

High-Throughput Analysis of Oligonucleotides using Automated Electrospray Ionization Mass Spectrometry

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Introduction

- Tens of thousands of oligos produced daily in HT oligo synthesis labs
- Efficient and reliable confirmation of all synthesized products desired
- To date, MALDI-ToF has been QC method of choice
- Demand for long (e.g., 70-mer) oligos for use in oligo arrays is increasing
- Long and/or fragile oligos have been problematic by MALDI-ToF
- We have developed a totally automated system using LC/MS with:
 - >1000 sample per 24 hr throughput
 - capability to analyze long (>100 mer) or fragile oligos
 - < 100 ppm mass accuracy & precision across entire mass range
 - capability for detailed LC/MS profiling on the same system

Experimental Systems

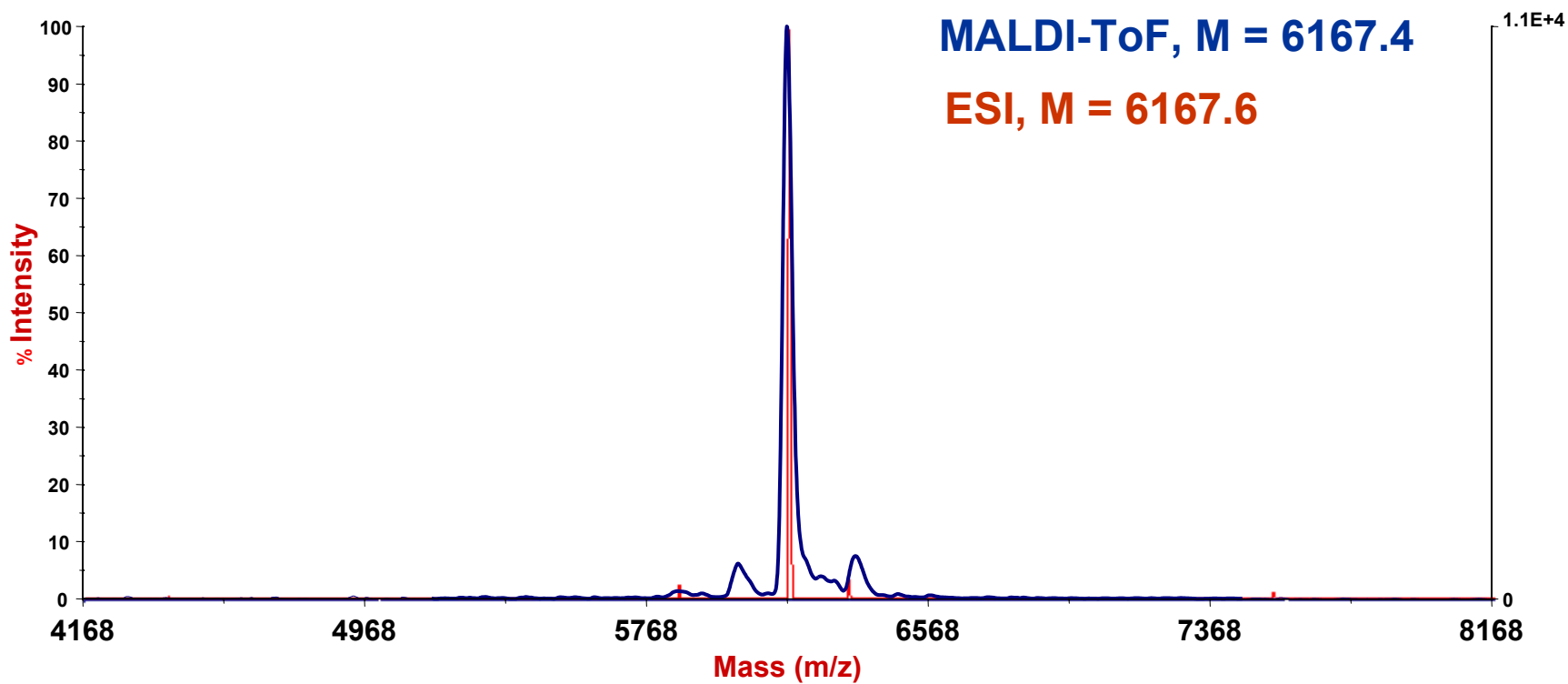
- MALDI-ToF
 - ABI Voyager DE, linear +ve mode, external calibration
 - 3-HPA – ammonium citrate matrix
- LC/MS (Oligo HTCS)
 - ThermoFinnigan TSQ7000, -ve ion mode
 - Xcalibur data system
 - Michrom Paradigm MS4 HPLC
 - HFIP / TEA “lite” mobile phase w/trace EDTA
 - LEAP CTC-PAL autosampler
 - *ProMass* auto biomolecule deconvolution processing software

ProMass Auto ESI Deconvolution Approach

- Uses a simple scoring algorithm to determine the charge of every peak in the raw mass spectrum (Zhang & Marshall, JASMS 1998)
- No deconvolution artifacts
- Incorporates signal processing and score normalization to improve reliability of deconvolution even on very noisy data
- Can confirm presence of target masses

