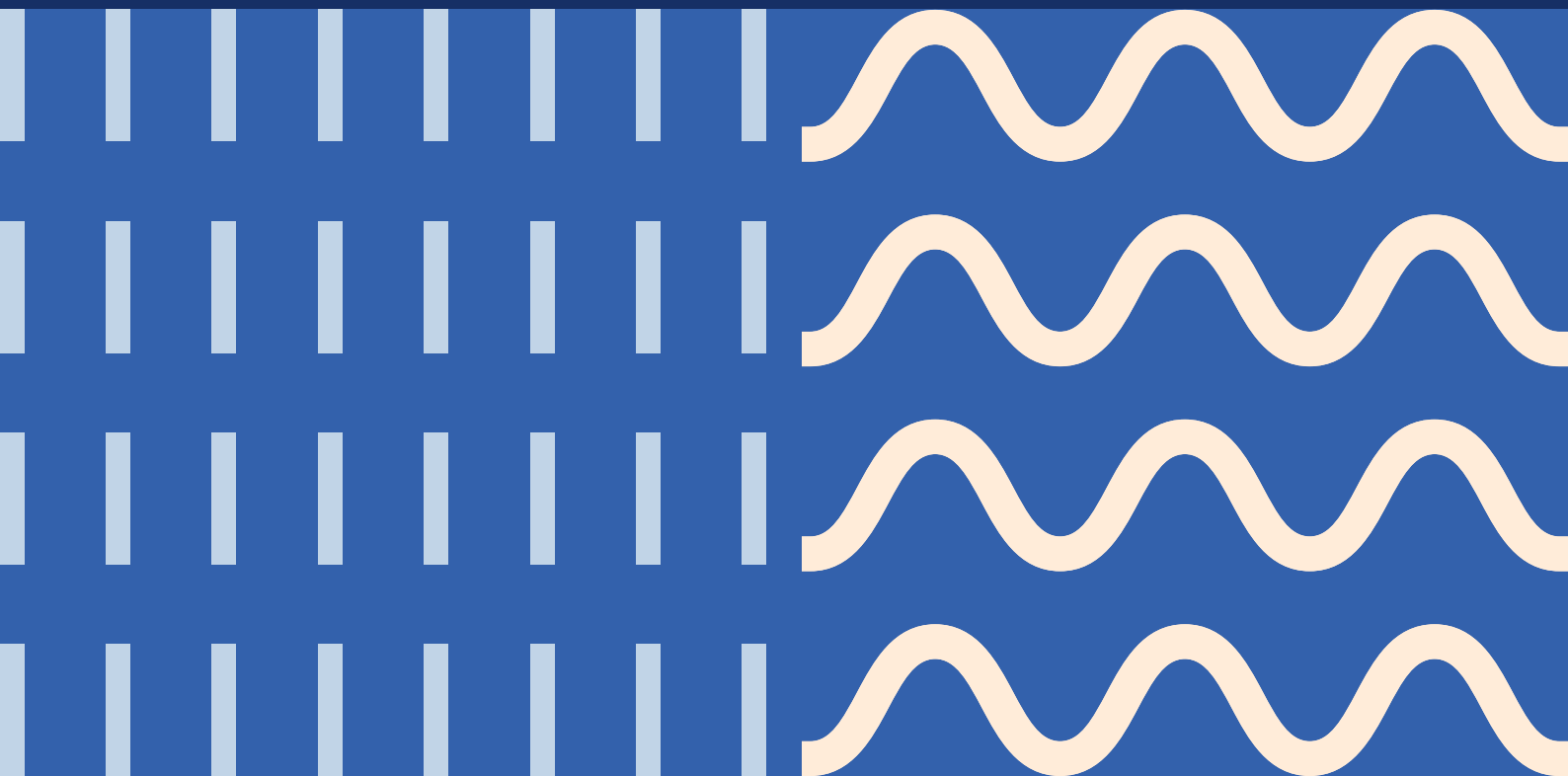
Hongene Biotech

Chemoenzymatic ligation

Redefining therapeutic siRNA and sgRNA manufacturing



Precision, scalability and sustainability for the next generation of oligonucleotide therapeutics

Chemoenzymatic ligation is set to transform therapeutic oligonucleotide manufacturing, offering a precise, scalable alternative to traditional solid-phase synthesis (SPOS).

