Introduction to Oligo HTCS Vanquish Automated Oligo LCMS System

Novatia, LLC

What is the Oligo HTCS Vanquish and what can it do?

- Automated ESI/MS that is capable of high-throughput analysis (~2400 samples/24 hrs (36 sec/sample)
- Can confirm targeted masses, as well as identify expected impurities and degradants
- Has routine mass accuracy of:
  - *± 0.01% with LTQ-XL MS*
  - *± 3 ppm (0.0003%) for Orbitrap Exploris MX using high resolution/accurate mass*
- Can analyze oligos >120 bases in length
- Standard system can switch from HT to detailed LC/MS mode automatically from within a run sequence
- Automatically processes data from HT or detailed runs and produces web-based reports
- Can estimate purity based on LC/UV/MS data

Oligo HTCS System Components

- Mass Spectrometers
  - *LTQ-XL ion trap, robust, sensitive, and easy-to-use MS systems (recently out of production)*
  - ***New** Orbitrap Exploris MX, benchtop high resolution accurate mass instrument*
- Oligo HTCS Vanquish includes:
  - *Vanquish Dual pump w/degasser*
  - *Vanquish Split Sampler, 4 tray capacity: 96-well, 384-well, or vial trays*
  - *2 x column compartments, 3 x 6-port valves*
  - *Optional Charger module for more plate capacity (9 or 20 tray options)*
- ProMass Deconvolution Software
  - *Automated charge deconvolution and reporting of average mass for oligonucleotide mass spectral data*
  - *ProMass HR determines exact (monoisotopic) mass within ± 3 ppm for isotopically resolved data*
- Xcalibur software control for ease-of-use
- Analytical methods
  - *Optimized for HT and detailed LC/MS analysis using negative ion detection*
  - *Optimized chemistries (buffers) for thorough desalting of samples*

ProMass Software Features for Oligo MS Analysis

- Integrated with Thermo Xcalibur data system
- *ZNova* algorithm for "*artifact-free"* charge deconvolution for isotopically unresolved data
- ProMass HR with PPL ReSpect deisotoping for automated exact mass analysis up to ~120 bases on Orbitrap instruments
- Automated deconvolution of spectra in LC/MS data processed from the MS sample list
- Target mass search capability
  - *calculated from oligo sequences, including multiple sequences*
  - *user-supplied explicit masses*
- Can confirm the presence of common impurities and degradants
  - *3', 5', or internal oligo fragments*
  - *depurination products*
  - *side reactions*
  - *adducts*
- Can estimate the sample purity from the LC/MS or LC/UV/MS data
  - *Correction of purity estimates based on co-eluters and known adducts*
- Web-based (HTML), Excel, and XML output

Oligo HTCS Vanquish with LTQ-XL

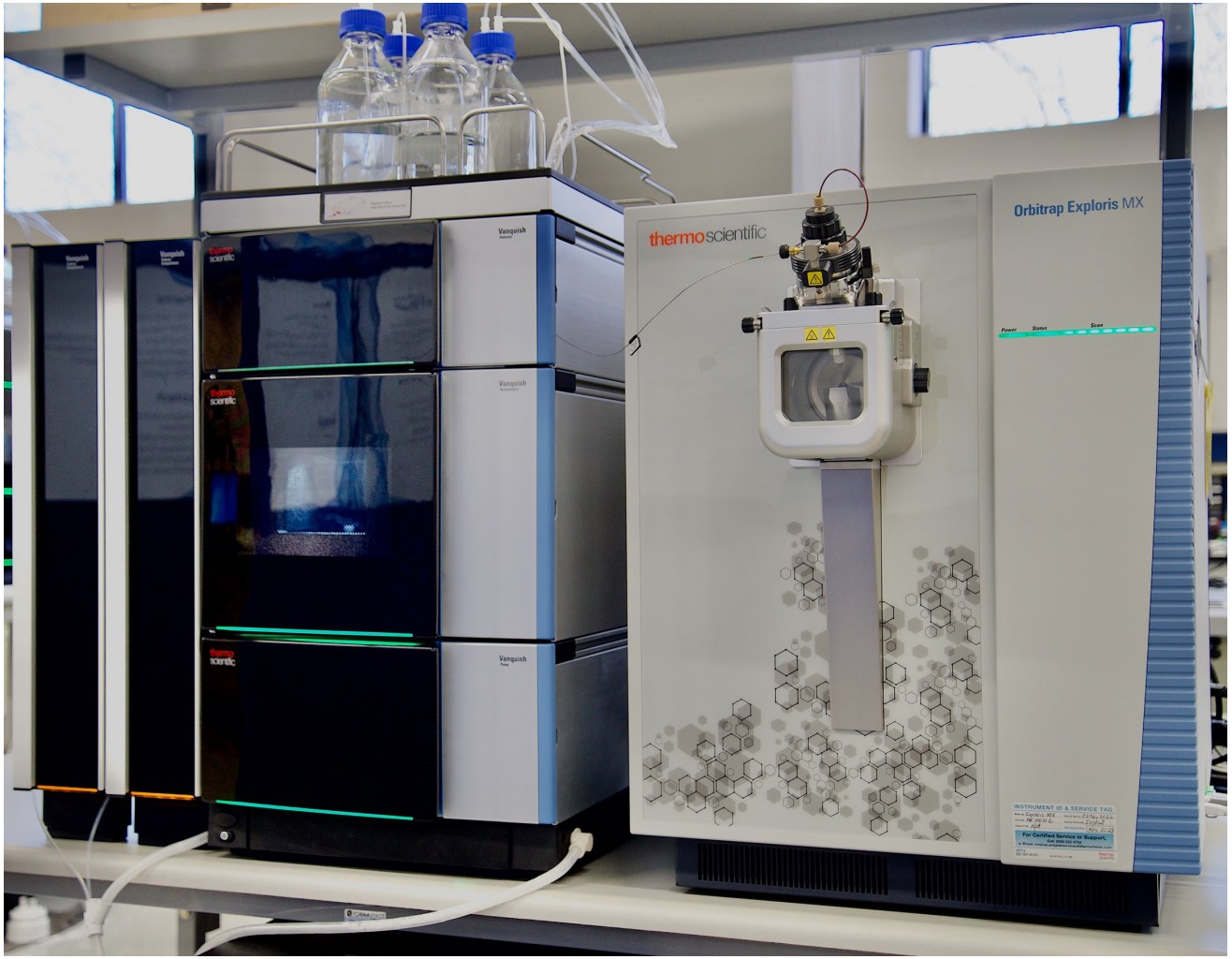
Vanquish UPLC with
Dual Pump F
Split Sampler FT
UV or DAD detector
Dual Column Ovens
3 x 6-port Valves

