RNA Synthesis Using Ligation for Improved Scalability and Reduced Manufacturing Cost

RNA Leaders EU, 2025

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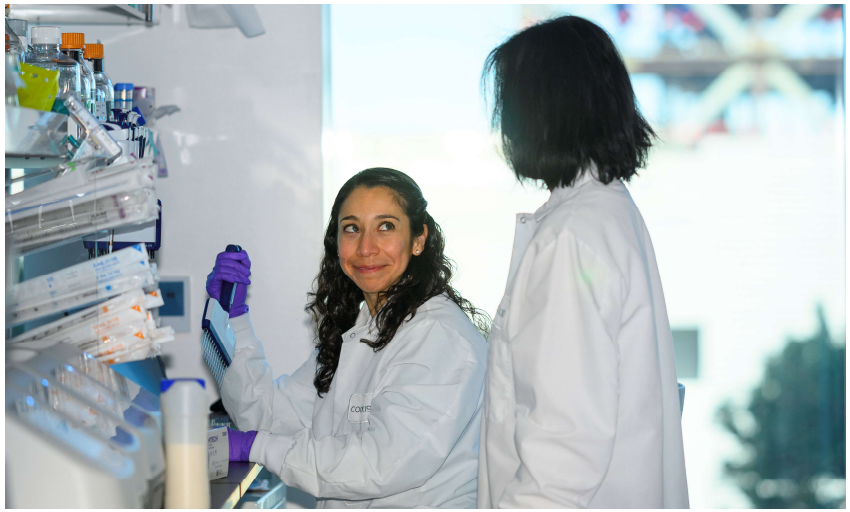
CODEXIS®

Codexis: Enzymatic Solutions for Efficient and Scalable Therapeutics Manufacturing

Company Overview

Founded: 2002
Headquarters: Redwood City, CA
Total Employees: ~200
Stock Ticker: CDXS



Business Snapshot

50+ commercialized engineered enzyme products

Pharmaceutical Biocatalysis: Foundational business in small molecule manufacturing

- → **Codexis enzymes are used to manufacture 16 approved therapeutic drugs**
- → Work recognized by multiple **Green Chemistry Challenge Awards** from American Chemical Society

ECO Synthesis™ Manufacturing Platform: enzymatic siRNA synthesis to meet future demand for RNAi therapeutics

- → dsRNA fragment ligation
- → Sequential enzymatic synthesis

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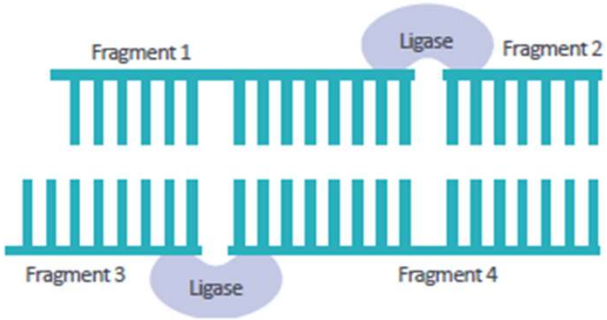
Step into the Future of RNAi

Codexis manufactures siRNA through enzymatic synthesis technologies. We apply appropriate enzymatic tools tailored to your siRNA asset needs.

