

Summary:

Digested oligonucleotide characterization of mRNA PQAs

IPRP Separation using the Phenomenex bioZen Oligo column

Byos Digested Oligonucleotide sequence mapping and 5' cap quantitation

Byos Oligo PolyA Tail deconvolution and mass matching

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

- eGFP mRNA was digested with **hRNase4** and analyzed by IP-RPLC-MS
- 70-minute IP-RPLC gradient using the **Phenomenex bioZen 2.6 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)**

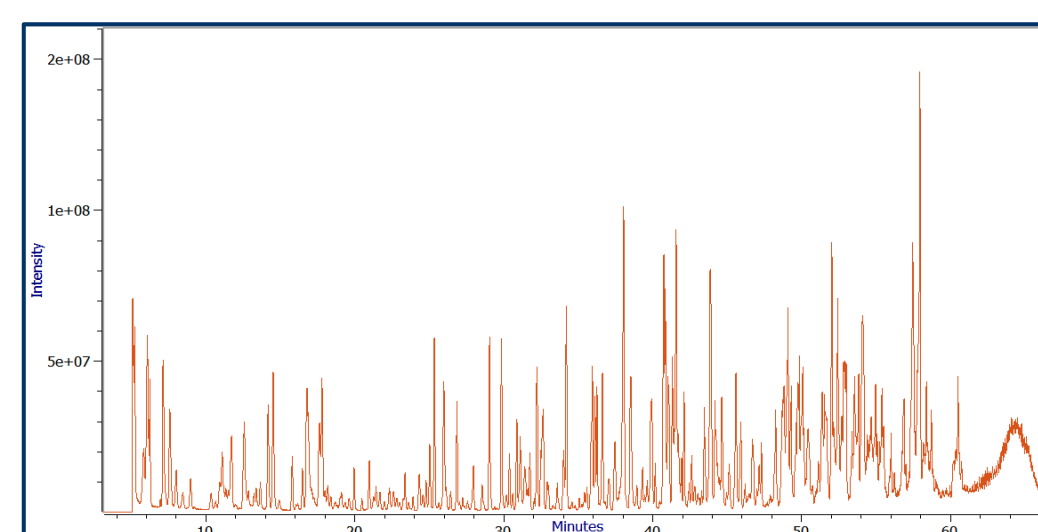


Fig. 1: TIC Chromatogram of hRNase4 digested eGFP.

