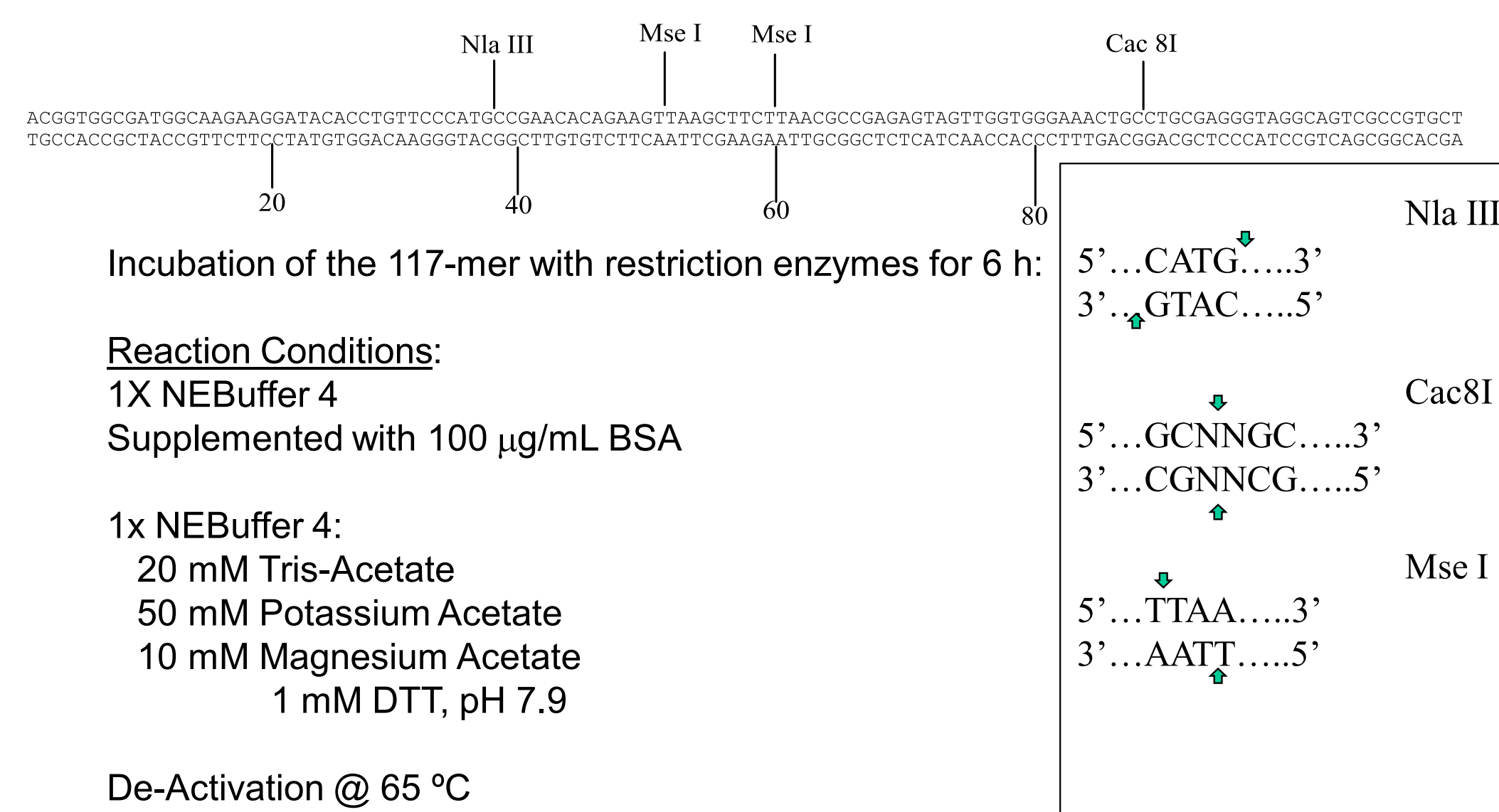


## Abstract

- Purpose** To determine the sequence of a 117-mer DNA duplex from *Lactobacillus johnsonii* F19785 using high-resolution mass spectrometer. Three restriction enzymes are used to process the target oligonucleotide into oligomers suitable (<40 nt) for MS analysis. A series of ten oligonucleotides are produced.
- Methods** This study utilized a hybrid linear ion trap, the Thermo Scientific LTQ XL™ Orbitrap mass spectrometer (LC-ESI-MS/MS) used in conjunction with an Accela™ high-speed chromatographic system. Development began with restriction enzyme mapping followed by U-HPLC separation using reversed phase C18 columns (Phenomenex Acquity UPLC OST column). The mobile phase compositions were 1,1,1,3,3,3-hexafluoro-2-propanol/triethylamine (pH 8.3) in methanol. Sequence analysis was carried out