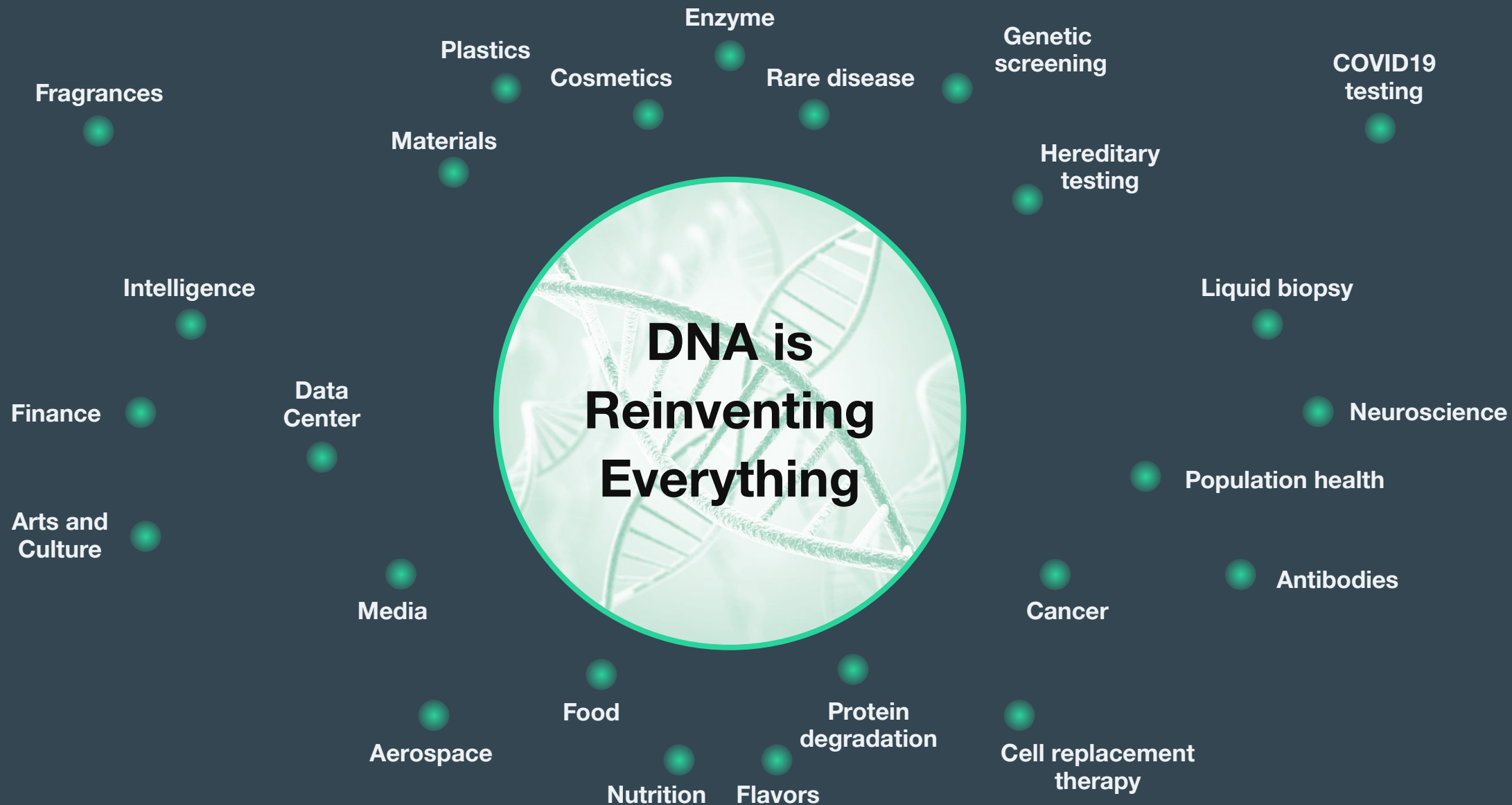




# DNA Synthesis Development and Application:

“HOW TO GROW ALMOST ANYTHING”

