Process Development for Enzymatic Synthesis of RNAi Therapeutics

Derek Gauntlett – Sr. Director ECO Process Development

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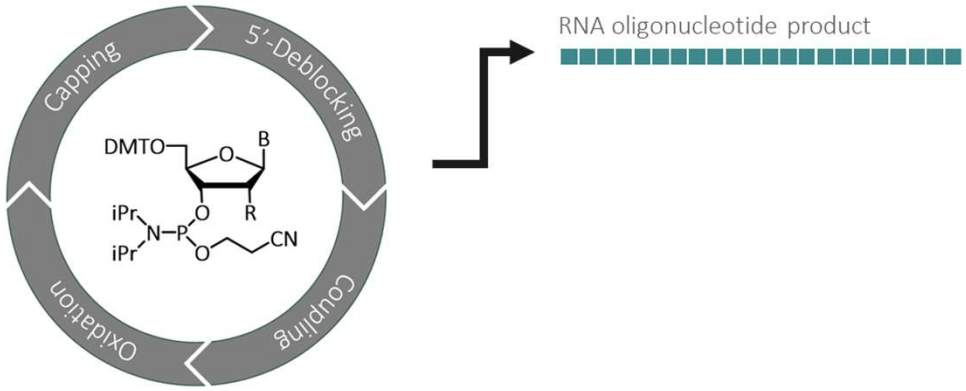
RNA Synthesis – An Evolving Manufacturing Landscape



	Phosphoramidite Chemistry
Development status	>40 years; mature w/ incremental improvements
Product quality	Lower
Product yield	Lower
ESG	>3000 kg organic solvent/kg API

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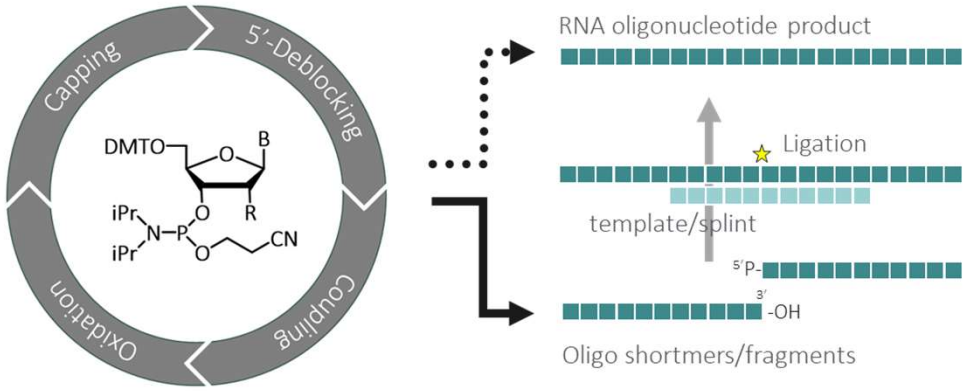
RNA Synthesis – An Evolving Manufacturing Landscape



	Phosphoramidite Chemistry	Enzymatic Ligation
Development status	>40 years; mature w/ incremental improvements	Ready for manufacturing
Product quality	Lower	Higher
Product yield	Lower	Higher
ESG	>3000 kg organic solvent/kg API	Partially aqueous

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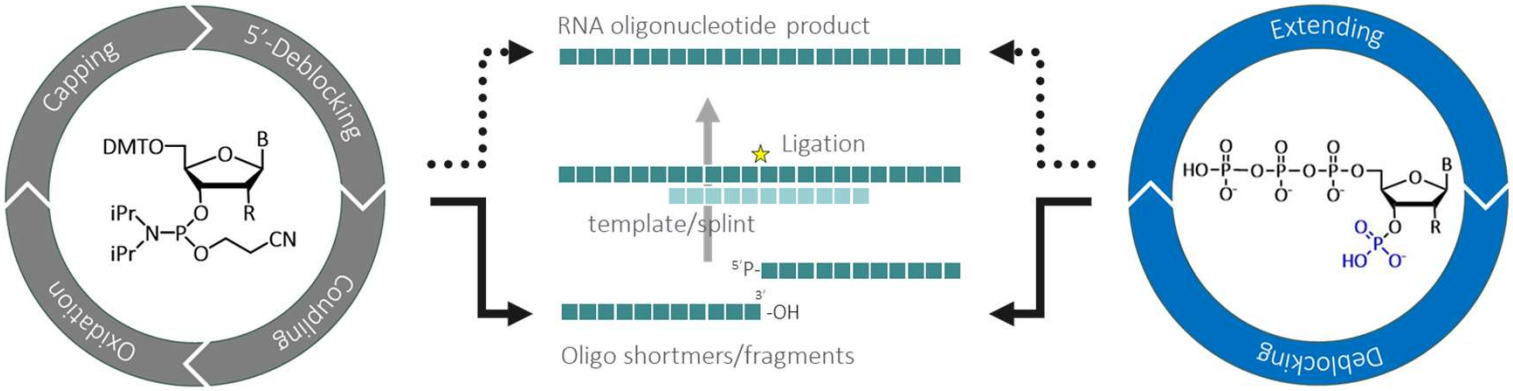
RNA Synthesis – An Evolving Manufacturing Landscape



	Phosphoramidite Chemistry	Enzymatic Ligation	Enzymatic Sequential Synthesis
Development status	>40 years; mature w/ incremental improvements	Ready for manufacturing today	Operational prototype w/ path to manufacturing
Product quality	Lower	Higher	Higher w/ potential for more
Product yield	Lower	Higher	Higher w/ potential for more
ESG	>3000 kg organic solvent/kg API	Partially aqueous / organic	Fully aqueous

