

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

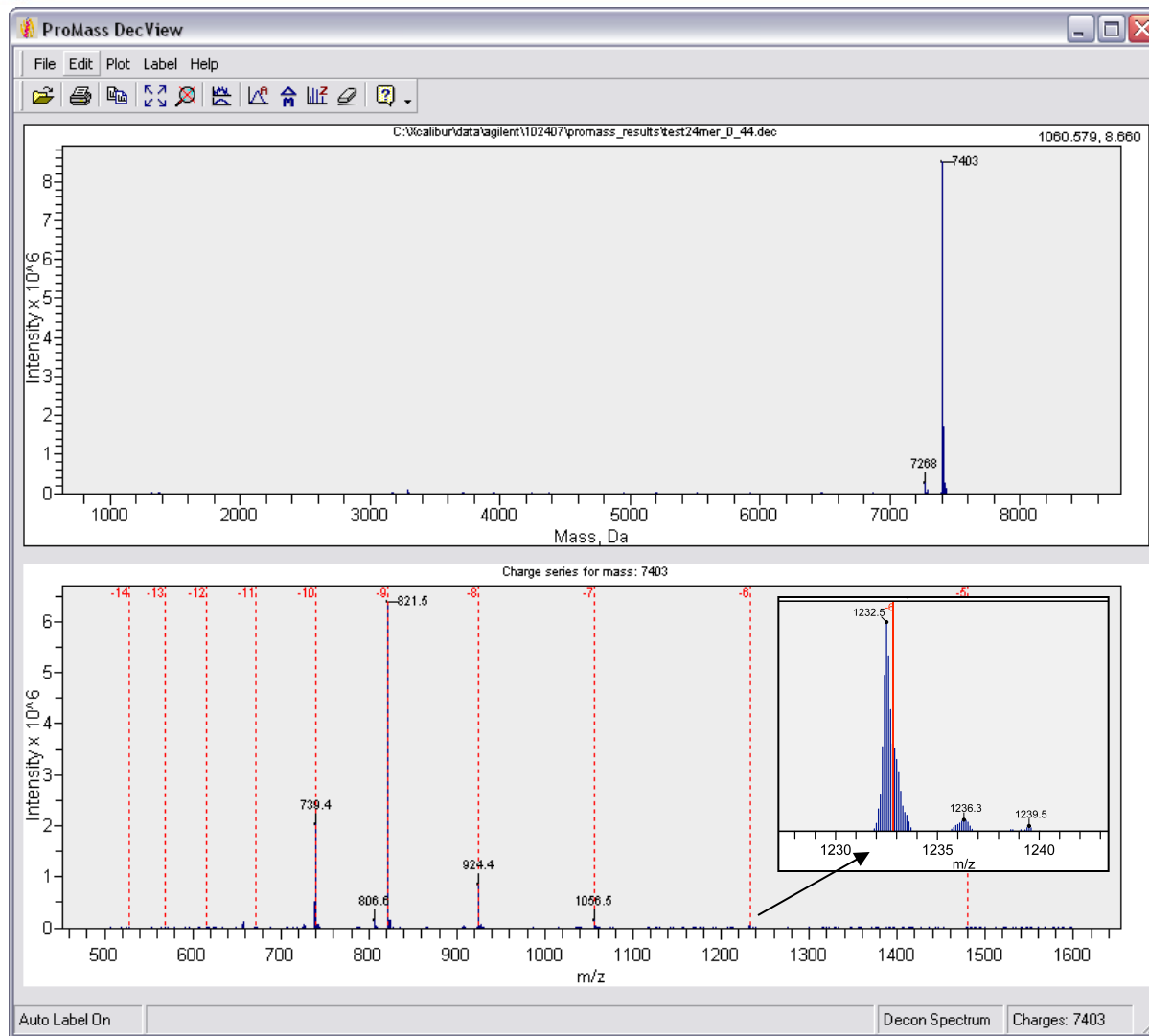
1. First confirm the intact mass of oligonucleotide by ESI/MS.
2. Select a low charge state ion for MS/MS (typically 4⁻ to 6⁻ charge state).
3. Obtain the MS/MS product ion spectrum on a LTQ-Orbitrap ion mass spectrometer (shown at right) using mass resolution of 30,000 FWHM.
4. Use Positive Probability, 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