LTQ-XL
MS

~2400 sample/day max throughput

Oligo HTCS Vanquish MX

Vanquish UPLC with Orbitrap Exploris MX



~2400 sample/day max throughput

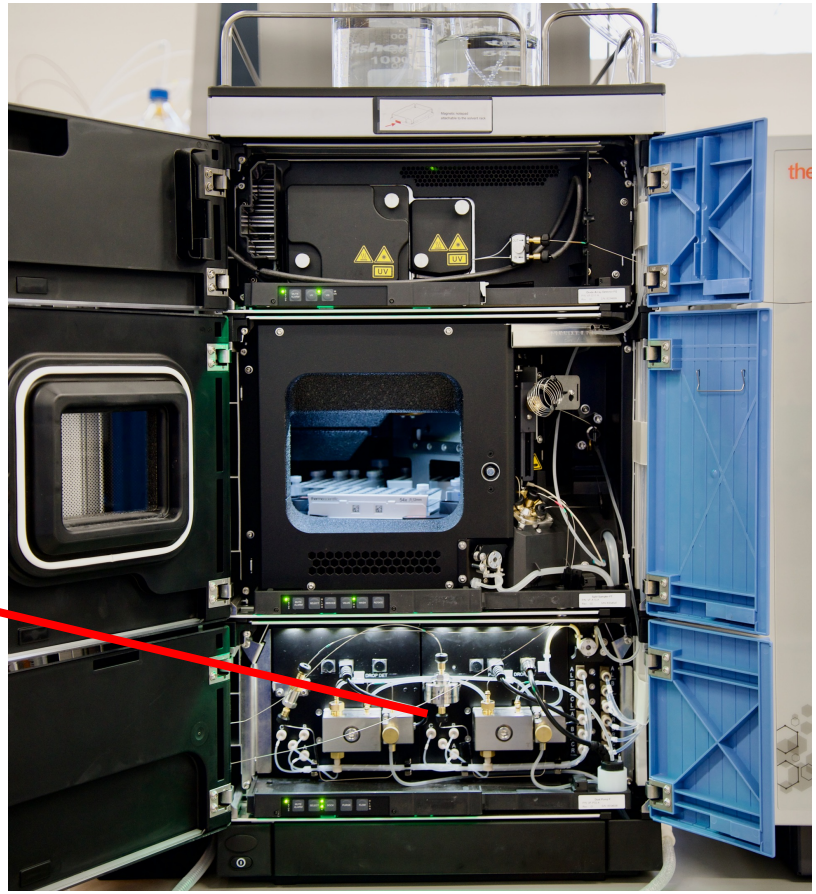
Vanquish UHPLC and Column Compartments

Dual Column Compartments
3 x 6-port Valves

Vanquish dual pump UHPLC

Vanquish UHPLC Dual Pump

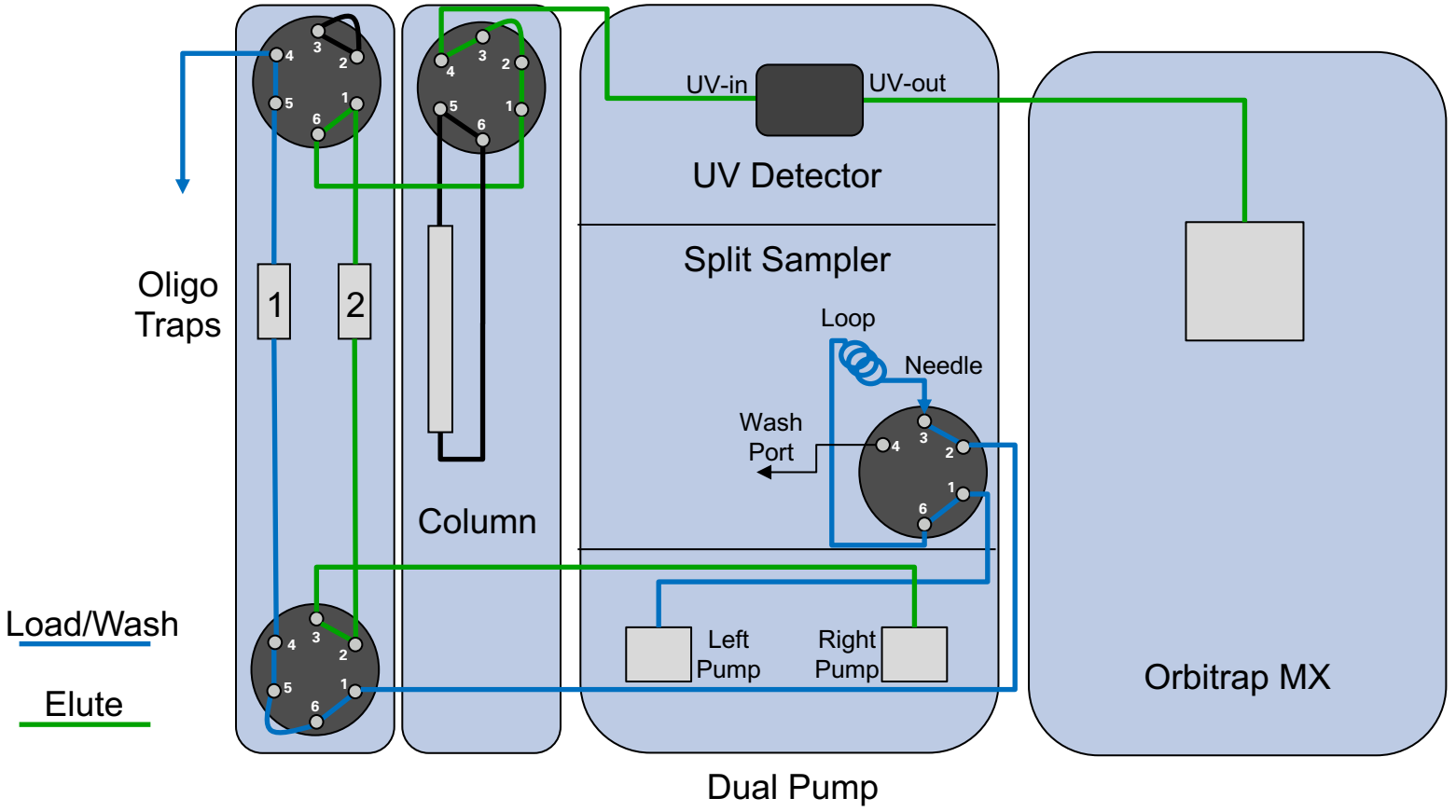
Dual pump
Left Pump for loading/washing
Right Pump for eluting