EMILY LEPROUST, PH.D., CEO and CO-FOUNDER

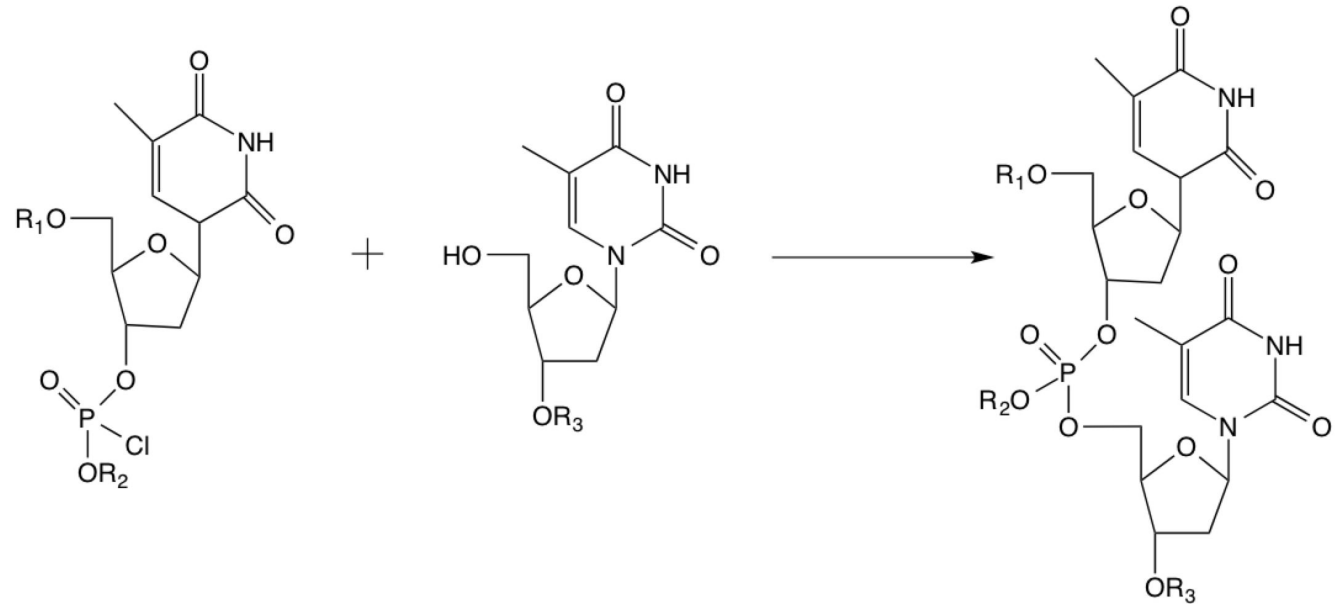




# History of DNA Synthesis - Chemistry Development

**1955**

Synthesis of the first dinucleotide  
by Michelson



1955

1960

1965

1970

1975

1980

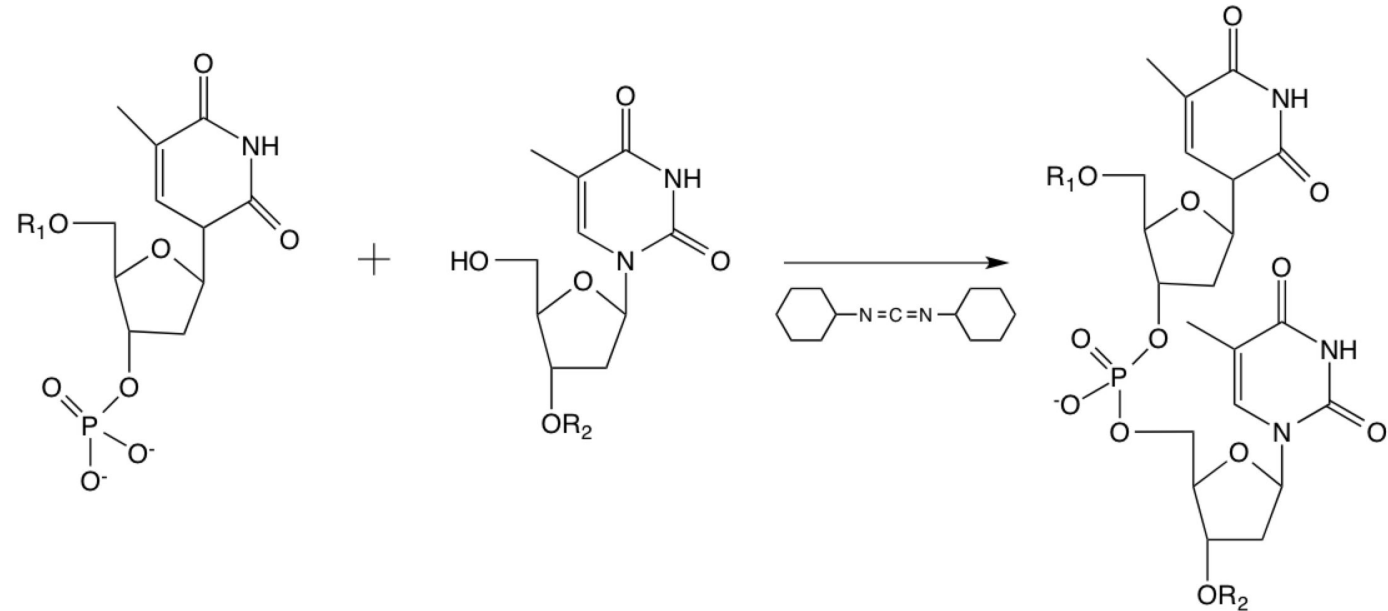
1985



# History of DNA Synthesis - Chemistry Development

**1955**

Synthesis of the first dinucleotide  
by Michelson



1955

1960

1965

1970

1975

1980

1985

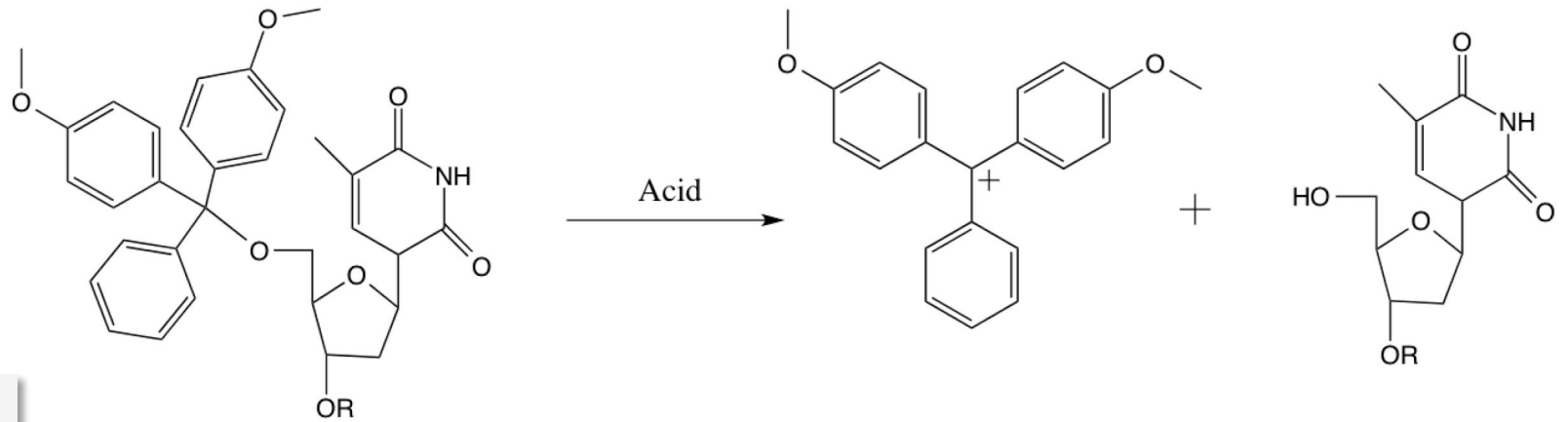
**1956**

Phosphodiester  
method by Khorana

# History of DNA Synthesis - Chemistry Development

**1955**

Synthesis of the first dinucleotide by Michelson



**1961**

On/Off protection scheme for sequential synthesis by Khorana

1955

1960

1965

1970

1975

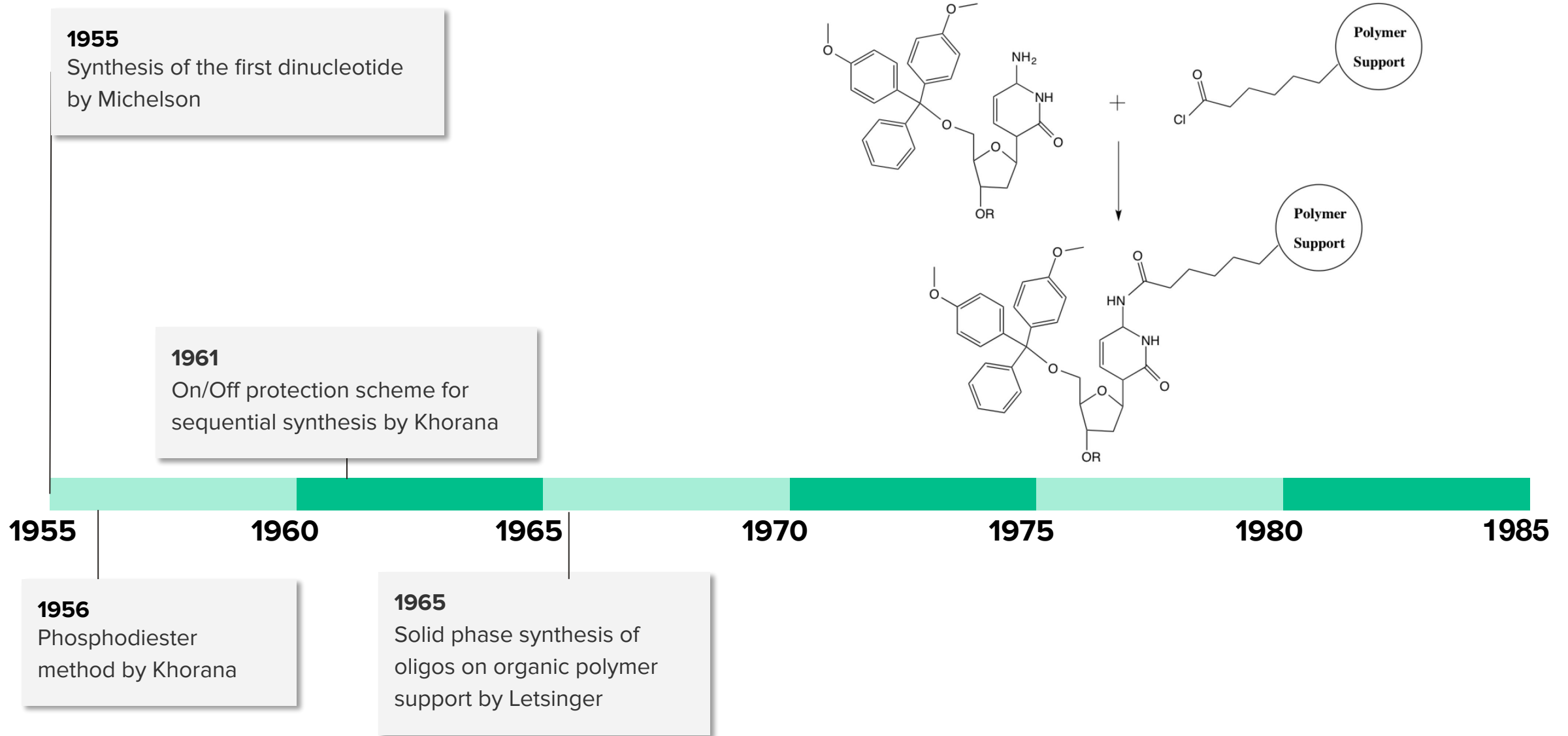
1980

1985

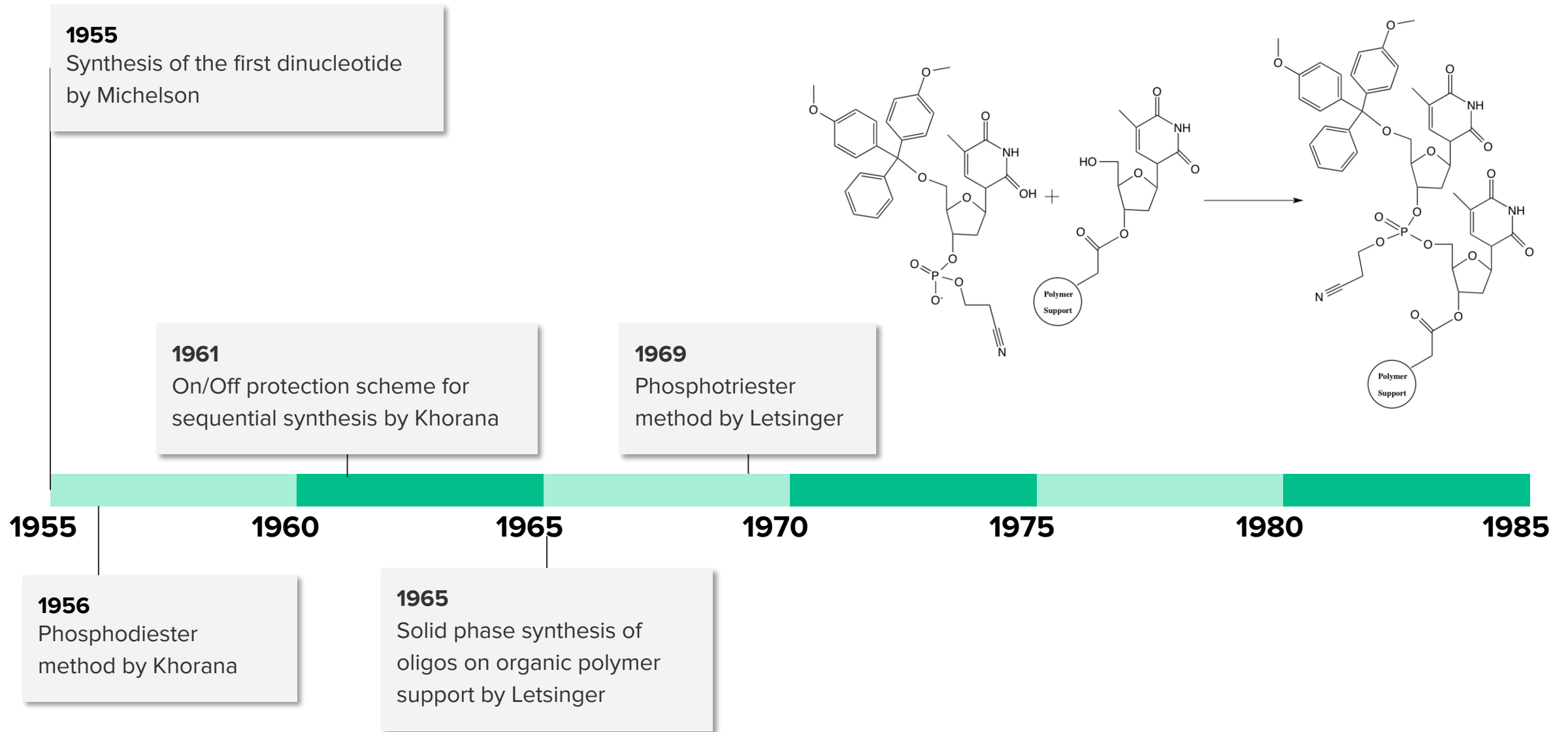
**1956**

Phosphodiester method by Khorana

# History of DNA Synthesis - Chemistry Development

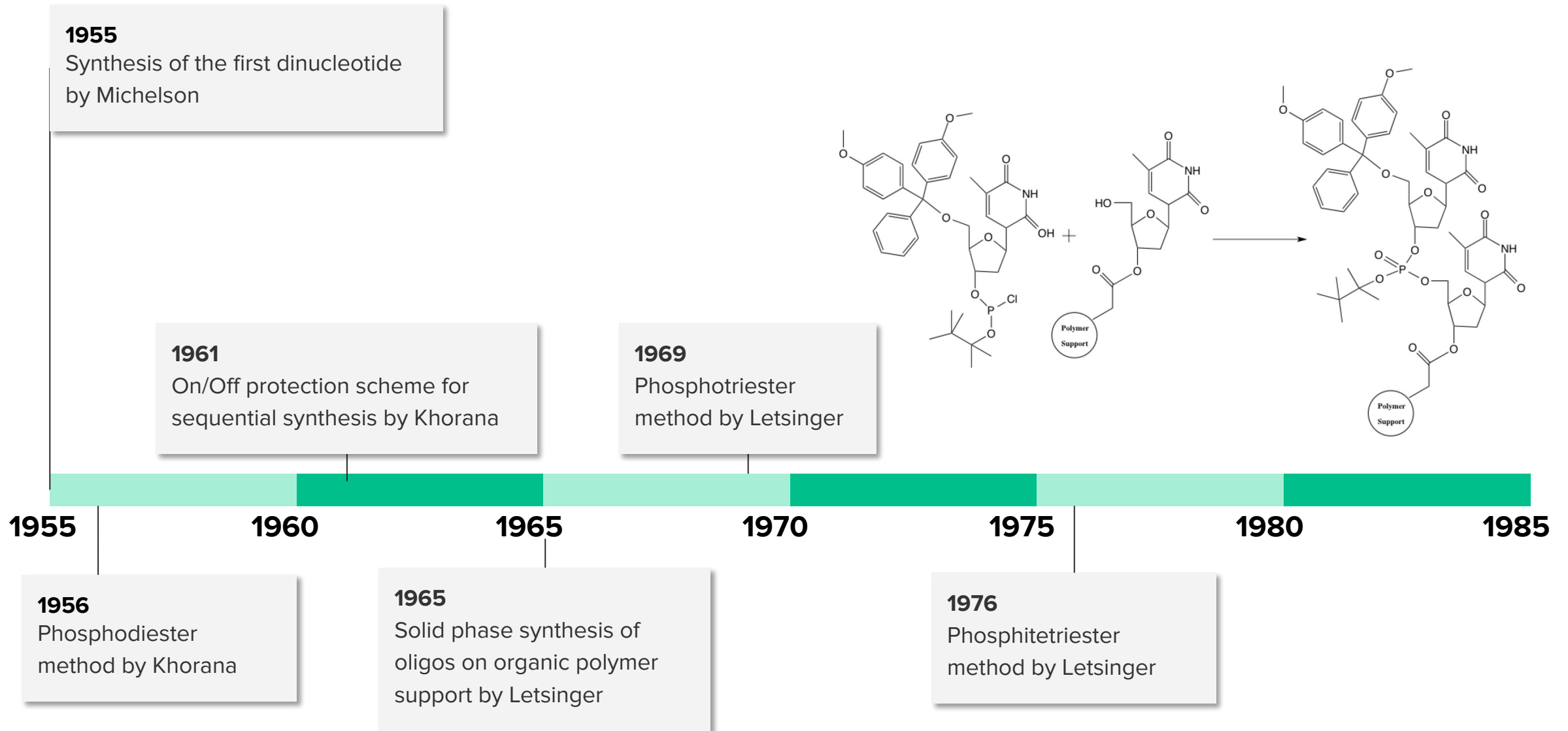


# History of DNA Synthesis - Chemistry Development





# History of DNA Synthesis - Chemistry Development



# History of DNA Synthesis - Chemistry Development

**1955**

Synthesis of the first dinucleotide by Michelson

**1961**

On/Off protection scheme for sequential synthesis by Khorana

**1969**

Phosphotriester method by Letsinger

**1981**

Phosphoramidite method by Caruthers

**1956**

Phosphodiester method by Khorana

**1965**

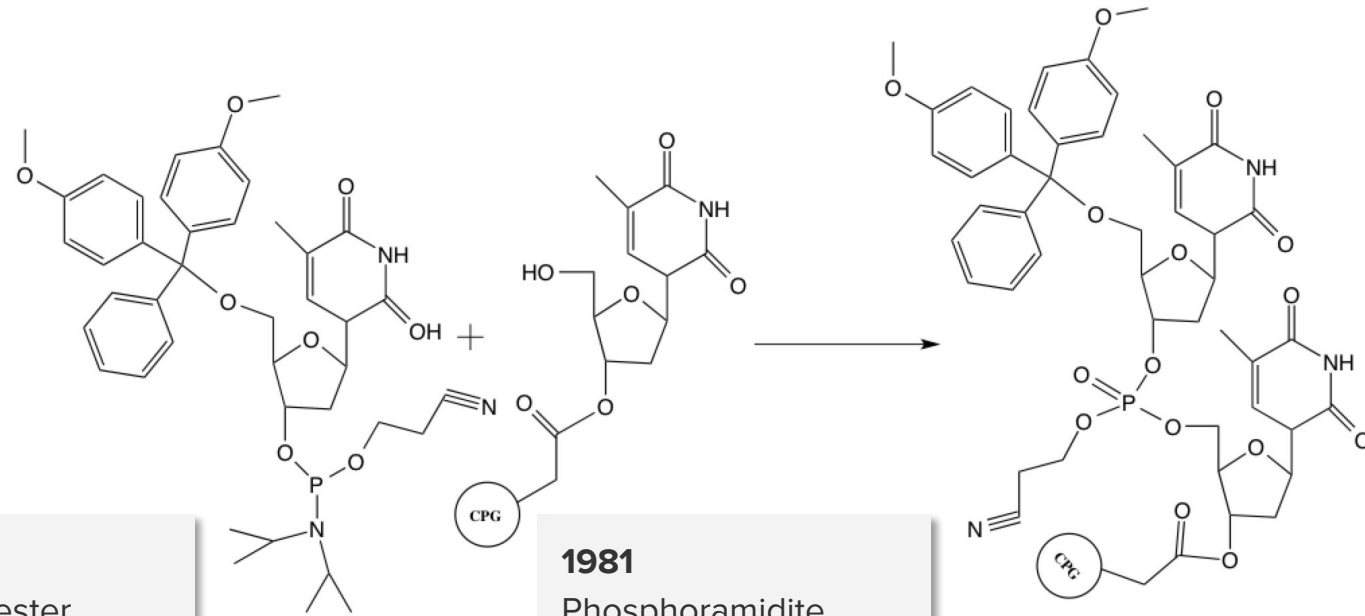
Solid phase synthesis of oligos on organic polymer support by Letsinger

**1976**

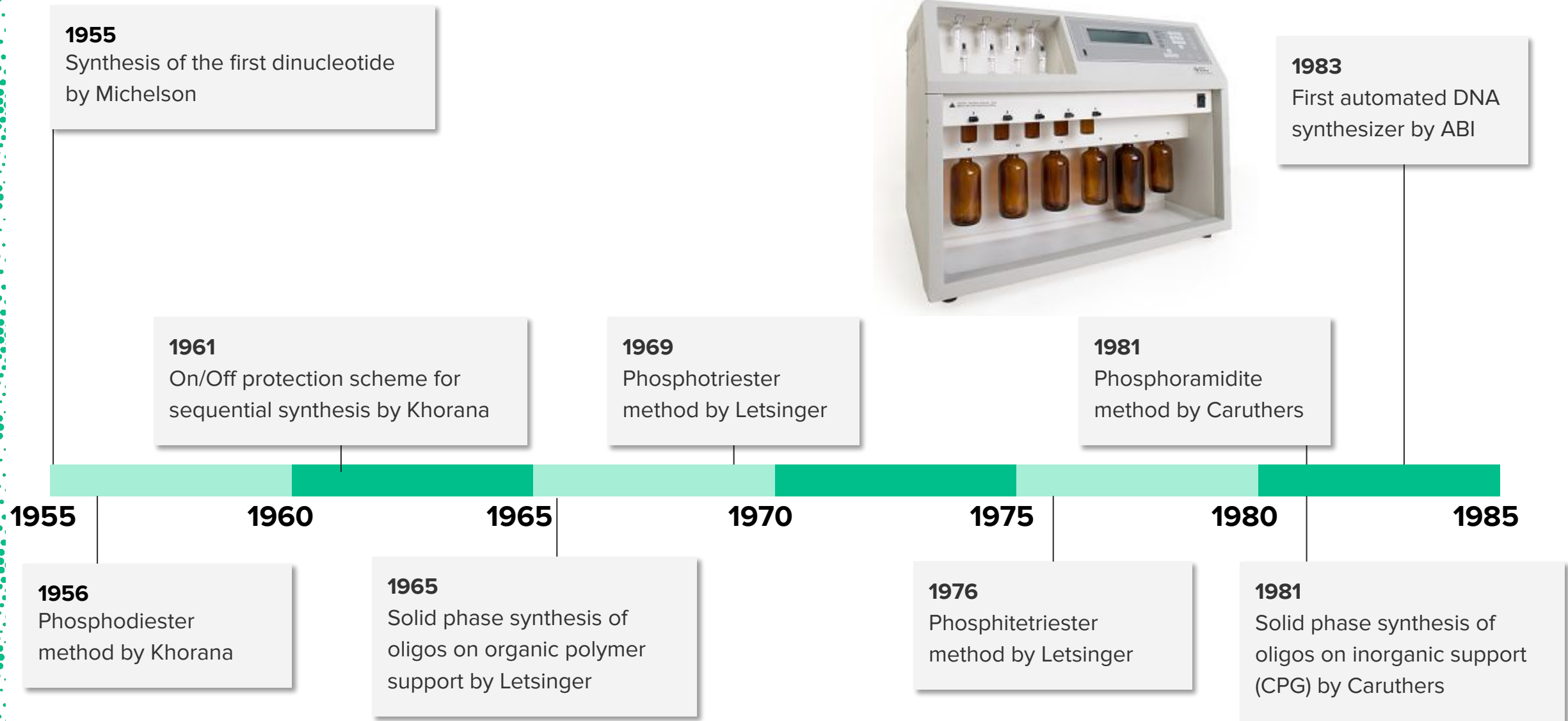
Phosphitetriester method by Letsinger

**1981**

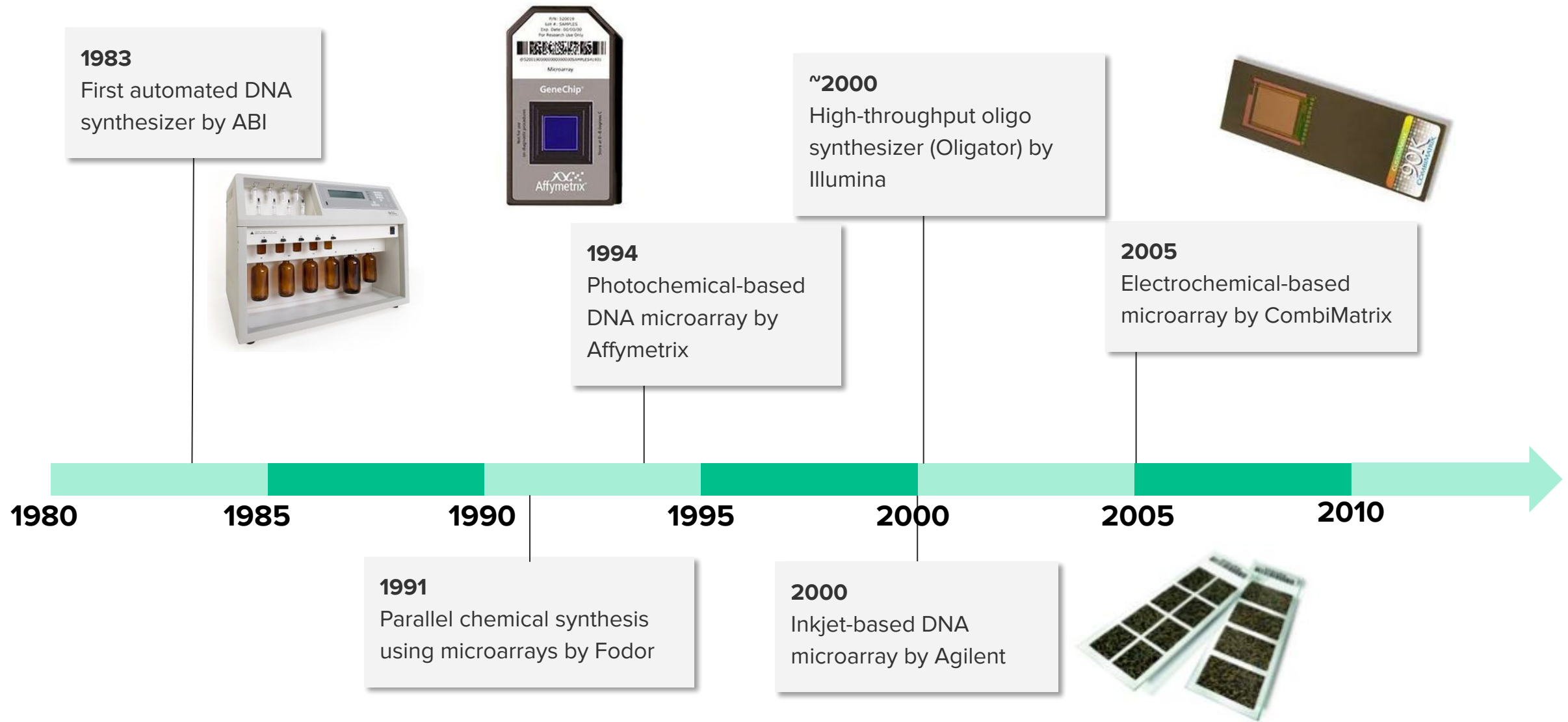
Solid phase synthesis of oligos on inorganic support (CPG) by Caruthers



# History of DNA Synthesis - Engineering



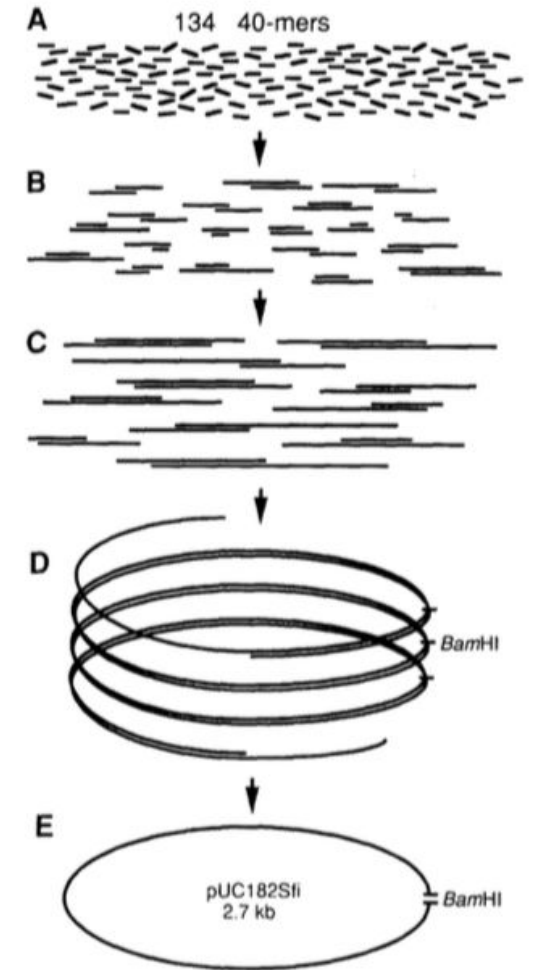
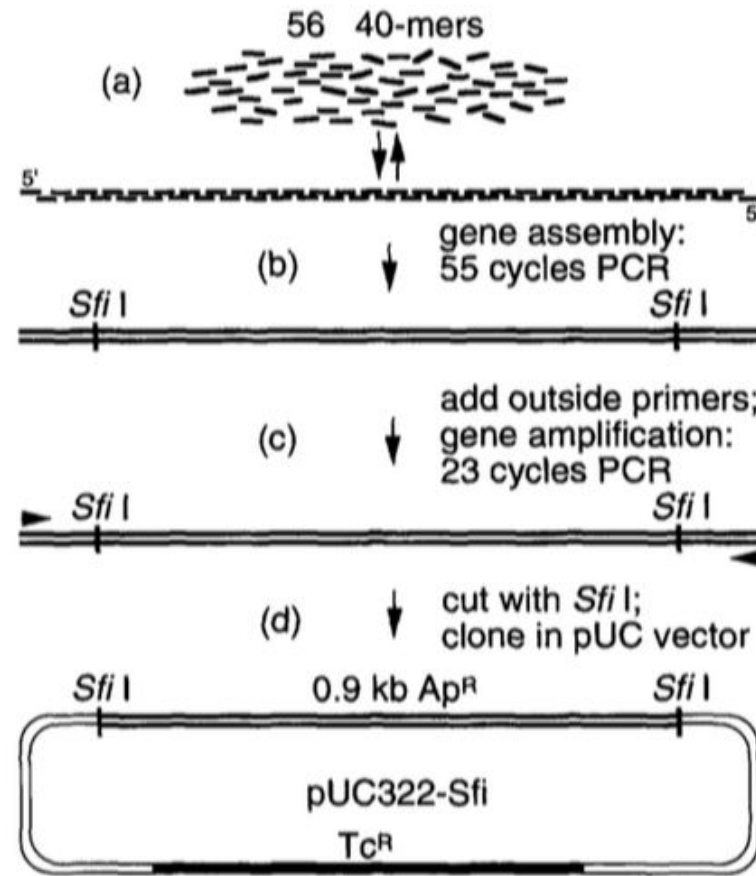
# History of DNA Synthesis - Engineering





# Classical Gene Synthesis

Stemmer (1995) *Gene* 164:49



# Synthetic DNA is the Future of Everything

## Writing the Future

At Twist Bioscience, we work in the service of customers who are changing the world for the better. In fields such as medicine, agriculture, industrial chemicals, and data storage, by using our synthetic DNA tools, our customers are developing ways to better lives and improve the sustainability of the planet.