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Progress Achieved on the ECO Synthesis™ Manufacturing Platform

Demonstrating the Power of Combining Enzyme Engineering & Process Development in One Year

Nov 2023 TIDES EU

Incorporation efficiency: **~92%**

FLP at 8mer (max. 6) = **72.3%**

Accepting mod. nucleotides: **2'-OMe, 2'-F, PS**

Dec 2023

Gram-scale synthesis (N+6 w/ fully modified nucleotides)

May 2024 TIDES USA

Incorporation efficiency: **>98%**

FLP at 8mer (max. 14) = **92.7%**

Accepting mod. nucleotides: **2'-OMe, 2'-F, PS, dT, Conjugation**

Nov 2024 TIDES EU

Incorporation efficiency: **>98%**

FLP at 8mer (max. 16) = **94.9%**

Accepting mod. nucleotides: **2'-OMe, 2'-F, PS, dT, Conjugation**

Therapeutic Sense and Antisense Fully Synthesized

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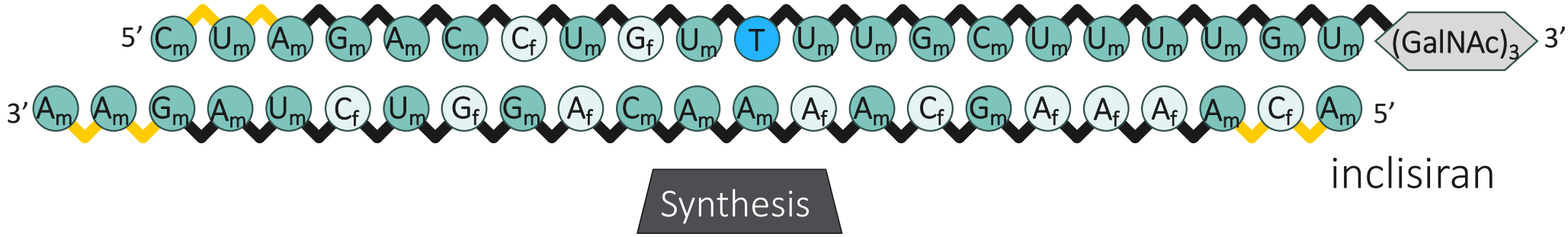
ECO Synthesis™ Technology Enables Manufacturing Versatility

4-Synthesis approaches to demonstrate ECO Synthesis™ toolbox for manufacturing RNA.



- **Route 1:** ECO Sequential Synthesis - Sequential synthesis of both sense and antisense strands (21-mer & 23-mer), including enzymatic ligation of targeting moiety (GalNAc)3.
- **Route 2:** PAC + dsRNA ligase – Chemical synthesis of four single strand fragments by phosphoramidite chemistry (PAC), followed by dsRNA ligation to full length product - Inclisiran.
- **Route 3:** PAC / ECO + dsRNA ligase – Four single strand fragments synthesized by either ECO Synthesis™ technology or PAC, followed by dsRNA ligation to full length product - Inclisiran.
- **Route 4:** ECO + dsRNA ligase – Four single strand fragments synthesized by ECO Synthesis™ technology including enzymatic ligation of (GalNAc)3 on the sense strand, followed by dsRNA ligation to full length product - Inclisiran.

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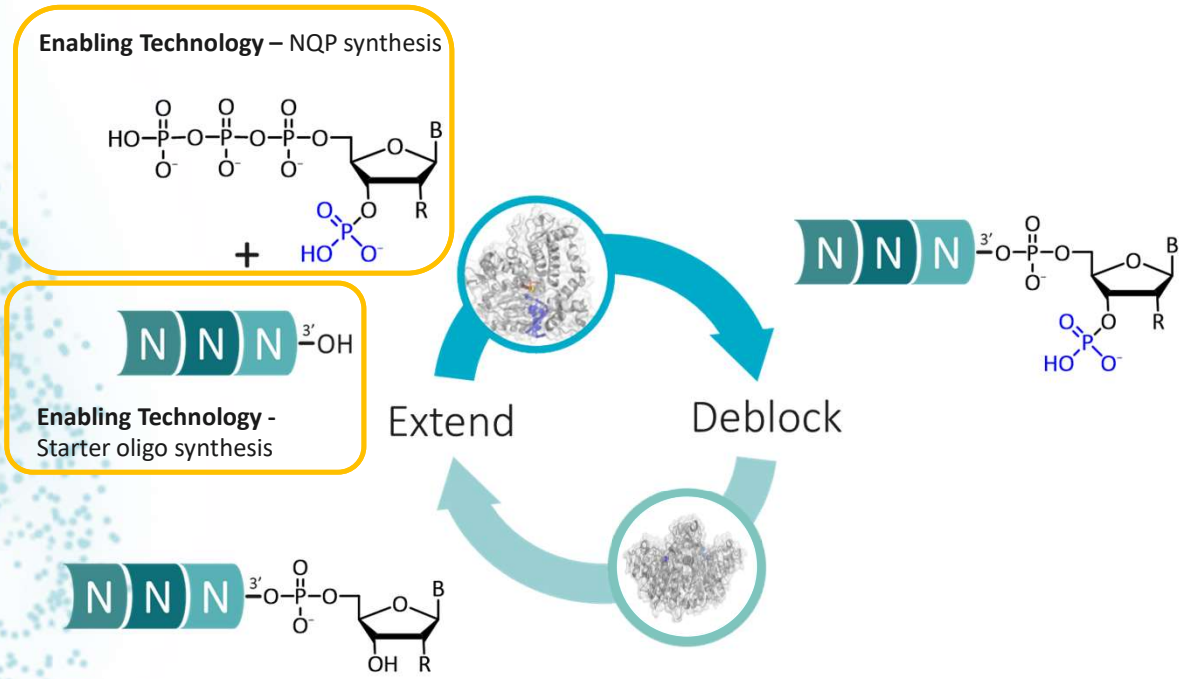
Route 1: ECO Sequential Synthesis