Vanquish dual pump UHPLC

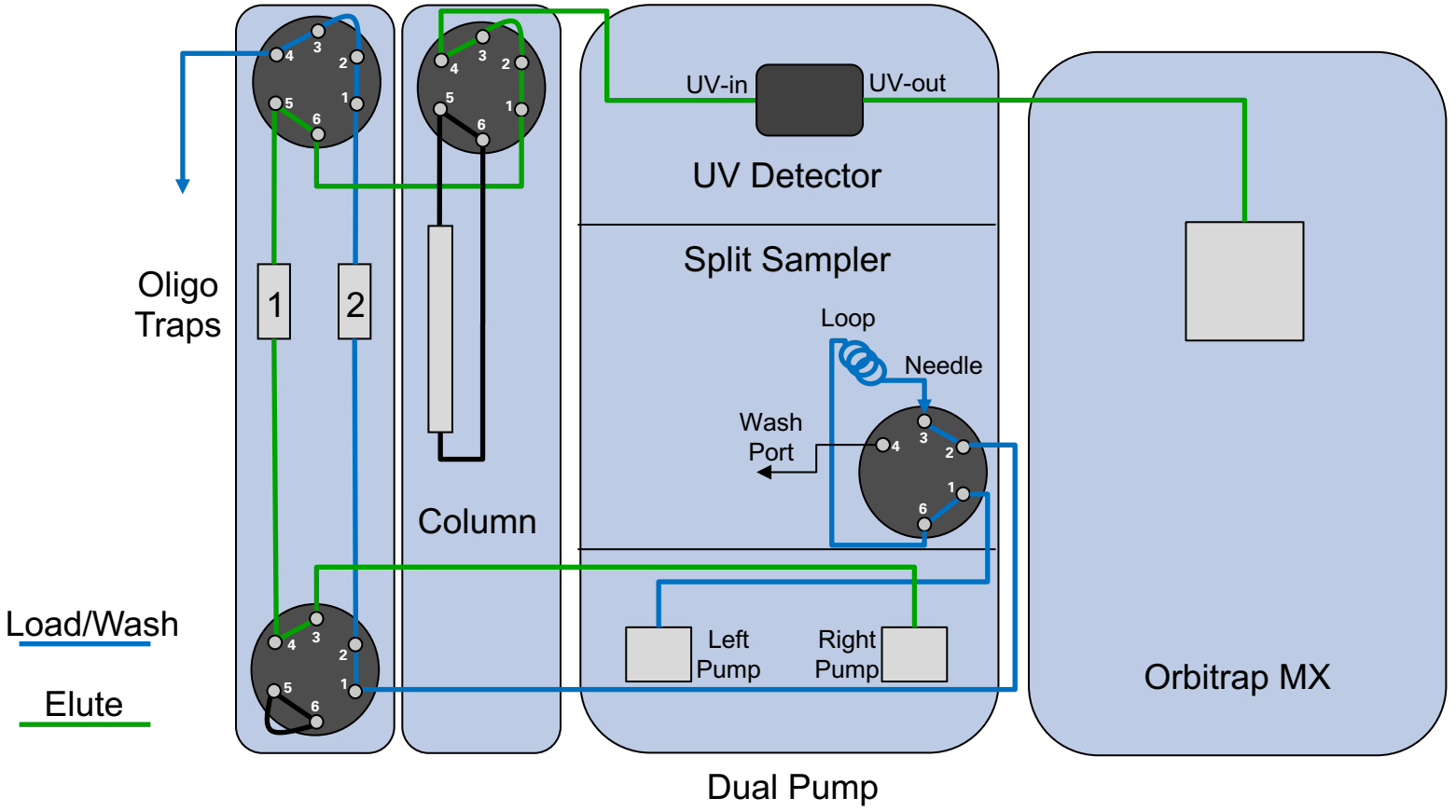
Oligo HTCS Vanquish – HT Desalting Mode

Trap 1 Desalting, Trap 2 Eluting



