Hongene Biotech

Your questions about chemoenzymatic ligation answered

As the RNA therapeutics field rapidly evolves, drug developers are seeking new ways to produce high-quality oligonucleotides more efficiently and sustainably at a larger scale. Traditional solid-phase synthesis (SPOS) is reaching its limits in scale and cost, creating a growing need for next-generation manufacturing technologies.

Chemoenzymatic ligation is emerging as a transformative solution that combines the precision of enzymatic assembly with the reliability of chemical synthesis. Here we answer your questions about what the technology is, why it matters and how it will shape the future of RNA manufacturing.

Q1: What is chemoenzymatic ligation?

Chemoenzymatic ligation is a hybrid oligonucleotide synthesis strategy that constructs full-length siRNA or sgRNA by enzymatically joining shorter, chemically synthesized oligonucleotide fragments (also called blockmers). This approach leverages the reliability of solid-phase oligonucleotide synthesis (SPOS) for fragment production, combined with the precision of enzymatic ligation using RNA ligases.

Because ligases selectively join adjacent fragments with correctly positioned functional groups, a 5′-phosphate and a 3′-hydroxyl, many common synthesis-related impurities are excluded from the final product. The result is a high-efficiency, high-fidelity assembly process that enhances product purity, improves yield and enables scalable manufacturing of complex oligonucleotide therapeutics.

Q2: Why is chemoenzymatic ligation important for the industry?

It offers a scalable, cost-effective alternative to SPOS, which struggles with yield, impurity control and sustainability at large scale. By enabling high-efficiency assembly from short, clean fragments, chemoenzymatic ligation mitigates the economic and environmental burdens of traditional synthesis.

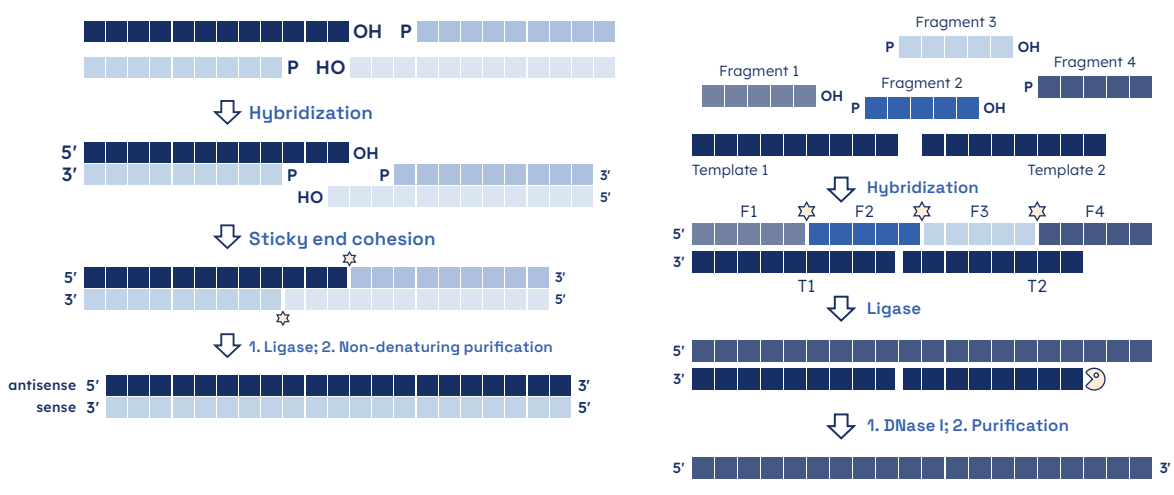
This approach is especially critical as siRNA programs expand into cardiometabolic diseases, where annual demand may exceed several tons within 10 years, and as oligonucleotides become longer and more complex, as for sgRNA and pegRNA for gene editing. Ligation-based manufacturing meets these challenges with modularity, precision and efficiency.

Q3: How is chemoenzymatic ligation being applied in RNA manufacturing today?

At Hongene, two tailored workflows have been implemented: sticky-end ligation for double-stranded siRNA, and splinted ligation for longer, single-stranded oligonucleotides like sgRNA and pegRNA. These workflows are readily adaptable to stainless steel batch reactors or single-use bioreactors, enabling flexible, scalable deployment across GMP manufacturing infrastructure.

In a major milestone, chemoenzymatic ligation has recently moved from concept to clinical deployment and is now used by Hongene to produce GMP-grade siRNA for therapeutic programs. It is ideally suited for large-scale clinical supply.

Hongene's chemoenzymatic ligation platform processes



Q4: What are the main advantages?

