an Applied DNA Sciences company

LineaRx™

DNA made simple

The Future of RNA Therapies Produced with LinearDNA™ IVT Templates

Forward Looking Statement

The statements made by Applied DNA in this presentation may be "forward-looking" in nature within the meaning of Section 27A of the Securities Act of 1933, Section 21E of the Securities Exchange Act of 1934 and the Private Securities Litigation Reform Act of 1995. Forward-looking statements describe Applied DNA and its affiliates' future plans, projections, strategies and expectations, and are based on assumptions and involve a number of risks and uncertainties, many of which are beyond the control of Applied DNA. Actual results could differ materially from those projected due to its history of net losses, limited financial resources, limited market acceptance, the uncertainties inherent in research and development, future clinical data and analysis, including whether any of Applied DNA's or its partner's therapeutic candidates or programs will advance further in the preclinical research or clinical trial process, including receiving clearance from the U.S. Food and Drug Administration (FDA), United State Department of Agriculture (USDA) or equivalent foreign regulatory agencies to conduct clinical trials and whether and when, if at all, they will receive final or conditional approval from the FDA, USDA or equivalent foreign regulatory agencies, the unknown outcome of any applications or requests to FDA, USDA or equivalent foreign regulatory agencies, whether results from preclinical studies will be predictive of the results of later preclinical studies and clinical trials, the unknown ability to manufacture therapeutic grade DNA via PCR in large quantities, the fact that there has never been a commercial drug product utilizing PCR-produced DNA technology approved for therapeutic use, and various other factors detailed from time to time in Applied DNA's SEC reports and filings, including our Annual Report on Form 10-K filed on December 9, 2021, its Quarterly Report on Form 10-Q filed on February 10, 2022 and May 12, 2022, and other reports it files with the SEC, which are available at www.sec.gov. Applied DNA undertakes no obligation to update publicly any forward-looking statements to reflect new information, events, or circumstances after the date hereof or to reflect the occurrence of unanticipated events, unless otherwise required by law.

Applied DNA Sciences

The PCR Company

DNA Manufacturing

Proprietary, highly efficient and scalable linDNA (linear DNA, the product of PCR) manufacturing process for use in nucleic acid-based therapies

LineaRx™

Clinical MDx Testing Services

Detection of DNA via PCR to alter clinical outcomes

ACL

Applied DNA Clinical Labs LLC

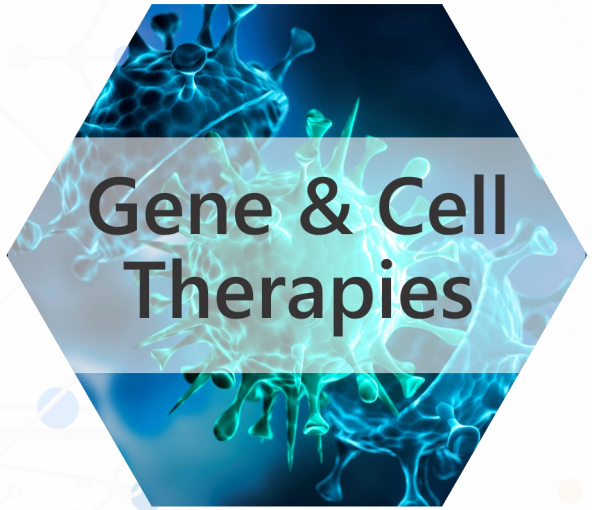
Supply Chain Integrity

Using the immutable power of DNA to help ensure the flow of ethical, sustainable, safe and authentic materials throughout the global marketplace

CertainT®

LineaRx™

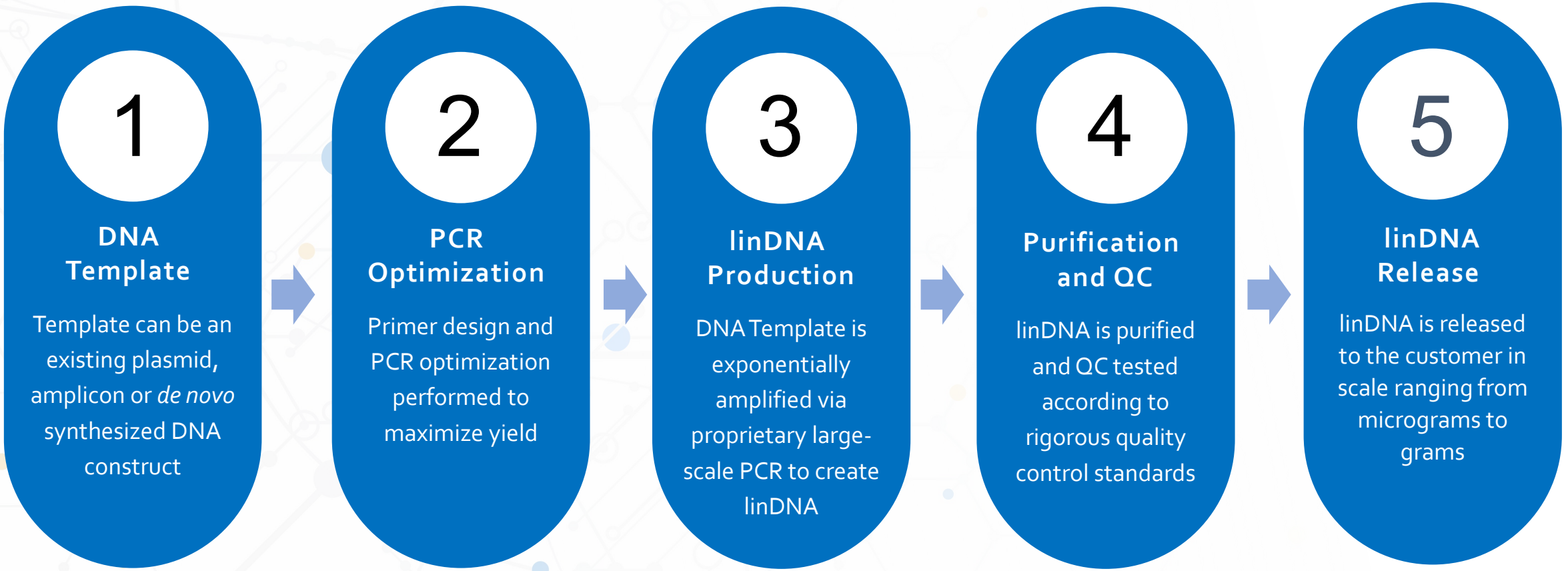
The Common Denominator of Next-Generation Therapies



