

A decorative graphic consisting of a trail of small, light blue particles that curves across the top of the slide, resembling a comet tail or a molecular path.

Two Enzymatic Approaches for Large-scale siRNA Synthesis

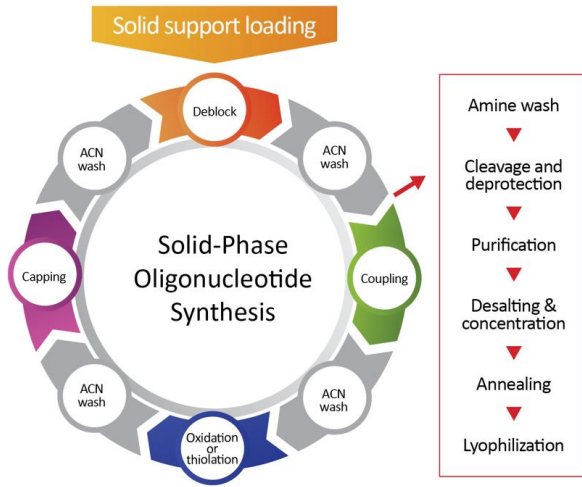
TIDES USA, 2024

Mathew Miller, PhD

CODEXIS[®]

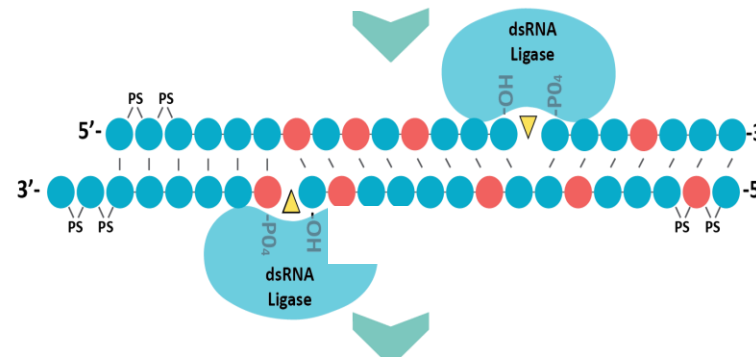
Enzymatic Solutions for siRNA Synthesis are Increasing

Chemical



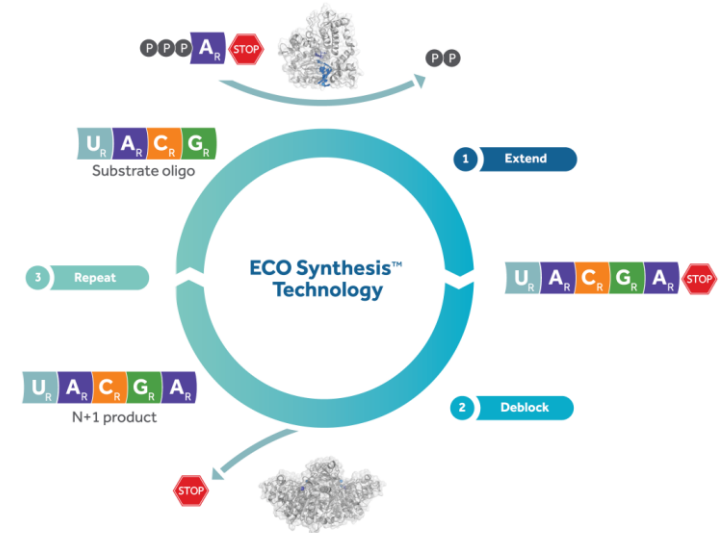
Traditional siRNA Synthesis using Phosphoramidite Chemistry (PAC)

