Oligonucleotide Sequence Confirmation by High Resolution Ion Trap Tandem Mass Spectrometry

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Overview

- It is important to confirm the structure of oligonucleotides to be used in diagnostic and therapeutic applications.
- The use of modified oligonucleotides often precludes the use of enzymatic digestion combined with mass spectrometry for sequence confirmation.
- Tandem mass spectrometry can be used to reliably confirm the sequence of DNA and RNA, including modified oligos.
- Novatia uses a unique approach that utilizes high resolution hybrid LTQ-Orbitrap mass spectrometry and novel deisotoping and charge deconvolution software for MS-based oligonucleotide sequencing.
- Applications include sequence confirmation of known sequences, as well as denovo sequencing and identification of modified residues.

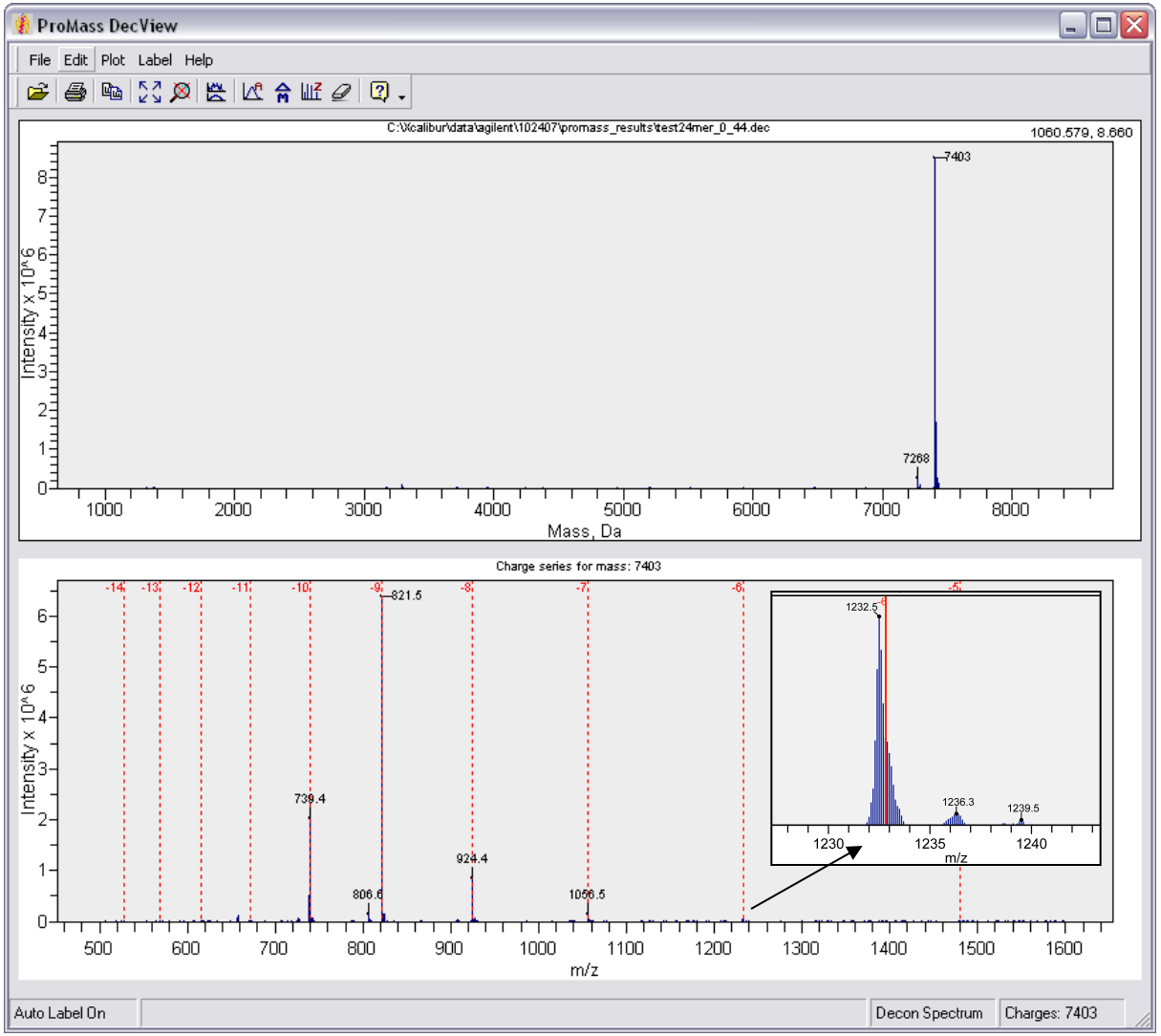
General Approach

1. First confirm the intact mass of oligonucleotide by ESI/MS.
2. Select a low charge state ion for MS/MS (typically 4^- to 6^- charge state).
3. Obtain the MS/MS product ion spectrum on a LTQ-Orbitrap ion mass mass spectrometer (shown at right) using mass resolution of 30,000 FWHM.
4. Use Positive Probability, Ltd. (PPL) Respect deconvolution software to deisotope the MS/MS product ion spectrum and obtain a simplified fragment spectrum yielding exact masses.
5. Compare the “simplified” product spectrum to the list of predicted fragments calculated from the expected oligo sequence or “read” the sequence from the spectrum.

Novatia’s LTQ-Orbitrap high resolution mass spectrometer

Mass Determination of Intact Oligo

24-mer DNA, 7402.8 Da



Deconvoluted mass spectrum indicating mass of 7403 Da, obtained with Novatia's ProMass Deconvolution software

ESI mass spectrum with charge states labeled and 6^- ion inset

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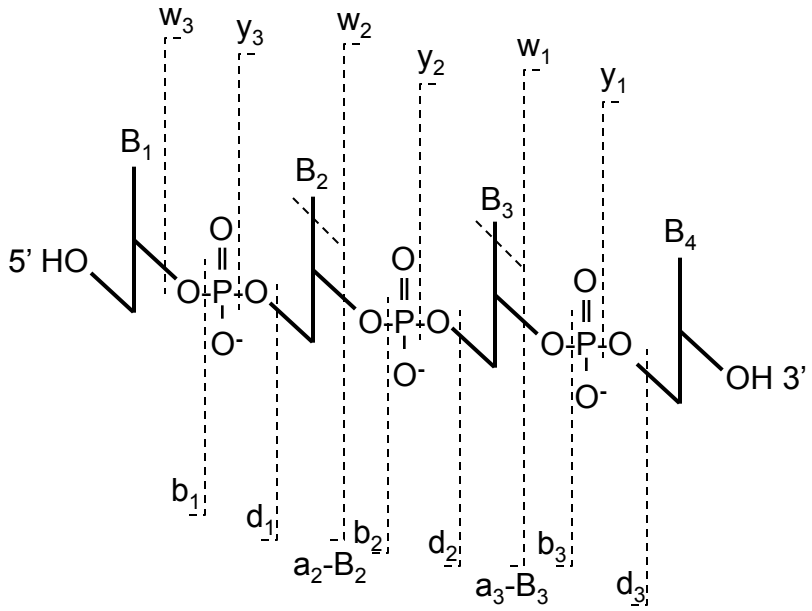
Oligonucleotide MS/MS Fragmentation Scheme

Oligonucleotides fragment along the phosphate backbone producing a set of ions containing the 5' terminus (**a-B**, **b**, and **d**) and another set of ions containing the 3' terminus (**w** and **y**). Losses of H_2O from these ion types are also common with **d** and **w** ions. The **a-B** ion is somewhat unique in that it involves base loss in addition to cleavage at the phosphate.



Reference: McLuckey et. al, JASMS, **1992**, *3*, 60-70.