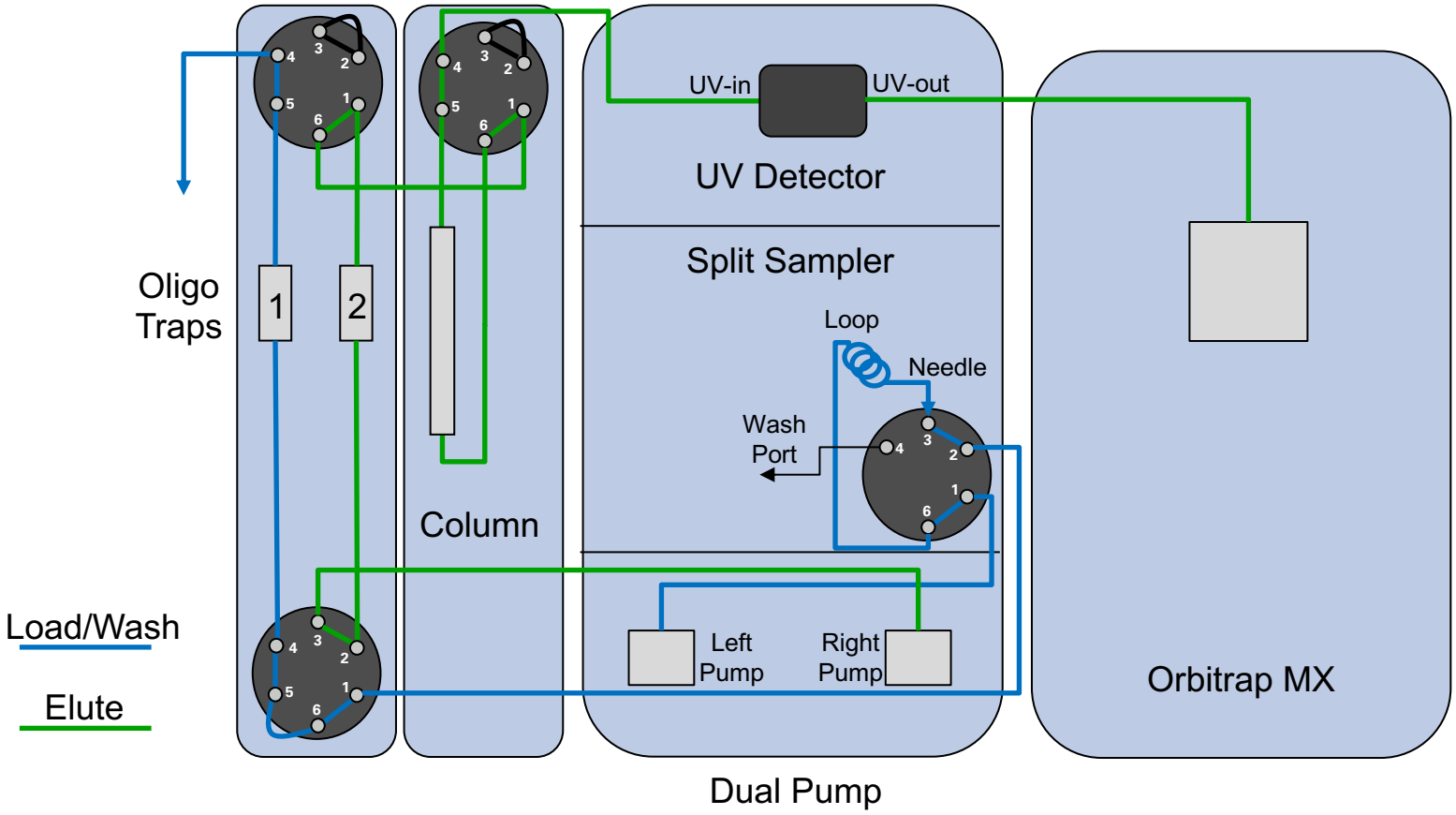
Oligo HTCS Vanquish – HT Desalting Mode

Trap 2 Desalting, Trap 1 Eluting

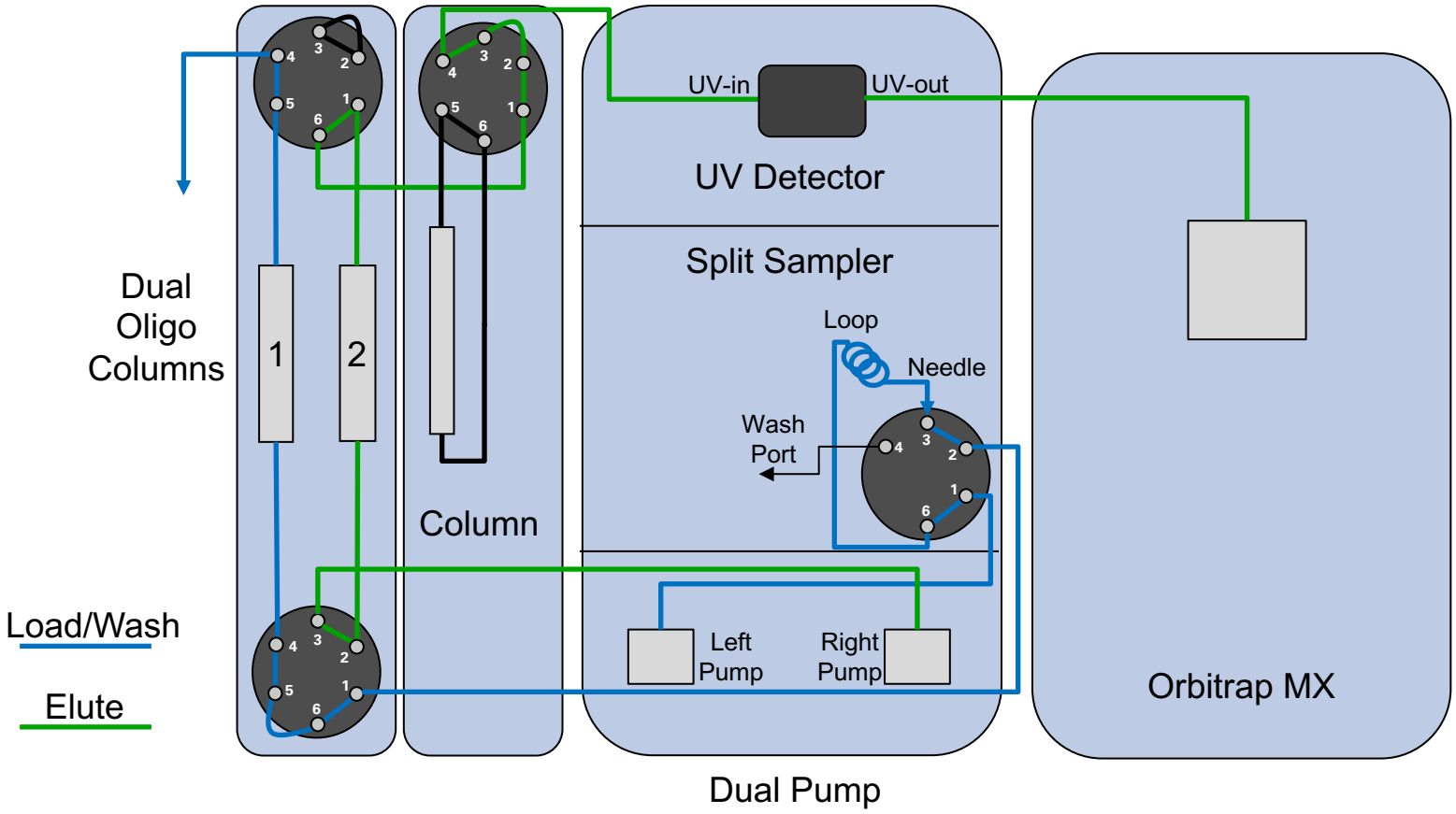