LineaRx™

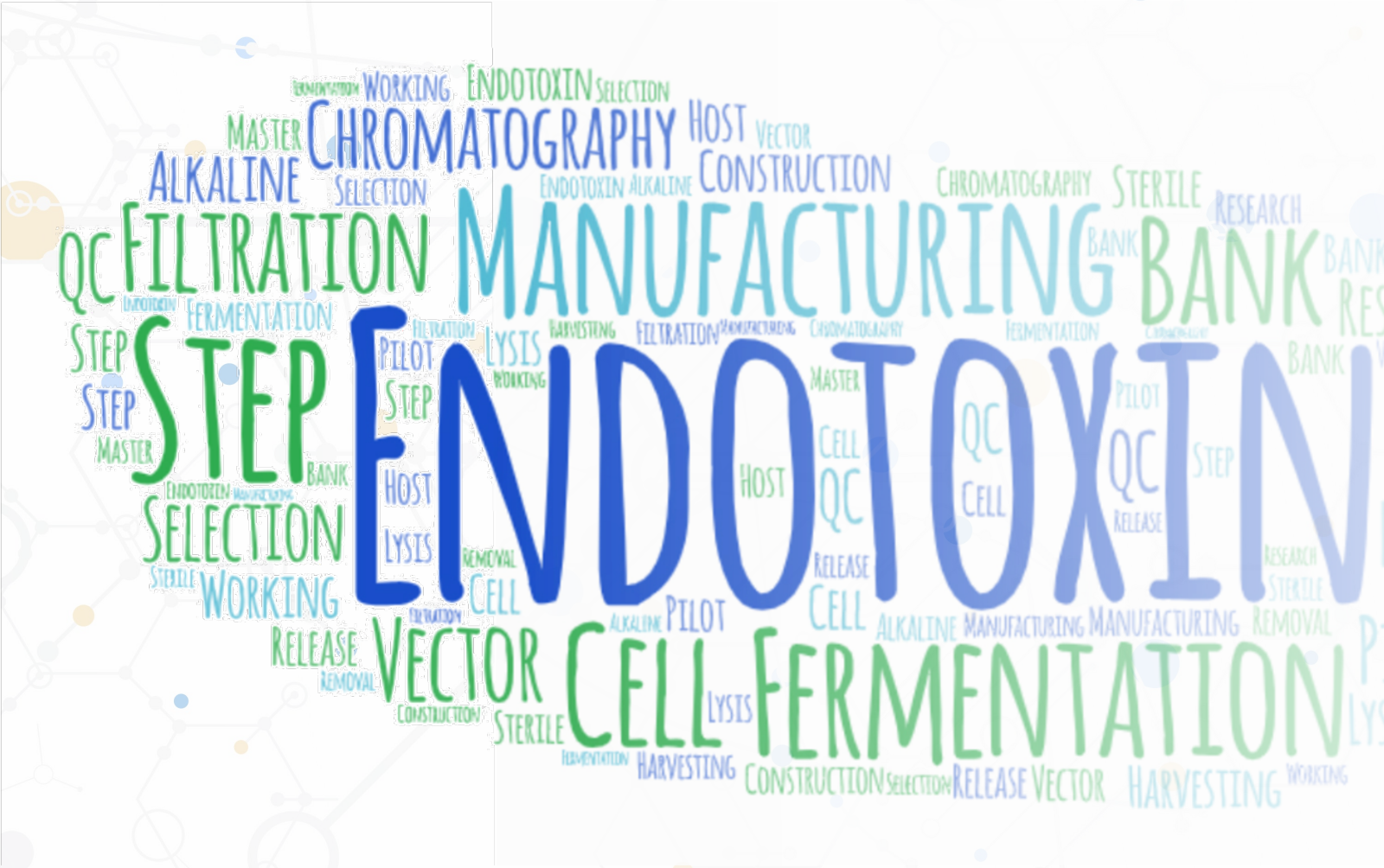
The LinearDNA™ (linDNA) Platform

DNA Made Simple



Process can be completed in under 2 weeks

Plasmid DNA – A Complex Workflow



- Based on 40-year-old technology
- Long lead times
- Poor batch-to-batch consistency and batch failures
- Regulatory scrutiny
- Adventitious DNA sequences in backbone, including antibiotic residence gene
- Complex sequences are unstable

linDNA

The Enzymatically-Produced Alternative to pDNA

Linear, blunt-ended dsDNA construct

- Perfectly suited for IVT templates
- Ready for IVT upon receipt – no need to digest or purify

Manufactured using high-fidelity DNA Polymerase

- Error rate in 10^{-7} – 10^{-6} range[1]
- Error rate for T7 RNA polymerase in the 10^{-4} range[2]

From 100bp to >20kb

- Ability to support small RNAs to large RNAs such as self-replicating RNA (srRNA)

[1] Potapov V, Ong JL. Examining Sources of Error in PCR by Single-Molecule Sequencing. PLoS One. 2017 Jan 6;12(1)

[2] Jianbin Huang, Luis G. Brieba, and Rui Sousa. Biochemistry 2000, 39, 38, 11571–11580

