Hongene Biotech

Why chemoenzymatic ligation matters in RNA manufacturing

The demand for RNA therapeutics is accelerating, but conventional solid-phase oligonucleotide synthesis (SPOS) is limited in scale, yield, and sustainability.

This visual guide illustrates how Hongene's chemoenzymatic ligation platform offers a next-generation solution: enabling high-purity siRNA and sgRNA manufacturing at scale, with improved scalability and yield, and reduced environmental impact and cost.

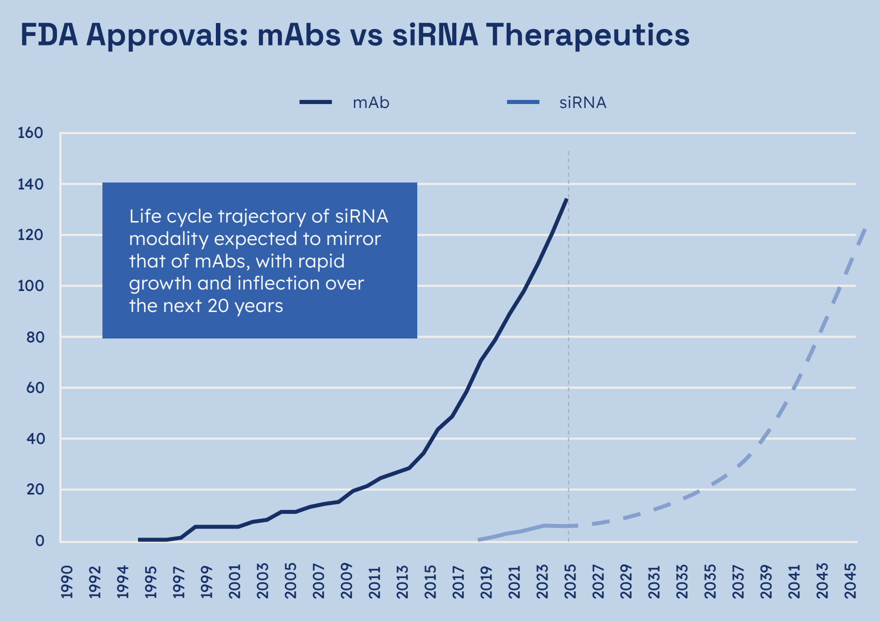
Market potential of siRNA drugs

Market demand for siRNA drugs and CMC challenges expected to grow significantly over the next decade

Cardiovascular disease area	Example siRNA drug	Target gene	Development phase	Possible approval
Hypercholesterolemia	Inclisiran	PCSK9	4	Approved
Hypertriglyceridemia	Plozasiran	APOC3	4	Approved
Elevated Lp(a)	Olpasiran	LPA	3	- 2027
Hypertension	Zilebesiran	AGT	3	- 2030
NASH/MASH	Rapirosiran	HSD	2	- 2030
Obesity	Multiple	INHBE	1/2a	2030s

- Several disease areas have potential to reach >10M US patients
- Five could be approved by 2030
- Our research suggests >10 t siRNA API could be required by early 2030s

FDA Approvals: mAbs vs siRNA Therapeutics



The Antibody Society. Therapeutic monoclonal antibodies approved or in regulatory review. (Sep 2025); Antibody therapeutics product data - The Antibody Society.