using through MSn analyses number. ESI mass spectra were acquired in negative ion mode. Fragment ions were calculated using in-house OligoSeq Ion Calculator and compared with observed fragments.
- Results** Confirmation of sequence was determined. With the proper use of restriction enzymes, nucleotide sequencing may be a useful tool for the identification of longer oligonucleotides such as aptamers or tRNA.
- Conclusion** The advantage using an LTQ-Orbitrap to confirm the sequence identity of the oligonucleotides is its ability to provide high-resolution mass data with the LC-MS analysis. Finally, the method employed novel validated software for mass calculation of all oligonucleotides.

## Experimental

*Lactobacillus johnsonii* is a member of the acidophilus group of lactobacilli. Because of their probiotic properties, including attachment to epithelial cells, immunomodulation, and competitive exclusion of pathogens, representatives of this group are being intensively studied.



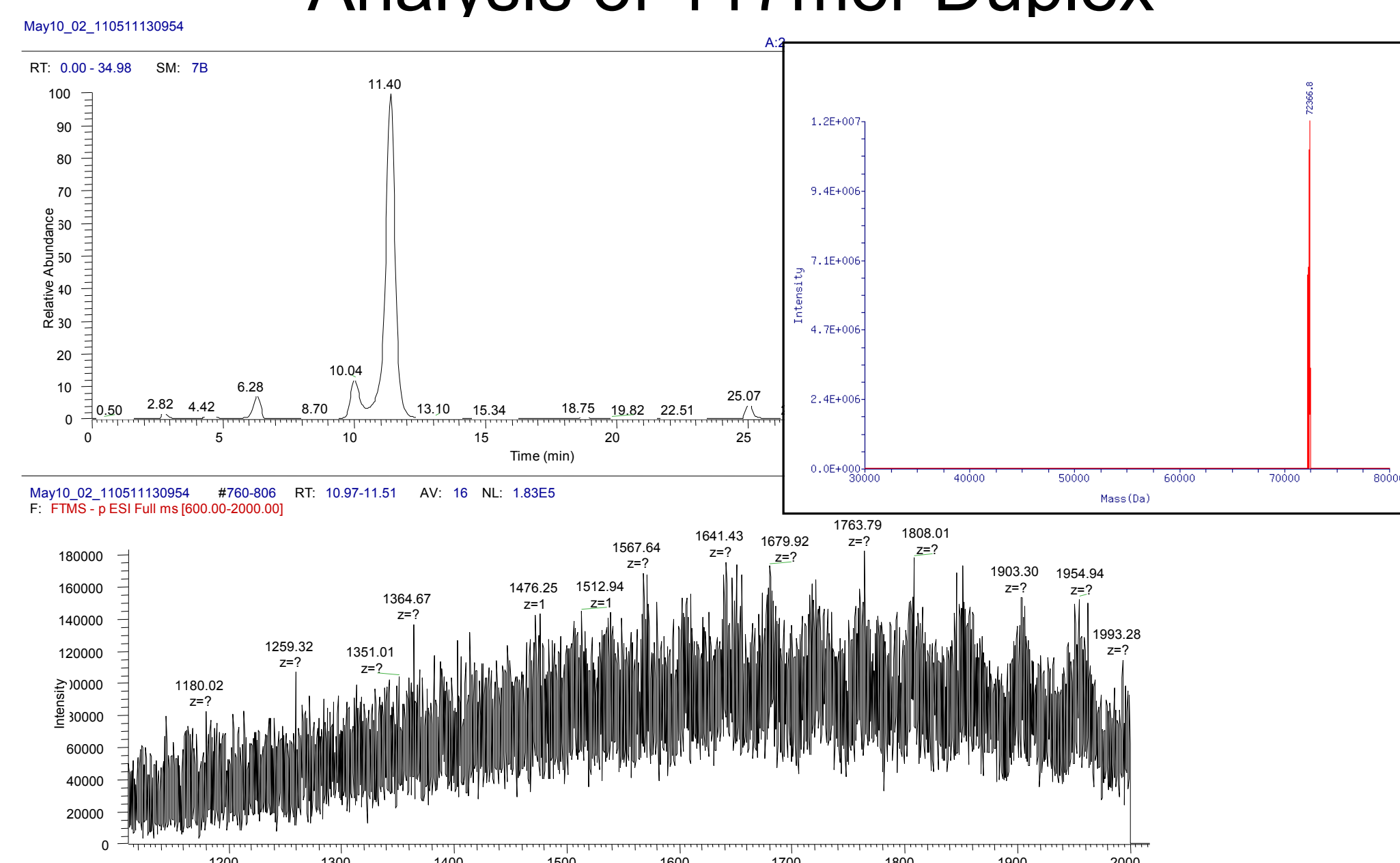
LTQ-Orbitrap with Accela - Direct Infusion Conditions DNA Solution – Oligonucleotide in 5% Triethylamine/25%CH<sub>3</sub>CN and 70% DNase, RNase-free Water Infused at 10 µL/min; LC flow 200 µL/min 50% CH<sub>3</sub>OH/Water with 100 mM; HFIP/8.6 mM TEA added Tuned for maximum LC/MS sensitivity; **Mass Spectrometer Parameters:** Electrospray Voltage: 2.0-2.2 kV Capillary Voltage: -202.5 V; Sheath Gas: 60 arbitrary units; Aux N<sub>2</sub> Gas: 0 arbitrary units; Capillary Temp: 200 °C; Xcalibur 2.1 on Wm XP Platform; ProMass 2.5 SR-1; Accela Parameters: High Pressure Mode (7000 psi to 16000 psi); Column Compartment: Ambient; Autosampler; Temperature: 5°C Flow Monitored 260 nm