### **Chemicals**

Sustainability



### **Food**

Food Security



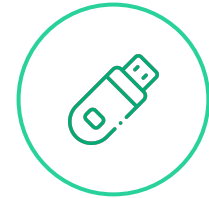
### **Therapeutics**

Health



### **Diagnostics**

Precision Medicine



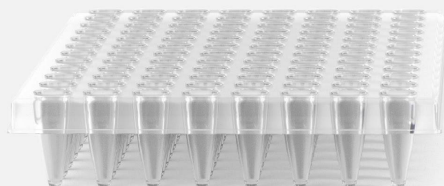
### **Data Storage**

Preserving Heritage

# Game-Changing Throughput, Quality & Affordability

Everyone Else

**1** oligo per **well**



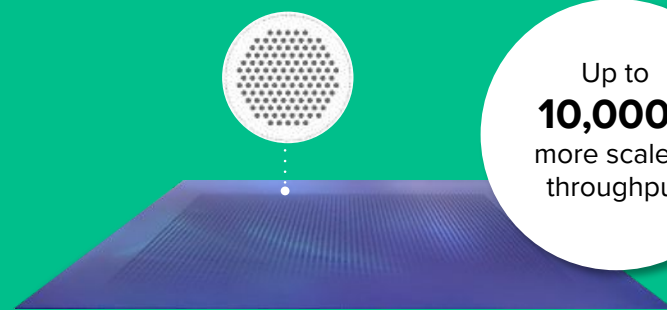
96-well plate makes

**1 gene**

VS



**1M** oligos per **chip**



Up to  
**10,000x**  
more scale &  
throughput

Twist Silicon Platform  
can make **9,600 genes**

**Our state of the art**  
commercial infrastructure



- Proprietary software
- Robotics
- Integrated ecommerce platform with order tracking
- Manufacturing execution system



# We Continue Loading MORE On The SAME chip

## Rapid Growth, Unmatched Scale, High Margin Expansion

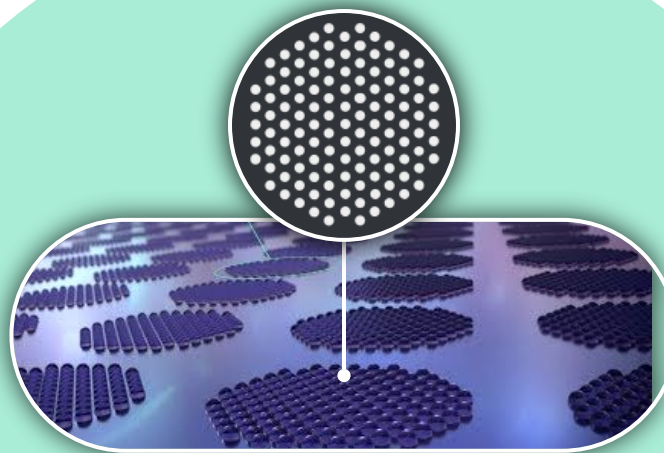
**DNA on Silicon Platform**

**MORE**  
**Products**

**MORE**  
**Customers**

**MORE**  
**Applications**

**MORE**  
**Markets**



T W I S T  
BIOSCIENCE



# Solutions That Work Together

## 2021

### NGS APPLICATIONS

Synthetic Controls  
UMIs/Adapters  
3000 UDIs

- Exomes
- Fusion
- Methylome
- MRD500
- Precision Dx
- Long read PGx/dark genes
- Spatial genomics barcoding

### PANELS

- DNA Custom
- RNA Custom
- MethylSeq Custom
- MRD 10k
- MRD Express (coming soon)

### LIBRARY PREP KITS

- EF/MF lib prep kits
- cfDNA lib prep kits
- FlexPrep lib prep kits
- RNA lib prep kits
- EMSeq lib prep kits
- PCR-free WGS kits (coming soon)
- TrueAmp DNA lib prep kits (coming soon)

### DNA SYNTHESIS & PROTEIN SOLUTIONS

Oligo Synthesis

Oligo Pools

Gene Fragments / Long GF

Engineered Enzymes

Variant Libraries

+ Multiplexed Gene Frag / Gene Pools

Clonal Genes / Express Genes

In Vitro Discovery

In Vivo Discovery

AI Enabled Discovery

In Silico Discovery

IgG Proteins

Antibody Characterization

Linearized IVT Template

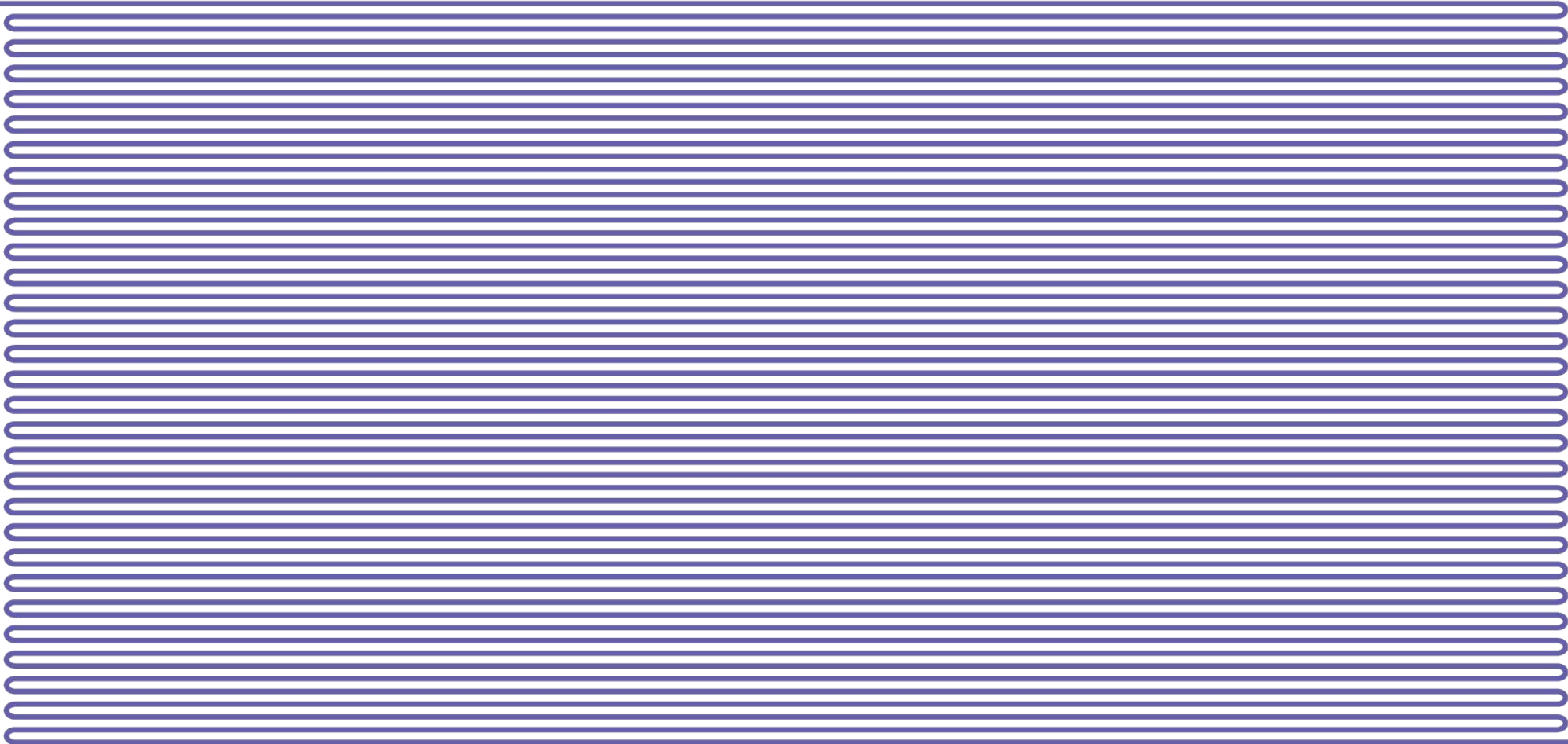
HT mRNA (Early access)

**MORE**  
on the same chip

# Manufacturing One Gene is Equivalent to Driving:



TWIST BIOSCIENCE  
**0.092 miles** (0.15 km)



STANDARD 96-WELL  
PLATE APPROACH

**59 miles**  
(95 km)



1 horizontal line = 1 mile driven

From the EPA Greenhouse Gas Equivalencies Calculator EPA 2024

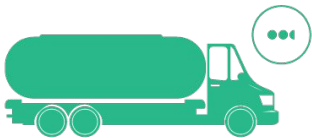
# Twist's Oligo CO<sub>2</sub>e

(Specific to NGS TE Panels):

Twist CO <sub>2</sub> e	Industry Standard
180,000 kg CO <sub>2</sub> e*	470,000,000 kg CO <sub>2</sub> e*



STANDARD 96-WELL PLATE APPROACH  
6,222 tanker trucks  
worth of gasoline

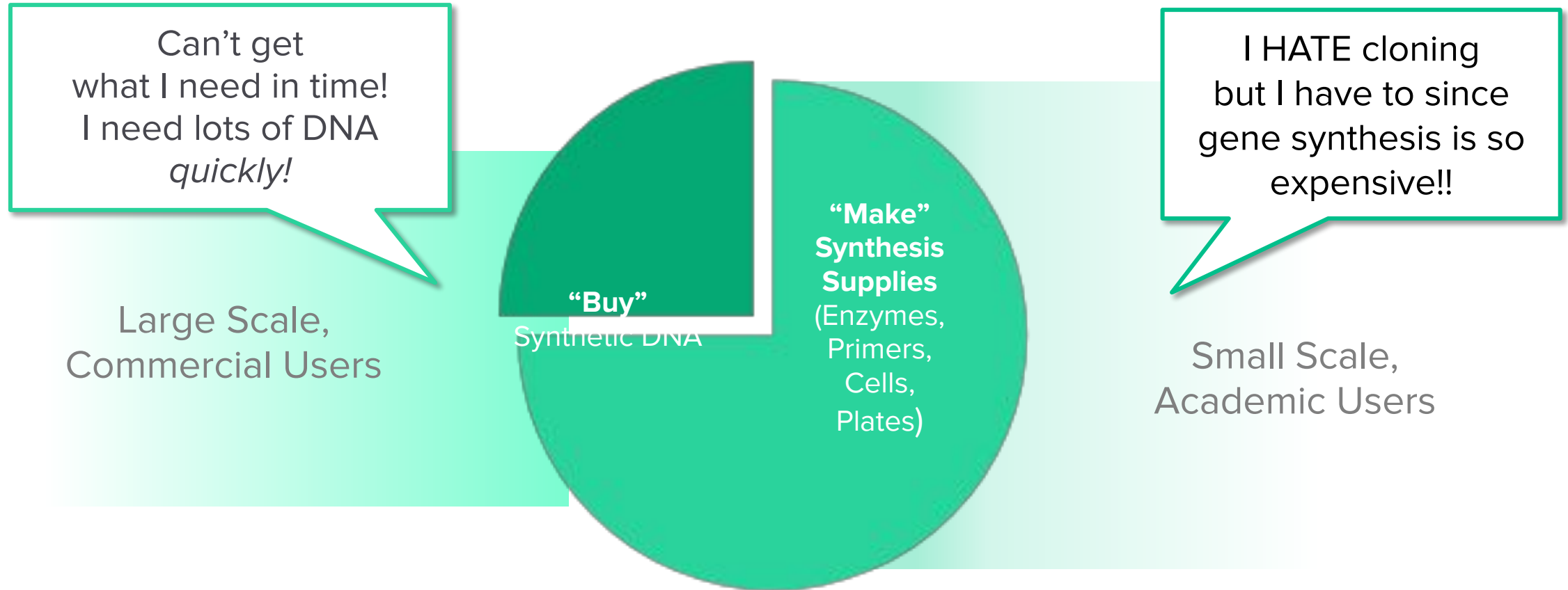


TWIST BIOSCIENCE  
2.4 tanker trucks  
worth of gasoline

# Genes and Clonal Genes

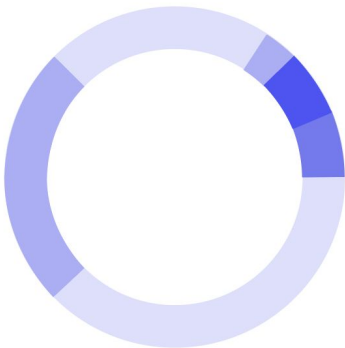


# DNA Users are Buyers and/or Makers



# Gene Fragments and Clonal Genes

Earlier slide said 5 KB



## Gene Fragments Available up to 5KB

Fragments with industry leading low error rate of 1:7,500.

GENE FRAGMENTS

**Up to 5 kb**

2 business days. No one else comes close.

CLONAL GENES

**0.3 to 5 kb**

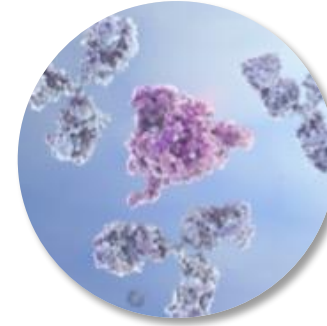
10 business days  
**Express Genes:**  
4-7 business days

## Clonal Genes

Sequence perfect genes cloned into a Twist stock vector or a custom vector of your choice

Academic get express genes for no additional cost.

# Twist Synthetic Clonal Genes



**Antibody-Based  
Drug Development**



**Gene Editing:  
Donor DNA Synthesis**



**Pathway Assemblies**



**Gene Therapy**

# You Handle the Brilliant Research.

We will handle the cloning.

## Onboard your own vectors



Upload your vector map and define your insertion site.



Ship us your vector.



We sequence and bank the vector for you.



We clone Twist Genes into your vector.

|----- TAT – 10 BUSINESS DAYS -----|

## Catalog vectors



Cloning vectors



pET bacterial expression vectors



Mammalian expression vectors



Antibody expression vectors



Lentivirus shuttle vectors



IVT template vectors

Never clone another gene again.

With our cloning service, clone genes into your vectors without time, cost or bandwidth limitations.

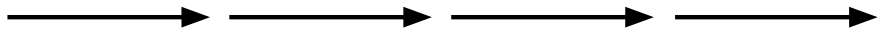
Go from order to shipment of your cloned sequences in 4 – 7 business days with Twist Express Genes.\*

\*Turnaround time starts at 4–7 business days and increases to 7–10 business days for 10 µg–100 µg and 100 µg–1 mg DNA prep scales.

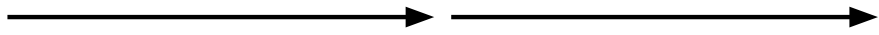
# Got Extremely Difficult Sequences?

Challenge accepted. Challenge complete with Ultra-complex Genes.

Sequences with Short Tandem Repeats



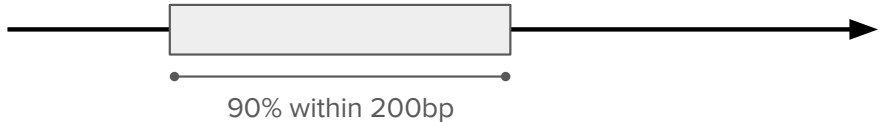
Long Repeats



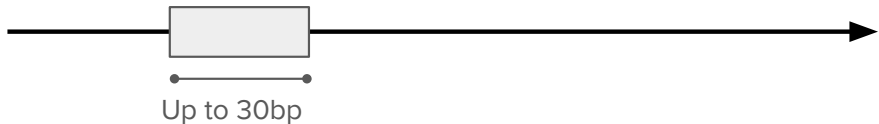
Inverted Repeats (hairpins)



High GC/AT content



Homopolymers



- Know immediately if we can or cannot make a sequence in our ordering portal
- Get sequences containing complexities within 10 business days
- Explore more sequence space: Design complex promoters, UTRs, long linkers, secondary structure

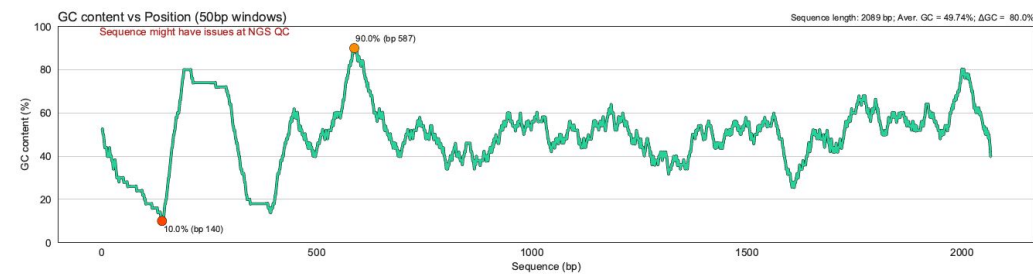
\*Target date. Early access turnaround time may be subject to delays

