixture components

alpha & beta chains, bovine & porcine proteins

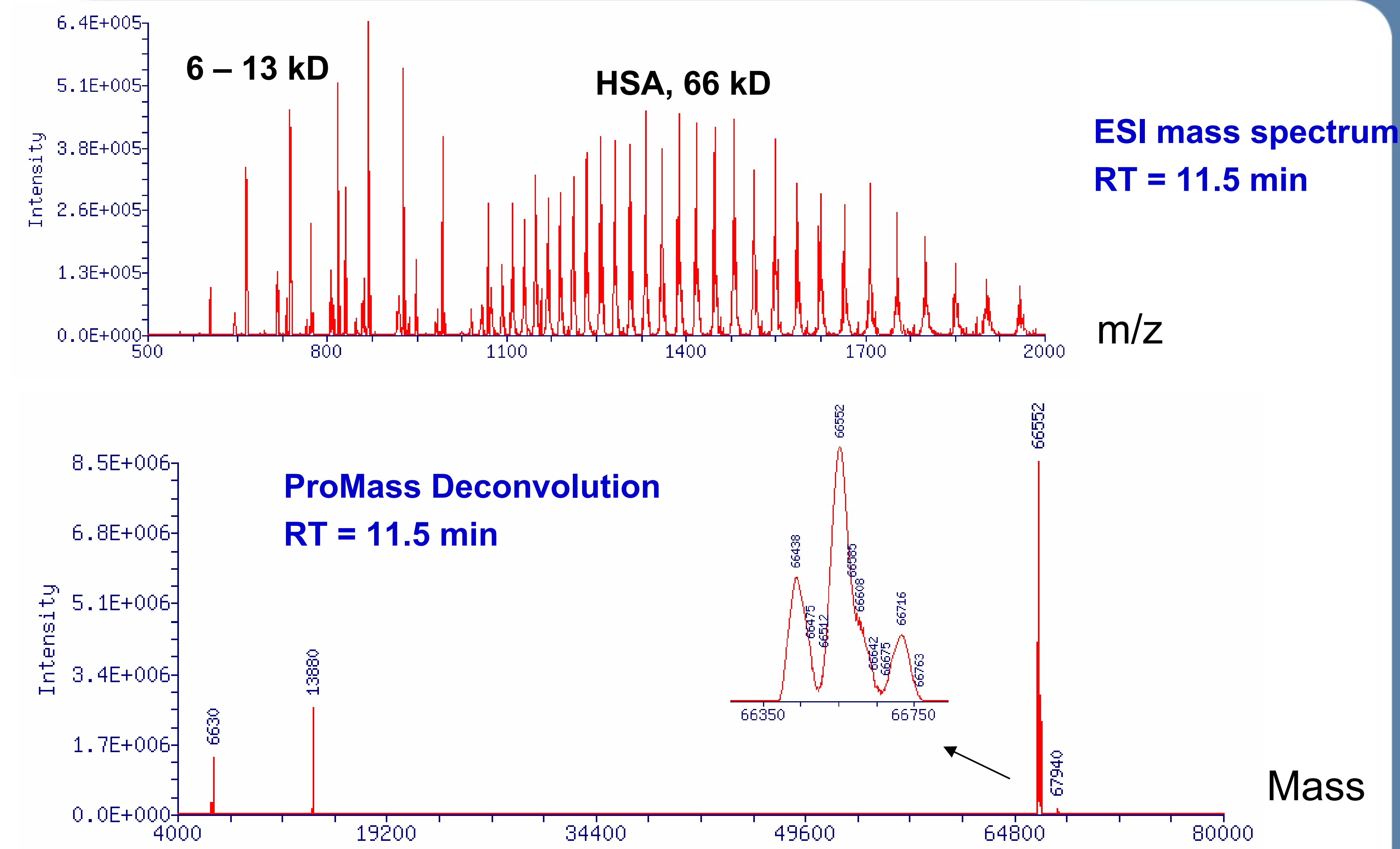


Novatia

ProMass Deconvolution of Coeluting Components

Example of overlapping mixture components over wide MW range

mixture is from supernatant from precipitated human serum



Novatia

ProMass HomePage

ZNova **Parameter Setup**
And Manual Processing

Browse the latest *automated* processing event

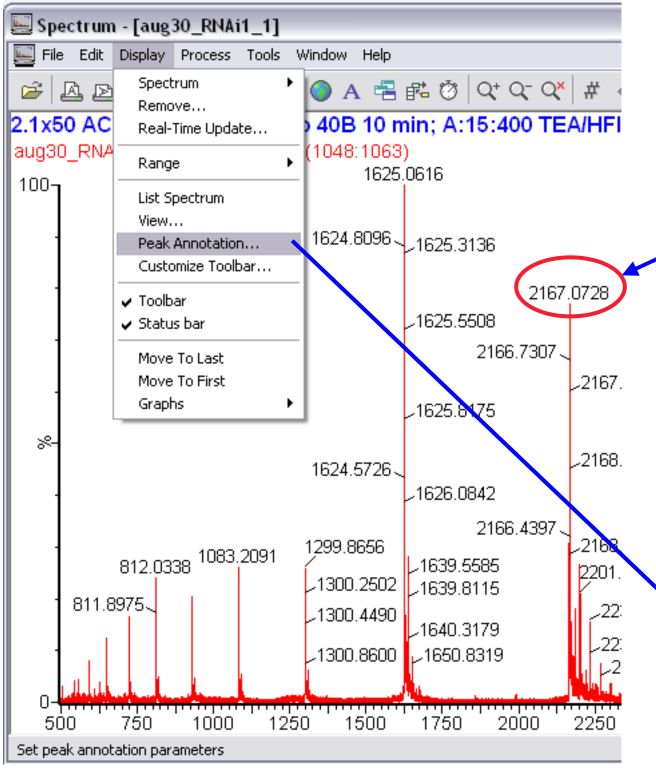
ProMass Deconvolution Modes

- Manual Deconvolution
  - *Interactive processing of individual spectra copied from the MassLynx Spectrum application*
  - *Fast processing via a simple copy/paste operation*
  - *Very simple web-based spectrum report output*
  - *Interactive browsing with ProMass DecView*
- Automated Deconvolution
  - *Processing driven from MassLynx sample list*
  - *Allows deconvolution of entire LC/MS data sets*
  - *Full reporting of targeted components in web-based report*
  - *ProMass parameter file for deconvolution parameters*
  - *ProMassBridge parameter file for chromatogram peak-picking and spectral pre-processing parameters*
  - *Highly detailed web browser-based summary and sample reports*

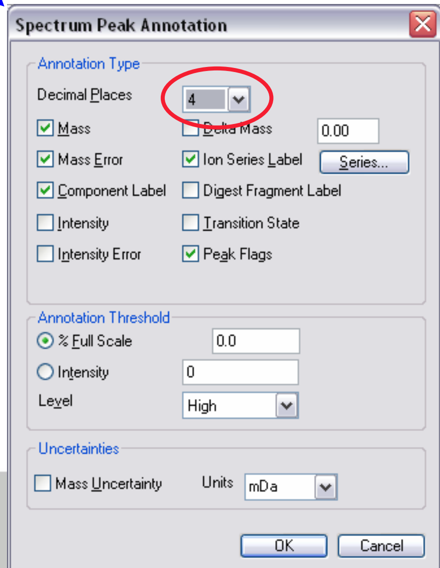
ProMass Manual Deconvolution Mode

- Manual deconvolution mode is performed by “copying” a spectrum from the MassLynx Spectrum window and “pasting” the data into the ProMass application.
- Manual deconvolution mode is useful for *tuning* ProMass parameters or for quickly deconvoluting a spectrum from a selected LCMS chromatogram peak.
- Manual deconvolution *does not* include the advanced reporting features that are available in the automated deconvolution mode.

Preparing MassLynx for Manual Deconvolution



- Setup MassLynx for spectrum export
  - *In the MassLynx spectrum window, ensure that 4 decimal places are being displayed on spectrum annotated masses*
  - *If not, select Display | Peak Annotation…*
  - *Set decimal places to 4 and click OK*
  - *MassLynx should remember these settings*



ProMass Manual Deconvolution Overview

- From the MassLynx Spectrum application, display the desired spectrum.
- If more than one spectrum is displayed, click the target spectrum to select it.
- Click the Copy button on the MassLynx Spectrum toolbar, or click *Edit | Copy Spectrum List*.