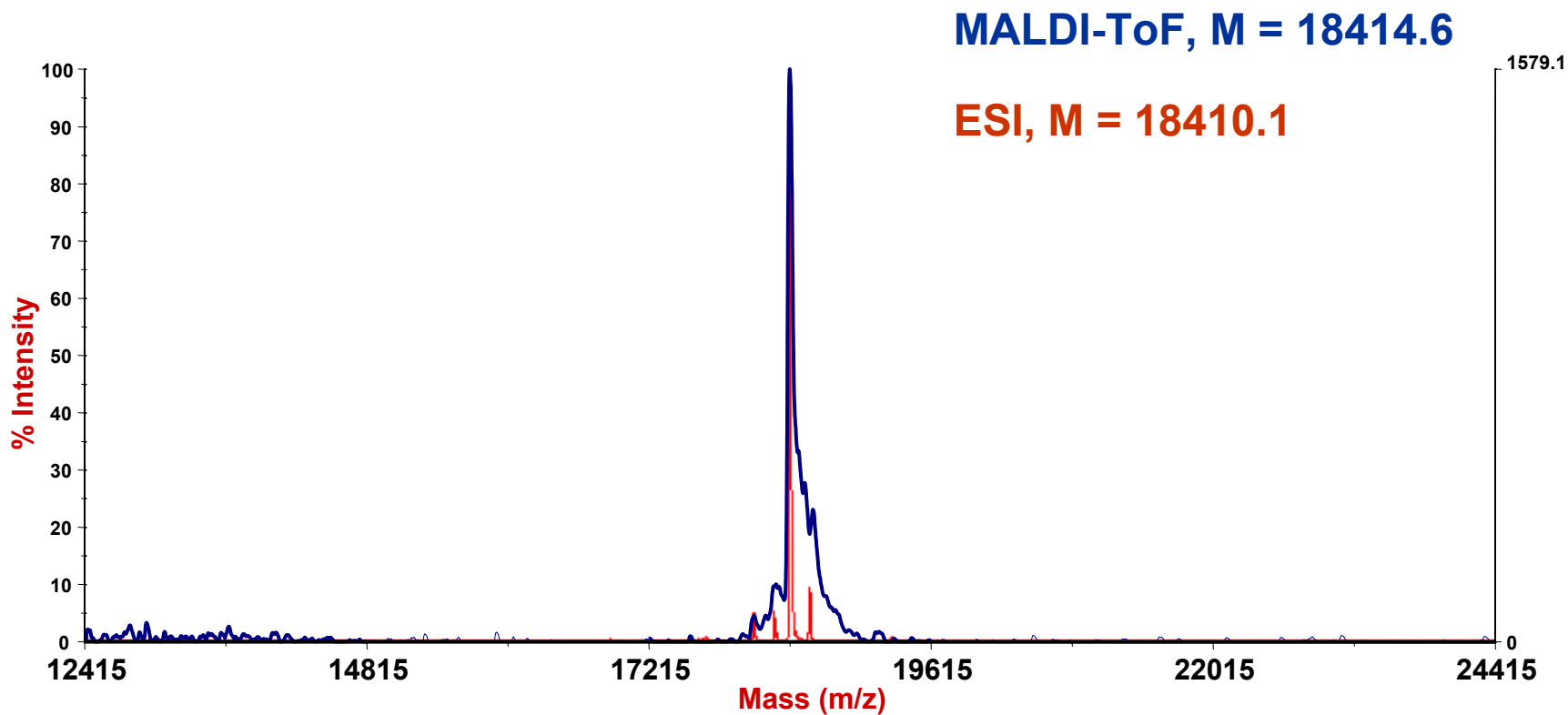
MALDI and ESI MS Data of a 20-mer Oligo

M = 6168.4



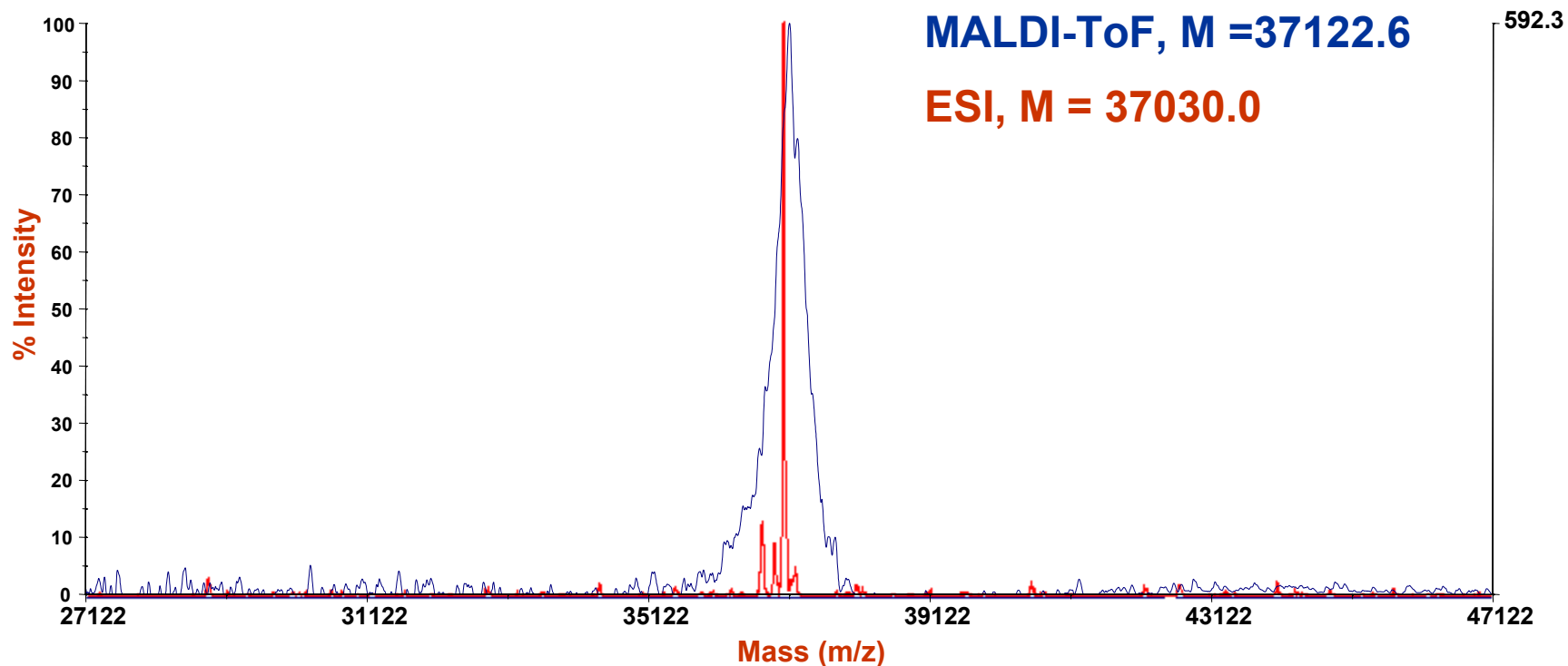
MALDI and ESI MS Data of a 60-mer Oligo

M = 18410.0

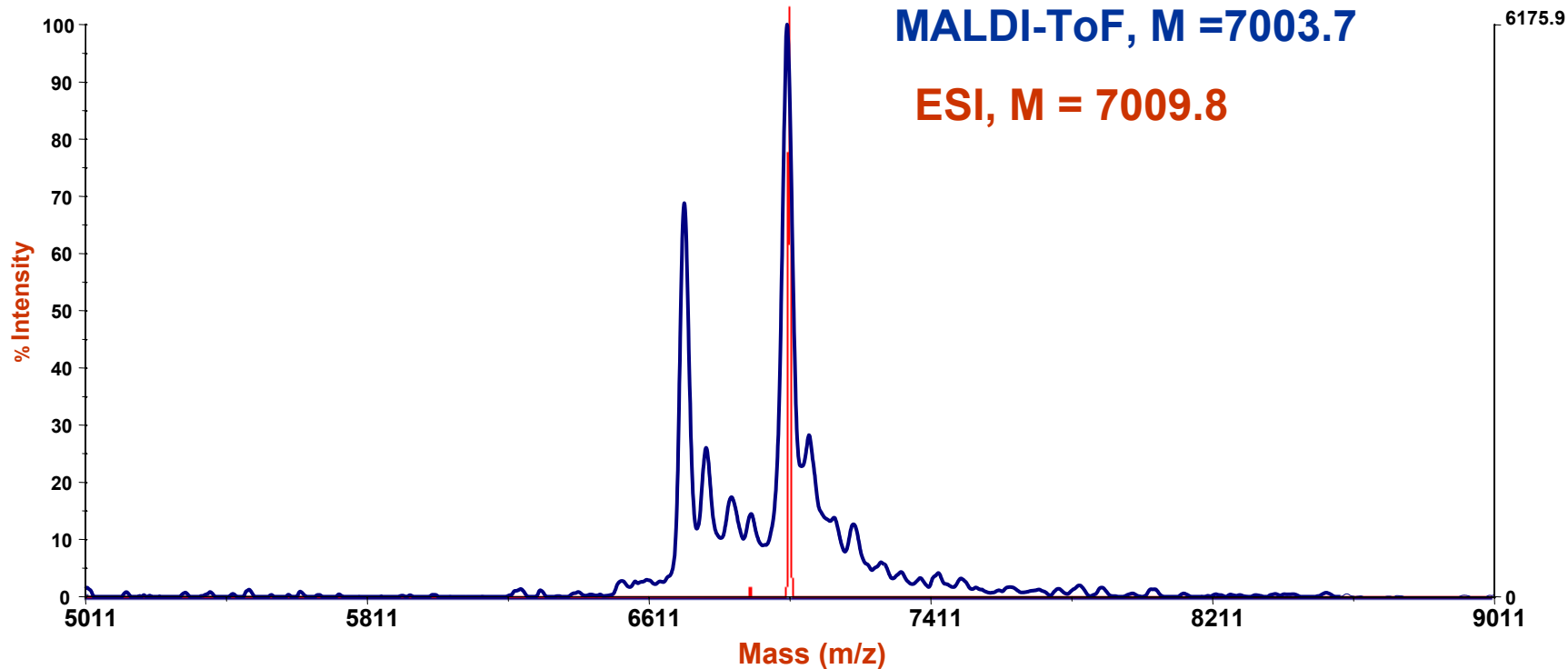


MALDI and ESI MS Data of a 120-mer Oligo

M = 37031.0



MALDI and ESI MS Data on Fragile Dual-labeled Probe Black Hole Quencher™ M = 7011.8



