Engineered Enzymes to Overcome Scalability and Sustainability Challenges of Nucleic Acid Therapeutics Manufacturing

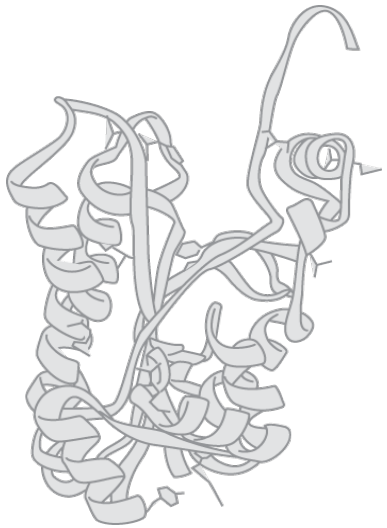
Mathew Miller, PhD Associate Director, Life Sciences Technology and Applications

Stephanie Forget, PhD Senior Scientist I

CODEXIS®

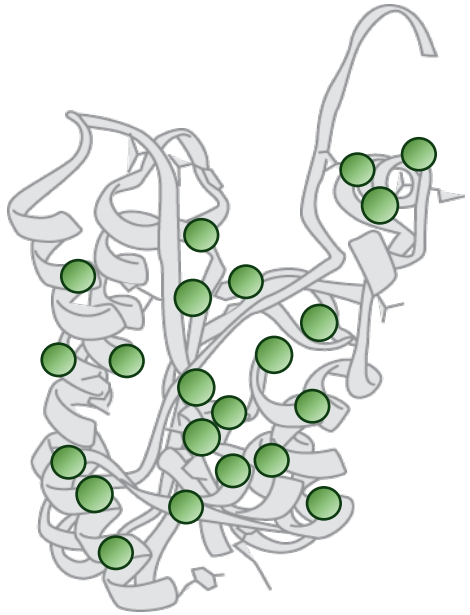
Codexis core business pillars

Based on CodeEvolver® platform to accelerate enzyme discovery and commercialization



Enzymes
from Nature

CodeEvolver®



Commercially
Relevant Enzyme

Value
Creating
Products

Pharmaceutical Manufacturing
enzymes for small molecule production

Life Sciences
enzymes for NGS applications and DNA/RNA synthesis

Biotherapeutics
enzymes as oral drugs; engineered transgenes and capsids for gene therapy

CODEXIS®

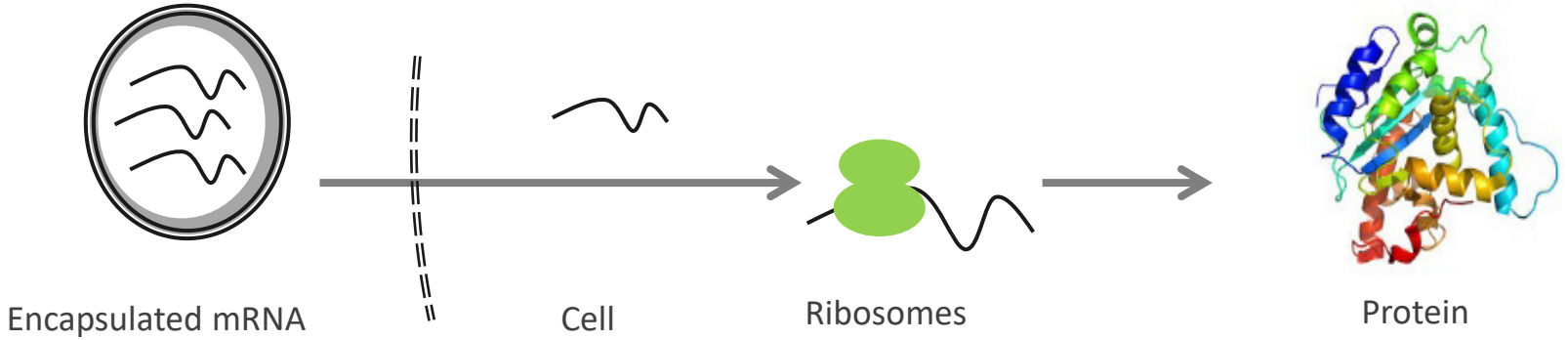
About the Speaker

Mathew Miller, Ph.D.

Associate Director of Life Science Technology and Applications, Codexis

Mathew joined Codexis in 2009. He is currently focused on enzymes used in mRNA manufacturing, including directed evolution for T7 RNA polymerase. Matt has previously lead protein engineering projects for next generation sequencing and enzymatic DNA synthesis. He received his Ph.D. from the University of California, San Francisco.

mRNA is a highly versatile therapeutic modality with manufacturing challenges



Applications

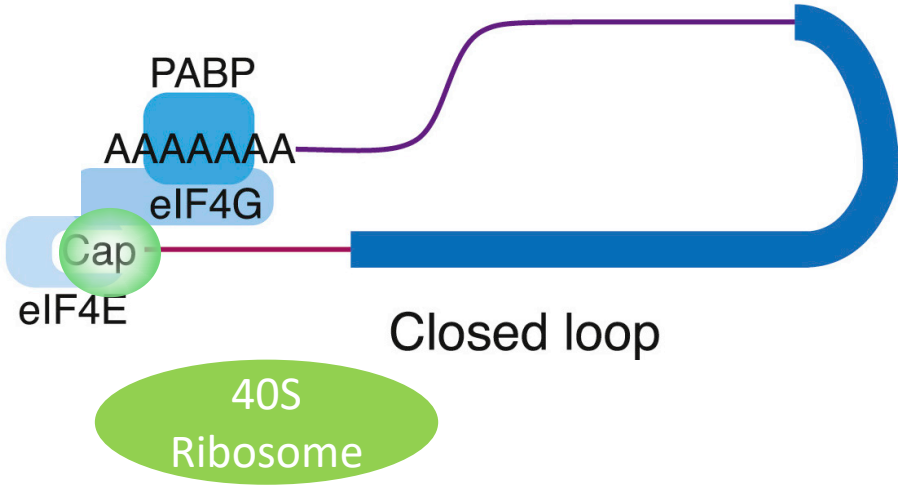
- Vaccines (SARS-CoV2, others)
- Cancer vaccines (Immuno-oncology)
- Gene editing (CRISPR/Cas, others)
- Therapeutic proteins

mRNA manufacturing challenges

- mRNA capping efficiency
- Undesired side products (dsRNA)
- mRNA yield
- Downstream processing

Eukaryotic mRNA cap: structure and function

7-Methylguanosine (7mG) Cap:



Cap functions:

- Prevents recognition of mRNA as foreign/non-self
- Stabilizes mRNA
- Initiates translation

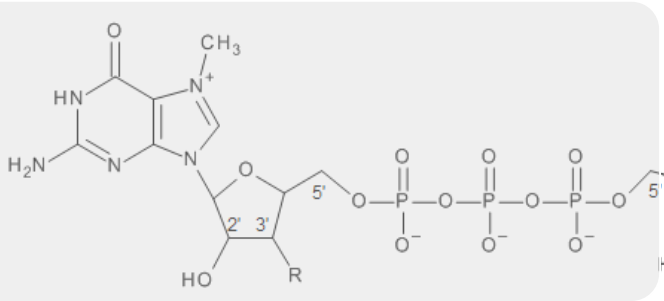
CODEXIS®

Vicens, Q., Kieft, J. S. & Rissland, O. S. Revisiting the Closed-Loop Model and the Nature of mRNA 5’–3’ Communication. *Mol Cell* **72**, 805–812 (2018).

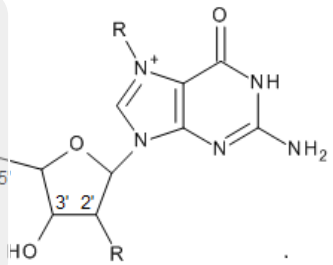
Co-transcriptional capping

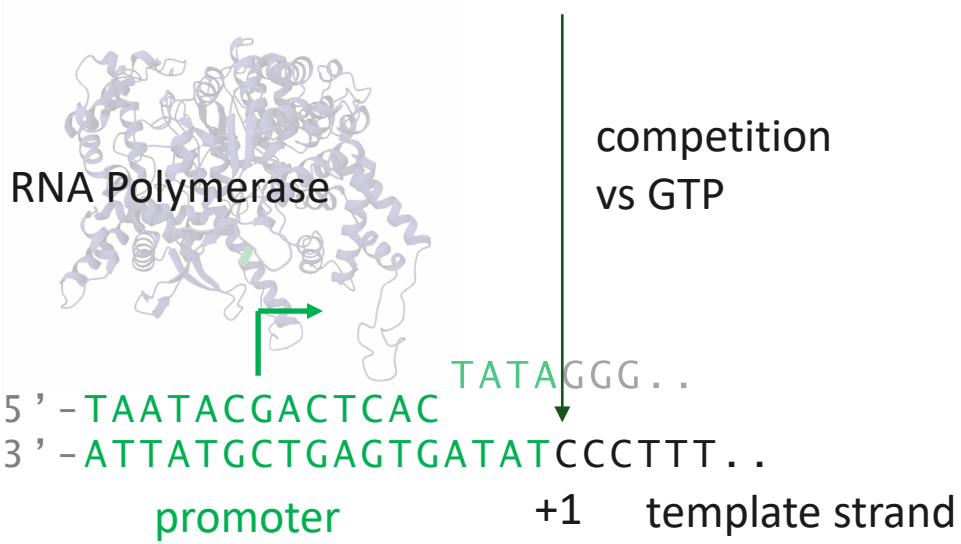
Dinucleotide cap analog:

N7-Methyguanosine



Guanosine





Cap analog is the most expensive reaction component

Increased ratio of cap to GTP ensures:

- Increased capping efficiency
- Decreased mRNA yield
- High cost of production

CODEXIS®

RNA Polymerase Evolution Target

Enzyme Engineering targets:

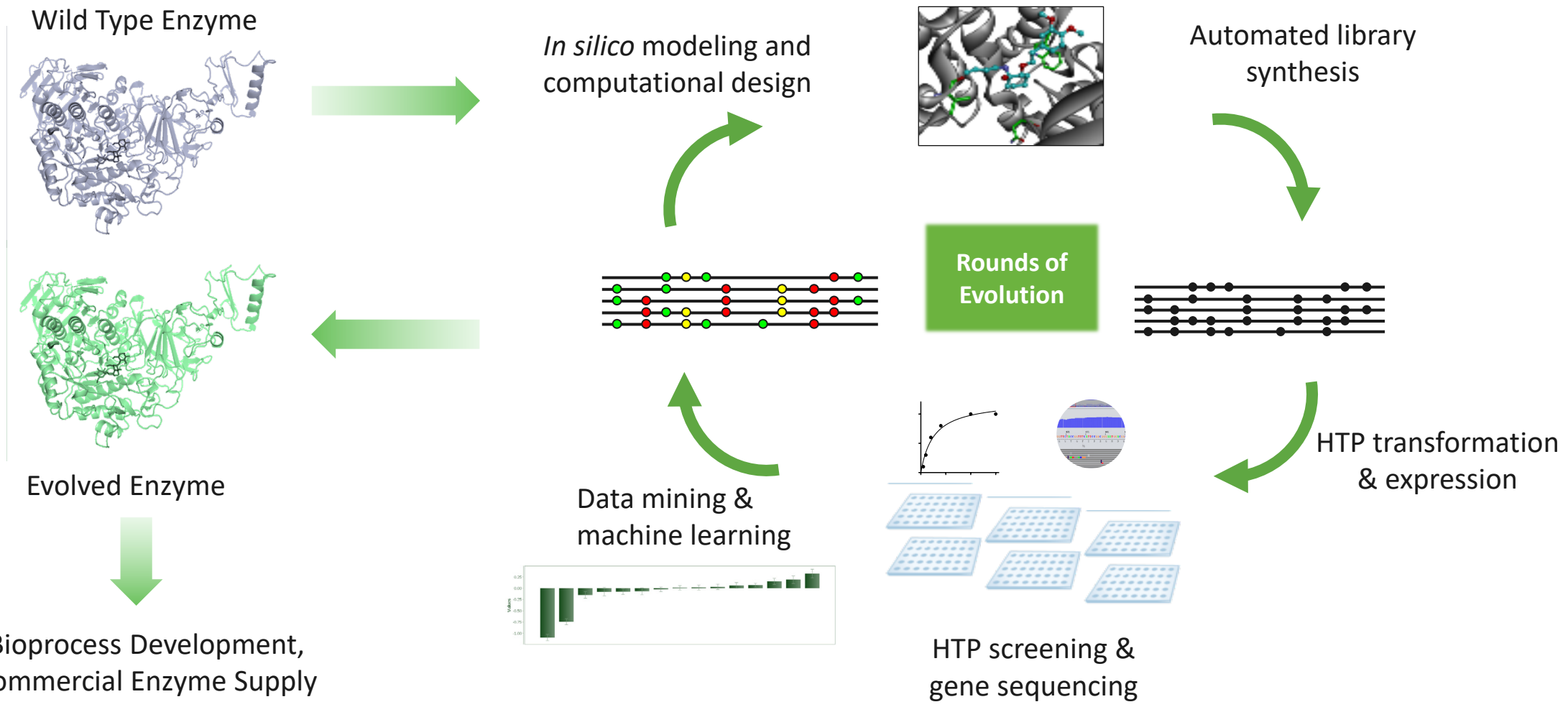
Increased capping efficiency →

- Reduce cost of the process

Reduced dsRNA generation →

- Increase mRNA safety and efficacy
- Reduce cost of the process

CodeEvolver® technology rapidly generates enzymes with desired properties through iterative rounds of enzyme optimization

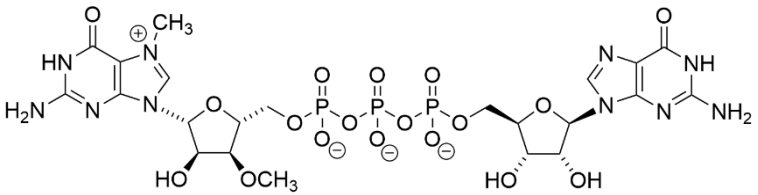


CODEXIS®

Co-transcriptional capping: ARCA cap analog

ARCA

Dinucleotide



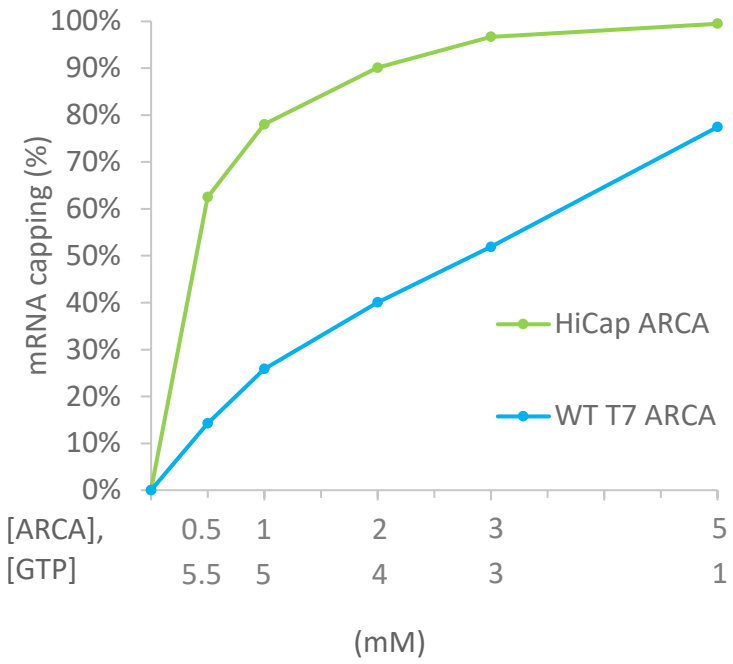
- Inefficiently incorporated by WT T7RNAP

CODEXIS®

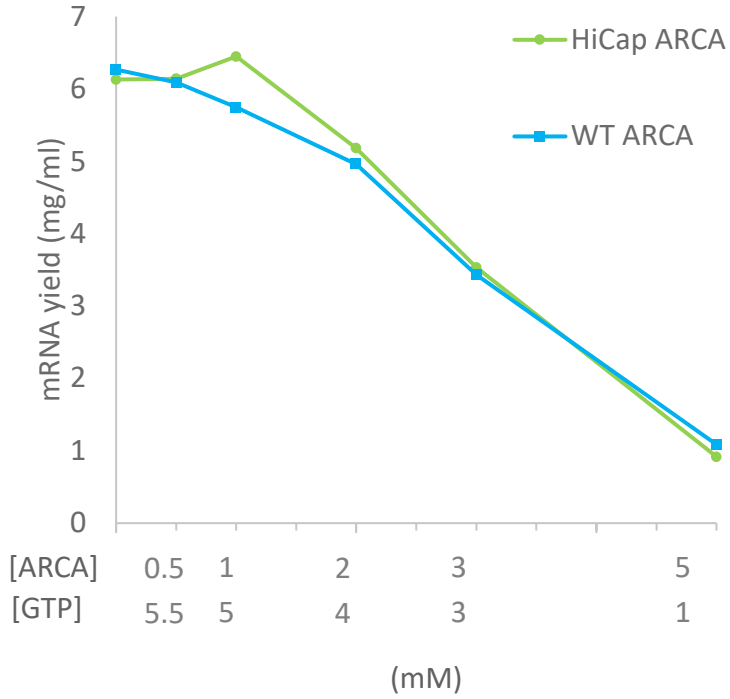
Codex® HiCap RNA Polymerase

Increased dinucleotide analog capping efficiency (ARCA)