Hybrid



PAC fragments + Ligation

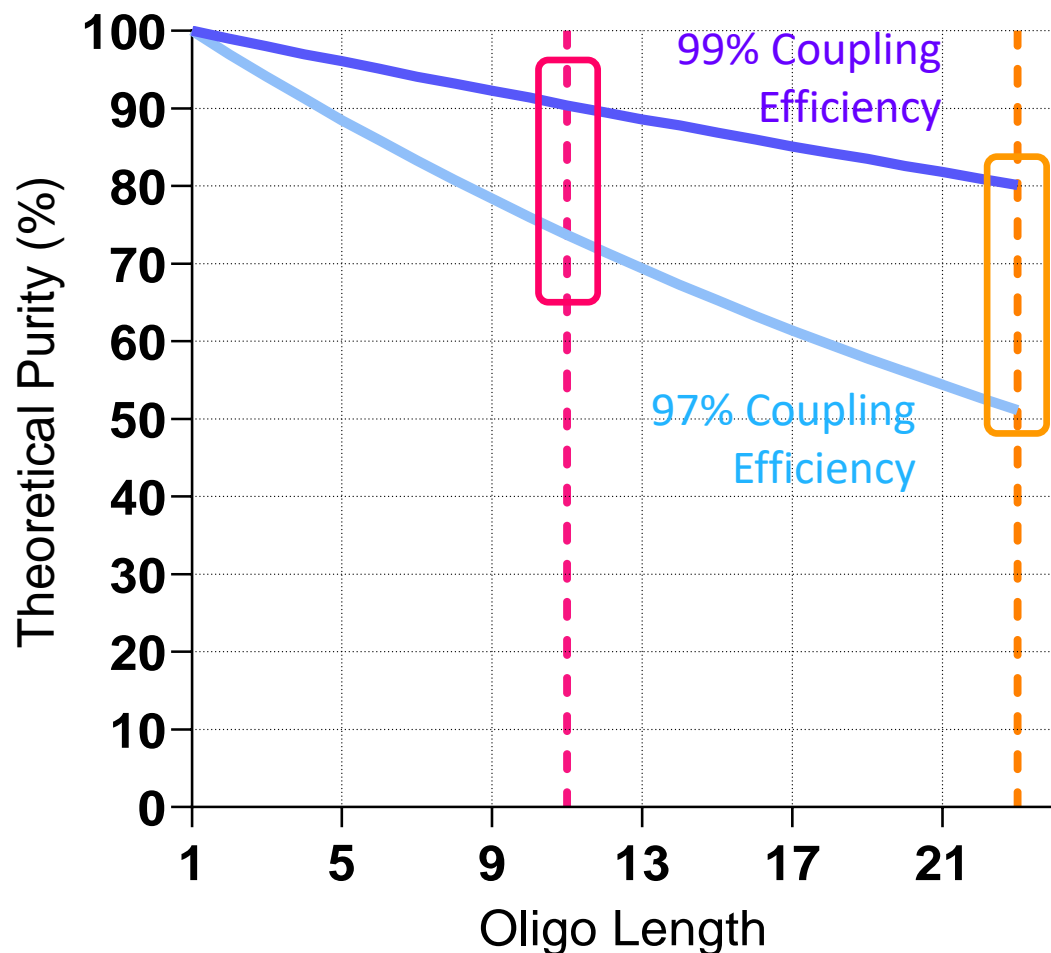
Enzymatic



ECO Synthesis™ (Enzyme-Catalyzed Oligonucleotide Synthesis) Manufacturing Platform