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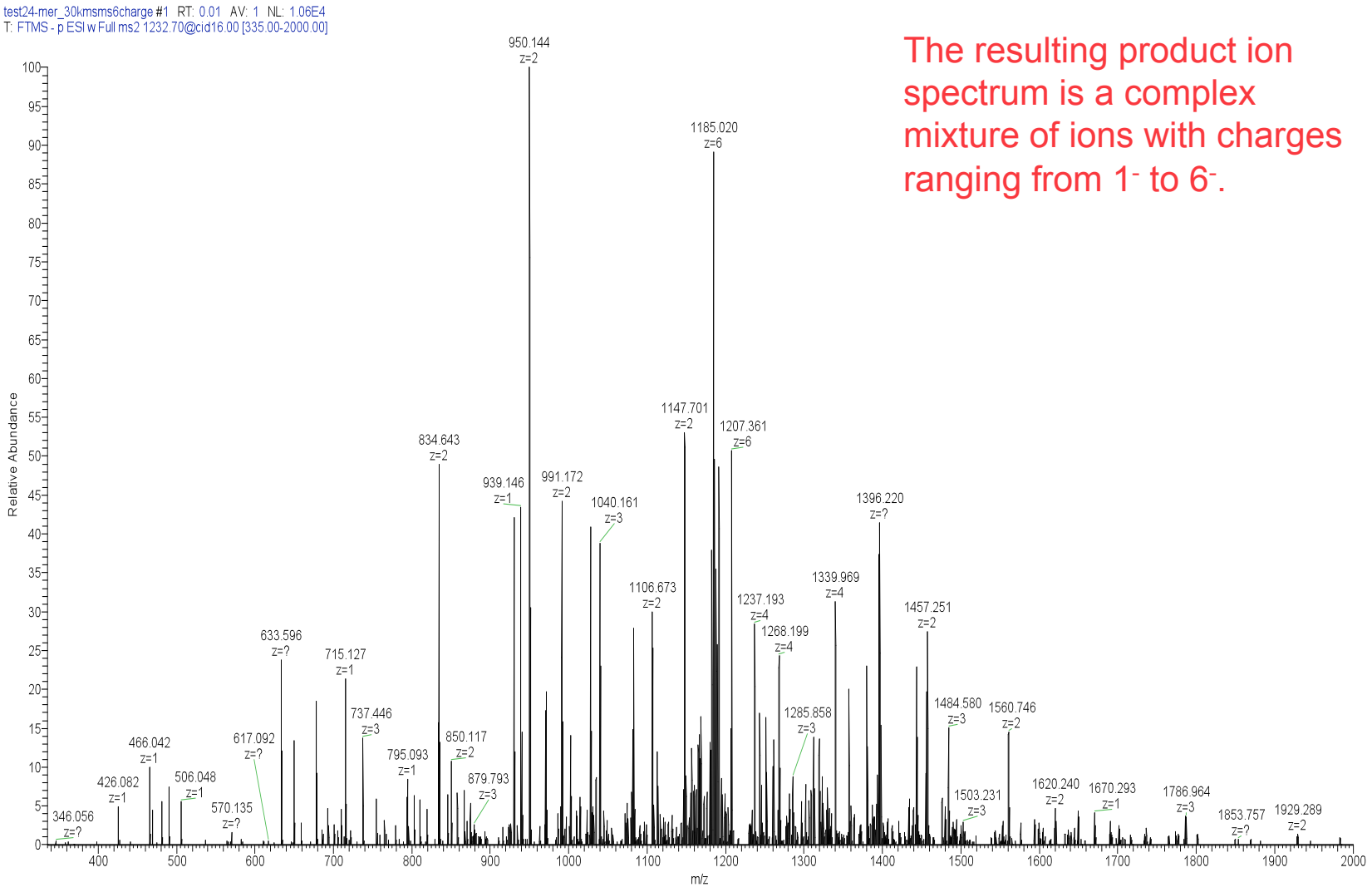
LTQ-Orbitrap MS/MS Product Ion Spectrum of 24-mer DNA

MS/MS of 6^- charge state, m/z 1232.5, MW 7402.8 Da, 30,000 FWHM resolution



The resulting product ion spectrum is a complex mixture of ions with charges ranging from 1^- to 6^-.

