

Oligo Factory Enhances Oligonucleotide Synthetic Services through a Differentiated Partnership

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INTRODUCTION

Oligonucleotides are short DNA or RNA molecules that have a wide range of applications in research, from qPCR to functional genomics to targeted therapeutics. Synthesized in the laboratory by solid-phase chemical synthesis, these small segments of nucleic acids are manufactured as single-stranded molecules with any customized sequence. Research and commercial laboratories that require quick access to user-specified oligos often rely on external vendors for their custom oligonucleotide needs. Supported by a strong technological solution and long-standing partnership with Waters Corporation, Oligo Factory developed an innovative RNA/DNA oligonucleotide purification methodology to drive manufacturing process improvements. This case study explores how Oligo Factory partnered with Waters to pioneer a fresh approach to oligo purification by using low-pressure operation on the Waters LC Prep 150 System resulting in improved product quality, operational efficiency, ROI, and, ultimately, customer satisfaction.

INNOVATIVE MINDSET

Oligo Factory was founded in 2006 by individuals with expertise in oligo synthesis technology and the oligo service business. Oligo Factory emphasizes science and product excellence to provide custom oligos and high-quality purified DNA and RNA at medium- to large-scale quantities to the research and life science communities. The company consists of a growing team of skilled chromatographers, synthesis chemists, instrumentation engineers, and commercialization specialists with more than 60 years of combined experience. Oligo Factory is passionate about continuous investments in technology and people to innovatively manufacture large-scale products with maximized yield and purity, such as large-scale PCR oligos, primers, probes, guide RNAs, antisense oligos, aptamers, PTOs, natural RNA and DNA oligos, and chimeric oligos. They have also synthesized oligos with almost every modification, including (but not limited to) 2'-OMe RNA, 2'-MOE RNA, 2'-Fluoro RNA, LNA, C3/C6/C12-Aminos, C3/C9/C18-Spacers, PEG, FAM, ROX, Cy3, Cy5, BHQ, Alexa, C3/C6-Thiols, and other unique formulations.

The Differentiating Benefits of Partnering with Oligo Factory

- **Superior customer relationships and communication:** Oligo Factory treats every custom product with ultimate care and attention to ensure results for customers' research or products. Their proactive communication and excellent technical and customer support are focused on customer success.
- **Experience and Insight:** Oligo Factory's services and products are built to serve all the DNA/RNA synthesis markets and applications. Their world-class oligonucleotide synthesis combined with decades of knowledge, enables them to tackle any challenging synthesis or oligo product needs.
- **Reproducible quality:** Oligo Factory's proprietary synthesis and purification systems deliver high-quality oligos at the purest levels available addressing customers' research to production needs.

CLIENT SUCCESS THROUGH QUALITY AND DIFFERENTIATION

For more than 15 years, Oligo Factory has served customers ranging from research institutions to multi-national pharmaceutical and life science companies spanning the globe. Oligo Factory differentiates itself by developing partnerships with customers at a project level – from custom design to unique purification needs and quality assessments – and by understanding customers' desired downstream results. They recognize that customer service entails a bi-directional conversation that enables both parties to work together to find the best solution to accommodate any request. Oligo Factory's scientists and technicians have the responsiveness, experience, and chemistry background to optimize projects to save customers costs without diminishing quality. Their ability to synthesize custom oligos with quick turn-around time, as well as their ability to establish personal relationships with customers is driving client acquisition and retention in today's competitive biotech market.

Lastly, Oligo Factory was proud to be awarded ISO 9001:2015 certification in 2022, and they are also working on meeting the requirements for ISO 13485:2016 quality systems standards. As Oligo Factory continues to invest in its culture, technology, and quality standards, they are enhancing the regulatory framework that will be needed to move to GMP manufacturing and becoming cGMP compliant by early 2024.



Figure 1. Oligo Factory recently moved into a new oligo manufacturing facility that increased its production capacity by more than 12x.

- **Cost savings:** Oligo Factory provides mass production of oligos with high purity from milligram to gram to kilogram quantities at consistent, sufficient recovery and productivity at a price that helps customers to grow.
- **Significant productivity and quick turn-around time:** This is realized by increasing the injected mass load using Oligo Factory's unique use case of the Waters Prep 150 LC Systems also enabling efficiency and cost savings.
- **Proactive supply chain management:** Oligo Factory preemptively manages its monomer and supports vendors to mitigate the impact of any supply chain issues. They strategically anticipate problems and order their customary supplies before they are needed.

A PARTNERSHIP THAT STIMULATES INNOVATION

Improving the processes that contribute to the quality and enhanced delivery time of custom oligo products is a way to create scalable and sustainable change, and the right process improvements can be enhanced by adopting a strong foundation with the right technology partner. In collaboration with Waters, Oligo Factory has modified the functionality of a fleet of Waters LC Prep 150 Systems with custom-packed glass anion exchange (IEX) columns to run at low pressures and low flow rates for the purification of larger-scale synthesis. They also work closely with other parts of Waters' business, including consumable products, technical support, and training. However, the consultative partnership is more than just Oligo Factory establishing Waters as the single supplier of their separation solutions – it also entails a true collaboration with Waters to provide any scientific or application support needed to help maintain the caliber, purity, and timeliness of their products.

A UNIQUE TECHNOLOGY OPTIMIZATION STRATEGY

SYNTHESIS

Oligo Factory further differentiates itself through industry-leading innovation in proprietary automated oligonucleotide synthesizer development. The oligo synthesis instruments, optimized for mid- to large-scale quantities, are built in-house and utilize proprietary software. These instrument platforms satisfy the needs of customers migrating from research to commercial scales.