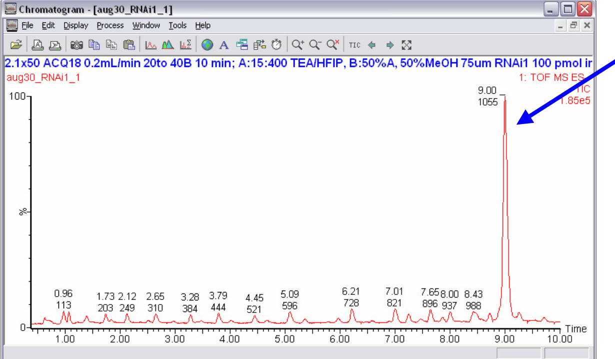
- Launch ProMass and click *Build Params*
- Set or restore a default set of ProMass parameters
  - *New users can click the defaults button on the toolbar and select an appropriate molecule type.*
- Click the *Paste/Process* spectrum button
- After deconvolution is complete, ProMass results are displayed in the default web browser window

ProMass Basic Deconvolution Settings

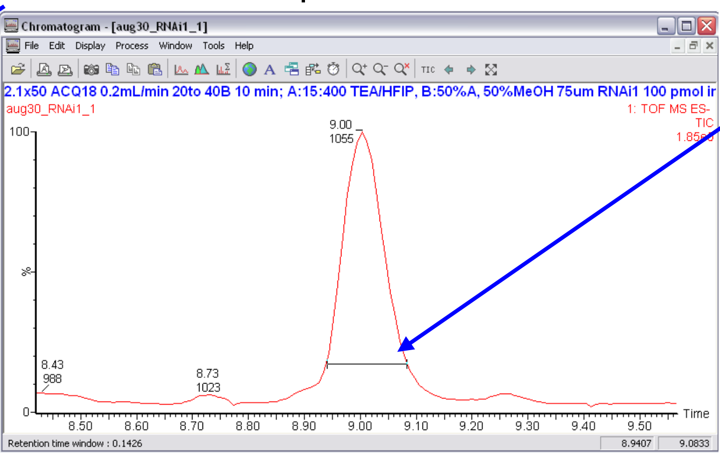
In Basic deconvolution mode, one can easily process spectra by restoring a set of default parameters and setting input and output mass ranges.

Manual Processing Example

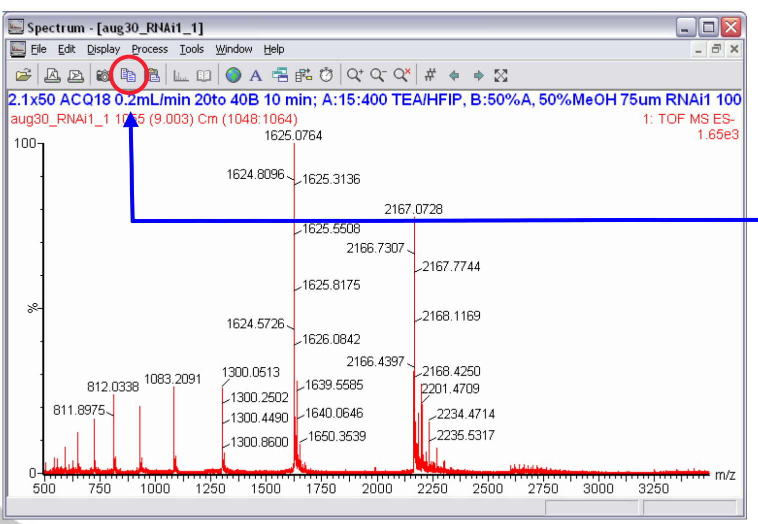
Oligonucleotide from LCMS chromatogram



Goal: Select this peak for deconvolution.



Step 1: Right mouse click and drag across the peak to combine (add) scans for the desired peak in the LCMS chromatogram.



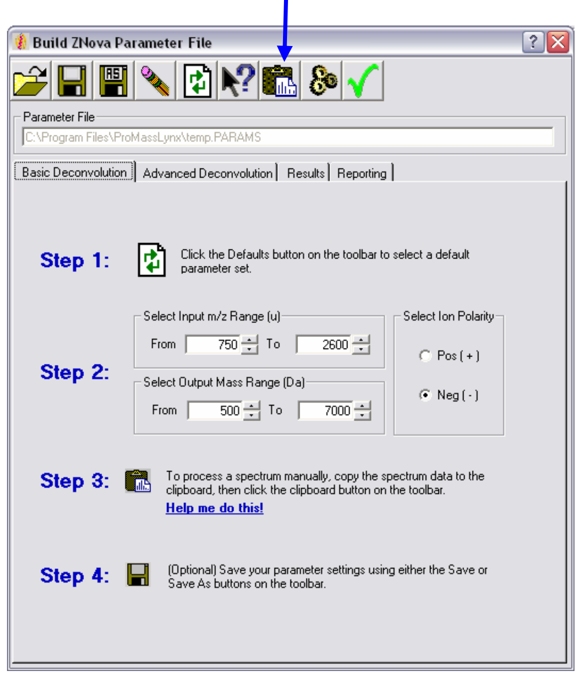
Step 2: After the desired spectrum is displayed, click the copy button. *Note: you can preprocess the spectrum in MassLynx to remove baseline or subtract background before exporting data to ProMass.*

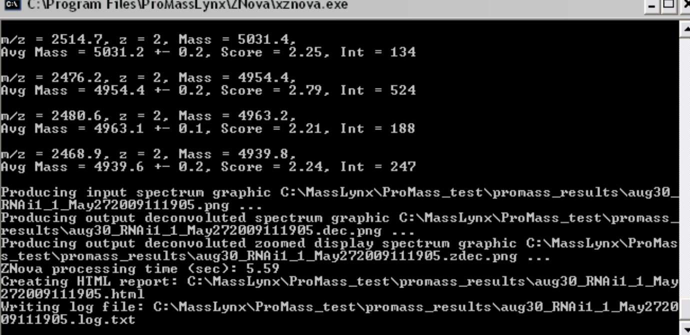
Step 3: Launch ProMass and click *Build Params* button.

Manual Processing Example, continued…

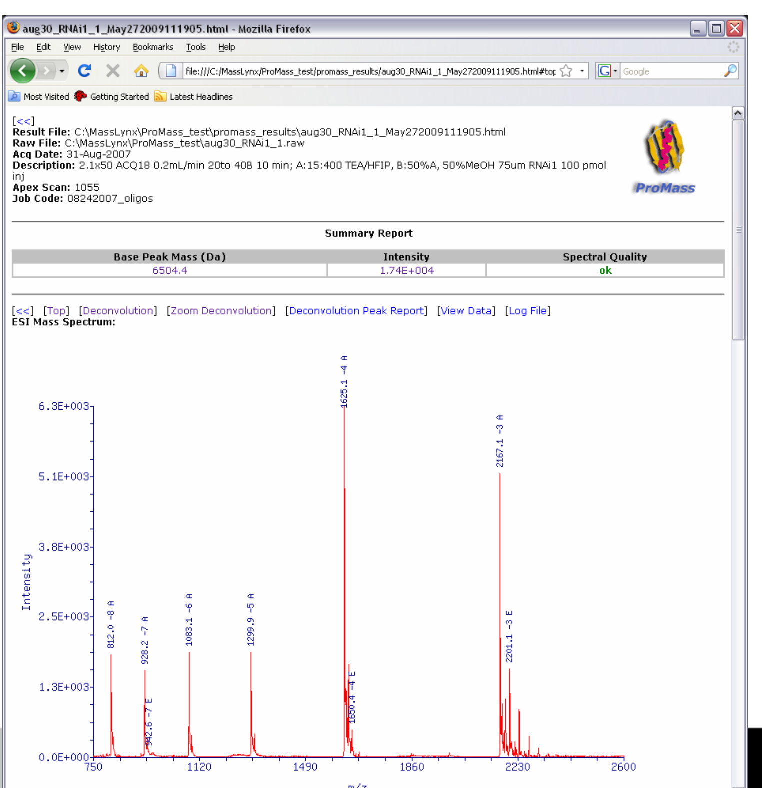
Oligonucleotide from LCMS chromatogram

Step 4: From the *Basic Deconvolution* tab set the input and output mass ranges. Select the ion polarity mode, then click the "Paste" button.





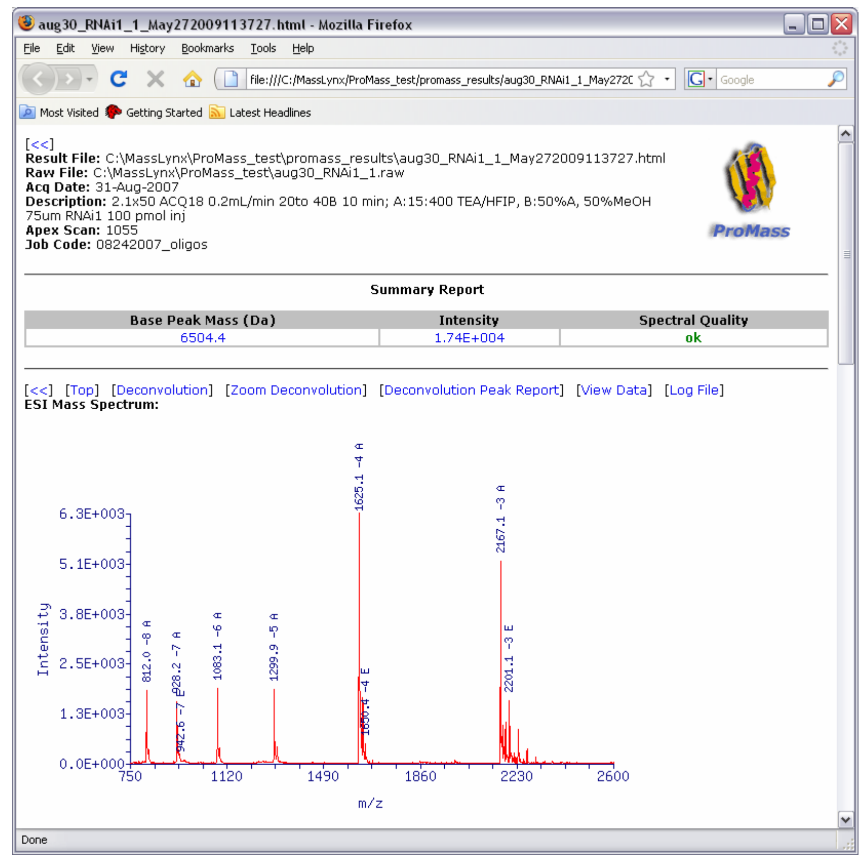
Processsing: During processing, the ProMass console window is displayed (or may run minimized in the task bar).