Oligo HTCS Vanquish – LC/MS Mode

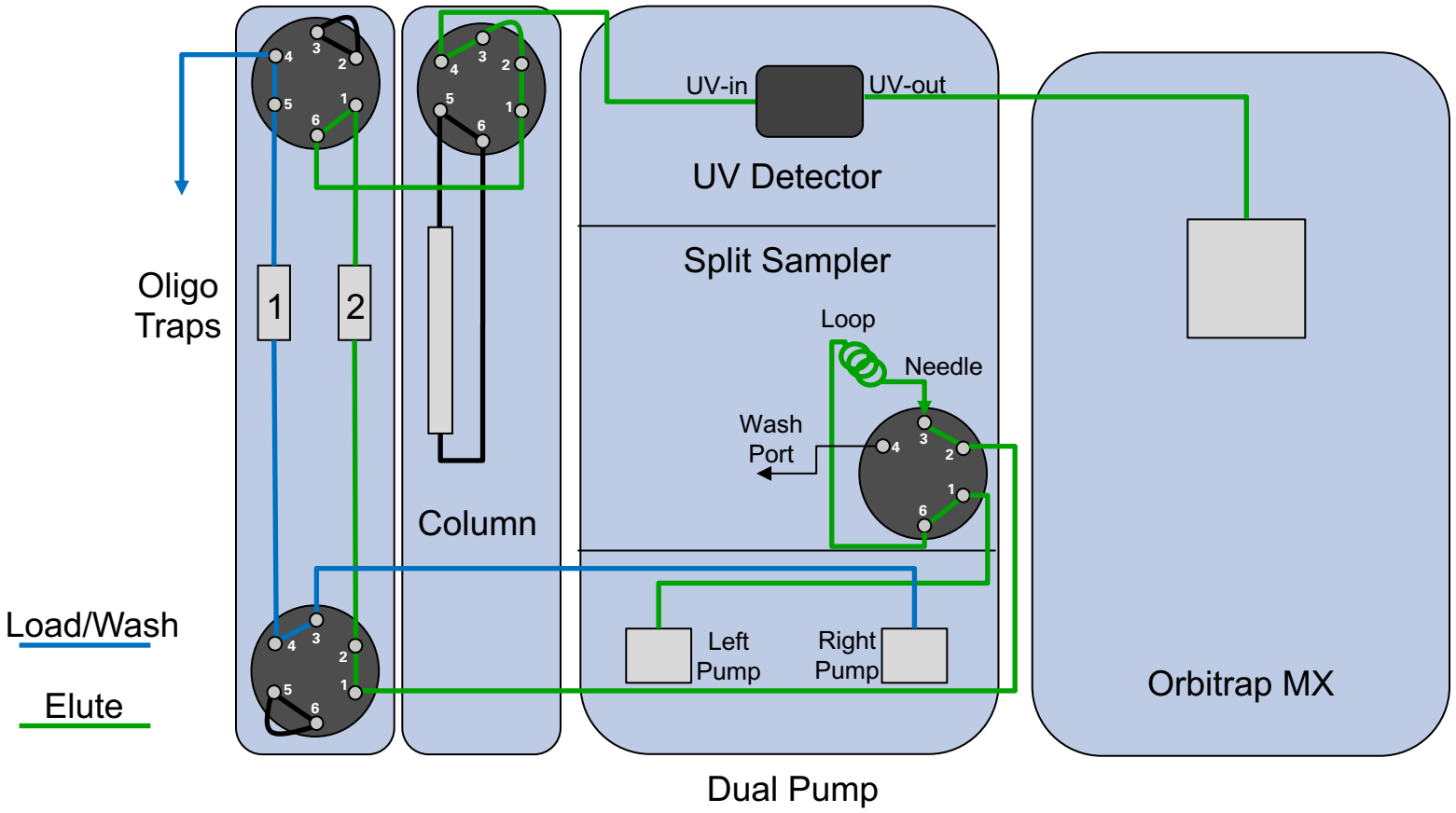
Gradient running through trap 2 and column



