



Introduction

Characterization of mRNA PQAs is necessary to ensure safety and efficacy. LC-MS/MS digested oligonucleotide sequence mapping can characterize the 5' cap, oligo sequence, and 3' PolyA tail in a single assay.

Methods



Oligo
Identification, annotation of Oligonucleotide molecules



Digested Oligonucleotides
Enzymatically digested oligonucleotides

eGFP mRNA was digested with **hRNase4** and analyzed by IP-RPLC-MS

70-minute IP-RPLC gradient using the **Phenomenex bioZen2.6 µm Oligo 150 x 2.1 mm column** (PN: 00F-4790-AN) and DDA Neg mode using the Thermo Q-Exactive Plus

Data processing with **Byos Digested Oligonucleotide** and **Byos Oligo** (v5.6)

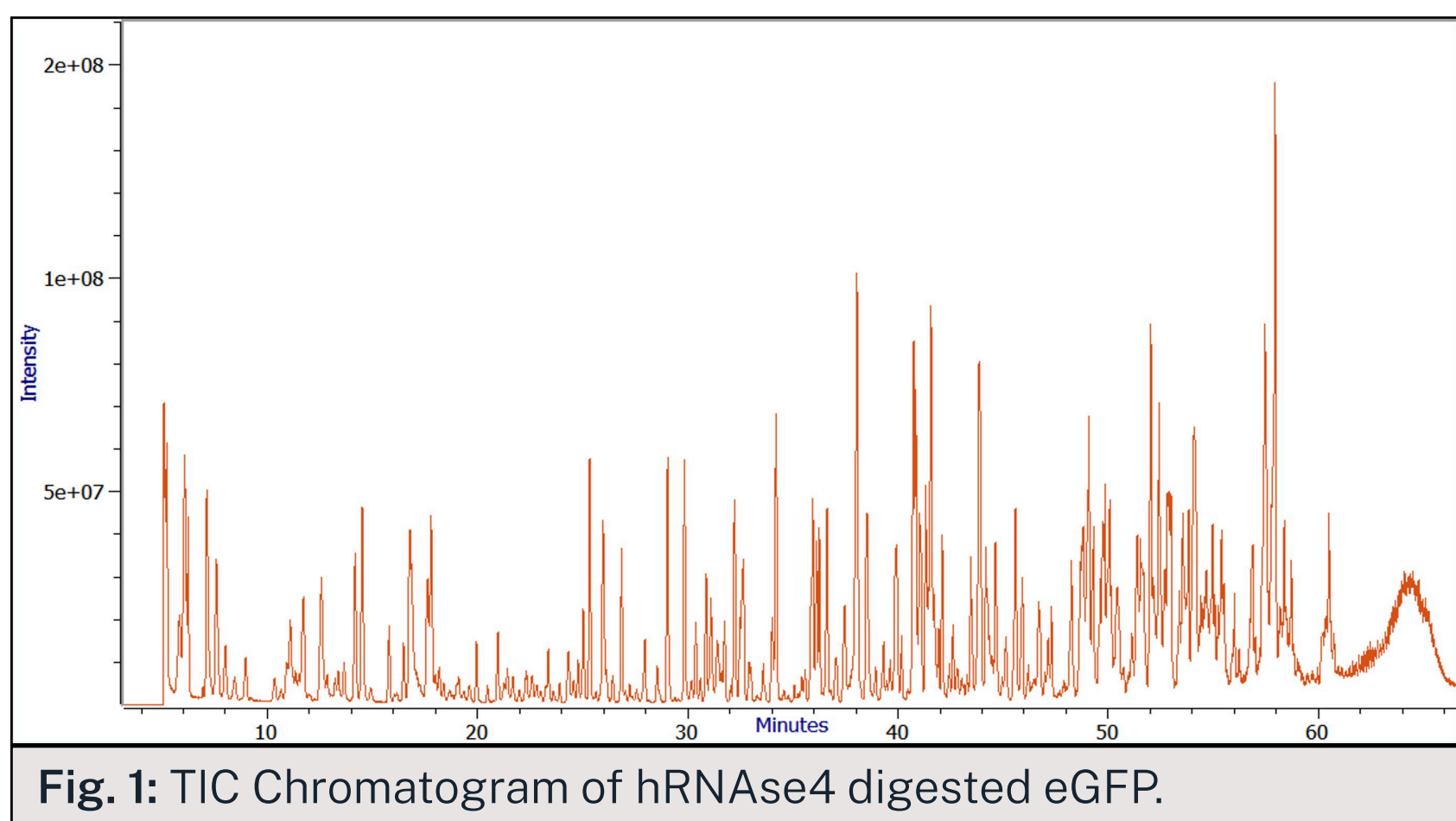


Fig. 1: TIC Chromatogram of hRNase4 digested eGFP.