**... and Genes up to 10 kbp!**

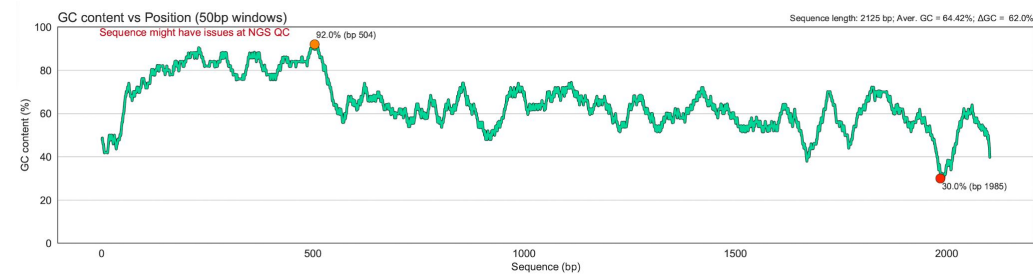


# Twist Can Now Synthesize Otherwise “Impossible Genes” With The Following Complexities (part 1):

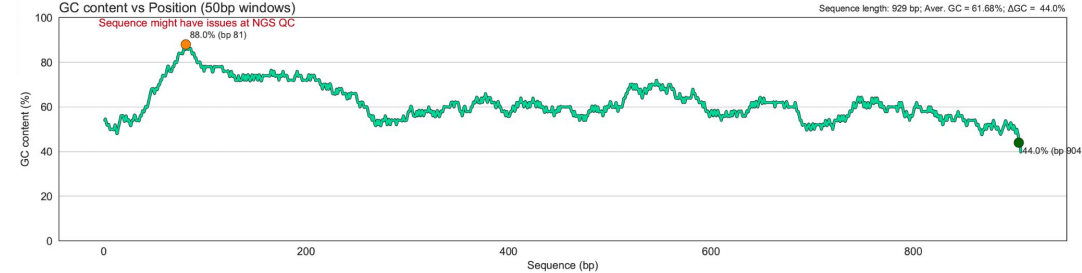
Extreme changes in GC content



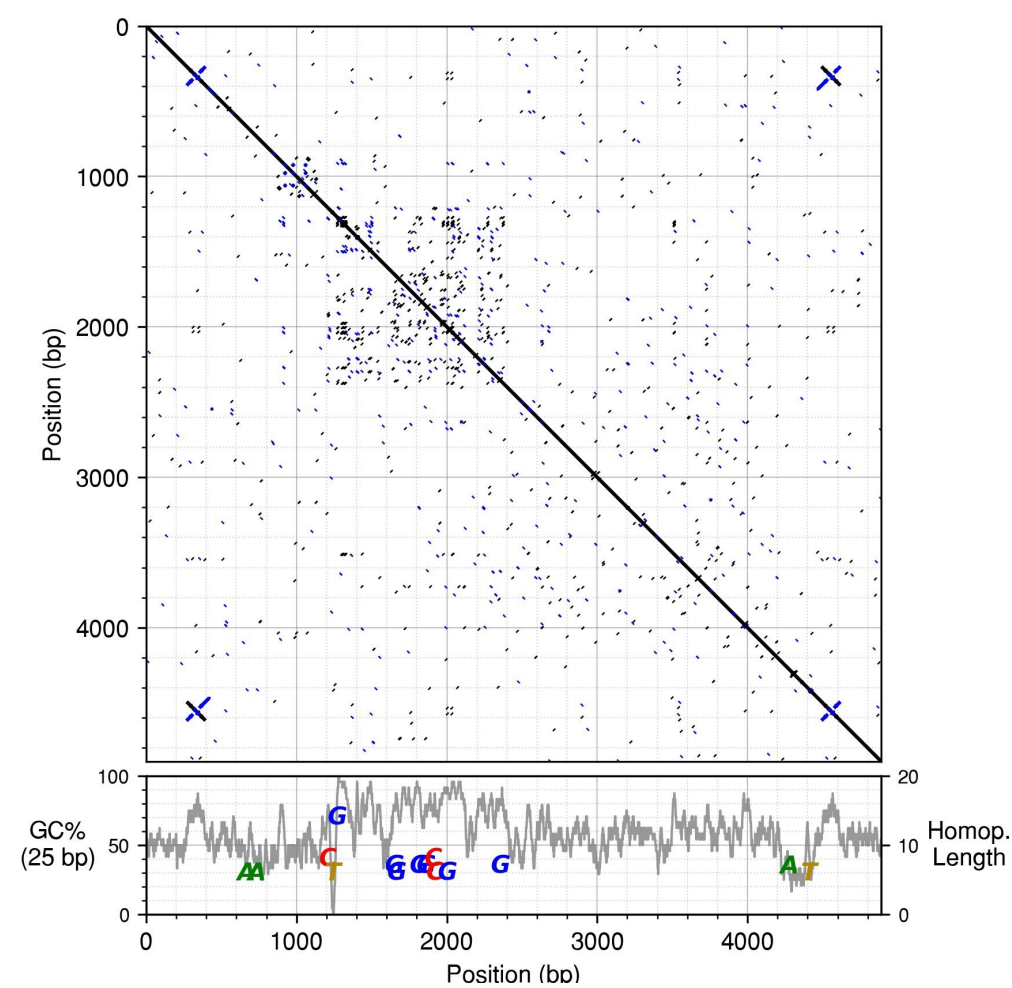
Regions of high average GC content



High average GC content



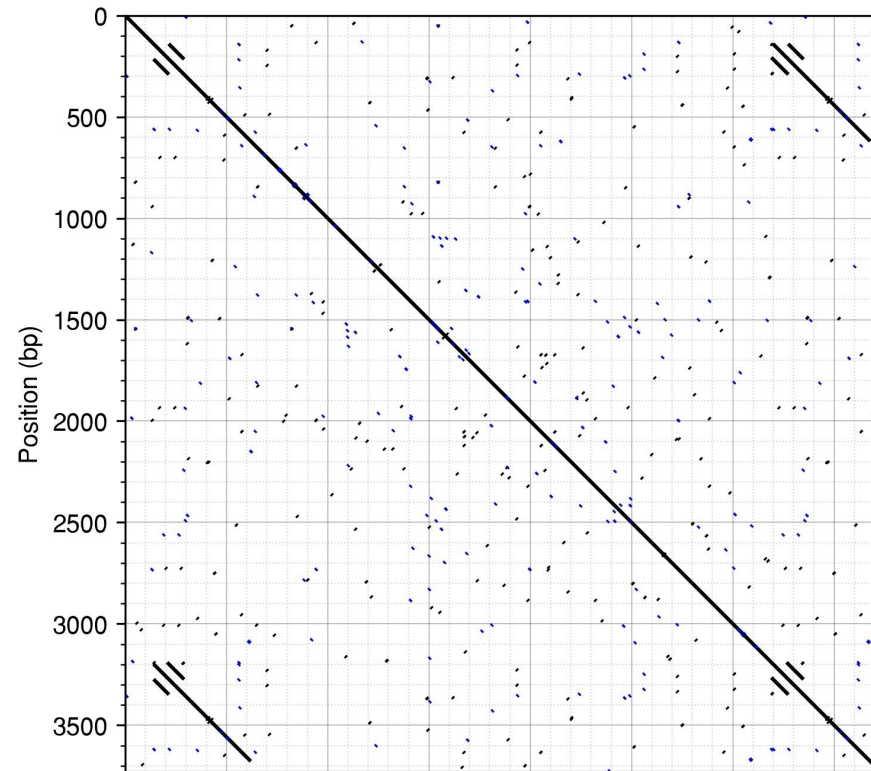
Hairpins and secondary structure



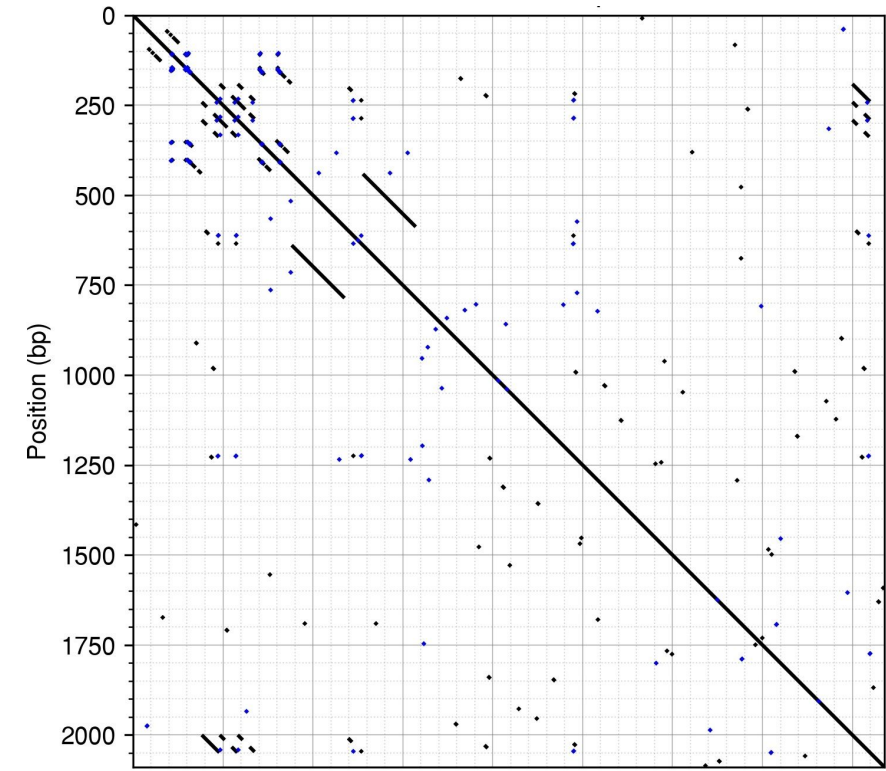


# Twist Can Now Synthesize Otherwise “Impossible Genes” With The Following Complexities (Part 2):

Long terminal repeats

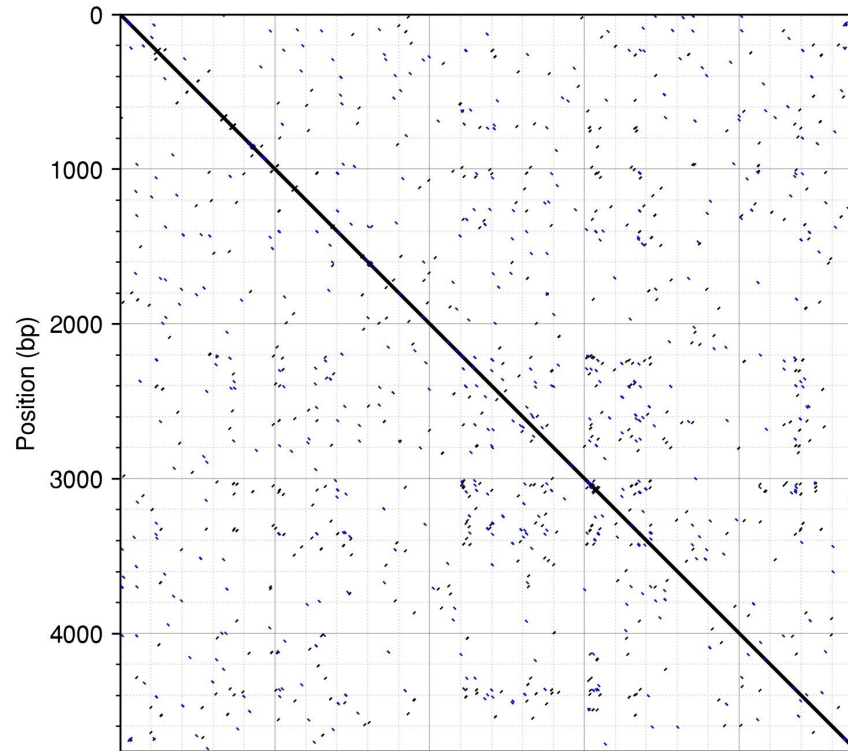


Long internal repeats and tandem repeats

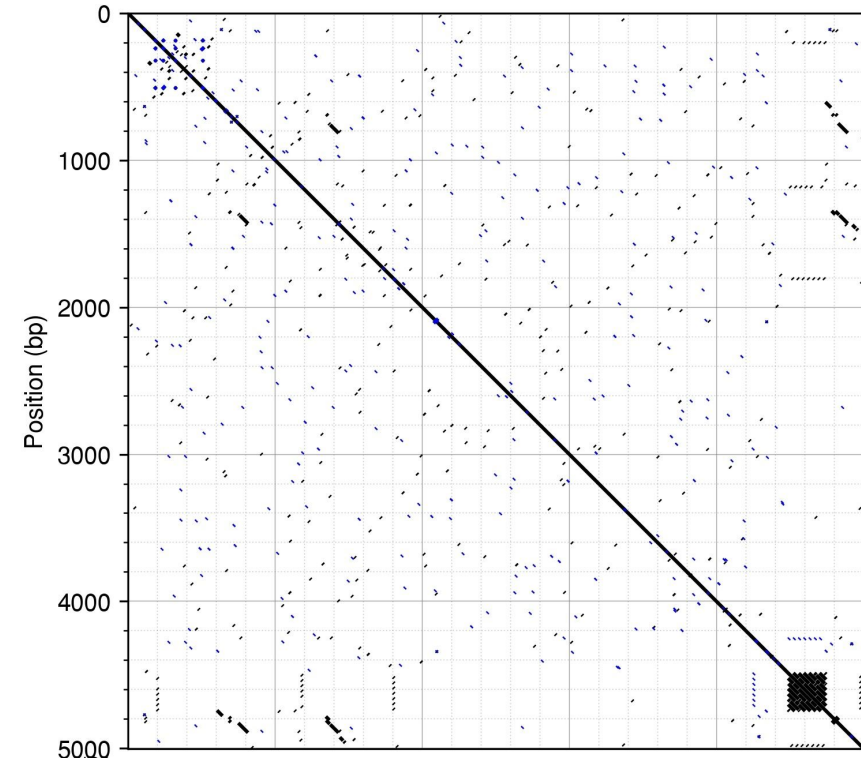


# Twist Can Now Synthesize Otherwise “Impossible Genes” With The Following Complexities (Part 3):

Clusters of repeats



Clusters of long repeats



# Reliable Synthesis for Complex DNA

## PROBLEM

1. Users have **little choice** on complex clonal DNA providers in the market.
2. Current providers say “yes” with **unclear timelines**. Sequences in the same order can take weeks to months.
3. Providers make their complex sequences in China, leading to **IP security concerns**.

## OUR PLATFORM

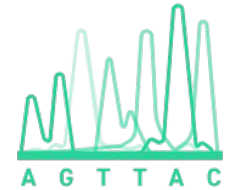
Ultra-long  
oligo synthesis



Enzymatic  
assembly



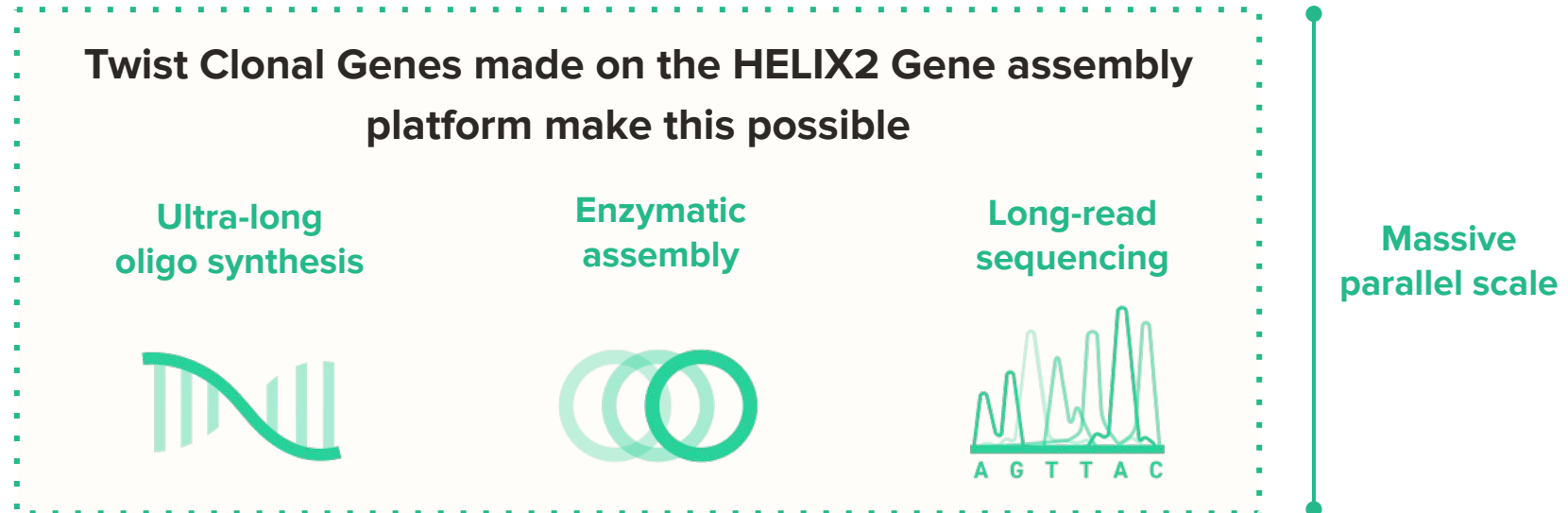
Long-read  
sequencing



1. With our synthesis platform, our Clonal Gene Synthesis workflow can reliably deliver sequence perfect clonal genes to users in 4-15 business days, **regardless of sequence complexity or order scale**.
2. Users have the best shot on goal to make every gene sequence they need in **predictable timescales** by coming to Twist Bioscience.

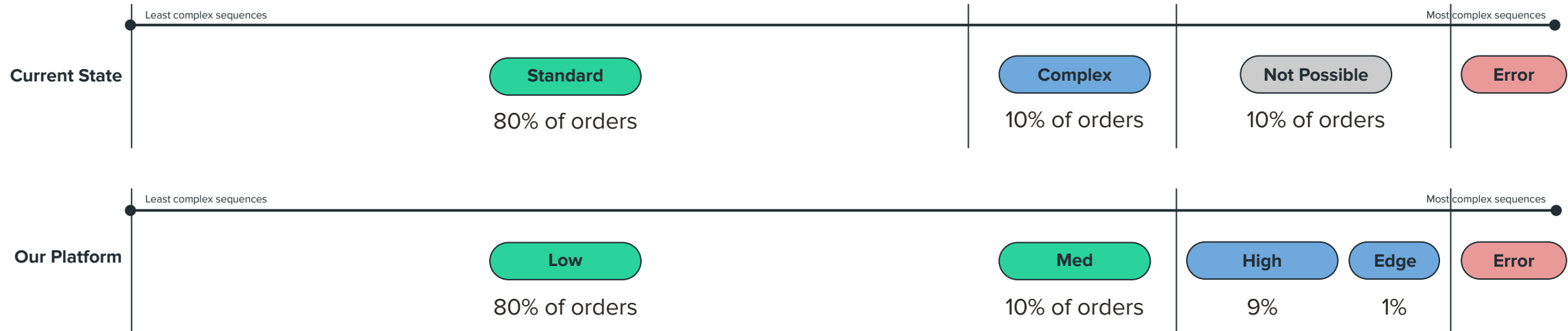
# Twist Clonal Gene Synthesis Overview

Twist provides a US-based gene synthesis ecosystem that integrates seamlessly and affordably into users experimental workflows and supply chain. With Twist Clonal Genes, users can expect **100% accurate, cloned DNA delivered in 4-15 business days, regardless of order scale or complexity.**



# Framework

- Our updated platform enables us to accept “almost everything” customers need, and allows us to deliver every gene we accept in a reliable turnaround time.
- We don’t market as the product. The product is Clonal Genes and is the proof and validation of the expanded complex synthesis capabilities.





# Complex Motifs We Can Now Make

## Sequences with Short Tandem Repeats



**Definition:** Short sequences of DNA (3-9 base pairs) that repeat multiple times in a head-to-tail manner, with up to 100 % of homology between repeats.

**Synthesis Limitations:** We can make up to 100 bp

**Applications:** Synthesis of specific runs of CAG repeats in Huntingtin protein. GS Linkers in proteins.

## Long Repeats



**Definitions:** Regions  $\geq 10$  bp and 100% homology that repeat more than once within a single sequence

**Synthesis Limitations:** We can make long repeats of 200 bp

**Applications:** Neoantigens, Protein engineering, mRNA secondary structure design

## Inverted Repeats (hairpins)

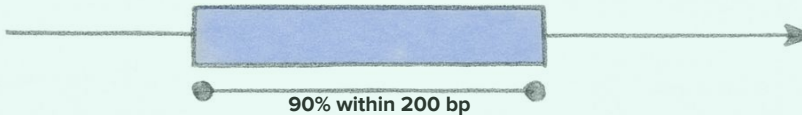


**Definitions:** Repeats complementary to each other (repeats forwards, then backwards), with up to 100% of homology between repeats.

**Synthesis Limitations:** We can make inverted repeats up to 150 bp long

**Applications:** functionalized exonic sequences, functional mRNA, DNA origami, effectors, promoters

## High/LOW GC/AT content



**Definitions:** Local GC content  $\geq 90\%$  or  $\leq 10\%$

**Synthesis Limitations:** local GC content within a 50 bp window between 10% and 90%

**Applications:** Nuclear transfer sequences, functional mRNA

## Homopolymers



**Definitions:** Runs of an identical bases up to 30bp..

**Synthesis Limitations:** We cannot make sequences that have runs of an identical base longer than 30bp

**Applications:** Poly-A tails

... and Genes up to 7 kbp!



# Let Twist Build Genes For You!



## Your Genes, Your Way

You design what you need whether it's ready to clone Gene Fragments or ready to use Clonal Genes, with options for various prep scales, endotoxin free or normalization we can get you your DNA your way



## Industry Leading Price and Performance

Low synthesis cost enables more science, and performance ensures experimental success, saving you time and money so you can accelerate your research.