Hybrid Strategy

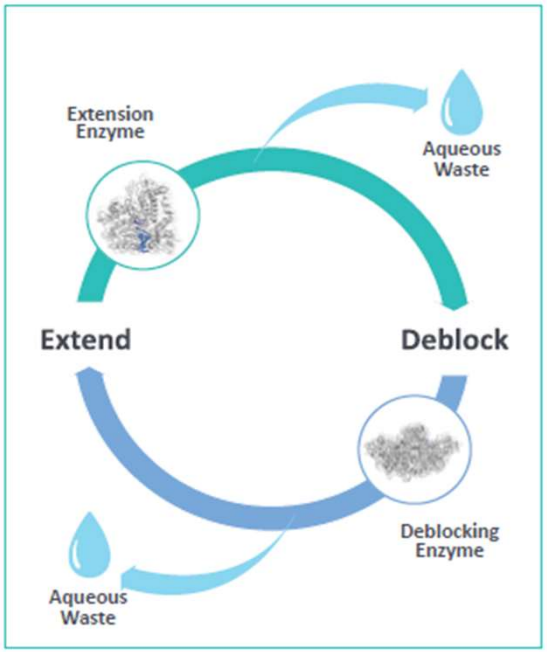
Ligation using ecoRNA™ Ligase

Chemically or enzymatically synthesized fragments



Fully Enzymatic

Enzymatic siRNA Synthesis



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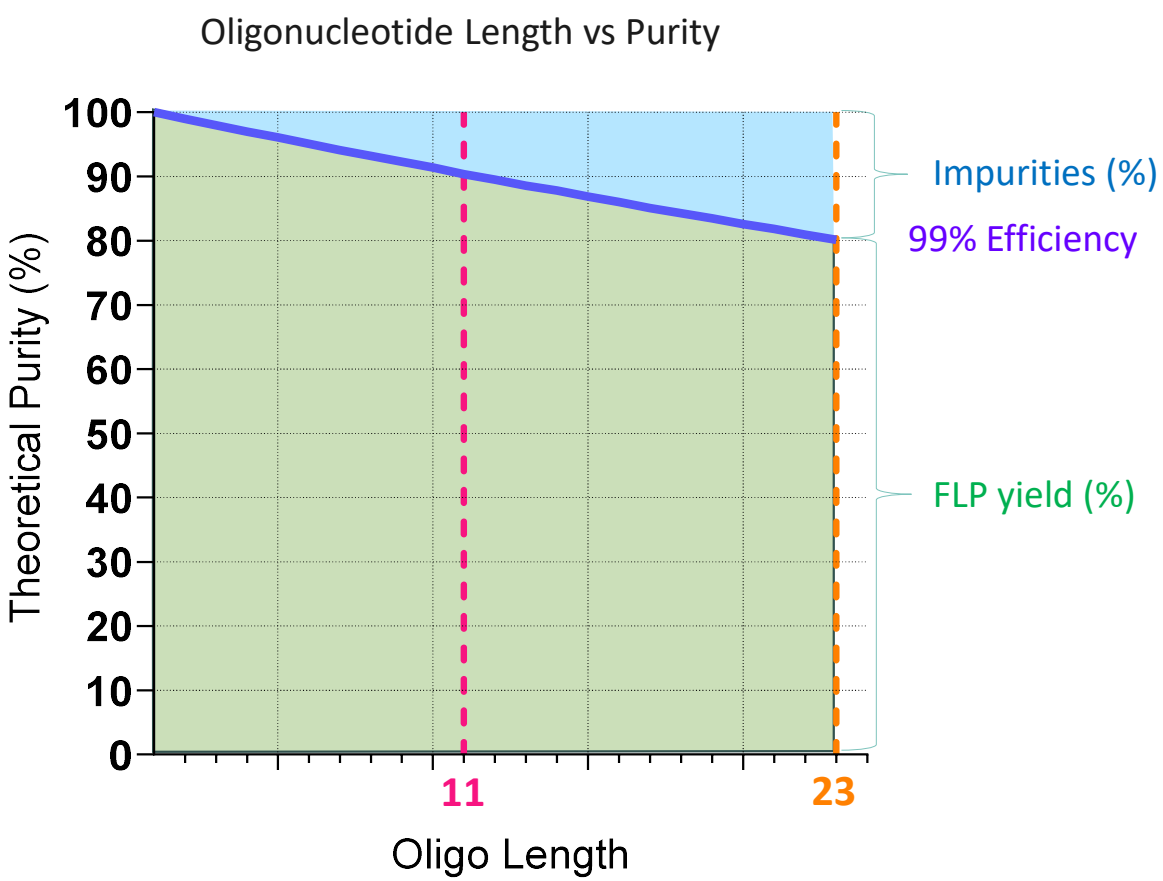
Oligonucleotide Purity Declines with Length



Advantages of Shorter Oligonucleotides:

- Higher yield of the full-length product (FLP)
- Fewer impurities
- Amenable to solid *or* liquid-phase synthesis
- Simpler purification (if necessary)
- Reduced risk of batch failure

