High-throughput Oligonucleotide Analysis using RapidFire/TOF MS and OligoSearch Software

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The production of oligonucleotides (oligos) continues to accelerate due to their expanding utility in multiple applications. Because the processes to make oligos are imperfect, products are frequently contaminated with impurities that require identification for regulatory purposes or for safety and process improvement. Oligos are often analyzed using LC/MS and typical run times require several minutes. The annotation of observed impurities can also require a significant amount of time, especially in the absence of software to assist data interpretation. Taken together, these factors can make the characterization of oligos quite tedious and time-consuming. Consequently, techniques that expedite and simplify the analysis of oligos can be widely useful. A complete workflow for the high-throughput acquisition, analysis, and review of oligo data is described here.

## Oligo Acquisition Methods and Results

RapidFire Conditions		
Cartridge	PLRP-S, 8 um 1000A, 4 ul bed volume	
Inj. Vol.	10 uL	
Pump 1	Water + 7.5 mM TEA + 200 mM HFIP	0.6 ml/min
Pump 2	50% methanol + 7.5 mM TEA + 200 mM HFIP	0.6 ml/min
Pump 3	50% methanol + 7.5 mM TEA + 200 mM HFIP	0.6 ml/min
State 1	Aspirate sample	200 msec
State 2	Load/wash (desalt)	9,500 msec
State 3	Extra wash	0 msec
State 4	Elute (inject)	4,500 msec
State 5	Reequilibrate	500 msec

| MS Conditions	
Ion Polarity	Dual AJS Negative
Data Storage	Both (Centroid and Profile)
Gas temperature	275 ºC
Drying gas flow	12 L/min
Nebulizer gas	35 psi
Sheath gas temperature	325 ºC
Sheath gas flow	12 L/min
Capillary voltage	3500V
Nozzle voltage	1000V
Fragmentor	175 V
Skimmer	65 V
Oct 1 RF Vpp	750 V
Mass Range	400 – 3200 m/z
Acquisition Rate	4 spectra/sec

Analytical Methods.

Oligo sampling and acquisition was conducted on an Agilent RapidFire/6545 Q-TOF system in (-) MS mode. The sample-to-sample cycle time was ~15 seconds enabling the data for 384 samples to be collected in just over 1.5 hrs.
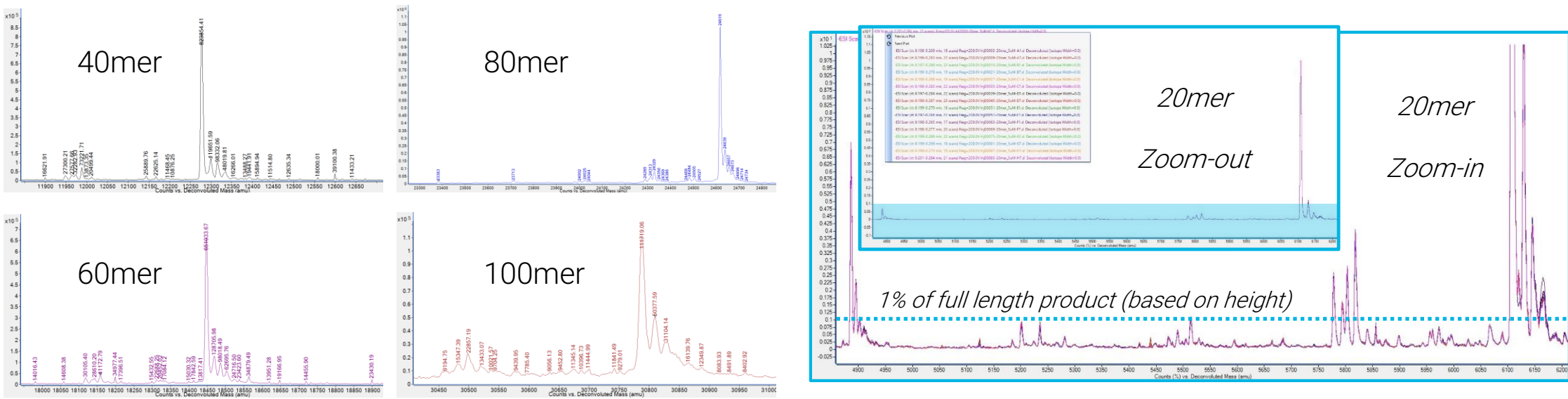
The high-throughput acquisition method is versatile and robust.

