

IDENTIFYING SUSTAINABLE PATHWAYS TOWARD OLIGONUCLEOTIDE THERAPEUTICS

Source: Ajinomoto Bio-Pharma Services
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After nearly 50 years of research and development, oligonucleotide therapeutics are starting to make their mark on the pharmaceutical scene. Over the next several years, the market is poised to grow significantly, with 16 oligonucleotide drugs approved in Japan in 2022.¹ As interest in therapeutic oligonucleotides accelerates, manufacturers and researchers are working to identify and develop processes that address the significant environmental burdens of oligonucleotide manufacturing while saving resources and improving scalability.

Waste, chemical hazards, and energy efficiency are all important considerations as manufacturers set out to increase production volumes of therapeutic oligonucleotides. In response to this priority, Aji Bio-Pharma, a leading research, development, and manufacturing organization, has developed two sustainable solutions for oligonucleotide production: AJIPHASE® liquid phase synthesis, which reduces the use of raw materials, and the enzymatic method, which simplifies the purification process. As you examine the methodologies that work best for your company's needs, explore innovations that maintain scalability and efficiency while reducing waste and consumption.

The Environmental Impact of Oligonucleotide Manufacturing

Despite their major contributions to the world economy, pharmaceutical companies have been one of the leading causes of the global carbon footprint, producing approximately 1.9 Mt CO₂ per annum, noticeably higher than other industries.² Green chemistry, which focuses on implementations that can minimize the generation of hazardous substances, is one viable option to reduce pollution. In

2016, the American Chemical Society (ACS) Green Chemistry Institute Pharmaceutical Roundtable (GCIPR) identified the development of greener operations for oligonucleotide therapeutics as a critical unmet need. The ACS GCIPR oligonucleotide sub-team used process mass intensity (PMI) to determine the sustainability of oligonucleotide manufacturing. PMI is a measure of material efficiency and sustainability that determines the quantity of raw materials (kg) required to produce one kg of isolated API. PMI takes into consideration all materials used, including water. However, it does not account for energy usage, environmental impact, or starting material complexity.

In Figure 1, Graph A (by material type) shows that roughly equal amounts of organic material and water are used in oligonucleotide production, with wash solvent making up half of the organic materials.³ Graph B (by process stage) shows that the synthesis and purification stages make the most significant contributions to the PMI and that the purification stage is responsible for approximately half of the material used.³ Thus, improvements to the purification stage could make oligonucleotide manufacturing significantly greener. PMI, however, treats the

