

Summary

Vendor neutral data processing and reporting software for oligonucleotide impurity analysis

Comparison of novel progressive deconvolution data processing

Different relative quantitation approaches compared including UV, MS, and UV-MS

Accelerating oligonucleotide LC-MS data impurity analysis and reporting

Introduction

Mass spectrometrists are often tempted to use the latest tools, irrespective of the datasets. In this poster we confirm that wide depth and breadth of Oligonucleotide analysis may need a more nuanced selection of tools depending on the desired outcome. We compare the applicability of a 'Progressive Deconvolution' [PD] algorithm with a 'Trace Peak' [TP] algorithm. PD was developed to maximise the amount of low-level information and provide peak areas based on detector response for identified species, whereas TP is used for deconvoluting a time-based section of m/z data.

Figure 1: Trace Peak Centric (TPC) Data Processing – HiRes 40-mer

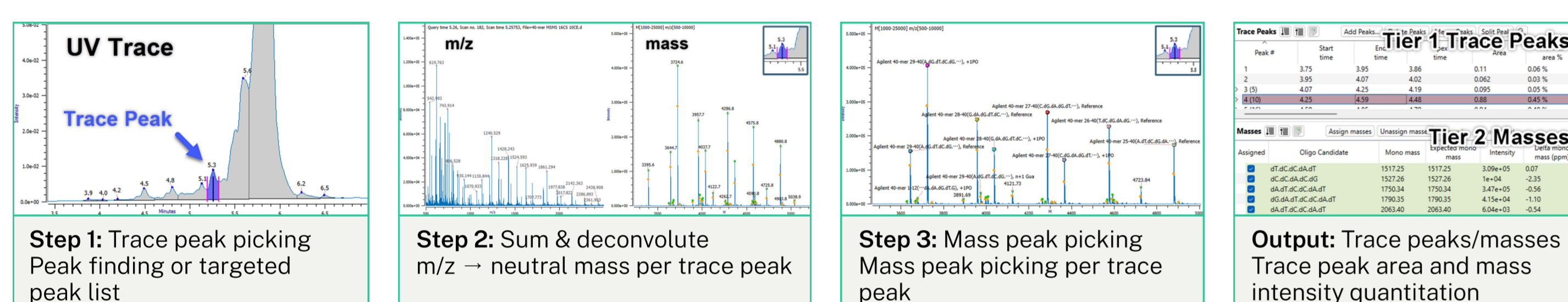
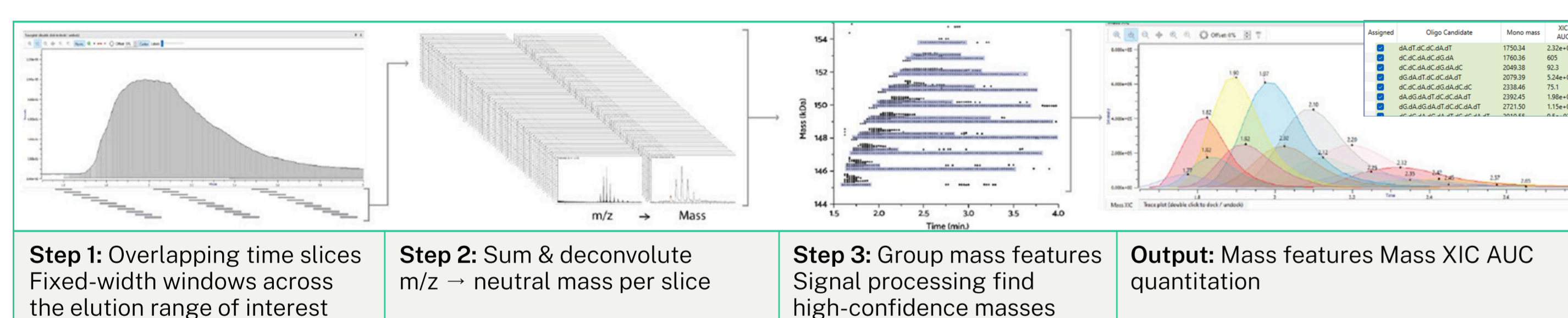


