Hongene Biotech

The Evolving Landscape of Oligonucleotide Manufacturing: Meeting Future Demand with Ligation Technologies

David Butler, Chief Technology Officer

20th Annual Oligonucleotide Therapeutics Society Meeting, Montreal, October 6th 2024

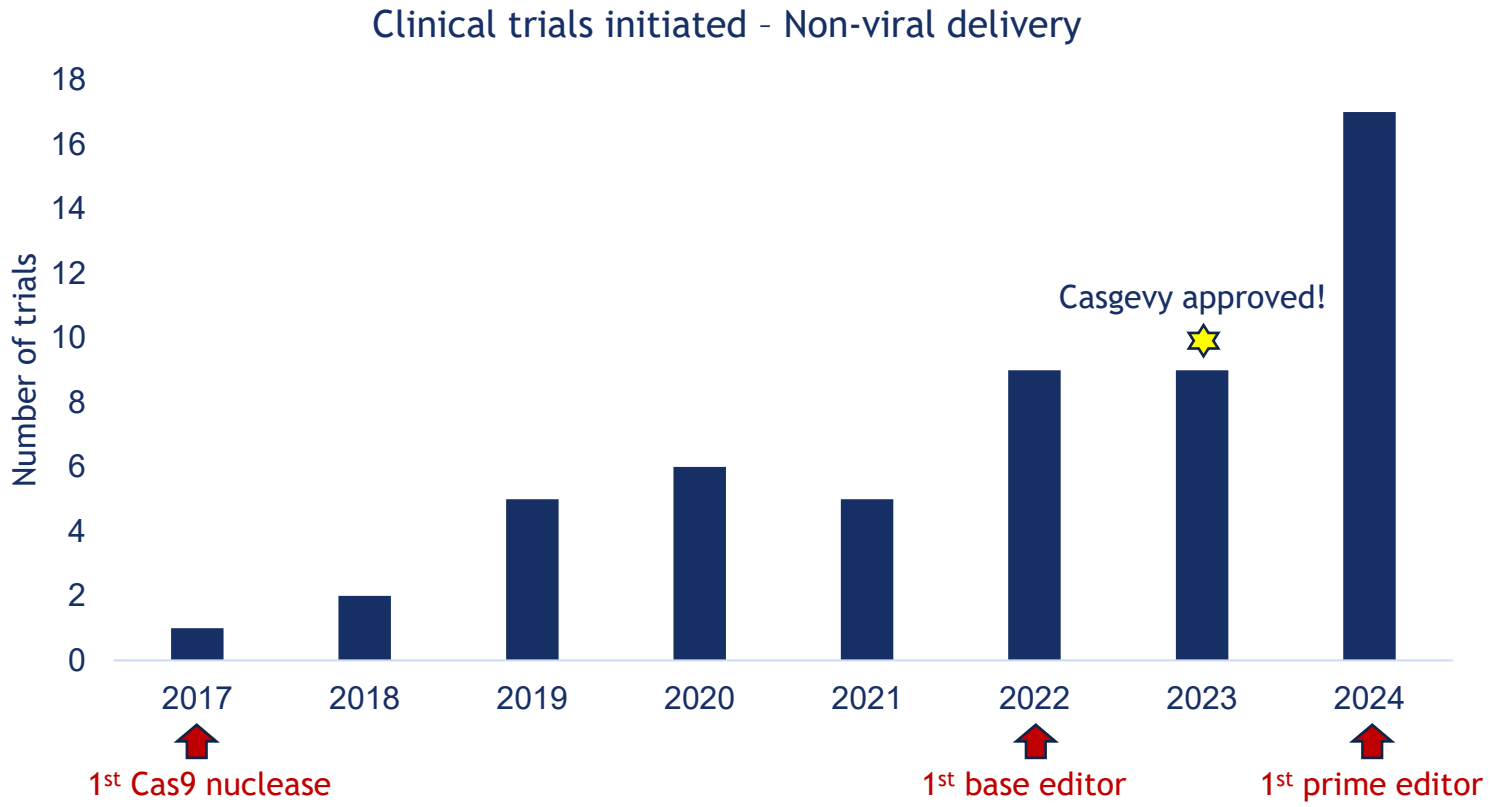
Disclosures

- Dr. Butler is an employee of Hongene Biotech Corporation and serves on the board of directors of Akte Therapeutics

Presentation overview

- The evolving market for sgRNA and siRNA therapeutics
- Three generations of oligonucleotide synthesis technology
- Mechanism, properties, and immediate applications of ligation technology
- Examples of products synthesized by ligation at Hongene
- CMC features of ligation
- Current and future oligonucleotide fragment manufacturing processes
- Future directions
- Conclusions
- Acknowledgements

Gene editing drugs – evolving requirements for sgRNA



Rapid evolution of GE drug modalities[1,2]

- Cas9 nuclease
- Base editors
- Transposases/ Recombinases
- Epigenome editors
- Prime editors