By combining the accuracy of enzymatic reactions with the control of chemical synthesis, this approach supports the production of high-purity oligonucleotides at scale, reducing costs and minimizing environmental impact. Whilst this technology can be applied to oligonucleotide constructs generally, it is ideally suited for siRNA and long sgRNA.

Why choose chemoenzymatic ligation?

Chemoenzymatic ligation of oligonucleotide fragments overcomes many of the limitations of SPOS, offering a powerful, cost-effective approach for the production of high-purity oligonucleotides.

Key benefits

Exceptional purity: Consistently high quality mediated by selective rejection of shortmers and other impurities.

Scalability and flexibility: Supports batch-mode manufacturing from milligrams to hundreds of kilograms, reducing costs and accelerating timelines.

Cost efficiency: Reduces raw material waste and downstream processing costs, supporting large-scale cost-sensitive therapeutic applications.

Sustainability: Lower environmental impact through reduced reagent consumption and improved atom economy.

Technology spotlight: Precision ligation methods

At Hongene, our chemoenzymatic ligation platform supports the production of next-generation therapeutics oligonucleotide constructs, with superior purity, scalability, and efficiency. These methods form the foundation of our advanced oligonucleotide manufacturing capabilities, supporting a wide range of therapeutic applications.

Splinted ligation — Precision for long oligonucleotide constructs

Splinted ligation is designed for long oligonucleotide constructs. It uses DNA splints to guide precise ligation, ensuring accurate fragment assembly and high sequence fidelity.

Key features

- >90% purity, achievable even for chemically complex, long RNA requiring multi-fragment assembly.
- Ideally suited for synthesis of sgRNA and pegRNA for CRISPR-based gene editing applications.

Hongene's real-world impact – 100mer G211 sgRNA

Therapeutic context: The G211 sequence is a highly modified model 100mer sgRNA designed** for CRISPR-based gene editing, where high purity is critical for therapeutic efficacy and minimization of unwanted off-target effects.

Our manufacturing success: We produced the G211 sgRNA using a two-step splinted ligation strategy, achieving 96.8% purity after HPLC.

Why it matters: This highlights our ability to produce long, complex oligonucleotide constructs with outstanding quality, supporting traditional and next-generation CRISPR-based applications, including prime editing.

**'TTR' *Finn* et al, *Cell Reports*, 2018.

Sticky-end ligation – High-purity siRNA products at scale

Sticky-end ligation is ideally suited for large scale manufacturing of siRNA duplexes, providing products with exceptional purity and impurity control.

Key features

- >95% siRNA purity with no new ligation-related impurities
- Broad chemical compatibility, including 2'-OMe, 2'-F, GalNAc and PS/PO backbones
- Scalable manufacturing processes adaptable to batch reactors

Hongene's real-world impact – Inclisiran (siRNA)

Therapeutic context: Inclisiran (LEQVIO®) was selected as a model for high volume siRNA assets for treating cardiovascular disease

Our manufacturing success: We produced inclisiran using our four-fragment sticky-end ligation strategy, achieving 97% purity, with no detectable ligation-related impurities after HPLC purification.

Why it matters: This demonstrates our ability to produce high-purity, clinically relevant siRNA duplexes with scalable, cost-effective manufacturing.

Hongene's real-world impact – Divalent siRNA containing exNA

Therapeutic context: Divalent siRNAs containing exNA represent a promising new class of oligonucleotide therapeutics, offering enhanced efficacy and target selectivity through modified backbone chemistries[1].

