



Custom
Oligonucleotides
Quality Control, Quality Assurance

Sigma® is recognized as the world's leading supplier of custom DNA and RNA oligonucleotides for the global life science research community. Our ability to guarantee oligo performance is directly related to our comprehensive understanding of oligonucleotide chemistry and synthesis platforms, our analytical systems and our experience in methods development.

Our Quality Commitment

Quality is an integral part of our manufacturing process as highlighted in **Figure 1**. Sigma analyzes all oligonucleotides by mass spectrometry, ensuring that our customers receive only the highest quality products. Complementary techniques, such as chromatography and electrophoresis, can also be employed to verify specifications are met. Our sizeable investment in state-of-the-art analytical equipment provides industry-leading tools to develop and monitor our processes. Sigma's careful selection of software tools for peak integration, waveform deconvolution and signal processing ensures the data produced will be meaningful, useful, and practical.

Having the right tools is only half the solution – Sigma's team of analytical chemists, molecular biologists and nucleic acid scientists is what truly sets us apart. Our team works together to create analytical methods to provide our customers with solutions to support their research, product development and commercial requirements.

Starting Materials & Chemistry

To guarantee the highest quality oligonucleotides, we start with the highest quality raw materials. Sigma-Aldrich is world renowned for producing high-quality chemicals, reagents and solvents. Critical raw materials are sourced from within Sigma-Aldrich and include: phosphoramidites, solid phase synthesis supports, reagents and solvents. Upon receipt of critical raw materials, incoming quality control is performed and may include water content analysis (Karl Fischer titration), phosphoramidite identification, solid support verification and acceptance based upon Certificate of Analysis. Additionally, a rigorous supplier qualification program is part of our quality management system, which allows Sigma to manage supplier relations and performance.

Chemistry protocols have been optimized to ensure > 99.0% coupling efficiency for each nucleotide addition during synthesis. Protocols are finely tuned to synthesize both short (< 35 mer) and long (>110 mer) oligonucleotides. Oligonucleotide synthesis is performed using the established phosphoramidite chemistry. We routinely investigate new chemistries including protecting groups, activators and synthesis supports to continually improve oligonucleotide quality and expand our product offering.

Instrumentation & Synthesis

Our in-house Engineering team includes mechanical, electrical and software professionals who design and qualify our proprietary synthesis and processing equipment. Instruments are qualified through an established design review process as well as Installation Qualification and Operational Qualification (IQ & OQ) programs. An extensive preventive and prognostic maintenance program for instruments provides optimal performance, ensuring consistency and reliability. Our LIMS system continuously monitors the progress of each oligo in real-time and nonconforming products are flagged for quarantine.

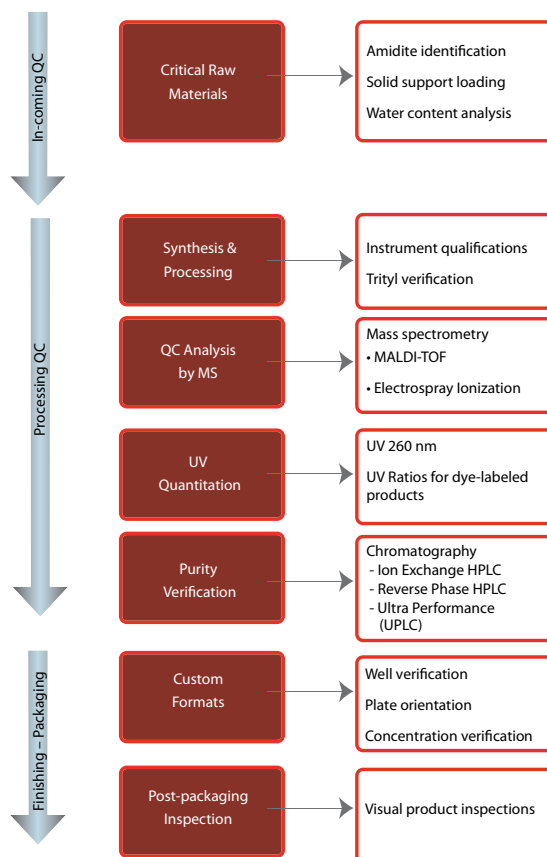


Figure 1. Production Process & In-process Controls

Quality Analysis by Mass Spectrometry

Mass Spectrometry is the technology of choice for analyzing oligonucleotide synthesis. It enables the most sensitive detection of low levels of by-products, which can affect performance such as:

- Incomplete coupling (N-1)
- Depurination
- Side-chain protecting groups
- Acrylonitrile adducts

Every oligonucleotide is thoroughly characterized by mass spectrometry, ensuring the highest quality.