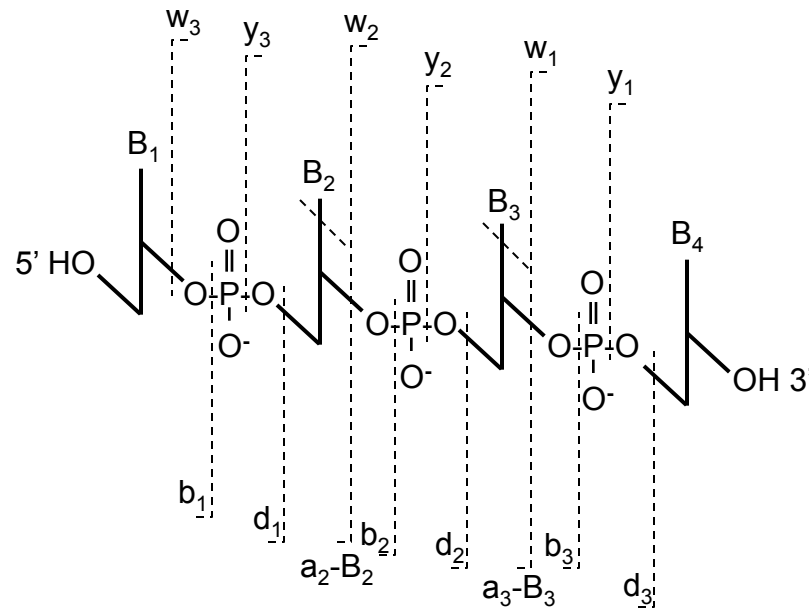
ESI mass spectrum with charge states labeled and 6⁻ ion inset



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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 H₂O 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.

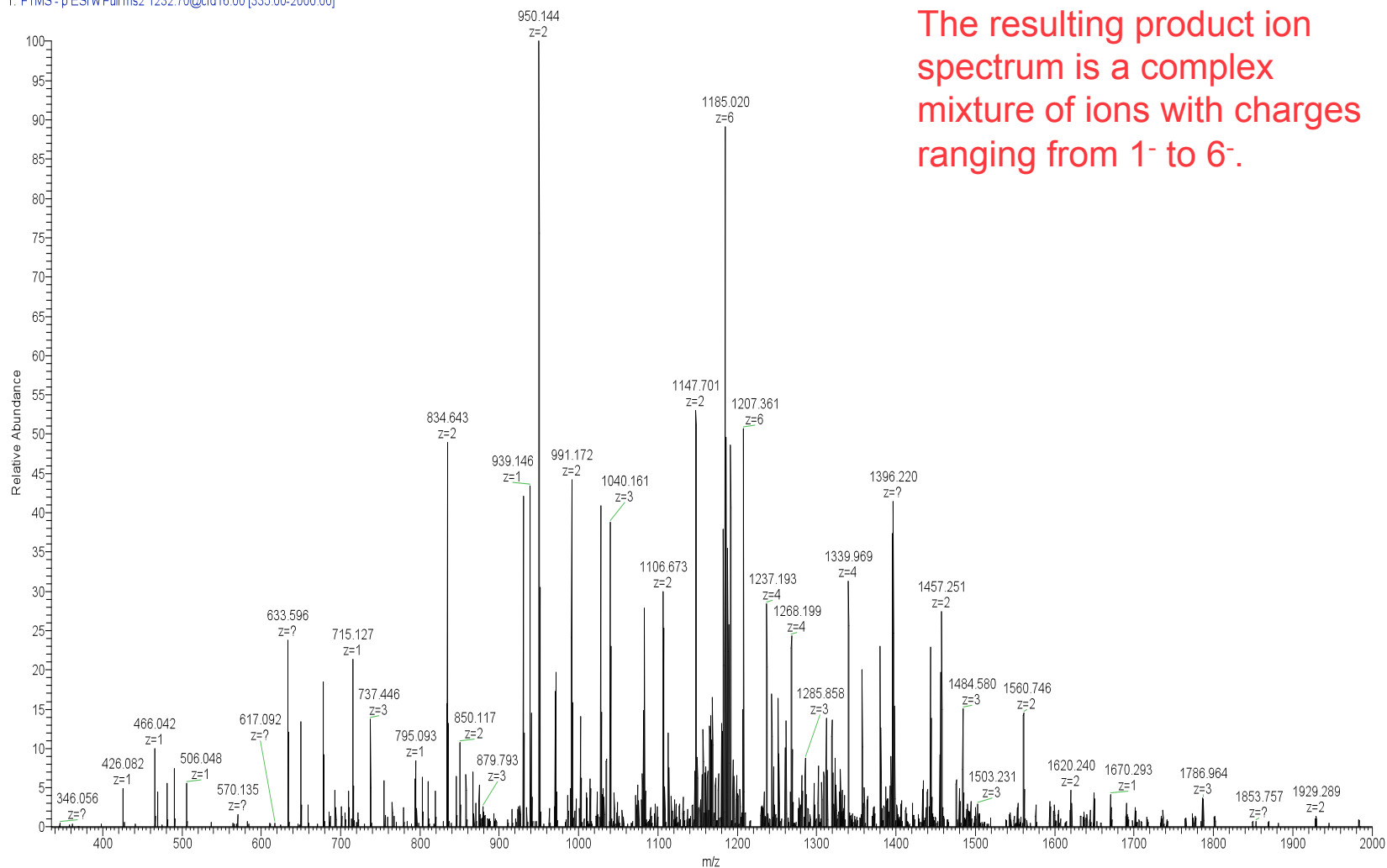


Reference: McLuckey et. al, JASMS, **1992**, 3, 60-70.