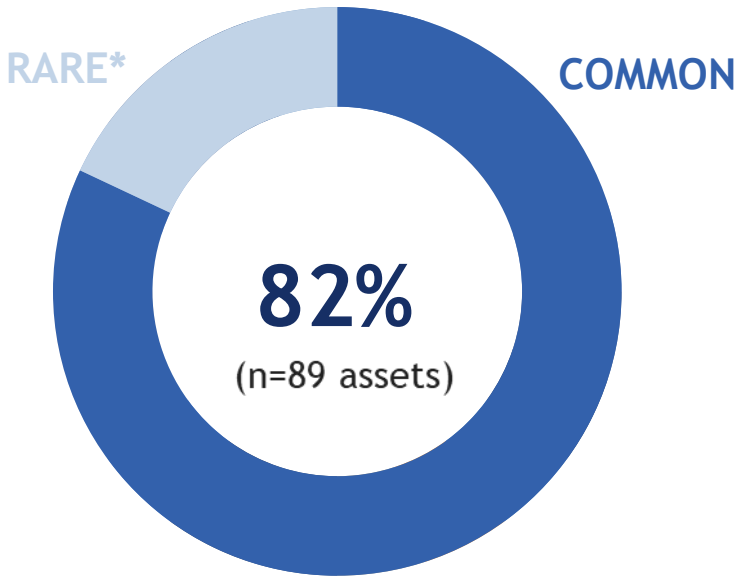
Trend
Longer sgRNA up to ~250 nt
&
More complicated oligo chemistries

Data sourced from Beacon database, 26th September 2024

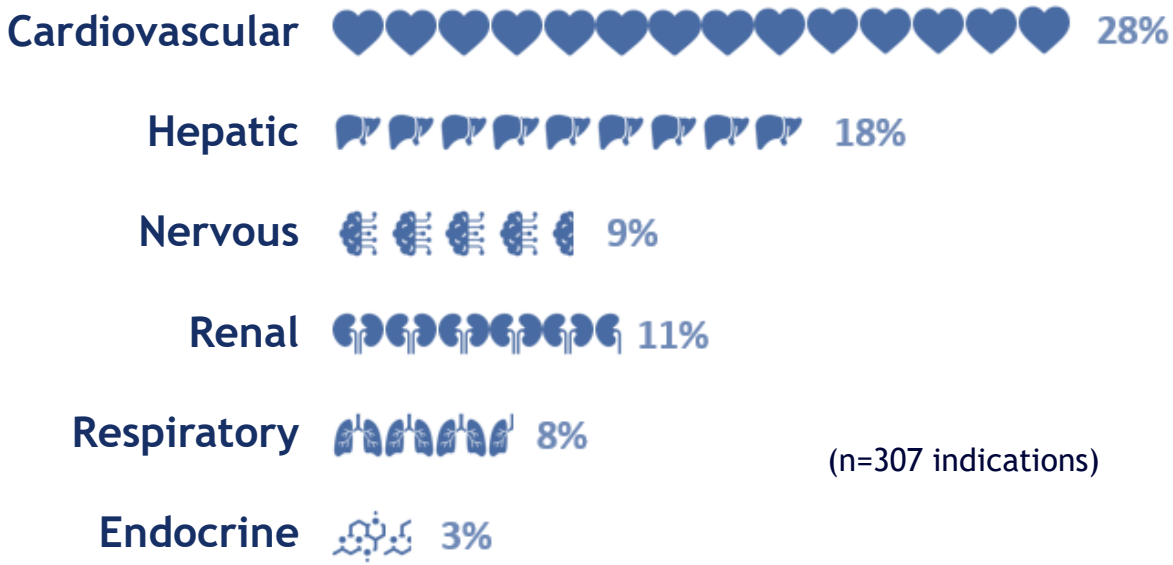
1. A.V. Anzalone et al, *Nat Biotechnol*, 2020; 2. M.G. Durrant et al *Nature*, 2024.

Disease focus for siRNA drugs in clinical development

82% of clinical siRNA assets target at least one common disease indication



More than ¼ clinical stage siRNA disease indications are cardiovascular



(n=307 indications)