Figure 2: Progressive Deconvolution (PD) Data Processing – HiRes 40-mer

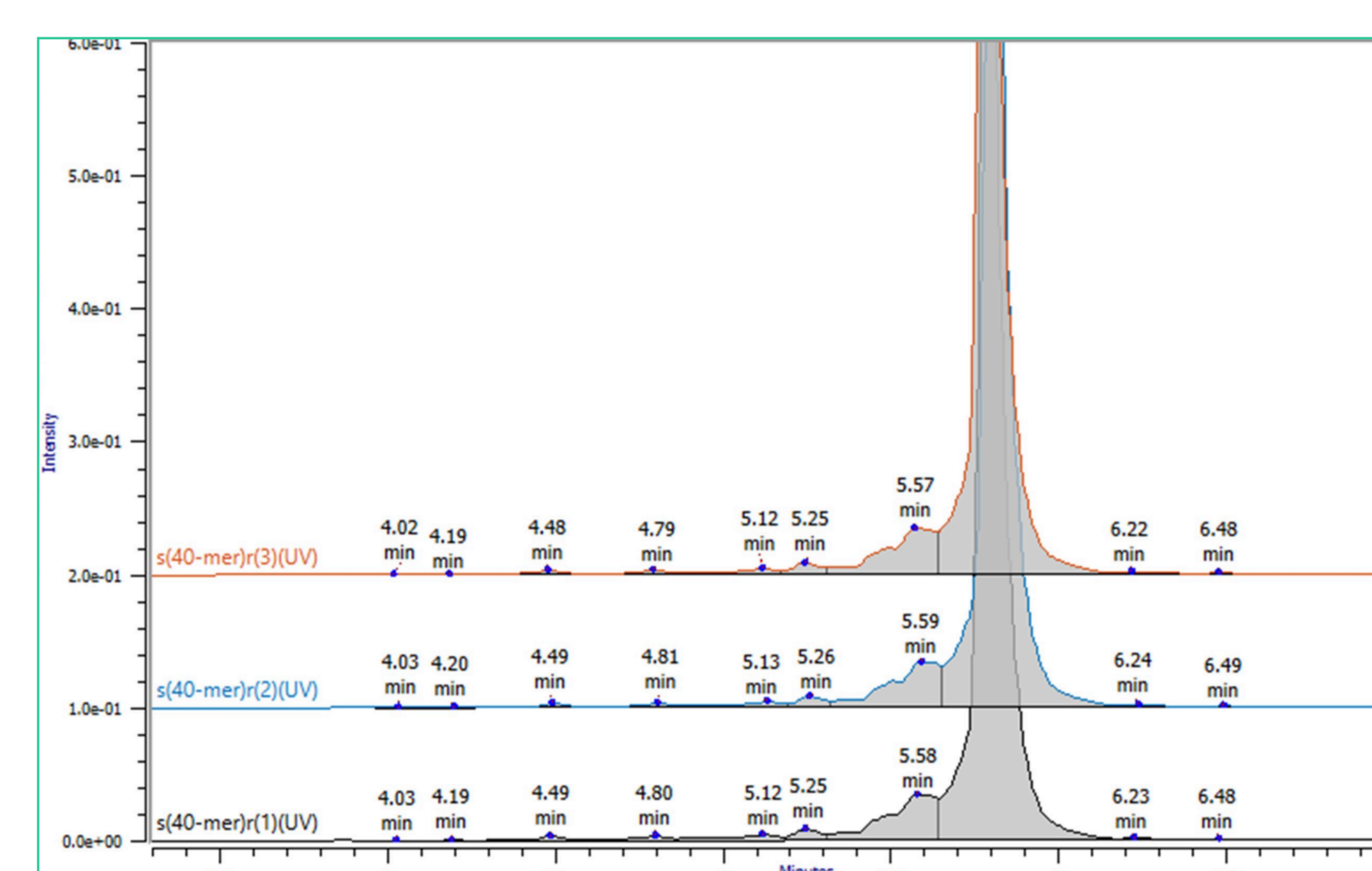


Methods

High Resolution Data: Triplicate Agilent DNA 40mer LC-HRMS datafiles were analyzed using Byos Oligo (v6.0) with TPC and PD workflows (Figures 1 and 2). Monoisotopic masses were matched to the FLP and defined impurity types (clips, shortmers, longmers, substitutions, and chemical modifications) within 10 ppm. Relative quantitation was estimated using UV-only, UV-MS, and MS-only approaches. Automated report generation was performed using Byos template-driven reporting. Low Resolution Single Quad data was analyzed with TP for a 9kDa Oligonucleotide. In the low resolution data there was no MSMS information available to align XICs and so the function could be switched off.

Results – UV relative quantitation (TPC)

