AJINOMOTO

BIO•PHARMA SERVICES

THE POWER TO MAKE®

FROM RESEARCH TO COMMERCIAL QUANTITIES:
A SEAMLESS SUPPLY OF OLIGONUCLEOTIDES

Oligonucleotides are poised for high growth over the next five years, with the market doubling in value during this timeframe. This growth is driven by the high application potential of oligonucleotides to be used in the treatments of a variety of medical conditions, the growing number of FDA approved oligonucleotide drugs, an increased focus on customized or personalized medicine, and a continued emphasis on the development of therapeutics for rare disease.

As a result, contract development and manufacturing organizations (CDMOs), such as Ajinomoto Bio-Pharma Services, are seeing increased demand for oligonucleotide synthesis. However, with this developing market comes some considerable challenges for drug manufacturers in the ability to cost-effectively, quickly and easily scale from research amounts to commercial needs.

In this white paper, we will discuss how Ajinomoto Bio-Pharma Services meets this seamless supply challenge by providing development, scale-up and synthesis capabilities of research to commercial quantities of customized oligonucleotides.

THE POWER TO MAKE®

OLIGONUCLEOTIDES

Custom oligonucleotide manufacturing involves making changes to nucleobases, the sugar backbone, or the phosphodiester bonds to create a specific DNA or RNA molecule, creating for example, BNA (bridged nucleic acids) and LNA (locked nucleic acids). These chemical modifications have led to the development of new drugs for various diseases and therapies that previously were difficult to treat using conventional medical treatments.

Additional nucleic acid technologies include the custom synthesis of antisense oligonucleotides (ASO), small interfering RNA (siRNA) and microRNA (miRNAs), which are used to modulate gene and protein expression, aptamer RNAs, which modulate protein functions, as well as CpG oligodeoxynucleotides (CpG ODN), which are used as vaccine adjuvants.

Given the broad variety of platforms and modalities, oligonucleotides hold a strong promise and a growing portfolio for researchers and biopharma drug companies in developing effective, targeted, next-generation therapeutic drugs and vaccines.

SOLID PHASE SYNTHESIS

Custom oligonucleotide manufacturing is accomplished through traditional solid phase synthesis (chain elongation) using fully automated oligonucleotide synthesizers, which can manufacture to specific quantity batches. The customized configurations on these machines allow for synthesis flexibility and yield improvement, at any scale from µg to 20 kg quantities.

Once the oligonucleotide has been synthesized, an appropriate deprotection method is selected based on the optimal conditions of the specific oligonucleotide. Purification by HPLC or MPLC is then completed to ensure the correct purity and the oligonucleotide goes through desalination by ultrafiltration to remove any excess salts. From there, the oligonucleotide can be lyophilized or filled as a sterile solution.

Before the oligonucleotide is released to the customer, analytical testing is completed to ensure product quality. This testing can include visual inspection, molecular weight confirmation, sequence analysis, UV measurements, and tests for purity, sodium and endotoxin.

Solid phase oligonucleotides can be synthesized as either *in vivo* or GMP grade. *In vivo* grade is suitable for pre-clinical studies, while GMP is required for clinical and commercial products. Ajinomoto Bio-Pharma Services has the capability to synthesize oligonucleotides in *in vivo* grade and with GMP compliance to meet a wide range of product needs.

LARGE SCALE SYNTHESIS

Historically, scale up to large scale production of oligonucleotides has been a challenge due to the increase of manufacturing costs. Using smaller scale synthesizers to develop numerous batches of oligonucleotides leads to cost-inefficiencies from reagents and materials, making it difficult to bring a commercial scale drug therapy to market.

Recently, large scale synthesizers for kilogram quantities of oligonucleotides have been made available in the market, which has enabled larger, more cost-efficient production batches. In 2019, Ajinomoto Bio-Pharma Services opened a large scale oligonucleotide API manufacturing facility, in order the better serve the increasing demand of the oligonucleotides market.

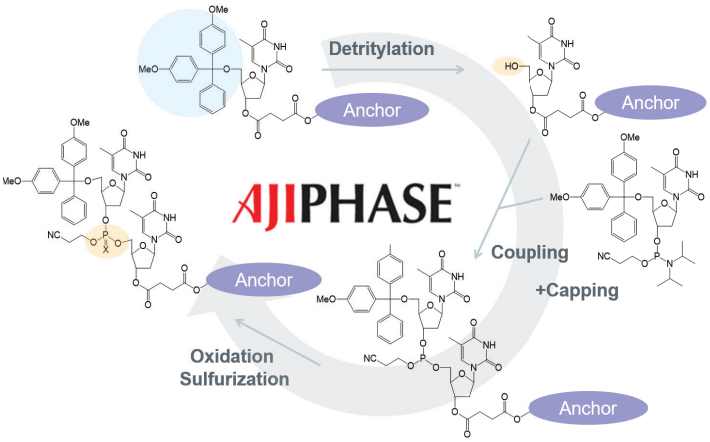
This 2,000 m^2 manufacturing facility provides a Class 100,000 clean area for cGMP manufacturing of oligonucleotide APIs, a multipurpose room to custom synthesize heavily modified oligonucleotides, and an OligoProcess synthesizer, for large scale solid phase oligonucleotide manufacturing. This customized synthesizer provides the capability to produce up to 20 kg quantities, making it suitable for late stage clinical trials and commercial production. The facility also houses R&D labs for the development of novel oligonucleotide manufacturing technologies.