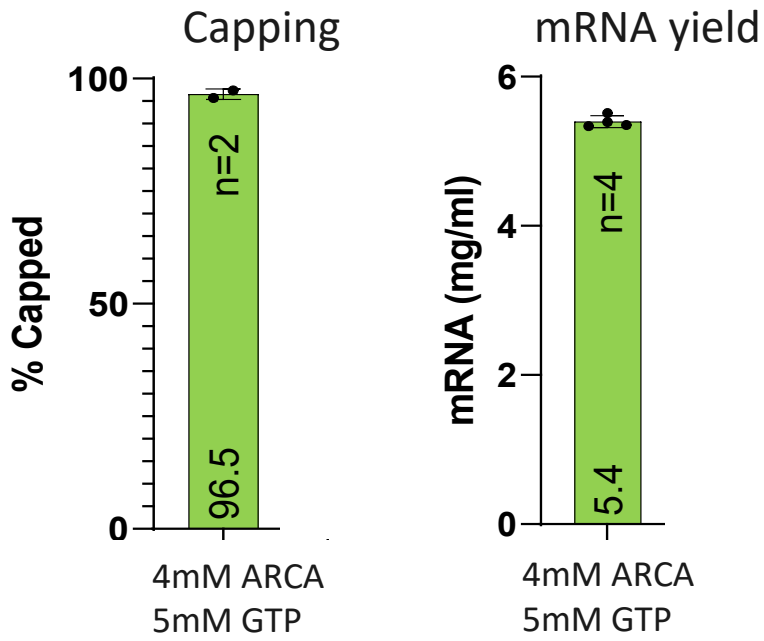
Capping efficiency



mRNA yield



Optimized condition:



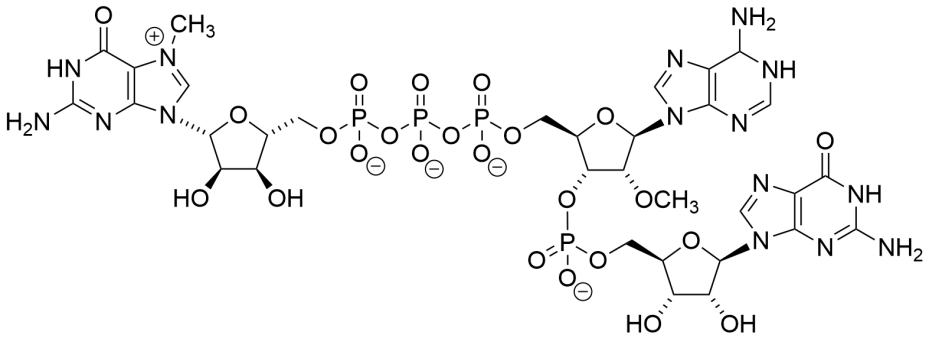
- Allows higher capping efficiency for a given concentration of ARCA, GTP
- Allows for >95% capping efficiency in batch IVTs without GTP starvation, preserving yield and lowering cost

CODEXIS®

Co-transcriptional capping: CleanCap® AG cap analog

CleanCap®
Reagent AG

Trinucleotide

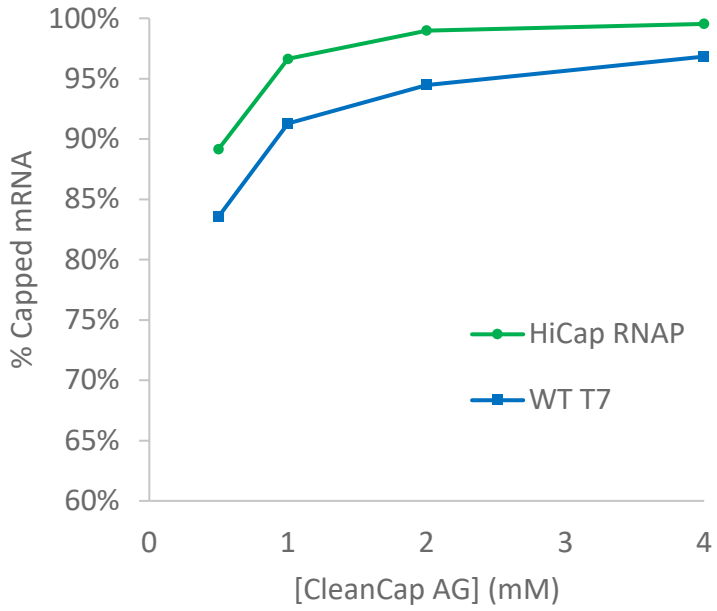


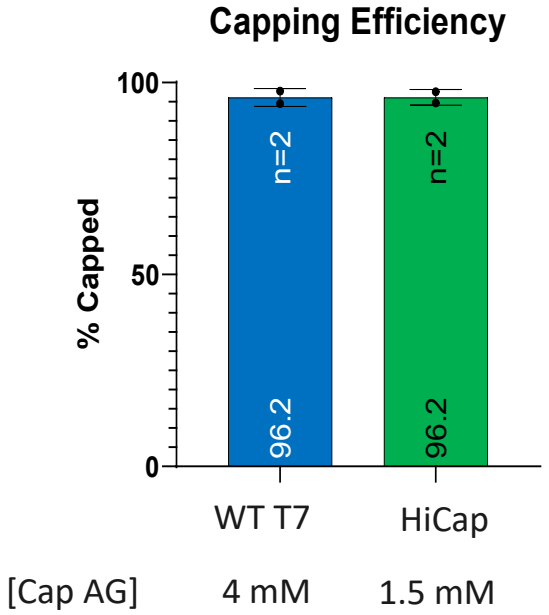
- Higher incorporation by WT T7RNAP
- Native Cap-1 structure
- More expensive to produce and use

Codex® HiCap RNA Polymerase

Increased trinucleotide (CleanCap® AG) capping efficiency

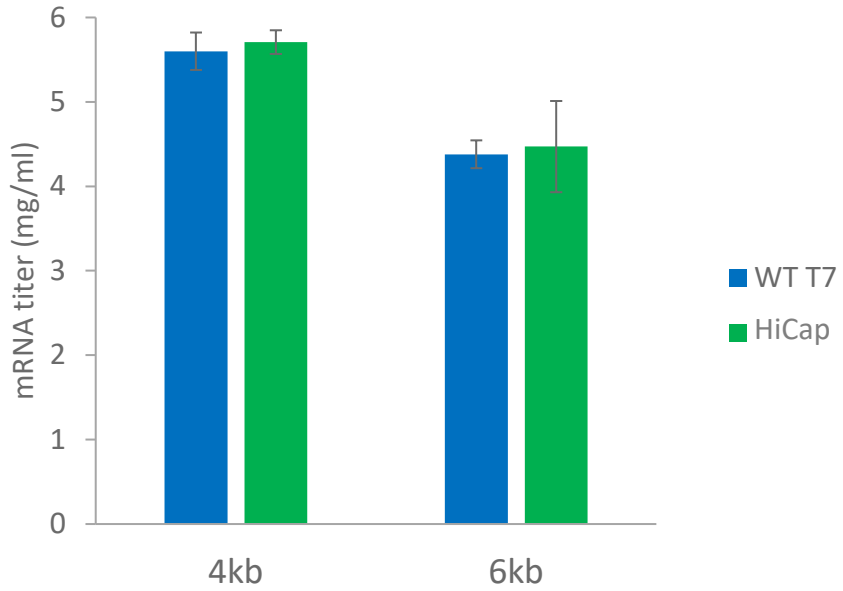
Capping efficiency





mRNA yield

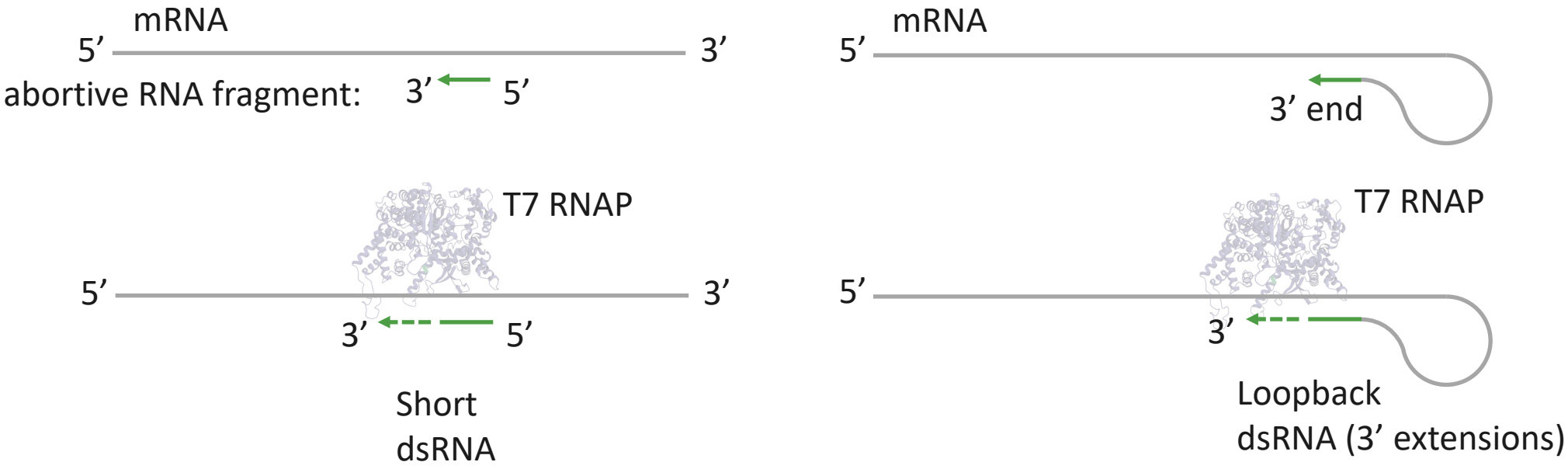
1.5mM CleanCap AG, 5mM NTPs (N1Mψ)