Figure 3: UV Trace Peak Integration and % Relative Peak Area

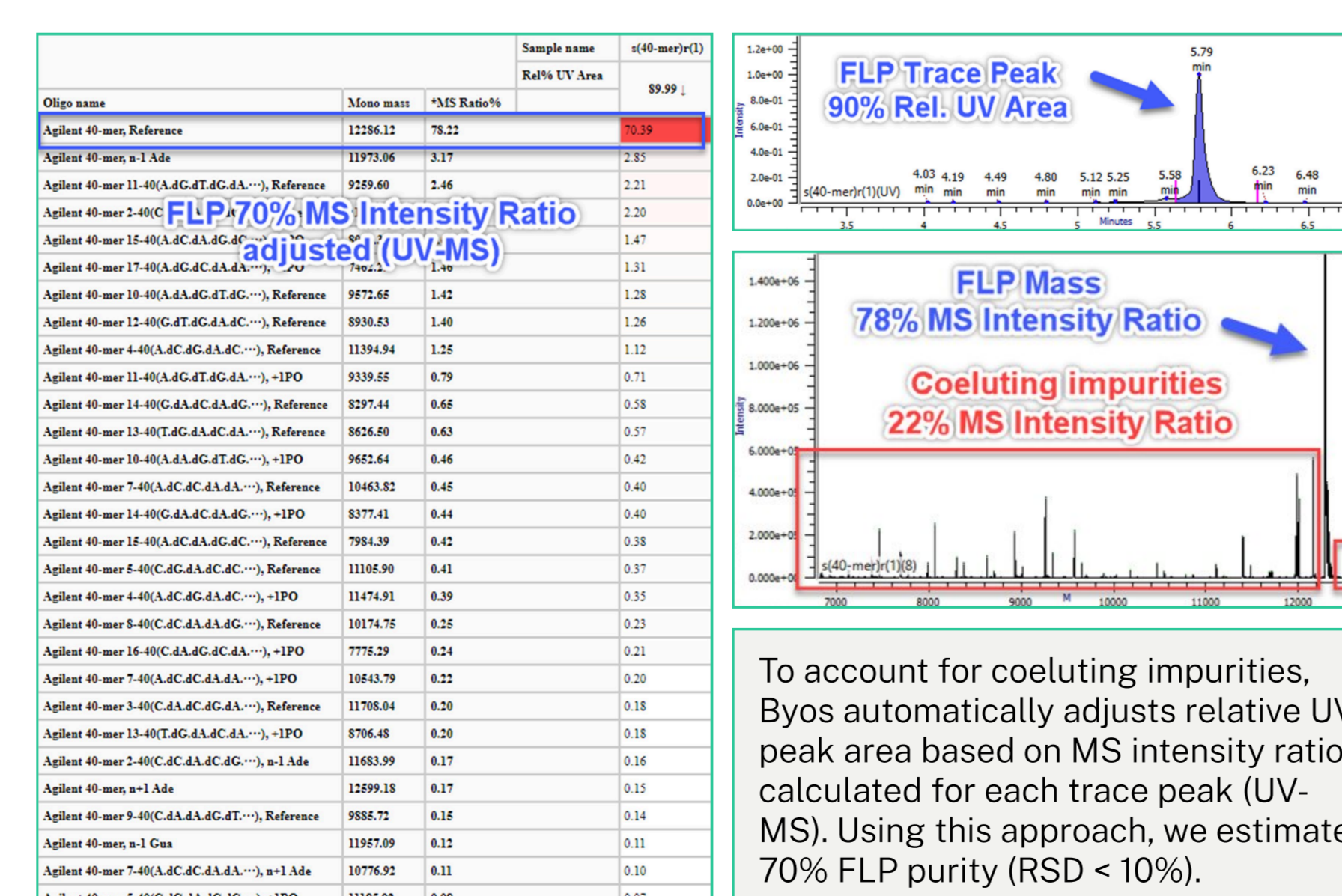


10x UV trace peaks were found per sample and integrated using Byos AutoCompute trace peak finder.