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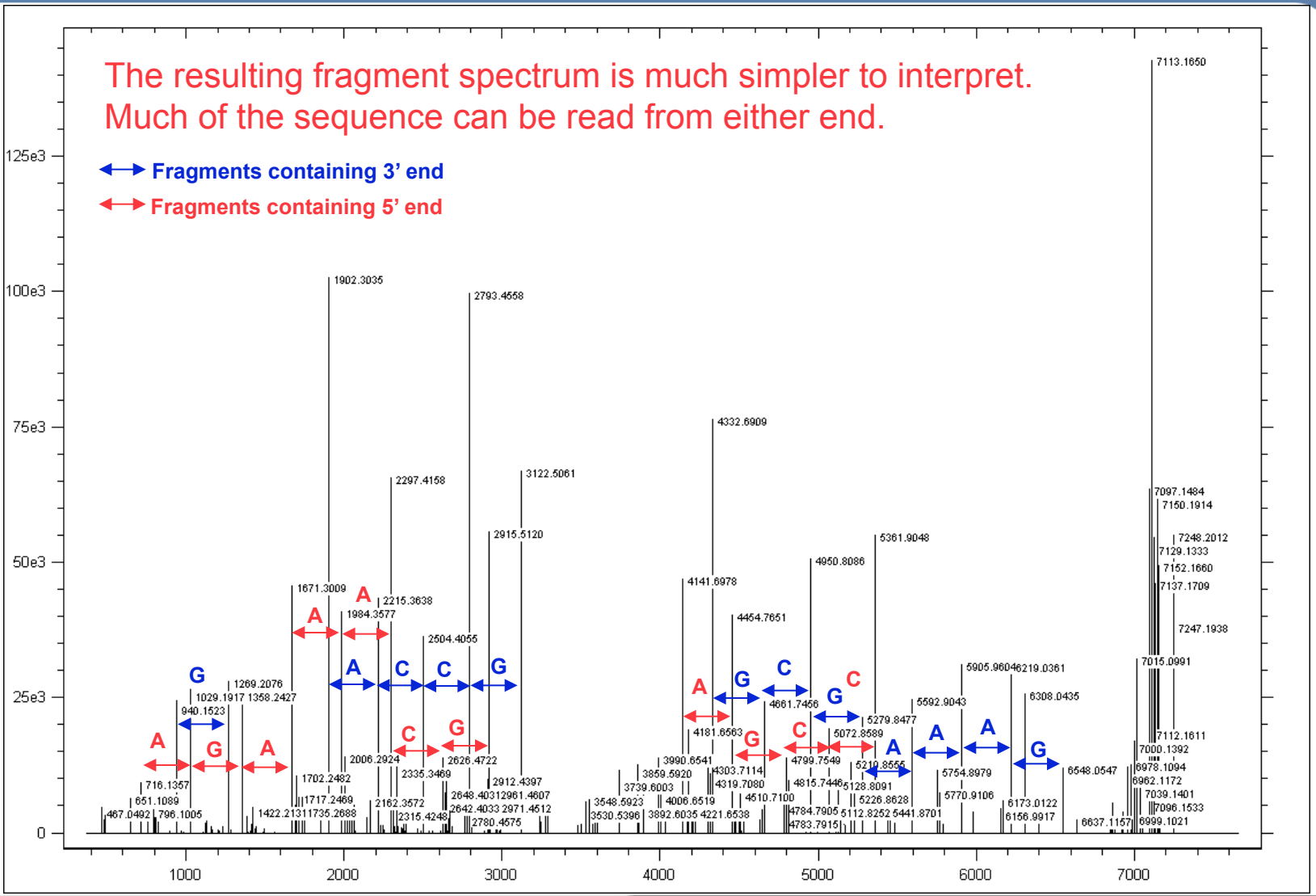
Product Ion Scan After Charge Deconvolution and Deisotoping

24-mer DNA after processing with PPL Respect algorithms