PURIFICATION

Following automated synthesis, Oligo Factory uses multiple purification methods to isolate pure full-length oligos. Anion exchange high performance liquid chromatography (HPLC) is the separation chemistry of choice for most oligo purifications, as separation by charge yields the purest oligos with maximum chromatographic resolution. Reverse phase HPLC is used for some oligos, dependent on modifications and potential higher-order structures. Multiple, different oligos ranging in length from 5-100 nucleotides with varying modifications can be purified in a single day at various scales. This variation in oligo structure necessitates a flexible purification platform, so that universal buffers and methods can be easily adapted to best fit the characteristics of the oligo and the final oligo purity required for the project.

UNIQUE LOW PRESSURE – LOW FLOWRATE PREPARATIVE SCALE INNOVATION

Oligo Factory is using a fleet of Waters LC Prep 150 Systems configured with 2545 Quaternary Gradient Modules (QGM), 2489 TUV Detectors, and Waters Fraction Collector III's designed to isolate full-length product from crude oligos synthesized in the lab. The specialized prep systems are controlled by the Waters FractionLynx Application Manager running in Waters MassLynx™ Software. This configuration provides great flexibility in selecting purification methods and fraction collection conditions. FractionLynx and MassLynx Software offer options for custom fraction collection bed layouts, as well as custom collection vessels. This gives the laboratory team the ability to adjust the collection method based on oligo composition and scale. The Waters 2545 QGM typically includes 1/4" flexible solvent lines as standard, but for Oligo Factory's unique large-volume sample loading configuration the standard 1/4" tubing does not provide the flexibility required in the workspace, so the solvent lines were exchanged with 1/8" tubing and fittings. Taking advantage of MassLynx Software's reinjection feature, the sample is introduced through one of the solvent lines in the method to re-inject a sample multiple times, eliminating the use of an injector. By utilizing a solvent line for automated sample introduction, manual injections are not used, so there is no need for a chemist to periodically inject material. Fraction pooling then collects the fractions repeatedly into the same location, which helps to decrease analytical time after purification. Auto-collection on the fraction collector is based on peak detection, so collection begins at the same point for each individual injection. A custom collection rack is programmed to accommodate the large volume collection required by this unique application.

"The team at Waters has been instrumental in our Purification Lab expansion and strategizing our unique use case of purifying custom oligos at scale. We look forward to growing the partnership with other Waters software solutions and instrumentation in the near future."

Chris Boggess, Oligo Factory CEO

PURITY DETERMINATION

Oligo synthesis involves a large number of individual reactions which may lead to impurities such as truncated oligos so purity must be determined following purification. The purity of the pooled fractions is determined by anion exchange chromatography using Waters Gen-Pak FAX Columns on Waters ACQUITY™ UPLC™ H-Class PLUS Bio Systems running on Waters Empower™ Software. The analytical method can separate failure sequences from the full-length product peak, and the peaks for oligos differing by a single base can be visually observed on the resulting chromatogram. Customers define purity specifications depending on their downstream needs. Through purification, the whole crude oligo is split into fractions and each fraction is analyzed, then the higher-purity material is pooled and desalted via ultrafiltration. During the final QC process, Oligo Factory confirms molecular weight by mass spectrometry (MS) as well as IEX HPLC analysis. Following analysis and ultrafiltration, the final product is vacuum-filtered through a 0.22-µm filter, followed by lyophilization and packaging for shipment.



Figure 2. Oligo Factory scientists analyze samples in the company's new facility.

References

1. Gilar, M. et al., HPLC and UPLC Column for Analysis of Oligonucleotides, Waters Application Note, [720002376EN](#), 2020.
2. Ivleva, V., UPLC-MS Analysis of Interfering RNA Oligonucleotides, Waters Application Note, [720002412EN](#), 2008.
3. [Oligo Factory Opens New State-of-the-Art Oligo Manufacturing Facility](#), Business Wire, 2023.

INNOVATION PARTNERSHIPS: THE WAY FORWARD

For those in custom contract manufacturing like Oligo Factory, growth typically involves innovating by integrating new technologies to expand capacity and/or introduce new capabilities into existing workflows. While equipment can be purchased from a variety of vendors, growing companies need more than just a technology supplier – they need a consultative business partner who will work with them to find solutions to challenges that arise during any phase of their process, from technology pre-sale to post-implementation. Optimizing oligo manufacturing equipment, consumables, and chemistries is a critical aspect of process development. Knowledge of the equipment, the process, and the product are paramount, and successful projects rely on experts embedded in a vendor's organization at every customer touchpoint. Close cooperation between instrument suppliers and users is a key to success, as the life sciences and biopharma industries address the ever-growing need for process improvement, speed-to-market, and scientist training. Waters has proven to be a true outcome-based partner that helps Pharmaceutical Contract Organizations (COs) with their innovation strategy to expand technical capabilities, increase operational efficiencies, and minimize risks to maintain competitiveness and differentiate their lab's quality and services to drive business. That innovation can include helping COs to fearlessly embrace easy-to-use, in-house mass spectrometry detection for molecular weight confirmation, and by helping labs digitize their data management solutions for future compliance readiness. Using the example of Oligo Factory's unique use case of commercially available instrumentation, COs can become more competitive by offering more efficient and innovative lab services, and a customer-centric vendor partnership can help COs add differentiating analytical functionality to attract new customers and maintain existing ones.

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