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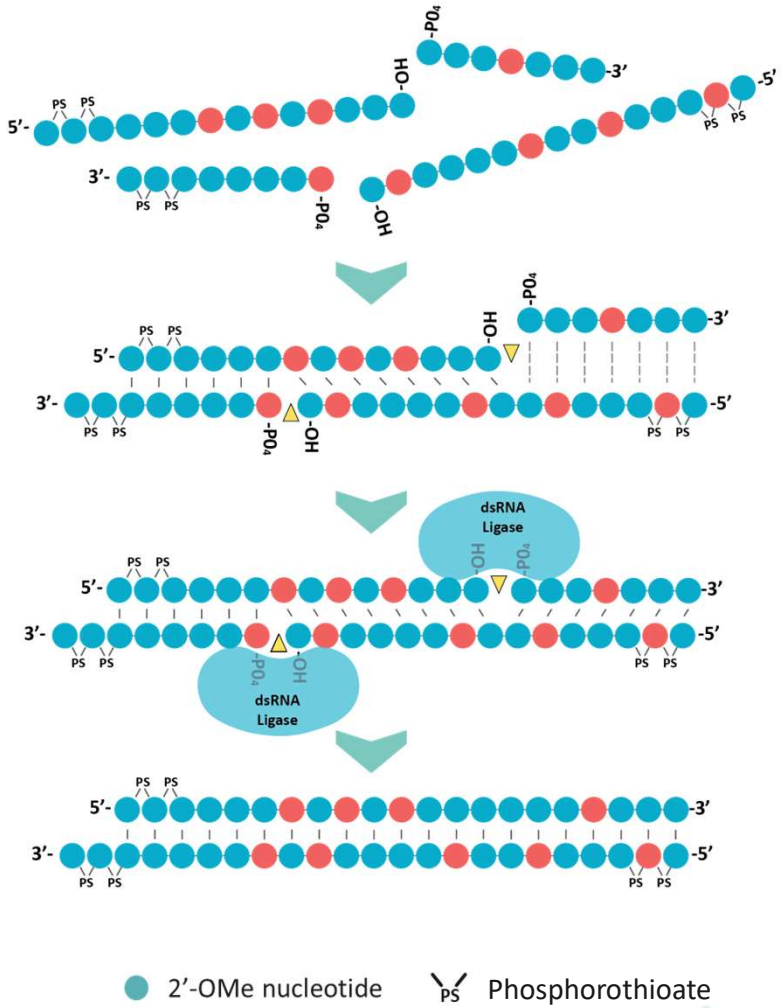
Fragment Ligation Strategy for siRNA Manufacturing