Rising demand, outdated methods

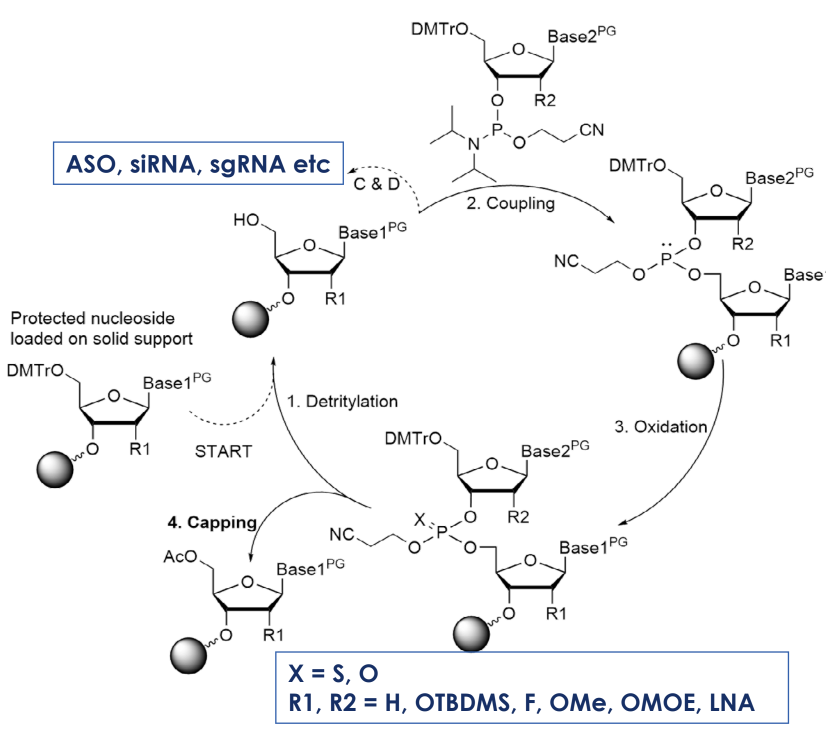
- Demand for cardiometabolic siRNA drugs could reach >10 tons in the next decade.
- Traditional SPOS is currently limited to batch sizes of ~10 kg. Manufacturing larger volumes requires scaling out.
- Over 3,000 kg of raw material are consumed to produce 1 kg of API. (B. Andrews et al, JOC, 2021)
- Further efficiency gains are constrained by SPOS platform maturity.

Solid Phase Oligonucleotide Synthesis (SPOS)

The current paradigm for oligonucleotide manufacture (Generation 1 technology)

- Solid support with reagents flowing over the synthesis bed
- Linear synthesis consisting of 160+ steps for a 21mer siRNA duplex
- Yield depends on modality ~50% for siRNA, purities ~90%, amazing achievement
- But scale limited to <10 kg per batch
- Decades of process improvements now achieving diminishing returns
- Suitable for meeting market demands of rare disease indications, more challenging for widespread disease indications
- ~80% of drugs in pipelines target at least one non-rare disease indications[1]

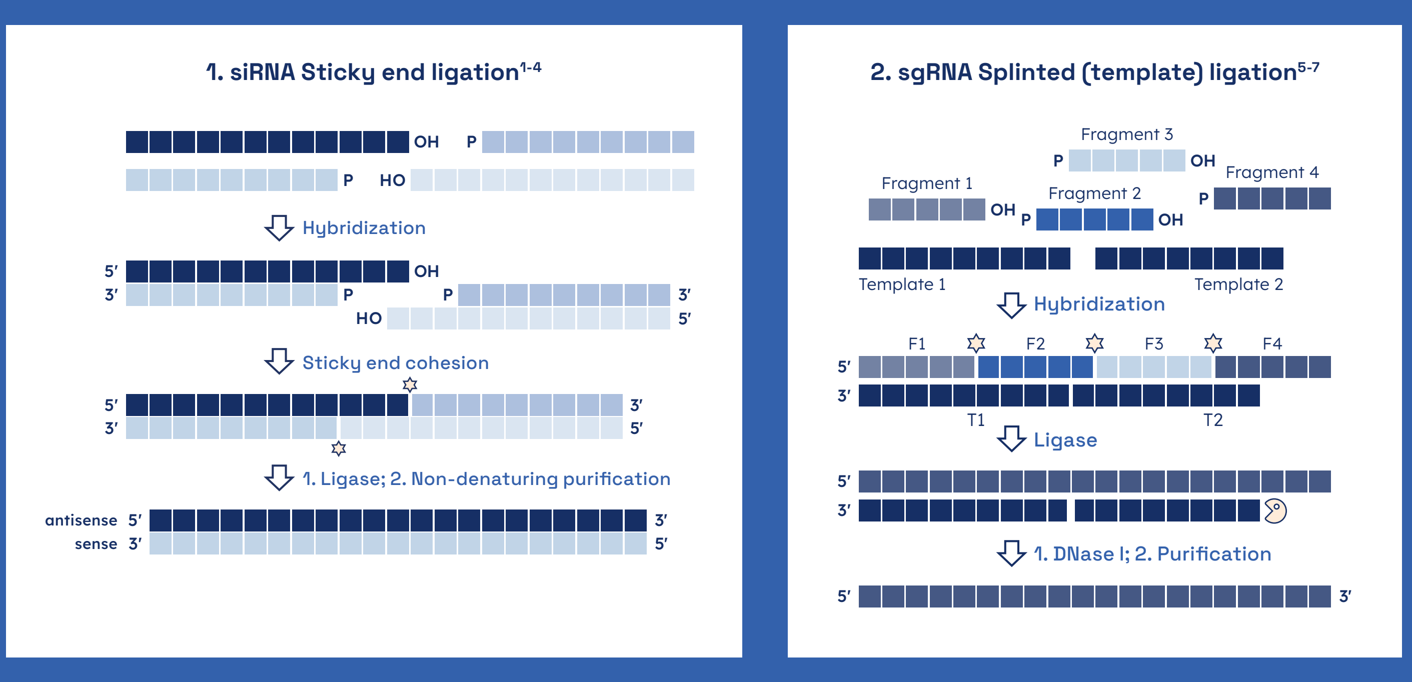
1 Hanson Wade (Beacon Intelligence), Beacon, 2025