**Rare diseases defined via* https://www.orpha.net/en/disease/list/a

Data sourced from Beacon database, 30th August 2024

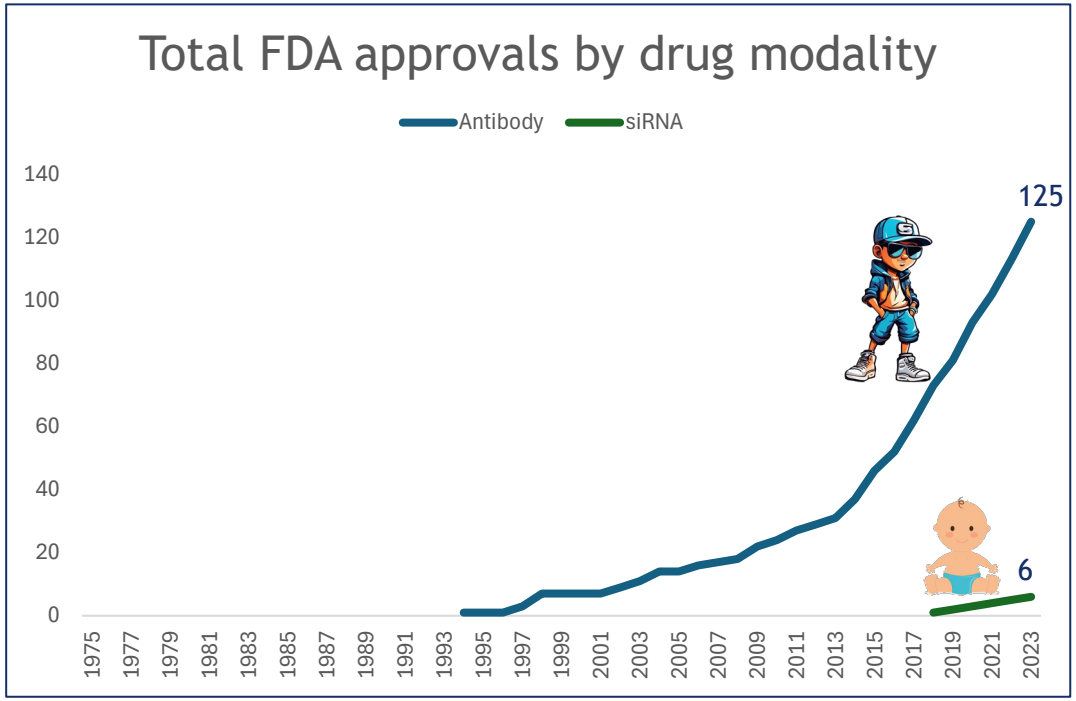
Market potential of siRNA drugs

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

Cardiovascular disease area	Lead siRNA drug	Target	Phase	Possible approval
Hypercholesterolemia	Inclisiran	PCSK9	4	Approved
Hypertriglyceridemia	Plozasiran	APOC3	3	~2026
Elevated Lp(a)	Olpasiran	LPA	3	~2027
Hypertension	Zilebesiran	AGT	2	~2030
NASH/MASH	Rapirosiran	HSD	2	2030s
Obesity	*Multiple*	INHBE	Preclinical	2030s

- Several disease areas have potential to reach >10M US patients
- Inclisiran + 3 could be approved by 2030
- Research suggests >10 t API required by early 2030s

siRNA is in its infancy in its life trajectory as a therapeutic drug modality



The Antibody Society. Therapeutic monoclonal antibodies approved or in regulatory review. (Aug 2024); Antibody therapeutics product data - The Antibody Society

Three generations of oligonucleotide synthesis technology

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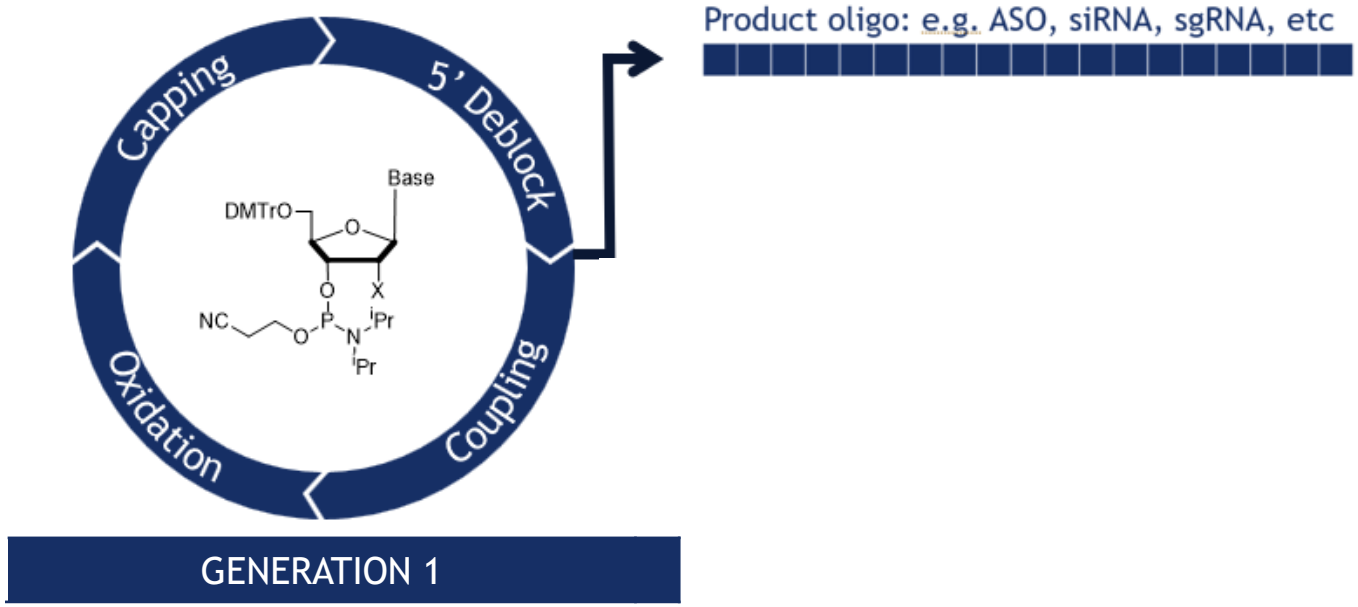
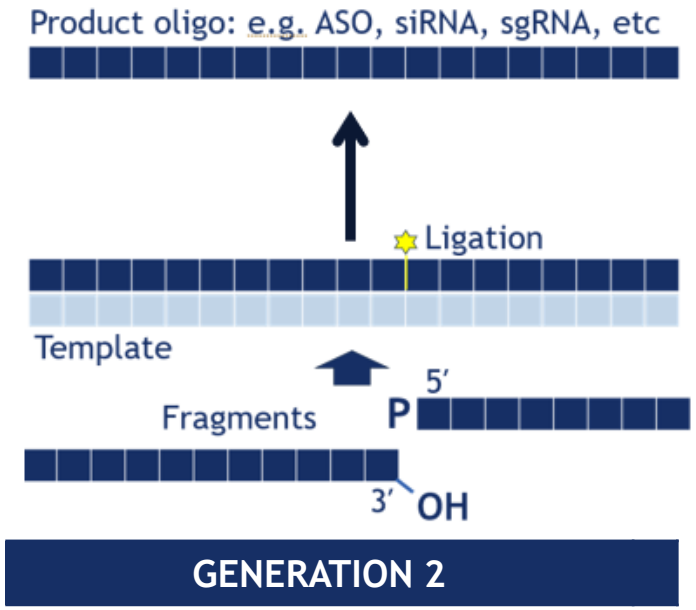
Generation 1: Chemical synthesis cycle