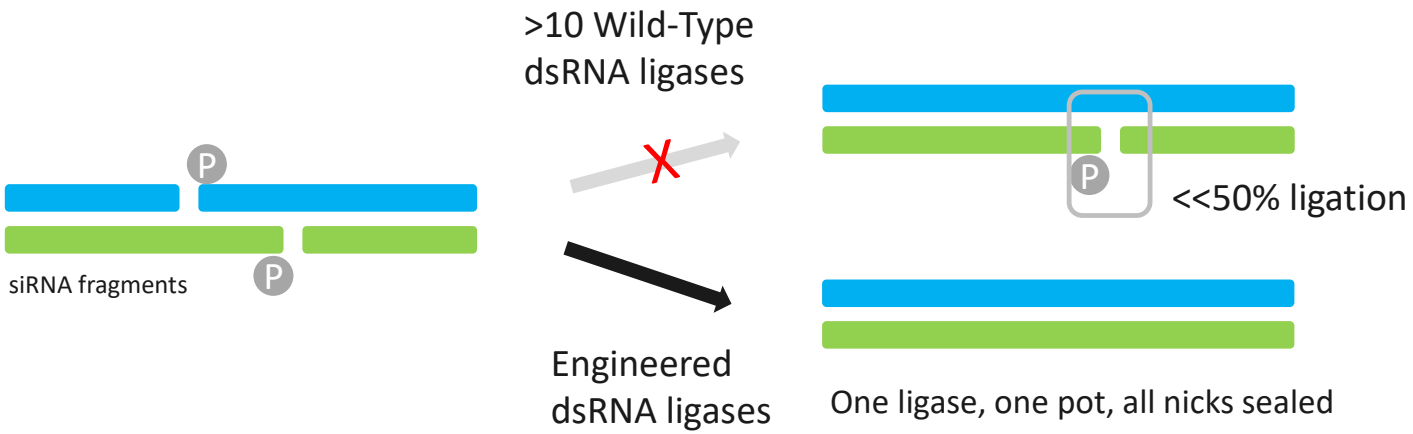


Wild-type dsRNA ligases are inefficient for my process.

How can Codexis enable an efficient ligation-based workflow?

Case Studies: Engineered Ligases Enable Lower Manufacturing Costs

Drives Valuable Economics Through Improved Performance for Two Large Pharmas



WT ligases did not efficiently convert all nicks in a given partner's substrate set

Engineered ligases achieved **target conversion (>90%) for *all* nicks** in substrate design

Custom Engineered dsRNA Ligase Improvements:

- Increased conversion of multiple nicks with one enzyme → Enabled the ligation route
- Increased substrate loading → Increased volumetric productivity
- Increased expression and enzyme stability for scalable expression → Robust enzyme supply/manufacturability

Valuable Economics:

Enzyme delivered at scale

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I see potential in ligation-based siRNA manufacturing.

How can I accelerate and de-risk my process development?

RNA Ligase Screening Services Workflow

1

Fragment design maximizes:

- Average fragment length (purity, yield)
- Fragment annealing
- Ligase activity

2

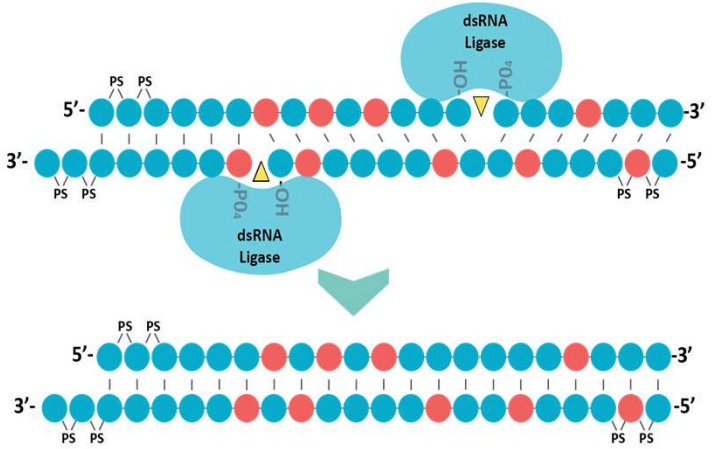
Screening Codexis' library of engineered dsRNA ligases

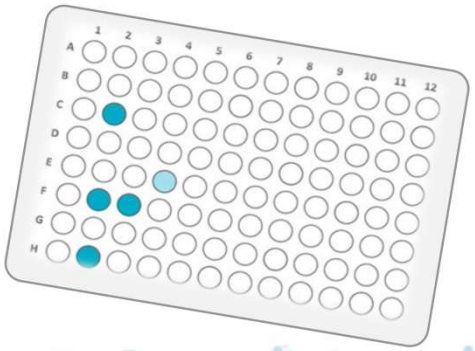
- Conversion on challenging substrates
- In-process robustness

3

Results and recommendations:

- Specific ligases for your application
- Ligases for expression scale-up





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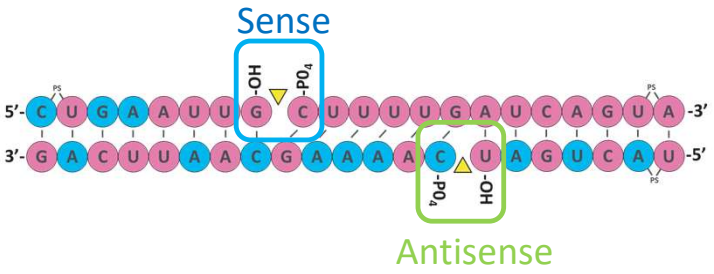
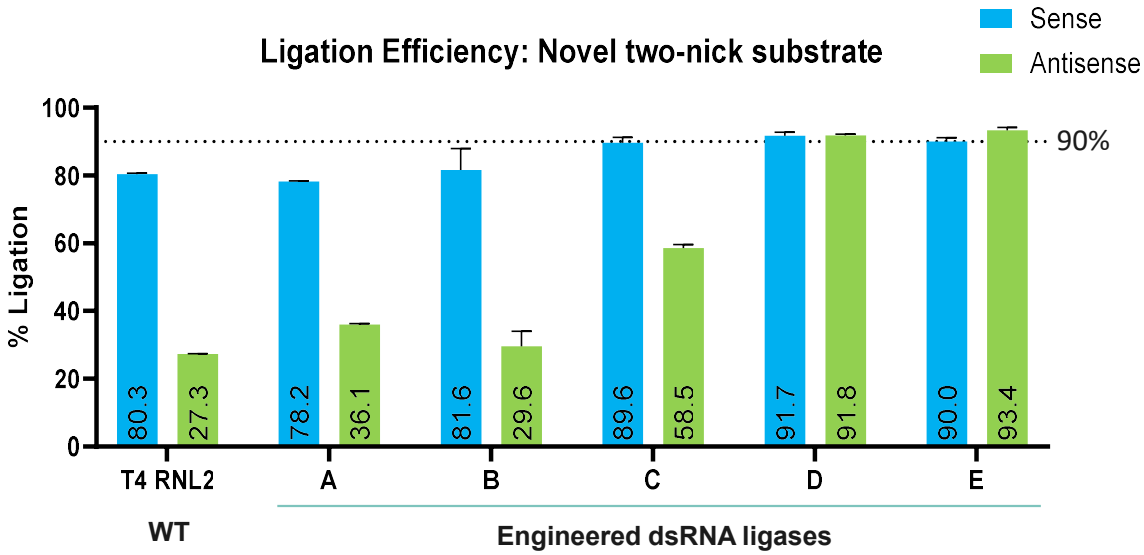
Single Engineered Ligase Converts Multiple Nicks in a One-pot Reaction

Novel two-nick substrate:





- Screening and Optimization services used to identify hits on novel 2-nick substrate
- A **single ligase** in a **one pot** reaction seals **all nicks** in the substrate design at >90% efficiency