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

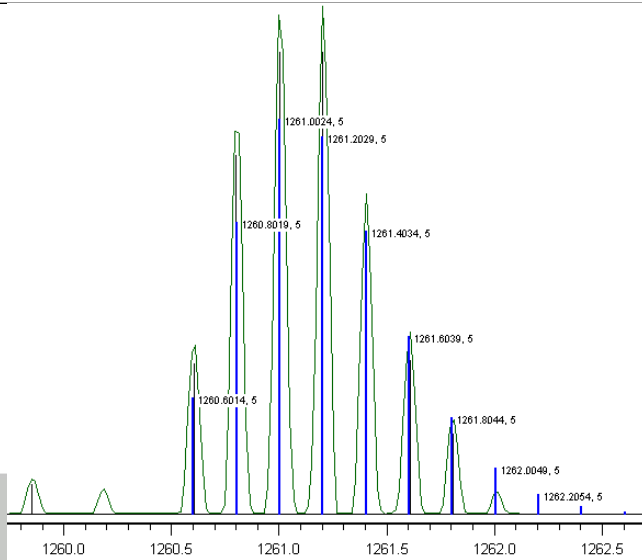
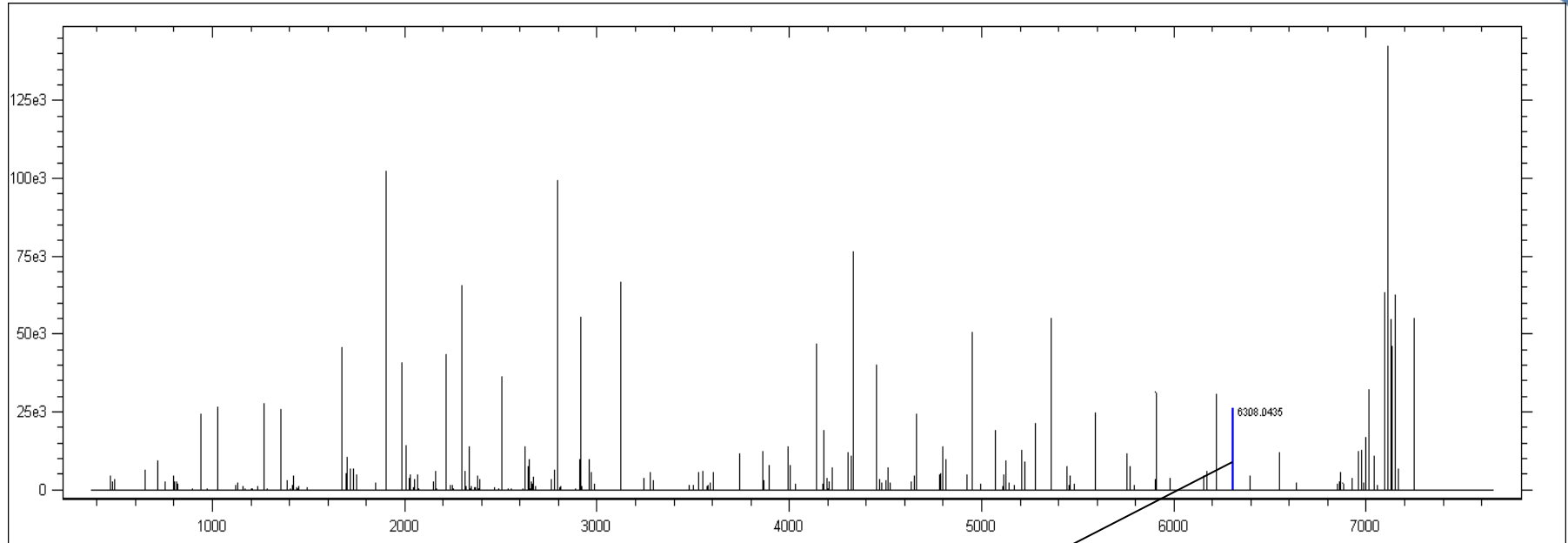
MS/MS of 6⁻ charge state, m/z 1232.5, MW 7402.8 Da, 30,000 FWHM resolution

test24-mer_30kmsms6charge#1 RT: 0.01 AV: 1 NL: 1.06E4
T: FTMS - p ESI w Full ms2 1232.70@cid16.00 [335.00-2000.00]



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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 ₂ O	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-H₂O** 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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