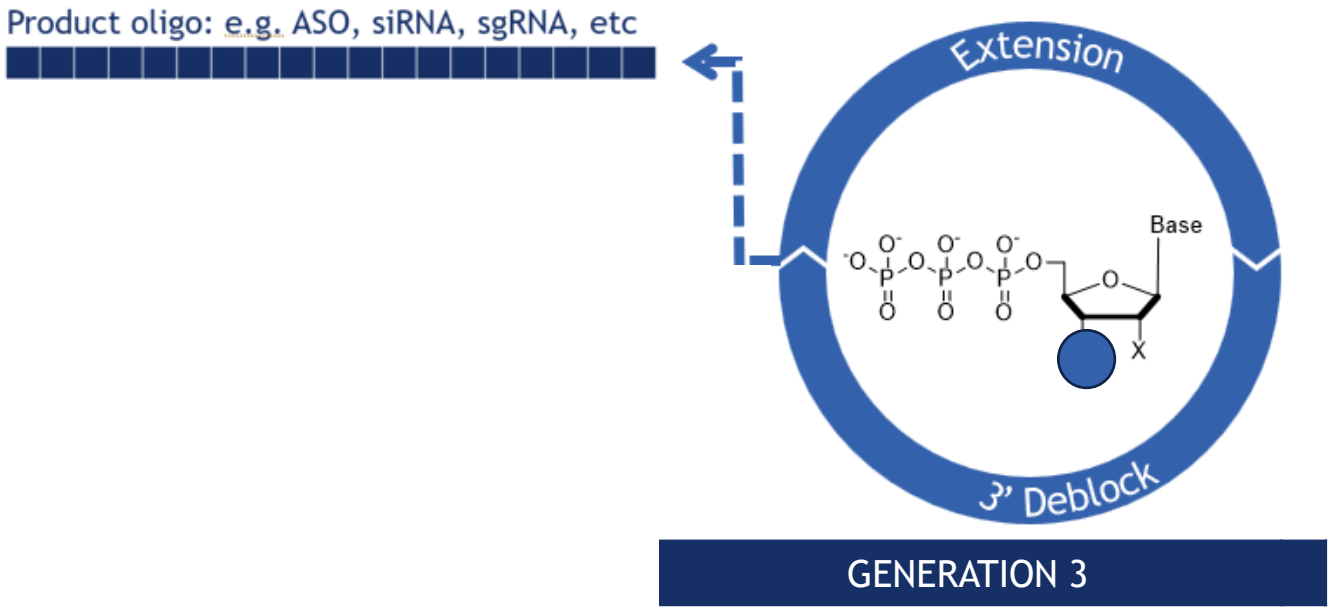
Three generations of oligonucleotide synthesis technology