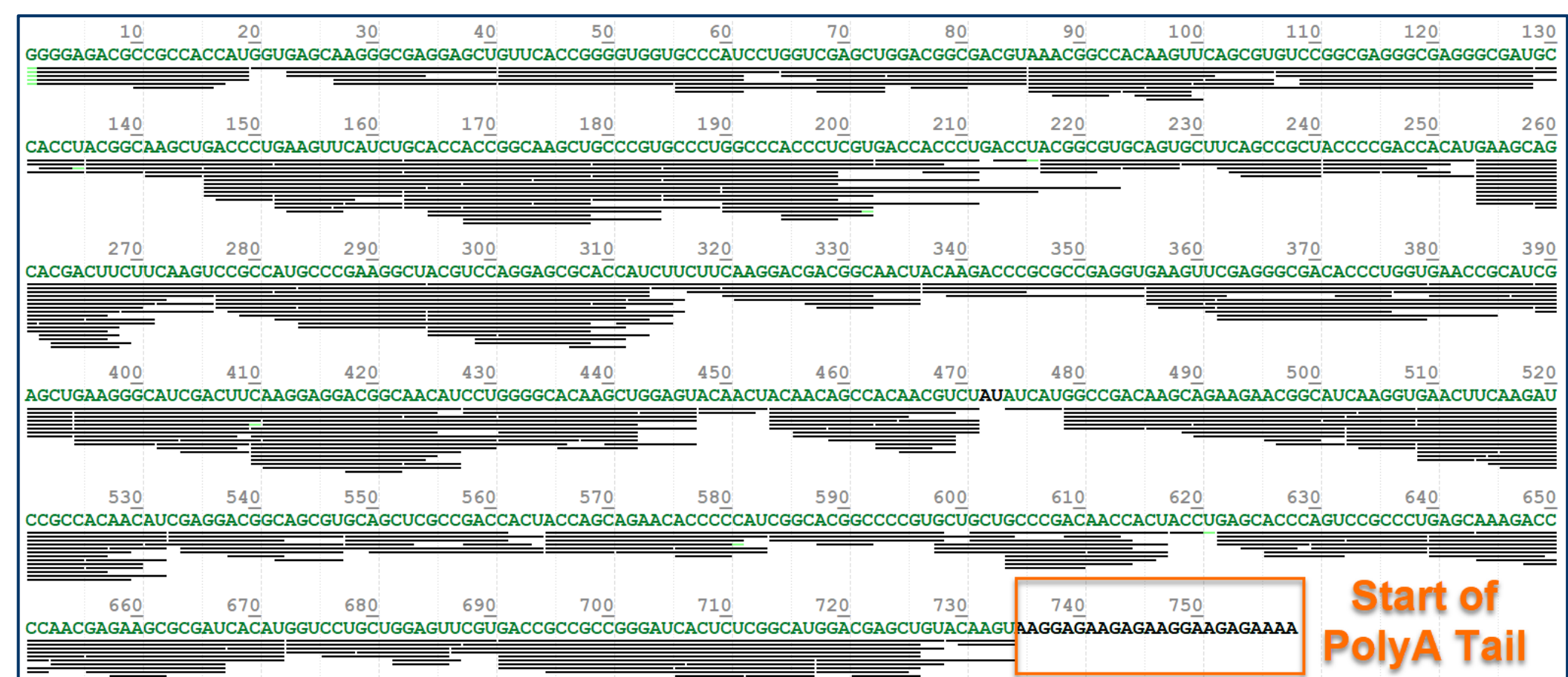
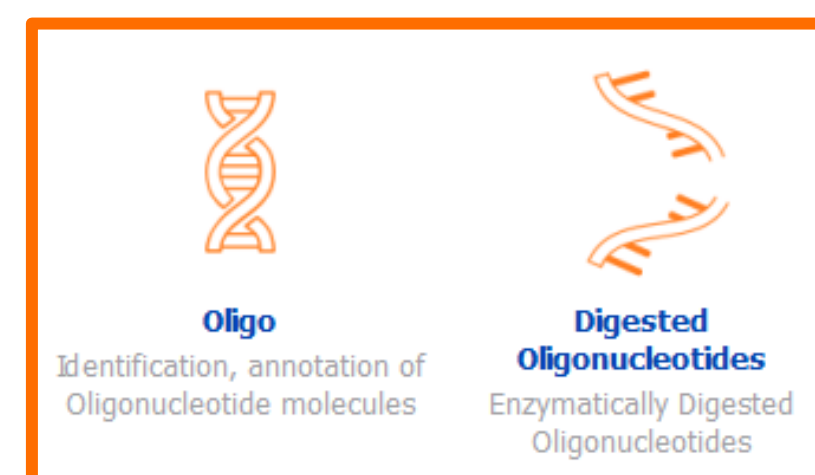


Fig. 2: eGFP mRNA Sequence coverage map.