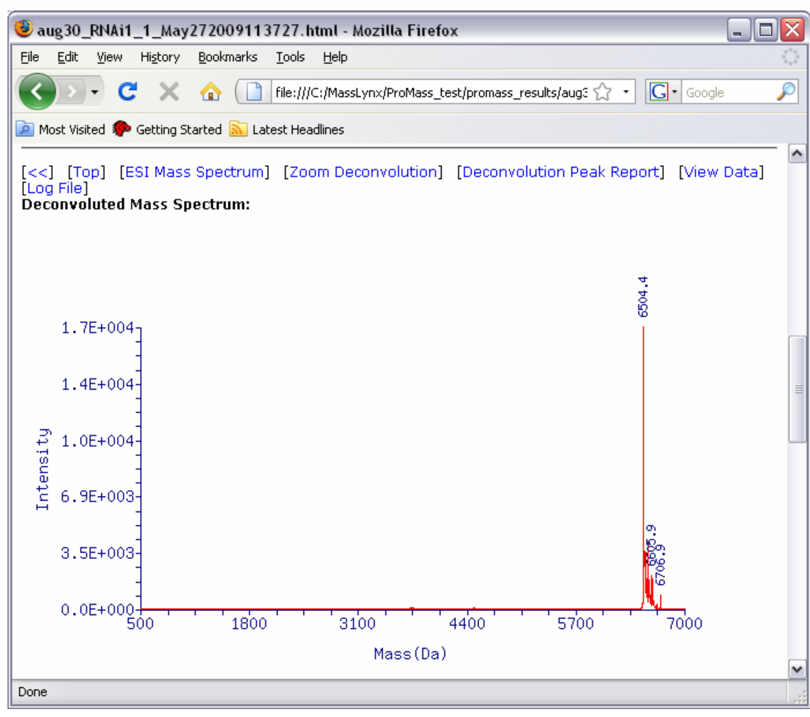


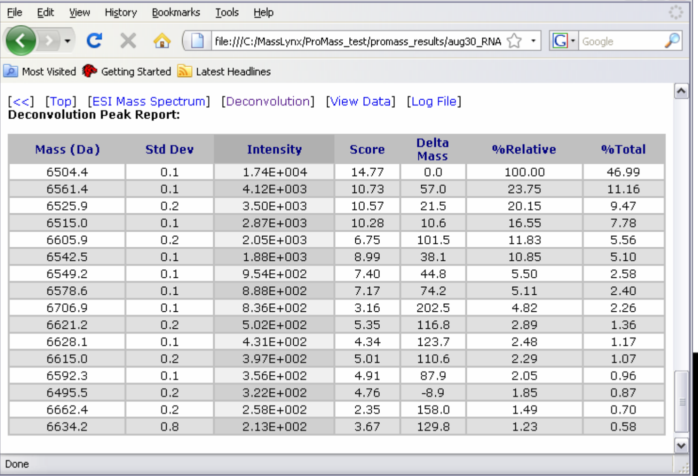
Results: After processing is complete, the ProMass spectrum report is automatically launched in the default web browser.

Manual Processing Example, continued

Oligonucleotide from LCMS chromatogram






aug30_RNAi1_1_May272009113727.html - Mozilla Firefox

[<<] [Top] [ESI Mass Spectrum] [Deconvolution] [View Data] [Log File]

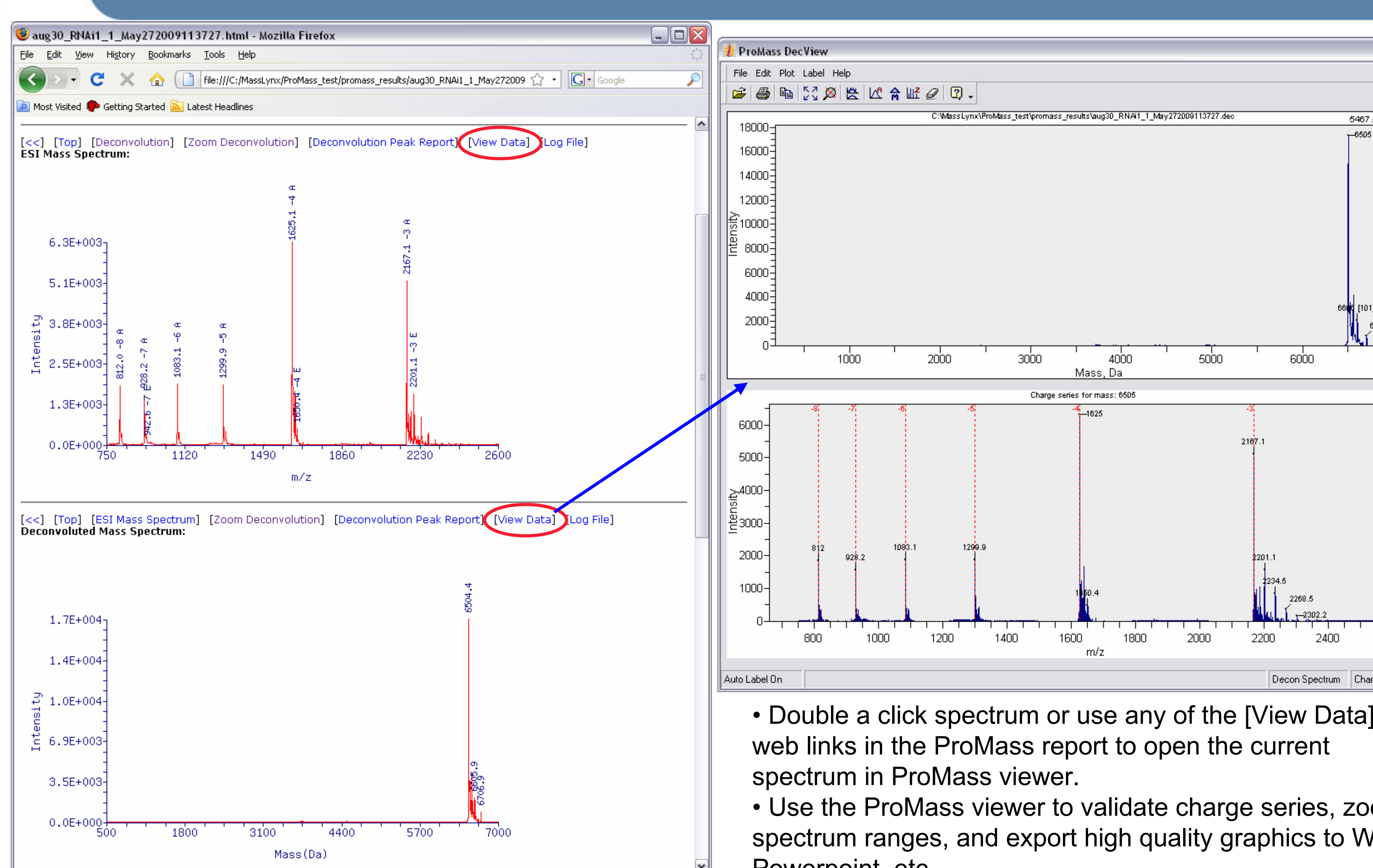
Deconvolution Peak Report:

Mass (Da)	Std Dev	Intensity	Score	Delta Mass	%Relative	%Total
6504.4	0.1	1.74E+004	14.77	0.0	100.00	46.99
6561.4	0.1	4.12E+003	10.73	57.0	23.75	11.16
6525.9	0.2	3.50E+003	10.57	21.5	20.15	9.47
6515.0	0.1	2.87E+003	10.28	10.6	16.55	7.78
6605.9	0.2	2.05E+003	6.75	101.5	11.83	5.56
6542.5	0.1	1.88E+003	8.99	38.1	10.85	5.10
6549.2	0.1	9.54E+002	7.40	44.8	5.50	2.58
6578.6	0.1	8.88E+002	7.17	74.2	5.11	2.40
6706.9	0.1	8.36E+002	3.16	202.5	4.82	2.26
6621.2	0.2	5.02E+002	5.35	116.8	2.89	1.36
6628.1	0.1	4.31E+002	4.34	123.7	2.48	1.17
6615.0	0.2	3.97E+002	5.01	110.6	2.29	1.07
6592.3	0.1	3.56E+002	4.91	87.9	2.05	0.96
6495.5	0.2	3.22E+002	4.76	-8.9	1.85	0.87
6662.4	0.2	2.58E+002	2.35	158.0	1.49	0.70
6634.2	0.8	2.13E+002	3.67	129.8	1.23	0.58

ProMass Spectrum Report: The report shows relevant sample information in the header, the ESI mass spectrum (above), the deconvoluted mass spectrum (above right), a zoom deconvolution around the most abundant mass (not shown), and a sorted deconvolution peak report (right).