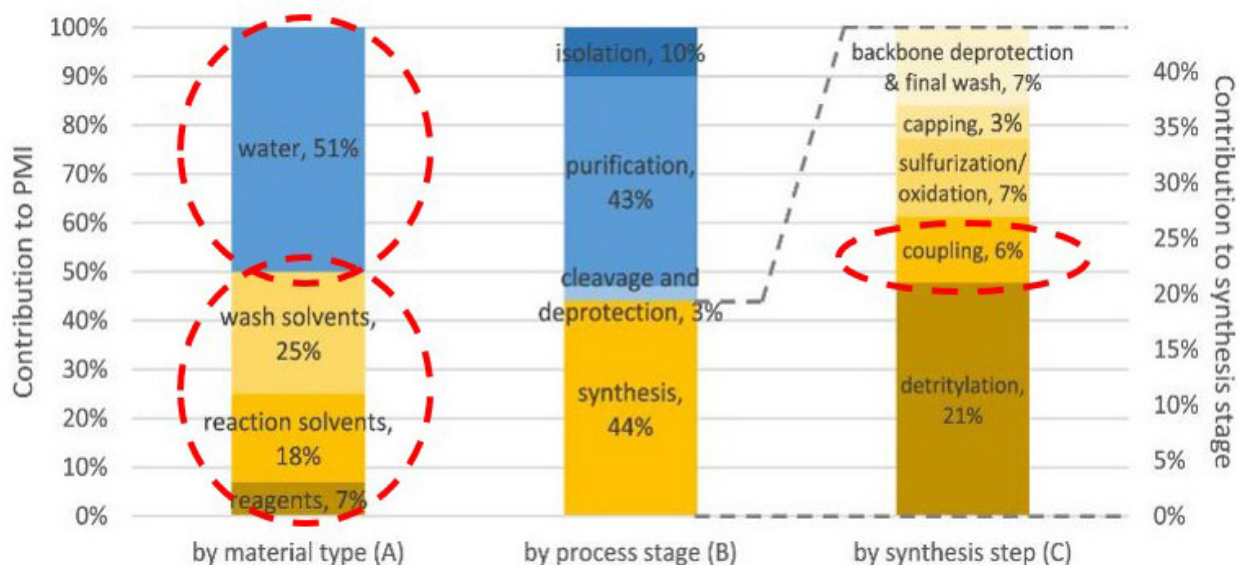


Fig. 1 – PMI findings from the ACS GCIPR oligonucleotide sub-team.⁹

environmental impact of water and organic solvents equally, while organic solvents account for significantly more degradation.

An alternate index for estimating the greenness of chemical procedures is an emission factor, which may

be a more accurate estimate of the environmental impact of oligonucleotide manufacturing. Figure 2 demonstrates how to determine how much CO₂ is emitted to produce the necessary quantity of bulk API. To estimate the emission factor of certain products, account for the input and output sources shown in Figure 2.

$$\text{Emission Factors (kg CO}_2\text{e/kg)} = \frac{\text{Quantity of CO}_2\text{ emission (kg)}}{\text{Quantity of bulk API out (kg)}}$$

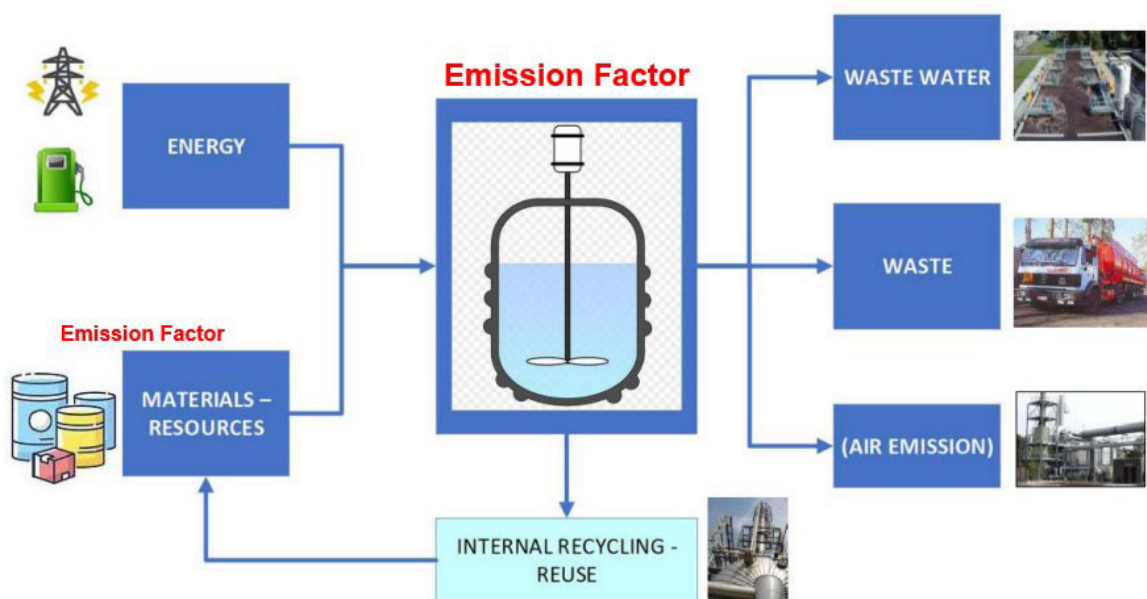


Fig. 2 – How to determine the emission factor.

Now, let's explore two oligonucleotide production technologies offered by Aji Bio-Pharma and their environmental impacts: AJIPHASE[®] synthesis for general oligonucleotide therapeutics and the enzymatic method for siRNA, specifically.