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

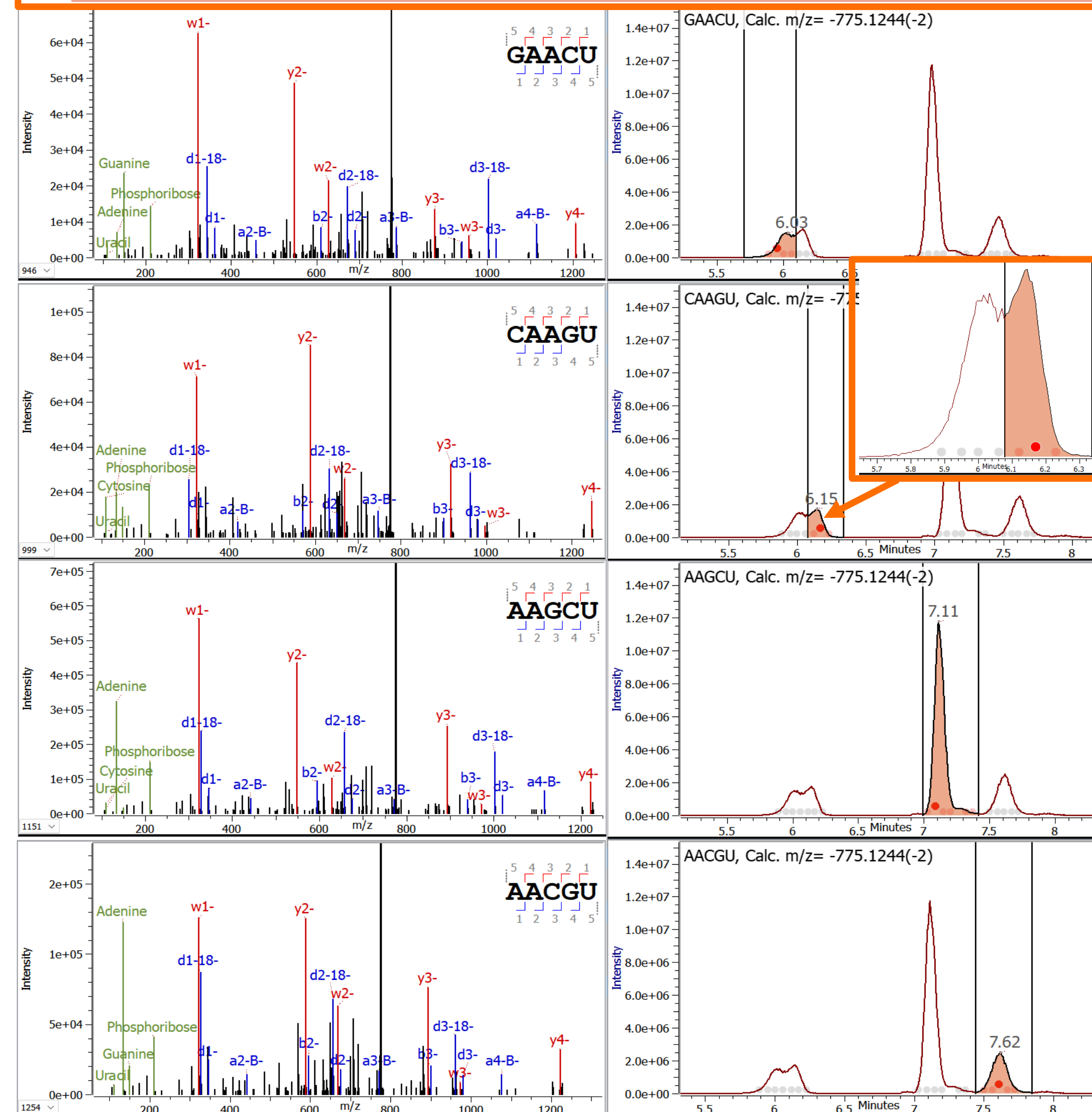


Fig. 3: MS/MS spectra and XICs of four oligo isomers. The Oligo Table at the top includes columns useful for assessing oligo assignments. Figures and tables are part of the **Byos Digested Oligonucleotide** investigation view.

Results - Oligo Sequence Mapping

- >99% eGFP seq. coverage (Fig. 2)
- Byos automatically assigns oligo isomers by MS/MS (Fig 3). IPRP separation is critical for reliable isomer assignment.
- 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.
- Grey/Red dots in XIC Plot show isomeric OSMs. Grey dots represent assigned OSMs (see Fig 3 zoom in to the left).