Oligonucleotide Synthesis Experiences Purity Declines with Length

Cumulative effects of <100% coupling efficiency on oligonucleotide purity



11-mer → 73 – 91% purity
9 – 27% impurities

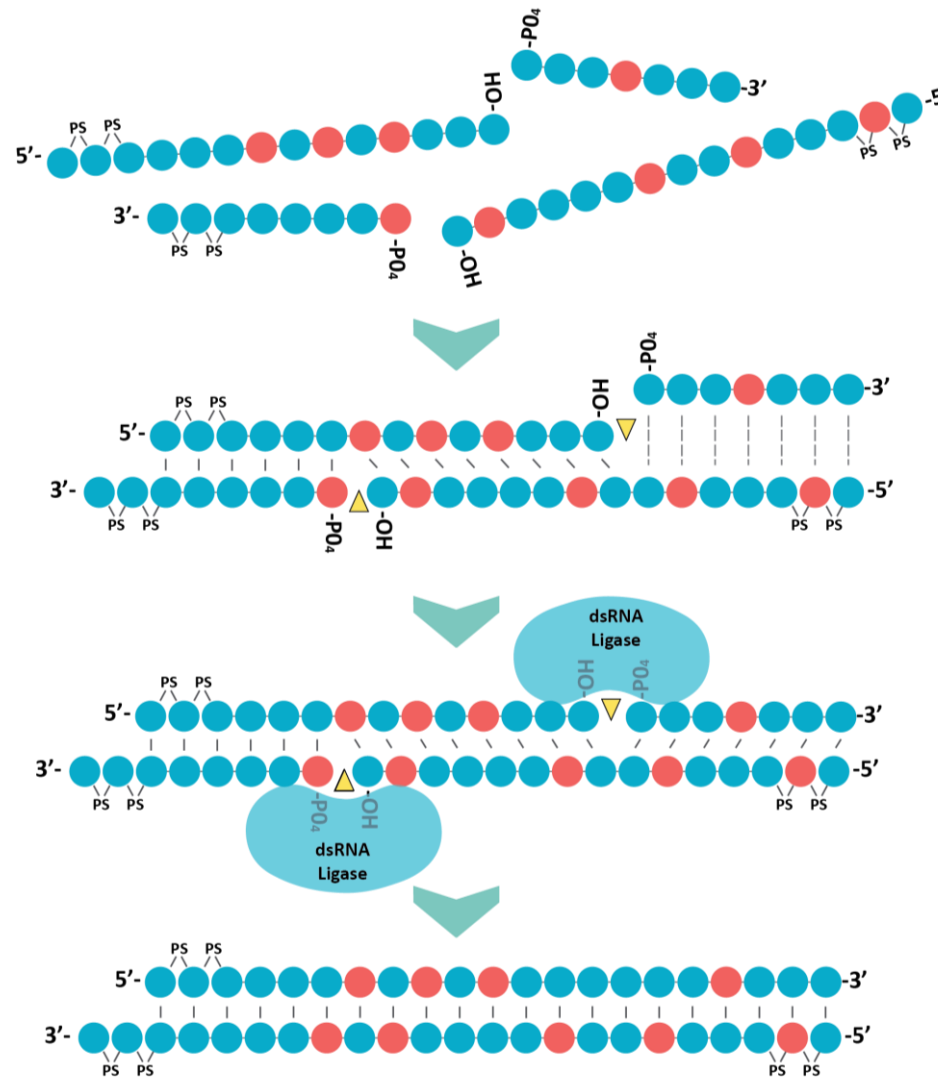
23-mer → 51 – 80% purity
20 – 49% impurities

Advantages of synthesizing shorter oligonucleotide sequences:

- Higher yield of the full length product (FLP)
- Fewer impurities
- Simpler purification
- Reduced risk of batch failure

An option for enzymatic methods – a necessity for chemical synthesis

Fragment Ligation Strategies are Increasing for siRNA Manufacturing



ssRNA Fragments

Annealing

Ligation

Full-length dsRNA

● 2'-OMe nucleotide \diagdown_{PS} phosphothioate
● 2'-F nucleotide ▼ Ligation site

Engineered Ligases Enable Lower Manufacturing Costs

Wild Type dsRNA Ligase

Low Activity

- Poor ligation efficiency on clinically-relevant molecules
- Low activity requires high enzyme loading

Limited Scalability

- Low substrate loading
- Require larger reaction volume and capital investment

High Cost

- Low expression & high enzyme loading results in high enzyme cost
- Low substrate loading increases fixed & variable production cost

Codexis Engineered dsRNA Ligase

High Activity

- ✓ Higher ligation efficiency results in higher yield of siRNA
- ✓ Less enzyme required for target conversion

Scalable

- ✓ Higher substrate loading for increased volumetric productivity
- ✓ Scalable manufacturing of engineered ligase

Valuable Economics

- ✓ Reduced enzyme cost-in-use
- ✓ Reduced reaction volume and capital requirements

Codexis Center of Excellence for Enzymatic RNA Synthesis

Services Offered

Screening & Optimization

- Design of RNA fragments for optimal ligation
- Identification of lead ligase by high-throughput screening of large libraries of engineered RNA ligases
- Optimization of ligation protocols



Enzyme Customization

- Tailored ligase performance under process conditions via CodeEvolver® technology-driven enzyme engineering
- Research-scale enzyme production under appropriate quality systems to support customer-led process development