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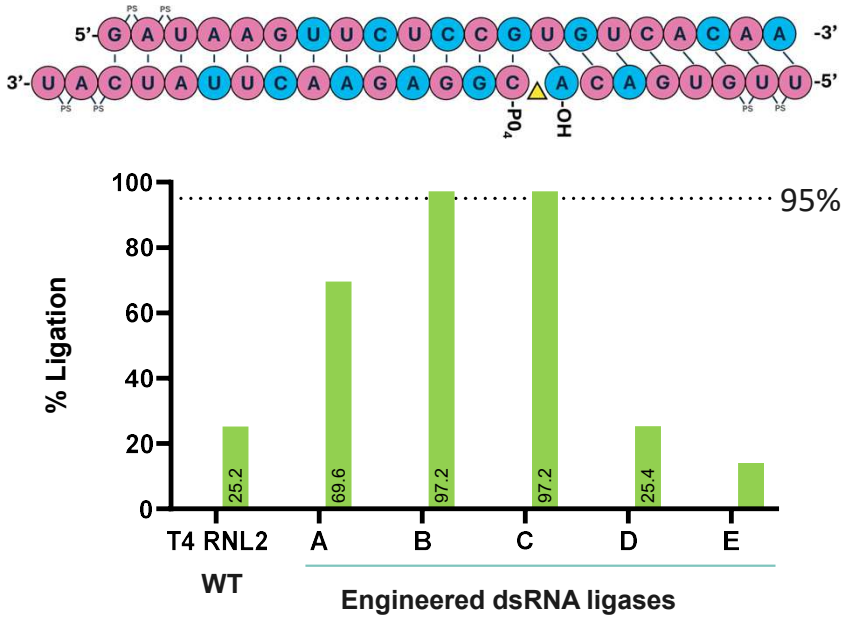
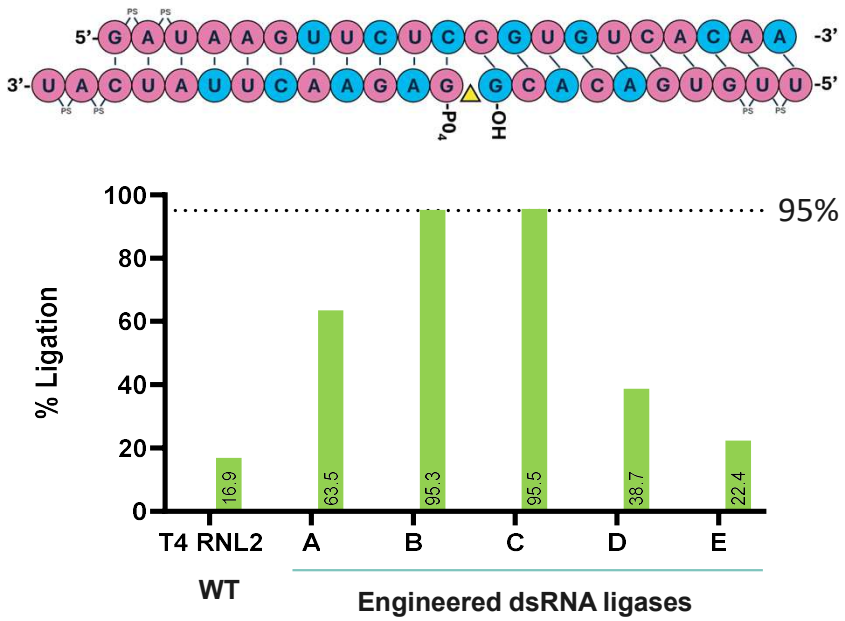
Engineered Ligases Outperform WT on Multiple Substrates

Novel substrate, nicks in two positions





- Multiple hits identified in our screening process using 2 novel substrates
- Significant improvements in ligation efficiency relative to WT for select library variants
- The optimal ligase varies based on the substrate

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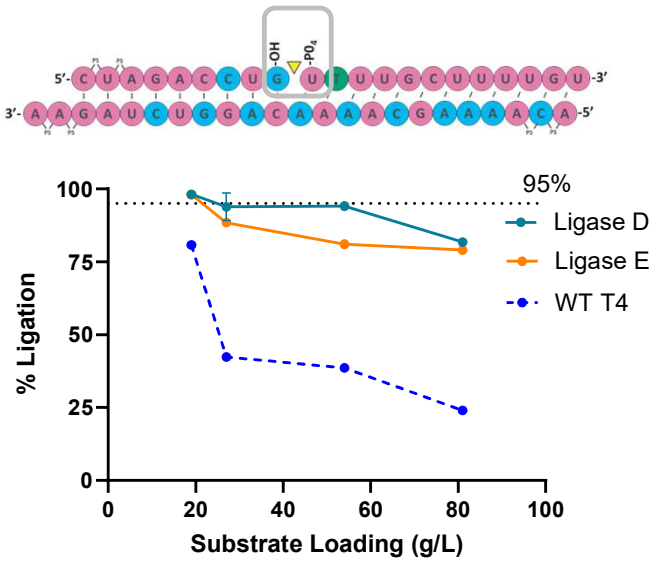
Engineered Ligases Deliver Robust In-Process Performance