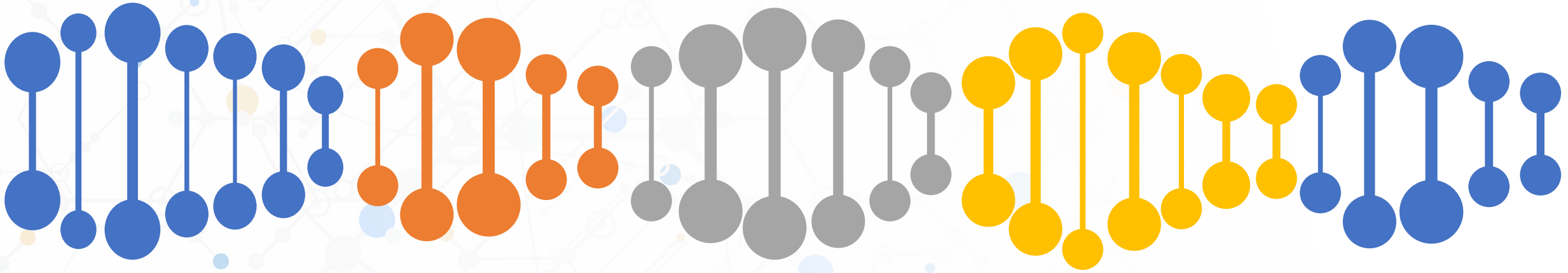
linDNA Advantages

Purity

Produce Only The
DNA You Want

Flexibility

Chemical Modifications
Various templates



Simplicity

Simplify
IVT Workflows

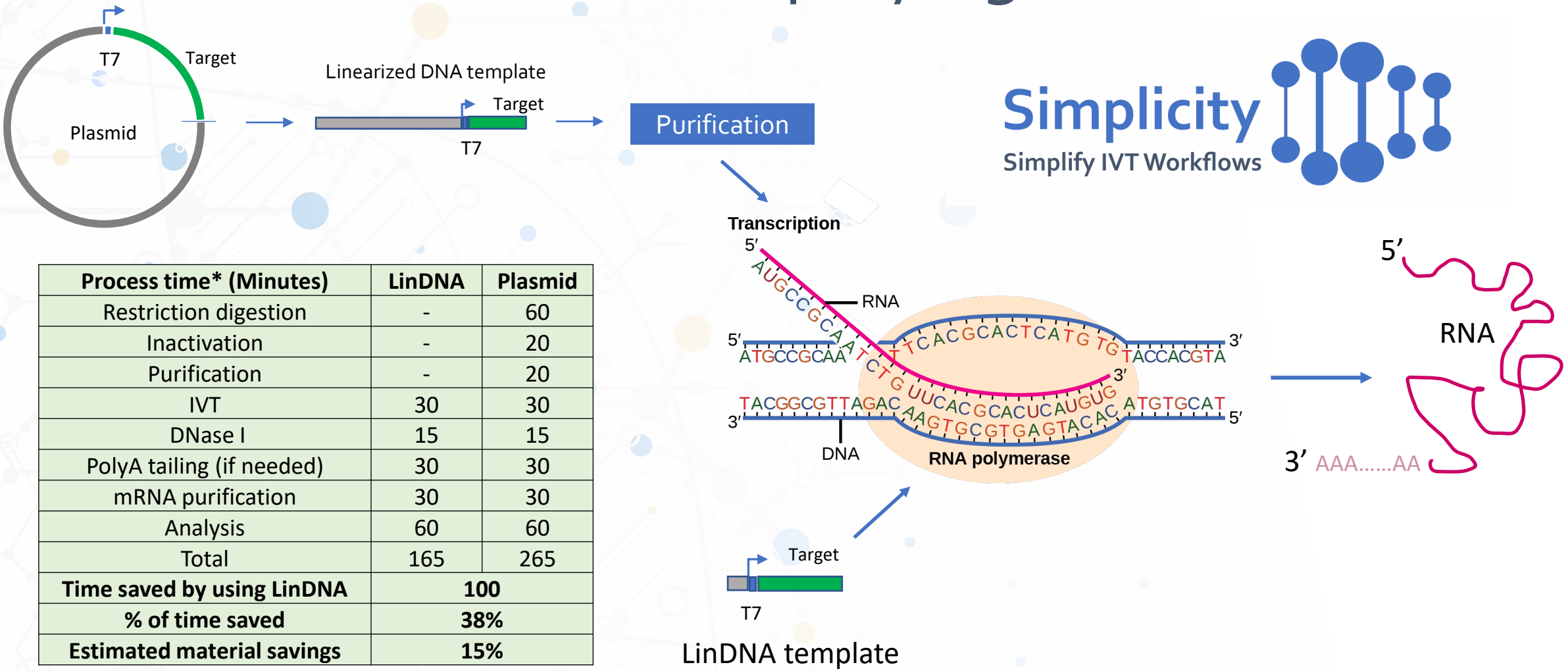
Speed

No More Waiting
For DNA Templates

Scalability

Micrograms -> Grams

linDNA – Simplifying IVT



Simplicity
Simplify IVT Workflows

Process time* (Minutes)	LinDNA	Plasmid
Restriction digestion	-	60
Inactivation	-	20
Purification	-	20
IVT	30	30
DNase I	15	15
PolyA tailing (if needed)	30	30
mRNA purification	30	30
Analysis	60	60
Total	165	265
Time saved by using LinDNA	**100**	
% of time saved	**38%**	
Estimated material savings	**15%**	

* Based on HiScribe™ T7 ARCA mRNA Kit (NEB)

linDNA – Simplifying IVT

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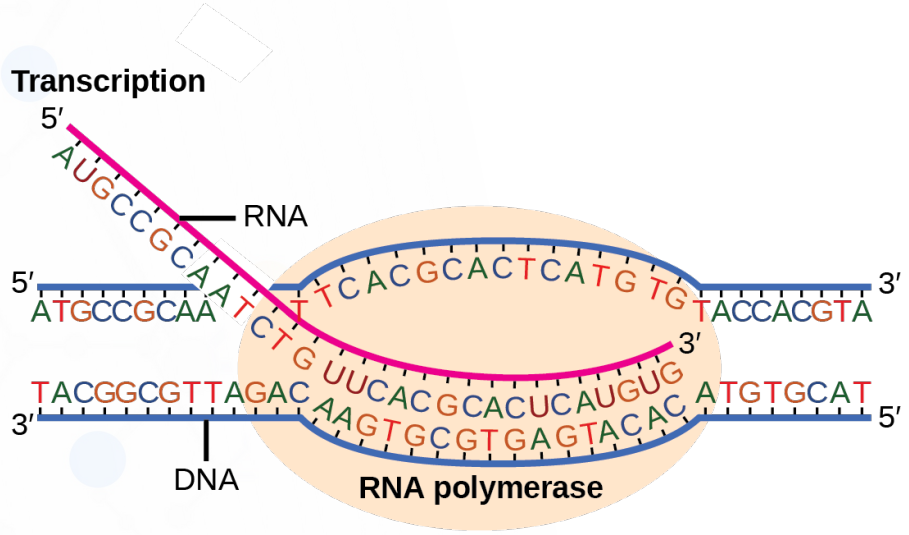
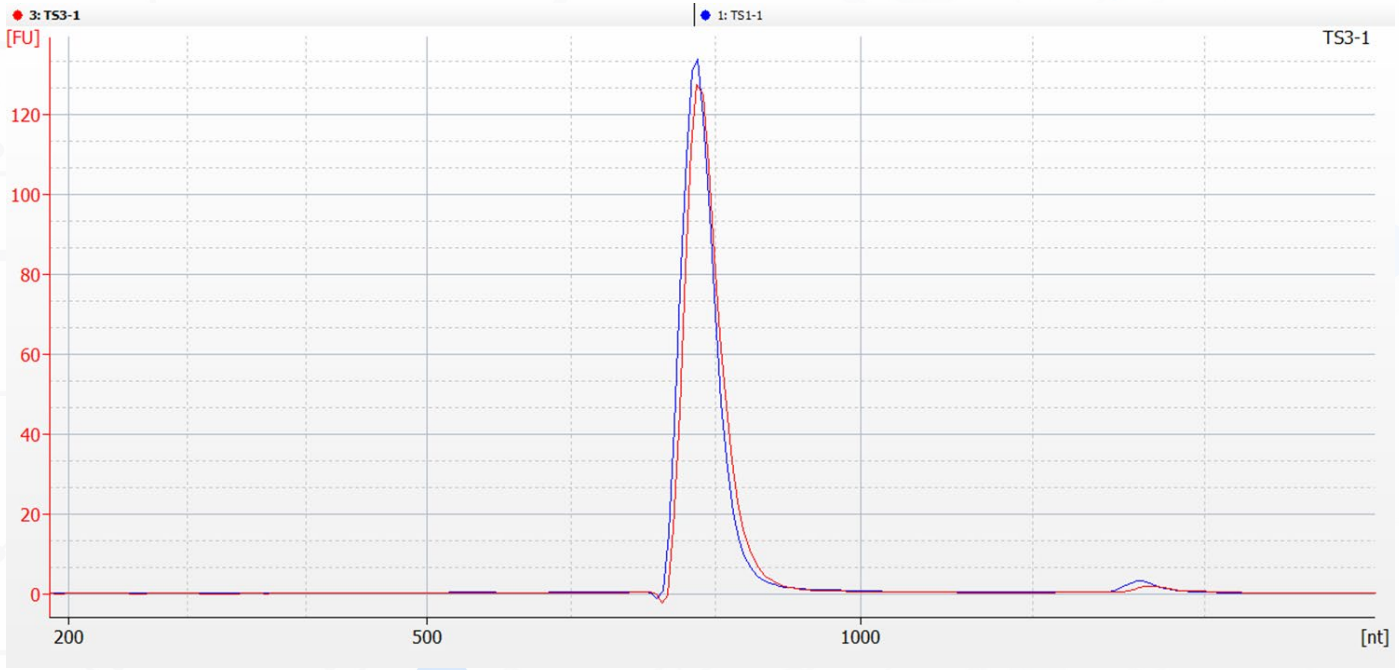


* Based on HiScribe™ T7 ARCA mRNA Kit (NEB)

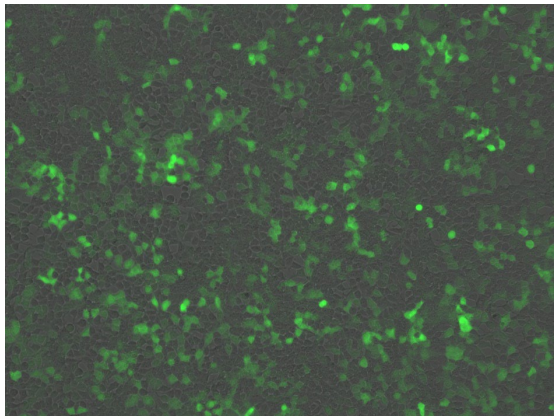
Less Steps- Identical RNA Profile and Expression