The Benefits of AJIPHASE[®] Synthesis

There are currently two different methods of oligonucleotide manufacturing: solid-phase synthesis and AJIPHASE[®] synthesis. The traditional method, solid-phase synthesis, is widely used and can synthesize the target oligonucleotide quickly. That said, it can also lead to challenges: it requires a dedicated solid-phase synthesizer, has limited manufacturing volume, and uses significant raw materials, including monomers and organic solvents. To solve these problems, Aji Bio-Pharma developed a large-scale, liquid-phase synthesis method called AJIPHASE[®].

The key to AJIPHASE[®] technology is a compound called the anchor. Solid-phase synthesis is carried out on a resin composed of insoluble particles. Oligonucleotides are elongated from the resin in a liquid/solid two-layer system. On the other hand, AJIPHASE[®] uses a soluble anchor instead of a resin and all reactions are carried out in a liquid homogeneous system. The anchor enables oligonucleotides to dissolve in certain solvents even if they are getting longer. Figure 3 demonstrates the complete AJIPHASE[®] process.

Initially, the anchor is dissolved in non-polar solvents, then the elongation reactions are anchored.

These are the same reactions that occur in solid-phase synthesis as part of the phosphoramidite method – the mainstream method of oligonucleotide synthesis. Following the reaction, organic solvent is poured into the solution to precipitate the target oligonucleotide. Finally, the accessories and byproducts are removed by filtration, and the intermediate with anchor is linked to another monomer within this cycle. This is repeated with each nucleotide in the sequence until the fully protected oligonucleotide has been assembled. Because the elongation reaction is the same, there is no difference in the purity profiles of the resultant oligonucleotide.

Perhaps the most important advantage of AJIPHASE[®] is the manufacturing volume. Solid-phase synthesis has a limitation on the production volume dependent on the synthesizer. Because any standard reaction tank can be used, AJIPHASE[®] technology has no limitation. In a homogeneous system, chemical reactions proceed well, reducing reagent consumption. Since the intermediates are soluble, it is possible to monitor and optimize each step in a similar manner to standard small molecule chemistry, minimizing the use of excess reagents. Furthermore, the AJIPHASE[®] process has only one washing step per one mer elongation, whereas solid-phase has several washing steps per one mer elongation. However, the AJIPHASE[®] manufacturing period is longer because solid-phase can be conducted automatically by programming. Figure 4 shows a side-by-side breakdown of their advantages and disadvantages.

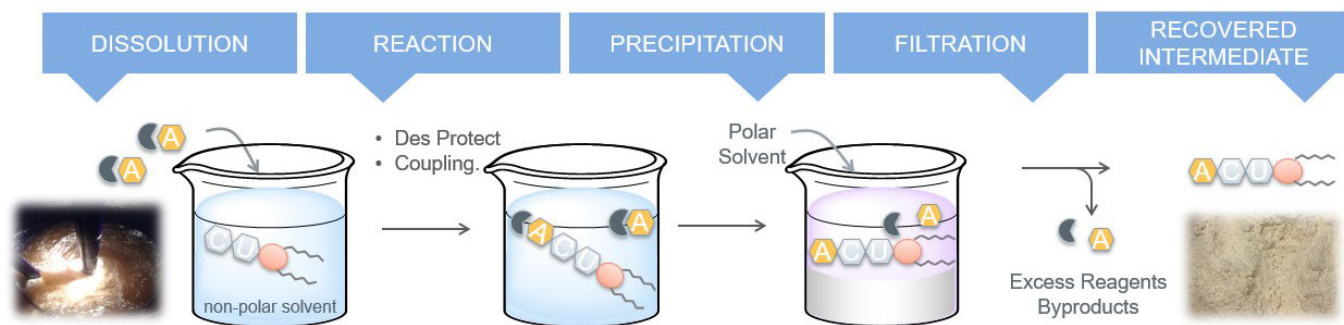


Fig. 3 – Step by step AJIPHASE[®] synthesis.