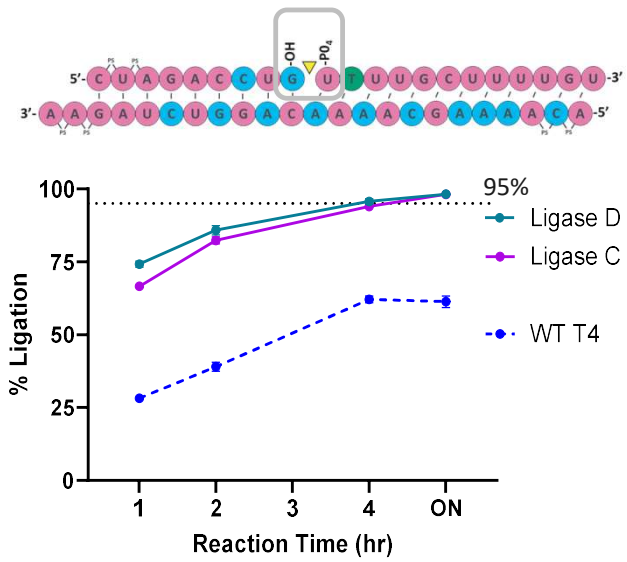
High Substrate Loading



Markedly improved tolerance to high substrate loads

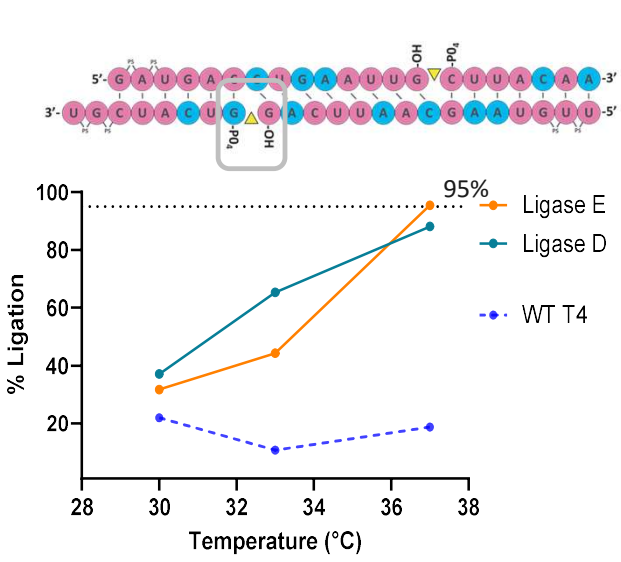
>90% conversion at 54 g/L RNA

Faster Reaction Times



Higher overall conversion and faster reaction kinetics

Temperature Robustness



In-reaction thermostability

Improved conversion at elevated temperatures

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How can I accelerate and de-risk my initial ligase screen?

Can We Accelerate Early PoC By Selecting Nicks to Suit the Ligase

Standard Work-flow: Screen ligase variants optimal for a given set of shortmers of targeted duplex RNA

RNA fragments (shortmers) Target duplex RNA product

Challenge: Ligation efficiency can vary significantly depending on the ligase being utilized and its substrates.

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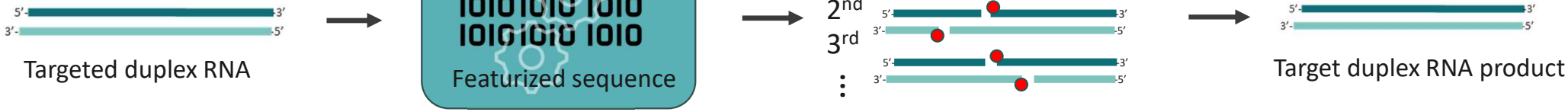
Can We Accelerate Early PoC By Selecting Nicks to Suit the Ligase

Standard Work-flow: Screen ligase variants optimal for a given set of shortmers of targeted duplex RNA

RNA fragments (shortmers) → Target duplex RNA product

ML Enhanced Workflow: Model aimed at predicting activity of ligase variants for targeted duplex RNA



Targeted duplex RNA → Optimizing combination of RNA shortmer designs & ligase variants → Ranked designs → Target duplex RNA product

Significantly accelerating lead identification of ligase & shortmer pairings by maximizing enzyme-nucleic acid compatibility while minimizing screening of large collections on ligase variants and multiple RNA shortmer designs

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Why Partner with Codexis?

Our expertise in process development enables adoption of next generation enzymatic oligo synthesis technologies.

The partnership to enable your innovations

The ECO Synthesis™ Innovation Lab discovers what's possible

The technology to scale

Achieve greater scale and higher purity through an aqueous manufacturing route

The expertise to power your project

Our 20+ years of enzyme expertise accelerates process development for enzymatic siRNA manufacturing

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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, Operational

Coming soon

Learn more about enzymatic siRNA manufacturing services

Booth 26

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Thank You

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