ECO Synthesis™ Technology - Overview

Oligonucleotide synthesis by sequential incorporation of modified nucleotides via a two-step extend & deblock protocol



Key Performance Indicators for ECO enzymes:

- Non-native Substrates
- Enzyme Promiscuity
- Productivity / Coupling Efficiency
- Enzyme Robustness

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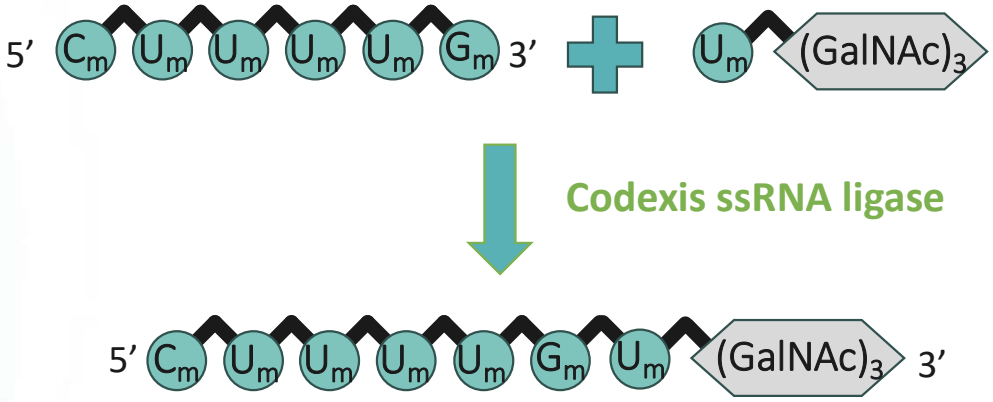
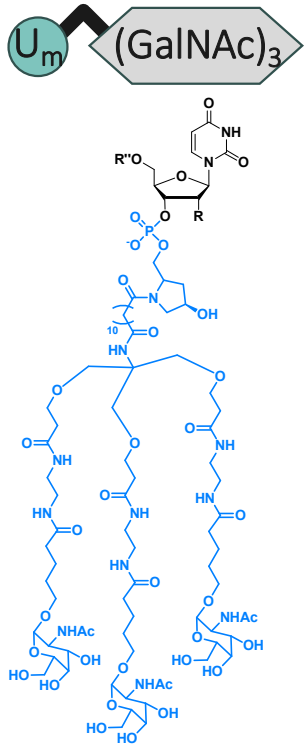
Route 1: ECO Sequential Synthesis

Conjugation of Tissue Targeting Moieties

L96 for inclisiran sense strand

- Codexis has engineered a manufacturable, highly active single-stranded (ss)RNA ligase with broad tolerance for conjugated nucleotides.
- Successful incorporation of L96 donor and inclisiran oligo as acceptor at mM concentrations.





Enzymatic conjugation enables incorporation of tissue targeting moieties allowing manufacturing pathways for therapeutically relevant siRNA

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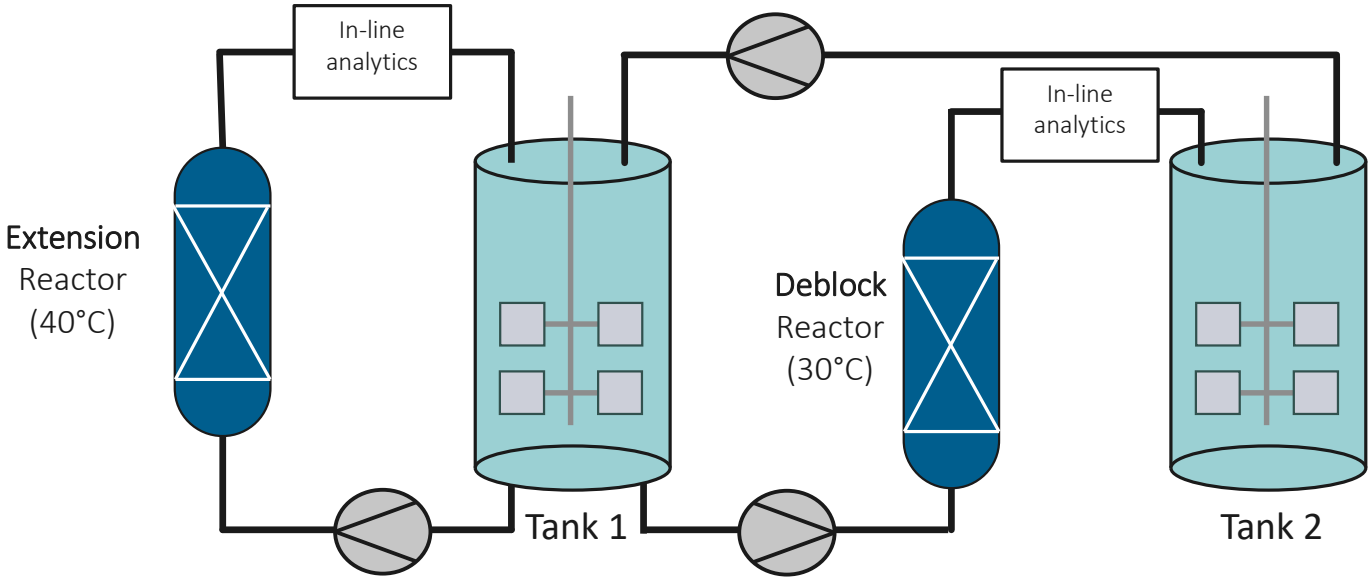
ECO Synthesis™ Process Overview