Better quality and consistency

Only fragments with correct terminal functionalities are ligated, reducing the incorporation of synthesis-related impurities.

Lower waste

Shorter SPOS fragments can be manufactured in high yield, minimizing reagent use and chemical waste.

Modular design

Enables efficient synthesis of longer RNAs (e.g., sgRNA, pegRNA) not easily produced via traditional SPOS.

Improved scalability

Solution-phase ligation is well-suited to large-volume stainless steel or single-use bioreactor systems.

Chemical compatibility

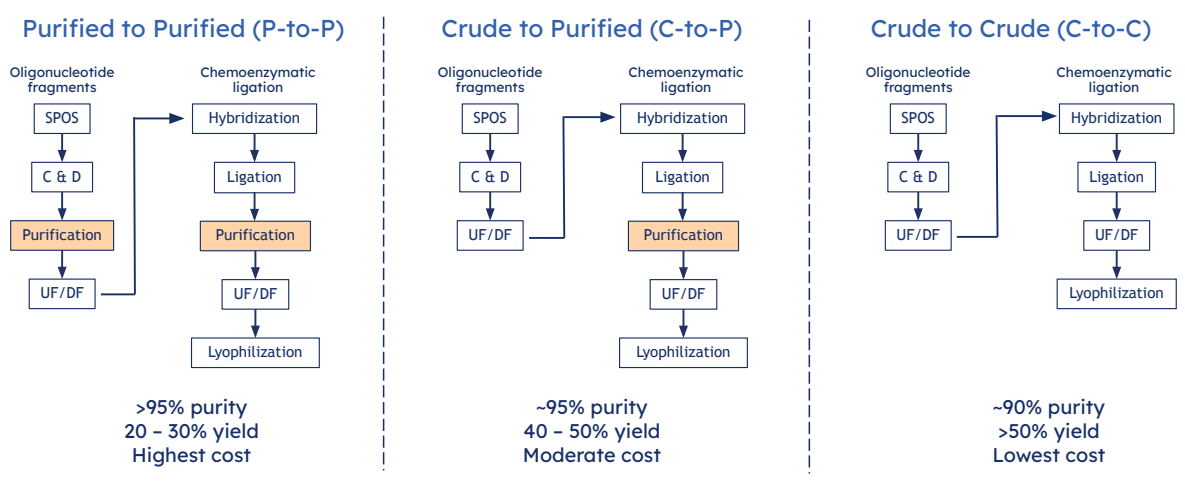
Supports all standard siRNA chemistries, including PO, PS, MsPA backbones, 2′-OMe,2′-F modifications and GalNAc conjugation.

Q5: Is chemoenzymatic ligation cost-effective?

Yes, because the process is inherently high-yielding and scalable: fragments are synthesized in high yield via SPOS, and the ligation step proceeds with near-quantitative efficiency. As a result, overall yields are significantly higher than those achieved for synthesis of the full-length oligonucleotide using SPOS alone.

This efficiency reduces reagent and solvent usage, minimizes downstream processing and supports a lower cost of goods. Innovations such as improved fragment manufacturing, elimination of chromatography (e.g., C-to-P and C-to-C process) and enzyme engineering will further enhance cost-effectiveness, positioning chemoenzymatic ligation as a highly competitive solution for future commercial-scale oligonucleotide production.

Elimination of chromatography for cost control



Q6: How does chemoenzymatic ligation enhance environmental performance and support global goals?

Chemoenzymatic ligation reduces environmental impact by lowering reagent and solvent usage compared to full-length SPOS. High-yield fragment synthesis and near-quantitative ligation minimize waste generation for the overall process.

As the platform evolves and manufacturing volumes increase, we expect to realize efficiencies of scale that will further improve sustainability of resource-efficient oligonucleotide production, aligned with global environmental objectives.

Q7: What does this mean for drug developers and partners?

Chemoenzymatic ligation offers a robust, scalable platform for advancing RNA therapeutics from discovery through GMP manufacturing. Its modular design supports rapid scale-up, consistent batch quality and seamless integration into modern CDMO operations.

For drug developers, this means reduced development risk, accelerated timelines and a defined pathway to large-scale clinical supply. With demonstrated GMP deployment, the technology supports reliable, regulatory-compliant manufacturing across siRNA, sgRNA and emerging oligonucleotide modalities.

Bring your RNA ambitions to life

Partner with a team that combines deep technical expertise, vertically-integrated operations and a forward-thinking approach to RNA manufacturing innovation.

Connect with Hongene to start the conversation.

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