Novatia

Interactive Viewing with the ProMass Viewer



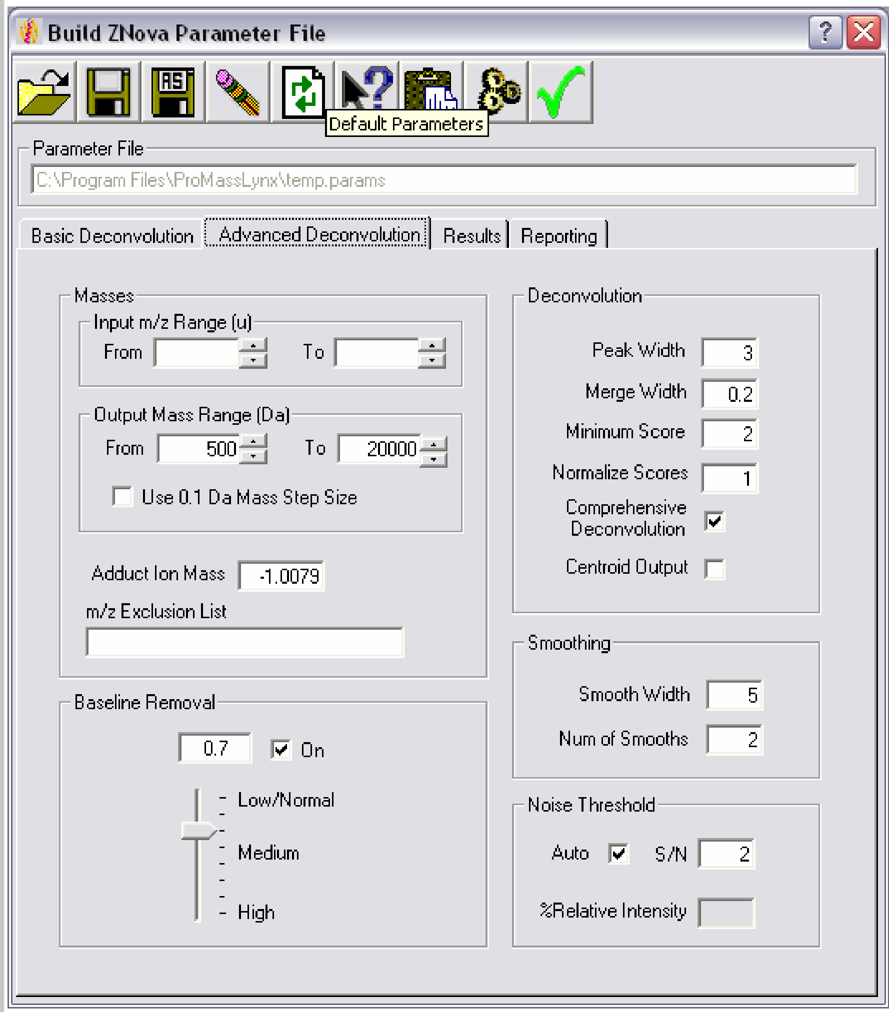
- Double a click spectrum or use any of the [View Data] web links in the ProMass report to open the current spectrum in ProMass viewer.
- Use the ProMass viewer to validate charge series, zoom spectrum ranges, and export high quality graphics to Word, Powerpoint, etc.

Novatia

ProMass DecView Viewer Tips

- Top spectrum is always deconvoluted, bottom is ESI
- Click a spectrum pane to make it active for zooming, unzooming, etc.
- Click on a deconvoluted mass label to show charge states for the selected mass – useful for validating results.
- See ProMass DecView help for more information.

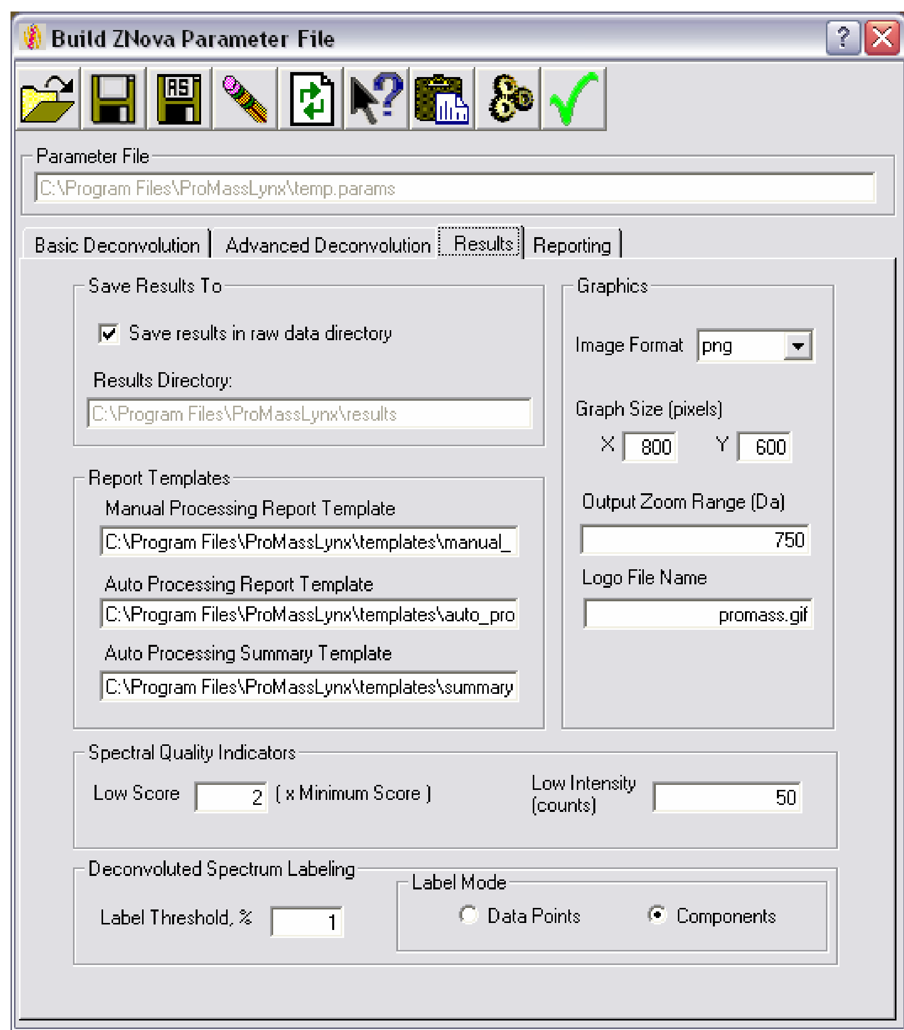
ProMass Advanced Decon Settings



Parameters Guide:

- Set input/output mass ranges
- Use 0.1 Da step size for < 5000 MW
- *Adduct Ion Mass* -1.0079 for negative ions
- Adjust *Baseline Removal* to get a flat baseline (look at raw ESI spectrum in ProMass report)
- Set *Peak Width* to match m/z peak width (at base) in original mass spectrum
- *Merge Width* ~10-20% of *Peak Width,* has an effect on reported centroided masses
- Use *Comprehensive* decon for most applications where there could be mixture overlap
- Most other settings can be set using defaults

ProMass Results Settings



What the settings do:

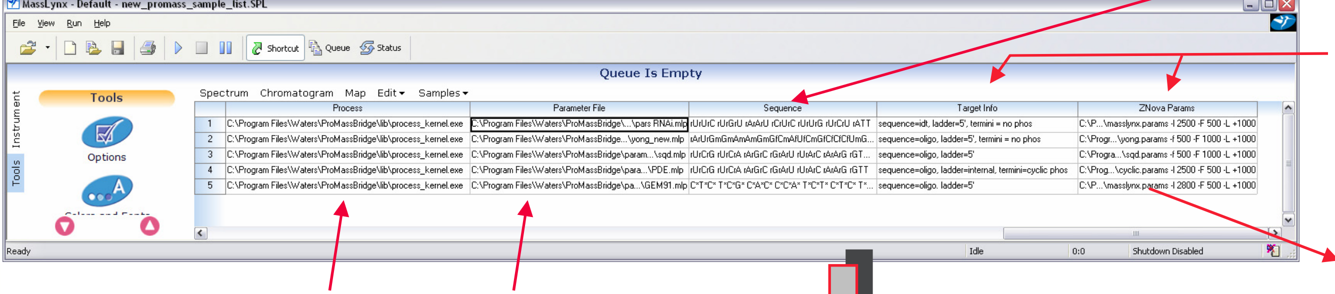
- Determine where results are stored
- Customize the graphics output - size, image format, logo.
- Allow user to set the width of the zoomed spectrum, or to set an explicit zoom range
- Specify report template for web-based output
- Spectral quality indicator settings for flagging low intensity or low ZNova score results
- Set labeling parameters – use *Data Points* labeling mode if *Components* labeling does not give you enough detail, especially when a wide peak width setting is used.