Route 1: ECO Sequential Synthesis



Process Characteristics:

- Enzymes immobilized within reactor
- Oligo in solution with aqueous reaction system

Process Conditions:

- [Oligo]: 6mM
- [NQP]: 1.5 mol eq.
- > 4-hour cycle time per NQP deblock/extension

Note: ongoing enzyme engineering and process development

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Sequential Enzymatic Synthesis - By the Numbers

Route 1: ECO Sequential Synthesis

Sense and antisense strands synthesized using ECO Synthesis™ manufacturing platform

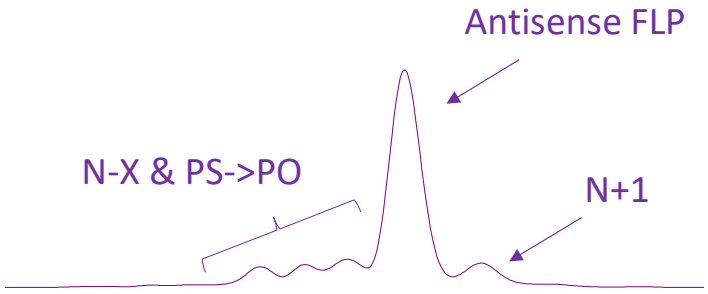
Compound	RNA Starter Length	Number of Extensions	Added Monomer	Avg. Coupling Efficiency (%)
Sense Strand	7	14	2'F / 2'OMe / L96	98.4
Antisense Strand	7	16	2'F / 2'OMe	98.0

Antisense Purity

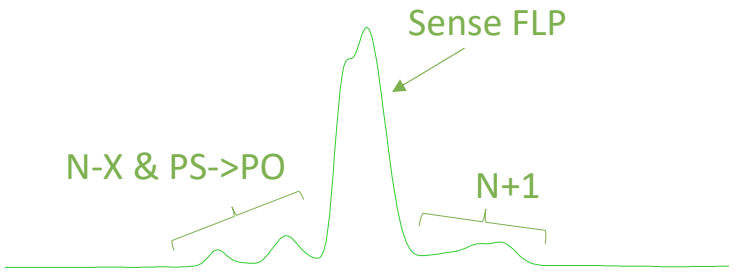
ID	Area %
N-1	5.3
PS->PO	6.2
N-1 & PS->PO	7.8
FLP	73.9
N+1	6.8

Sense Purity

ID	%Area
N-2	2.6
N-1 & PS->PO	6.9
FLP	80.2
N+1	10.3



Antisense LC-UV260nm Spectra



Sense LC-UV260nm Spectra
note - diastereomer resolution of FLP

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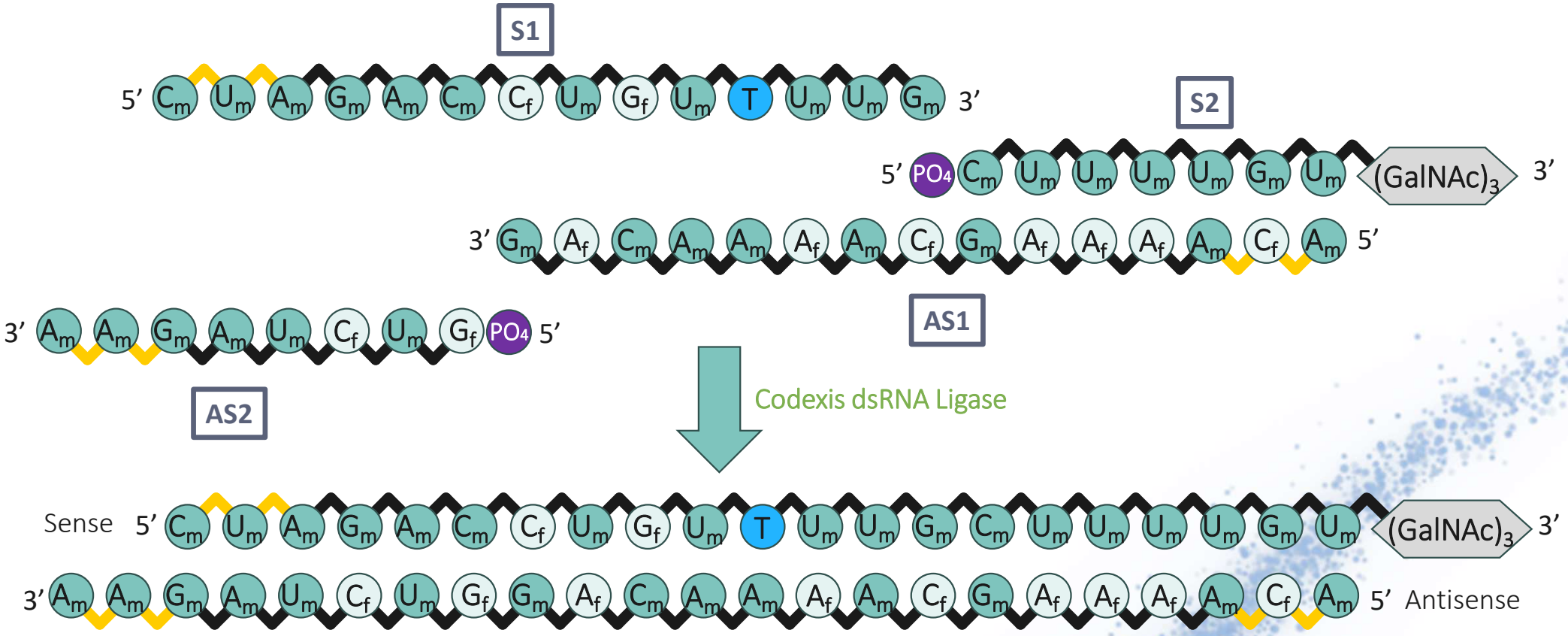
Ligation Disconnection Strategy