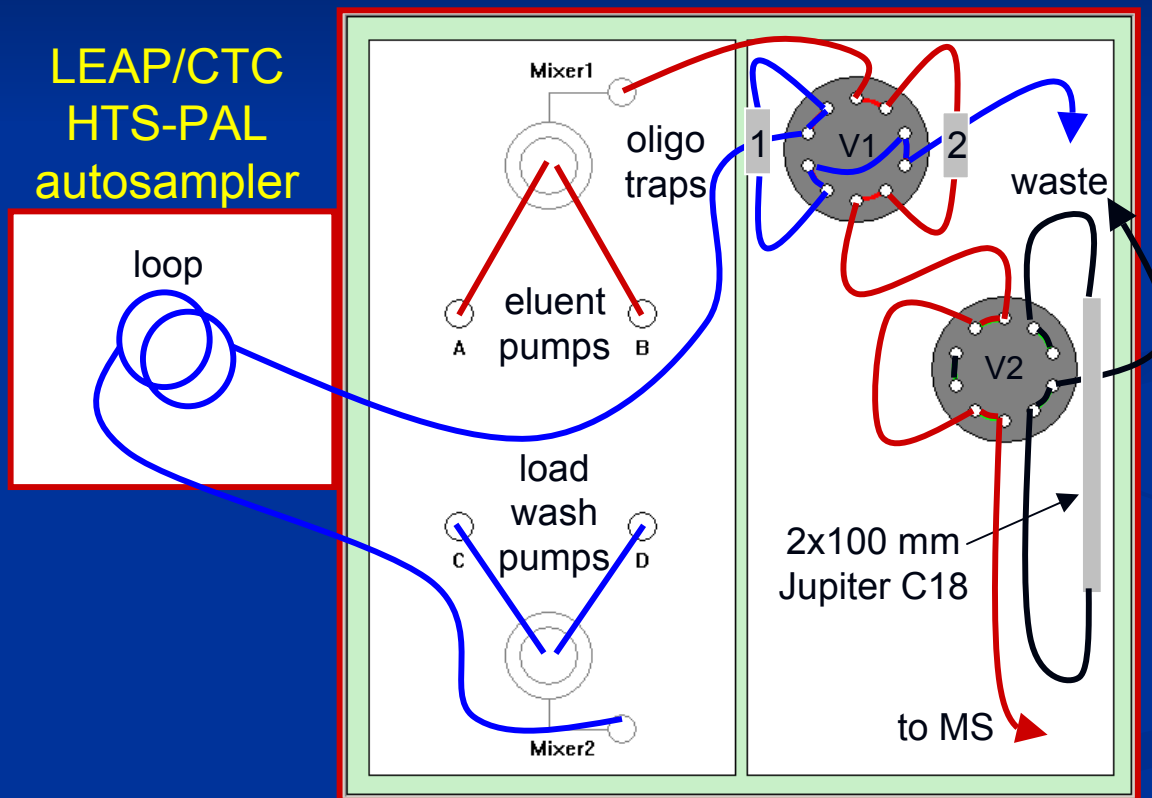
Mass Accuracy of 10 to 120-mers

Oligo	MW	MALDI	Error (ppm)	ESI	Error (ppm)
10-mer	2993.0	2992.1	-300.7	2993.1	33.4
20-mer	6168.1	6167.4	-113.5	6167.6	-81.1
40-mer	12300.0	12298.6	-113.8	12299.7	-24.4
60-mer	18410.0	18414.6	249.9	18410.1	5.3
80-mer	24661.1	24664.7	146.0	24661.0	-4.1
100-mer	30951.1	30978.3	878.8	30950.6	-16.2
120-mer	37031.0	37121.6	2446.6	37030.0	-27.0

Oligo HTCS Plumbing Scheme

Shown in High-throughput QC Mode

Michrom Paradigm MS4 HPLC



- Dual trap column configuration
- Eluent pumps A/B, 200 uL/min
- Load/wash pumps C/D, 1 mL/min
- V1 toggled for each run
- V2 bypass
- One trap is equilibrated while other is eluting
- 1.4 min/sample = 10 x 96 well plates/day
- 1.7 min/sample = 8 x 96 well plates/day with pre-dilution & mixing steps

ProMass Automated Data Processing

Xcalibur Sample Sequence
defines samples to be analyzed

Optional Amino acid or
nucleotide sequence

Parameter Setup
defines parameters for
deconvolution

Xcalibur Processing Method
defines how peaks are selected

**Peak picking,
auto decon-
volution, and
report
generation**

File Name	Proc Meth	ZNova Params	BioSequence
1 1451oflb	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	CATGCATGAAAGTACTCGGGAGCTA
2 1451ona	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	CACAGCATGCATGAAAGTACTTC
3 637oflb	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	AAGCCATGCATGAAAGTACTTC
4 637ona	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	GAGCATGCATGAAAGTACTTCACA
5 637ona02	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	AACAACCATGCATGAAAGTACTTC
6 7-2probeC3SS	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	TATATACATGCATGAAAGTACTTC
7 7-2probeC6SS	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	CACACATGCATGAAAGTACTTCACA
8 7-1probeC3SS	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	TCCTTCATGCATGAAAGTACTTCAG
9 7-1probeC6SS	C:\Xcalibur\methods\oligo_top3	P'D:\Znova\oligo.params	GCATGCATGAAAGTACTTC

Build ZNova Parameter File

Parameter File: C:\Program Files\ProMass\Xcal\Temp.params

Basic Deconvolution | **Advanced Deconvolution** | Results | Reporting

Masses: Input m/z Range (u) From [] To []

Output Mass Range (Da) From [500] To [20000]

Adduct Ion Mass: -1.0079

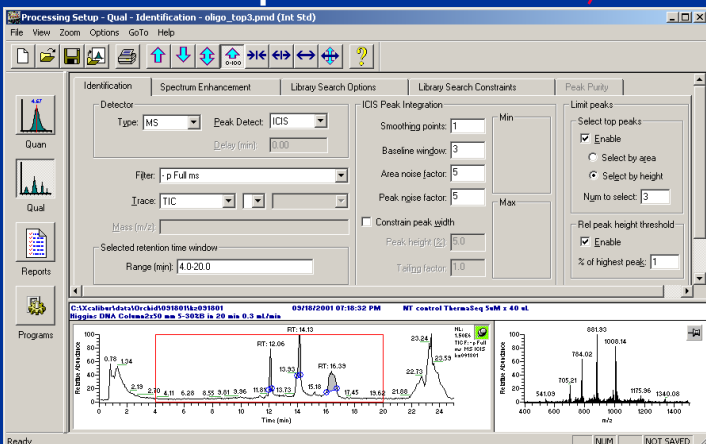
m/z Exclusion List: []

Deconvolution: Peak Width [2], Merge Width [0.5], Minimum Score [2], Normalize Scores [1], Comprehensive Deconvolution [checked], Centroid Output []

Baseline Removal: [0.5] [checked], On [], Low/Normal [], Medium [], High []

Smoothing: Smooth Width [3], Num of Smooths [2]