Generation 2: Chemoenzymatic ligation of fragments

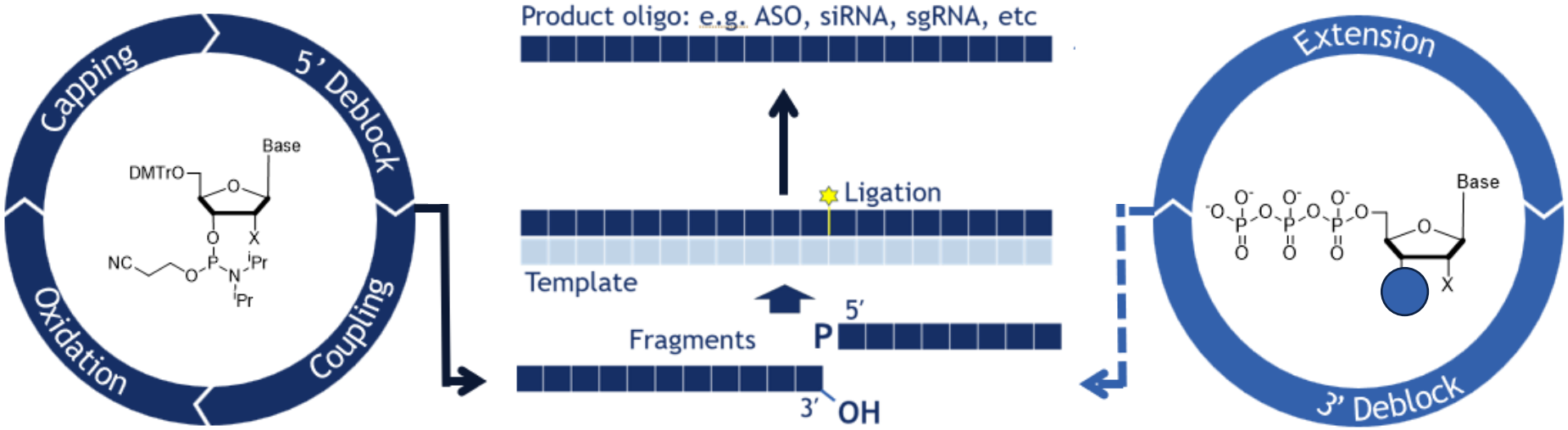


Three generations of oligonucleotide synthesis technology

Generation 3: Enzymatic synthesis cycle



Chemoenzymatic ligation process currently dependent on Generation 1

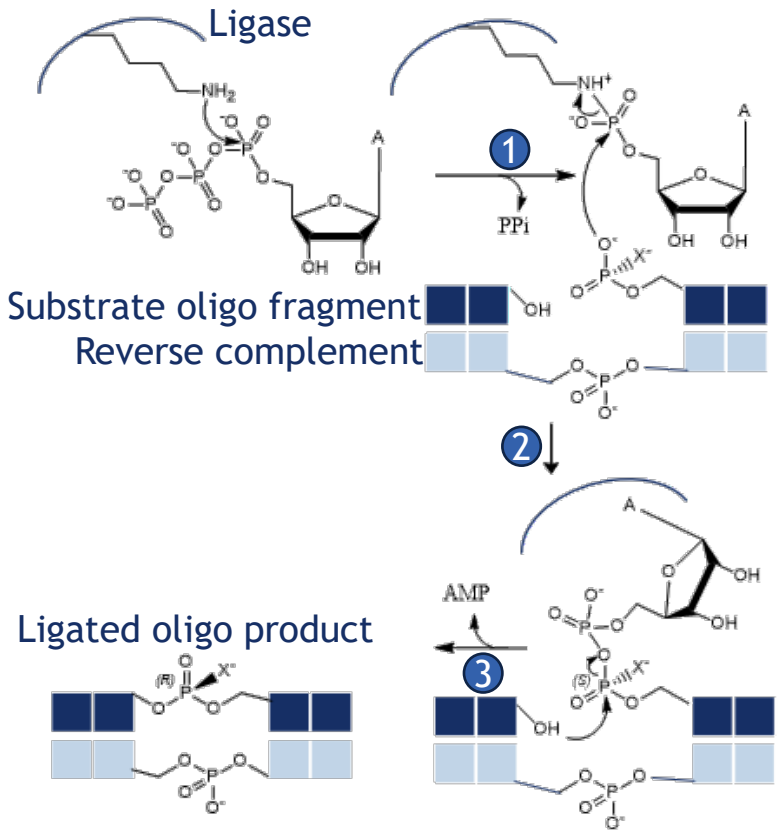


	GENERATION 1		GENERATION 2		GENERATION 3	
Raw materials	Phosphoramidites		Oligonucleotide fragments		NTPs, 3'-protected NTPs, enzymes	
Development status	>40 years, current paradigm		Ready for manufacturing		Very early[2,3,4,5]	
Product purity	Lower		Higher		Enzymes need to be engineered	
Product yield	Lower		Higher		Enzymes need to be engineered	
PMI	>3,000 kg RM/kg API[1]		Lower (partly aqueous)		Lowest (aqueous)	

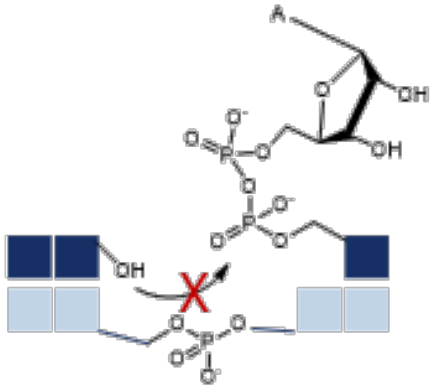
1. B. Andrews et al, *JOC*, 2021; 2. E.R. Moody et al, *Science*, 2023; 3. N. Sabat et al, *Front Chem*, 2023; 4. D. Wiegand et al, *Nat Biotechnol*, 2024; 5. S. Forget et al, *bioXriv*, 2024.