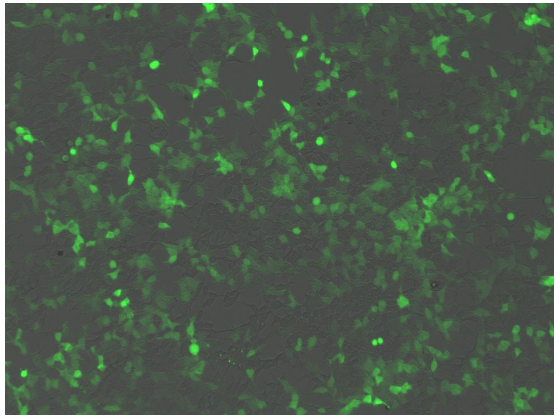
mRNA from linDNA IVT Template

mRNA from pDNA IVT Template

IVT performed via HiScribe™ T7 ARCA mRNA Kit (NEB)



linDNA template-based RNA of GFP transcript

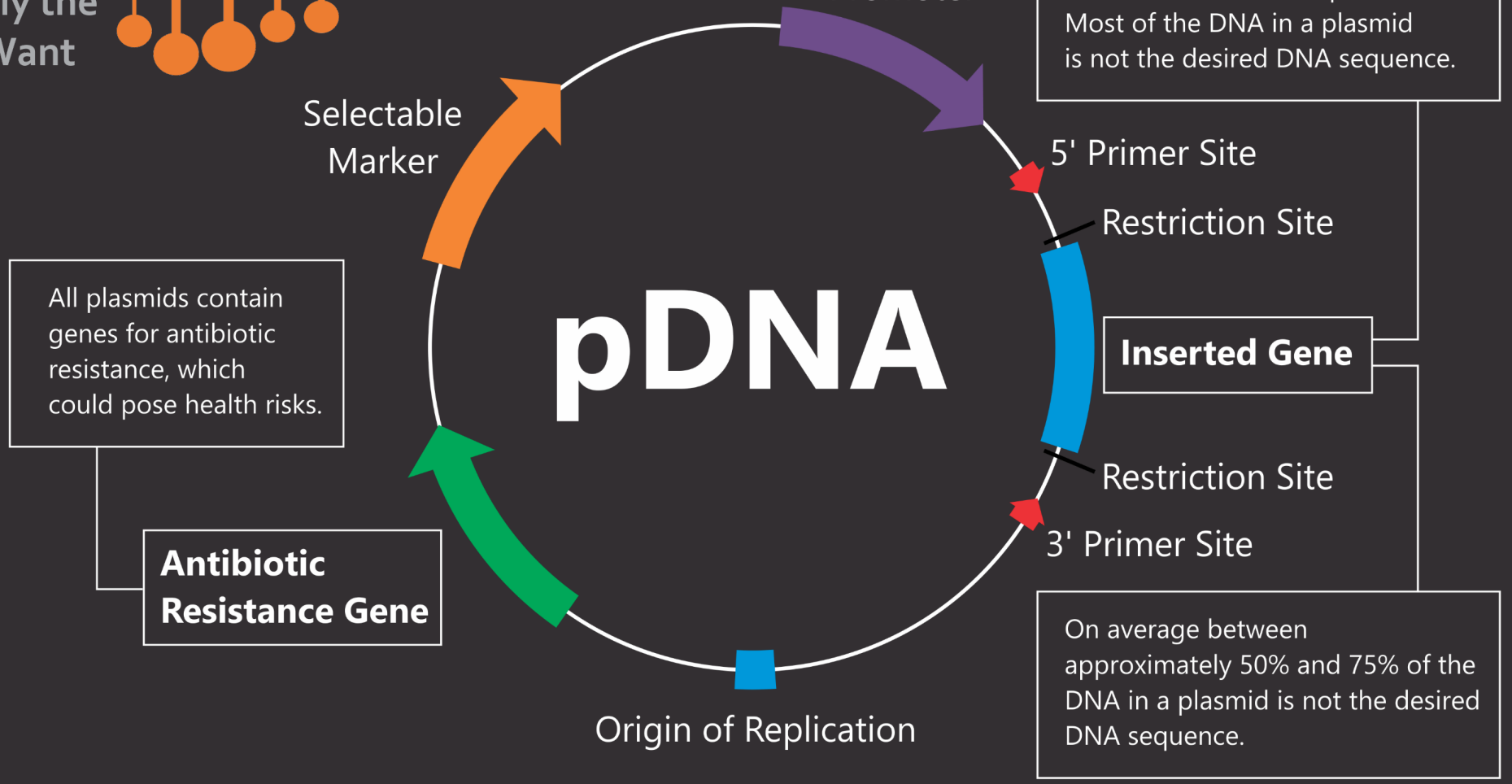


Plasmid template-based RNA of GFP transcript

Produce Only the DNA You Want

Purity

Produce Only the DNA You Want

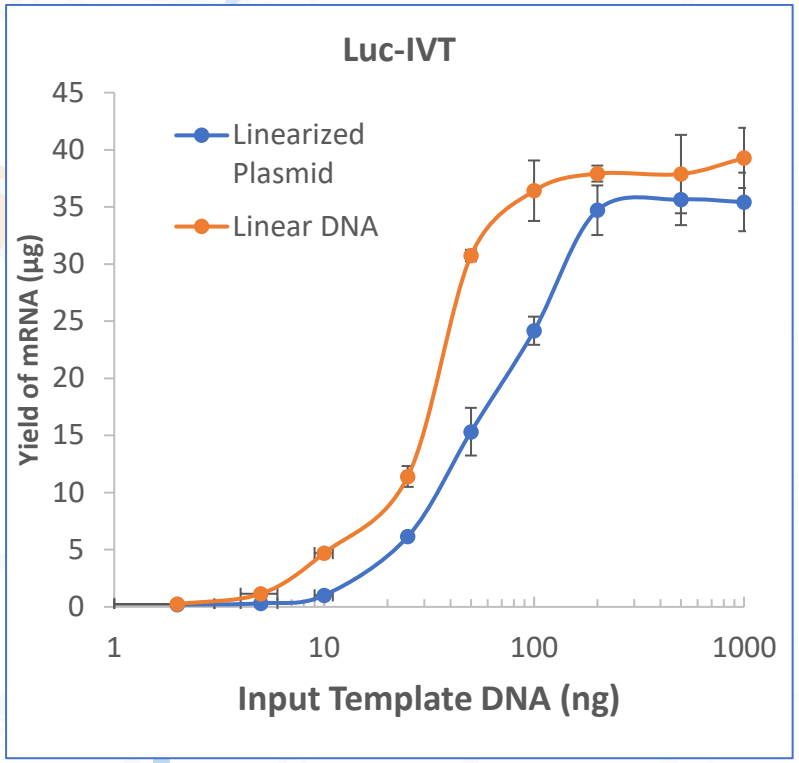


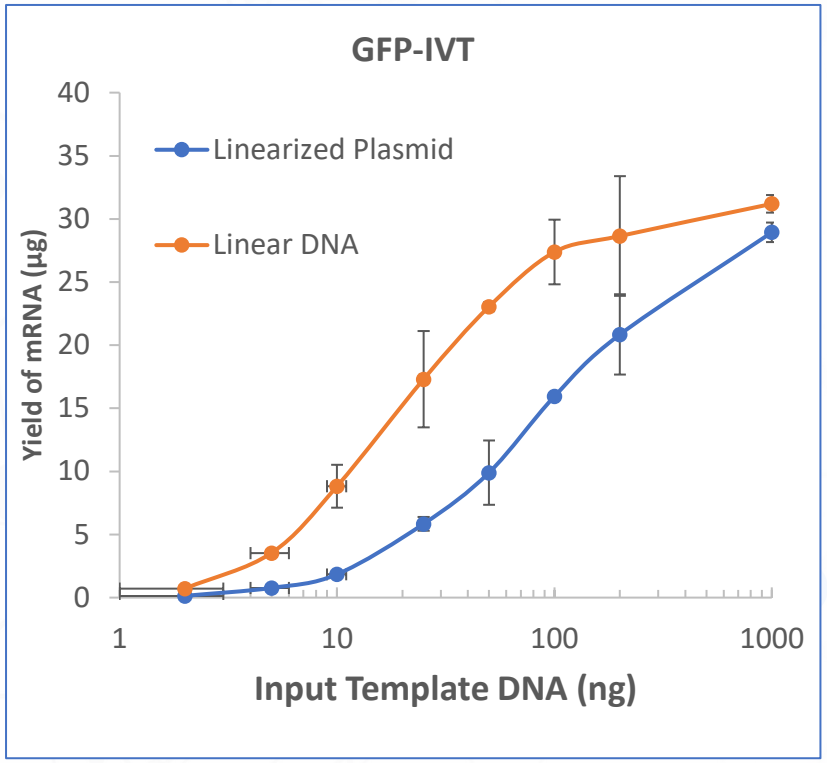
Only a small portion of a plasmid is the desired DNA Sequence. Most of the DNA in a plasmid is not the desired DNA sequence.

All plasmids contain genes for antibiotic resistance, which could pose health risks.

On average between approximately 50% and 75% of the DNA in a plasmid is not the desired DNA sequence.

Less is More – Copy Number Advantage





- At high DNA inputs, IVT kit likely oversaturated by linDNA