Noise Threshold: Auto [checked], %Relative Intensity []

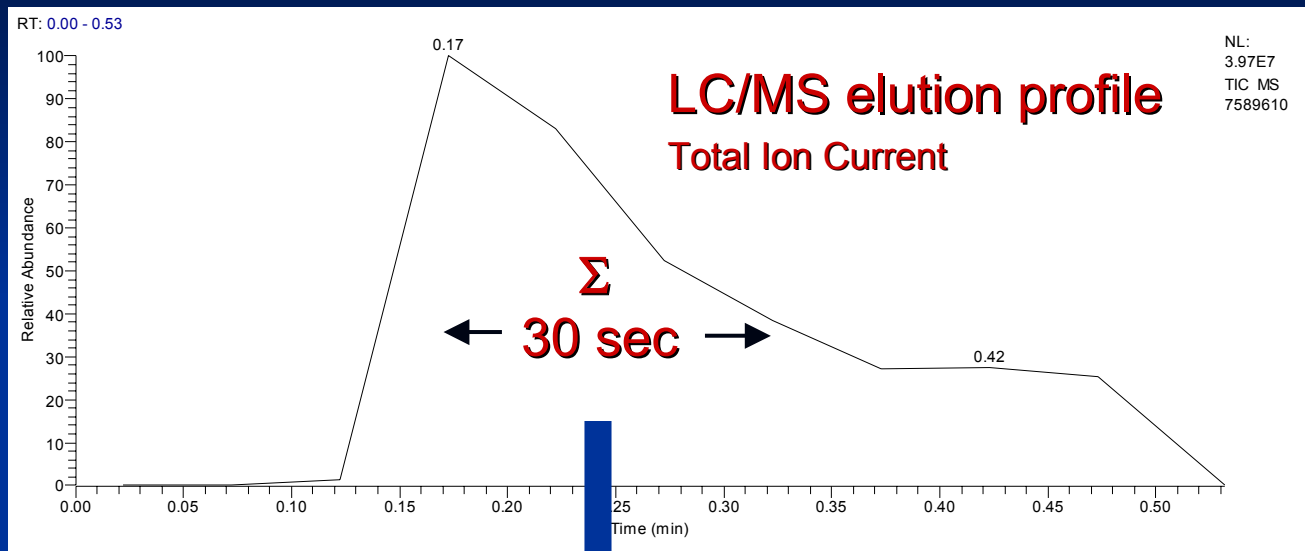


ProMass Sample Browser

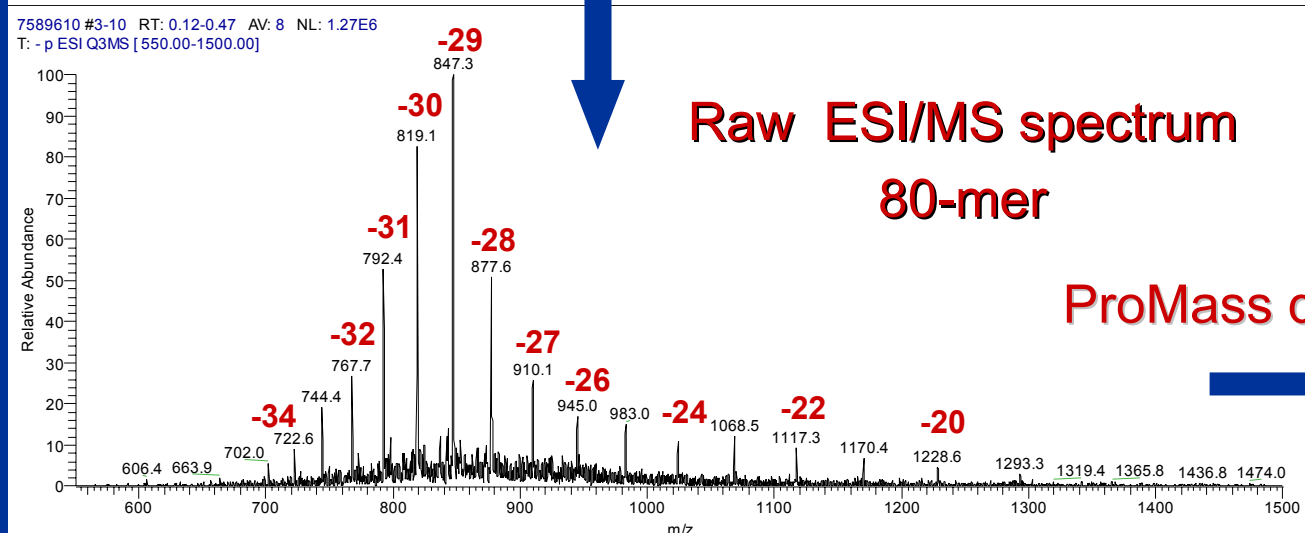
Sample ID	Sample Comments	Target Masses	Result Code
oligo01	test oligo 01 TEA/HFIPa pH 7.3 / 70% MeOH, PLEP tracers/ablate, 0.2 mL/min	[5348.7]	[]
oligo02	test oligo 02 cgc/CATCAACCCAC	[5573.9]	[]
oligo03	test oligo 03 cgc/ACCTAAGCACTGGG	[5494.6]	[]
oligo04	test oligo 04 CCGGGGCTGACCCACAGC	[5387.5]	[]
oligo05	test oligo 05 TATTTAATATATTACAGACACATTGCTG	[5616.3]	[]

HTML Results Report
including plate view,
spectra, chromatograms,
and tabular results

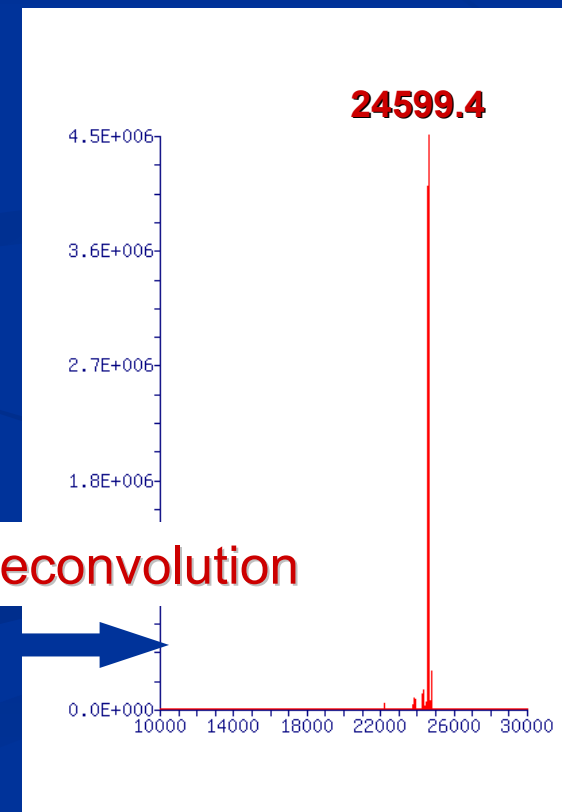
Automated Oligo ESI Mass Spectral Processing