The single acquisition method demonstrated good data quality on all oligo sizes (20, 40, 60, 80, and 100mer DNA, purchased from IDT DNA) over all purity levels tested. MaxEnt deconvoluted spectra for unpurified 40, 60, 80, and 100mer (above left) all showed the detection of truncation species and salt adducts, which are commonly observed by LC/MS. Also, as expected, the quality of the oligo products decreased as their size increased. To test the reproducibility of the method, 96 replicates of a 20mer were processed and the results for every 6th injection (n=16) were overlaid (above right). The deconvoluted spectra superimposed nearly perfectly. The zoom-in of the low abundance species also showed that many impurities, smaller than 1% of the main peak, were reliably detected.
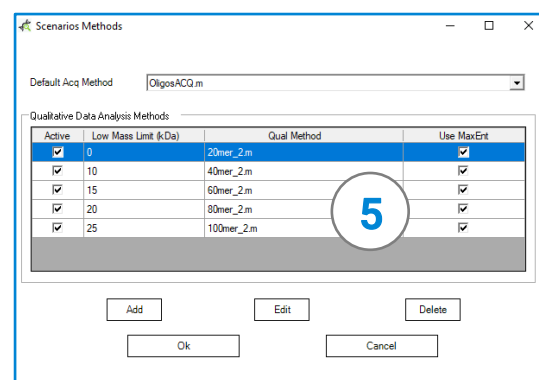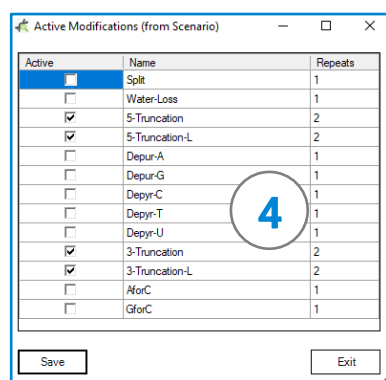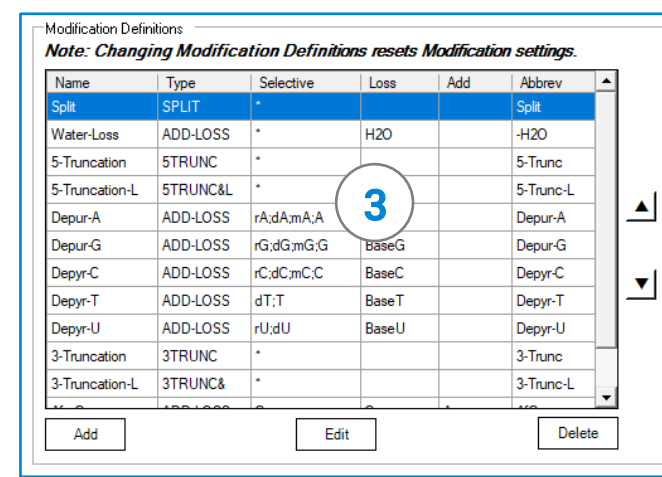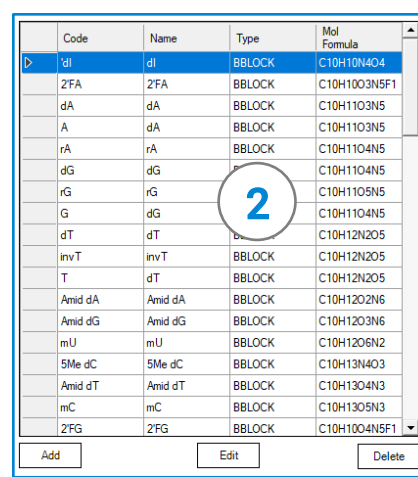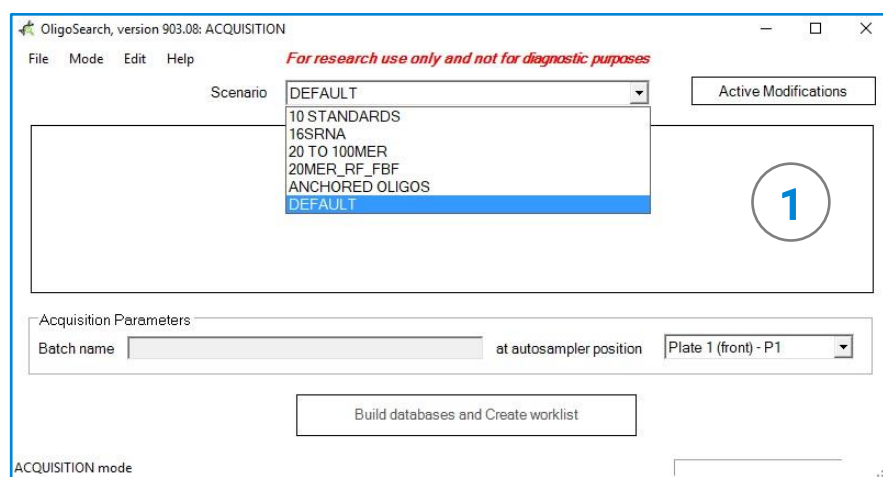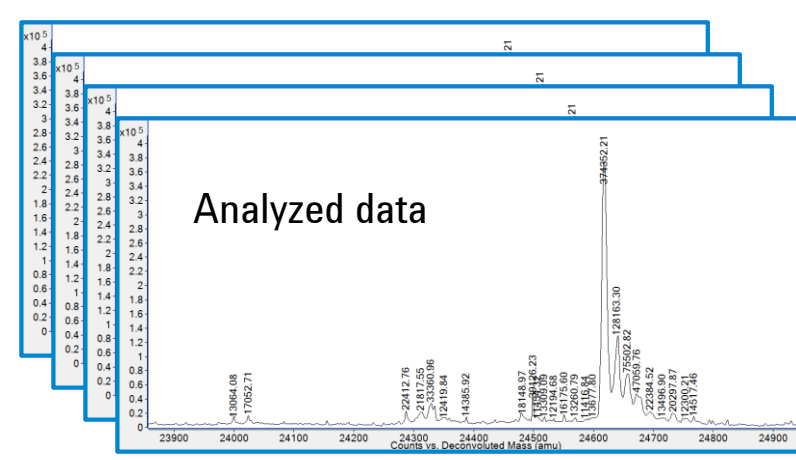
OligoSearch makes oligo processing easier.