- Ability to significantly down-titrate linDNA and still produce RNA yields equal or better than pDNA
- On average linDNA can be used at 25%-50% of pDNA to produce comparable RNA yield

IVT performed via HiScribe™ T7 ARCA mRNA Kit (NEB)

linDNA Purity & Efficiency

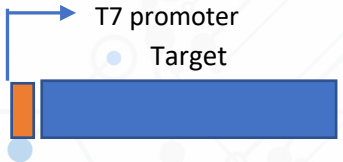
comparison of mRNA Expressions

IVT Templates of PCR-based LinearDNA™ and Plasmid DNA were used for mGFP mRNA expression studies

DNA templates

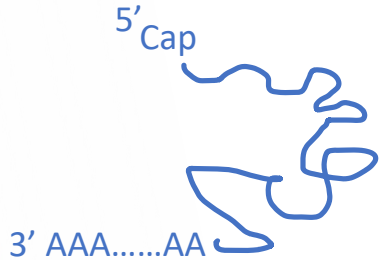
Poly (A) mRNA

1. PCR-based Linear DNA

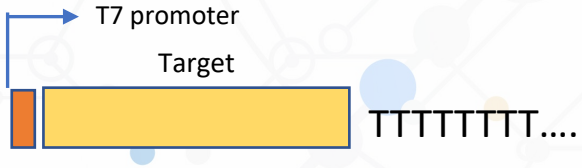


IVT

Poly(A) addition

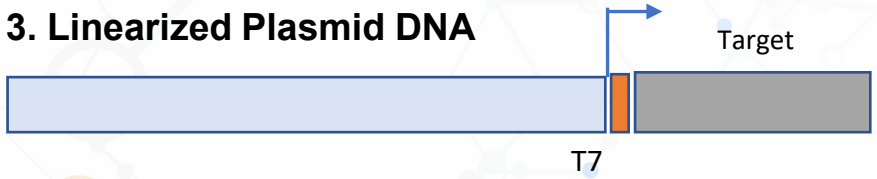


2. PCR-based Linear DNA with Poly(T)



IVT

3. Linearized Plasmid DNA



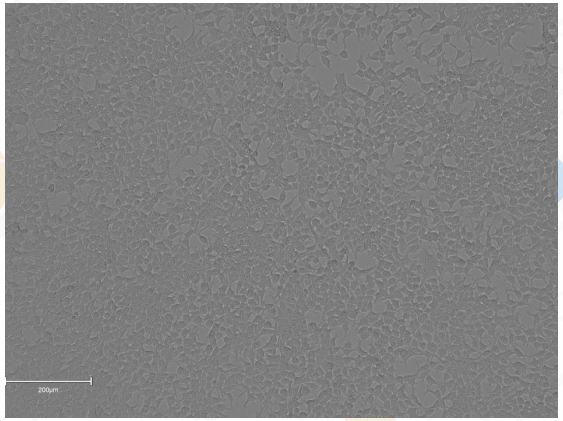
IVT

Poly(A) addition

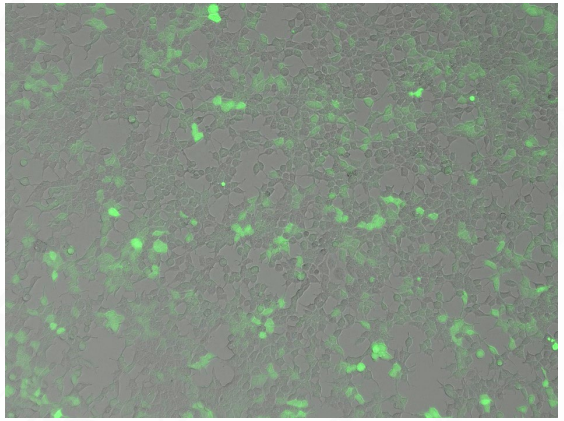
linDNA Purity and Efficiency

Fluorescence microscopy images and analysis of mGFP mRNA expression in HEK293T cells