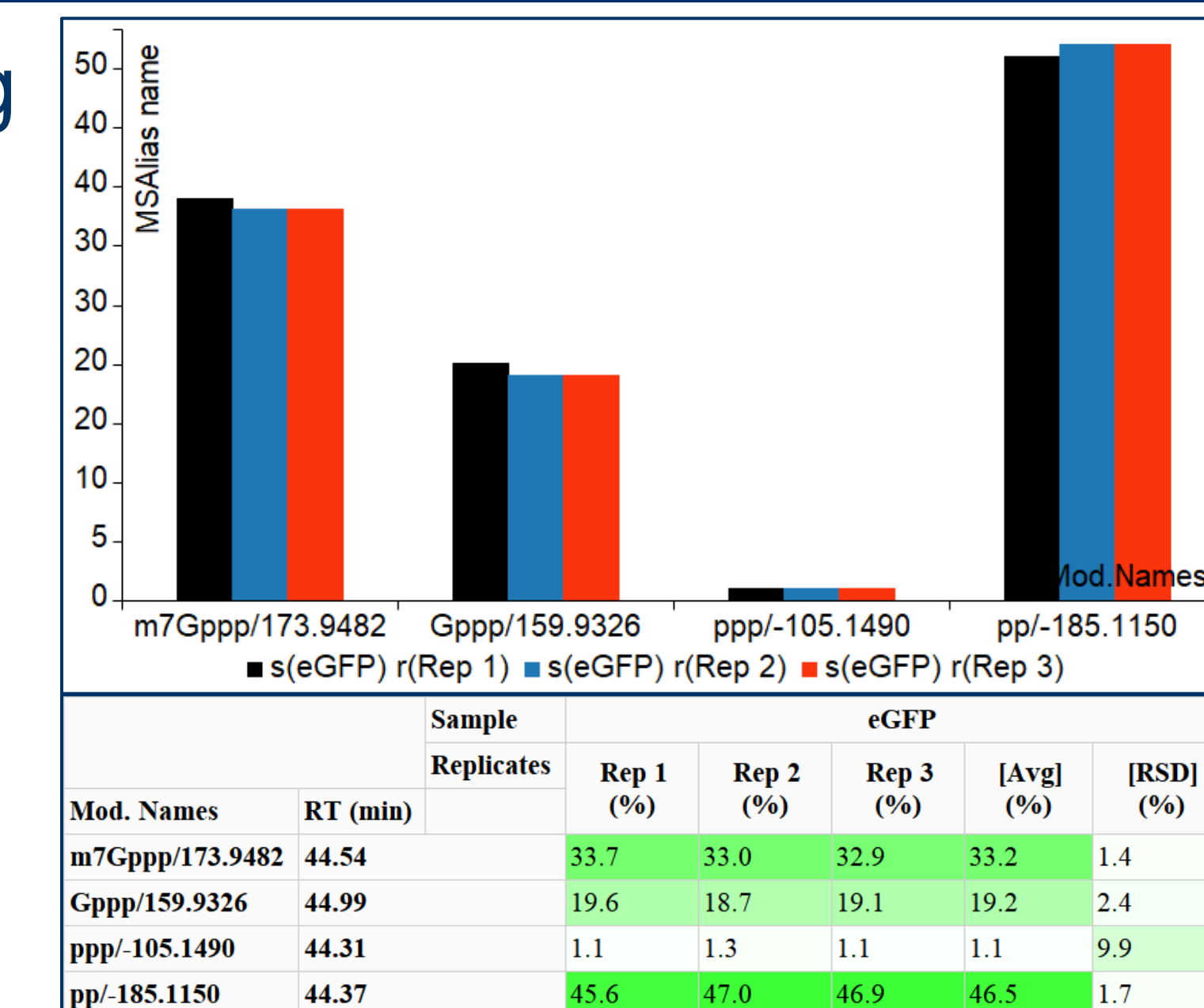


Fig. 4: Bar graph and table for relative abundance of 5' cap species (n=3) prepared with Byos Reporting.