OligoSearch is a stand-alone application that makes oligo processing easier by building sample-specific analysis methods and impurities databases (Find-by-formula and MaxEnt deconvolution). OligoSearch also provides worklists that, when brought into MassHunter, instruct the acquisition (for LC/MS) and/or analysis (for LC/MS and RapidFire/MS) of large sample sets in a fully automated way.
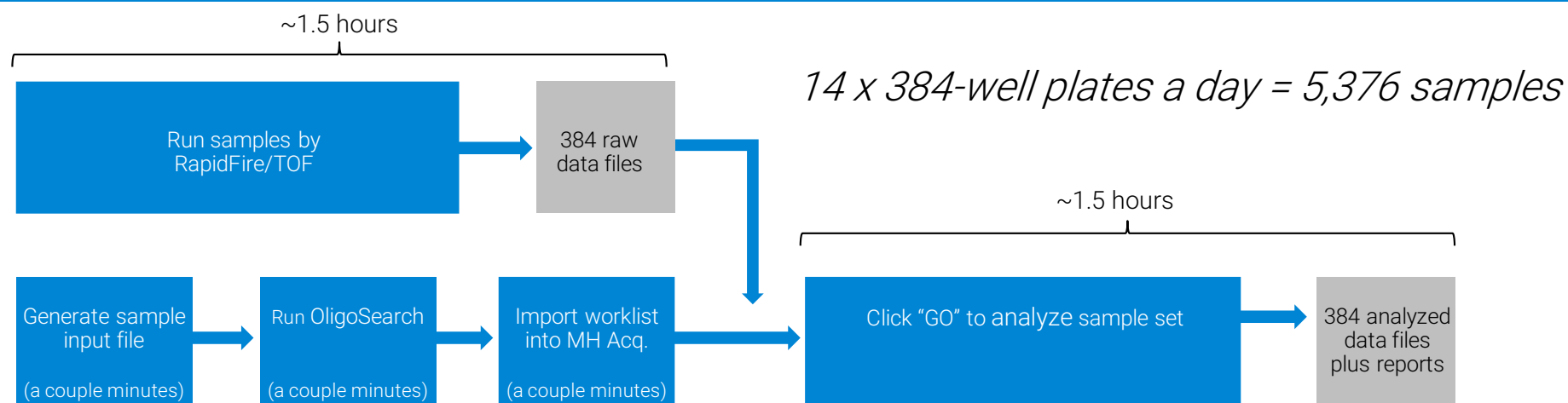
OligoSearch can be set up as follows: (1) Select or create the "scenario" which maintains project settings. (2) Supplement the default oligo components database with specialized/proprietary oligo sugars, linkers, tags, and nucleobases. (3) Supplement the default modifications definitions with additional impurity types to be interrogated. (4) Select which modifications to be included in the sample databases. (5) Indicate the acquisition and template analysis methods to use. Once the OligoSearch scenario is configured, the application is ready for routine use..