Route 2: PAC + dsRNA ligase

Two nick / four-fragment approach used for the generation of inclisiran



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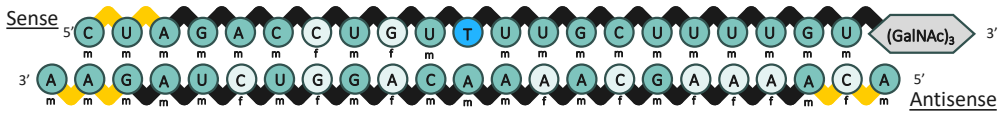
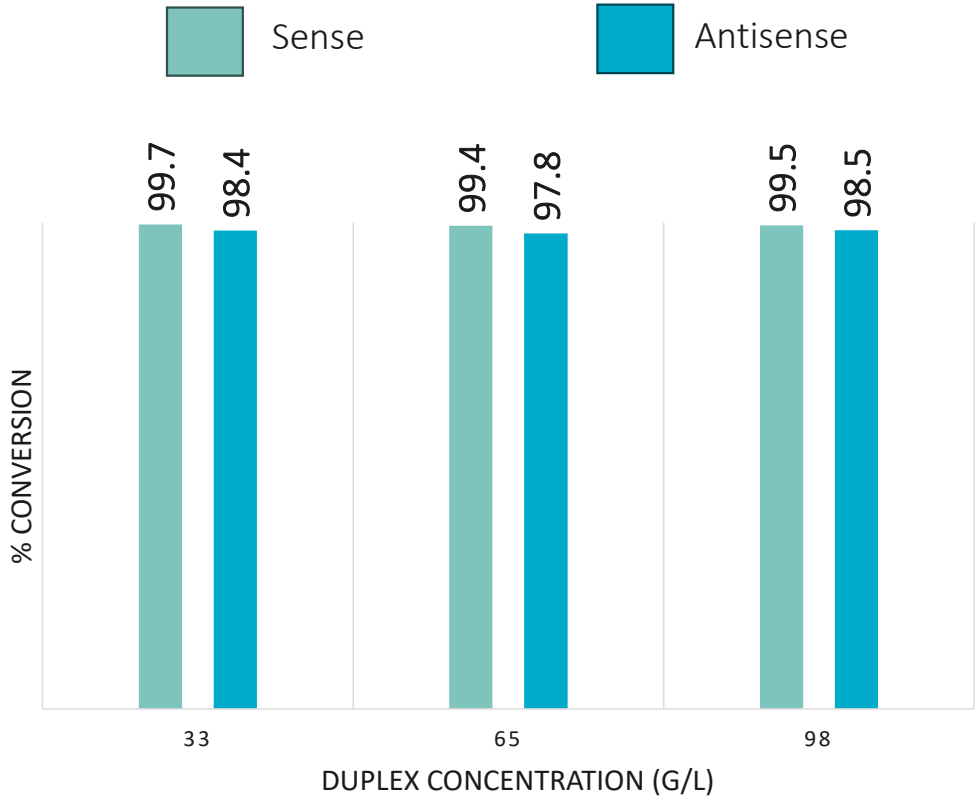
Ligation Using PAC Fragments - inclisiran

Route 2: PAC + dsRNA ligase

Low concentrations of Codexis dsRNA ligase with PAC fragments result in high conversion yields

Parameter	Condition
Substrate (g/L)	6 to 98
CDX Ligase (mg/mL)	0.1
Buffer Composition	Aqueous / TRIS
Incubation Temperature (°C)	33
Incubation Time (h)	6