We employ two different types of Mass Spectrometry (MS) to accomplish our 100% commitment to quality control. Each instrument type produces a determination of the oligonucleotide's composition through direct molecular weight measurement. While MALDI-TOF instruments typically have a higher throughput, the length limitation and lower performance for modified oligonucleotides limits the type of oligonucleotide which can be analyzed using this technique. As a natural complement to MALDI-TOF, Electrospray Ionization (ESI) MS provides the ability to analyze longer oligonucleotides as well as those oligonucleotides containing photo-labile modifications which can be problematic for MALDI-TOF analysis.

Matrix-Assisted Laser Desorption Ionization, Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS uses laser light in conjunction with a chemical matrix to impart a charge to the sample (ionization) in question and then accelerates the charged ions through a flight tube to the detector, which measures particle counts as a function of time, see **Figure 2**. The time-of-flight (TOF) is directly proportional to the mass of the molecule and has mass resolution of approximately 0.03%, i.e., resolution of +/- 3 Da on a 10 kDa oligonucleotide.

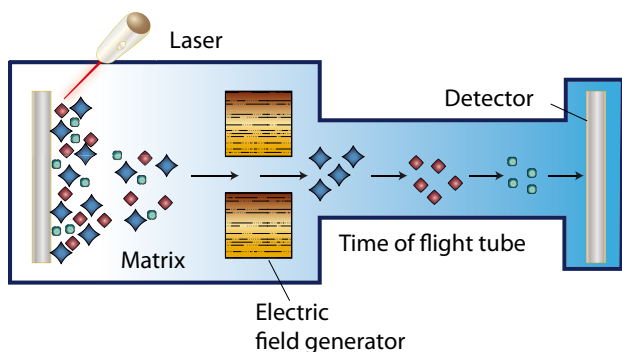


Figure 2. MALDI-TOF Principle

MALDI-TOF MS is ideally suited for primers and high throughput requirements, when speed is essential. Though a powerful and efficient technology to characterize oligonucleotides of lengths below 50 bases, MALDI-TOF MS has the disadvantage that the ionization efficiency, and therefore the resolution of the procedure, decreases rapidly for samples greater than 50 bases (>13 kDa). Furthermore, the laser source used to generate the charge could be detrimental to the analysis of modified oligonucleotides that are photosensitive. ESI-MS can be utilized to bypass these limitations.

Electrospray Ionization Mass Spectrometry (ESI-MS)

Applications requiring long oligonucleotides (>50 bases) e.g., microarray applications, cloning and/or gene synthesis, have increased the need for instrumentation that can accurately characterize these molecules. The method of choice for these oligonucleotides is ESI-MS, see **Figure 3**. The target molecules are ionized into multiple charge states producing a waveform that can be de-convoluted into parent peaks. As only the charge state will vary for the ions, oligonucleotides with high molecular weights can be analyzed using this method. Additionally, the inherently milder ionization conditions make this analytical technique a great tool for the analysis of labile compounds such as common quenchers, e.g., dabcyI and BHQs, used in dual-labeled fluorogenic probes. The ESI-MS systems have mass resolution of approximately 0.03%, i.e., resolution of +/- 3 Da on a 10 kDa oligonucleotide. For a comparison of MALDI-TOF and ESI, see **Figure 4** and the table summary.