Raw data

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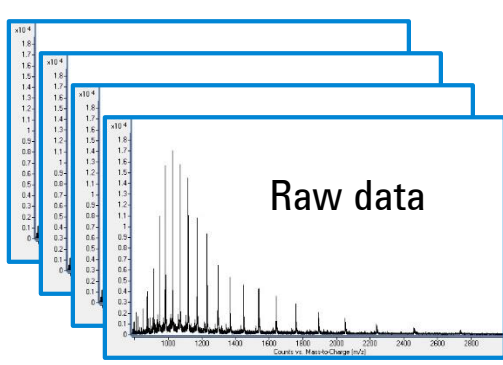
- Analysis methods including impurities databases
- Worklist that instructs the analysis of each sample in the set

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Analyzed data

- Select scenario
- Input sample sequences
- Click GO

OligoSearch

Reports with impurities annotated

OligoSearch enriches and facilitates the automation of data analysis.

OligoSearch must be given an input file that contains the well-plate location, laboratory notation, and sequence of each sample. OligoSearch then makes the analysis methods and impurities databases for each sample. OligoSearch also provides a worklist that relates these analysis methods to their corresponding data files. In doing so, OligoSearch not only removes the tedious and time-consuming steps of quality database production, but also enables the analysis of sample sets to be fully automated.

~1.5 hours

Run samples by RapidFire/TOF → 384 raw data files

14 x 384-well plates a day = 5,376 samples

~1.5 hours

Generate sample input file (a couple minutes) → Run OligoSearch (a couple minutes) → Import worklist into MH Acq. (a couple minutes) → Click "GO" to analyze sample set → 384 analyzed data files plus reports

Complete workflow for oligo acquisition and analysis using RapidFire/MS and OligoSearch.