- Codex® HiCap RNA Polymerase selectively incorporates CleanCap® AG
- Allows for higher capping efficiency *or* equivalent efficiency with lower cap concentrations (1.5mM), reducing cost

CODEXIS®

Double-stranded RNA side products



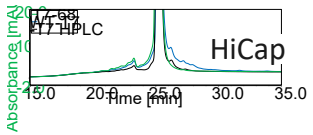
- dsRNA is generated by WT T7 via an RNA-dependent RNA polymerase activity
- Pattern recognition receptors (TLR3, RIG-I) recognize dsRNA and induce interferon signaling, causing inflammation
- dsRNA signaling inhibits protein translation
- dsRNA contamination impacts safety and efficacy of mRNA therapeutics
- **Polishing step required to remove dsRNA – adds complexity and cost**

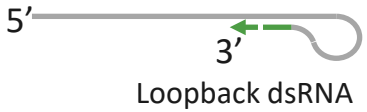
CODEXIS®

Codex® HiCap RNA Polymerase

Reduced dsRNA formation

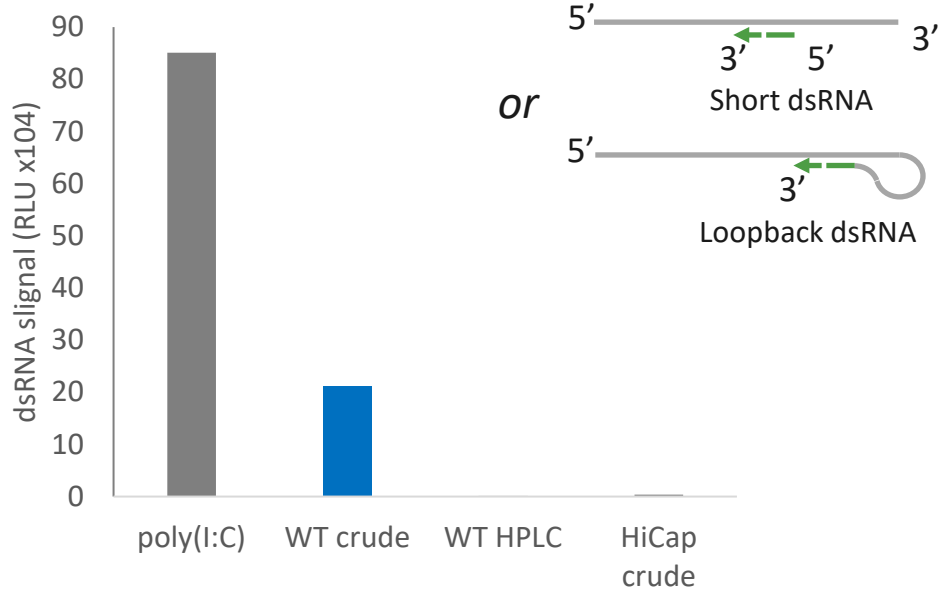
Analytical reverse-phase HPLC:





3’ extensions

dsRNA-specific Sandwich ELISA:

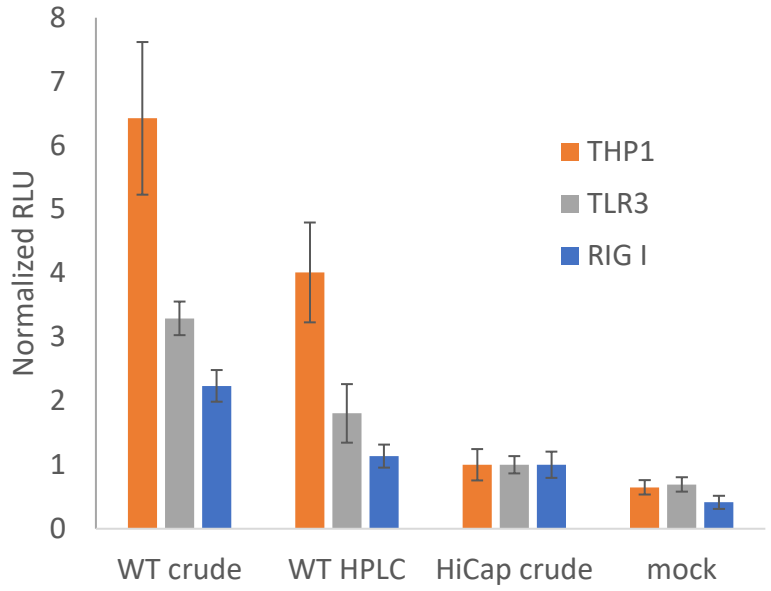


- 3’ extensions for the HiCap RNA Polymerase sample are significantly reduced vs the WT sample
- Residual dsRNA from HiCap RNA Polymerase without purification is similar to that from purified WT polymerase sample
- dsRNA sandwich ELISA shows ~50-fold reduction in total dsRNA side products
- **Lower dsRNA eliminates the need for polishing – simplified workflow and lower cost**

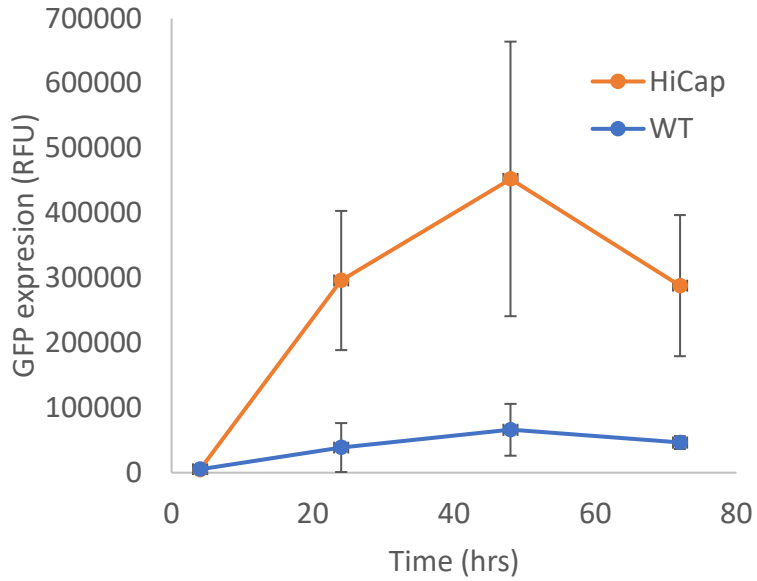
CODEXIS®

Reduced dsRNA formation correlates with reduced immune stimulation and increased protein translation

Reduced immune stimulation



GFP reporter translation post-transfection



- Codex® HiCap RNA Polymerase shows significant reduction in IFN signaling compared to WT T7
- Codex® HiCap RNA Polymerase shows significant increase in protein translation in HeLa cells
- Lower dsRNA improves safety and efficacy, using a simplified workflow

CODEXIS®

Codex® HiCap RNA Polymerase attributes

	Codex® HiCap RNA Polymerase	Wild-type T7 polymerase
Reduced dsRNA production	●●●	●
Co-transcriptional capping efficiency	●●●	●●
IVT yields	●●●	●●●
Reduced capping reagent	●●●	●
Manufacturing simplification	●●●	●
Finished product yield	●●●	●

Information previously presented by Tyler Goodwin, Ph.D., Head of Non-viral Delivery Development and Vector Core, Tune Therapeutics

Higher quality mRNA and improved manufacturing cost & efficiency

CODEXIS®

Engineered Enzymes to Overcome Scalability and Sustainability Challenges of Nucleic Acid Therapeutics Manufacturing

Mathew Miller, PhD Associate Director, Life Sciences Technology and Applications

Stephanie Forget, PhD Senior Scientist I

CODEXIS®

RNAi: High Demand / Constrained Supply

Demand Drivers

RNA delivering on promise of personalized medicines
(10+ FDA approved therapies have reached market in past 5yrs)

RNAi therapeutics as a modality is growing rapidly with >450 assets in pipelines

RNAi therapies are treating the previously untreatable diseases

Production & Supply Challenges

Chemical RNA synthesis produces **Millions** of liters of chemical waste… and growing!

Phosphoramidite synthesis will be challenged to meet demand of 1,000s kg of RNAi p.a. by 2030

Critical solvent supply constraints likely (Acetonitrile)

CODEXIS®

Enzymes are poised to spur innovation and disrupt RNAi manufacturing

Scalability

Enzyme catalytic activity enables multiple cycle use

Purity & Yield

Evolution targets >99% incorporation efficiency

Sustainability

Aqueous waste streams & lower solvent use

CODEXIS®

About the Speaker

Stephanie Forget, PhD

Senior Scientist I, Codexis

Technical lead for enzymatic RNA synthesis program. Obtained PhD in Chemistry at Dalhousie University, Canada. Studied biosynthetic enzymes involved in rare sugars found in natural products. Postdoctoral fellow at University of British Colombia, Canada. Studied oxidative enzymes through reaction profiling.