Mechanism and properties of ligation reaction

Reaction mechanism[1]



Important properties of ligation



- Some shortmers are rejected

- Fidelity (specificity)
- Efficiency (yield)
- Kinetics (rate)

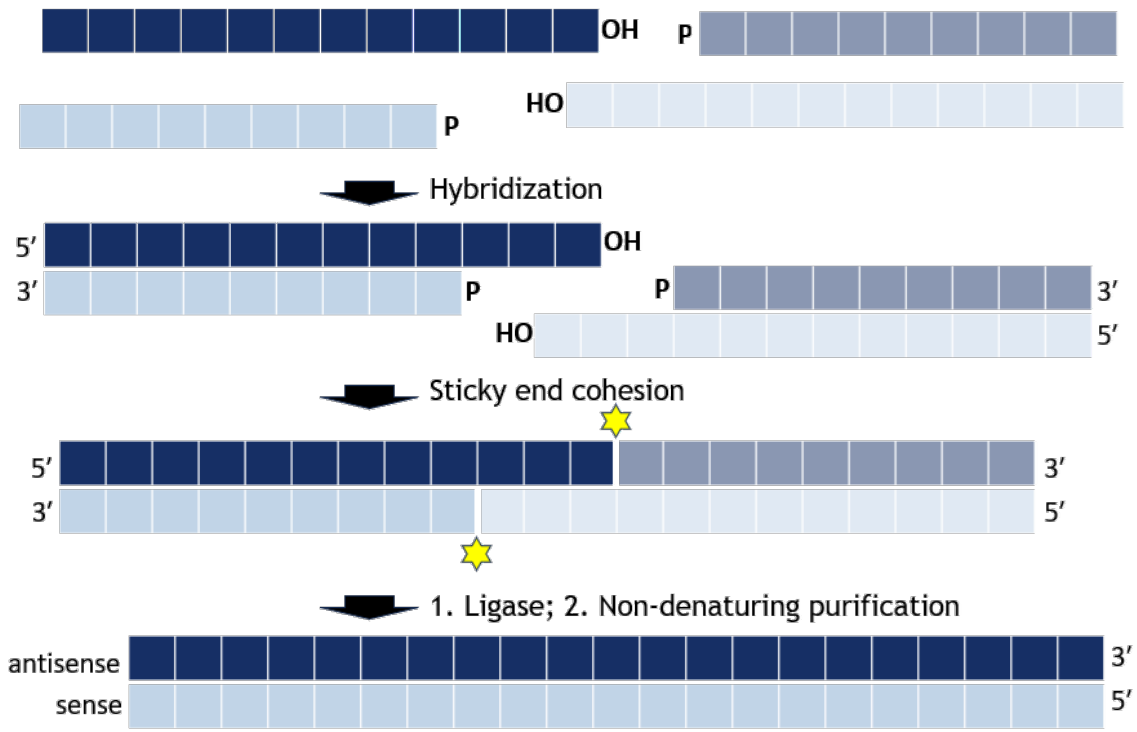
} Process optimization: (buffer, temp, time, conc, fragment length, pos^{n}....)

- Prochiral 5'-thiophosate → *Rp* PS stereochemistry in product[2]

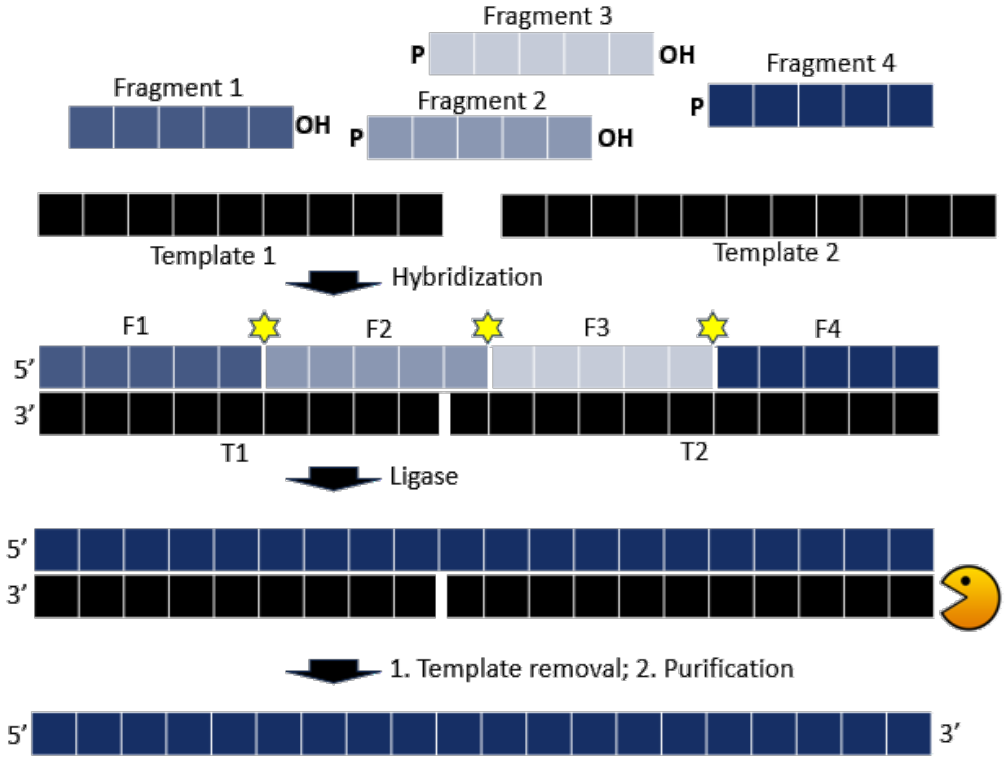
1. J. Nandakumar et al, *Cell*, 2006; 2. F. R. Bryant et al, *Biochemistry*, 1982.