Byos automatically calculates relative UV peak area, estimating 90% FLP purity (RSD < 10%).

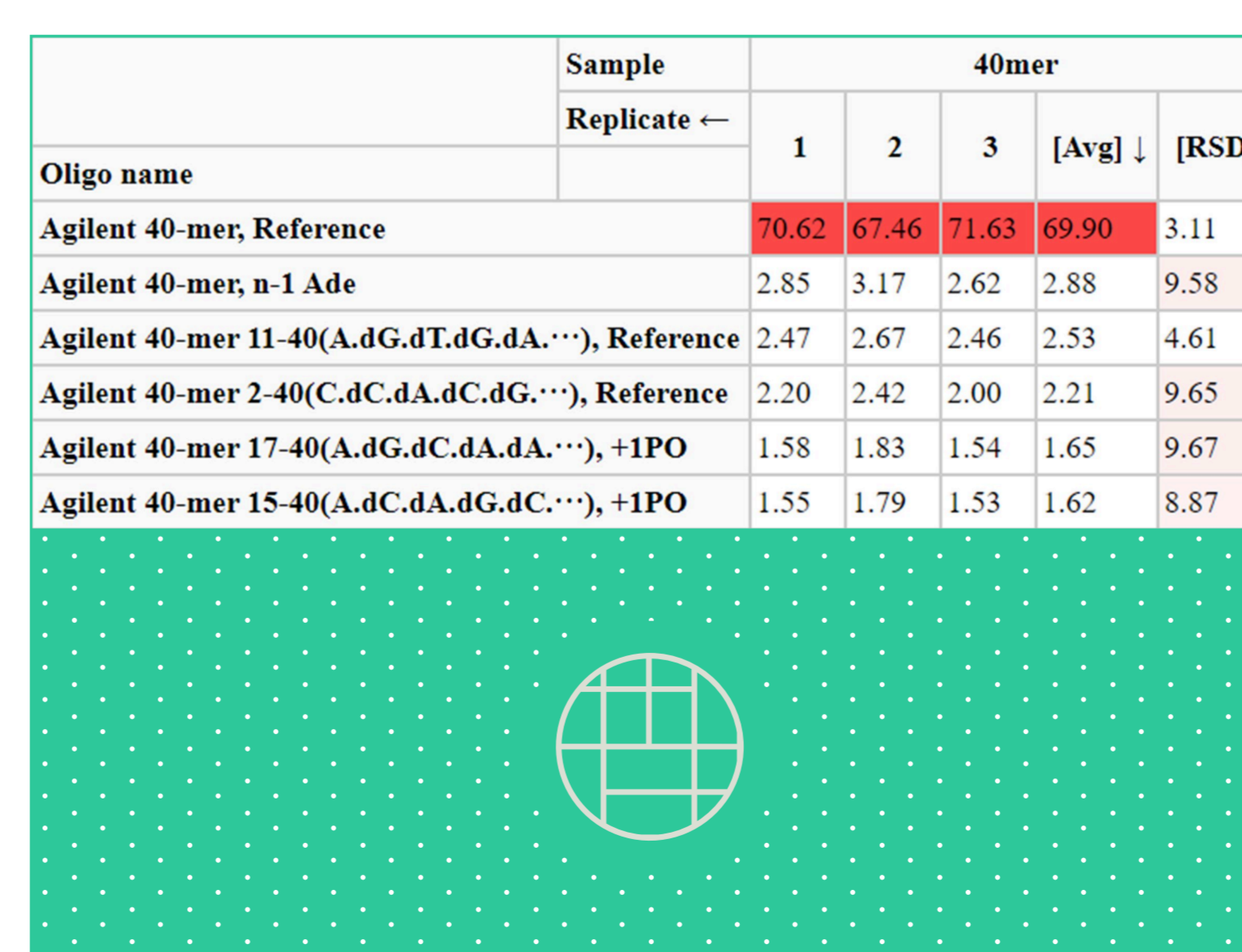
Results – UV-MS relative quantitation (TPC)