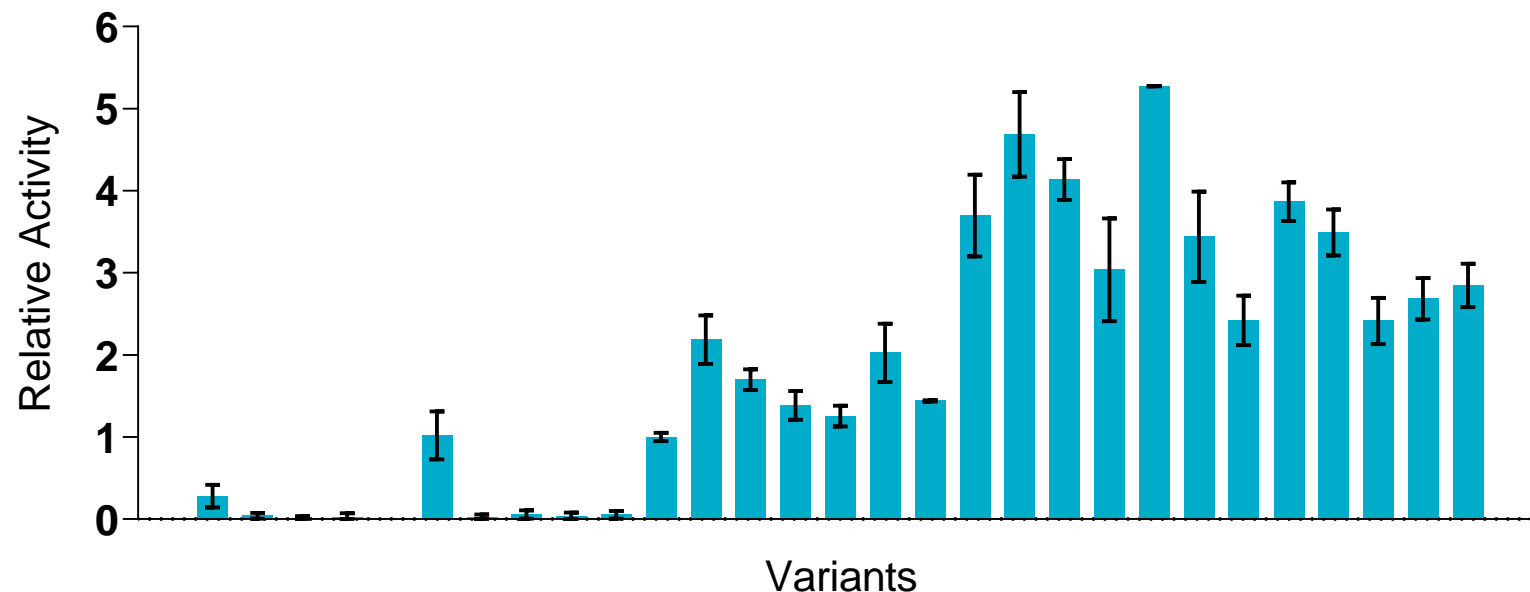
Research Grade RNA Production

- Codexis-led process development & manufacturing of research-grade RNA oligonucleotides
- Product impurity profile assessment (LC-MS)
- Tech transfer support to customer or CDMO of choice



dsRNA Ligase Services: Screening & Optimization

Evaluating **customer's fully modified dsRNA substrates** against **our ligase variant libraries**, consisting of hundreds of differentiated, engineered enzymes **at high substrate loadings**



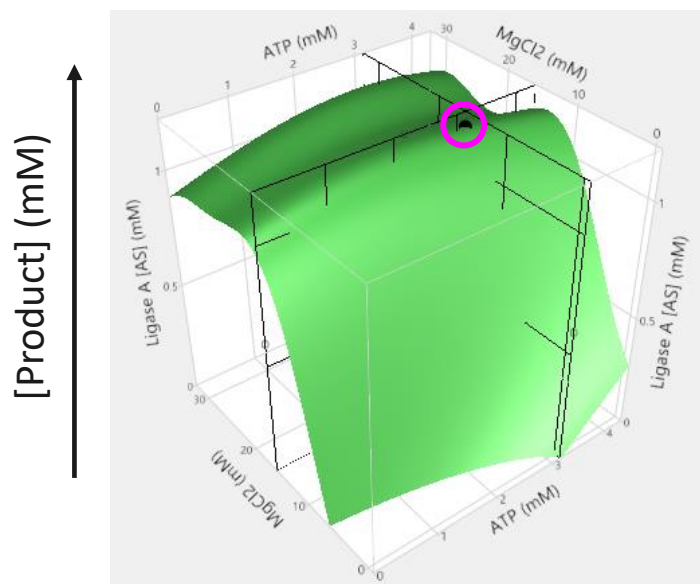
dsRNA Ligases engineered for:

