FlashNote

OligoQuest – Software for high confidence sequence confirmation of RNA and oligonucleotides

Advancements in gene therapy and approval of mRNA vaccines have dramatically increased the demand for well characterized RNA and oligonucleotides. OligoQuest supports this emerging need with advanced tools to analyze nucleic acid sequences by mass spectrometry.

OligoQuest

Challenge

RNA and oligonucleotide sequence accuracy is a critical attribute impacting molecular safety profiles (avoid off target effects or toxicity) and compound efficacy (correct activity and expression). While useful for basic synthetic oligos and simple RNA molecules, Next Generation Sequencing methods do not offer comprehensive characterization of highly modified RNA-based therapeutics.

The combination of isotopic fidelity with high resolution in instruments like the Bruker maXis II and timsTOF Pro 2 enable measurement of biopolymers in the 30-50 kDa range with sub-ppm mass accuracy. Robustness, sensitivity and most importantly high dynamic range in negative mode allow the detection and identification of impurities based on intact mass. These characteristics have benefited the characterization of analytes ranging from siRNA APIs, single guide RNA for gene editing (sgRNA) and mRNA specific assays (5' capping, polyA tail). However, intact mass is not always sufficient for unambiguous sequence assignments - MS/MS experiments, often coupled with enzymatic digests are required to fully establish the molecular identity of an LC-MS peak.

Solution

OligoQuest bridges the MS/MS data gap by adding powerful tools for the interpretation of RNA and oligonucleotide data to Bruker's BioPharma Compass suite.

A flexible sequence editor supports a wide range of nucleic acid building blocks, both natural and synthetic. Proven algorithms for peak detection and deconvolution of MS and MS/MS data obtained with high isotopic fidelity and ultrahigh resolution ensure correct mass assignments even in high dynamic range samples. Specifically, SNAP has been successfully used with top-down MS for the interpretation of spectra with large numbers of overlapping multiply charged ions. Finally, a powerful MS/MS annotation engine automatically computes and identifies terminal and internal fragments, allowing rapid analysis of complex MS/MS data in a straightforward, easy to interpret fashion.

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Decrease project risks with better characterization of single guide RNA and other modified nucleic acid sequences 100 nucleotides and beyond

"The introduction of this new suite of software, which will enable the routine analysis of larger and modified nucleic acids, will put us a step closer to realizing the broad range of analytical capabilities available now for protein analysis"

Dr. Dan Fabris, CEO of Ribodynamics and Harold S. Schwenk Sr. Professor at University of Connecticut, MA, USA

1. The high isotopic fidelity coupled with ultra-high resolution and high dynamic range creates high quality deconvoluted and annotated MS data, which can be reviewed in the software (Figure 1).