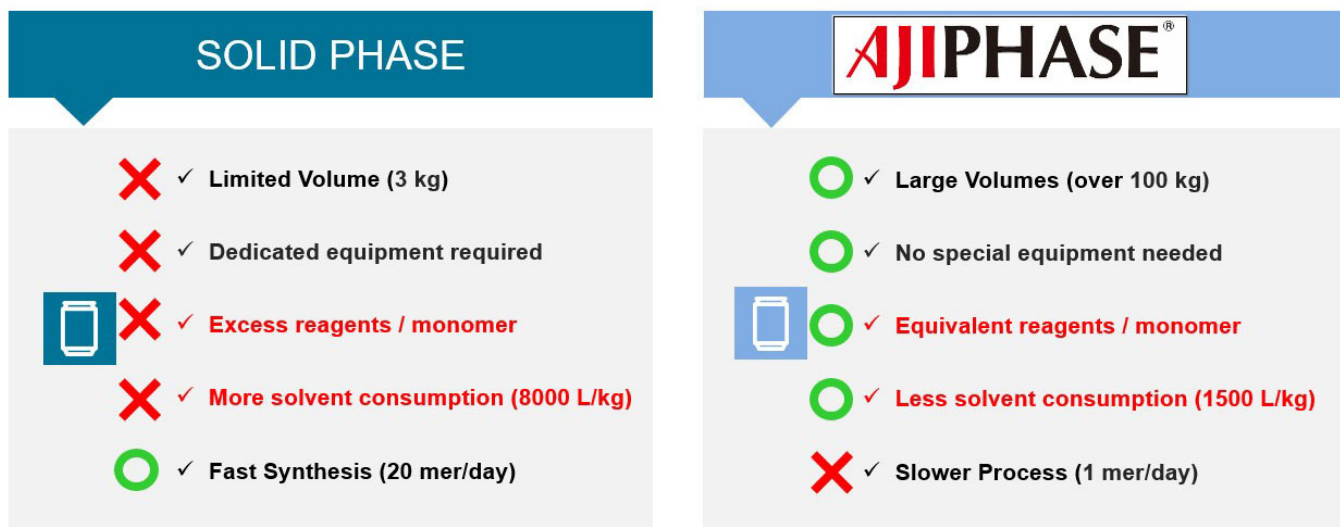


Fig. 4 – A breakdown of the advantages and disadvantages of solid-phase and AJIPHASE® synthesis.