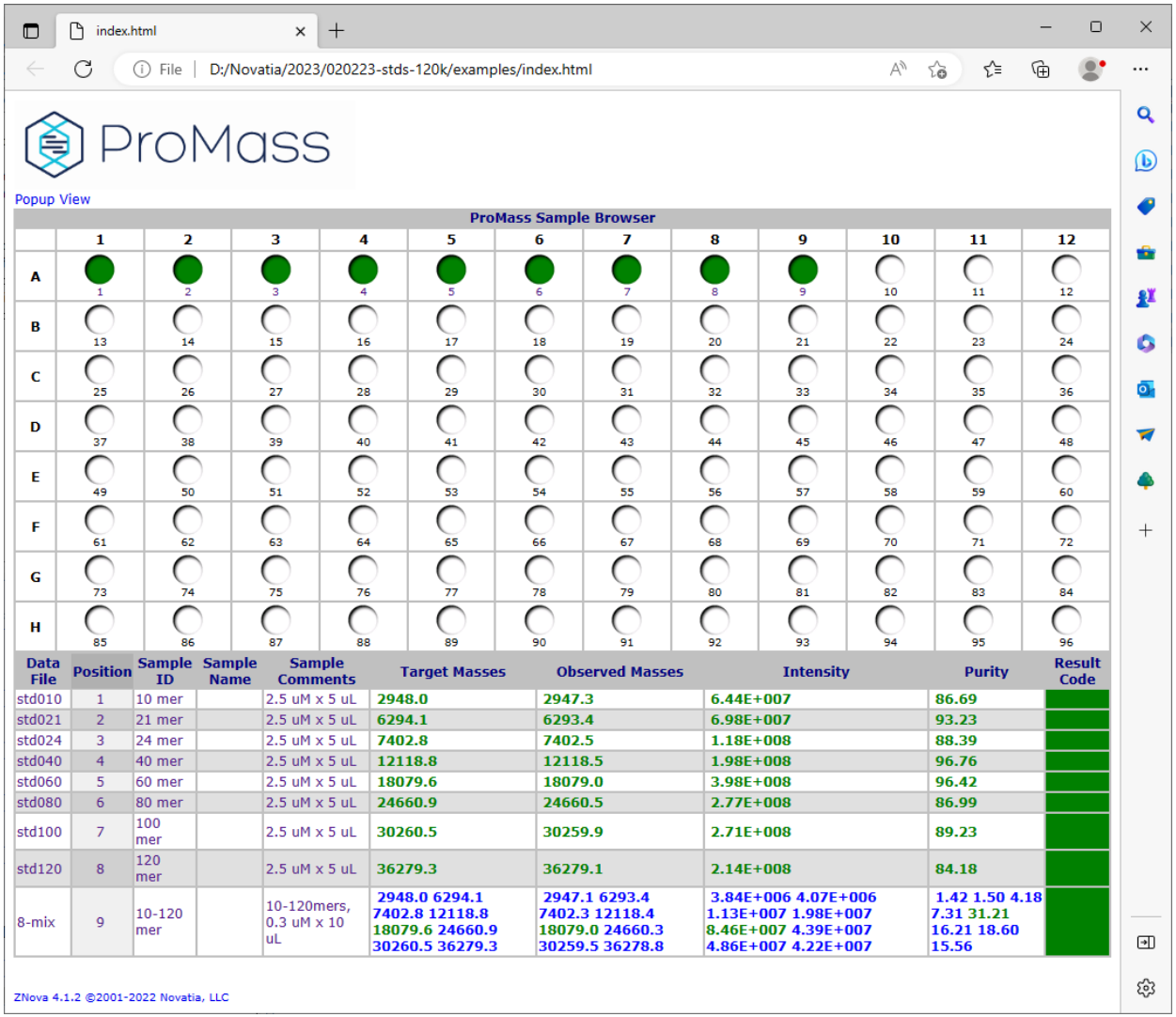
Dual Column LCMS Mode – Loading/Washing/Equilibrating
Column 1, Eluting Column 2



Direct Inject Mode – Injecting Trap 2, Cleaning Trap 1



Example Data: 10 – 120 mer oligonucleotides



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	Intensity	Purity	Result Code
std010	1	10 mer		2.5 uM x 5 uL	2948.0	2947.3	6.44E+007	86.69	
std021	2	21 mer		2.5 uM x 5 uL	6294.1	6293.4	6.98E+007	93.23	
std024	3	24 mer		2.5 uM x 5 uL	7402.8	7402.5	1.18E+008	88.39	
std040	4	40 mer		2.5 uM x 5 uL	12118.8	12118.5	1.98E+008	96.76	
std060	5	60 mer		2.5 uM x 5 uL	18079.6	18079.0	3.98E+008	96.42	
std080	6	80 mer		2.5 uM x 5 uL	24660.9	24660.5	2.77E+008	86.99	
std100	7	100 mer		2.5 uM x 5 uL	30260.5	30259.9	2.71E+008	89.23	
std120	8	120 mer		2.5 uM x 5 uL	36279.3	36279.1	2.14E+008	84.18	
8-mix	9	10-120 mer		10-120mers, 0.3 uM x 10 uL	2948.0 6294.1 7402.8 12118.8 18079.6 24660.9 30260.5 36279.3	2947.1 6293.4 7402.3 12118.4 18079.0 24660.3 30259.5 36278.8	3.84E+006 4.07E+006 1.13E+007 1.98E+007 8.46E+007 4.39E+007 4.86E+007 4.22E+007	1.42 1.50 4.18 7.31 31.21 16.21 18.60 15.56	