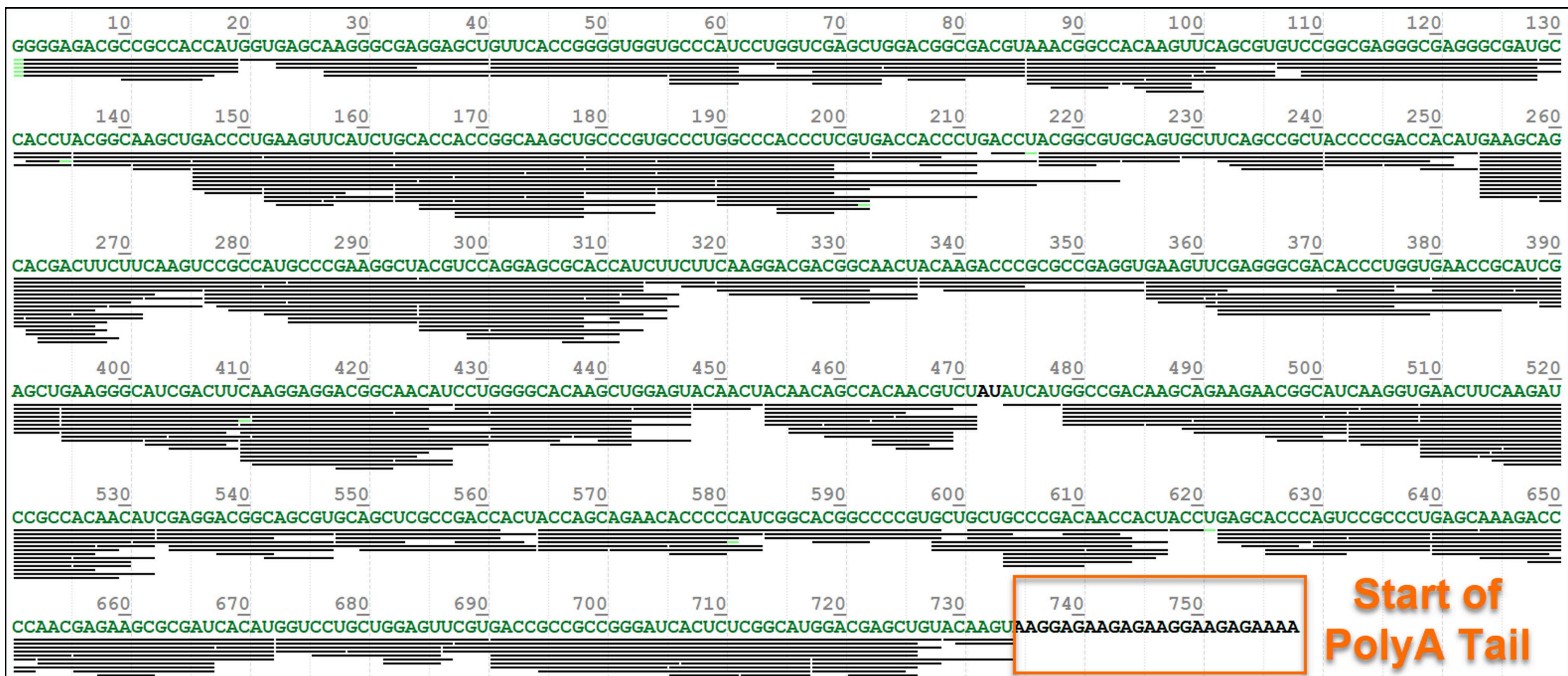


Fig. 2: eGFP mRNA Sequence coverage map.