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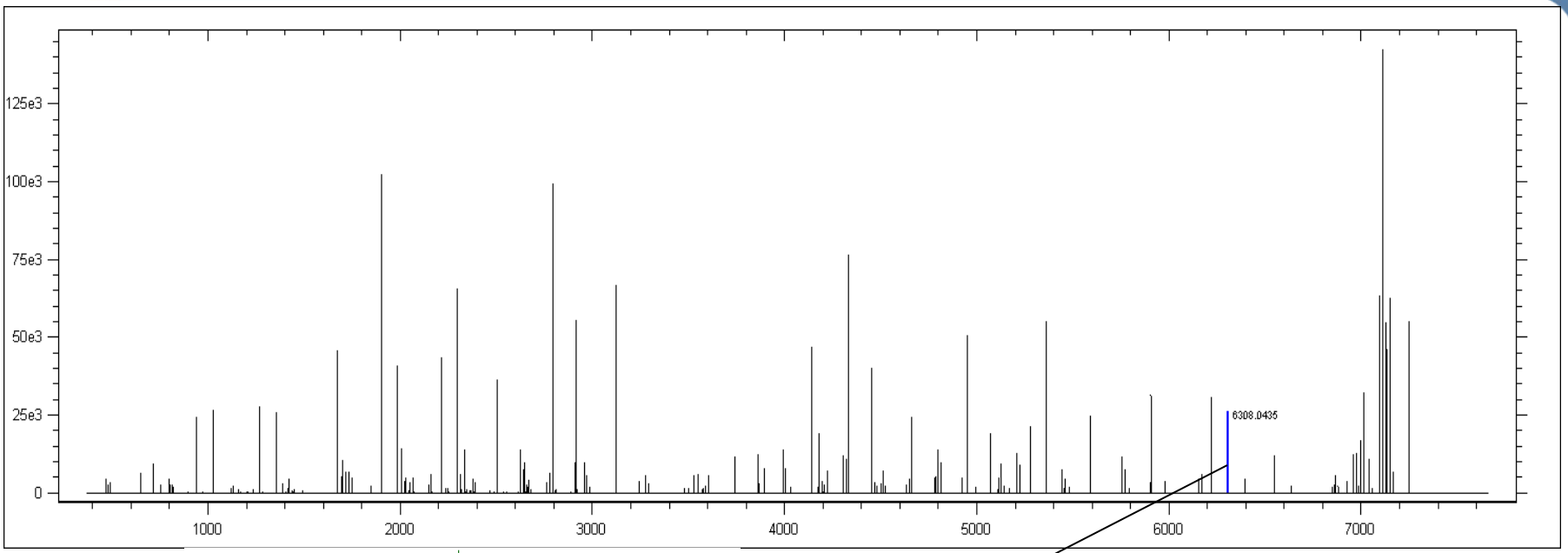
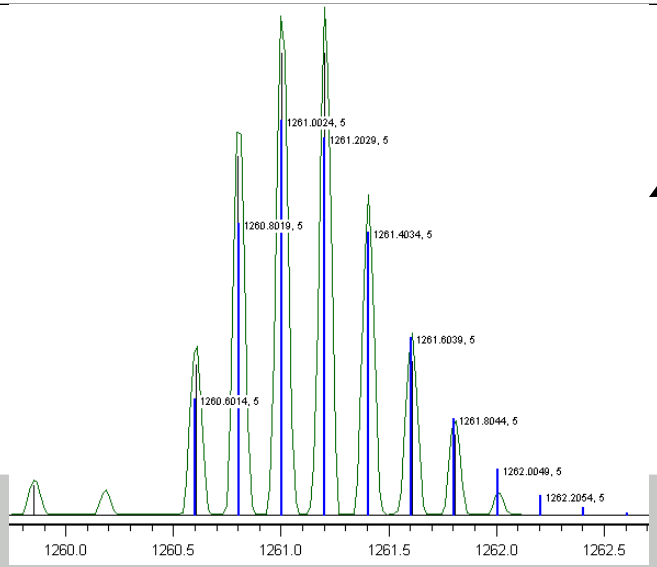
Raw MS/MS Data Inspection – 24-mer DNA