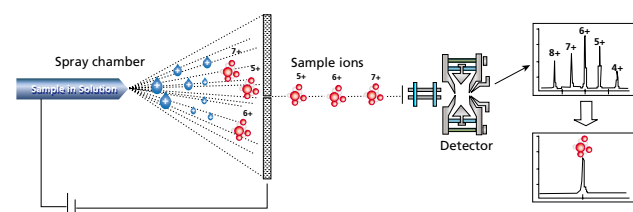


Figure 3. ESI/MS Principle

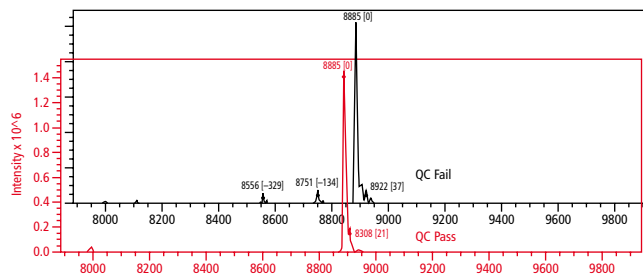


Figure 4. Pass/Fail Mass Spectra Comparison

Benefit Summary for MALDI-TOF vs. ESI-MS

Criteria	MALDI-TOF	ESI-MS
<50 bases	+	+
>50 bases	-	++
Photosensitive Modified Oligonucleotides	-	+
Degenerate (wobble) Oligonucleotides	-	+
Throughput	+++	+
N-1 Detection	+	+
Incomplete deprotection	+	+
Depurination	+	+
Mass accuracy	+	++

Quantitative Analysis of Oligonucleotides

Quantitative analysis of oligonucleotides is often required to verify the specifications have been met. MALDI-TOF and ESI-MS can provide a qualitative assessment of purity. Preferable methods for determining purity are ion exchange HPLC (IE-HPLC), reverse-phase HPLC (RP-HPLC), or ultra performance liquid chromatography (UPLC). The methods of choice, to fully characterize an oligo, depends on the nature of the oligonucleotide and in part on the final specifications.

Ion Exchange HPLC (IE-HPLC)

Ion exchange chromatography is the separation of different components (n-1, truncations, deletions, etc.) by differential exchange of ionic species, such as negatively charged oligonucleotides, with the ions bound to the separation phase, see **Figure 5**. This technique enables the analysis of oligonucleotides up to 40-50 bases with single nucleotide resolution. When coupled to UV-Vis or Photodiode array (PDA) detectors, samples containing modifications with an absorbance different from that of the oligonucleotide (260 nm) can be identified and thus easily quantified. However, the limited resolution obtained for oligonucleotides greater than 40 bases requires the utilization of different techniques, such as reverse phase HPLC or UPLC.

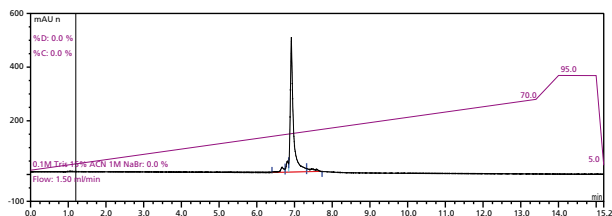


Figure 5. Ion-exchange Chromatogram

Ultra Performance Liquid Chromatography (UPLC)

Advances in particle chemistry performance, detectors, and systems, have transformed standard HPLC systems into ultra performance instruments. Such systems allow for the efficient separation of analytes; in this case, oligos of different lengths at a single base resolution for both short and long sequences. Efficiencies of the instrument allow for shorter separation times thus reducing the overall analysis time.

Reverse Phase HPLC (RP-HPLC)

Reverse phase chromatography results from the adsorption of hydrophobic molecules onto a hydrophobic solid support in a polar mobile phase. Decreasing the mobile phase polarity by using organic solvents reduces the hydrophobic interaction between the solute and the solid support resulting in selective elution. The more hydrophobic the molecule, the stronger it will adsorb onto the solid support (stationary phase), thus requiring more organic strength for elution of the molecule. Reverse phase systems can be configured with a wide variety of detectors, including UV, PDA and MS.

Reverse phase chromatography (complementary to IE-HPLC) is a powerful technique for separating a wide range of molecules.

LC-MS

For applications requiring specific purity profiles, or identification and quantification of by-products, we routinely develop extended LC-MS methods, see **Figure 6**. Unlike simple mass spectrometry measurements, the extended LC-MS methods combine the strengths of RP-HPLC with the precision of MS to fully characterize oligonucleotides and identify by-products. For complex mixtures, Selective-Ion-Monitoring (SIM) provides unequalled sensitivity for ultra-low level detection limits.