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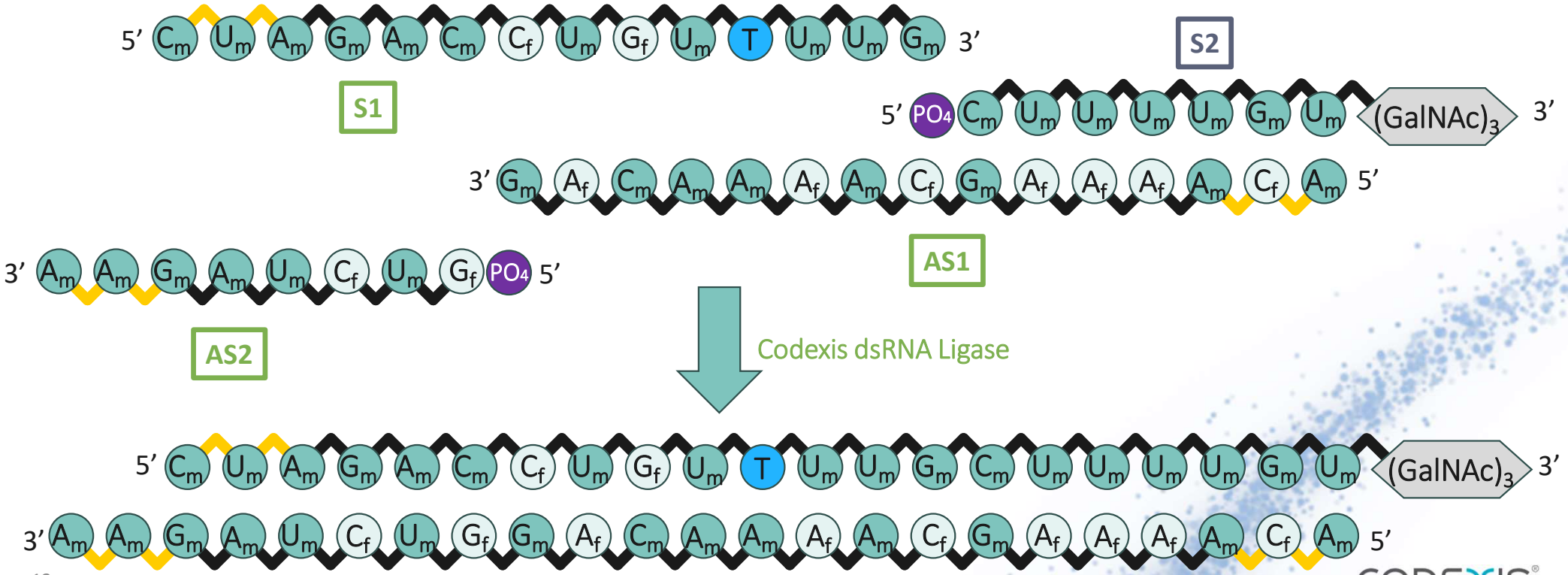
Ligation Using PAC + ECO Fragments - inclisiran

Route 3: PAC / ECO + dsRNA ligase

Two nick approach using a combination of PAC and ECO derived fragments for the generation of inclisiran

Example: 1 PAC / 3 ECO Disconnection



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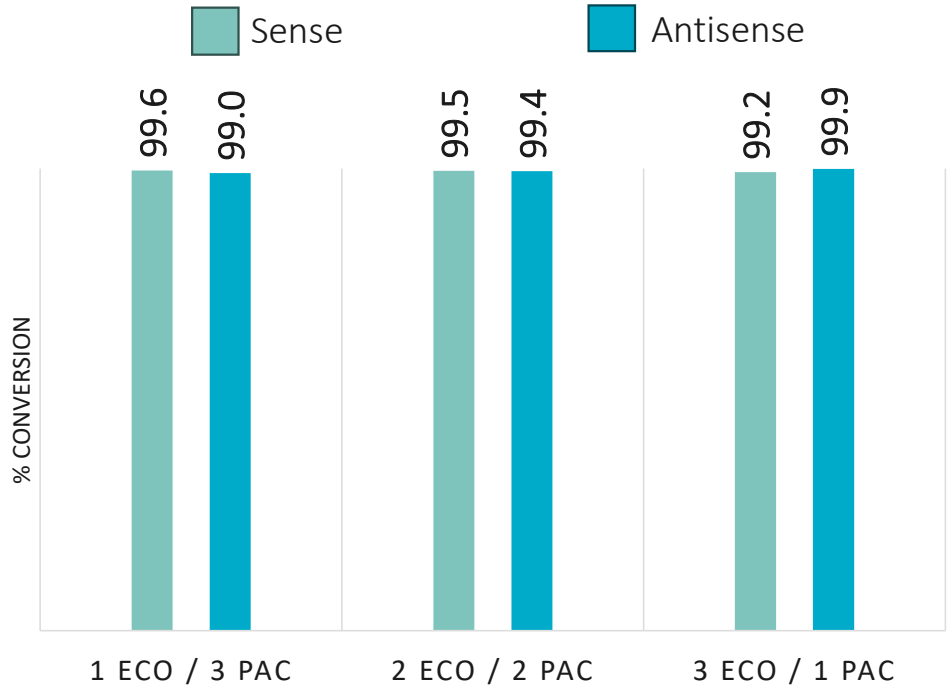
Ligation Using PAC + ECO Fragments - inclisiran

Route 3: PAC / ECO + dsRNA ligase

Using low concentrations of Codexis dsRNA ligase with PAC + ECO fragments result in high conversion yields

Process Conditions

Parameter	Condition
Substrate (g/L)	6
CDX Ligase (mg/mL)	0.1
Buffer Composition	Aqueous / TRIS
Incubation Temperature (°C)	33
Incubation Time (h)	6



Ligation is successful when using ECO and PAC synthesized fragments enabling both synthesis routes when manufacturing siRNA.