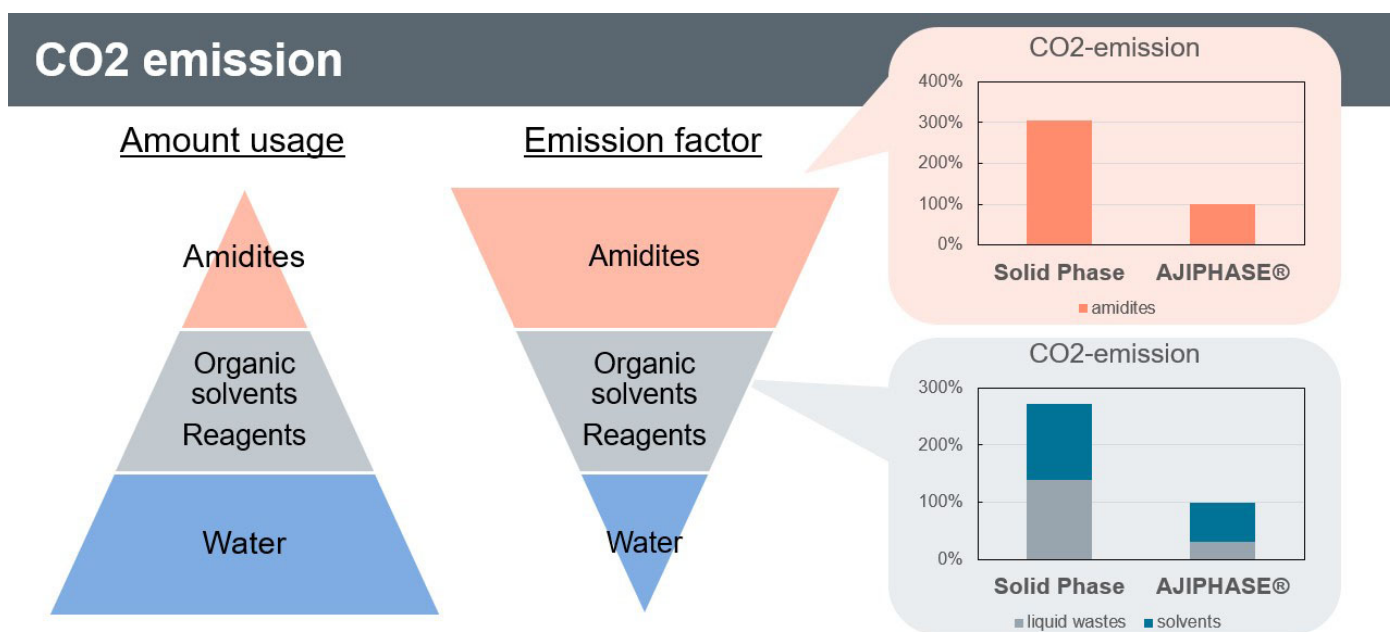


Fig. 5 – Graphs highlighting amount usage and emission factors for solid-phase and AJIPHASE®.

Figure 5 demonstrates the amounts of amidites, organic solvents, reagents, and water used in solid-phase and AJIPHASE® and their respective impacts on CO2 emission. Although only a relatively small amount of amidites are used, they have a major impact on CO2 emission. To produce amidites that are monomers of an oligonucleotide, several synthetic steps are required, increasing

the number of reagents consumed and thereby the emission factor. Solvent usage has a smaller but still significant impact on the emission factor due to the amount of solvent consumed throughout oligonucleotide manufacturing. The AJIPHASE® process reduces the amount of amidite and organic solvent required and, as such, emits much less CO2 than solid-phase.

By utilizing the advantages that come with solution-phased reaction, AJIPHASE® can be scaled up without any major obstacles and no special equipment or reactors needed. This robust technology can produce a better-quality product, reduce the amount of reagent consumption, and improve cost and CO2 emissions.

Enzymatic Method for Oligonucleotide Manufacturing

Historically, single-strand elongation was the standard in oligonucleotide manufacturing. However, in recent years, siRNA has become the fastest growing category of oligonucleotide therapeutics thanks to its ability to target a wide variety of diseases. siRNA is a class of double-stranded RNA that is typically between 19 and 23 mer. siRNA therapeutics are based on the biological process known as RNA interference (RNAi), a discovery that won the Nobel Prize in 2006 and established that “double-stranded RNA triggers suppression of gene activity in a homology dependent manner.”⁴

When comparing antisense oligonucleotide manufacturing – a single strand technique – and siRNA manufacturing, siRNA emerges as the more complicated due to its second strand, which requires an additional elongation and purification step as well as an annealing step. This can lead to increased impurities. To

simplify this process, there are a handful of environmentally friendly methods that use enzymes.

One approach, enzyme-catalyzed templated ligation, involves the assembly of short, unpurified oligonucleotide fragments on a complementary template in aqueous solution. Several of these template strands are attached to a soluble chemical “hub” molecule. Once the fragments are assembled, an engineered DNA ligase joins them together to form the full-length product. Uncoupled fragments that are impurities are selectively detached from the hub via heat and removed by a nanofiltration or diafiltration operation, while the product remains attached to the hub and template. At higher temperatures, the product detaches from the template and is recovered in a similar fashion, and the hub is recycled for further use. Reported purities are high enough to enable the removal of further purification steps that would have a significant impact on the emission factor.