Row#	Sequence	Calc.M	Apex Time (Posit)	Score	Delta Score	XIC area summed
> 1	U.GAACU.U	1552.26	6.03	416	40 - 143	1.37e+07
> 2	U.CAAGU.C	1552.26	6.15	372	89 - 112	1.13e+07
> 3	C.AAGCU.G	1552.26	7.11	362	33 - 43	6.63e+07
> 4	C.AACGU.C	1552.26	7.62	359	51 - 68	2.04e+07

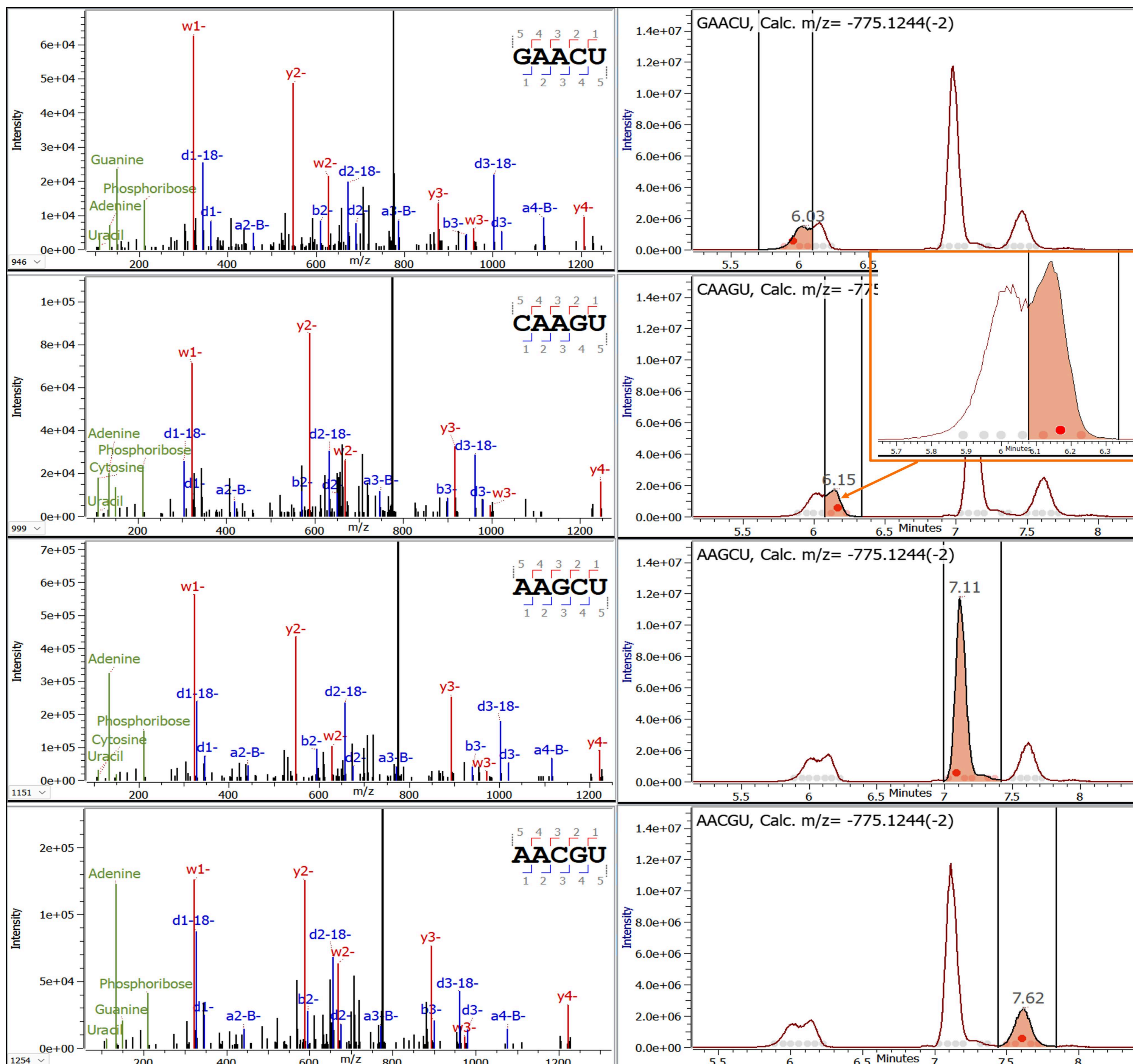


Fig. 3: MS/MS spectra and XICs of four oligo isomers. The Oligo Table at the top includes columns useful for assessing oligo assignments. Figures and tables are part of the Byos Digested Oligonucleotide investigation view.