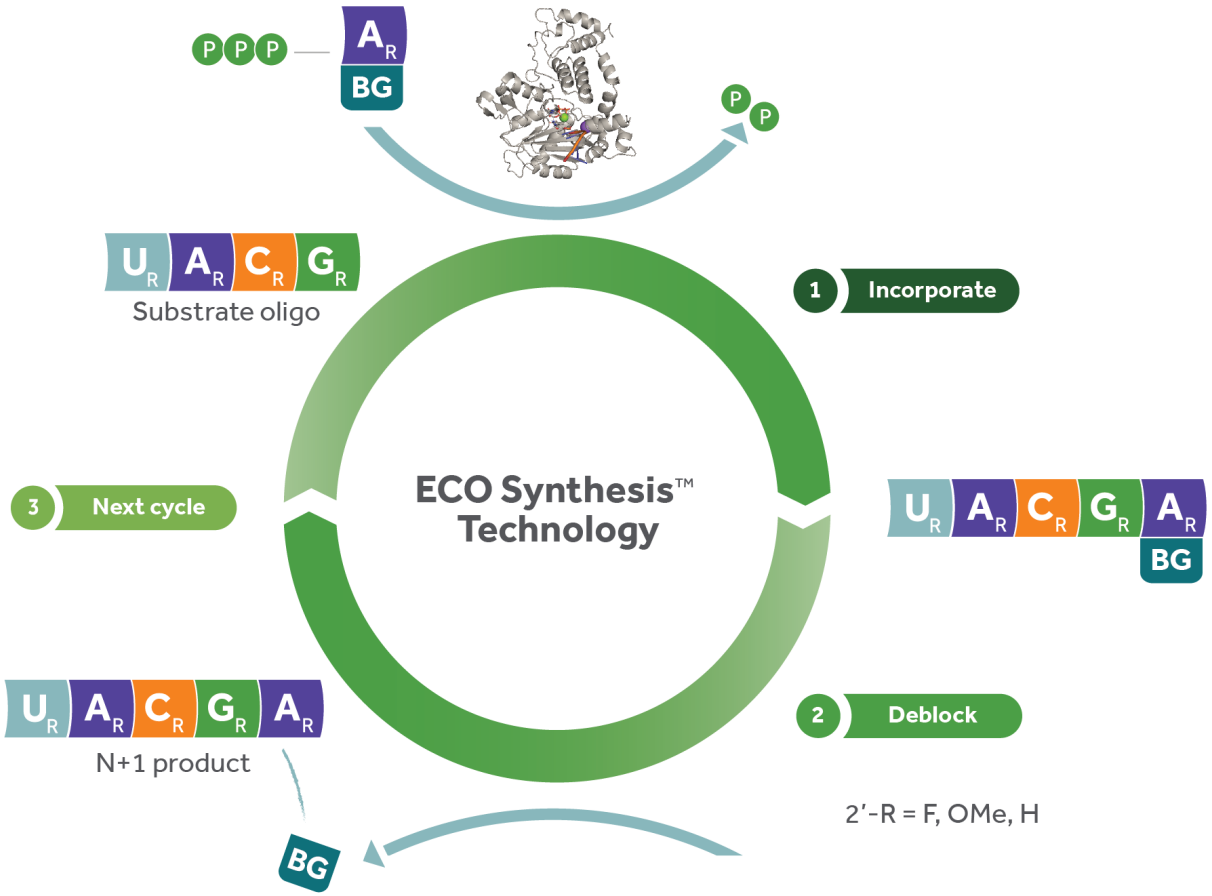
CODEXIS®

ECO Synthesis™ Technology

(**E**nzyme-**C**atalyzed **O**ligonucleotide Synthesis)

Codexis ECO Synthesis™ Technology

Enzyme-Catalyzed Oligonucleotide Synthesis for RNAi therapeutics



ECO Synthesis™ Technology

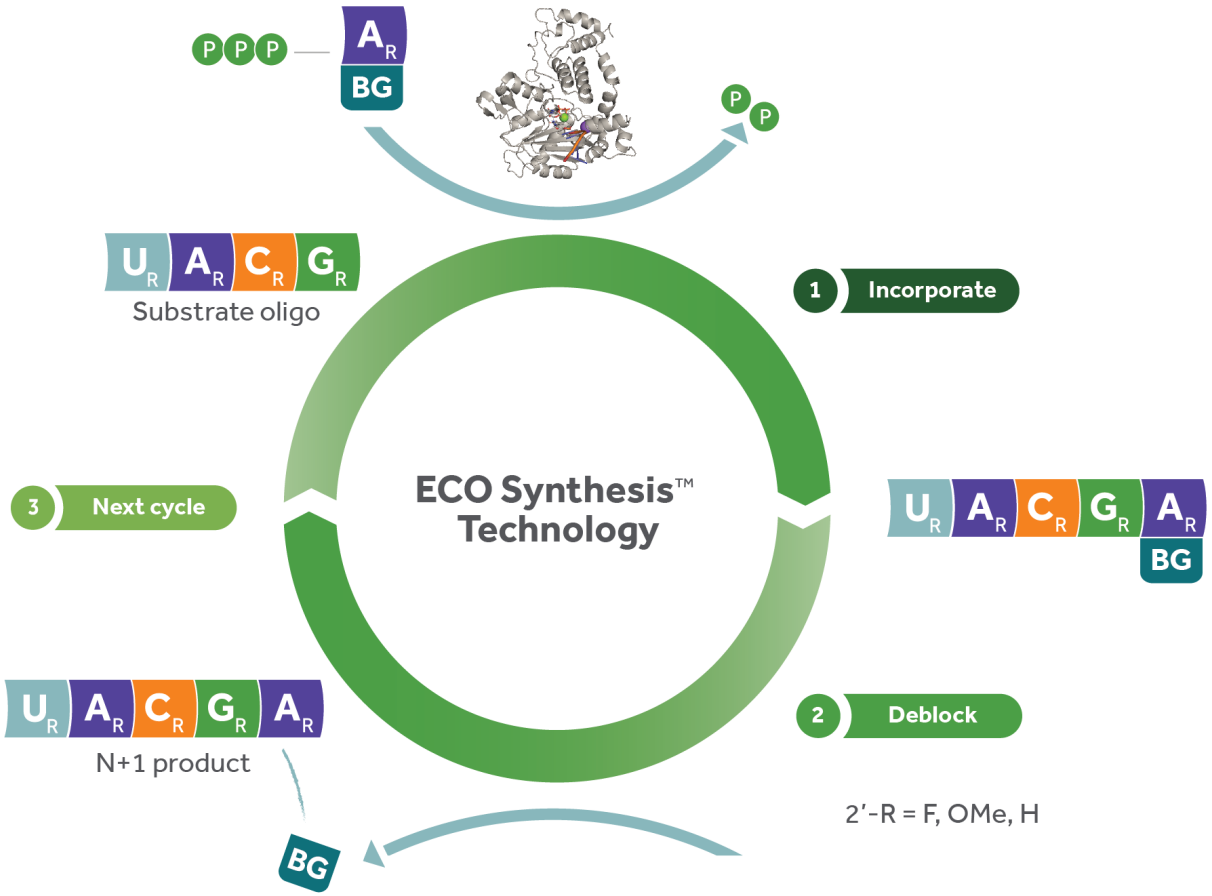
- Controlled addition of modified RNA bases (TdT)
- Deblocking of 3'blocking group (phosphatase)
- Supply of 3'blocked NTP substrates (multiple enzymes)

Final process in development

CODEXIS®

Codexis ECO Synthesis™ Technology

Enzyme-Catalyzed Oligonucleotide Synthesis for RNAi therapeutics



Enzyme Performance

- High incorporation efficiency (>99%)
- No sequence bias

At-Scale Process Requirements

- Controlled addition of monomers
- Low impurity production
- High volumetric productivity