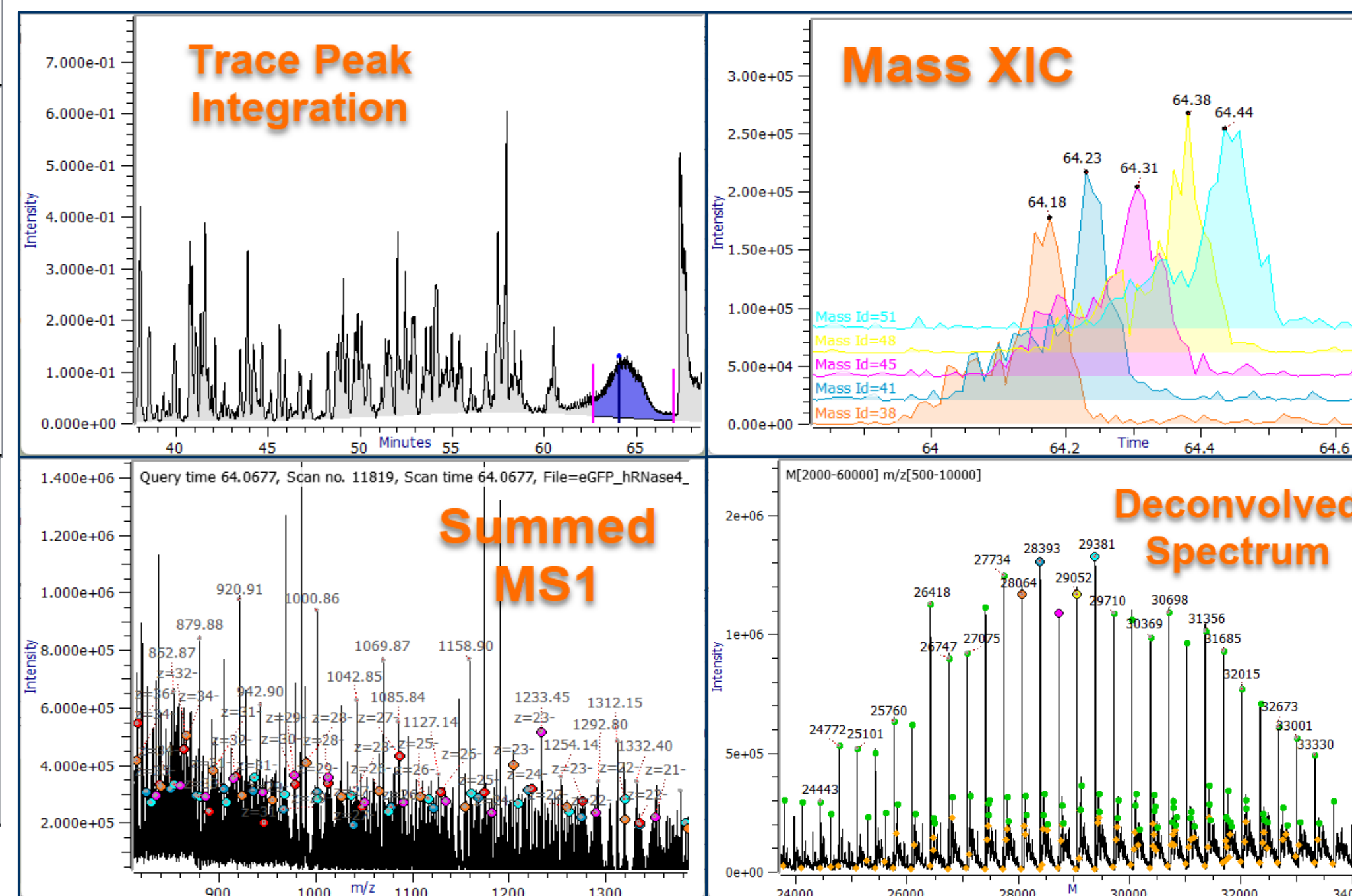


Fig. 5: **Byos Oligo** MS1 scan summation, deconvolution and Mass XIC plotting of the eGFP PolyA tail.

Results - 5' cap and 3' PolyA Tail

- m7Gppp 5' cap relative abundance of 33.2% %RSD=1.4% (n=3).
- Observed 5' cap intermediates Gppp (19%), ppp (1%), and pp (47%).
- PolyA tail lengths of 51-84 are mass matched in deconvoluted spectrum.
- Mass XIC plot shows LC resolution of PolyA tails.