## References

- Wegmann, U.; Overweg, K.; Horn, N.; Goesmann, A.; Narbad, A.; Gasson, M.J.; Shearman, C. "Complete Genome Sequence of *Lactobacillus johnsonii* F19785, a Competitive Exclusion Agent against Pathogens in Poultry" *J. Bacteriology*. 2009, 191, 7142-7143.
- Dai, G.; Wei, X.; Liu, Z.; Liu, S.; Marcucci, G.; Chan, K. K. "Characterization and quantification of Bol-2 antisense G3139 and metabolites in plasma and urine by ion-pair reverse phase HPLC coupled with electrospray ion-trap mass spectrometry" *J. Chromatogr. B*. 2005, 825, 201-213.
- Gilar, M.; Fountain, K. J.; Budman, Y.; Hoyokey, J. L.; Davoudi, H.; Gebler, J.C. "Characterization of Therapeutic Oligonucleotides Using Liquid Chromatography with On-line Mass Spectrometry Detection" *Oligonucleotides* 2003, 13, 229-243.

## Results

### Analysis of 117mer Duplex

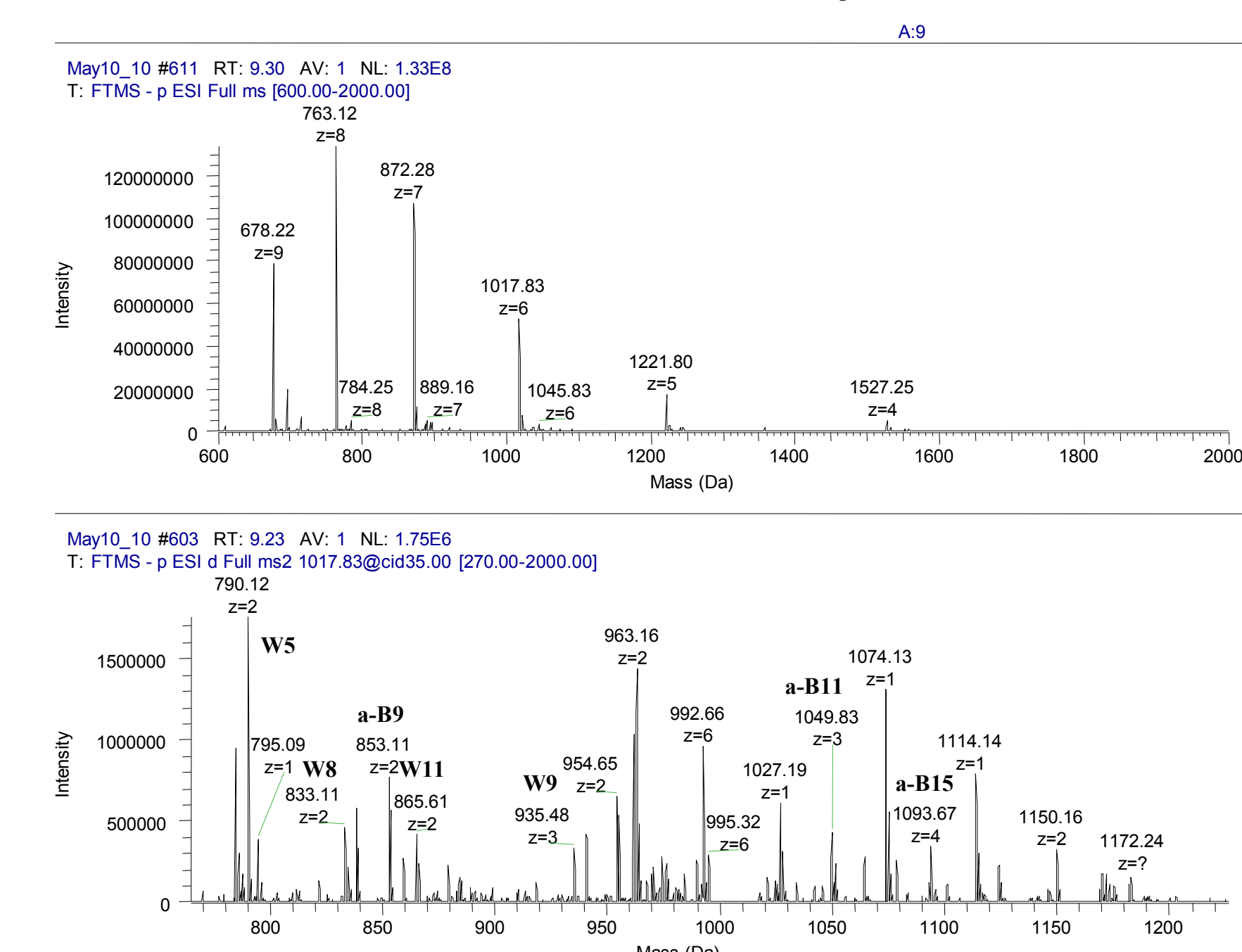


Full scale UV (top), FIMS (middle) and Mass Deconvolution (inset) of DNA Duplex 117-mer. Conditions: Sample Load: 5 µL, Flow rate: 477 µL/min, Temperature: 25°C, Mobile Phase A Composition: 100 mM hexafluoroisopropanol (HFIP)/ triethylamine (TEA) (pH 8.35) in RNase-free MQ H<sub>2</sub>O; Mobile Phase B Composition 100 mM hexafluoroisopropanol (HFIP)/ triethylamine (TEA) in CH<sub>3</sub>OH. Starting Composition: 98%A, Elution Gradient: 19.6% B / min (2% B to 100% B in 25 min), Monitored 260 nm, Phenomenex Column (50 mm x 2.1 mm), 1.9 micron particle size.