Deconvoluting Difficult Spectra

- Most (>80%) spectra can be deconvoluted successfully with “default” settings
  - *Try default settings first, then modify as needed*
- Noisy spectra, complicated mixtures, or spectra with a high baseline may require some parameter adjustment
  - *Adequately remove the baseline noise*
  - *Choose the correct peak width*
  - *For very noisy spectra, narrow your deconvolution range to focus the algorithm to where you expect masses to be present.*
  - *Noisy spectra may require manually setting the noise threshold*
- A *“blob”* spectrum with a peak at every mass may be impossible to deconvolute with any algorithm
- Use ProMass viewer to validate that observed charge series are real

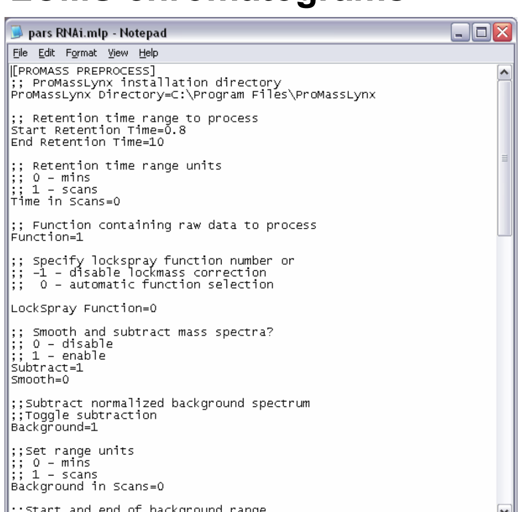
ProMass Automated Processing Workflow Overview

optional amino acid or nucleotide sequence

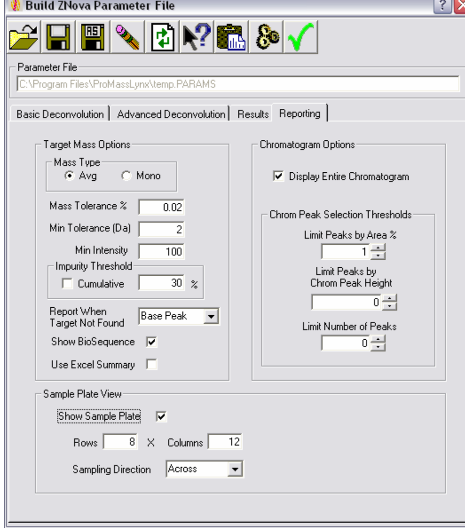


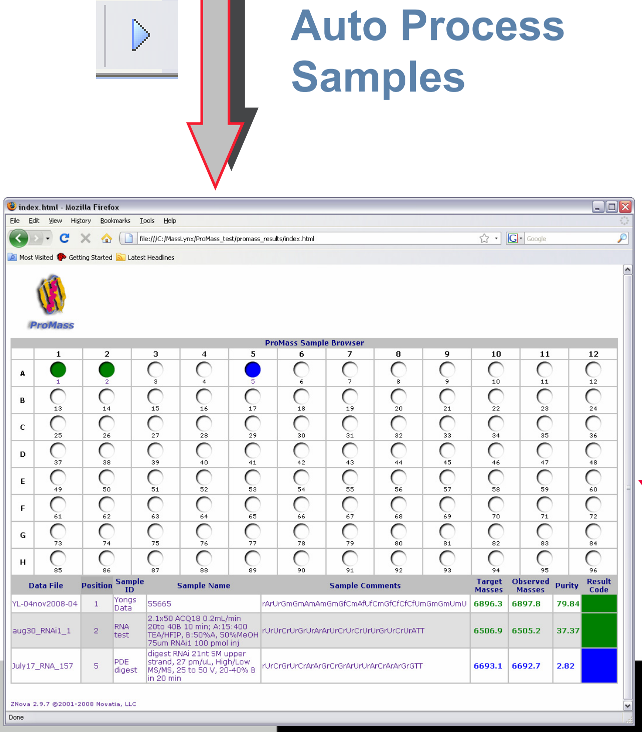
ProMass parameters and target mass settings

ProMassBridge program and parameter file for peak-picking LCMS chromatograms



Auto Process Samples



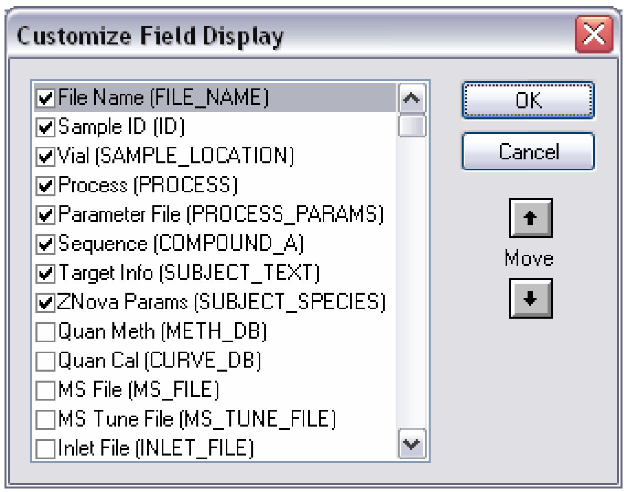


HTML Results Report including plate view, spectra, chromatograms, tables, etc.

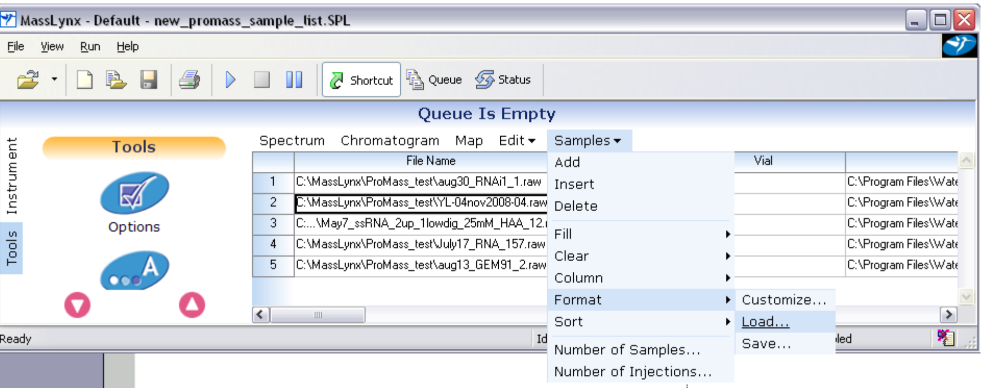
ProMass Automated Processing

- Basic Requirements:
  - *MassLynx sample list with associated data file(s)*
  - *Correct sample list fields formatted for ProMass processing*
  - *ProMassBridge software installed, process_kernel.exe executable file specified in the sample list "Process" field.*
  - *ProMassBridge parameter file*
    - Text file (.mlp or .olp) edited with notepad or other text editor
    - For setting various parameters for chromatogram peak selection and pre-processing (e.g., smoothing, baseline subtraction, LockSpray, etc.)
  - *Optional ZNova parameter file specified in "ZNova Params" sample list field for ProMass deconvolution settings*
  - *Optional Target Mass settings specified in "Target Info" sample list field*
  - *Optional sequence for oligonucleotide or protein in "Sequence" sample list field.*

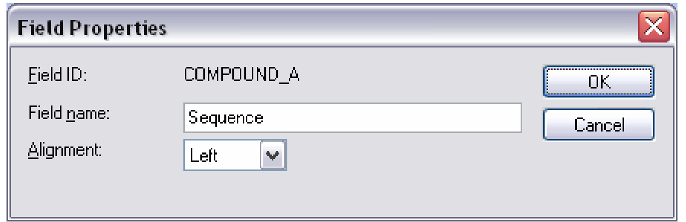
ProMass Sample List Fields



ProMass requires certain sample list fields to be present to allow for automated processing. The display above shows how the fields should be mapped. This dialog is activated by right-clicking a sample list column heading and selecting *Customize Display…*



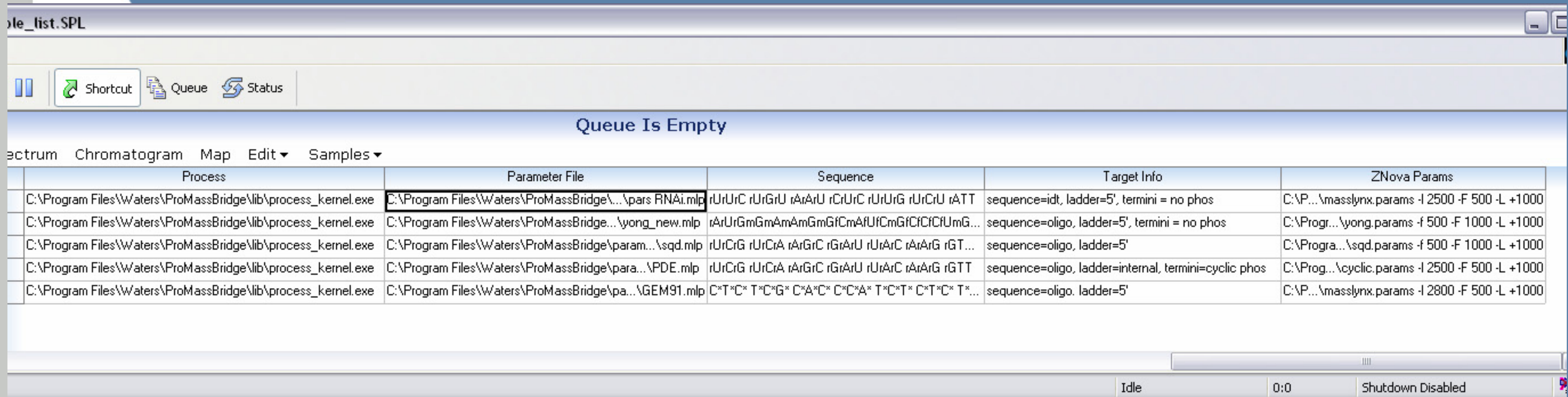
To load the ProMass sample list format, select *Samples* | *Format* | *Load…* as shown above from the sample list and browse for the ProMass sample list format type. Load promass.fmt from the ProMassLynx install directory.