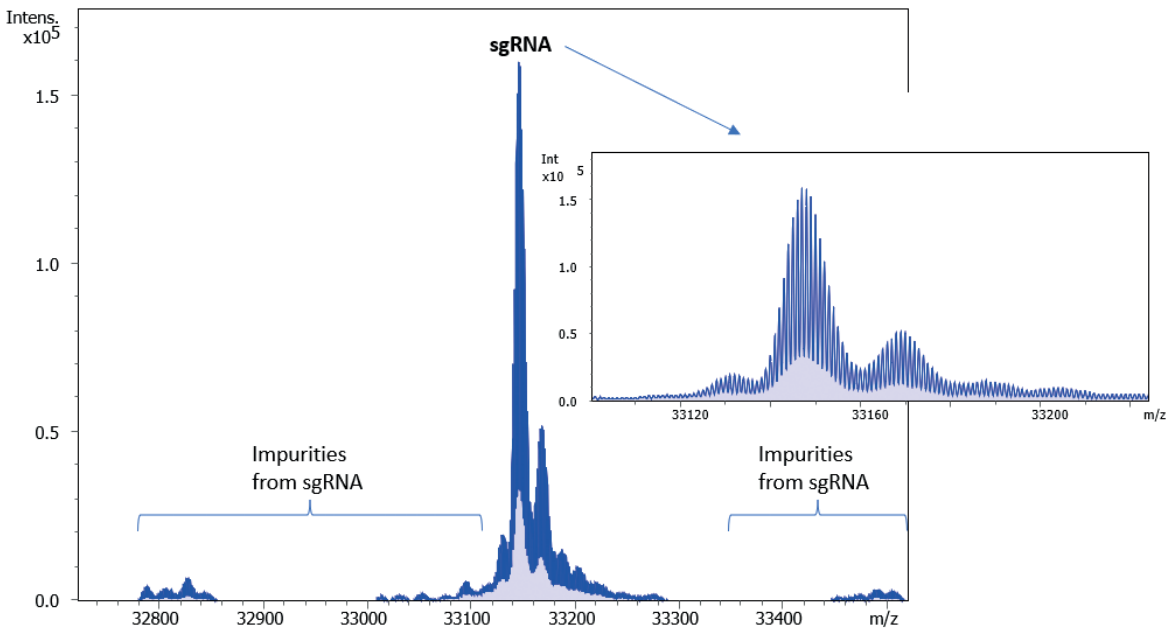
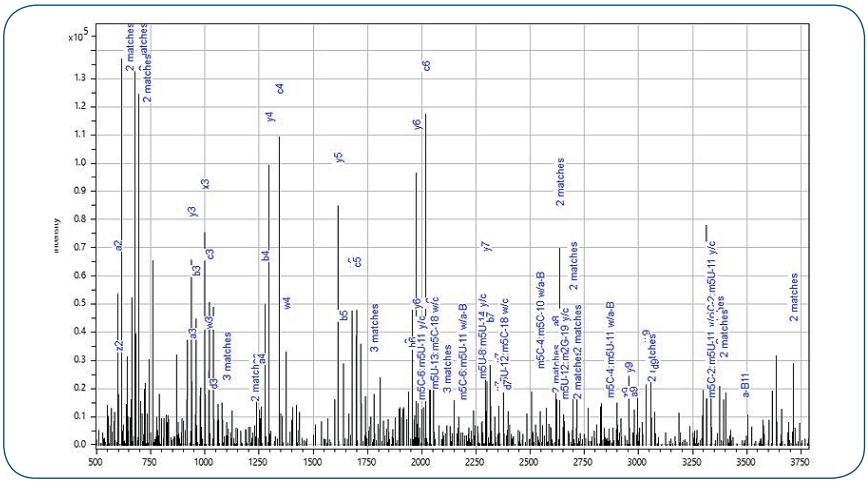


Figure 1: Deconvoluted spectrum for a sgRNA and associated low level impurities



2. MS/MS data is deisotoped with the SNAP algorithm and annotated by OligoQuest. The advanced peak picking helps with the confident interpretation of MS/MS data with complex and overlapping peaks. Matched peaks are annotated directly on the spectrum (Figure 2). Fragments with multiple explanations are denoted on the spectrum with the associated number of matches and not used analytically.

Figure 2: OligoQuest annotation of MS/MS data for a highly methylated 24 nt RNA (sample courtesy of Axolabs). Each brick represents a matching fragment ion confirming the respective nucleotide; mass errors (ppm) are displayed for 5´- or 3´- fragment ions.

3. A common challenge with longer molecules is the increased presence of internal fragments in the MS/MS data. Their interpretation is made difficult by the mass degeneracy due to the limited number of possible permutations between the basic building blocks. OligoQuest only annotates internal fragments with a unique explanation in a map view to complement the information that can be derived from terminal fragments (Figure 3).