Immediate applications of oligonucleotide ligation

1. siRNA
Sticky end ligation[1,2,3,4]



2. sgRNA
Splinted (template) ligation[5,6]



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.

Examples of products synthesized by ligation at Hongene

Molecule type	Oligonucleotide chemistry				Ligation method	Fragments		Splints		HPLC Purity[1]
	Length	2'-Ribose mods	Backbone	GalNAc		No.	Purif^{n}	No.	Purif^{n}	
siRNA[2]	21/21	2'-OMe, 2'-F	PS/PO	✓	Sticky end	2/2	UF/DF	-	-	95%[3]
siRNA	23/24	2'-OMe, 2'-F	PS/PO	✓	Sticky end	2/2	UF/DF	-	-	97%[3]
sgRNA	100	2'-OMe, 2'-OH	PS/PO	X	Splinted	3	HPLC	2	UF/DF	98%[4]
sgRNA	161	2'-OMe, 2'-OH	PS/PO	X	Splinted	5	HPLC	4	HPLC	96%[4]
oligo	44	2'-OMe, 2'-F	PS/PO/PN	X	Splinted	2	HPLC	2	HPLC	97%[3]

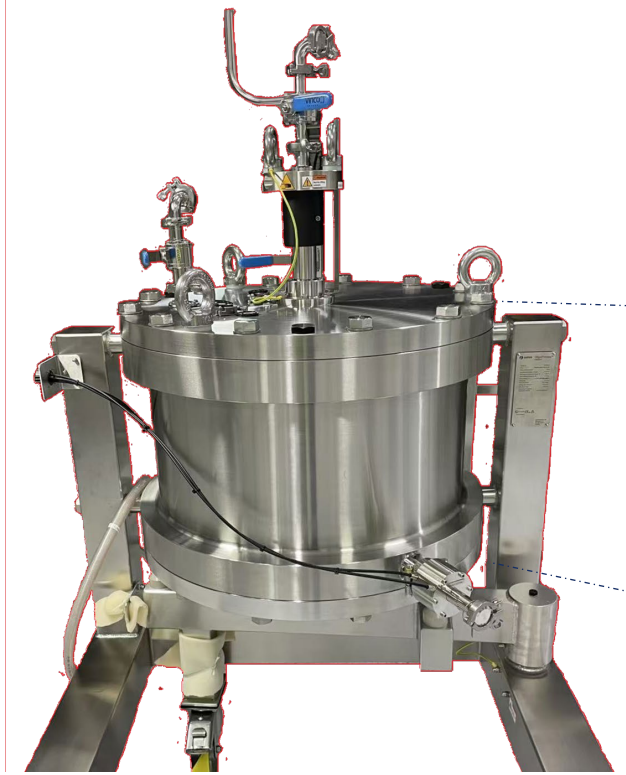
1. Denaturing UPLC method; 2. non-GMP engineering batch, GMP batch ongoing; 3. Data verified by customer; 4. Data verification ongoing.

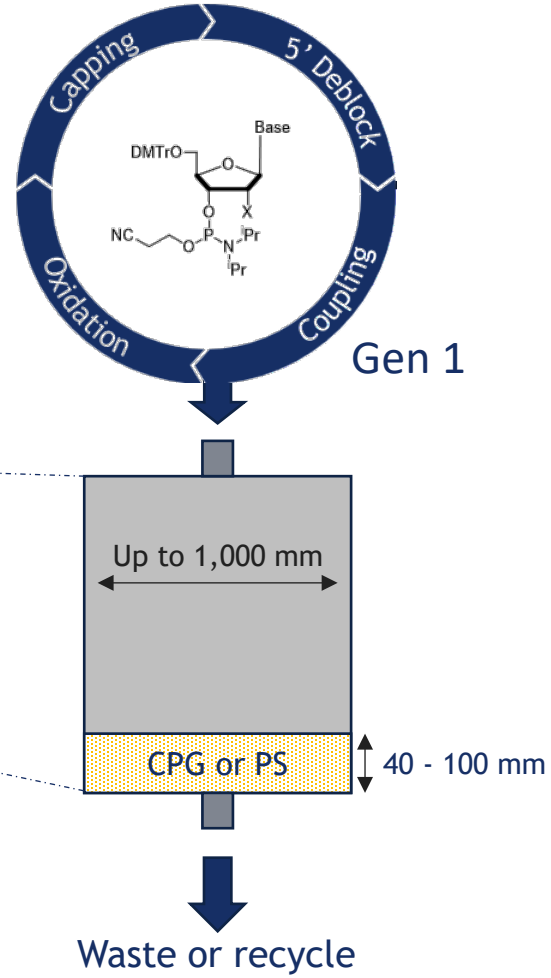
CMC features of ligation

- Quality
  - Shortmer impurities rejected during ligation
  - Higher purity compared to traditional generation 1 process
  - High yielding conversion of fragments
- Chemistry
  - Promiscuous to 2'-ribose chemistry
  - Tolerates PS & other backbone linkages
  - Tolerates ligands at either end
  - Modular and easily adapted for long oligonucleotides
- Manufacturing
  - Adaptable to batch mode and single use bioreactors (scalable)
  - Fragments and/or API may not require HPLC purification