Figure 4: UV-MS calculation example



To account for coeluting impurities, Byos automatically adjusts relative UV peak area based on MS intensity ratios calculated for each trace peak (UV-MS). Using this approach, we estimate 70% FLP purity (RSD < 10%).

Figure 5: UV-MS FLP and top 5 impurities



Results – MS relative quantitation (TP and PD) – 40-mer HiRes

Figure 6: MS only relative quantitation TPC vs. PD (top 10 masses detected by abundance)

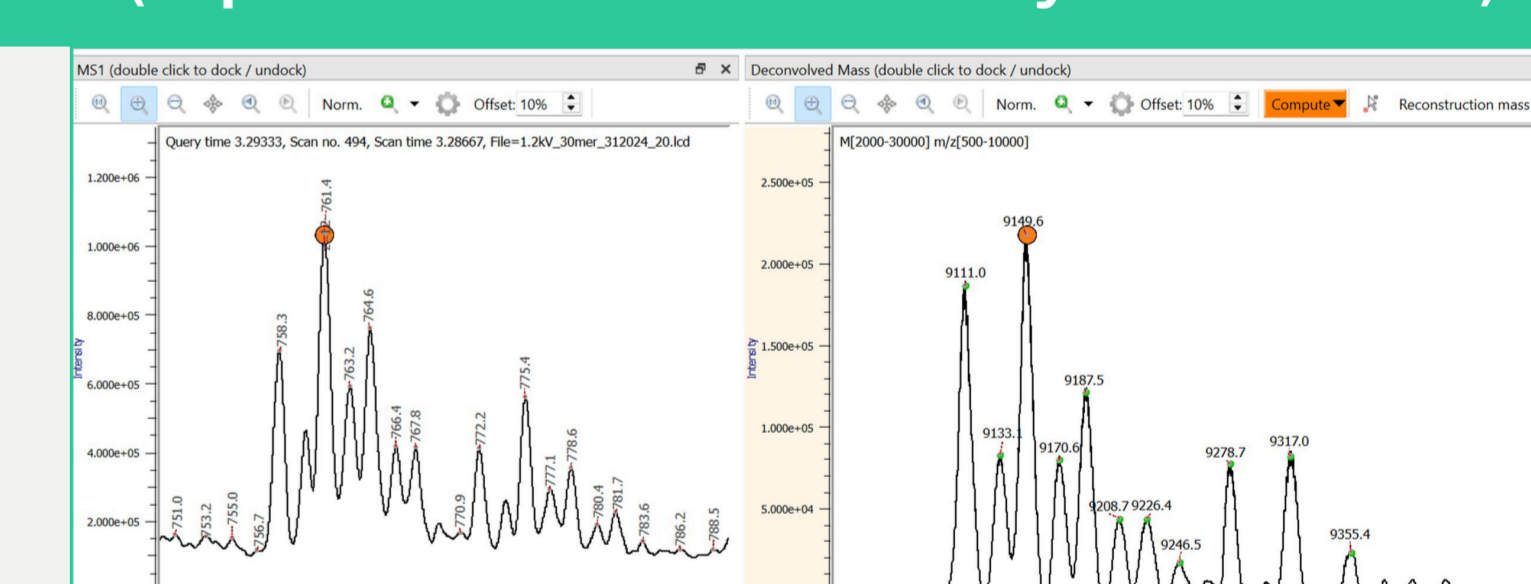
Relative quantitation using deconvoluted mass intensity (TPC) and XIC AUC (PD). Intensity yielded superior reproducibility (%RSD < 20%). The lower reproducibility observed with PD XIC AUC is likely due to limited MS scans per chromatographic peak; faster acquisition rates or broader LC peaks would improve reliability.