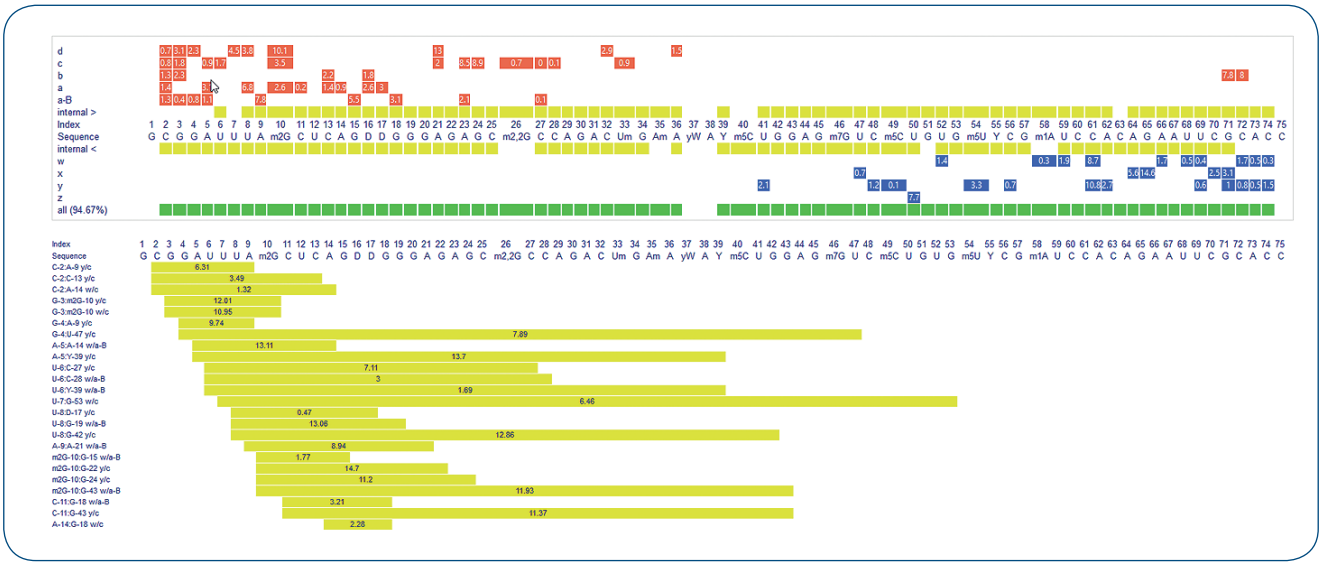


Figure 3: Internal fragments with unique explanations complement the sequence coverage for based on terminal fragments tRNAphe (75 nt, data courtesy of University of Connecticut, Dan Fabris lab)

4. The entire analysis can be automated with a Bruker Elute (Figure 4), the tools provided with the software suite simplify tasks such as method version control, user management and data security.

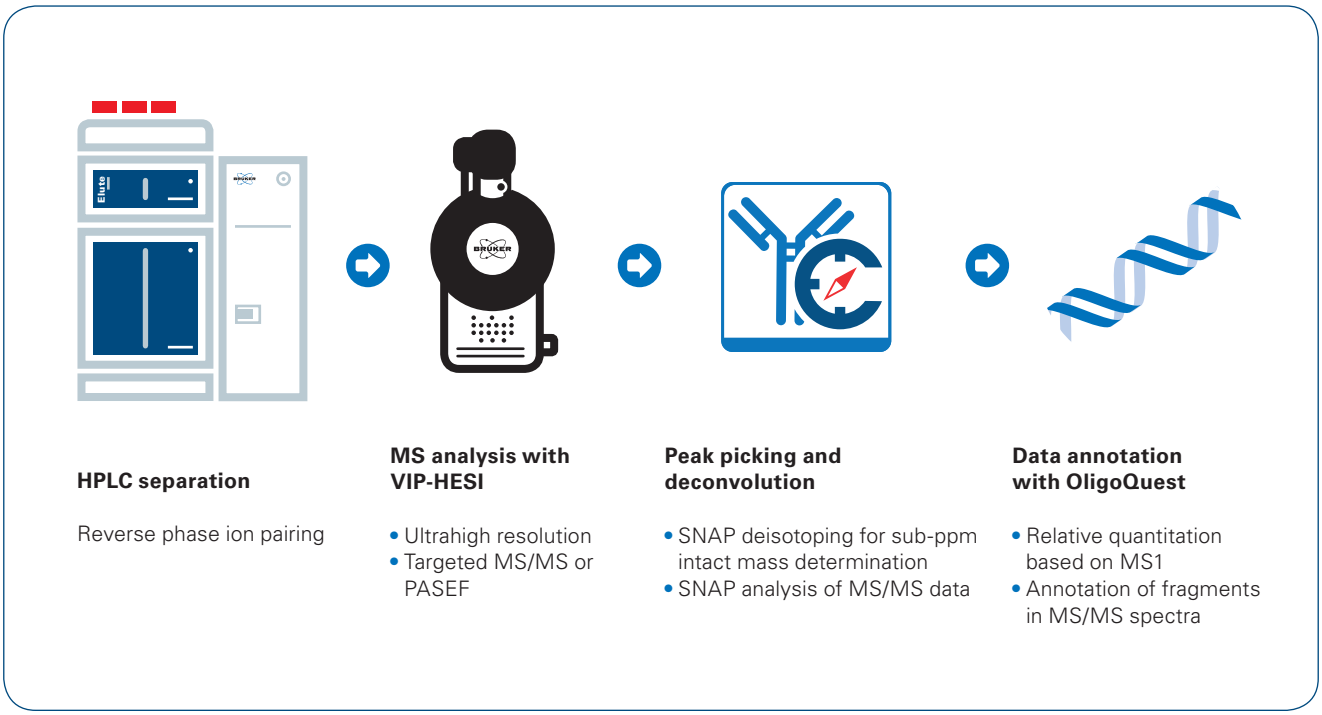


Figure 4: OligoQuest analytical workflow

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