Results - Oligo Sequence Mapping, 5' cap and 3' PolyA Tail

- >99% eGFP seq. coverage (Fig. 2)
- Byos automatically assigns oligo isomers by MS/MS (Fig 3). IPRP separation is critical for reliable isomer assignment.
- Grey/Red dots in XIC Plot show isomeric OSMs. Grey dots represent assigned OSMs isomers. (see Fig 3 zoom in to the left).
- Delta Score:** MSMS Score difference between the assigned oligo and the next best scoring oligo/isomer (isomer “uniqueness” score).
- Key isomer distinguishing fragment ions observed in annotated MS/MS spectra.

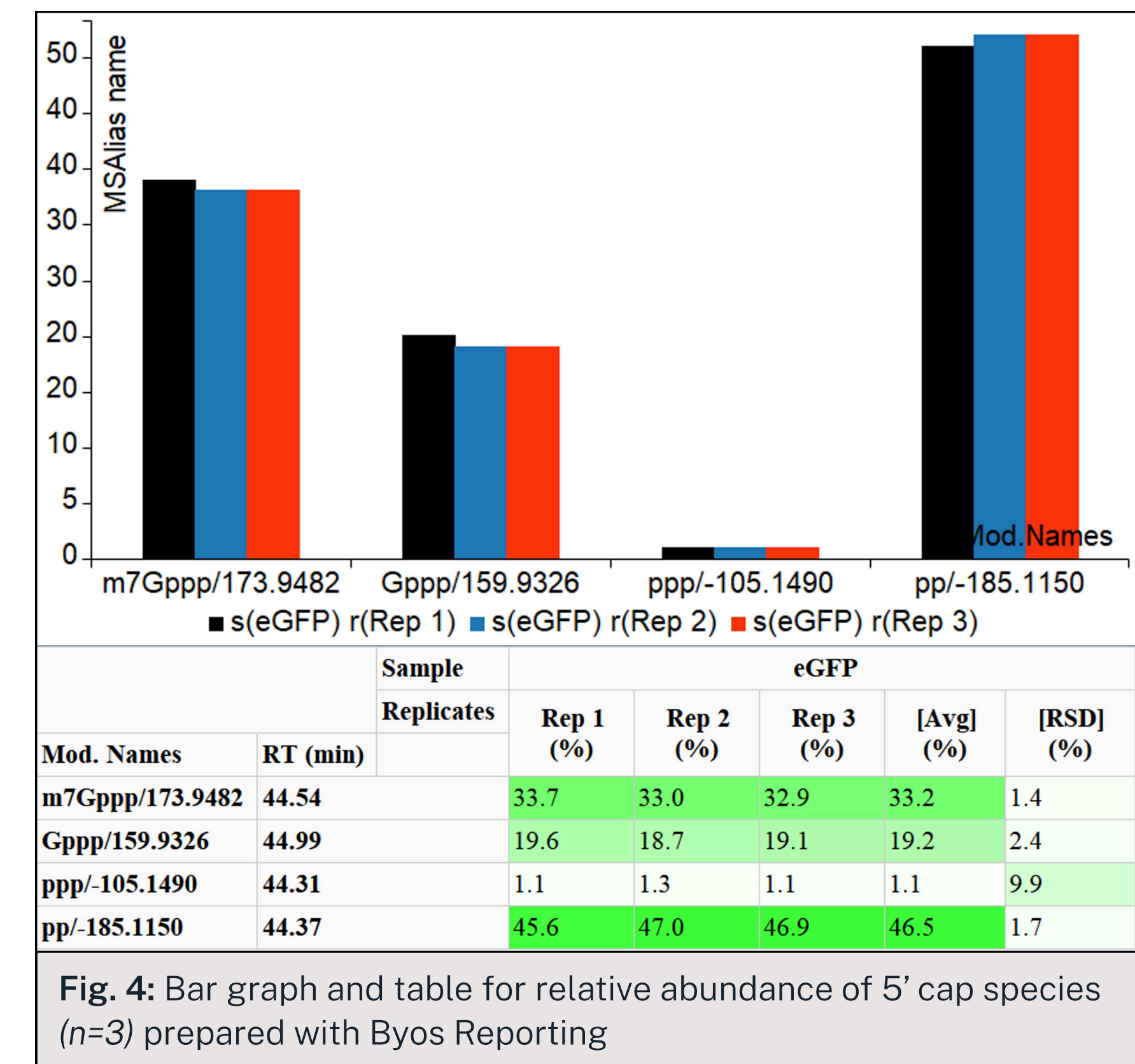


Fig. 4: Bar graph and table for relative abundance of 5' cap species (n=3) prepared with Byos Reporting