Smarter oligonucleotide assembly through ligation

- Tailored workflows for siRNA (sticky-end ligation) and sgRNA (splinted ligation).
- Oligonucleotide fragments (blockmers) are synthesized in high yield using traditional SPOS.
- Produces duplex siRNA at ≥97% purity and long sgRNAs and pegRNAs at ≥90% purity.
- Compatible with standard chemistries: 2′-OMe, 2′-F, 2′-H, Vinyl phosphonate, GalNAc, MsPA, PO and PS.
- Eliminates many shortmer impurities inherent to SPOS by rejecting nonligatable species.

Hongene's ligation process for siRNA and sgRNA manufacture



1. H.G. Khorana, Pure Appl Chem, 1968; 2. A. Sosic et al, Bioconj Chem, 2014; 3. S. Paul et al, ACS Chem Biol, 2023; 4. S. Kajimoto et al, AEM, 2022; 5. M. Moore et al, Methods Enzymol, 2000; 6. N.Sabat et al, Nat Commun, 2024; 7. Bigatti et al, OPRD, 2025.

Proven performance at scale

Hongene's ligation platform is GMP-compliant and already supports clinical oligonucleotide manufacturing:

- >1 kg of PCSK9 siRNA drug substance produced under GMP to support clinical development
- sgRNA constructs manufactured for CRISPR applications with high yield and purity.
- No new ligation-related impurities observed. Impurities are SPOS-related and all below 1.0%

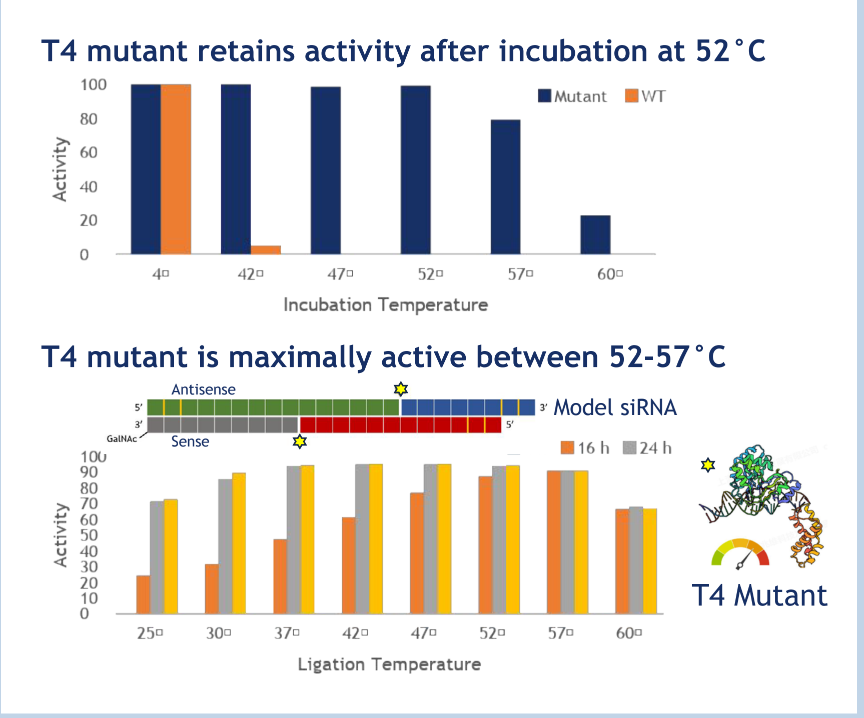
Table 1. Summary of exemplary siRNAs synthesized by ligation