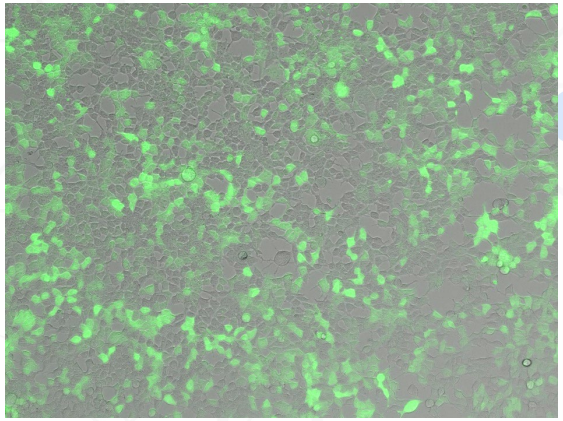
Transfection Reagent Only



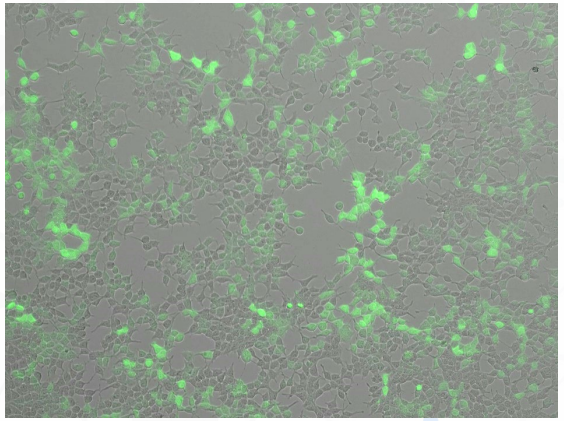
1 mRNA generated from Linear DNA



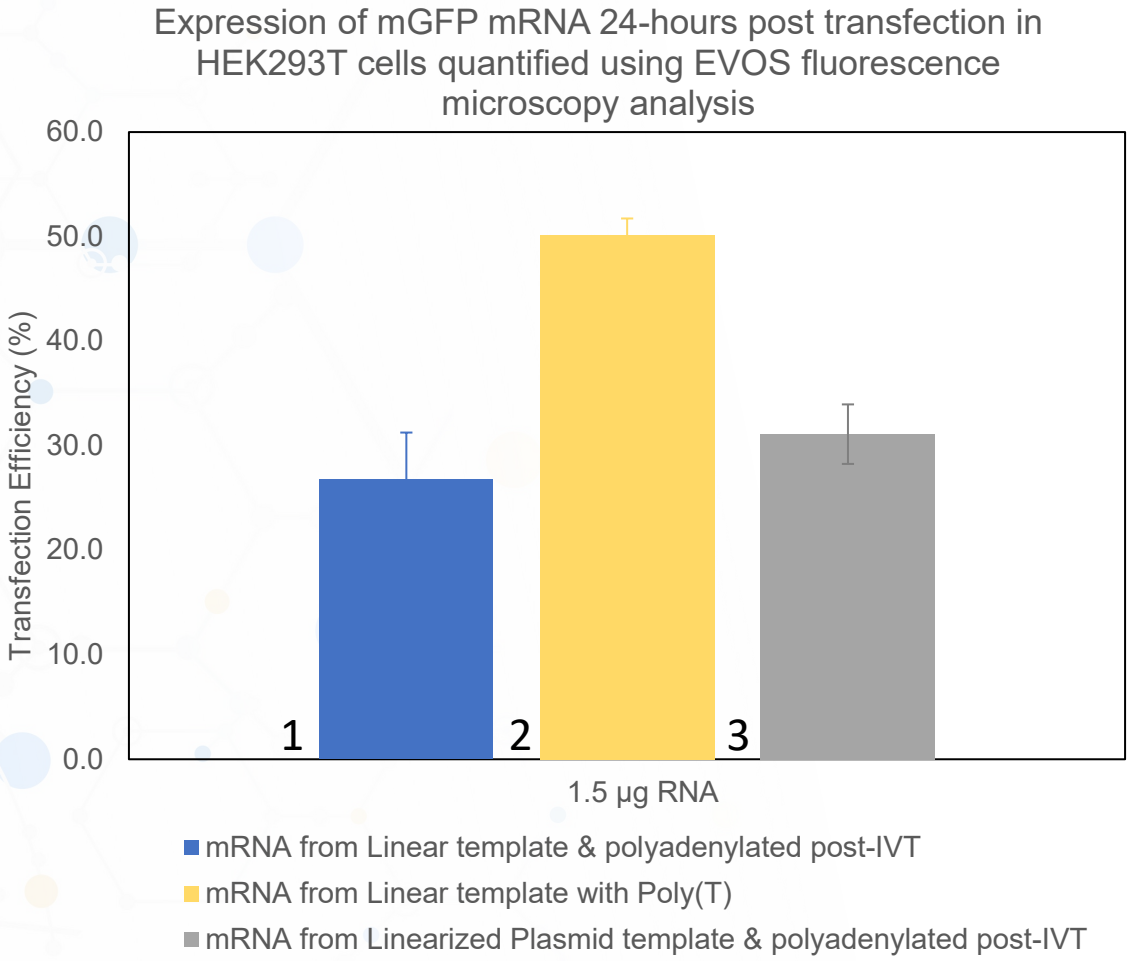
2 mRNA generated from Linear DNA with Poly(T)



3 mRNA generated from Linearized Plasmid



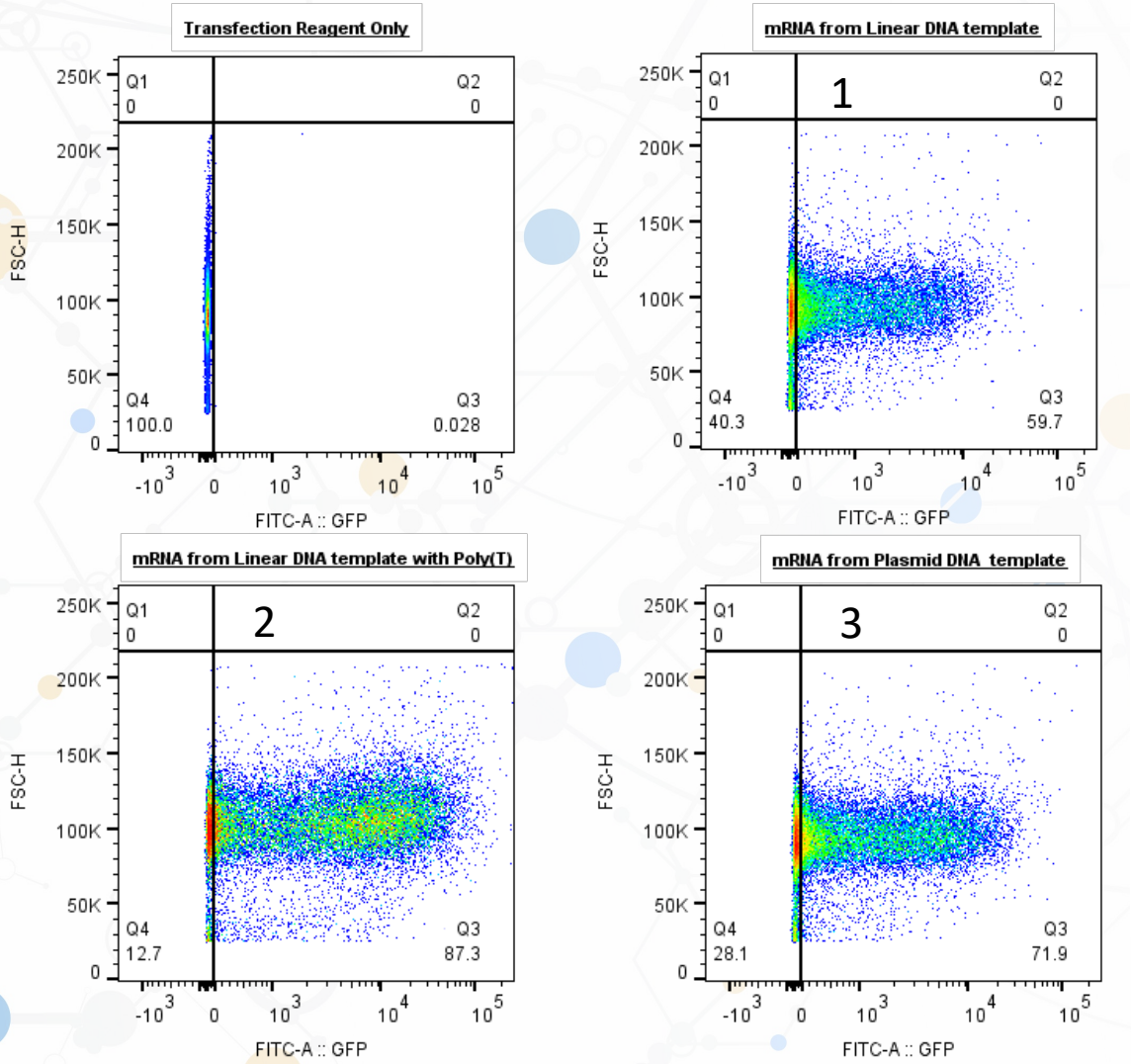
Overlay of images from GFP and bright-field channels 48-hrs post-transfection