Scalable & Economical Enzyme Manufacturing

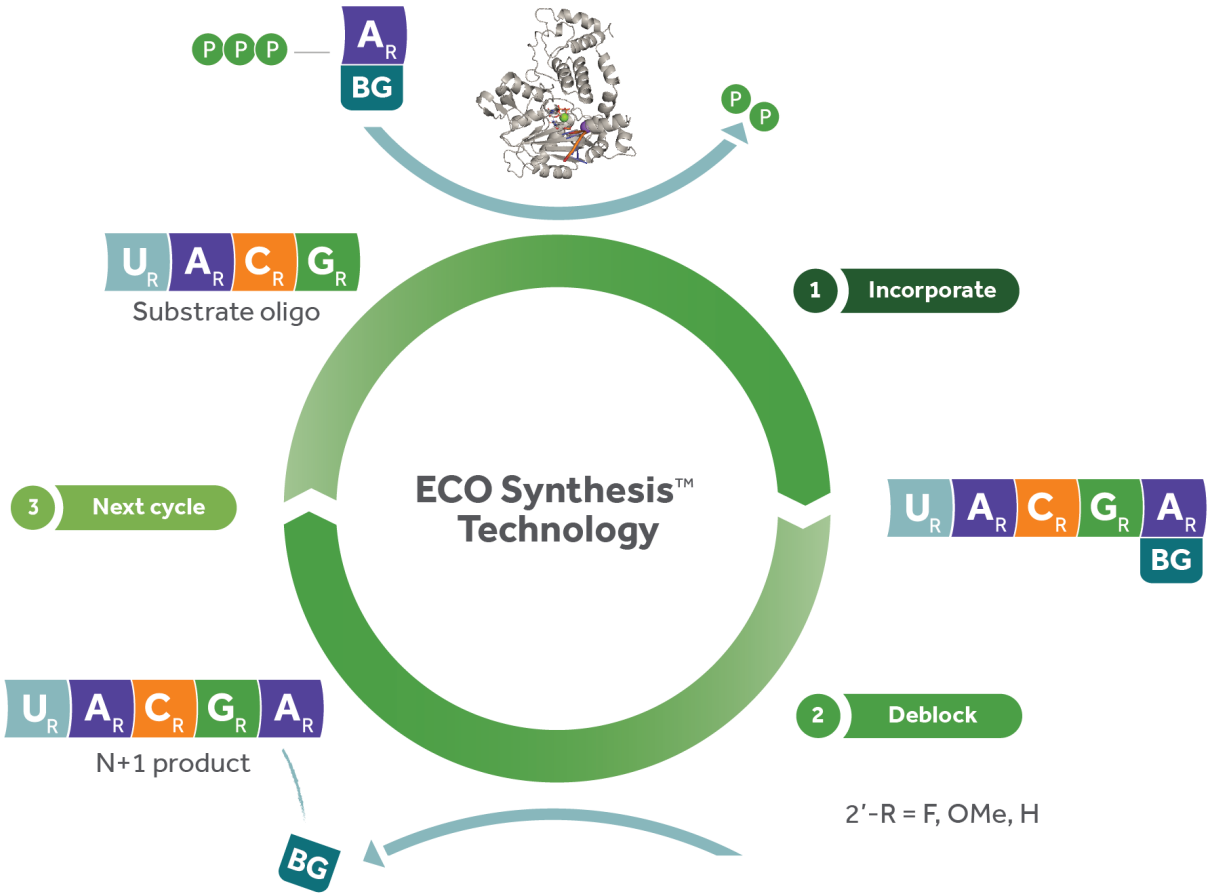
- High manufacturing yields
- Scalable supply of nucleotide triphosphates

Final process in development

CODEXIS®

Codexis ECO Synthesis™ Technology

Enzyme-Catalyzed Oligonucleotide Synthesis for RNAi therapeutics



Enzyme Performance

- High incorporation efficiency
- No sequence bias

At-Scale Process Requirements

- Controlled addition of monomers
- Low impurity production
- High volumetric productivity

Scalable & Economical Enzyme Manufacturing

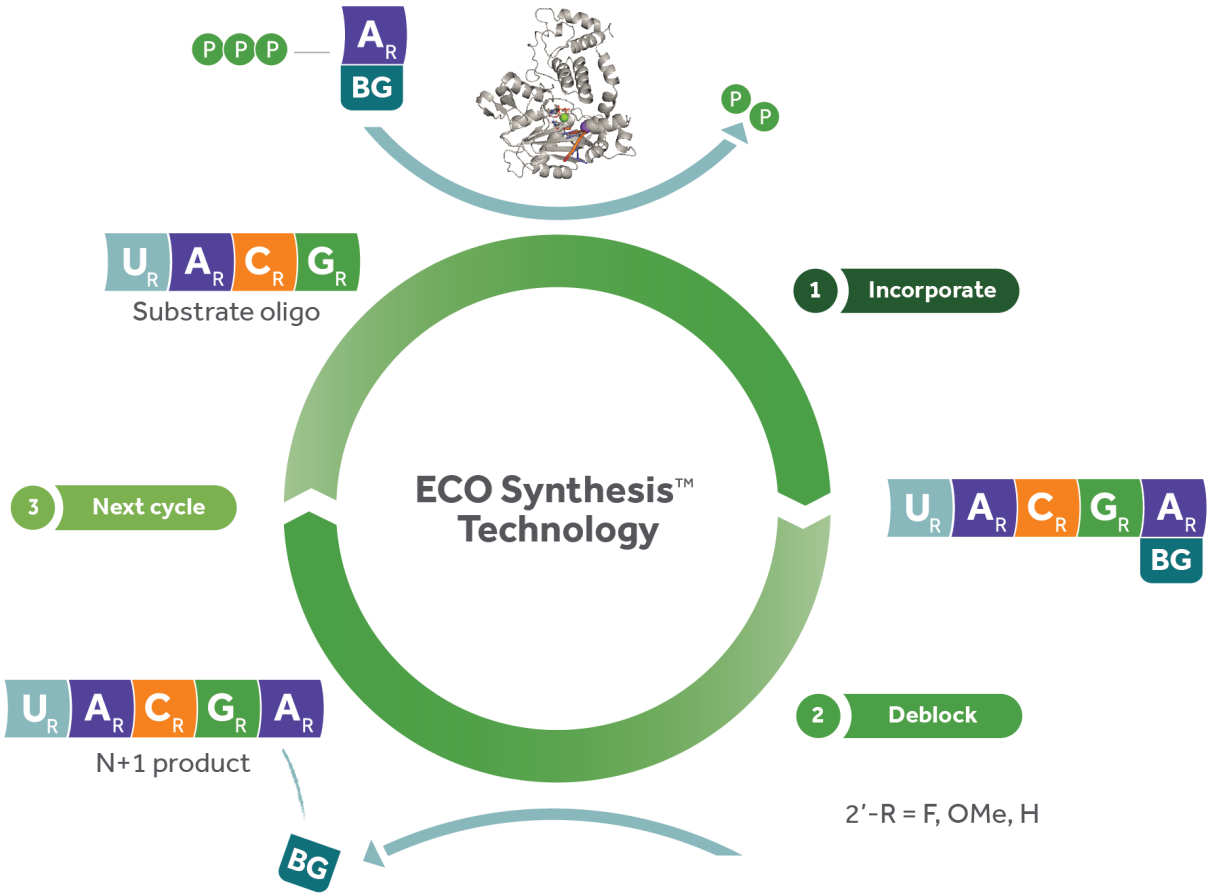
- High manufacturing yields
- Scalable supply of nucleotide triphosphates

Final process in development

CODEXIS®

Codexis ECO Synthesis™ Technology

Enzyme-Catalyzed Oligonucleotide Synthesis for RNAi therapeutics



Enzyme Performance

- High incorporation efficiency
- No sequence bias

At-Scale Process Requirements

- Controlled addition of monomers
- Low impurity production
- High volumetric productivity

Scalable & Economical Enzyme Manufacturing

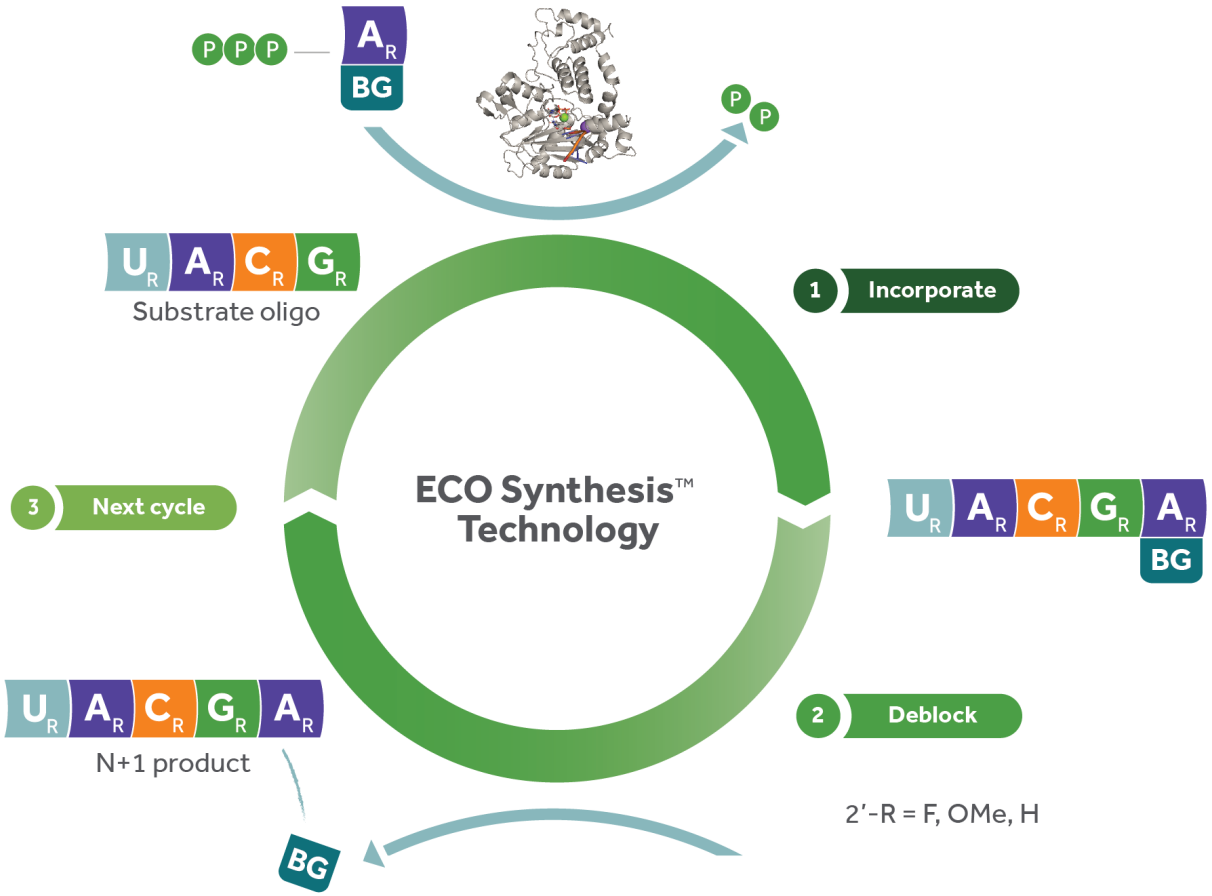
- High manufacturing yields
- Scalable supply of nucleotide triphosphates