When using OligoSearch with a RapidFire system, the acquisition and analysis steps are not directly coupled. This decoupling increases throughput by allowing the data from plate x to be analyzed while the data for plate x+1 are acquired (and so on) and creates a more versatile solution for data reprocessing. Assuming an average and rate-limiting acquisition time of 15 seconds per sample, the data for up to fourteen 384-well plates (5,376 samples) can be acquired and analyzed per day.

Here, the color-coding object was tested by designing a plate containing widely differing oligo purity
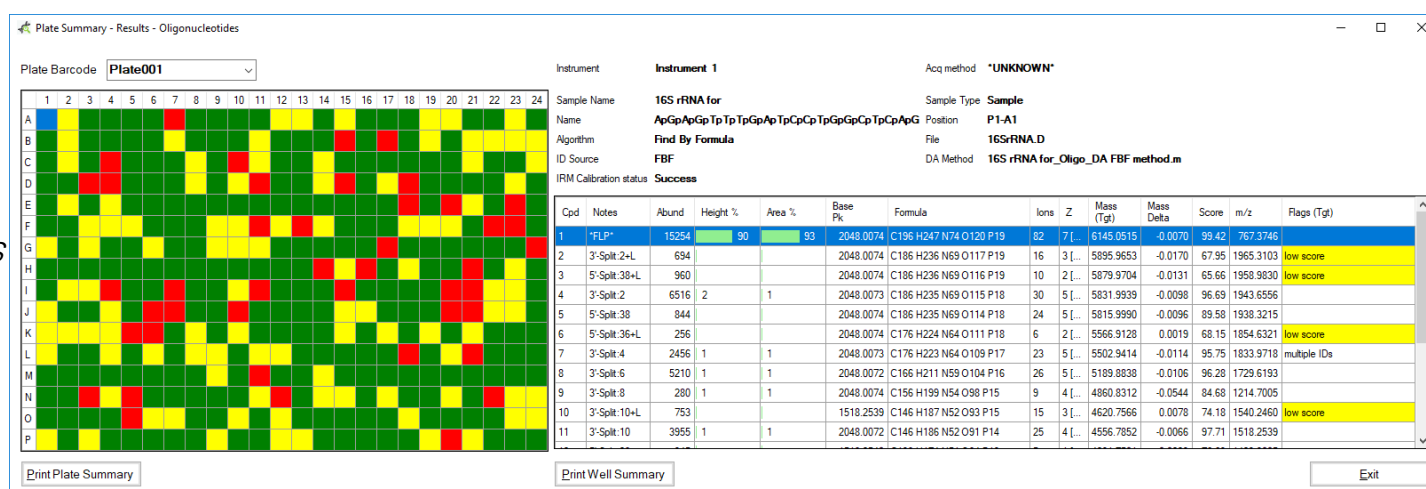
OligoResults is a stand-alone application that provides a summary of well-plate results.

OligoResults makes the review of oligo data simple by providing a summary of well-plate results. OligoResults looks at the CSV report for each sample, calculates the purity of the target, and color codes that location according to user-defined ranges (e.g. <85%, 85-90%, and >90%). Selecting a well-plate location brings up the corresponding annotated impurities report.

A high-throughput acquisition method for oligos was developed using a RapidFire/6545 system

The results showed good mass accuracy, resolution, isotopic fidelity, and dynamic range – revealing large numbers of impurities, some more than 100-fold less abundant than the target oligo.

OligoSearch simplified the analysis of oligos by providing (1) methods to annotate the impurities found in each sample and (2) a worklist to automate the processing of large sample sets.

Automated data analysis could run in parallel with acquisition of the next plate, enabling 14 x 384-well plates (5,376 samples) to be acquired and analyzed in a 24-hour day.

OligoResults simplified the review of processed samples by providing a color-coded summary of well-plate results.

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