As a manufacturing alternative, solution (or liquid) phase synthesis provides advantages to large scale solid phase synthesis through cost reduction, scalability and purity. However, depending on the type of oligonucleotide (natural DNA/RNA, LNA, BNA, 2'-F, 2'-OMe, etc.), the physiochemical properties of some oligonucleotides can make using solution phase synthesis incompatible. To overcome this obstacle, PEG and other anchor molecules can be used to negate the lipophilic and insolubility of the intermediary oligonucleotide.

AJIPHASE® SYNTHESIS

One such approach is AJIPHASE®, a hybrid of solid and solution phase syntheses that uses an anchor to make the molecules very soluble in non-polar solvents, providing a homogenous mixture. After the reaction occurs, the anchor is filtered out along with the excess reagents and byproducts, providing the recovered intermediate, all in one reactor.

This advanced technology allows Ajinomoto Bio-Pharma Services to manufacture commercial quantities of various oligonucleotides under GMP. When compared to traditional solid phase synthesis, the AJIPHASE® technology uses less solvents and reagents, while providing high purity and equivalent quality, high yield batch sizes.



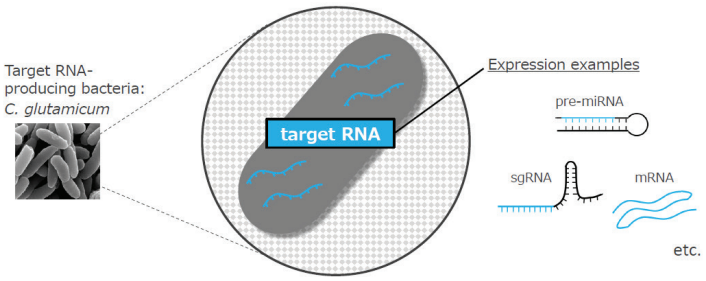
FUTURE ADVANCEMENTS

Overall, the future of oligonucleotide synthesis and the ability to successfully develop large scale quantities of oligonucleotides that can be used in drug therapies are both dependent on a CDMO's ability to effectively scale up oligonucleotides to manufacture at a lower cost, while maintaining a high yield and purity.

This becomes especially important in long chain RNA, as yield goes down as the chain length increases. To manufacturing long chain RNA, enzymatic condensation is used to combine long chimera DNA/RNA by fragment enzymatic ligation methods. This gives high purity and accurate molecular weight, but at a high cost to manufacture in quantities larger than research scale. Therefore, new manufacturing technologies to ensure high scalability at a lower cost is incredibly important.

The Research Institute of Biosocience Products and Fine Chemicals, a R&D arm of Ajinomoto Co., Inc., is working on a novel RNA producing system based on microbial fermentation of *Corynebacterium glutamicum*. Ajinomoto Co., Inc. has a long history and vast experience using *C. glutamicum* in various bio-manufacturing applications.

In this application, *C. glutamicum* is used as a recombinant RNA producing host strain. This fast growing, robust bacterium offers high productivity of target RNA, is non-pathogenic and non-endotoxic, has a clarified genome sequence and low endogenous RNase cellular activity. Using microbial fermentation, *C. glutamicum* provides a novel RNA producing system for the bio-production of various types of long chain RNA (> 100 mer) with high scalability and yield at a low cost with high purity profiles.



Ajinomoto Bio-Pharma Services has invested significant resources to build manufacturing platforms that meet the needs of the oligonucleotide market, from research (µg – mg) to commercial (g – 20 kg) quantities and everything in between. Our goal is to provide clients with the confidence that they can cost-effectively, easily, and quickly develop, scale up, manufacture and aseptically fill their oligonucleotide from beginning to end. By selecting a CDMO that possesses extensive oligonucleotide experience, a structure that meets your stage of production, and the range of service capabilities to meet your needs, you will find yourself more prepared to seamlessly move your oligonucleotide-based drug therapeutic out of the lab and into the hands of patients, who need it most.

Ajinomoto Bio-Pharma Services Oligonucleotide API Manufacturing Center at the Osaka, Japan campus.

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