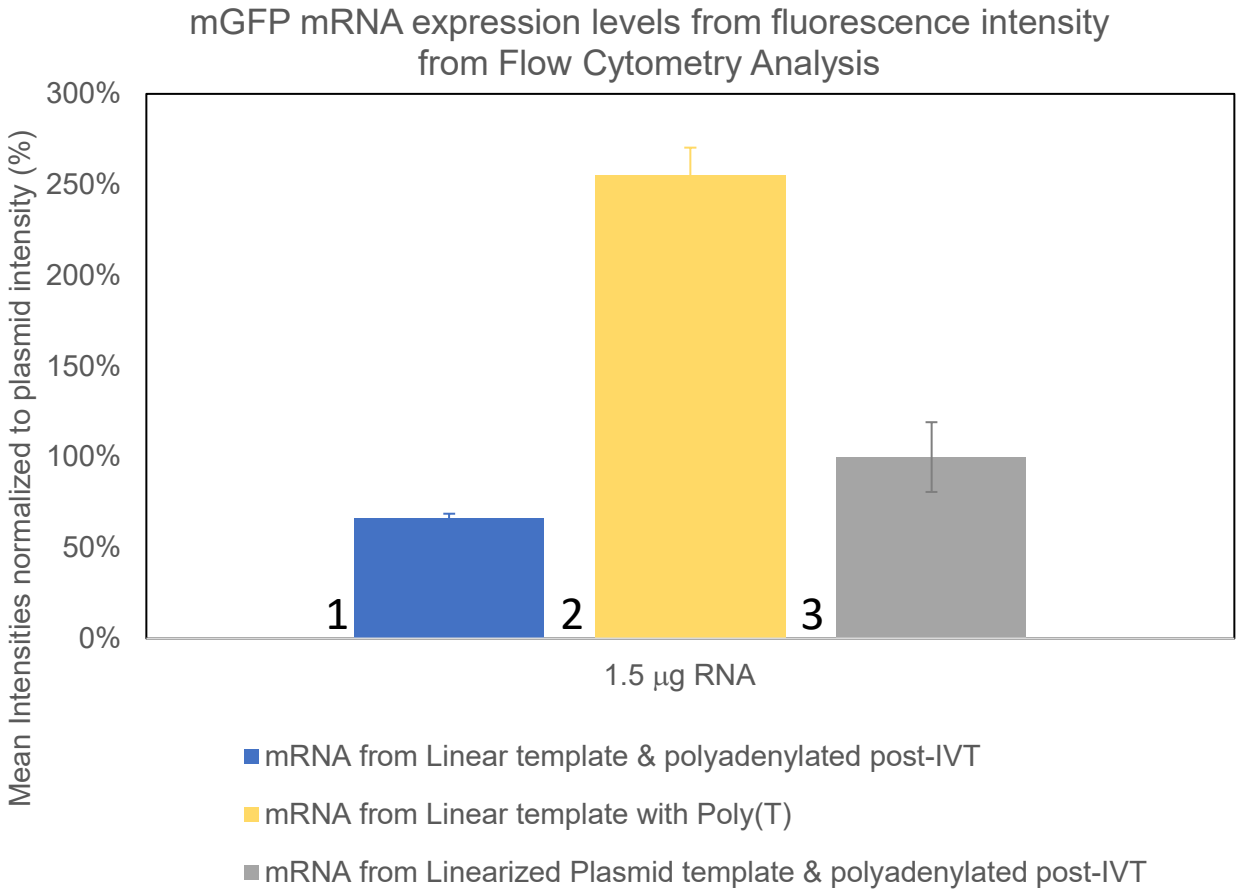


All mRNAs are polyadenylated, through either Poly(T) template or post-IVT Poly(A) addition.

linDNA Purity and Efficiency

Flow Cytometry Analysis of mGFP mRNA expression in HEK293T cells transfected using Messenger Max 72-hours post-transfection





All mRNAs are polyadenylated, through either Poly(T) template or post-IVT Poly(A) addition.

linDNA Purity and Efficiency
comparison of mRNA expressions

Equimolar mRNA from Poly(T) linDNA template encoding mGFP yields approximately 2.5-fold more expression over mRNA from plasmid template based on flow cytometry data, saving time and adding capacity.

Reduced dsRNA Production

dsRNA detected by dot blot[1,2]

1. dsRNA Control
2. Negative control, ssRNA
3. RNA produced from unmodified PCR amplicon
4. RNA from linDNA IVT template
5. RNA from linearized pDNA IVT template
6. RNA from linDNA IVT template with polyT sequence

- dsRNA is cytotoxic
- linDNA IVT templates produced significantly less dsRNA than conventional PCR amplicons
- dsRNA reduction achieved via use of proprietary sequences in linDNA IVT template upstream of T7 promoter
- dsRNA from linDNA and pDNA comparable

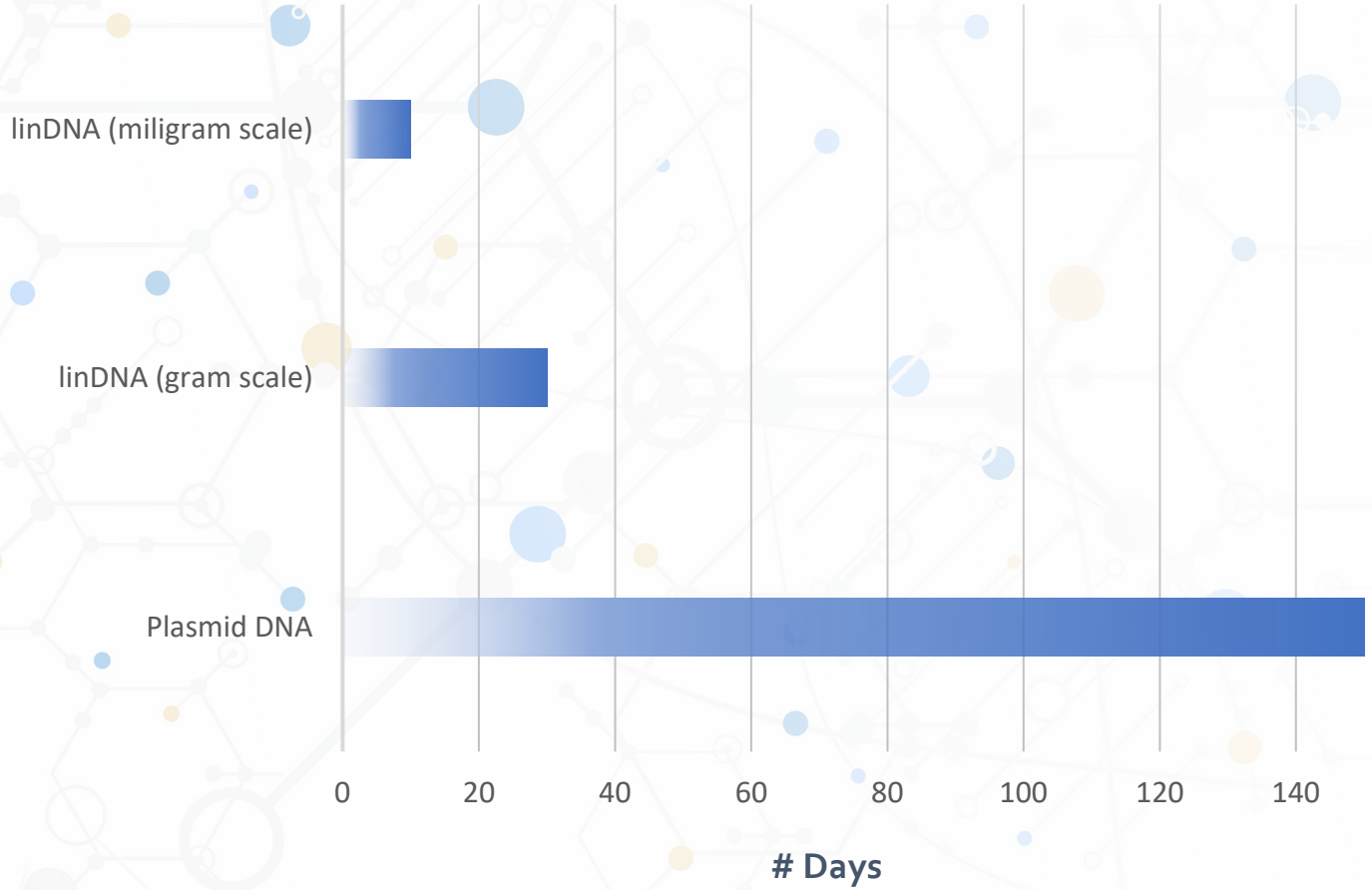
[1] Baiersdörfer M, Boros G, Muramatsu H, Mahiny A, Vlatkovic I, Sahin U, Karikó K. A Facile Method for the Removal of dsRNA Contaminant from In Vitro-Transcribed mRNA. Mol Ther Nucleic Acids. 2019 Apr