Another option is Aji Bio-Pharma’s enzymatic method for manufacturing siRNA that focuses on the double-stranded structure. As demonstrated in Figure 6, the target siRNA is divided into four fragments. The division points are determined to ensure that each fragment overlaps well during the annealing step and then enzymatic ligation is performed to obtain the target siRNA. Often, the product contains some synthetic impurities

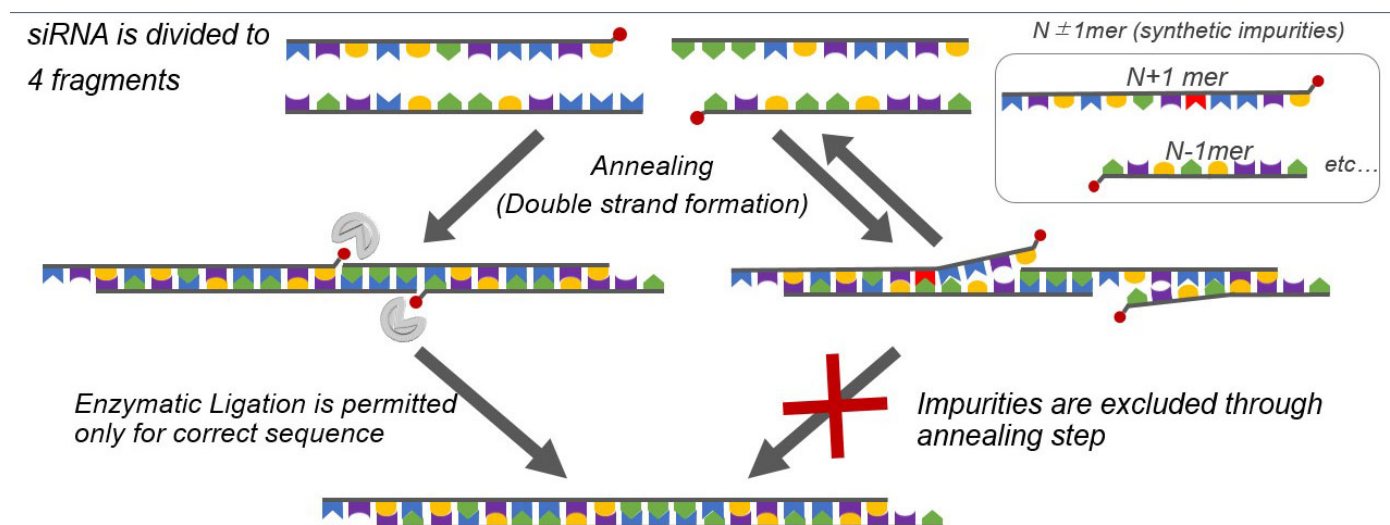


Fig. 6 – An example of Ajinomoto’s enzymatic method.

such as N ± one mer. These impurities are difficult to remove by liquid chromatography purification because they are structurally similar to the target sequence. However, with Aji Bio-Pharma's enzymatic method, these impurities are excluded because they cannot be overlapped into two strands during the annealing step. Enzymatic ligation is permitted only for the correct sequence. As a result, the target product can be obtained with high purity and a simplified purification process. Furthermore, these four fragments can be manufactured in parallel, shortening the production period.

Figure 7 shows the difference in total impurities between the conventional and enzymatic methods. Generally, the reactivity becomes worse in the latter half of the elongation, and impurities tend to increase. The enzymatic method can decrease impurities significantly, even prior to the enzymatic reaction, which will further reduce impurities. As a result, not only can purification steps be reduced, but the burden and period of final purification will be

smaller, reducing the amount of organic solvent used and significantly improving the emission factor.

Conclusion

As the oligonucleotide market grows dramatically, it is crucial to curtail negative environmental effects. As evidenced by emission factors, the consumption of monomers and solvents has a major impact on CO₂ emissions. In response to this, Aji Bio-Pharma is approaching sustainable oligonucleotide manufacturing with its own unique technologies: AJIPHASE[®] synthesis and the enzymatic method. AJIPHASE[®] technology reduces the consumption of raw materials such as monomers and organic solvents and allows for larger manufacturing quantities, while the enzymatic method simplifies purification and reduces consumption of organic solvents. Both technologies significantly reduce emission factors, which makes them viable options to support the surge of oligonucleotide therapeutics.