Molecule	GMP	Oligonucleotide chemistry				Synthesis strategy[1]	Yield[2]	Purity[3]
		SS/AS	2′-Ribose modifications	Backbone	GalNAc			
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/TEG	x	P-to-P	19%	97%
C-to-P siRNA	–	21/23	2′-OMe, 2′-F	PS/PO	✓	C-to-P	43%	96%
CDMO siRNA	N	19/21	2′-OMe, 2′-F	PS/PO	✓	C-to-P	~960 g	95%
CDMO siRNA	Y	19/21	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. % Yields based on theoretical MEC, calculated from lowest yielding fragment; 3. Denaturing IPRP-UPLC method.

A more sustainable path forward

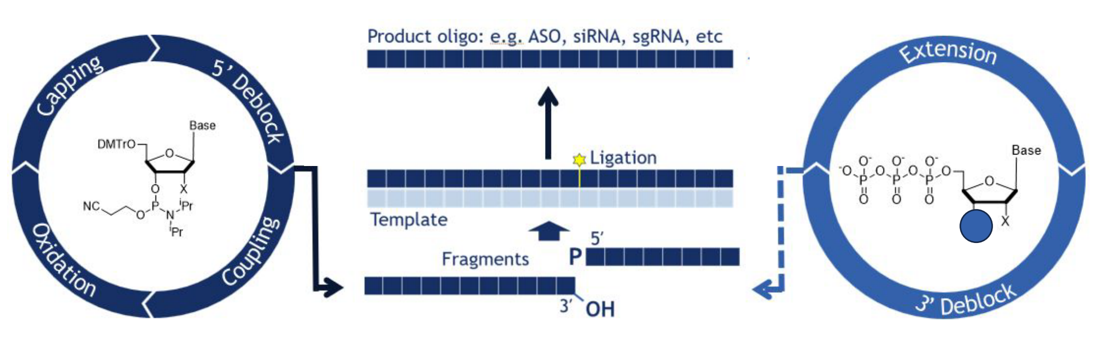
- Hongene is a member of the ACS Green Chemistry Institute Roundtable, advancing best practices in oligonucleotide sustainability.
- Enzyme-catalyzed ligation occurs in aqueous media with reduced solvent use and improved atom economy.
- Crude-to-purified (C-to-P) and crude-to-crude (C-to-C) workflows eliminate chromatography, cutting both cost and environmental burden.
- Enzyme engineering will further reduce waste and energy demands vs SPOS, as part of Hongene's commitment to environmentally responsible manufacturing.



Built to scale with innovation at its core

- Scalable from preclinical R&D to multi-kilogram GMP API supply.
- Vertically integrated CDMO services, including in-house raw material synthesis.
- Supporting Generation 2 manufacturing today and Generation 3 (fully enzymatic synthesis) capabilities tomorrow.

Three generations of oligonucleotide synthesis technology



	Generation 1	Generation 2	Generation 3
Raw materials	Phosphoramidites	Oligonucleotide fragments	NTPs, 3′-protected NTPs, enzymes[2-5]
Development status	>40 years, current paradigm	Supporting clinical development	Early
Product purity	Lower	Higher	Enzymes need to be engineered
Product yield	Lower	Higher	Enzymes need to be engineered
Sustainability	>3,000 kg RM/kg API[1]	Better (partly aqueous)	Best (aqueous)

1. B. Andrews et al, JOC, 2021; 2. E.R. Moody et al, Science, 2023; 3. N. Sabat et al, Front Chem, 2023; 4. D. Wiegand et al, Nat Biotechnol, 2024; 5. S. Forget et al, NAR, 2025.

Hongene: Pioneering ligation technology for next-generation RNA medicines.

Scalable. Sustainable. Clinically proven.

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