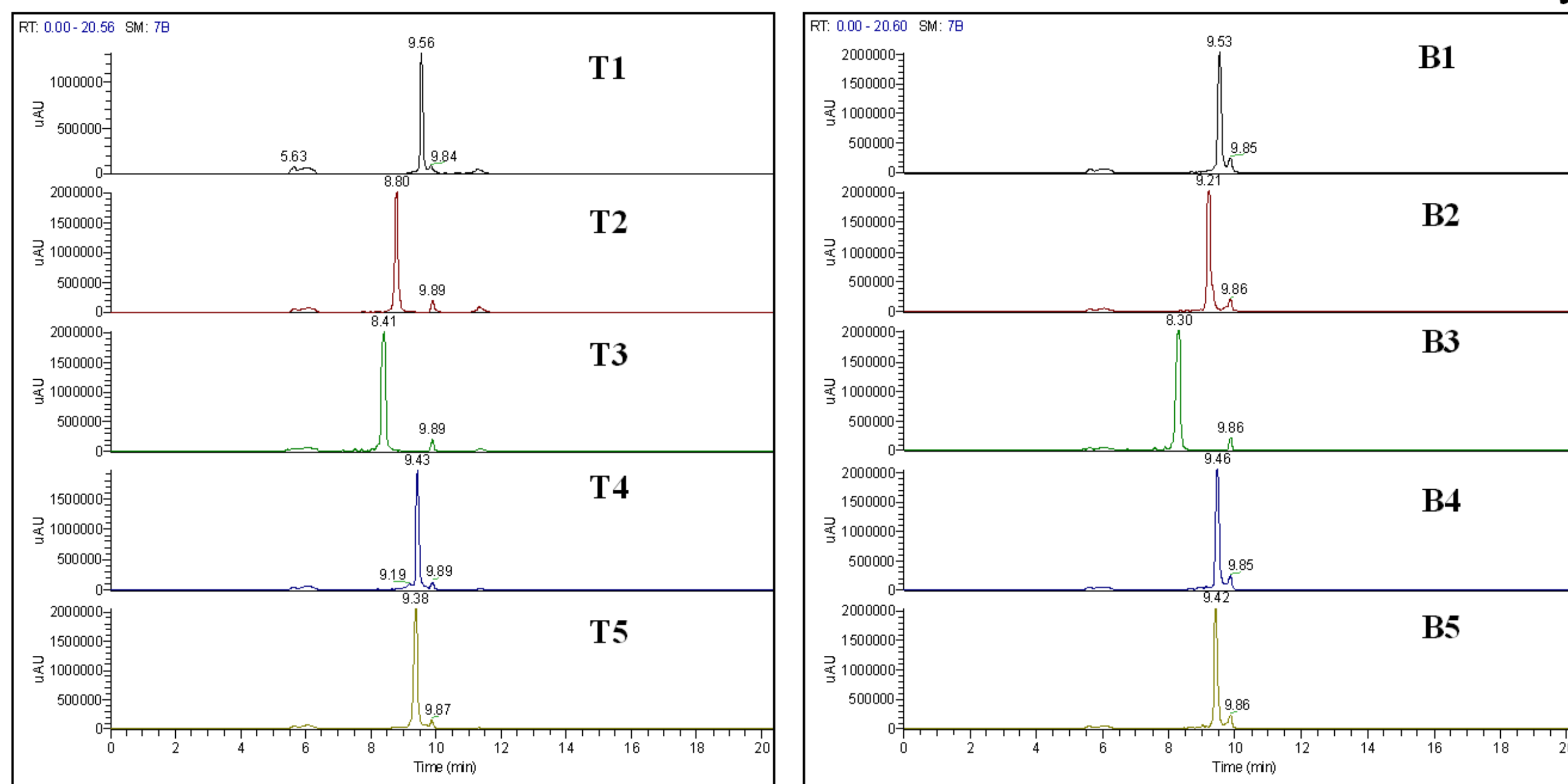
### Table of All Oligonucleotide Fragments

Fragment	Theoretical Mass(Da)	Observed Mass (Da)	Sequence (5' to 3')
T1	11751.7	11752.7	ACGGTGGCGATGGCAAGAAGGATACACCTGTTCCCATG
T2	4265.0	4265.8	CCGAACACAGAAGT
T3	2687.9	2688.8	TAAGCTTCT
T4	9624.4	9625.3	TAACGCCGAGAGTAGTTGGTGGGAACTGCC
T5	7754.4	7755.0	TGCGAGGGTAGGCAGTCGCCGTGCT
B1	10369.0	10369.7	GGAACAGGTGTATCCTCTTGCCATCGCCACCCTG
B2	6113.3	6114.0	TAACCTCTGTGTTGGCCATG
B3	2746.3	2746.9	TAAGAAGCT
B4	8789.0	8789.7	GGCAGTTCCTCCCAACTACTCTCGGCGT
B5	7572.0	7572.9	AGCACGGCGACTGCTACCTCCGCA

