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Climbing the oligonucleotide ladder toward rapid and wide-ranging oligonucleotide analysis using MALDI-MS.

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Results obtained from gel electrophoresis workflows can be acquired in *seconds* harnessing MALDI-MS.

1. Introduction

Biopharmaceutical and precision medicine technologies continue to transform drug design, life science research, and clinical diagnostics, demanding mass spectrometry (MS) techniques for fast, high-throughput oligonucleotide analysis requiring mass and sequence confirmation. Matrix-assisted laser desorption/ionization (MALDI-MS), provides a departure from toxic, time-consuming verification using gel electrophoresis and ethidium bromide. MALDI-MS techniques are amenable to rapid sample preparation and high-throughput studies, able to provide results in seconds.

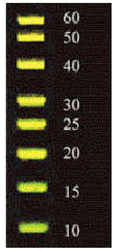

Figure 1. An oligonucleotide ladder used for mass verification in oligonucleotide gel electrophoresis.

2. Methods and Instrumentation

2-1. Sample Preparation

Oligonucleotide length standards were obtained as the 10/60 ladder from Integrated DNA Technologies (San Diego, CA). One µL volume was spotted onto Fleximass Stainless Steel MALDI Target Plates (Shimadzu Scientific Instruments, Columbia, MD). The matrix 0.5M 2',4',6'-trihydroxyacetophenone (THAP) was used and dissolved in 50% water, 50% acetonitrile. Diammonium citrate was added to chelate sodium ions.

2-2. Instrumentation

Benchtop MALDI-8020 technology was used to detect the prepared oligonucleotide mixture (Figure 2).

3. Results

3-1. Oligonucleotide ladder molecular weights

Nucleic acid sequences for each oligonucleotide length standards were obtained. Average [M+H]+ values were calculated using the Mongo Oligo Mass Calculator v2.08 from The RNA Institute, University at Albany, State University of New York. All oligonucleotides consist of single-stranded DNA and were unmodified with a hydroxyl group at both the 5' and 3' ends (Table 1).

MALDI-8020 Specifications	
Laser	355 nm, solid state, 200 Hz
Mass Range	*m/z* = 1 – 500,000
Resolution	>5000
Sensitivity	250 fmol protein 250 amol peptide
Ionization	Positive (+)

Figure 2. Benchtop MALDI-8020 instrument and specifications.

Length	Sequence	Average [M+H]+
10mer	ATC GCG GAT T	3044.053
15mer	GCT GCG ACG AGG CTG	4635.066
20mer	ATC GCG GAT TAG CAC TAC GT	6118.052
25mer	ATC TCG GAT TAG CAC TAC GCA TCG G	7643.039
30mer	ATC GCG GAT TAG CAC TAC GCA TCG GTT ACA	9192.051
40mer	ATC GCG GAT TAG CAC TAC GCA TCG GTT ACA AAC GAG TAC C	12275.063
50mer	ATC GCG GAT TAG CAC TAC GCA TCG GTT ACA AAC GAG GAC CTG ATG CAC TT	15380.074
60mer	ATC GCG GAT TAG CAC TAC GCA TCG GTT ACA AAC GAG GAC CTG ATG CAC TTT GAC AGC ATG	18494.098

Table 1. Oligonucleotide sequences and expected *m/z* values to be detected.

4-2. Simultaneous MALDI-MS detection of multiple oligonucleotide length standards in positive mode

Typical oligonucleotide analyses using MALDI-MS require negative ionization mode to achieve detection of nucleic acids. Paired with the described matrix conditions and the benchtop MALDI-8020, detection allowed for a *m/z* calibration range starting with the lowest calibrator at *m/z* = 3043.5 for oligonucleotide length of 10 nucleic acids and the highest at *m/z* = 9190.0, containing 30 nucleic acids.

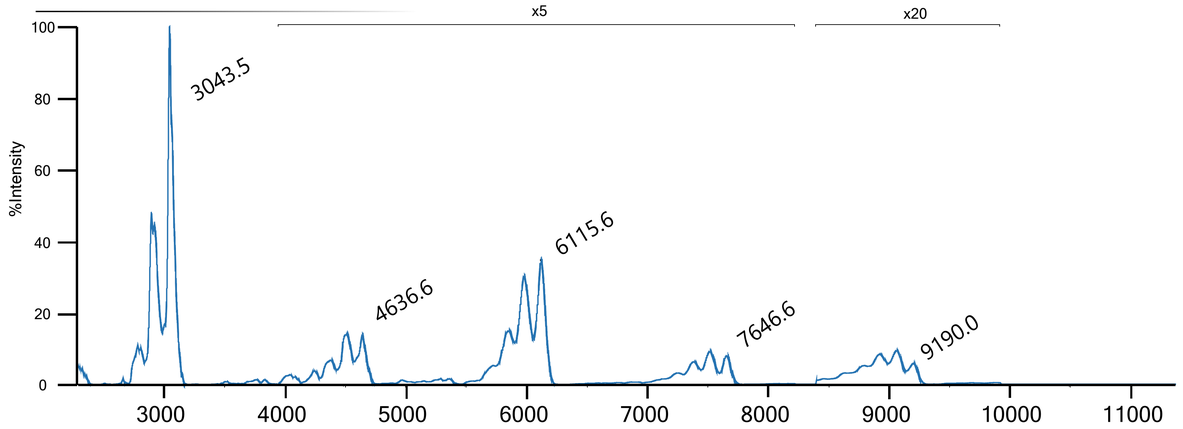

Figure 3. Observed positive ionization mode mass spectra of 10 – 60mer oligonucleotide standard. Of the 8 oligonucleotide lengths provided, 5 were detected using THAP and diammonium citrate as matrix.

4-3. Instrument Acquisition and Processing Parameters

MALDI-8020 employs software designed to guide the user workflow sequentially from data acquisition to processing, demonstrated in Figure 4.

MALDI-8020 Acquisition Parameters	
Mass Range	2000-20000
Laser Power	150
Accumulation Rate	30 shots @ 200 Hz
Pulsed Extract (*m/z*)	12360
Blanking Mass (*m/z*)	1500

MALDI-8020 Processing Parameters	
Baseline Subtraction	2000
Smoothing Method	Gaussian
Smoothing Filter Width	200
Peak Width	1
Peak Delimiter Method	Threshold Apex

Figure 4. Data acquisition and processing parameters used to generate mass spectra above.

5. Conclusions

- Moving from an agarose gel matrix to a MALDI matrix, this work aims to bridge time-consuming electrophoresis workflows to rapid MALDI analysis.
- Development of this study includes implementing a binary matrix for increased sensitivity of oligonucleotides.