Evidence for mass 6308.04

Raw data with superimposed modeled isotope distribution of fragment ion with 5^- charge at m/z 1261

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Predicted Deisotoped Fragments from 24-mer DNA

Observed fragments highlighted in green

n	5'	a-B	d-H_2O	w	y	3'
1	G		329.0525	**322.0566**	242.0903	T
2	C	**427.0893**	618.0989	**651.1091**	571.1428	G
3	A	**716.1357**	931.1565	**940.1555**	860.1892	C
4	G	**1029.1933**	1260.2090	**1269.2080**	1189.2417	G
5	A	**1358.2458**	1573.2666	1598.2605	1518.2942	G
6	A	**1671.3034**	1886.3242	**1902.3066**	1822.3402	T
7	A	**1984.3610**	2199.3818	**2215.3642**	2135.3978	A
8	G	**2297.4186**	2528.4344	**2504.4106**	2424.4442	C
9	C	**2626.4711**	2817.4807	**2793.4569**	2713.4906	C
10	G	**2915.5175**	3146.5332	**3122.5094**	3042.5431	G
11	T	3244.5700	3450.5793	3435.5670	3355.6007	A
12	C	**3548.6161**	3739.6257	**3739.6131**	3659.6468	T
13	T	3837.6624	4043.6717	4028.6595	3948.6931	C
14	A	**4141.7085**	4356.7293	**4332.7055**	4252.7392	T
15	G	**4454.7661**	4685.7818	**4661.7580**	4581.7917	G
16	C	**4783.8186**	4974.8282	**4950.8044**	4870.8381	C
17	C	**5072.8650**	5263.8746	**5279.8569**	5199.8906	G
18	A	**5361.9113**	5576.9322	**5592.9145**	5512.9482	A
19	T	5674.9689	5880.9782	**5905.9721**	5826.0058	A
20	G	5979.0150	6210.0307	**6219.0297**	6139.0634	A
21	G	**6308.0675**	6539.0832	**6548.0822**	6468.1159	G
22	C	6637.1200	6828.1296	6861.1398	6781.1735	A
23	G	6926.1664	7157.1821	**7150.1862**	7070.2199	C
24	T					G

Observations:

- DNA oligos produce **a-B** and **w** ions as the most abundant fragments.
- RNA oligos favor the production of **y** and **d-H_2O** ions.
- Fragmentation at the 3' side of **T** in DNA is often absent, but one can usually assume a T is present at these locations and proceed with the sequencing.
- Complimentary fragment ions from both ends of the molecule provide dual confirmation of the sequence for ~20 residues. This allows sequence confirmation of up to 40-mer sequences with this approach.
- Mass accuracy on the fragments is 1-3 ppm.

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