Expected Mass = 24600 Da
Mass Error = 0.6 Da

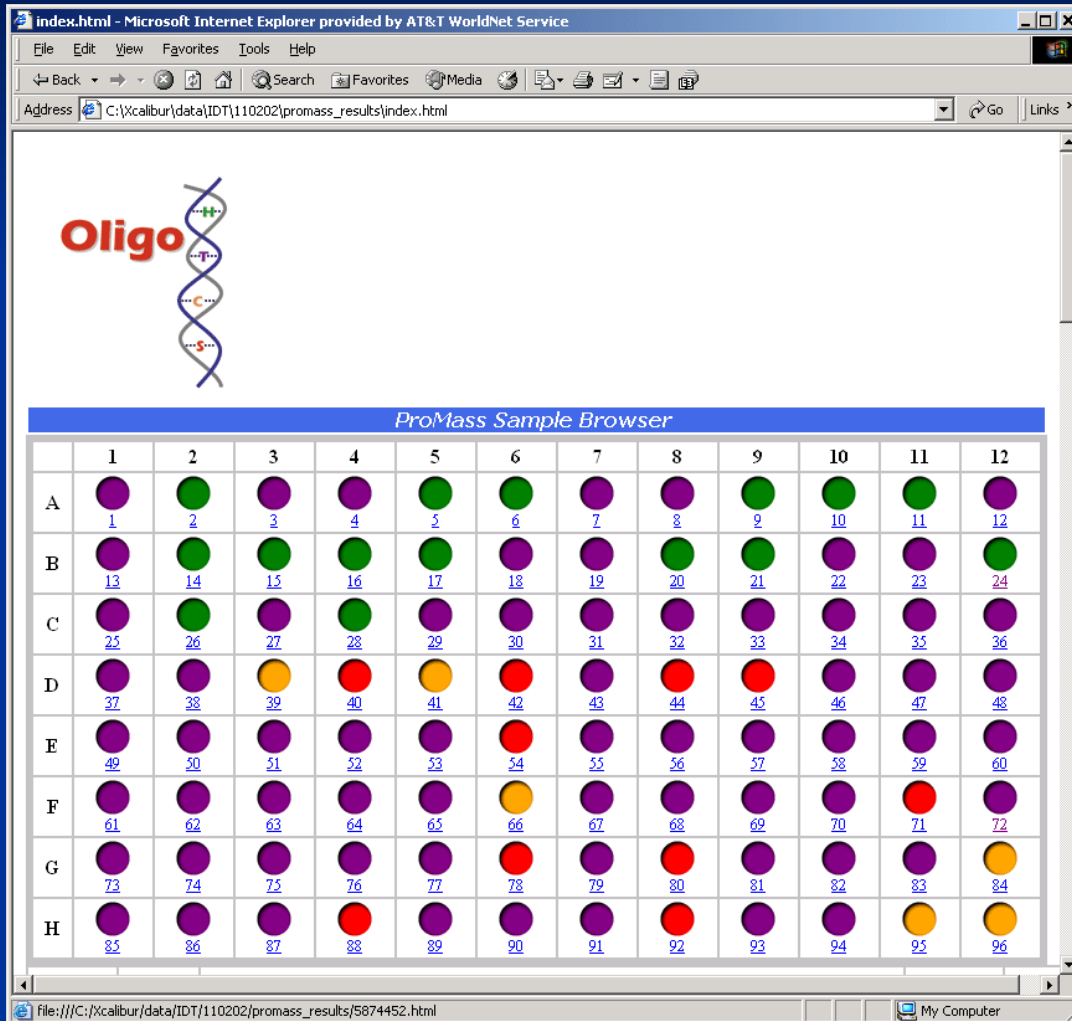


ProMass deconvolution







ProMass Sample Browser



96-well analysis of 50-130 mer oligos



- Color codes indicate presence or absence of target masses
- Color-coded wells are hyperlinked to detailed report

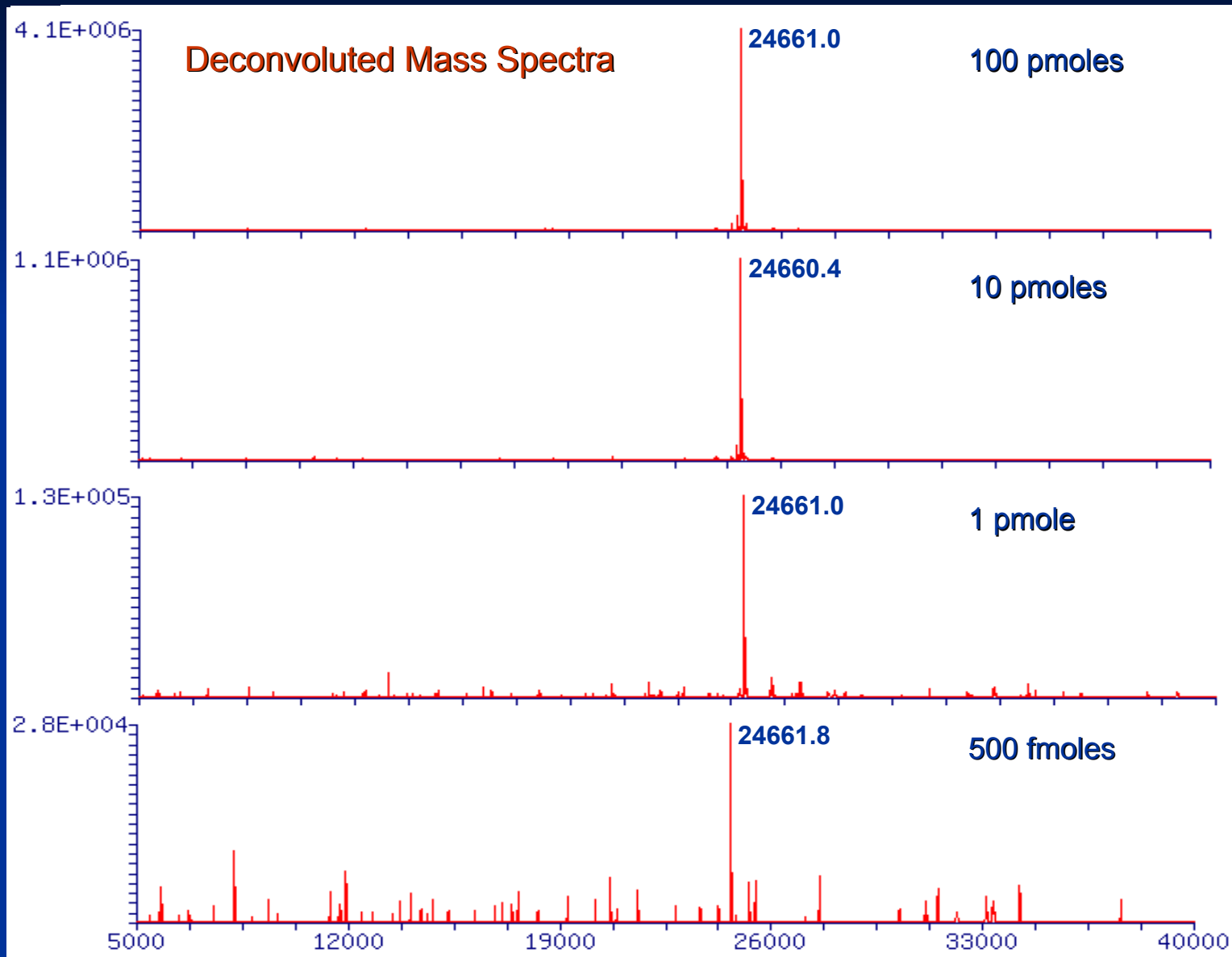
-  Target mass found as major component
-  Target mass found with other significant components
-  Target mass found but not as major peak
-  Target mass not found

96-well Plate-Level Mass Accuracy

- Average and Standard Deviation of Green  and Purple  wells from 3 plates run on different days
- **Plate 1 (70-mers):**
 - Average Mass Error: 0.0021% (21 ppm)
 - Standard Deviation: 0.0045% (45 ppm)
- **Plate 2 (70-mers):**
 - Average Mass Error: 0.0000% (0.4 ppm)
 - Standard Deviation: 0.0042% (42 ppm)
- **Plate 3 (50-130-mers):**
 - Average Mass Error: -0.0046% (-46 ppm)
 - Standard Deviation: 0.0046% (46 ppm)