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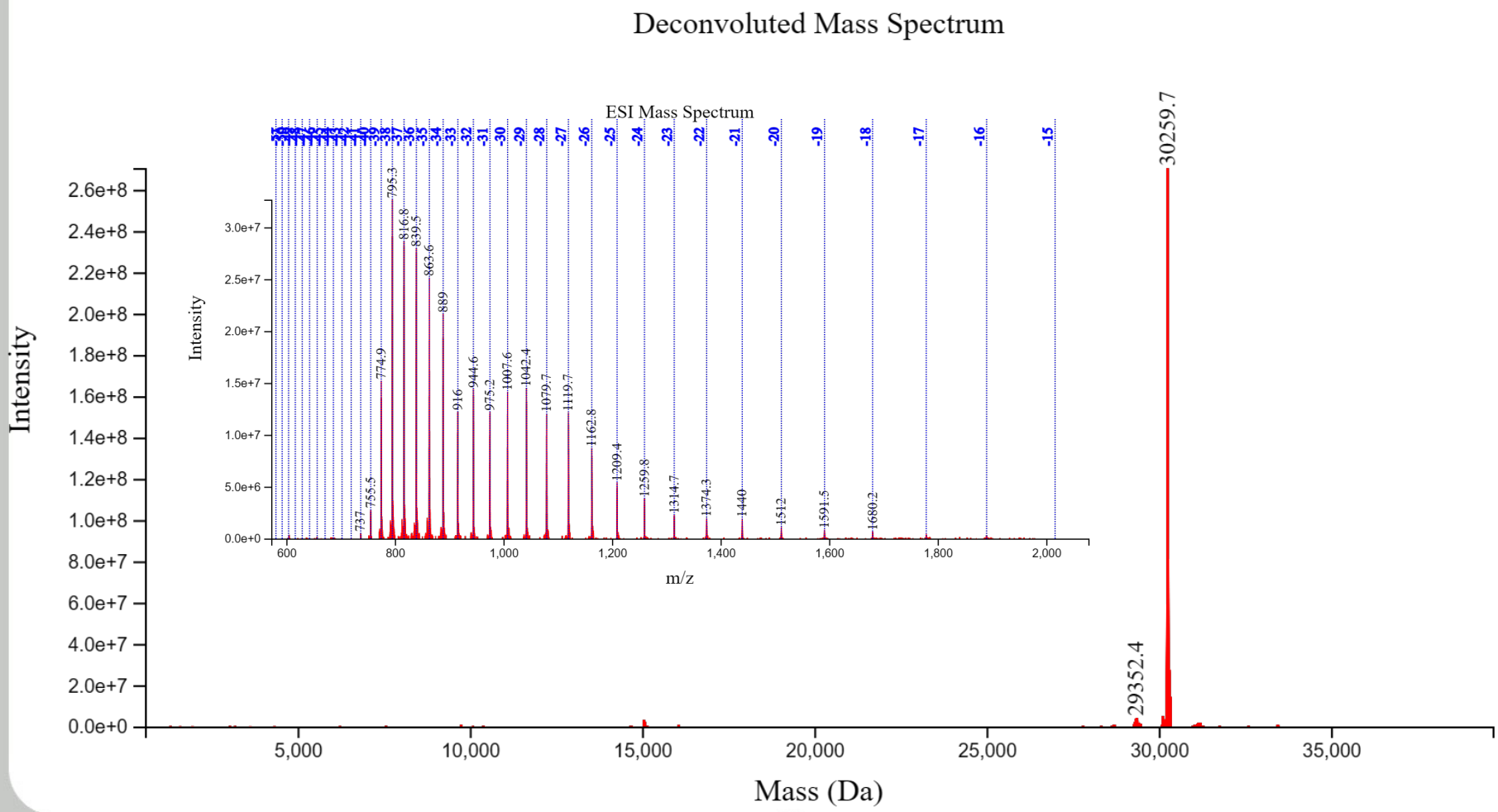
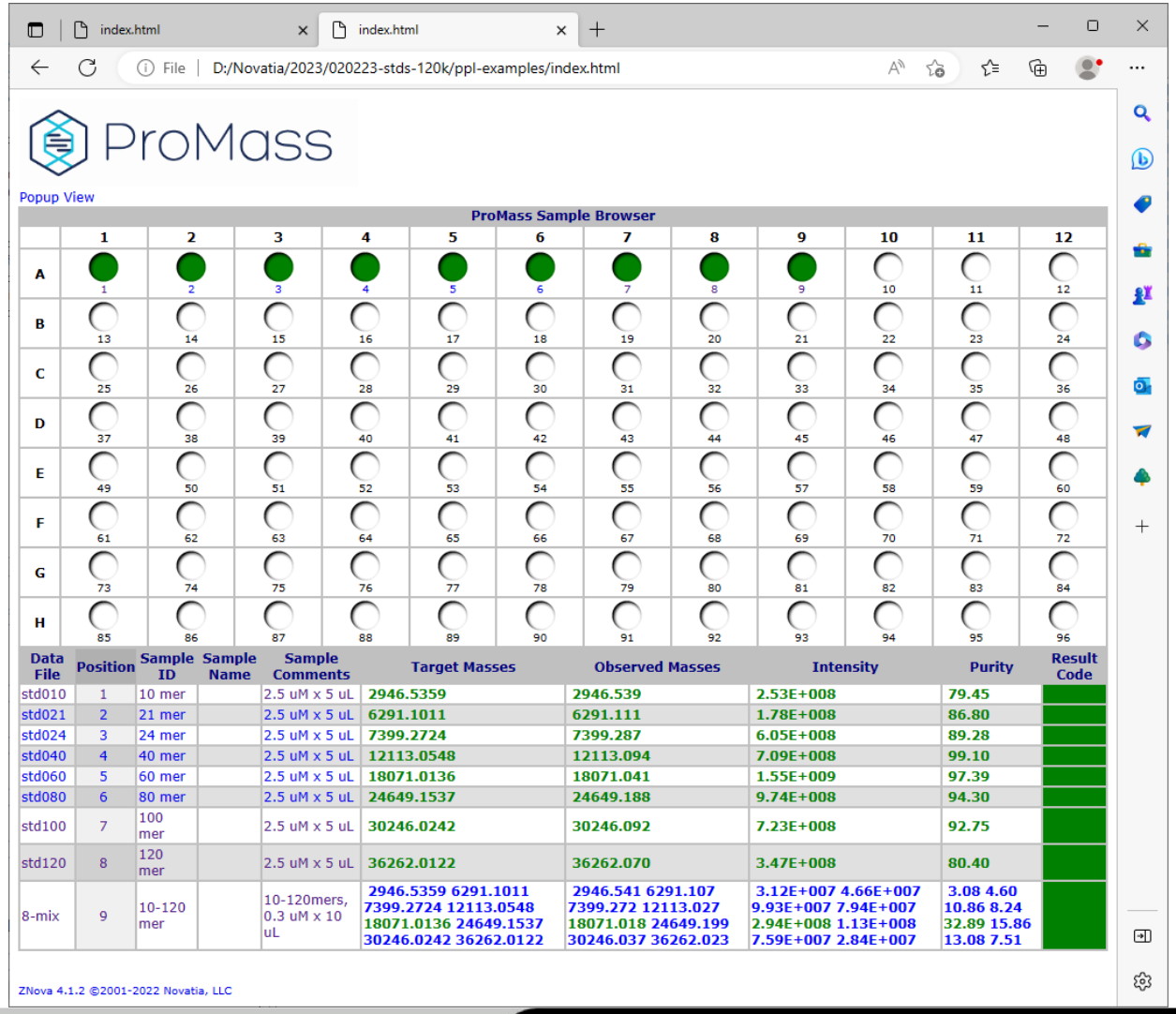
Oribtrap MX, 100 mer oligonucleotide