Current process for manufacturing oligonucleotide fragments

Solid-phase flow synthesizer





CMC challenges

- Scale
  - Limited to ~7kg per batch
  - Bed height & diameter limited by non-linear flow rates
  - 1 ton of API = **scale out** 140 x 7 kg batches
- Cost
  - Raw materials, equipment, suite time
  - 1 kg siRNA or 10 g sgRNA API price ~$1M
- Carbon footprint
  - Low atom efficiency (0.25 – 0.36)
  - High process mass intensity (PMI >3,000[1])
- Quality
  - Yield declines exponentially with oligo length
  - Impurities accumulate with oligo length
  - Low purity for longer oligos such as sgRNA

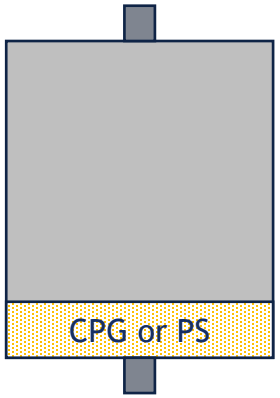
1. B. Andrews et al, *JOC*, 2021.

Meeting future scale requirements for oligonucleotide fragments

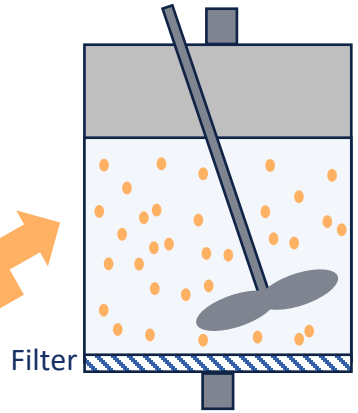
Solid Phase Oligo Synthesis (SPOS)

Liquid Phase Oligo Synthesis (LPOS)[1]

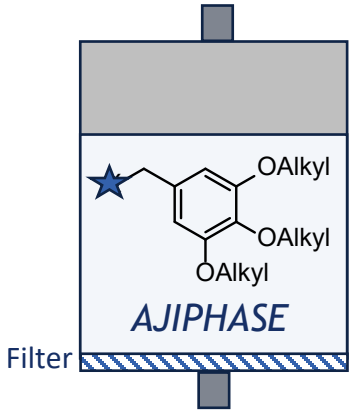
Solid-phase flow synthesizer



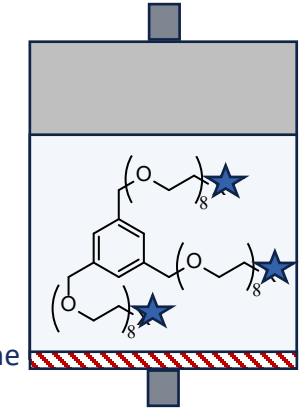
Batch reactor



Hydrophobic support[2,3]

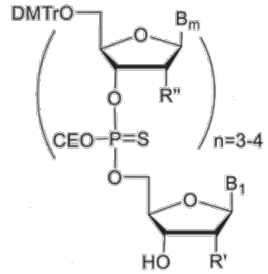


Organic solvent nanofiltration[4]



Others

- Mechanochemistry[5]
- Convergent fragment assembly[6]



BATCH SCALE <10 KG

THEORETICAL BATCH SCALE >100 KG

1. A.G. Molina and Y. Sanghvi, *Curr Protoc Nucleic Acid Chem*, 2019; 2. D. Takahashi, *Medchem News*, 2021; 3. Y. Okada et al, *JOC*, 2013; 4. J.F. Kim et al, *OPRD*, 2016; 5. J.D. Thorpe et al, *RSC Mechanochem*, 2024; 6. X. Zhou et al, *JOC*, 2022.

Future directions

- Continued optimization of chemoenzymatic ligation processes for siRNA and sgRNA
- Extend to other RNA modalities, including mRNA with discrete chemical modifications
- Control of stereochemistry to enable PS ASO ligation
- Optimization of existing, and development of new, fragment manufacturing methods
- Convergence of computational chemistry and AI for better Gen 2&3 enzymes
- Increased regulatory acceptance and wider adoption

Conclusions

- Market demand for oligonucleotide drugs is anticipated to grow significantly over the next decade
- This demand can be met by adoption of oligonucleotide fragment ligation technology
- Ligation technology is ready now for manufacturing sgRNA, siRNA and other oligonucleotides
- Advantages of ligation include improved quality, yield, environmental stability and scalability compared to purely chemical processes
- Oligonucleotide fragments are currently manufactured using scale-limited (<10 kg) solid-phase flow synthesizers
- Future demand will require adoption of scalable technologies that enable manufacturing of fragments in batch reactors

Acknowledgements

Hongene Oligo Team

- Jim Yang
- Frank Rao
- Wangqiyue Sun
- Hu Cheng
- Brian Lu

Hongene Biocatalysis Team

- Minzhi Wei
- Min Guo
- Zaiji Xiong
- Juan Xia
- Zhaoyue Fan
- Yanxing Liu
- Yumai Lu
- Liang Jiang
- Jianxiong Liu

Hanson Wade

- Ioana Panait
- Matty Slingsby
- Sophia Shamsi

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Thank you!

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