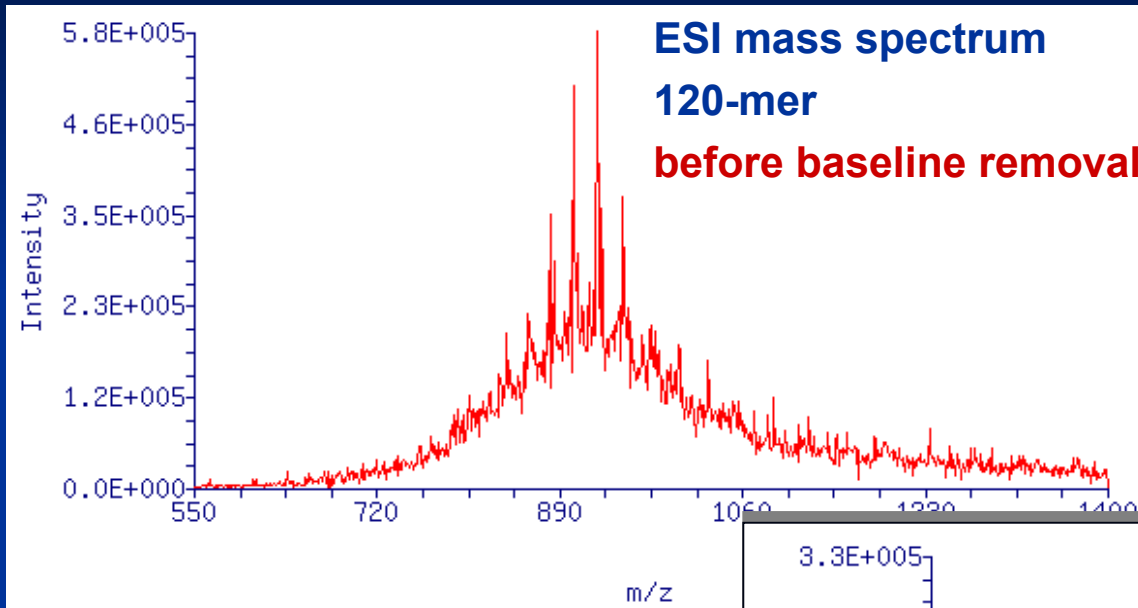
Oligo HTCS Sensitivity Study – 80 mer oligo

M = 24661.1



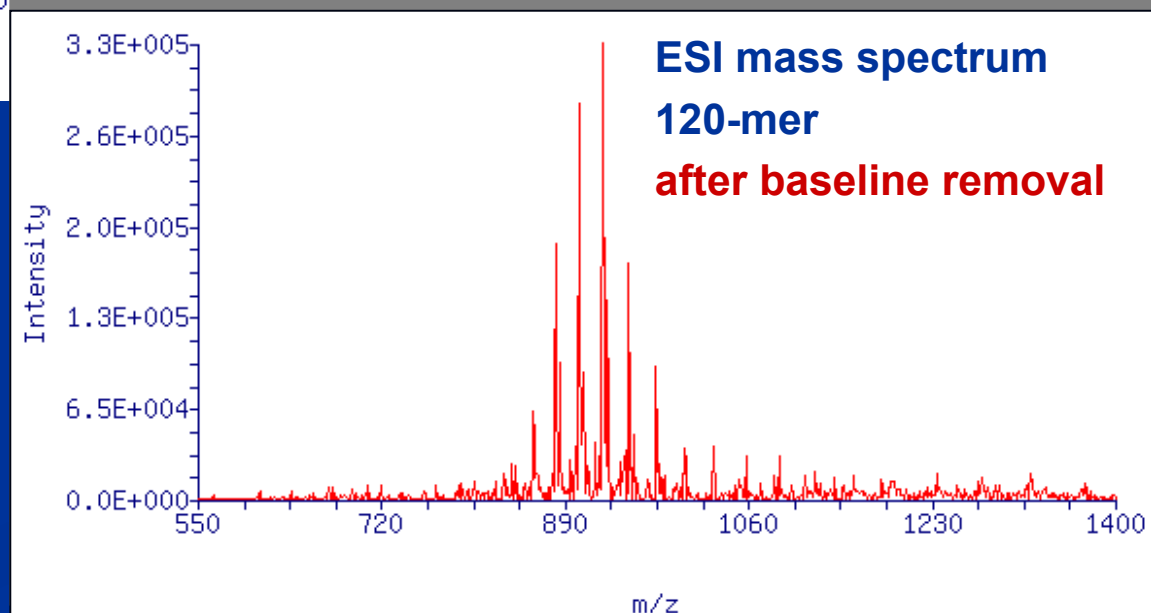
ESI Mass Spectra of Long Oligos

low purity makes analysis of long oligos challenging



- HT QC of crude synthesis products is highly desirable
- Low purity due to presence of multiple failure sequence products
- Causes baseline “hump” under ESI mass spectrum

Baseline removal is
key for reliable
deconvolution

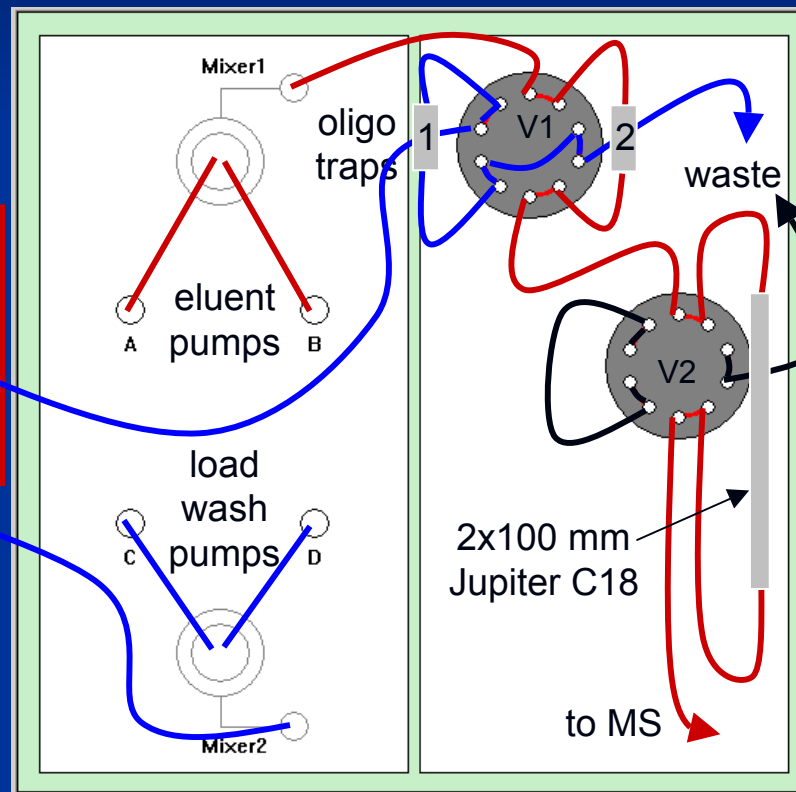
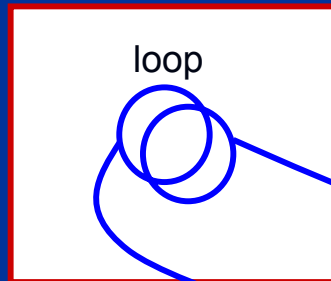