Example Data: Accurate Mass Determination



index.html | index.html

File | D:/Novatia/2023/020223-stds-120k/ppl-examples/index.html

ProMass

Popup View

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	Intensity	Purity	Result Code
std010	1	10 mer		2.5 uM x 5 uL	2946.5359	2946.539	2.53E+008	79.45	
std021	2	21 mer		2.5 uM x 5 uL	6291.1011	6291.111	1.78E+008	86.80	
std024	3	24 mer		2.5 uM x 5 uL	7399.2724	7399.287	6.05E+008	89.28	
std040	4	40 mer		2.5 uM x 5 uL	12113.0548	12113.094	7.09E+008	99.10	
std060	5	60 mer		2.5 uM x 5 uL	18071.0136	18071.041	1.55E+009	97.39	
std080	6	80 mer		2.5 uM x 5 uL	24649.1537	24649.188	9.74E+008	94.30	
std100	7	100 mer		2.5 uM x 5 uL	30246.0242	30246.092	7.23E+008	92.75	
std120	8	120 mer		2.5 uM x 5 uL	36262.0122	36262.070	3.47E+008	80.40	
8-mix	9	10-120 mer		10-120mers, 0.3 uM x 10 uL	2946.5359 6291.1011 7399.2724 12113.0548 18071.0136 24649.1537 30246.0242 36262.0122	2946.541 6291.107 7399.272 12113.027 18071.018 24649.199 30246.037 36262.023	3.12E+007 4.66E+007 9.93E+007 7.94E+007 2.94E+008 1.13E+008 7.59E+007 2.84E+007	3.08 4.60 10.86 8.24 32.89 15.86 13.08 7.51	

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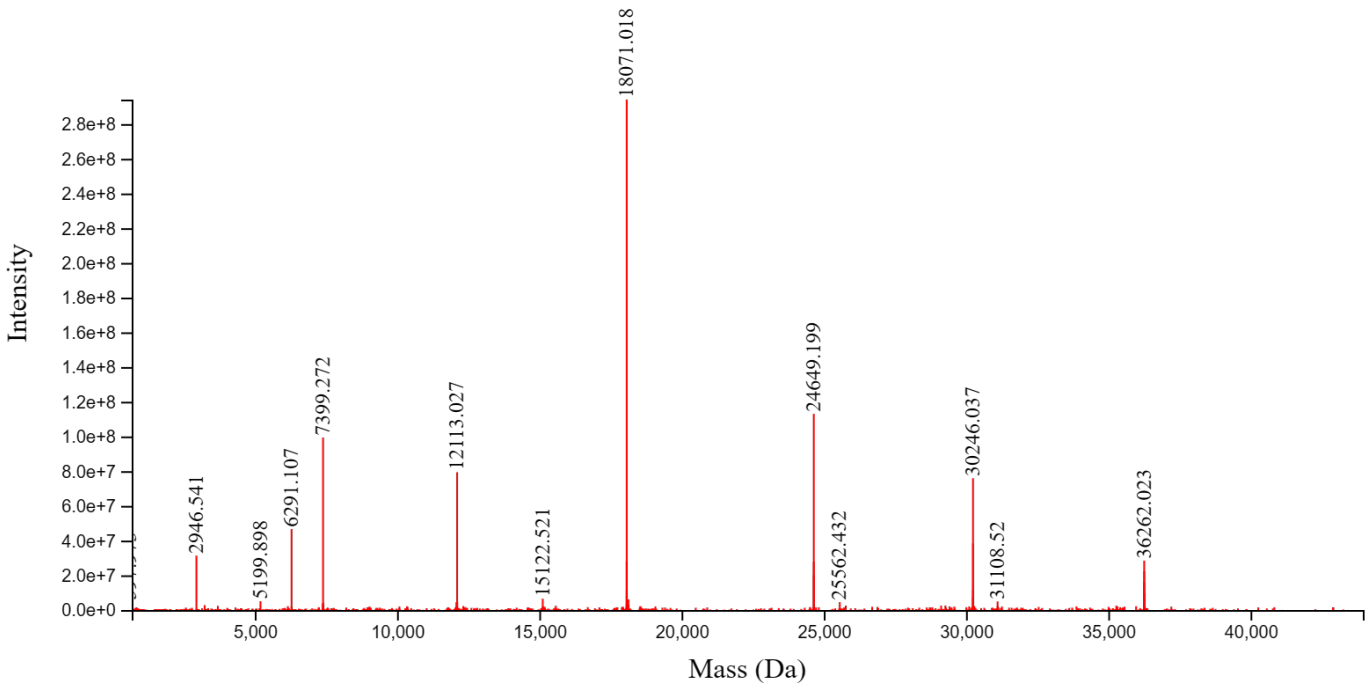
Accurate masses of 8-component mixture

Target Mass Summary

RT (min)	Target Mass (Da)	Observed Mass (Da)	Mass Error	Intensity	% Abundance (in Spectrum)	%Purity (Estimate)	Identity	Result Code
0.341	36262.0122	36262.023	0.0108 Da (0.3 ppm)	2.84E+007	2.81	7.51	Target Mass	
0.341	30246.0242	30246.037	0.0128 Da (0.4 ppm)	7.59E+007	7.49	13.08	Target Mass	
0.341	24649.1537	24649.199	0.0453 Da (1.8 ppm)	1.13E+008	11.15	15.86	Target Mass	
0.341	18071.0136	18071.018	0.0044 Da (0.2 ppm)	2.94E+008	29.03	32.89	Target Mass	
0.341	12113.0548	12113.027	-0.0278 Da (-2.3 ppm)	7.94E+007	7.83	8.24	Target Mass	
0.341	7399.2724	7399.272	-0.0004 Da (-0.1 ppm)	9.93E+007	9.81	10.86	Target Mass	
0.341	6291.1011	6291.107	0.0059 Da (0.9 ppm)	4.66E+007	4.60	4.60	Target Mass	
0.341	2946.5359	2946.541	0.0051 Da (1.7 ppm)	3.12E+007	3.08	3.08	Target Mass	

Deconvoluted Mass Spectrum