Total Impurities – Conventional vs Enzymatic Method

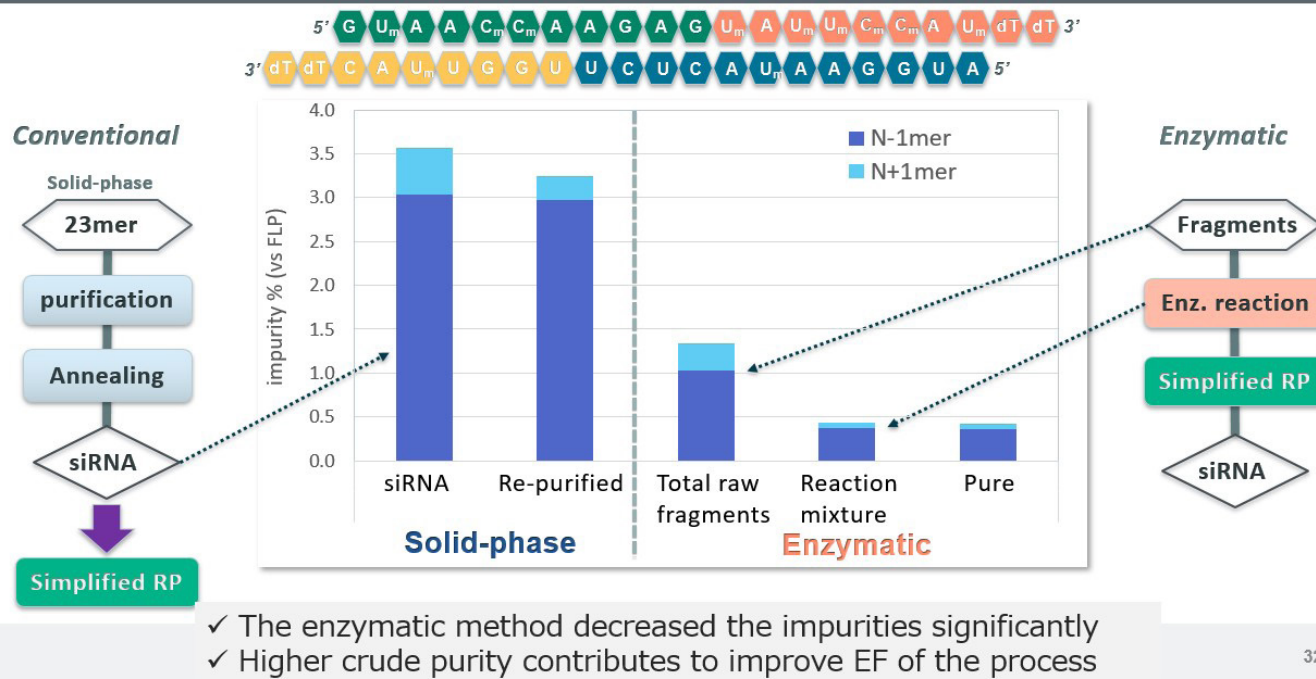


Fig. 7 – Comparison of impurities in the conventional vs. enzymatic method.

The content of this paper originally appeared in a webinar titled *Sustainable Approaches to Oligonucleotide Manufacturing*, led by Yuki Shinohara. You can view the full webinar [here](#).

Resources

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About Ajinomoto Bio-Pharma Services

Ajinomoto Bio-Pharma Services is a fully integrated contract development and manufacturing organization, with sites in Belgium, United States, Japan, and India, providing comprehensive process development services, cGMP manufacturing and drug product fill finish services of small molecule and large molecule APIs and intermediates. Ajinomoto Bio-Pharma Services offers a broad range of innovative platforms and capabilities to rapidly scale from clinical and pilot programs to commercial quantities, including: Corynec technologies, oligonucleotide synthesis, antibody drug conjugations (ADC), high potency APIs (HPAPI), biocatalysis, continuous flow manufacturing, and more. Ajinomoto Bio-Pharma Services is dedicated to providing a high level of quality and service to meet our clients' needs.