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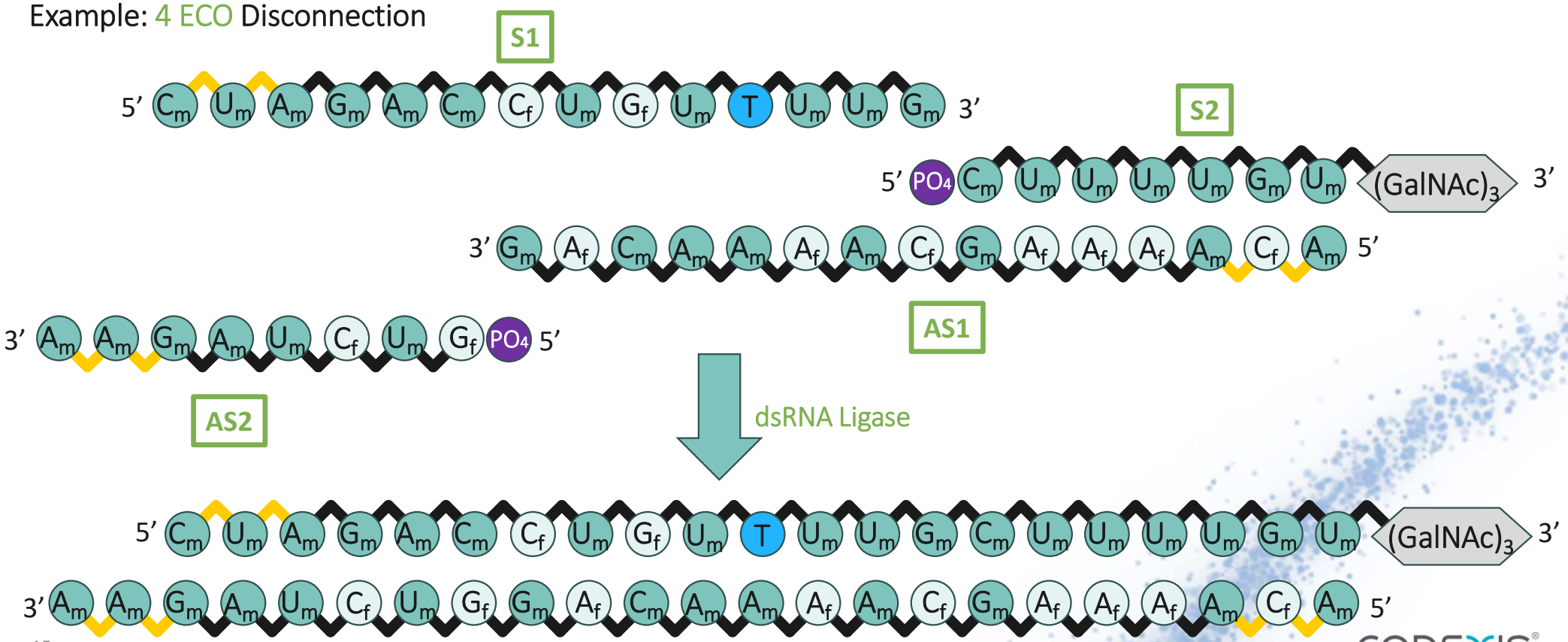
Ligation Using ECO Fragments - inclisiran

Route 4: ECO + dsRNA ligase

Two nick using 4 ECO derived fragment approach used for the generation of inclisiran

Example: 4 ECO Disconnection



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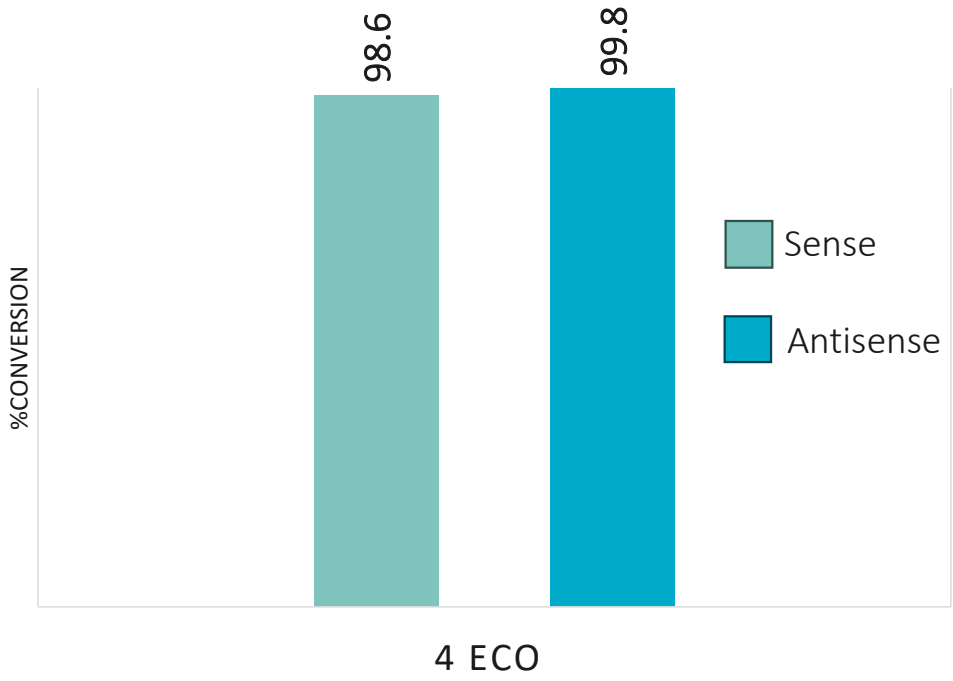
Ligation Using ECO Fragments - inclisiran

Route 4: ECO + dsRNA ligase

Low Codexis dsRNA ligase concentrations with ECO fragments result in high duplex yields

Parameter	Condition
Substrate (g/L)	6
CDX Ligase (mg/mL)	0.1
Buffer Composition	Aqueous / TRIS
Incubation Temperature (°C)	33
Incubation Time (h)	6



Ligation is successful when using ECO synthesized fragments pushing toward fully enzymatic synthesis of siRNA.