Oligo HTCS Plumbing Scheme

Shown in Detailed LC/MS Profiling Mode

Michrom Paradigm MS4 HPLC

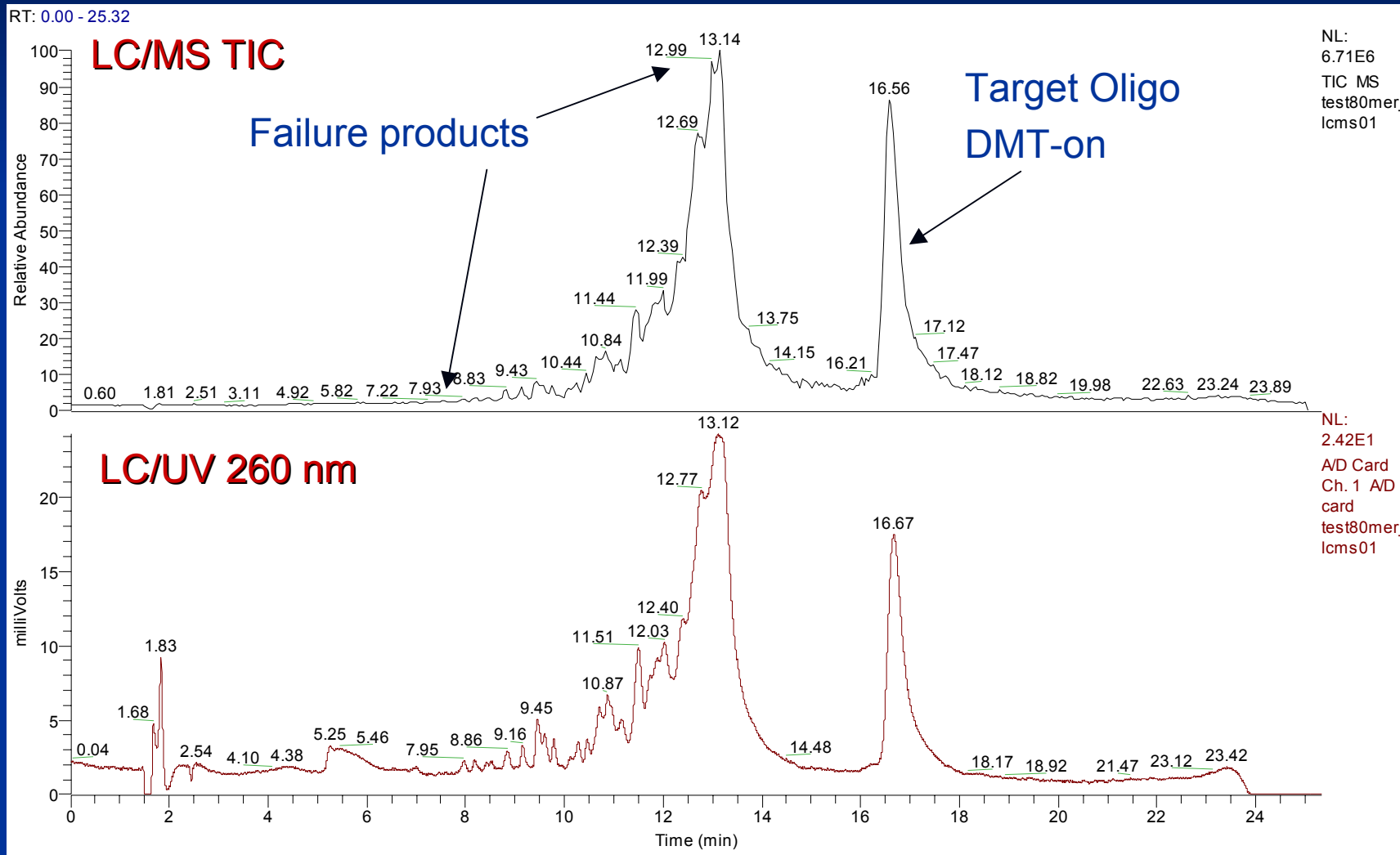
LEAP CTC-PAL
autosampler



- Switch from HT to detailed mode automatically
- V2 activates 2 x 100 mm C18 column
- Column wash w/ 100% D
- Useful for detailed profiling
- 5-40% B using 20 min gradient

LC/MS Analysis of Crude 80-mer Oligo

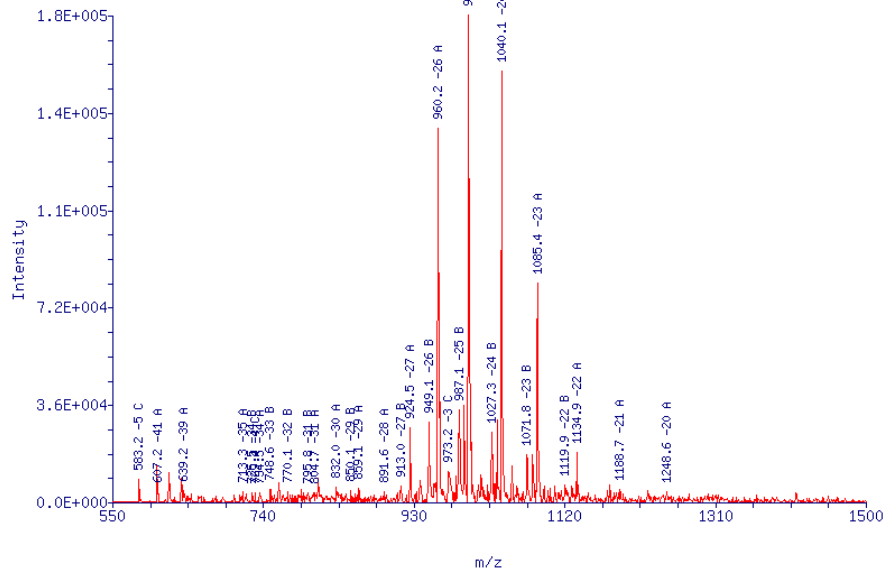
Jupiter C18 300A 2x100 mm, 5-40%B in 20 min