## Scalable Synthesis

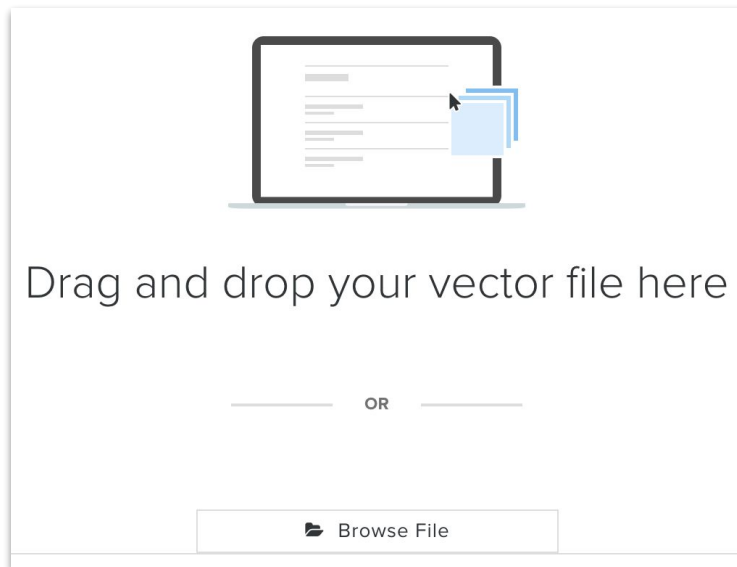
From one gene to a million we offer a scale of synthesis that is unmatched. Order as few or as many genes as you need.



# Easy Online Custom Vector On-Boarding


1

Simply input your vector sequence



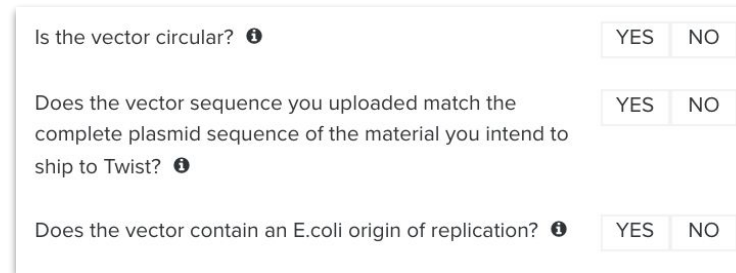
Drag and drop your vector file here

OR

 Browse File

2

Provide some information about the vector



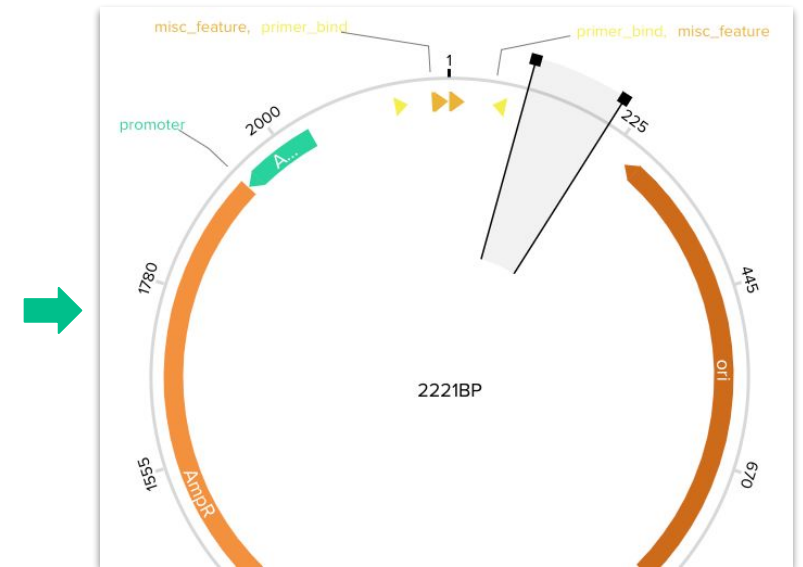
Is the vector circular? ⓘ YES NO

Does the vector sequence you uploaded match the complete plasmid sequence of the material you intend to ship to Twist? ⓘ YES NO

Does the vector contain an E.coli origin of replication? ⓘ YES NO

3

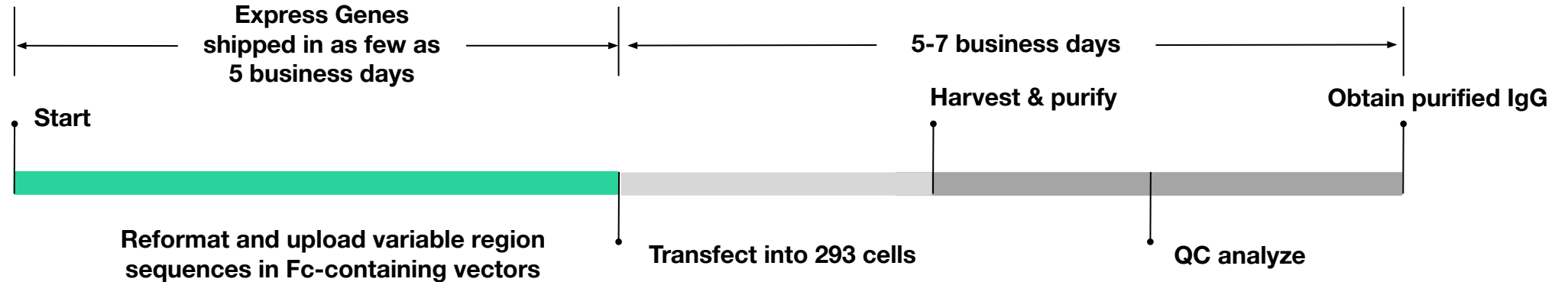
Select your insertion site



# Twist Express Genes Service | Enabling Faster Applications

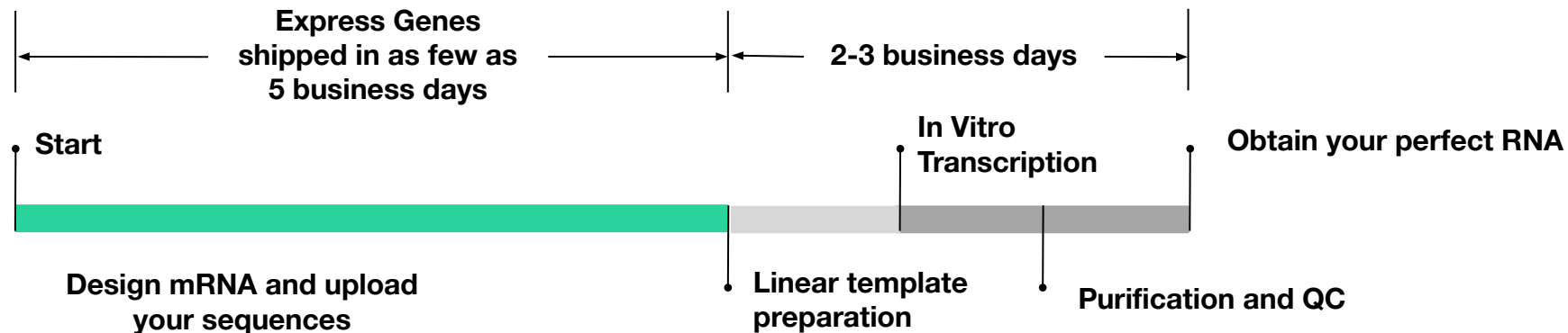
## Antibody Expression

Obtain your perfect IgG in as few as 10 business days



## mRNA Production

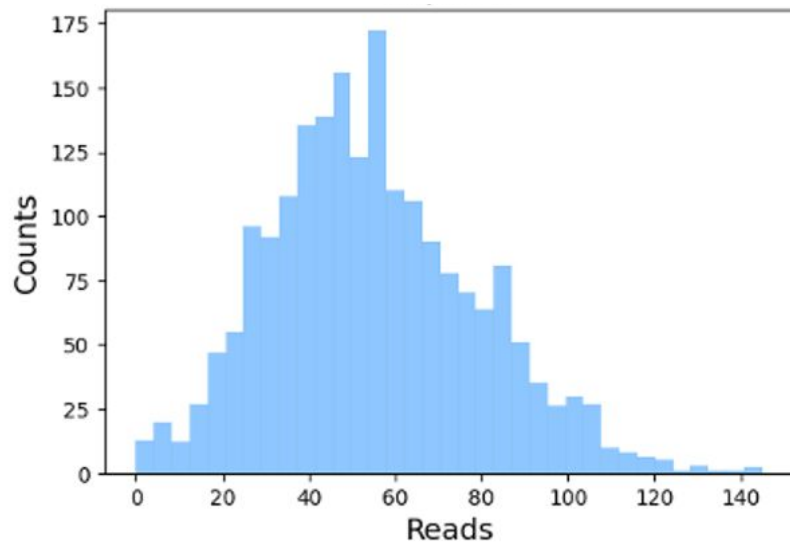
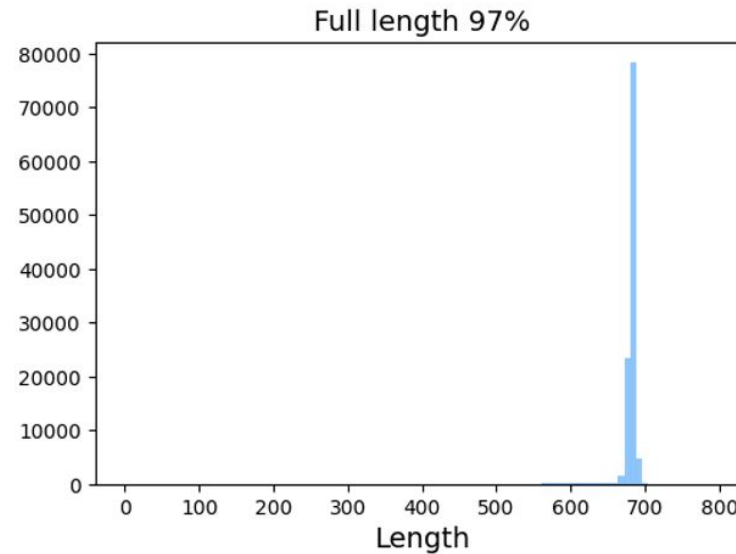
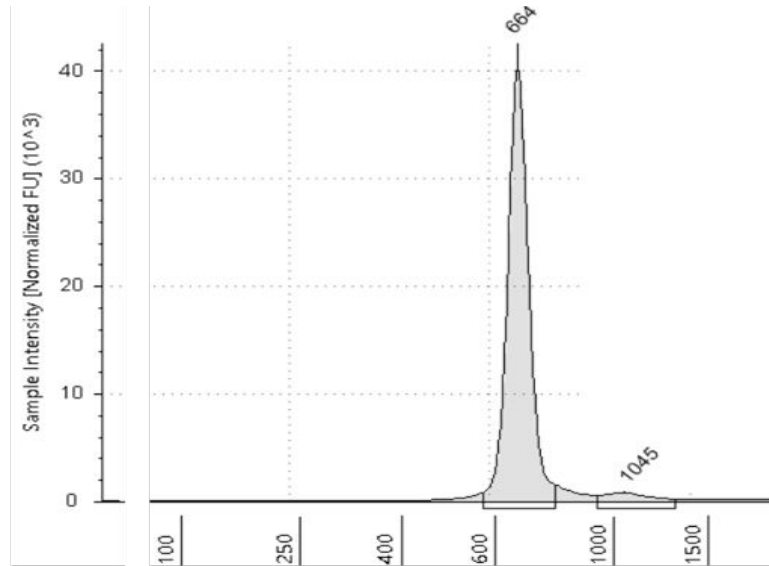
Obtain your perfect mRNA in as few as 7 business days



\*Turnaround time for Express Genes starts at 5-7 business days and increases to 8-12 business days for 10 µg - 100 µg and 100 µg - 1 mg DNA prep scales. Onboarding your own custom vector will incur 1-2 weeks additional turnaround time.

# Oligos and Multiplexed Gene Fragments

# We Continue to Push the Boundaries of Oligo Synthesis

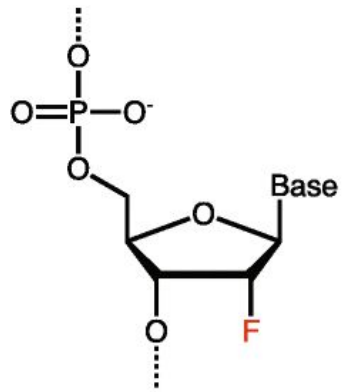


**With enhanced chemistry, we demonstrated the direct synthesis of **700mer** for the first time:**

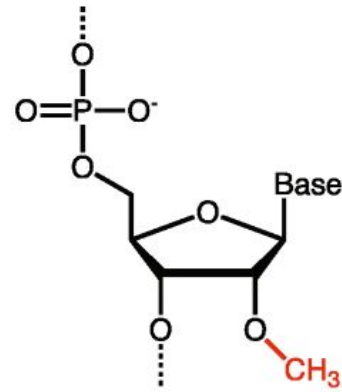
- Sharp and clean peak on fragment analysis
- 97% full length material per Nanopore sequencing analysis
- Uniform distribution among all oligos

# We Now have Validated Chemistry With Modified Nucleotides

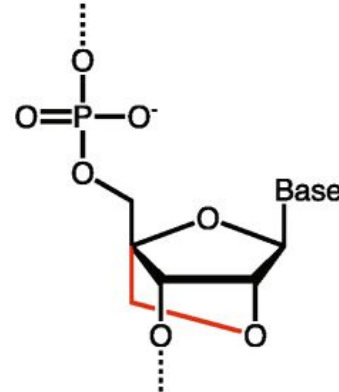
2'F RNA



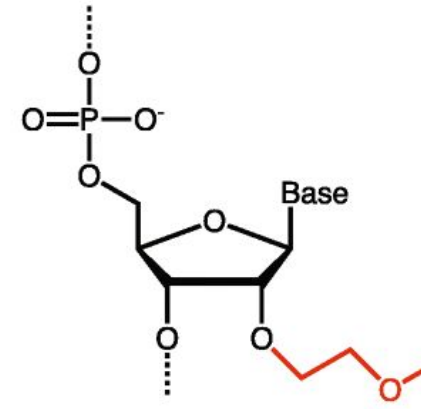
2'OMe RNA



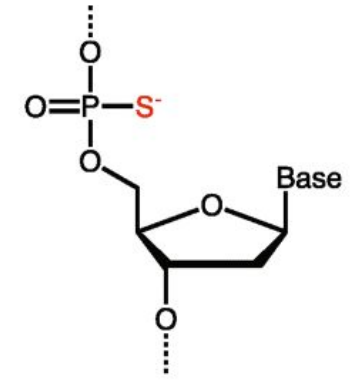
LNA



2'MOE



PS



Synthesis chemistry developed for modified nucleotides

Ability to synthesize ASO or siRNA, millions of them with various modifications

Quality of the synthesis measured by electrophoresis, NGS, and MS

Tag each modified sequence with unique barcode and/or molecular barcode (UMI) for NGS readout



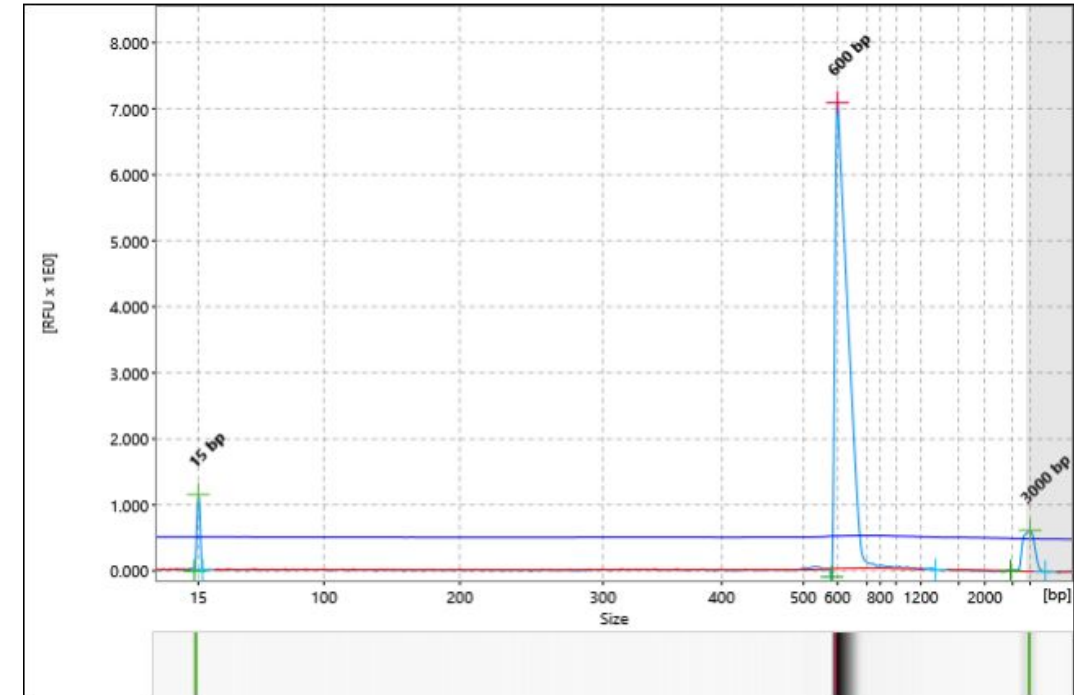
# Sometimes The Limitation is PCR, Not Synthesis

- Chimera formed during PCR of a pool
- GC bias during the amplification. Even 10% efficiency difference per cycle will make a large difference in final product

New solution:

Gene pools:

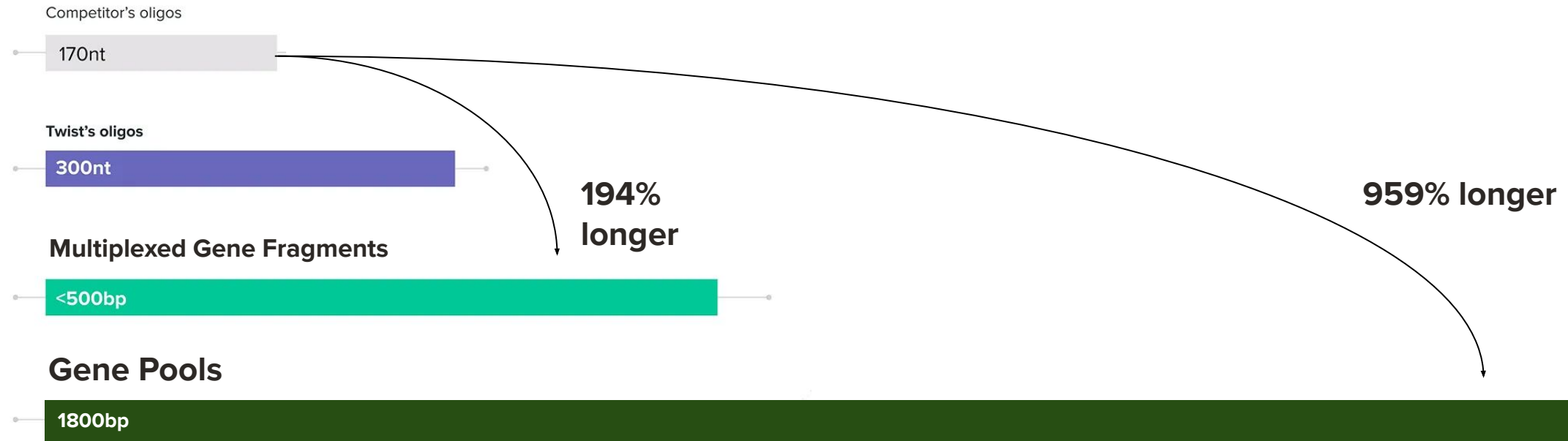
- Individually assembled gene fragments
- Pooled purification
- 1 in 3,000 bp error rate. Absolutely zero chimera artifacts
- Only Twist has the capacity to process tens of thousands assemblies



25,000 unique but highly similar 650bp sequences for TCR **zero** chimera

# Going From 7 cents Per Base, to 7 Bases Per Cent

Gene Pools up to 1.8kb available on early access



# Expanding What's Possible With An Oligo Pool

## Degenerate Bases and Mini-Pools

Degenerate base N is now accepted

N is equal mixture of A,T,C,and G

CGTTCCGCGC**NNNNN**NTGTTTCGTTAACTGTTGA

CGTT**N**CGCGCTCGTT**N**ATGTTTCGTTAACT**N**TTGA

T

OLIGO POOL PLATES

Oligo draft

IMPORT SEQUENCES

DESIGN SEQUENCES

SETTINGS

All Plates · Plate 1 of 4 < > View all plates v

Plate name

Plate

List

Download

Import

Sequence count

0

1-19

20-39

40-59

60-79

80-99

100-121

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
B	32	32	32	32	32	32	32	32	106	106	106	32	32	32	32	32	32	32	32	106	106	106	32	32
C	32	32	-	32	-	64	88	88	-	106	106	32	32	32	-	32	-	64	88	88	-	106	106	32
D	32	32	45	64	64	32	88	88	106	106	106	32	32	32	45	64	64	32	88	88	106	106	106	32
E	32	32	-	32	64	-	88	88	106	106	-	32	32	32	-	32	64	-	88	88	106	106	-	32
F	32	32	45	32	64	32	88	88	-	106	106	32	32	32	45	32	64	32	88	88	-	106	106	32
G	32	32	32	32	32	32	32	32	106	106	106	32	32	32	32	32	32	32	32	106	106	106	32	32
H	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14

Well name G13

X

Sequences

Longest

Shortest

Average

120

350 bp

350 bp

350 bp

Coding Sequence ⓘ

BP ⓘ

AGCTGTCAGTACGTAGATGACGACATC...