The individual sample list fields can be edited manually if you do not have the ProMass sample list format. The fields *must* be mapped as shown at the above left. To edit a column heading, right-click the heading and select *Properties…*The ordering of the fields is not important.

Novatia

Configuring MassLynx Sample List for ProMass



Process	Parameter File	Sequence	Target Info	ZNova Params
C:\Program Files\Waters\ProMassBridge\lib\process_kernel.exe	C:\Program Files\Waters\ProMassBridge\...\pars RNAi.mlp	rUrUrC rUrGrU rArArU rCrUrC rUrUrG rUrCrU rATT	sequence=idt, ladder=5', termini = no phos	C:\P...\masslynx.params -I 2500 -F 500 -L +1000
C:\Program Files\Waters\ProMassBridge\lib\process_kernel.exe	C:\Program Files\Waters\ProMassBridge...\yong_new.mlp	rArUrGmGmAmAmGmGfCmAfUfCmGfCfCfCfUmG...	sequence=oligo, ladder=5', termini = no phos	C:\Progr...\yong.params -f 500 -F 1000 -L +1000
C:\Program Files\Waters\ProMassBridge\lib\process_kernel.exe	C:\Program Files\Waters\ProMassBridge\param...\sqd.mlp	rUrCrG rUrCrA rArGrC rGrArU rUrArC rArArG rGT...	sequence=oligo, ladder=5'	C:\Progra...\sqd.params -f 500 -F 1000 -L +1000
C:\Program Files\Waters\ProMassBridge\lib\process_kernel.exe	C:\Program Files\Waters\ProMassBridge\para...\PDE.mlp	rUrCrG rUrCrA rArGrC rGrArU rUrArC rArArG rGTT	sequence=oligo, ladder=internal, termini=cyclic phos	C:\Prog...\cyclic.params -I 2500 -F 500 -L +1000
C:\Program Files\Waters\ProMassBridge\lib\process_kernel.exe	C:\Program Files\Waters\ProMassBridge\pa...\GEM91.mlp	C*T*C* T*C*G* C*A*C* C*C*A* T*C*T* C*T*C* T*...	sequence=oligo. ladder=5'	C:\P...\masslynx.params -I 2800 -F 500 -L +1000

- **Required Fields: *File Name, Vial (Bottle), Process (ProMassBridge executable), Parameter File (ProMassBridge parameter file)***
- *ZNova Params (optional):* for entering ProMass deconvolution parameters
  - *if not specified, default ProMass parameter set is used (znova.params in ProMassLynx\ZNova program directory)*
  - *"in-place" modifications to parameters can also be entered as command line arguments (e.g., -F, -L to set output decon mass range)*
- *Sequence (optional):* amino acid or nucleotide sequence
  - *For input sequences, masses are automatically calculated and treated as "target masses"*
  - *Sequence type must be specified in Target Info field (e.g., sequence=oligo)*
  - *Oligo sequences are entered in IDT base notation format (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/), allowing mixed DNA, RNA, LNA, phosphorothioates, 2-O-methyl RNA, 2-fluoro-RNA, and other custom modified or user-specified residues.*
- *Target Info (optional):* information about target masses
  - *Specify Sequence string type (e.g., sequence=oligo)*
  - *Specify other search directives (e.g., ladder=5', to search for 5' oligo sequence failures)*
  - *Enter explicit comma-separated target masses*

Novatia

ProMassBridge Parameter File

test.mlp - Notepad

File Edit Format View Help

```
[PROMASS PREPROCESS]
;; ProMassLynx installation directory
ProMassLynx Directory=C:\Program Files\ProMassLynx

;; Retention time range to process
Start Retention Time=0
End Retention Time=0

;; Retention time range units
;; 0 - mins
;; 1 - scans
Time in Scans=0

;; Function containing raw data to process
Function=1

;; Specify lockspray function number or
;; -1 - disable lockmass correction
;;  0 - automatic function selection

LockSpray Function=-1

;; Smooth and subtract mass spectra?
;; 0 - disable
;; 1 - enable
Subtract=1
Smooth=0

;;Subtract normalized background spectrum
;;Toggle subtraction
Background=1

;;Set range units
;; 0 - mins
;; 1 - scans
Background in Scans=0

;;Start and end of background range
Background Start=2
Background End=2.5
```

Set to limit range of processing, set both=0 to use whole chromatogram

Default range time in minutes

Function 1 is MS data

Set to '0' to enable Lockspray fxn

Set to '1' to enable spectral smoothing and baseline subtraction

Set to '1' to enable background subtraction

Default range time in minutes

Time range to use for background subtraction

- The ProMassBridge parameter file sets options for chromatogram peak picking and spectral preprocessing.
- The file is a text file that is edited with a text editor such as Notepad.
- Comments are specified with the semicolon (;) character.
- Options for smoothing, spectral subtraction (baseline removal), background subtraction, and LockSpray (for TOF data) processing are available.

ProMassBridge Parameter File, continued

test.mlp - Notepad

File Edit Format View Help

```
[CHROMATOGRAM]
;; Apex 3D peak detection parameters as in MassLynx

Smooth Iterations=2
Smooth Window=3
Auto Peak To Peak Baseline Noise=1
Peak To Peak Baseline Noise=20
Auto Peak Width at 5% Height=1
Peak Width at 5% Height=0.2
Baseline Start Threshold=0
Baseline End Threshold=0.5
Detect Shoulders=0

;; Use TIC or BPI for peak detection?
;; 0 - TIC
;; 1 - BPI
Use BPI=0

;; Spectrum smooth parameters if enabled
[SMOOTH PROMASS PREPROCESS]
; Smooth Type 2 means Savitzky Golay.
Smooth Type=2
Smooth Width=5
Number Of Smooths=2

;; Spectrum subtract parameters if enabled
[BACKSUB PROMASS PREPROCESS]
Type=1
Percent Below=65
Polynomial Order=5
Tolerance=0.01
Flatten Edges=1

;; Lockmass parameters
[MASSMEASURE PROMASS LOCKSPRAY PROCESS]
Do Subtract=0
Do Smooth=0

[BACKSUB PROMASS LOCKSPRAY PROCESS]
Type=1
Percent Below=25
Polynomial Order=5
Tolerance=0.01

[SMOOTH PROMASS LOCKSPRAY PROCESS]
Smooth Type=2
Smooth Width=3
Number Of Smooths=2

[TOFACM PROMASS LOCKSPRAY PROCESS]
Lock Spray Scans=5
Lock Mass=0.0
Mass Window=0.1
NP Multiplier=1.0
Resolution=9000.0

[CENTER PROMASS LOCKSPRAY PROCESS]
Centroiding Type=2
Top Percent=80.0
Use Areas=1
Min Peak Width Channels=4
```

Apex 3D peak detn parameters -defaults work well

Either TIC or base peak chromatogram can be used for processing

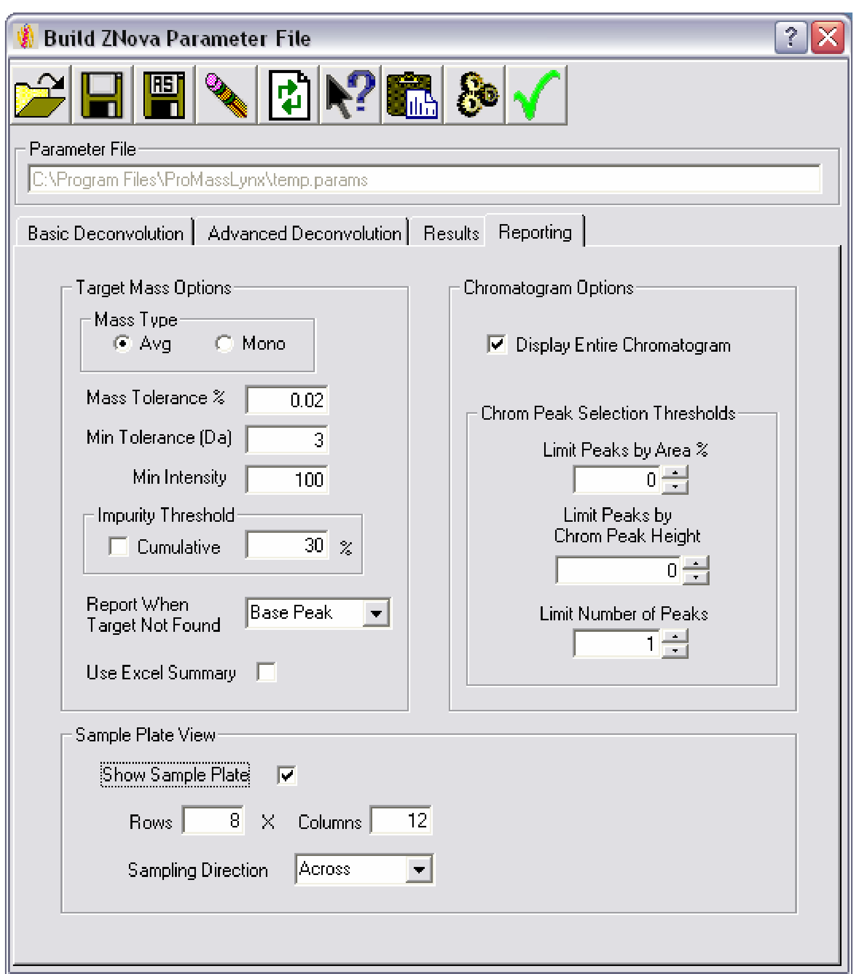
Spectrum smoothing options if enabled.