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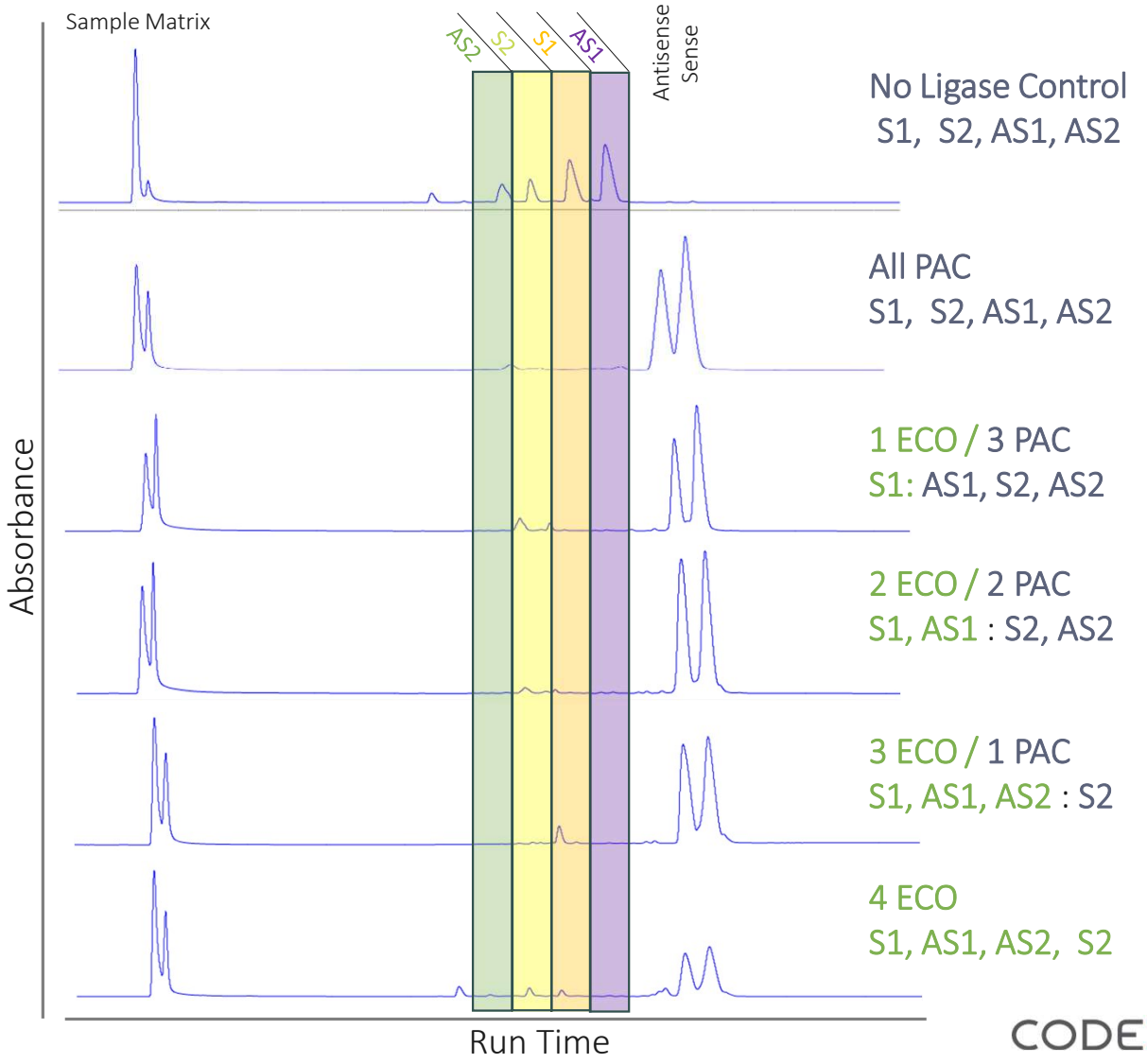
Adaptable Ligation via ECO & PAC Fragments for high yielding siRNA Duplex

Ligation Performance Takeaway

- Ligation using ECO and PAC fragments with Codexis dsRNA ligase yield high ligation efficiencies ≥ 98%.
- Similar impurity profiles observed

Note: process optimization can further improve duplex & impurity profile

Fragment Composition	% Ligation	
	Sense	Antisense
4 PAC	99.5	98.5
1 ECO / 3 PAC	99.6	99.0
2 ECO / 2 PAC	99.5	99.4
3 ECO / 1 PAC	99.2	99.9
4 ECO	98.6	99.8



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Ligation Impurity Profile - % Comparison Summary

Codexis demonstrated ligation of siRNA using 4 different synthesis routes to meet your needs

Ligation Pathway	Conversion (%)	Antisense Strand (%)	Sense Strand (%)	Crude Duplex Purity (%)	Notable Impurities
4 PAC	>98	53.3	41.6	91.5	AS1, S2
1 ECO / 3 PAC	>98	52.8	37.9	85.0	AS2, S2
2 ECO / 2 PAC	>98	51.6	44.1	88.8	AS2, S2
3 ECO / 1 PAC	>98	51.8	41.3	87.8	AS2, S2
4 ECO	>98	50.0	39.0	86.9	AS2, S2

Denaturing RP

Nondenaturing SEC

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Forward Looking for ECO Synthesis™ Manufacturing Platform

Codexis is positioned to supply therapeutic siRNA in 2025 from our Innovation Lab

- ECO Synthesis™ manufacturing platform has made significant progress since TIDES EU in November 2023
  - Ligation Services are available today!
- Continue process development and demonstrated scale up
  - Improving yield recovery across the synthesis via oligonucleotide recovery from resin / step
  - Decreased cycle times for volumetric productivity improvements
  - Drive bench scale to larger scale productions for both sequential and ligation siRNA processes
  - Assess automation processes for sequential synthesis and ligation
- Continue enzyme engineering on resin activity improvements

Step into the future of manufacturing RNA

Manufacturing – non-GMP

Preclinical GLP material

Manufacturing – cGMP

GMP – Phase I

GMP – Phase II

GMP – Phase III

GMP – Commercial

Codexis ECO Synthesis™ Innovation Lab

Coming soon

Learn more about enzymatic siRNA manufacturing services

Booth #709

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