- Substrate specificity
- Catalytic performance
- Manufacturability
- Stability

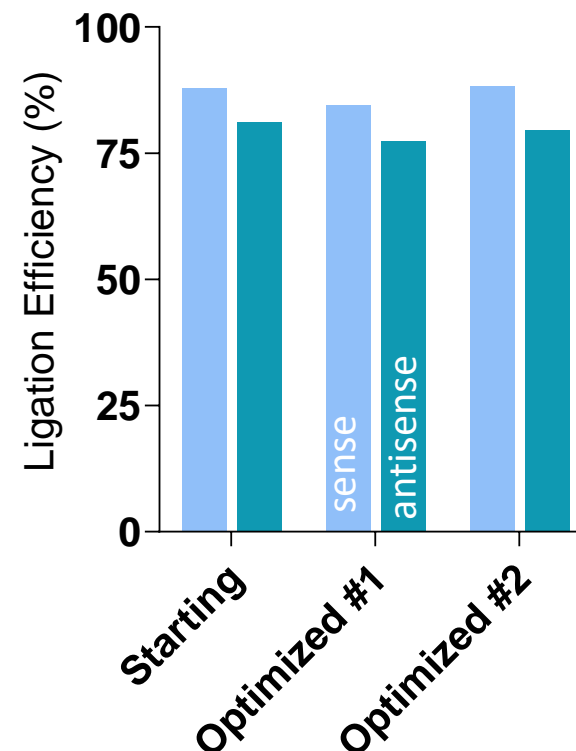
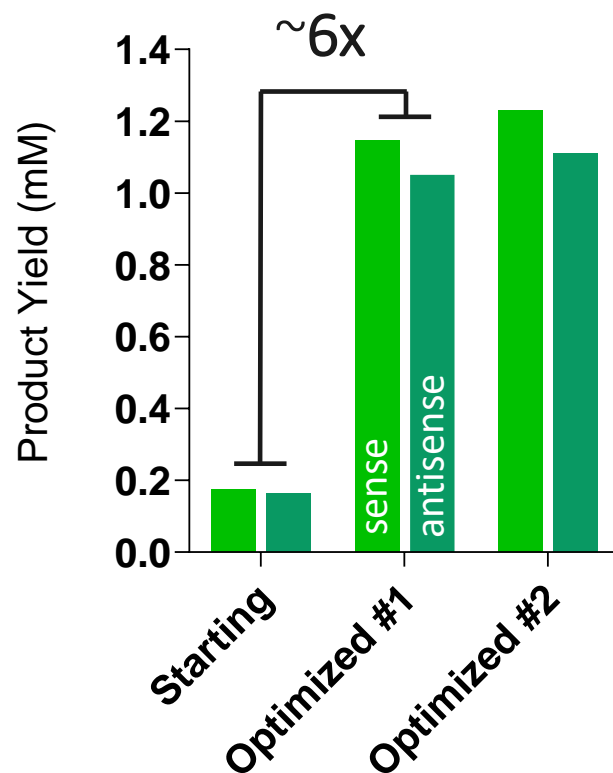
- Exemplary data on ligase variant performance with fully modified RNA model substrate at 20 g/L
- Significant differences in ligase activity at any given load
- Engineered ligase variants generally outperform wild type ligases

dsRNA Ligase Services: Screening & Optimization

Optimizing customer's fully modified dsRNA substrates with selected Codexis engineered ligases for maximum product yield and ligation efficiency at high substrate loadings



DOE response surface



- Response surface is specific/unique based on enzyme, substrates, and buffer (incl. Mg^{2+} , ATP)
- **Maintaining ligation efficiency at high substrate loading (1.2mM = 20 g/L) = increased volumetric productivity**