Our manufacturing success: We produced a model divalent exNA-containing siRNA construct, achieving 97% purity with no new ligation-related impurities, supporting rapid scale-up and cost-effective production.

Why it matters: This demonstrates our ability to support complex, next-generation oligonucleotide designs, including those with non-standard backbone chemistries like exNA, which are critical for emerging therapeutic applications.

Examples of siRNA constructs synthesized by ligation at Hongene

Chemoenzymatic ligation enables scalable manufacturing of siRNA therapeutics, supporting future requirements for high-purity API for cardiovascular and other large volume disease indications. This table exemplifies Hongene's capabilities, demonstrating the scalability, flexibility and precision of its ligation technology. Importantly, we have now translated the technology to our GMP facilities where we have manufactured siRNA at kilogram scale to support clinical trials.

		Oligonucleotide chemistry						
Molecule	GMP	SS/AS	2'-Ribose mods	Backbone	GalNAc	Synthesis strategy[1]	Yield[2]	Purity[3]
Inclisiran siRNA	-	21/23	2'-OMe, 2'-F, 2'-deoxy	PS/PO	✓	P-to-P	26%	97%
Divalent siRNA	-	16/21	2'-OMe, 2'-F, exNA, (E)-VP	PS/PO	✗	P-to-P	19%	97%
C-to-P siRNA	-	21/23	2'-OMe, 2'-F	PS/PO	✓	C-to-P	43%	96%
CDMO siRNA	No	ND	2'-OMe, 2'-F	PS/PO	✓	C-to-P	~960 g	95%
CDMO siRNA	Yes	ND	2'-OMe, 2'-F	PS/PO	✓	P-to-P	~1,020 g	97%

1. *P-to-P = HPLC purified fragments and purified siRNA; C-to-P = UF/DF processed fragments and HPLC purified siRNA;*
2. *Unoptimized yields based on theoretical MEC, calculated from lowest yielding fragment; 3. Denaturing IPRP-UPLC method*

Thermostable T4 RNA ligase — High-temperature ligation for complex constructs

A mutant thermostable T4 RNA ligase has been engineered to enable high-temperature ligation reactions. This enzyme supports a broad range of substrates and is particularly well-suited where elevated temperatures can be leveraged to improve product quality and yield.

Key features

- High thermal stability: Maintains activity at 52°C, supporting high-temperature ligation reactions
- Reduced secondary structures: Higher temperatures reduce RNA secondary structures, improving ligation efficiency
- Improved selectivity: Enhanced selectivity for fragments with optimal hybridization, reducing off-target ligation
- Cost-effective manufacturing: Potentially supports C-to-C ligation strategies, further driving down production costs

Reference

1. K. Yamada et al, *Nat Biotech*, 2024

Why partner with Hongene?

Specialist expertise: Nearly three decades of experience in nucleic acid chemistry, supported by dedicated R&D centers in the US and Asia.

End-to-end capabilities: Comprehensive support from raw materials to GMP oligonucleotide and mRNA DS and DP, reducing the need for multiple vendor handoffs

Global reach: Warehouses for distribution across key markets, ensuring reliable, localized support and rapid response times.

Commitment to quality: World-class personnel and QA/QC infrastructure delivering consistent, high-quality products.

Sustainability leadership: Member of the ACS Green Chemistry Institute, committed to reducing reagent waste and improving process efficiency for us and our partners.

Let's connect

Discover how Hongene's ligation platform can elevate your oligonucleotide program — from siRNA to sgRNA and beyond.

Let’s get started today.
Contact us to discuss your needs

+1-510-931-4711

product.info@hongene.com

www.hongene.com

Hongene Biotech

29520 Kohoutek Way,
Union city,
CA 94587

–

One Broadway,
14th floor Cambridge,
Boston, MA 02142

Hongene Biotech

www.hongene.com