350

AGCTGTCAGTACGTAGATGACGACATC...

350

AGCTGTCAGTACGTAGATGACGACATC...

350

AGCTGTCAGTACGTAGATGACGACATC...

350

AGCTGTCAGTACGTAGATGACGACATC...

350

AGCTGTCAGTACGTAGATGACGACATC...

350

Mini-Pools allow you to assign up to 121 sequences per well in a 384-well plate

# Precision Oligo Pools

The highest quality DNA input for your screen.

● **Get more space  
for innovation**

Get the space to do more with oligos up to 300 nucleotides, without pool size limits.

● **Screen once,  
screen right**

Our oligo pool's exceptional uniformity help improve your signal to noise ratio and reduce oversampling burden.

● **Be data  
confident**

Exceptionally low error rates and high full-length representation ensure that the pool you design is the pool you get.

Oligo Length	Up to 350 nt
Oligo Pool Size	No minimum, no maximum
Cloning Available	Yes
Oligo Pool Yield	>0.2 fmol average of each ssDNA oligo
Cloned Oligo Pool Yield	Starts at 50 µg total plasmid DNA
Uniformity	>90% oligos represented within <2.0x of the mean
Error Rate	Up to 1:3000

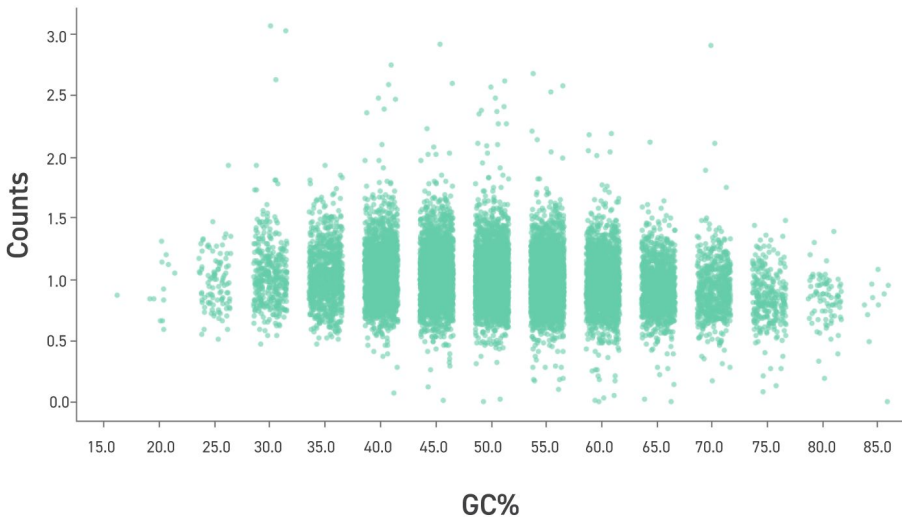
**Turnaround Time** 4 business days\*

*\*4 business days for Twist Oligo Pools up to 350 nt is based on internal data available as of 2025. This timeframe refers to the typical processing and handling time within our facilities before your order is handed over to the shipping carrier. Actual delivery times will vary depending on your location, the chosen shipping method, and the carrier's handling procedures.*

## We can clone oligo pools for you.

Don't waste time optimising complex pooled cloning! Have us clone your oligo pool instead and be screen-ready in weeks.

Our pooled cloning workflow has been refined to remove GC bias, maintain high uniformity and produce minimal dropouts.



# Oligo Based Therapeutics

## Introduction to Oligo-based Therapeutics

- Therapy to regulate gene transcription and protein expression
- Rapidly designed and produced, especially for newly identified targets

### Small RNA

siRNA, ASO, aptamer

Among approved drugs

- 60% ASO
- 30% siRNA
- 10% aptamer

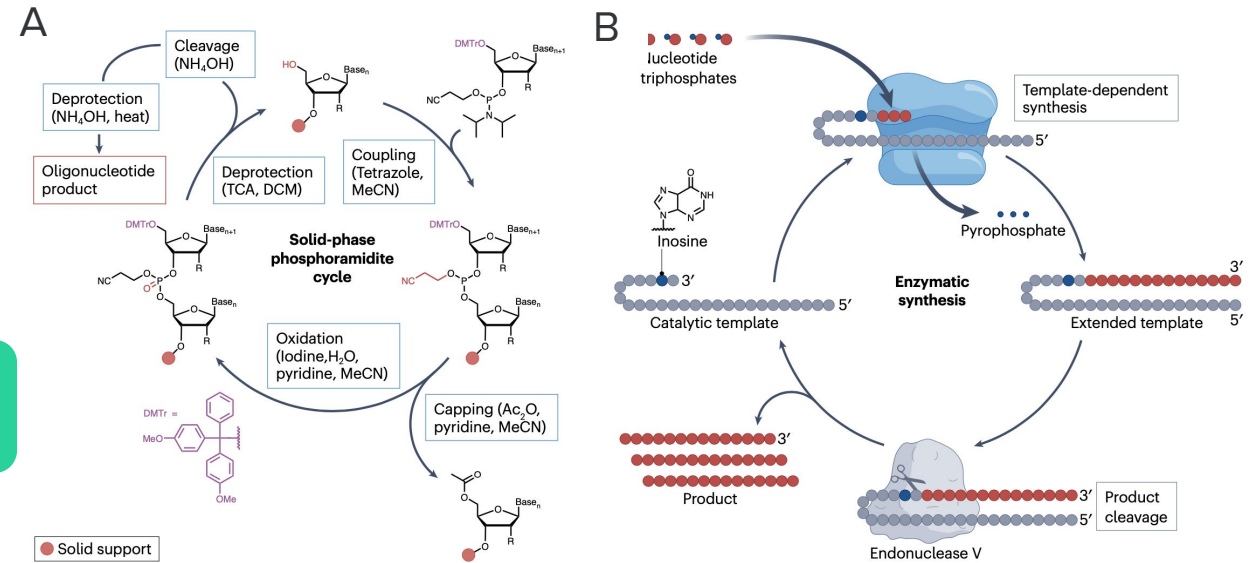
### Translatable RNA

mRNA, circRNA, saRNA

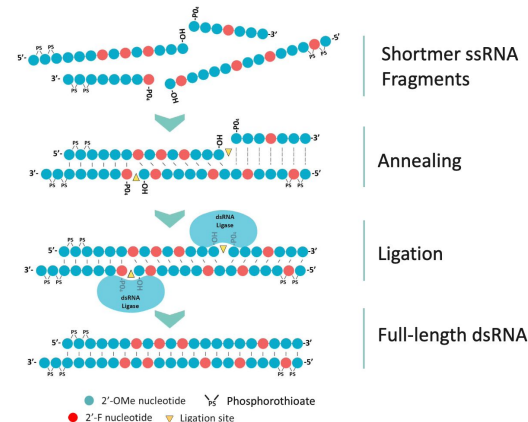
- Only COVID and RSV vaccine approved
- Personalized cancer vaccine

### CRISPR gRNA

guide RNA

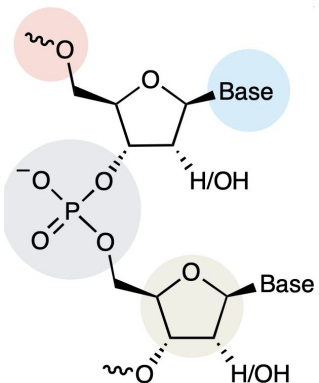


### C Fragment Ligation for siRNA Synthesis



### Modifications:

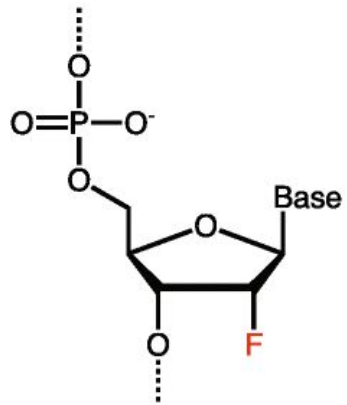
- Improve stability
- Increase binding affinity
- Decrease inflammatory response



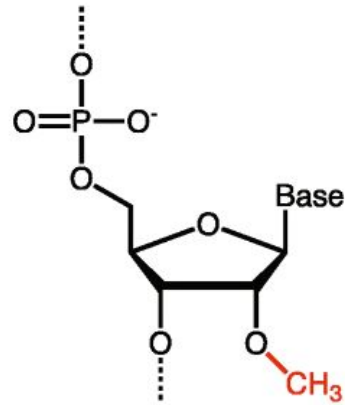
- Chemical synthesis on solid support
- Enzymatic synthesis via extension (TdT or KOD polymerase)
- Enzymatic synthesis via ligation (T4 DNA ligase)

# Validated Chemistry with Modified Nucleotides

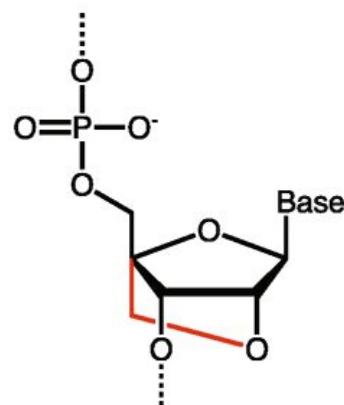
**2'F RNA**



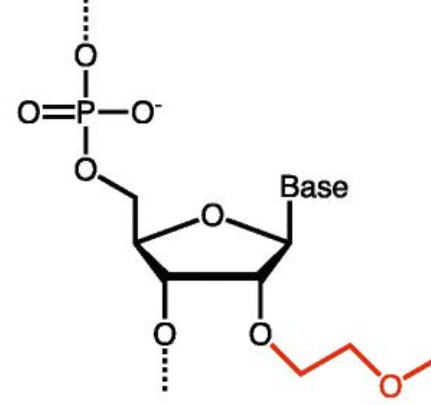
**2'OMe RNA**



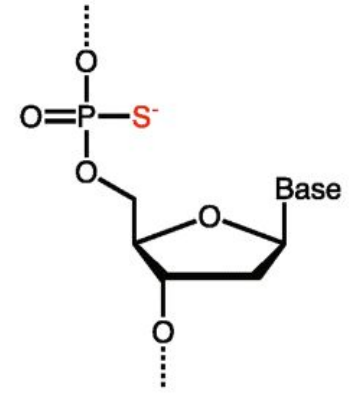
**LNA**



**2' MOE**



**PS**



- Synthesis chemistry developed for modified nucleotides
- Quality of the synthesis measured by electrophoresis, NGS, and MS
- Ability to synthesize ASO or siRNA portion on its own
- Tag each modified sequence with unique barcode and/or molecular barcode (UMI) for NGS readout



# Twist Oligo Pools for High Scale with Superior Performance

**Industry leading error rates and scalability. From hundreds to millions of oligos.  
The only limit is your imagination.**



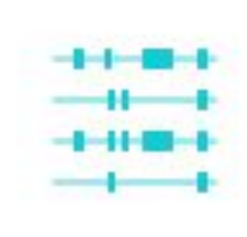
## **Precision Synthesis**

Exceptionally low error rates ensures the highest representation and full length pools while ensuring sequence integrity so the pool you design is the pool you get.



## **Maximized Screening Performance**

Exceptional uniformity improves signal to noise and enhances screening efficiency



## **Flexible Length and Pool Sizes**

Space to do more, longer homology, bar codes, structural motifs etc. Build a pool to meet your needs from a few hundred to millions of oligos.

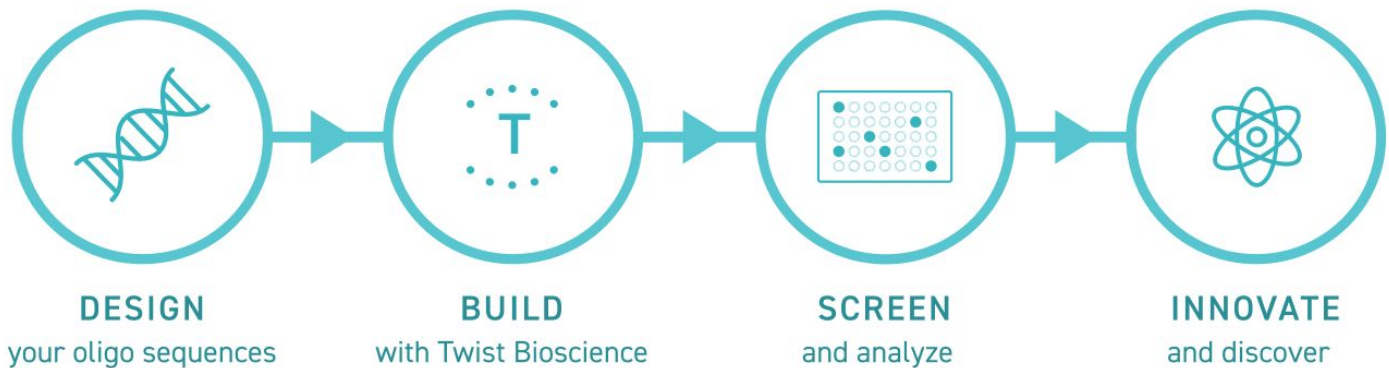
# Enhanced Oligo Pool offering

## Oligo Pools

Oligo Length	Up to 300nt
Oligo Pool Size	No limits on pool size
Yield	>0.2 fmol average of each oligo
Uniformity	>90% oligos represented within <2.0x of the mean
Error rate	Up to 1:3000
Turn-Around Time	As few as 3 days*
Price	Industry-leading pricing

## Cloned Oligo Pools

Oligo Length	Up to 250 nt for variable region (up to 300nt total including primer sites)
Oligo Pool Size	No limits on pool size
Yield	Up to 250µg of plasmid DNA
Uniformity	>90% oligos represented within <5x of the mean
Chimera rate	As low as 1.5%*
Turn-Around Time	As few as 4 weeks*
Price	Industry-leading pricing

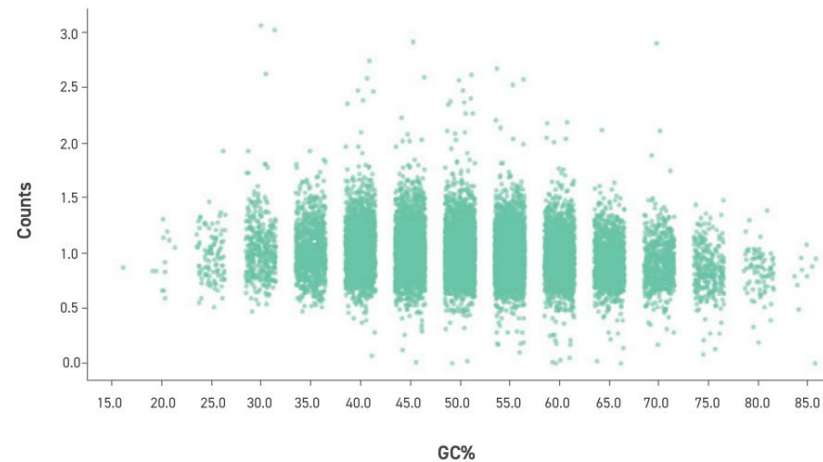


\*Turnaround time for the standard Oligo Pool offering varies based on length and sequence complexity. Oligo Pools at 20-121 nucleotides in length range from 3-6 business days, 121-200 nucleotides in length Baseline turnaround time for Cloned Oligo Pools, which includes cloning and amplification, of up to 100 and 300 nucleotides is 4-6 weeks and 6-8 weeks, respectively. Chimera rate, drop out rate, and uniformity will vary based on sequence complexity. range from 3-8 business days, and 201-300 nucleotides in length range from 5-10 business days.

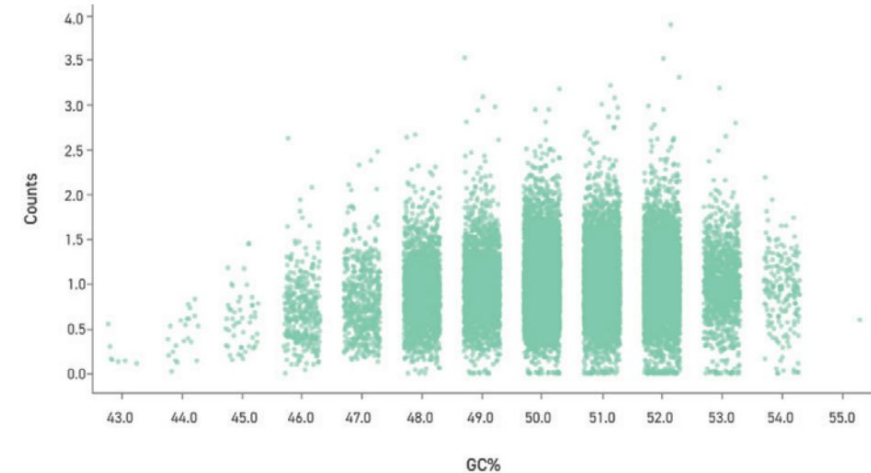
# Cloned Oligo Pools

## Maintained uniformity, regardless of GC

- GC plots of the two Oligo Pools from Figure 1 were created and show high GC content of each oligo (x-axis) and the normalized count for that particular oligo (y-axis) after amplification and cloning.
- Although, both Oligo Pools (2A and 2B) contain high GC content, limited bias with regards to oligo integration in relation to GC content, as well as low dropout rate and high uniformity were achieved.