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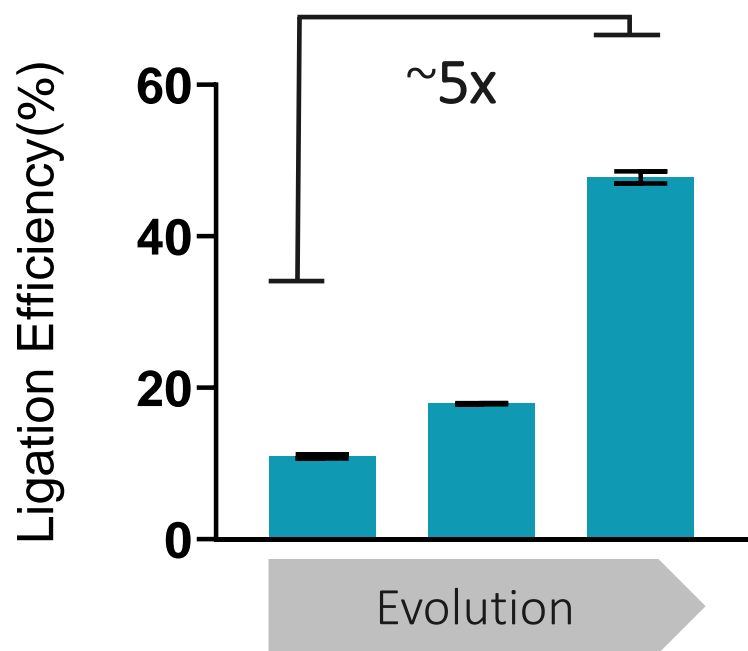
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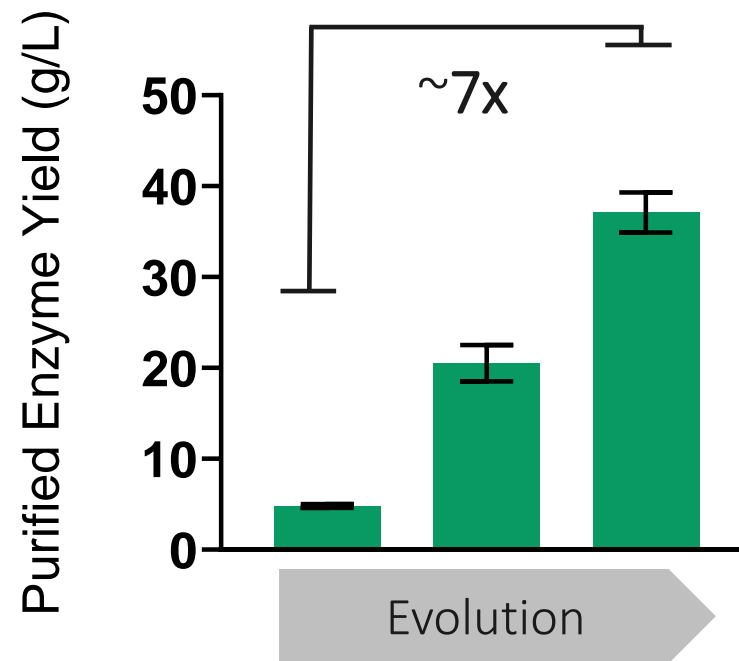


dsRNA Ligase Services: Enzyme Customization for Your Process

Tailoring enzyme performance to **Specific Substrates** or **Substrate Classes (Platform)** under **desired process conditions** via Codexis CodeEvolver® technology



Improving ligation efficiency at high substrate concentration



Improving process viability as a result of scalable dsRNA ligase manufacturability

Codexis Center of Excellence for Enzymatic RNA Synthesis

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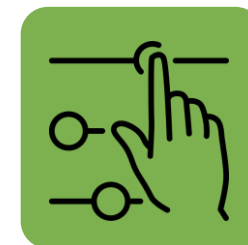
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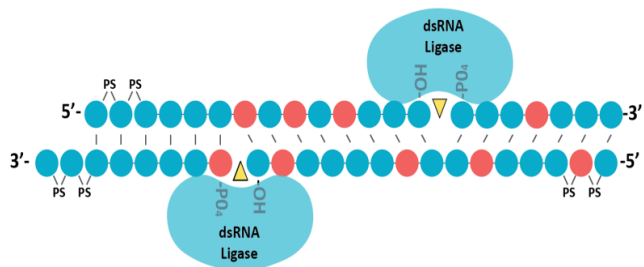
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Codexis Center of Excellence for Enzymatic RNA Synthesis

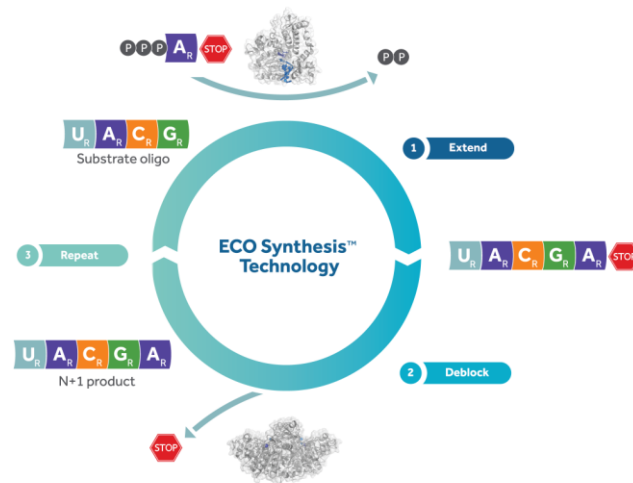
Your Pathway to Lower Cost and More Scalable RNA Manufacturing

Hybrid



RNA Ligase Services

Enzymatic



ECO Synthesis™
Manufacturing Platform



Booth #628