- m7Gppp 5' cap relative abundance of 33.2% %RSD=1.4% (n=3). (Fig. 4)
- Observed 5' cap intermediates Gppp (19%), ppp (1%), and pp (47%). (Fig. 4)

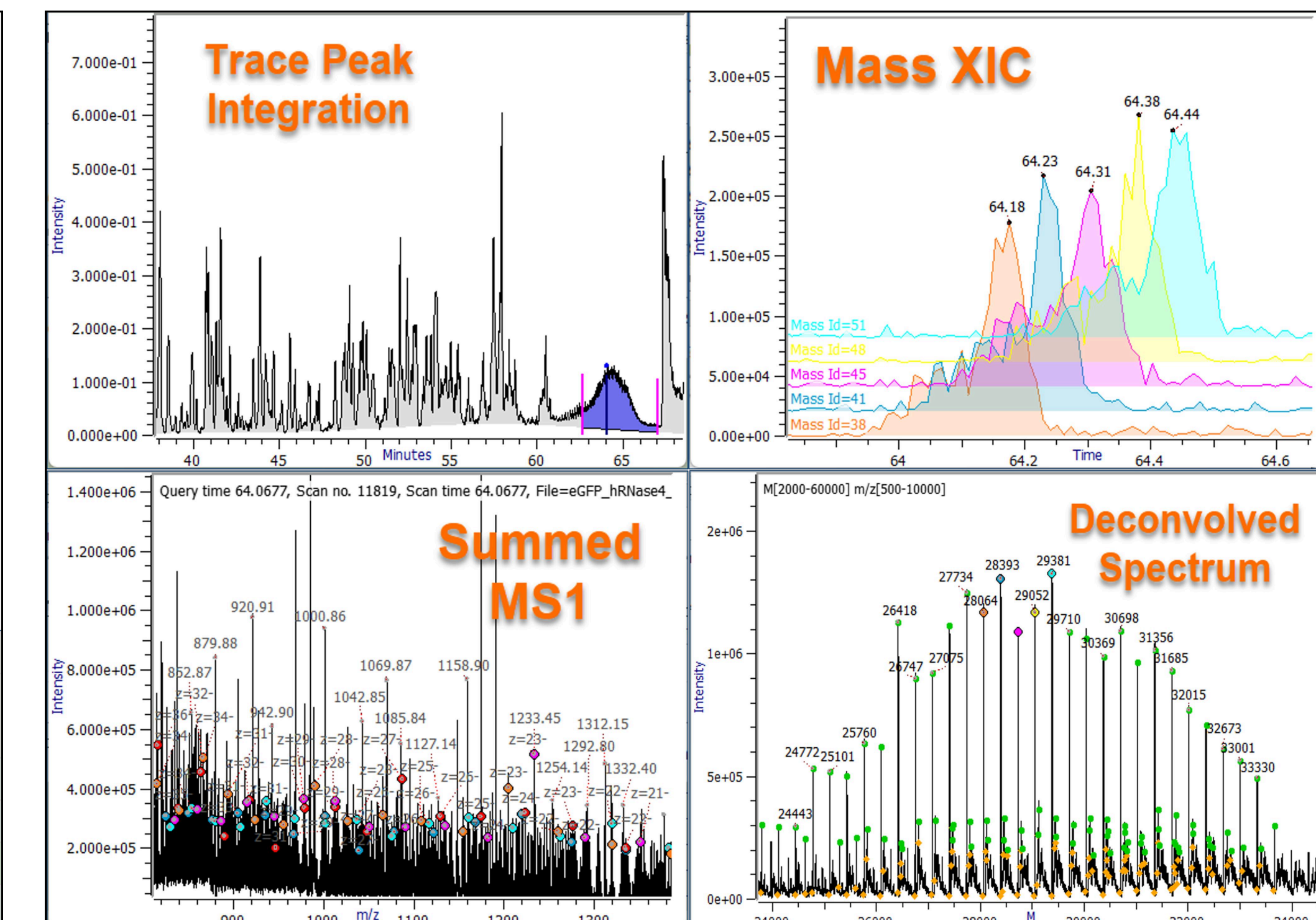


Fig. 5: Byos Oligo MS1 scan summation, deconvolution and Mass XIC plotting of the eGFP PolyA tail.

- PolyA tail lengths of 51-84 are mass matched in deconvoluted spectrum. (Fig. 5)
- Mass XIC plot shows LC resolution of PolyA tails. (Fig. 5)