**2A.** Uniformity for Cloned Oligo Pools that are comprised of sequences of 141 nucleotides in length with high GC content. Percentile: 2.08, Dropouts: 1



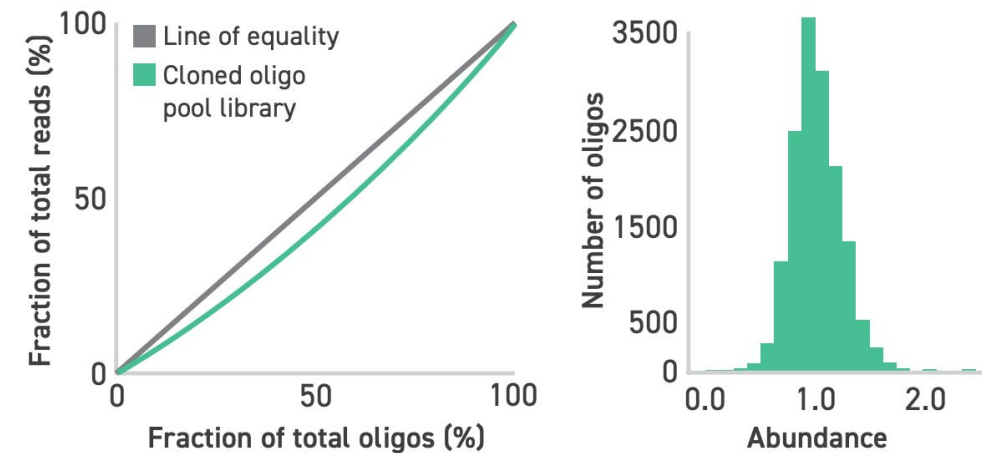
**2B.** Uniformity for Cloned Oligo Pools that are comprised of sequences of 300 nucleotides in length with high GC content. Percentile: 4.31, Dropouts: 77

# Cloned Oligo Pools

Uniformity is maintained regardless of length

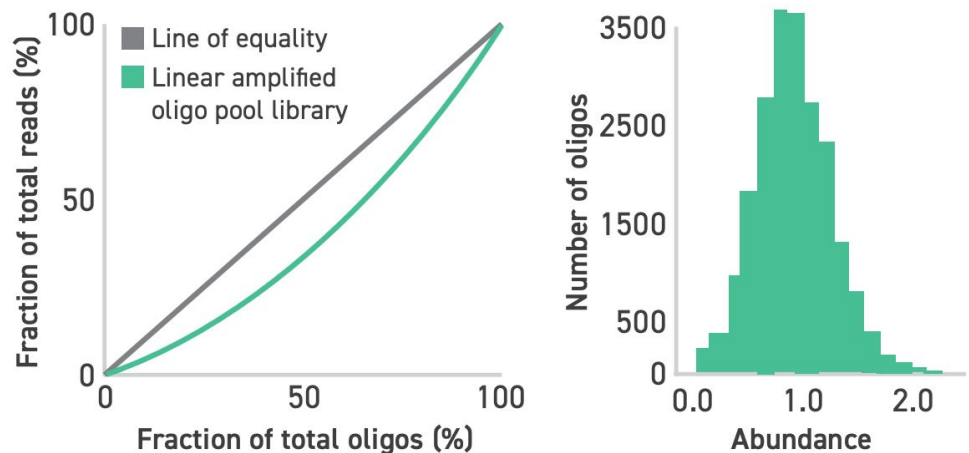
- Normalized read count of each of the mapped variants (X-axis) for a Cloned Oligo Pool of 141 nts (1A) and a Cloned Oligo Pool of 300 nts (1B)
- Abundance converging to 1 indicates that the Cloned Oligo Pools are distributed uniformly

1A. Cloned Oligo Pool Library Uniformity



% Oligos represented without error	96%
Dropouts	0%
Diversity	15154
95th/5th Percentile	2.08
90th/10th Percentile	1.7
Full Length Percentage	99%

1B. Cloned 300mer Uniformity: Dual Guide CRISPR Library

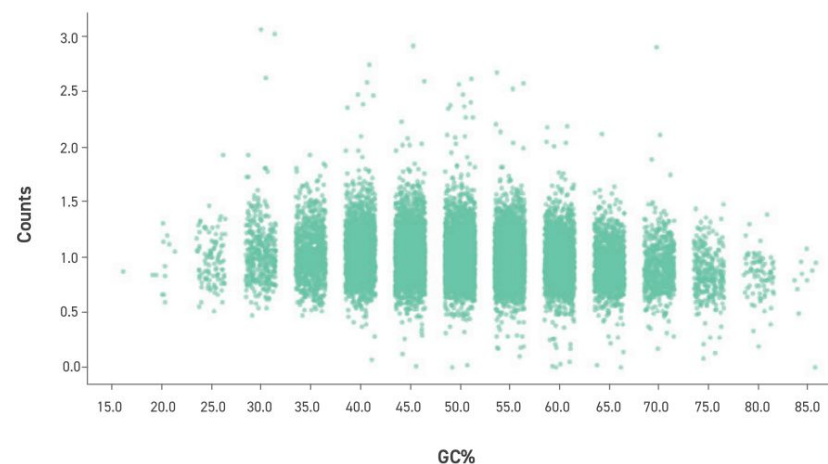


% Oligos represented without error	96%
Dropouts	0.204%
Diversity	21,554
95th/5th Percentile	4.31
90th/10th Percentile	2.92
Full Length Percentage	94.65%
Chimera Rate	3.20%

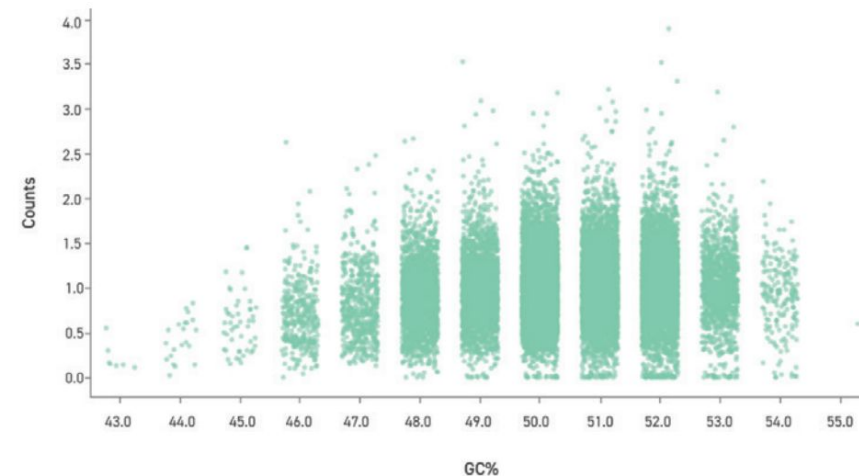
# Cloned Oligo Pools

## Maintained uniformity, regardless of GC

- GC plots of the two Oligo Pools from Figure 1 were created and show high GC content of each oligo (x-axis) and the normalized count for that particular oligo (y-axis) after amplification and cloning.
- Although, both Oligo Pools (2A and 2B) contain high GC content, limited bias with regards to oligo integration in relation to GC content, as well as low dropout rate and high uniformity were achieved.



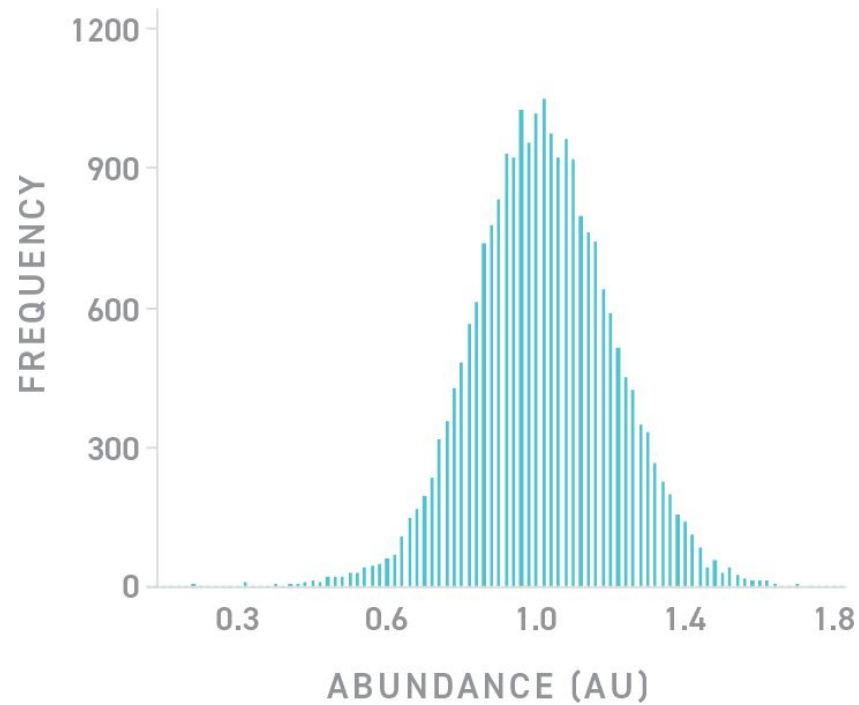
**2A.** Uniformity for Cloned Oligo Pools that are comprised of sequences of 141 nucleotides in length with high GC content. Percentile: 2.08, Dropouts: 1



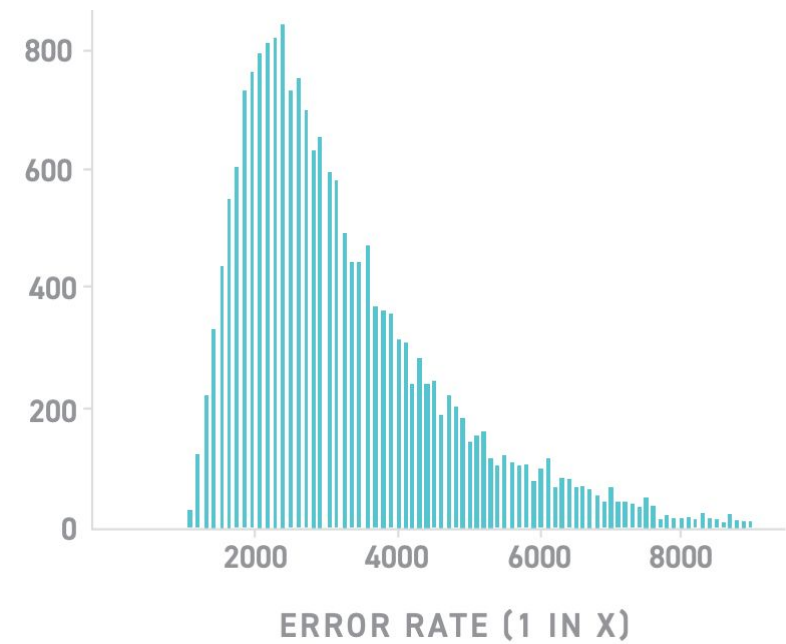
**2B.** Uniformity for Cloned Oligo Pools that are comprised of sequences of 300 nucleotides in length with high GC content. Percentile: 4.31, Dropouts: 77

# Oligo Pools Performance Advantages

## High Uniformity with 100% Oligo Representation

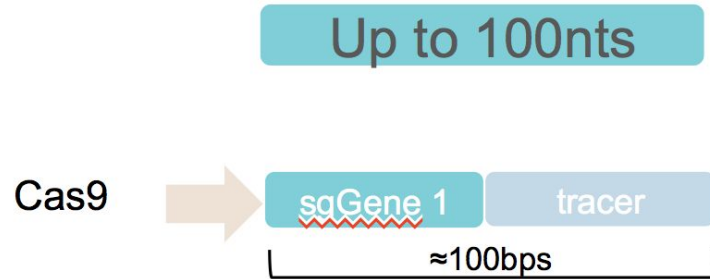


## High Quality 1:3000 Base Pair Error Rate





# Longer Oligos Allow for Cutting Edge CRISPR Screens

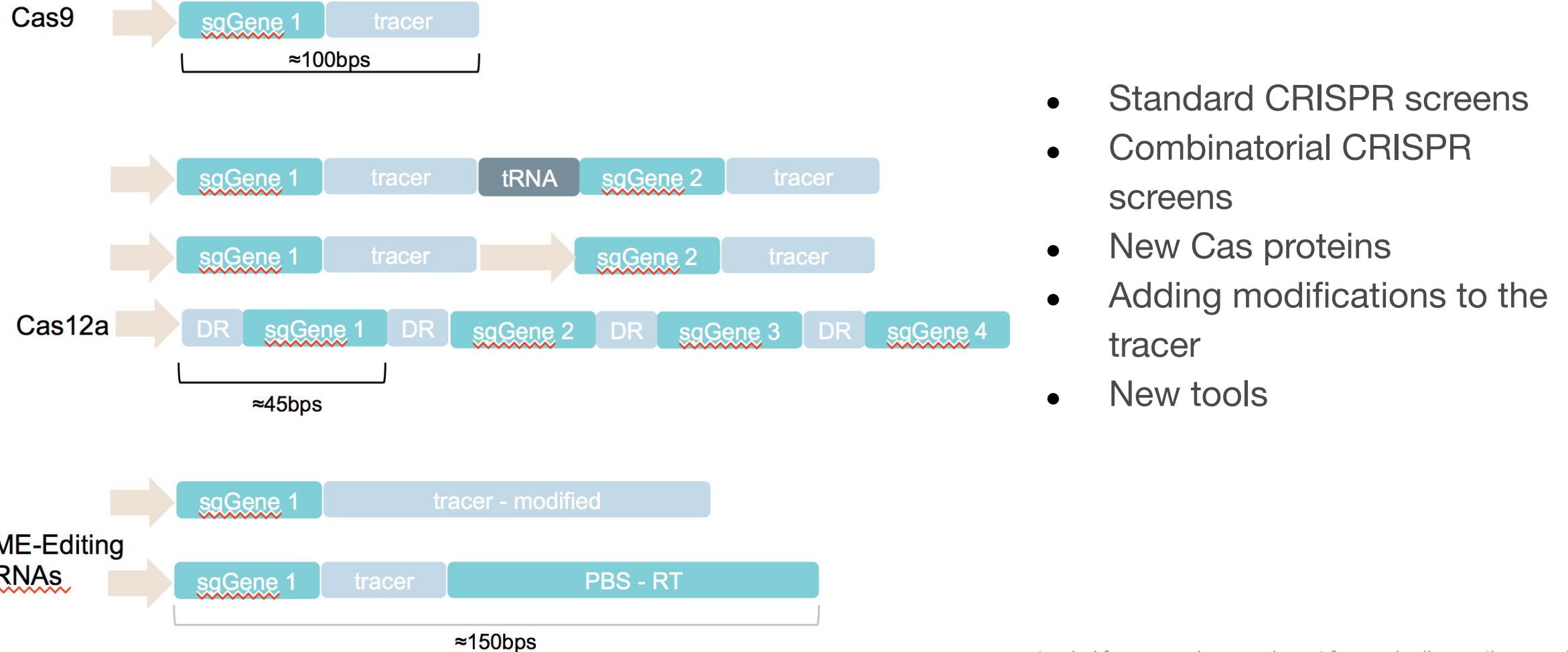


- Standard CRISPR screens

# Longer Oligos Allow for Cutting Edge CRISPR Screens

Up to 300nts

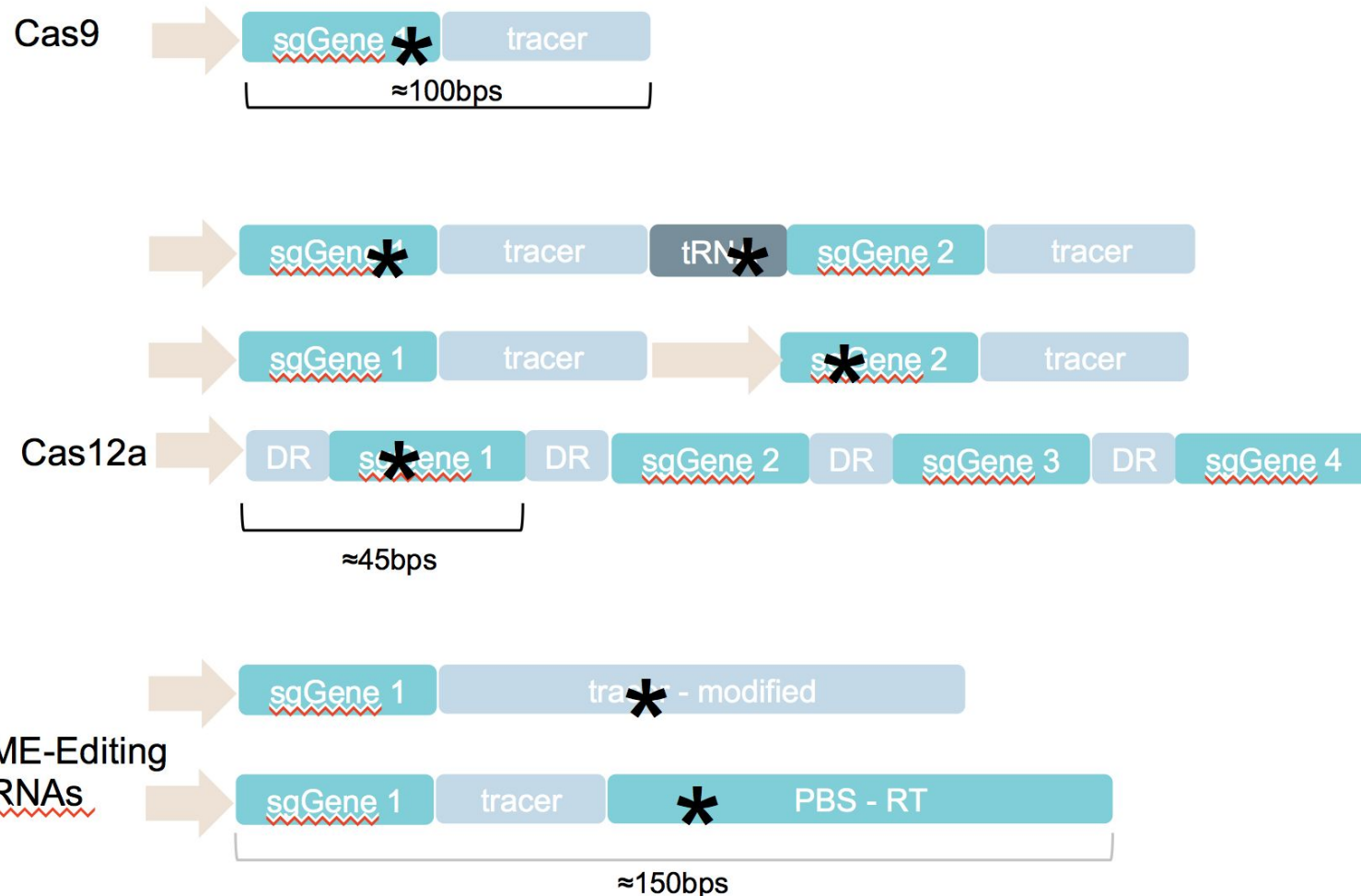
Error Rate: 1:3,000 nt



# Longer Oligos Allow for Cutting Edge CRISPR Screens

Up to 300nts

Error Rate: 1:3,000 nt vs 1:200nt



- Standard CRISPR screens
- Combinatorial CRISPR screens
- New Cas proteins
- Adding modifications to the tracer
- New tools