Final process in development

CODEXIS®

Codexis ECO Synthesis™ Technology

Enzyme-Catalyzed Oligonucleotide Synthesis for RNAi therapeutics



Enzyme Performance

- High incorporation efficiency
- No sequence bias

At-Scale Process Requirements

- Controlled addition of monomers
- Low impurity production
- High volumetric productivity

Scalable & Economical Enzyme Manufacturing

- High manufacturing yields
- Scalable supply of nucleotide triphosphates

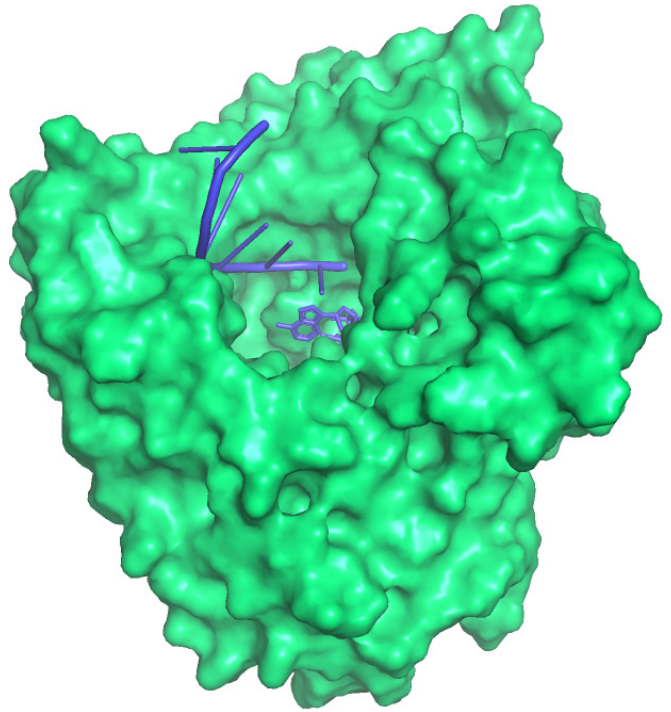
Final process in development

CODEXIS®

Enzyme Performance: A highly engineered TdT

Key Enzyme Challenges

- Does not naturally recognize modified RNA
- Poor soluble expression and stability
- Enzymes function close to physiological conditions
- Manufacturability for large scale enzyme needs



CODEXIS®

Enzyme Performance: A highly engineered TdT

% Incorporation efficiency of N+1 additions over multiple rounds of evolution

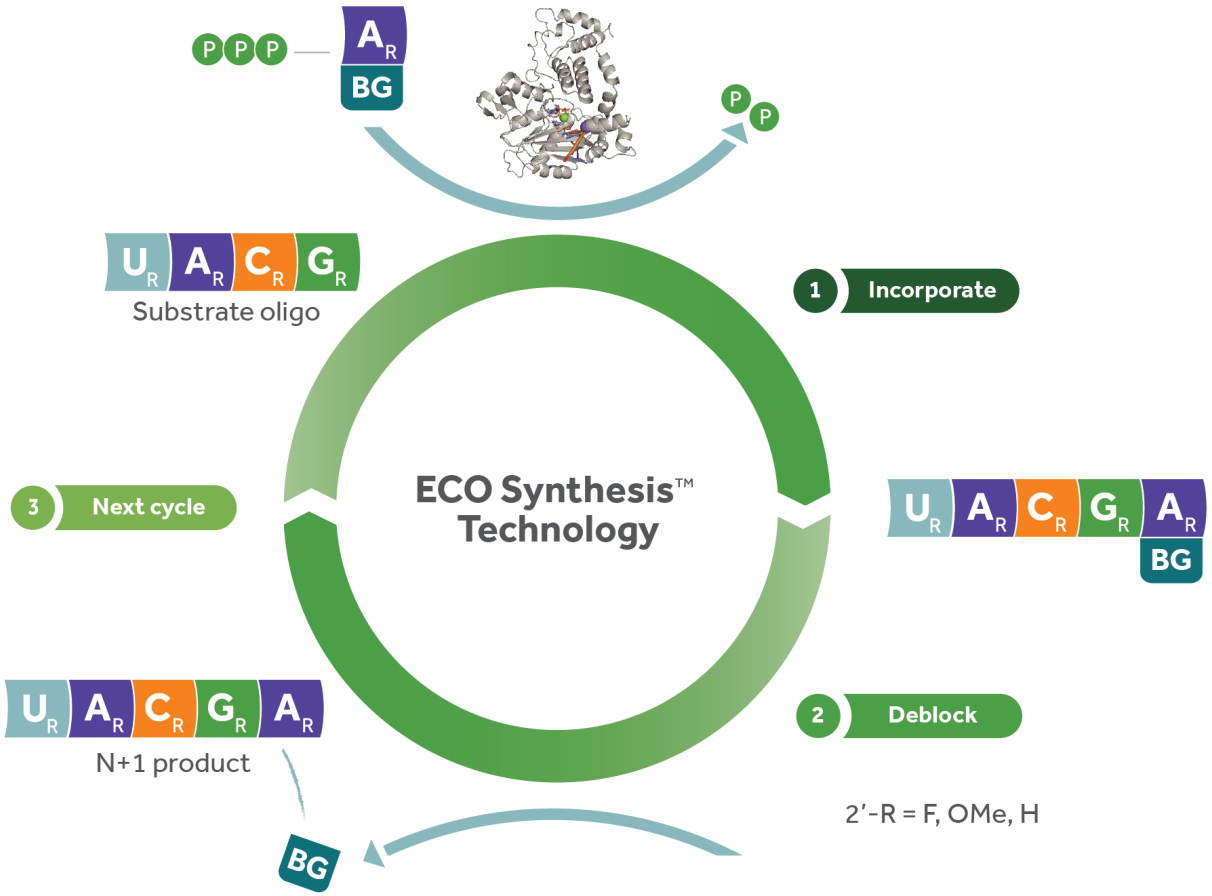
3'-Terminus Sequence	NTP	Starting TdT	Iterative Rounds of Evolution						
mAmCmU	fATP-3P	0	0	0	0	1	54	91	94
	fUTP-3P	0	0	0	0	1	56	93	94
	mCTP-3P	0	0	0	0	1	54	91	89
	mGTP-3P	0	0	0	0	1	24	87	78
	mATP-3P	0	0	0	0	1	39	88	66
	*mGTP-3P	0	0	0	0	0	0	2	55
	*mUTP-3P	0	0	0	0	0	0	19	24
mGmAmC	fUTP-3P	0	0	0	0	2	33	74	92
	mATP-3P	0	0	0	0	1	16	60	90
	fATP-3P	0	0	0	1	2	34	77	88
	mCTP-3P	0	0	0	0	0	13	66	86
	*mUTP-3P	0	0	0	0	0	0	11	79
	*mGTP-3P	0	0	0	0	0	0	3	64
	mGTP-3P	0	0	0	0	0	0	2	55
AT*mG	mATP-3P	4	1	4	49	75	82	47	56
AmU*mG		0	0	0	12	46	77	75	41
mAmU*mG		0	0	0	0	5	69	82	68
mAmUfG		0	0	0	0	0	2	1	66
mUmGmA	mATP-3P	0	0	0	1	2	38	82	86
mAfUCmC		0	0	0	0	4	58	88	86
mAmG(MOE)C		0	0	0	0	0	4	14	84
mC*mG*mA		0	0	0	0	2	39	75	75
mCmUmG		0	0	0	0	4	82	86	72
mAmUmC		0	0	0	0	2	57	84	63
mAmUfU		0	0	0	0	0	0	0	59
*mA*mA*mC		0	0	0	0	1	30	54	57
mAmUfC		0	0	0	0	0	1	1	51
mCmGmA	fATP-3P	0	0	0	1	4	82	93	92
*fAfGmA		4	2	4	19	50	79	85	85
mC*mG*mA		0	0	0	1	3	47	81	82
*fA*fAfG		0	0	0	0	0	44	56	70
fCfGfA		0	0	0	3	26	50	55	65
mU*fA*fA		0	0	0	0	3	11	17	34
fGmAfU		6	5	6	3	12	42	52	30
fC*fG*fA		0	0	0	0	4	14	30	14