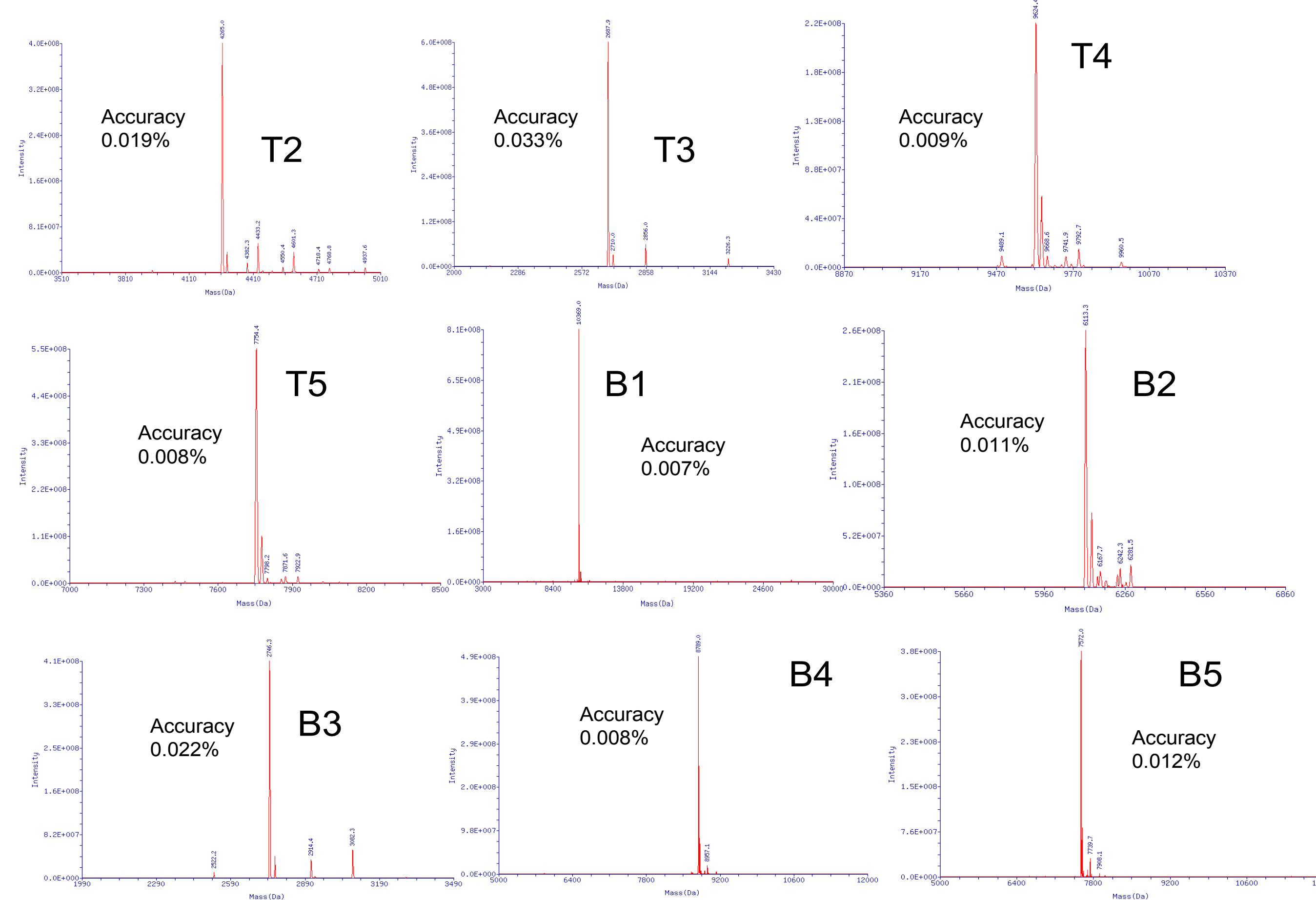
### MS/MS of B2 Fragment with Partial Fragments Labeled in the MS2 Spectrum



### U-HPLC and MS Analysis of DNA Fragments



Full scale chromatograms of DNA Oligonucleotides. Conditions: Sample Load: 5 µL, Flow rate: 477 µL/min, Temperature: 25°C, Mobile Phase A Composition: 100 mM hexafluoroisopropanol (HFIP)/ triethylamine (TEA) (pH 8.35) in RNase-free MQ H<sub>2</sub>O; Mobile Phase B Composition 100 mM hexafluoroisopropanol (HFIP)/ triethylamine (TEA) in CH<sub>3</sub>OH. Starting Composition: 98%A, Elution Gradient: 19.6% B / min (2% B to 100% B in 25 min), Monitored 260 nm, Phenomenex Column (50 mm x 2.1 mm), 1.9 micron particle size.



### U-HPLC and MS Analysis of 117-mer Duplex Digestion