ESI Mass Spectra of DMT-on Oligo

80-mer from LC/MS run, RT = 16.5 min

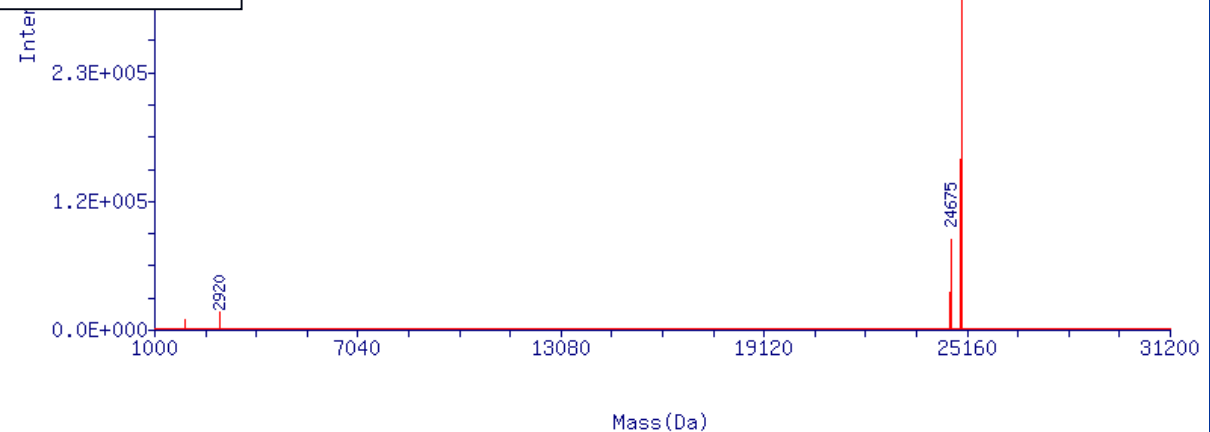
ESI/MS



ProMass Deconvolution

Expected Mass: 24988.4 Da

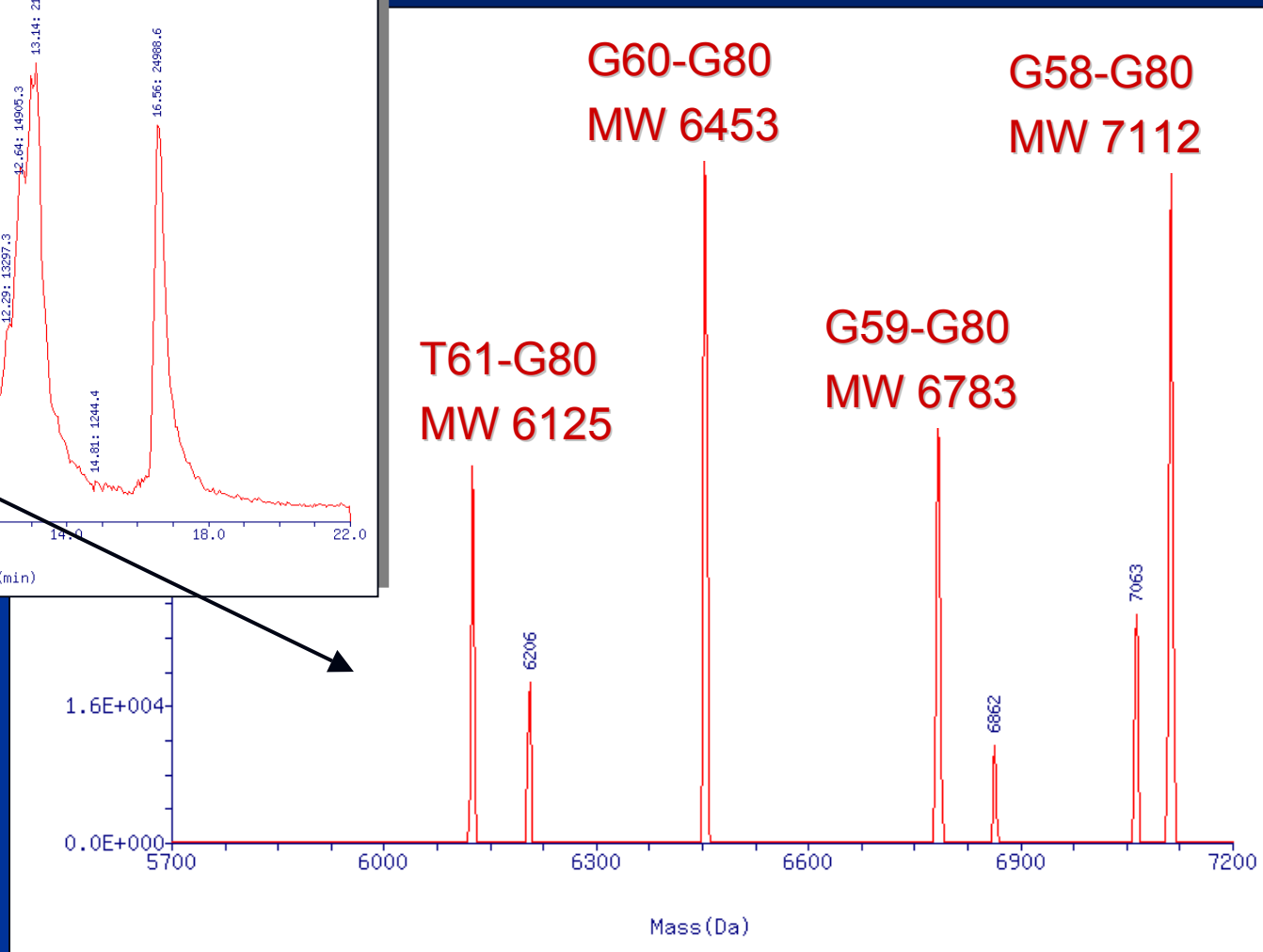
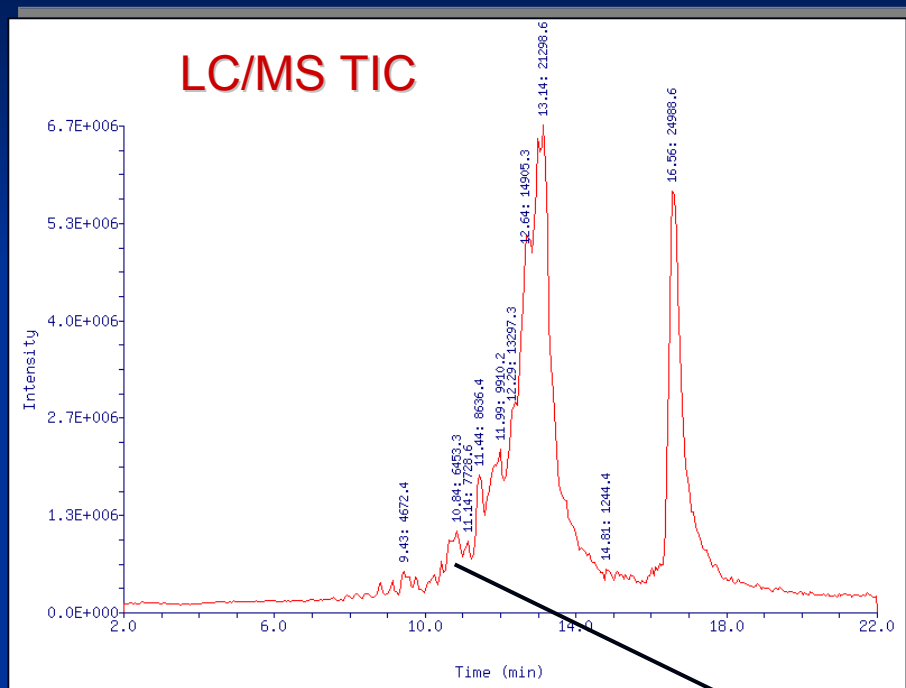
Observed Mass: 24988.6 Da



Detailed LC/MS Analysis of Failure Products

LC/MS of 80-mer

5'-TAATACGACTCACTATAGGGTAATACGACTCACTATAGGGTAATACGACTCACTATAGGG-3'



Conclusions

- A fully-automated system for HT oligo MS analysis was described
 - -ve ion detection with optimized mobile phase
 - High-speed on-line desalting
 - Automated ESI deconvolution and web-based reporting
 - Sample throughput of >1000 samples/day in HT MW confirmation mode
- MALDI-ToF and ESI-MS offer comparable mass accuracy below ~40-mers
- Better resolution with quadrupole ESI-MS vs. linear MALDI-ToF across entire mass range
- ESI-LCMS is the technique of choice for analysis of long and/or fragile oligonucleotides
 - “Softer” ionization
 - No degradation of mass resolution with increasing mass
 - Mass accuracy (< 100 ppm) across entire mass range
- Detailed LC/MS profiling capability on the same system via automatic valve switching

Acknowledgements

- Brian Elliott – IDT
- Co-workers – Jeff Whitney, David Detlefsen, Kathleen Anderson
- Kerry Nugent – Michrom BioResources