So far…

- ✓ Incorporation of relevant 2’-modifications with 3’ phosphate blocking group
  - ✓ 2’-deoxyfluoro and 2’-methoxy
- ✓ Incorporation of α-PS bonds
- ✓ Recognition of modified initiator sequences
- ✓ Improving incorporation efficiency of each nucleotide (goal >99%)

Note: All incorporating nucleotides contain 3’-blocking group; “*” denotes alpha PS-bond; “m, r, f” denotes 2’-OMe, 2’-OH, or 2’-F modifications, respectively

ECO Synthesis™ Technology: Controlled addition of monomers



Target Sequence:

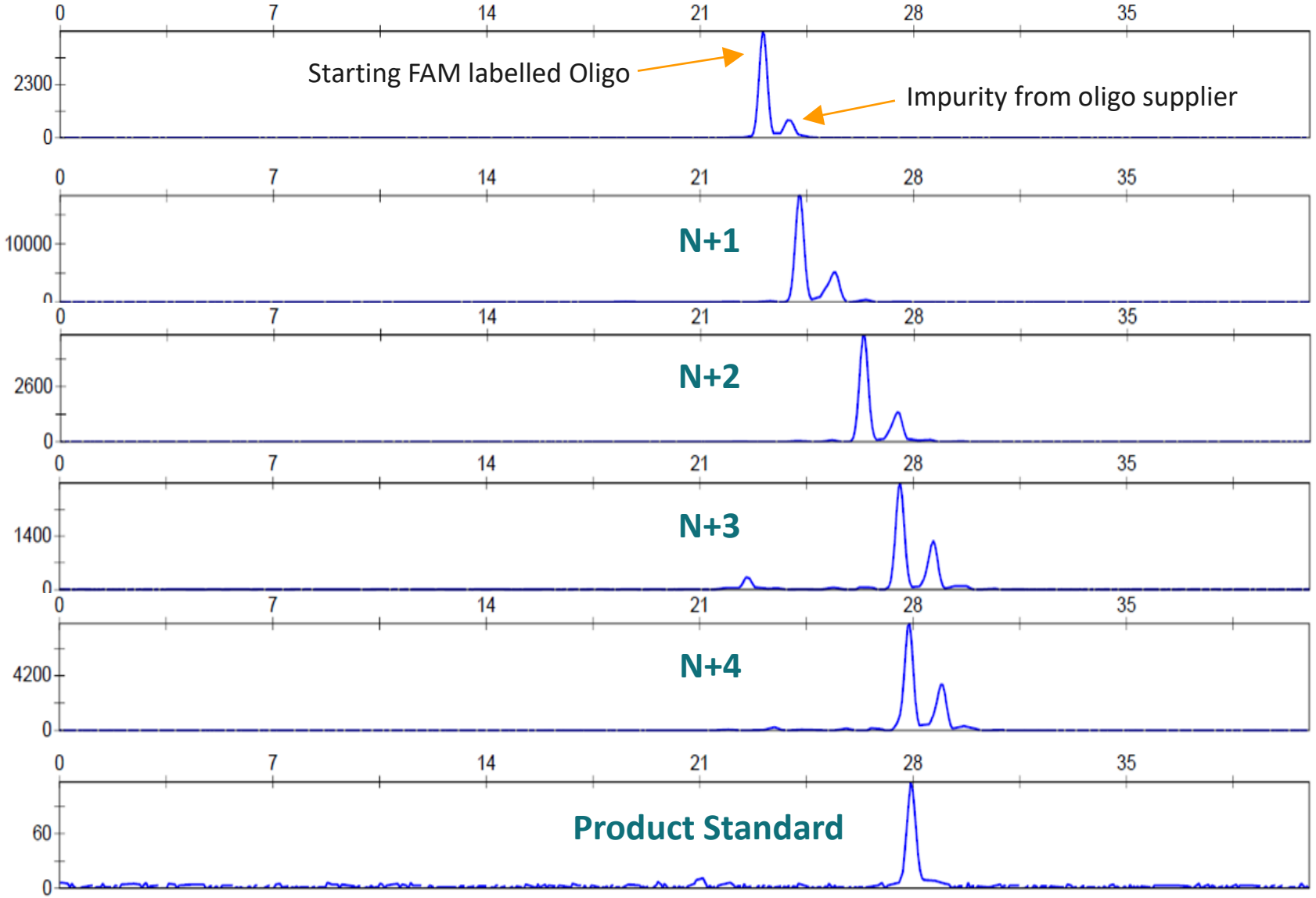
5' – Biotin/FAM...mA mU mA mA mG mA fA – 3'

- ✓ 4 Cycles for proof of concept
- ✓ Feasibility demonstrated for modified RNA synthesis

CODEXIS®

ECO Synthesis™ Technology: Controlled addition of monomers

Addition and deblocking with 5'-biotynilated-FAM…mAmUmA



5' …mA mU mA

5' …mA mU mA mA

5' …mA mU mA mA mG

5' …mA mU mA mA mG mA

5' …mA mU mA mA mG mA fA

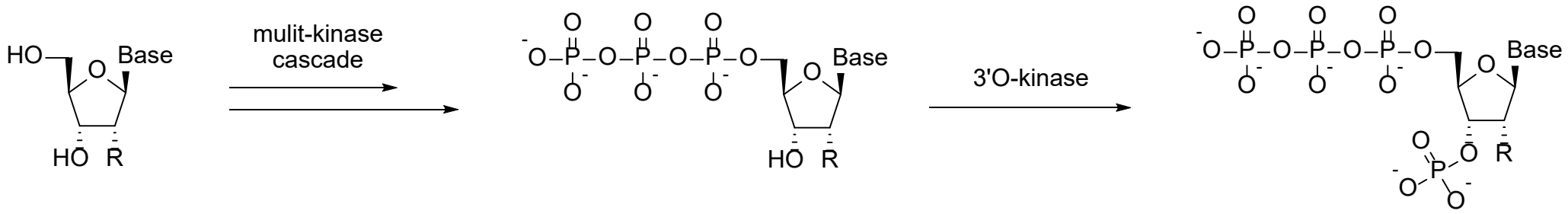
5' …mA mU mA mA mG mA fA

Not final process conditions

Coming soon…enzymatically-activated monomer supply

Demonstrated…

✓ Nucleoside → NTP conversion → 3’Blocked-NTP



“Two-step-one-pot” synthesis of 3’-blocked nucleotides

Provides scalable, sustainable, economic supply of required ECO Synthesis™ monomers

CODEXIS®

ECO Synthesis™ Technology: A vision for sustainable RNA synthesis

Accomplished to date

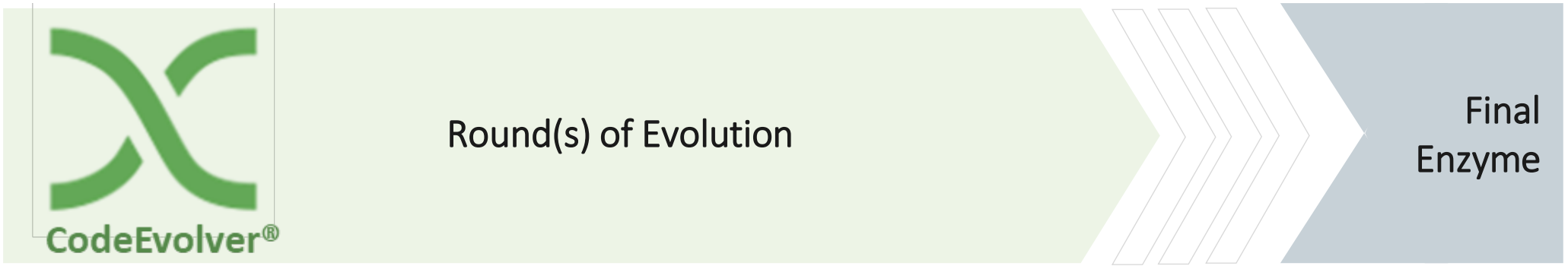
- ✓ Progress on critical TdT performance
- ✓ Proof of Concept for iterative nucleotide addition
- ✓ Concept for enzymatically-derived source of 3’-blocked nucleotides

Next Steps

- ❑ Increase % monomer incorporation to reduce impurities
- ❑ Sustainable & scalable supply of nucleotides
- ❑ Scale-up to process-relevant conditions
- ❑ Achieve gram-scale synthesis of modified RNA

CODEXIS®

Reimagine enzymes for mRNA manufacturing and RNAi synthesis



Codex® HiCap RNA Polymerase

- ✓ Low dsRNA impurity production
- ✓ High capping efficiency
- ✓ Improved manufacturing economics & efficiency

ECO Synthesis™ Technology

- ✓ New paradigm for synthesis for RNAi therapeutics
- ✓ Sustainable, scalable, economical
- ✓ Proof of concept demonstrated – More to come!

CODEXIS®