Spectrum baseline subtraction

These settings are available only when LockSpray processing is enabled.

- Peak detection is done with Apex 3D algorithm – use defaults with ProMass reporting parameter settings to limit number of peaks processed
- Either base peak (BPI) or TIC chromatograms may be used.
- Use high baseline subtraction settings for TOF data, as shown at left
- LockSpray parameters can be ignored unless LockSpray processing has been enabled.

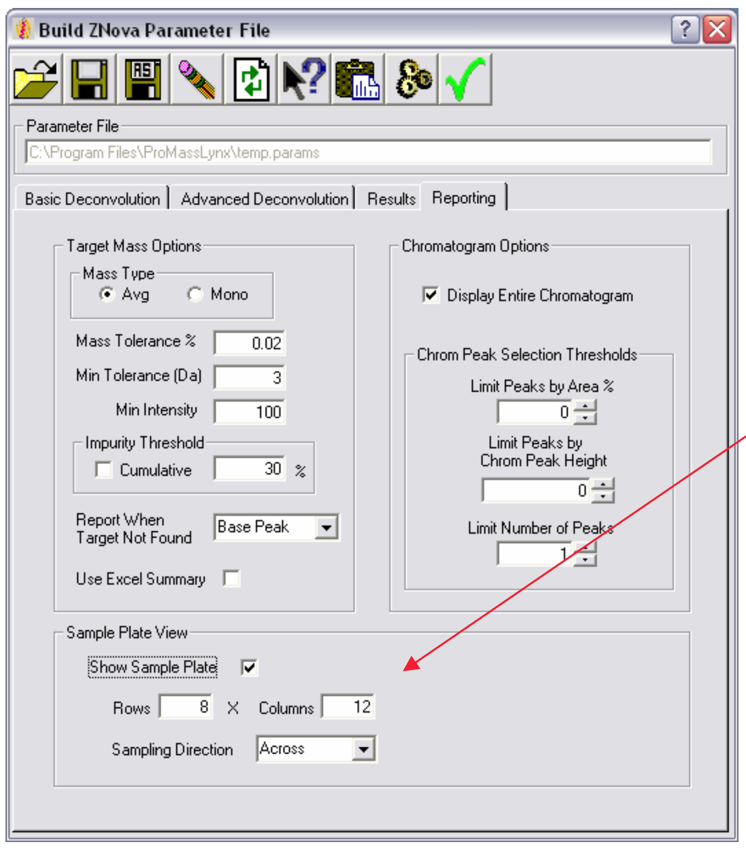
ProMass Reporting Settings



What the settings do:

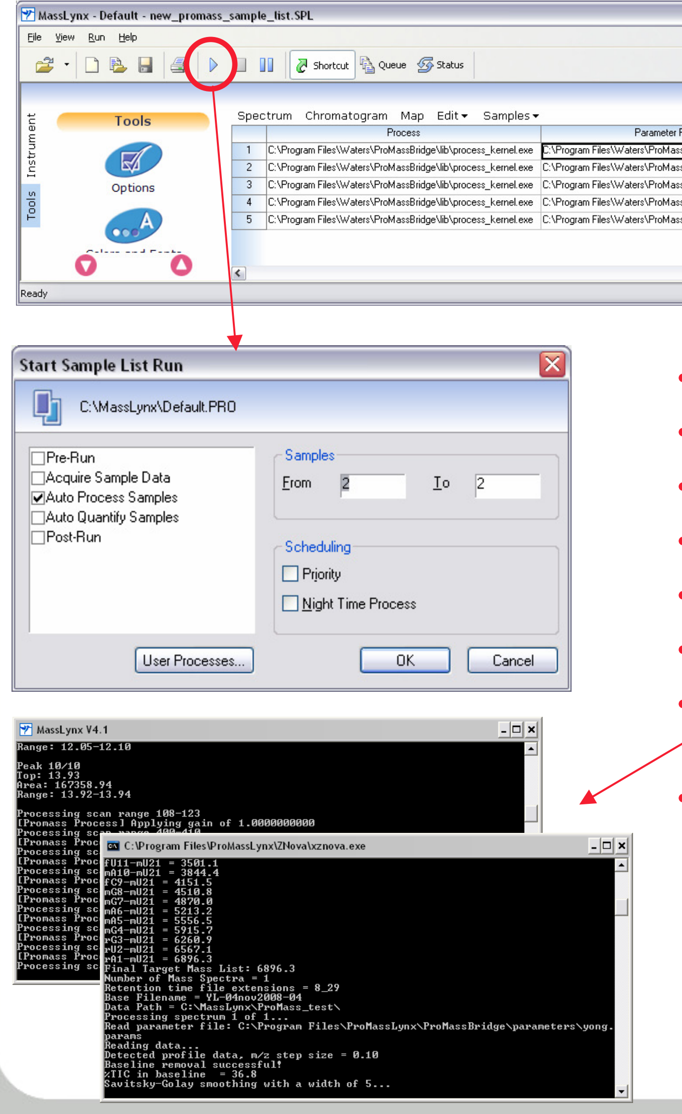
- *Reporting Tab settings ONLY affect automated processing report – does nothing to manual processing report*
- Determines Target Mass tolerance and minimum intensity for a confirmed target mass match
- Impurity levels which demote "green" to "purple" result color code
- Enables Sample Plate View or Excel Summary
- Set labeling behavior of chromatogram traces to display entire chromatogram or only processed range set with ProMassBridge settings
- Allows for setting of thresholds for chromatographic peaks selected for deconvolution, to limit peaks by area %, peak height or total number of peaks

ProMass Processing and Vial Referencing



- MassLynx allows many different types of vial referencing schemes when setting up an autosampler.
- In order to populate the ProMass plate view correctly, you should use *Sequential Discontinuous* vial referencing in MassLynx.
- If you select the *Horizonal Priority* sampling in MassLynx, sampling will occur across rows, otherwise sampling will occur down (by column).
- In the ProMass parameter file, select a *Sampling Direction* of *Across* when MassLynx Horizontal Priority is enabled. Otherwise, set the ProMass *Sampling Direction* to *Down*.
- ProMass can translate vial numbers consisting of just vial numbers (e.g., *1, 2, 3*…), tray: vial format (e.g., *1:1, 1:2, 1:3*…), and tray:96 well position format (e.g., *1:A1, 1:A2, 1:A3*…).
- Create a new raw data directory for each 96-well plate to prevent the ProMass plate view from being overwritten by identical vial numbers.
- See the MassLynx help for more information about vial referencing.

Auto Processing with ProMass



- Acquire data
- Configure sample list with ProMass format
- Hit the Start Run button
- Check the *Auto Process Samples* option
- Enter the sample rows to process in the *From* and *To* fields
- Click OK
- Processing should begin, indicated by appearance of ProMassBridge and ProMass console windows
- After processing is complete, launch ProMass and click the *ProMass Browser* button

ProMass Browser Summary

index.html - Mozilla Firefox

File Edit View History Bookmarks Tools Help

file:///C:/MassLynx/ProMass_test/promass_results/index.html

Most Visited Getting Started Latest Headlines

ProMass

ProMass Sample Browser

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

Data File	Position	Sample ID	Sample Name	Sample Comments	Target Masses	Observed Masses	Purity	Result Code
YL-04nov2008-04	1	SQD test 1	55665		6896.3	6898.0	66.08	
aug30_RNAi1_1	2	RNA test1	2.1x50 ACQ18 0.2mL/min 20to 40B 10 min; A:15:400 TEA/HFIP, B:50%A, 50%MeOH 75um RNAi1 100 pmol inj	rUrUrCrUrGrUrArArUrCrUrCrUrUrGrUrCrUrATT	6506.9	6505.2	34.78	
May7_ssRNA_2up_1lowdig_25mM_HAA_12	3	SQD test 2	50x2.1mm OST 1.7um, 0.2 ml/min, 10C,30-40%B in 10min, A 25mM HAA B100% ACN	rUrCrGrUrCrArArGrCrGrArUrUrArCrArArGrGTT rCrCrUrUrGrUrArArUrCrGrCrUrUrGrArCrGrATT	6607.0 6693.1	6605.8 6691.9	10.85 13.39	
July17_RNA_157	4	PDE digest	digest RNAi 21nt SM upper strand, 27 pm/uL, High/Low MS/MS, 25 to 50 V, 20-40% B in 20 min	rUrCrGrUrCrArArGrCrGrArUrUrArCrArArGrGTT	6693.1	6692.7	2.82	
aug13_GEM91_2	5	GEM91	2.1x50 ACQ18 0.2mL/min 19.5 to 23.5B in 20min; A:15:400 TEA/HFIP, B: MeOH 60C, 100 um	C*T*C*T*C*G*C*A*C*C*C*A*T*C*T*C*T*C*T*C*C*T*T*C*T	7776.3	7775.4	20.27	

ZNova 2.9.8 ©2001-2008 Novatia, LLC

Done

- ProMass Browser summary provides concise view of a sample list run.
- Detailed results are accessed by clicking sample wells or table row entries.
- Clicking table headings allows sorting of entries.
- Color codes indicate status of targeted components in data.