Oligonucleotide	Mono mass	Trace peak centric					Progressive deconvolution				
		Rep 1	Rep 2	Rep 3	Avg	%RSD	Rep 1	Rep 2	Rep 3	Avg	%RSD
Agilent 40-mer, FLP	12286.11	39.21%	37.20%	39.79%	38.73%	3.51%	38.38%	36.42%	37.35%	37.38%	2.62%
Clip (34-40)	2079.40	3.31%	3.33%	3.45%	3.36%	2.13%	3.79%	3.89%	5.55%	4.41%	22.40%
Clip (34-40)+3PO	2199.36	2.54%	2.62%	2.41%	2.52%	4.20%	3.44%	3.28%	3.39%	2.69%	
Clip (29-40)	3643.65	2.19%	2.39%	2.15%	2.24%	5.75%	2.45%	2.52%	2.34%	2.44%	3.87%
Clip (11-40)	9259.59	2.17%	2.25%	2.17%	2.20%	2.09%	0.71%	0.78%	1.23%	0.90%	31.16%
Clip (17-40)	7382.28	2.00%	2.18%	1.99%	2.04%	4.27%	2.39%	2.55%	2.36%	2.43%	4.02%
Clip (25-40)	4878.85	1.93%	1.95%	1.99%	1.96%	1.57%	1.68%	1.71%	1.06%	1.48%	24.68%
Clip (21-40)	6338.07	1.98%	1.99%	1.85%	1.94%	3.56%	1.45%	1.47%	1.30%	1.41%	6.57%
Clip (25-40)+3PO	4958.82	1.76%	1.80%	1.80%	1.79%	1.07%	1.99%	1.97%	1.94%	1.97%	1.30%

Results 2 – Low Resolution Dataset

Figure 6: MS only relative quantitation TPC vs. PD (top 10 masses detected by abundance)

Low resolution data was analyzed by TP – the deconvolution appropriately maintained the relative proportions of the peaks as shown in the zoomed section in Figure 7a. More complex mRNA data was similarly deconvoluted (Fig 7b). In both cases, there was no need to apply PD, as no additional identifications were made (and due to the nature of the data, no MSMS information was available).



Conclusion

One software workflow can be applied to datasets of different types. TP Benefits: Enables UV, MS, and UV-MS based quantitation. UV-MS provides a hybrid approach with advantages over UV-only and MS-only methods. Simpler, faster processing makes TP well-suited for high-throughput analysis. Also able to process single quad data in the same workflow with minimal adjustments. PD Benefits: Signal processing limits noise, enabling detection of very low abundance impurities. A single mass assignment per impurity simplifies interpretation, and avoids duplicate assignments seen across multiple trace peaks.

In the high Res data, both approaches successfully identified the FLP and >100 impurity masses, including n-1, n+1, clips (6–39mer), and other modifications. UV-only, MS-only, and UV-MS quantitation provide complementary approaches for estimating relative abundance. The analysis and reporting are automated using Byos Oligo. For this dataset PD demonstrates higher sensitivity (+31 impurity mass assignments), while TPC relative quantitation showed superior reproducibility compared to PD, which would benefit from more MS scans per chromatographic peak. TPC and PD offer unique and complementary advantages.