[2] 2µg RNA for each dot

Critical Quality Attributes

Test Purpose	Tests Performed
Identity	• Sequencing – Sanger or NGS • Agarose Gel Electrophoresis
Content & Purity	• Absorption Ratio 260/280 – Spectrophotometry • Concentration – Spectrophotometry A260 • Sizing – Bioanalyzer • Purity – High Performance Liquid Chromatography (HPLC) • Terminal filtration (sterile)
Safety	• Endotoxin Quantification (if requested by customer) • Sterility (if requested by customer)
Other	• Appearance – Visual Inspection • pH

linDNA - Speed of Manufacturing



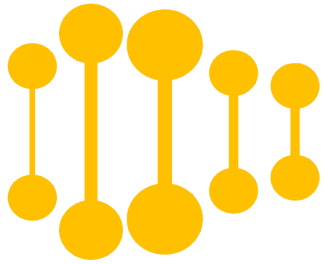
Speed

No More Waiting For DNA Templates

- The simplicity of the linDNA platform allows for rapid DNA manufacturing
- Unlike plasmid-based manufacturing, little optimization is needed for new DNA constructs
- Rapid manufacturing optimal for both R&D, clinical and commercial production workflows
- Excellent batch-to-batch consistency
- Highly-homogenous DNA product

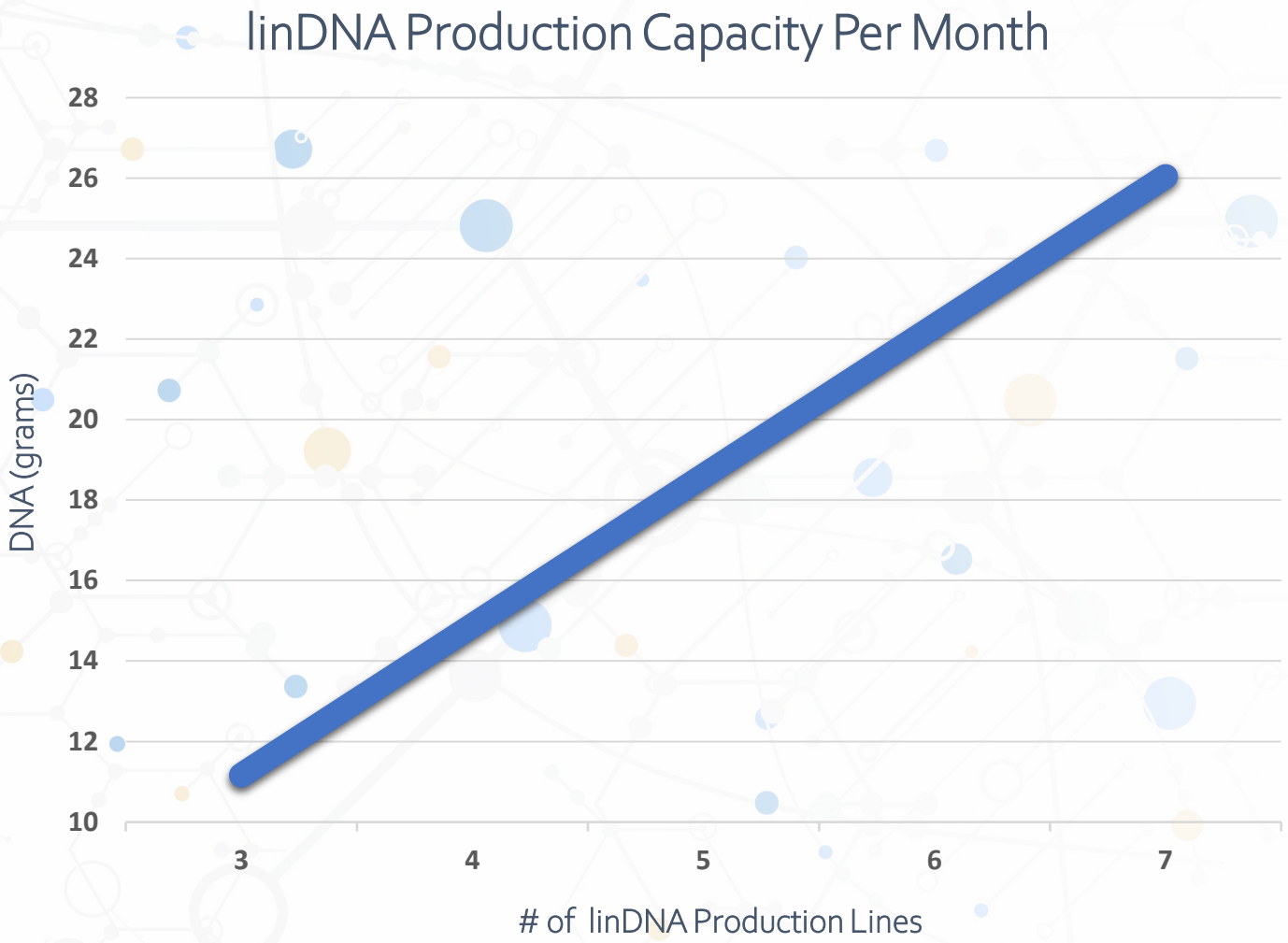
linDNA – Unparalleled Flexibility

Flexibility
Chemical Modifications
Various templates



Feature	linDNA	pDNA	Non-PCR Enzymatically Produced DNA
Usable for IVT Without Additional Modification Steps	✓		
Simple Chemical Modifications Can be Made to DNA	✓		
Completely pDNA-Free Workflow	✓		
Excels at Complex DNA Sequences (poly(T), ITRs)	✓		✓
Template Flexibility (pDNA, amplicons, *de novo* DNA constructs)	✓		
Endotoxin Risk Completely Eliminated	✓		

linDNA – Scalability



Scalability

Micrograms -> Grams

- Platform mimics benchtop instruments that are linearly scalable
- Large DNA manufacturing capacity in minimal footprint
- Currently operating 4 production lines, but can quickly add more

Getting Started with linDNA

linDNA Evaluation

Assess linDNA performance with your RNA production platform using off-the-shelf reporter constructs with user guides

Feasibility Assessment

Manufacture and supply of linDNA for customer's specific sequence of interest

Scale Up

Optimize customer's specific sequence for large-scale production on linDNA platform

Commercial Supply

Optimized large-scale production of customer's specific sequence to meet their desired scale and timelines

LinearDNA™ IVT Template Evaluation Kit

Each linDNA IVT template evaluation kit contains 25 µg of the following IVT templates:

- Circular plasmid IVT template
- linDNA IVT template without poly(T)
- linDNA IVT template with 120bp poly(T)

Order at **lineaRxDNA.com/mrna-summit** or visit the LineaRx booth.

LineaRx™
DNA made simple

Thank you!