Example ProMass Detailed Report

aug30_RNAi1_1.html - Mozilla Firefox

File Edit View History Bookmarks Tools Help

file:///C:/MassLynx/ProMass_test/promass_results/aug30_RNAi1_1.html

Most Visited Getting Started Latest Headlines

[<<]
Data File: C:\MassLynx\ProMass_test\aug30_RNAi1_1.raw
Sample Name: 2.1x50 ACQ18 0.2mL/min 20to 40B 10 min; A:15:400 TEA/HFIP, B:50%A, 50%MeOH 75um RNAi1 100 pmol inj
Sample ID: RNA test1
Position: 2
Instrument Method: MS: C:\MassLynx\Default.PRO\ACQUDB\DEFAULT INLET: C:\MassLynx\Default.PRO\ACQUDB\DEFAULT
Nucleotide:
rUrUrC rUrGrU rArArU rCrUrC rUrUrG rUrCrU rATT
Average Mass (Da): 6506.9
Monoisotopic Mass (Da): 6503.8

ProMass

Target Mass Summary

RT (min)	Target Mass (Da)	Observed Mass (Da)	Mass Error	Intensity	% Abundance (in Spectrum)	%Purity (Estimate)	Identity	Result Code
9.00	6506.9	6505.2	-1.7 Da (-0.026 %)	2.21E+004	58.47	34.78	Target Mass	

Sequence Ladder Summary

RT (min)	Calculated Mass (Da)	Observed Mass (Da)	Mass Error	Intensity	Sequence
9.00	6506.9	6505.2	-1.7 Da (-0.026 %)	2.21E+004	rU1-T21
0.97	1181.8	1181.0	-0.8 Da (-0.068 %)	3.81E+003	rU18-T21
1.06	1487.0	1486.1	-0.9 Da (-0.061 %)	2.51E+003	rC17-T21
2.12	2444.5	2443.4	-1.1 Da (-0.045 %)	2.14E+003	rU14-T21
1.73	2138.4	2137.3	-1.1 Da (-0.051 %)	1.99E+003	rG15-T21
2.65	2750.7	2749.4	-1.3 Da (-0.047 %)	1.90E+003	rU13-T21
6.22	4302.6	4301.1	-1.5 Da (-0.035 %)	1.83E+003	rA8-T21
3.79	3362.1	3360.4	-1.7 Da (-0.051 %)	1.64E+003	rU11-T21
5.09	3973.4	3972.0	-1.4 Da (-0.035 %)	1.62E+003	rU9-T21
1.39	1793.2	1792.1	-1.1 Da (-0.061 %)	1.59E+003	rU16-T21
3.28	3055.9	3054.7	-1.2 Da (-0.039 %)	1.46E+003	rC12-T21
4.45	3667.2	3665.6	-1.6 Da (-0.044 %)	1.29E+003	rC10-T21
7.65	5283.2	5281.1	-2.1 Da (-0.040 %)	1.23E+003	rG5-T21
7.01	4631.8	4630.3	-1.5 Da (-0.032 %)	1.19E+003	rA7-T21
7.25	4938.0	4936.7	-1.3 Da (-0.026 %)	8.86E+002	rU6-T21
8.00	5589.4	5587.2	-2.2 Da (-0.039 %)	8.86E+002	rU4-T21
8.43	5894.5	5892.7	-1.8 Da (-0.031 %)	4.92E+002	rC3-T21
8.73	6200.7	6198.9	-1.8 Da (-0.029 %)	4.73E+002	rU2-T21

Chromatogram Summary

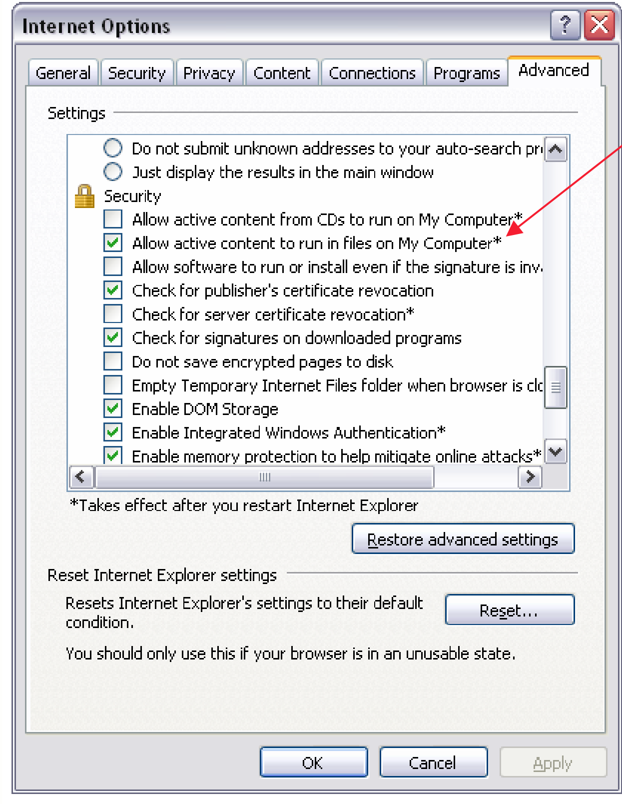
RT (min)	Base Peak Mass (Da)	Intensity	Spectral Quality	LC/MS Peak Area	LC/MS Area Percent
0.97	1181.0	3.81E+003	ok	5.91E+002	2.08
1.06	1486.1	2.51E+003	ok	4.38E+002	1.54
1.39	1792.1	1.59E+003	ok	2.88E+002	1.01
1.73	2137.3	1.99E+003	ok	3.95E+002	1.39
2.12	2443.4	2.14E+003	ok	3.99E+002	1.40
2.65	2749.4	1.90E+003	ok	4.84E+002	1.70
3.28	3054.7	1.46E+003	ok	3.55E+002	1.25
3.79	3360.4	1.64E+003	ok	4.91E+002	1.73
4.01	3449.6	4.62E+002	ok	1.70E+002	0.60
4.45	3665.6	1.29E+003	ok	4.23E+002	1.49
4.67	3754.5	4.36E+002	ok	1.70E+002	0.60
5.09	3972.0	1.62E+003	ok	7.20E+002	2.53
5.36	4061.2	4.60E+002	ok	2.20E+002	0.77

Done

- In this example an oligo sequence was specified in the *Sequence* field of the sample list, allowing calculation of the target mass.
- The Target Mass Summary table at the top of the report provides information about expected components.
- In this example, a Sequence Ladder Summary was produced using the *ladder=5'* command in the *Target Info* field of the sample list. This allows matching of all oligo sequence failures.
- The Chromatogram Summary table lists the most abundant deconvoluted mass (i.e., base peak mass) at each retention time.
- Report hyperlinks allow quick navigation to ESI and deconvoluted spectra for each retention time.
- As with manual processing, use the [View Data] hyperlinks in the report to interactively view mass spectra.

Novatia

Additional Tips for Using ProMass



- Set Internet Explorer security to *Allow active content to run in files on My Computer*, otherwise embedded Javascript in ProMass report pages may not run properly. *Available under Internet Explorer* | *Tools* | *Internet Options* menu item.
- ProMass can run in automated mode with the console window minimized. To do this, launch the ProMass home page, select from the Settings menu *ProMass Console* | *Hide*
- Previously processed data can be browsed by selecting the *File* | *Browse ProMass Files…* menu item from the ProMass home page.
- All mass definitions for nucleotide and amino acid groups are defined in the *znova_masses.ini* file. The user can easily add custom definitions to this file by editing with a text editor. The file is found in the ProMass installation ZNova directory.
- ProMass is currently optimized for unit resolution mass spectrometers. The algorithm works well on higher resolution data (e.g., Q-Tof), however, the resolution is not maintained in the deconvolution results. One can expect mass accuracies of +/- 0.01% for well-calibrated instruments when comparing to isotopic average expected masses.

Example Data

- Example data is provided in the \ProMassLynx\TestData directory.
- Sample data is included for oligos, a myoglobin LCMS, and a protein LCMS run.
- Open the sample list file (.spl) in one of the TestData directories to test automatic processing.

Resources and Feedback

- Novatia is continually working to improve ProMass and wants to be aware of any problems or suggestions to enhance the product.
- Please forward any feature requests and suspected software problems to Novatia.
- Contact Novatia at info@enovatia.com or (732)-274-9933