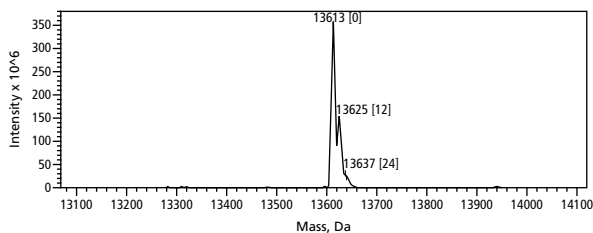


Figure 6. LC-MS Chromatogram

Quantitation by UV Spectroscopy

Oligonucleotides are quantified by UV Spectroscopy, i.e., OD at 260 nm. For accuracy, multiple measurements are made using automated UV-visible spectrophotometers equipped with path-length corrections. The extinction coefficient is calculated using the industry standard 'nearest-neighbor' method for determining the oligonucleotide quantity (μg and/or μmol).

siRNA Duplex Qualification

In addition to mass spectrometry measurements of siRNA simplexes, all siRNA duplexes are analyzed by gel electrophoresis (PAGE) to confirm proper duplex formation. The migration of the siRNA duplexes are compared to standards, ensuring RNA simplexes are properly annealed and ready for transfection.

Custom Formats

As automation continues to become a focus for many laboratories, delivery of oligonucleotide products in custom formats, such as 96 and 384-well plates, presents more challenges to ensure consistent quality. Product format, such as specific plates, concentration and the mixing of forward and reverse primers necessitates detailed specifications. Additional quality control measures are in place to verify accuracy for custom formats. These measures may include:

- Plate orientation and well position verification by mass spectrometry
- Mixed Primers, i.e. forward and reverse in a single well, verified by mass spectrometry, see **Figure 7**.
- Verification for machine readable barcodes (1D and 2D)
- Visual inspection of all master and daughter plates prior to shipment

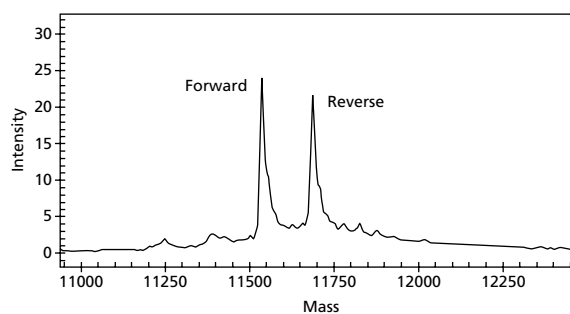


Figure 7. Mixed Forward and Reverse Primers by MS

Final Inspection

All products are subjected to a final visual inspection prior to packaging. Inspections provide a final assurance that finished products are in the correct formats, correctly labeled and ready for immediate use.



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Quality Management Systems Promise Quality Assurance

At the foundation of Sigma's manufacturing processes is a robust Quality Management System, which drives compliance to our ISO certification. This quality culture is integrated into all aspects of our business. The primary components of our Quality Management System include:

- Effective internal and external audits
- Corrective and preventive actions
- Change control and document control
- Training programs

Each component drives assurance for providing customers with the highest quality products and services.

Effective Internal and External Audits

We maintain the integrity of our ISO certification through a rigorous series of audits including:

- External registrar surveillance audits
- Internal audits
- Customer audits

Customers are welcome to audit any of our global manufacturing facilities for a first-hand view of our quality practices.

Corrective and Preventive Actions

Sigma strives to continually improve the effectiveness of our quality management system through the use of corrective and preventive actions. These procedures:

- Eliminate and prevent the causes of nonconformance
- Initiate process improvement
- Validate actions taken and measure effectiveness

Sigma maintains a database-driven system that aids in managing inputs from both internal and external customers.

Change Control and Document Control

When changes and improvements to processes are needed, our change control process assists in identifying possible risks as an outcome of the proposed change. These procedures allow for:

- Identification of documents within the changed process that need updating
- Identification and evaluation of risks
- Control of critical processes and procedures
- Rigid revision and approval process for procedures

Controlled documents are linked to our training database which ensures staff have been trained when processes and documents are altered.

Training Programs

Properly trained professionals sustain our precision-engineered processes. Training programs ensure:

- Critical skill sets are defined within each core process
- Training modules are administered to continually advance skill sets
- Programs are measured for effectiveness and skill sets are regularly assessed

Summary

The Sigma brand is synonymous with quality. Our continuous unwavering commitment to quality ensures we provide reliable, consistent products and services to meet our customer's research and/or commercial needs.

Let us ensure your next great discovery!

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