# Applications requiring even longer oligos: AI/ML Antibody, mRNA, CRISPR discovery and experimentation

Typical Pooled Oligos between 300-350 nucleotides



**Synthesizing full variable regions (VH or VL) requires multiple fragments to assemble**



**MPRA designs are forced to leave out sequences that would provide true genetic context**

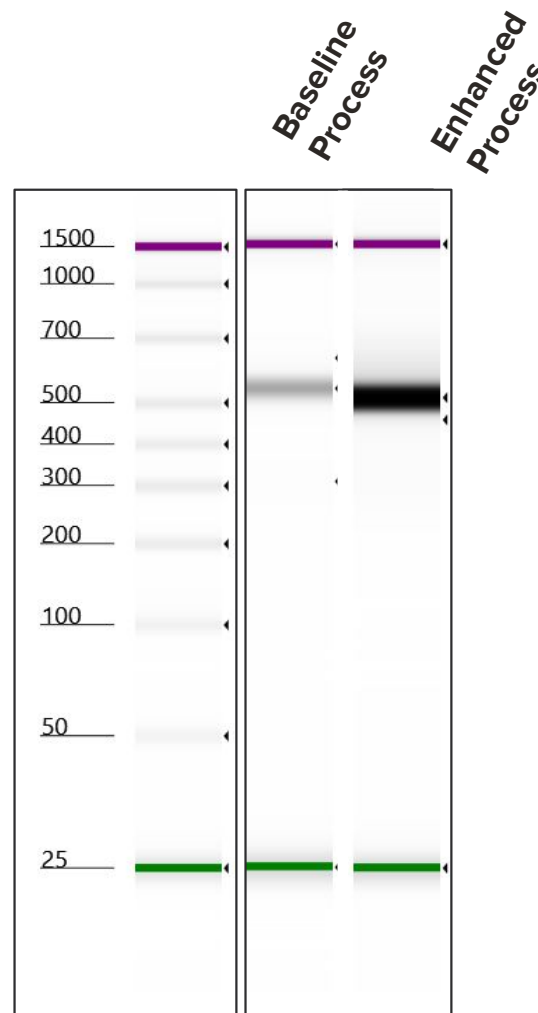
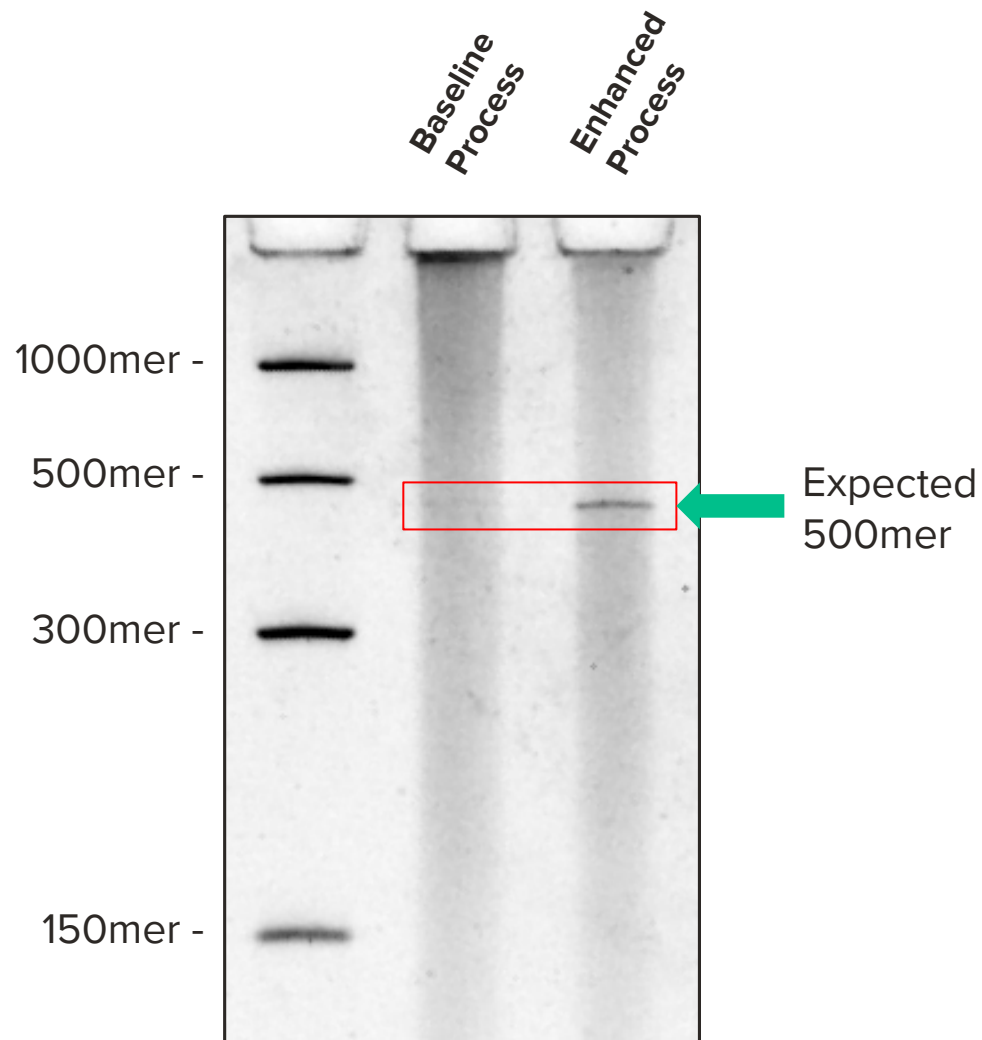


**Ultra-complex CRISPR Screens**



Multi-guide designs require assembly and cannot be synthesized entirely for research use only; not for use in diagnostic procedures

# We have made continued chemistry improvements for Multiplexed Gene Fragments

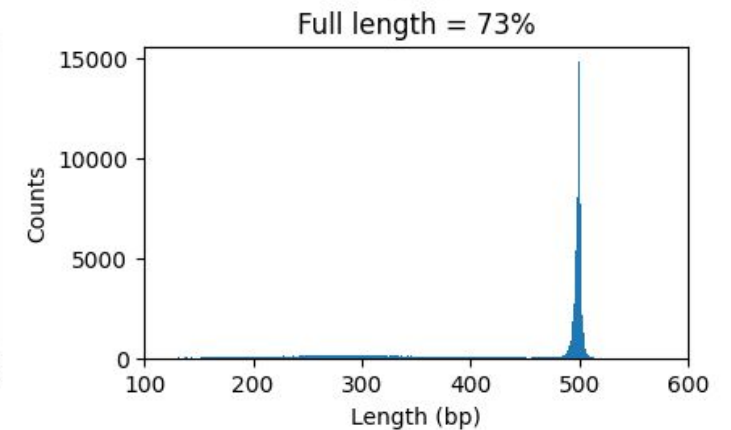
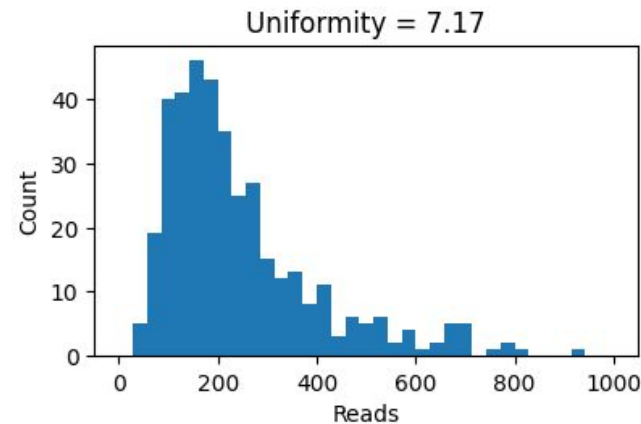
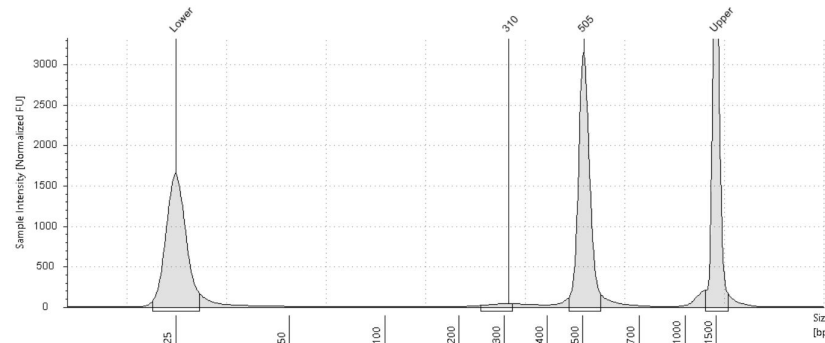


## With enhanced chemistry:

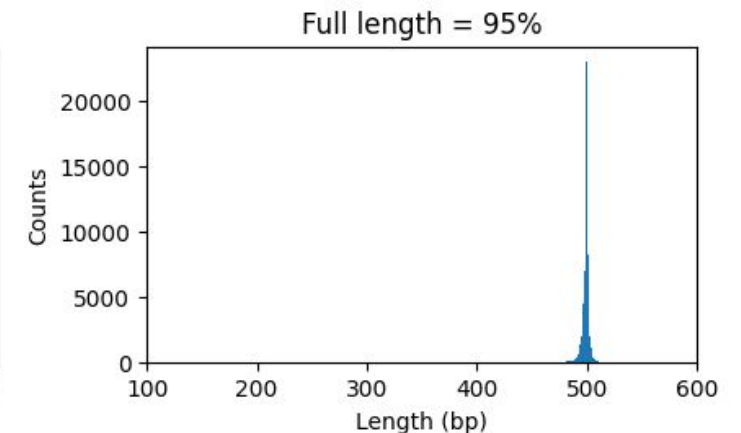
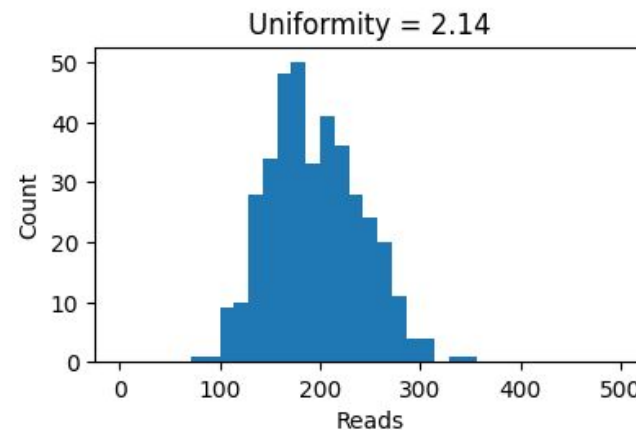
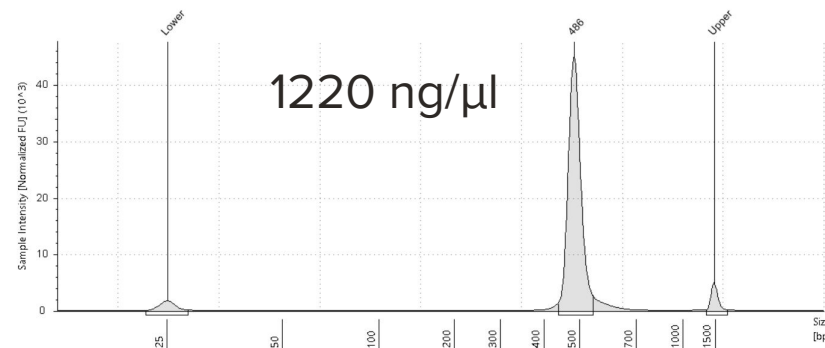
- Clear full length 500mer oligos on gel
- Maintaining 1 in 2,000 bp error rate
- >10 fold increase in PCR yield with better uniformity and more full length materials

# We Have Made Continued Chemistry Improvements for Multiplexed Gene Fragments

## Baseline Process:



## Enhanced Process:

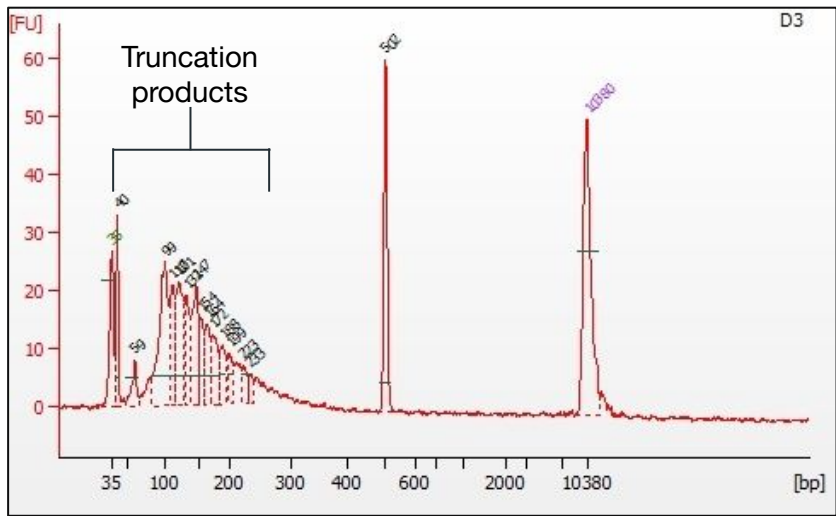


With enhanced chemistry, the PCR yield **increased by >10 fold** with better uniformity and more full length materials.

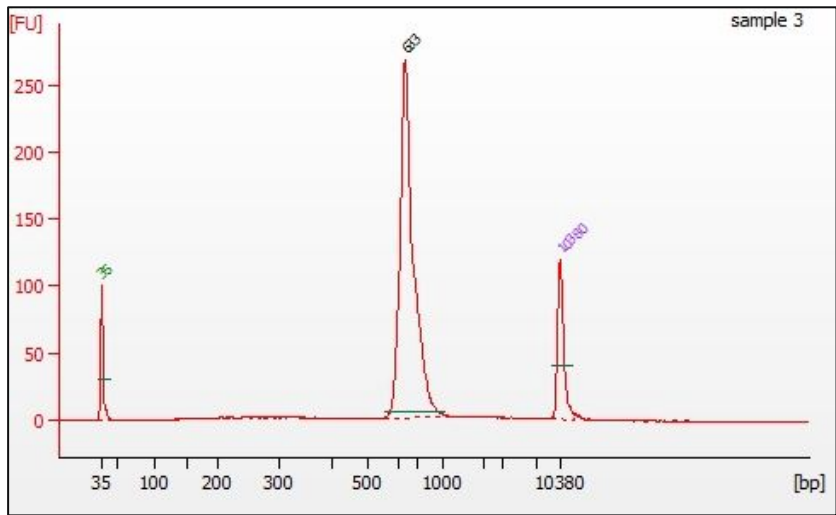


# Pushing the Boundaries of a 500 Nucleotide Synthesis

Recipe A

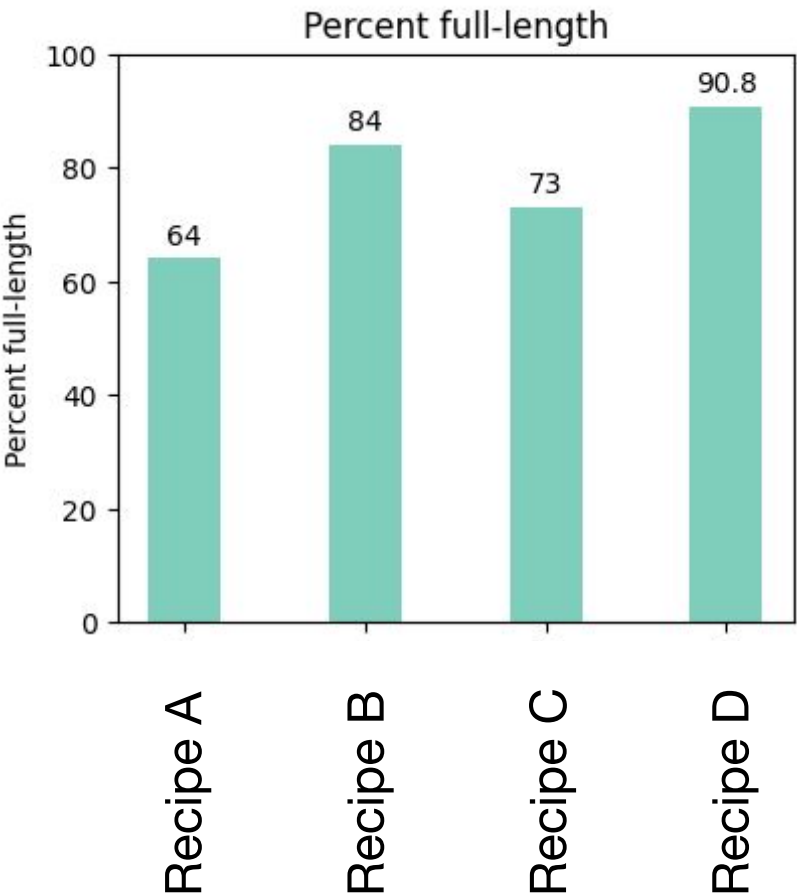


Recipe D



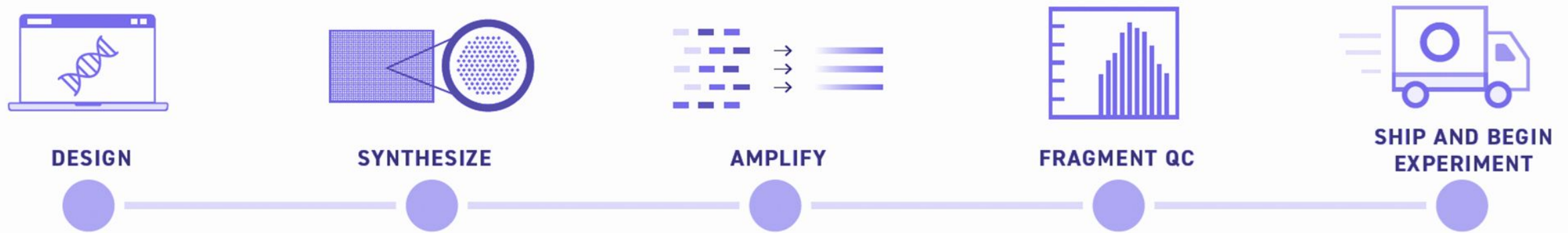
Post-amplification traces

ONT sequencing confirms higher full-length % post-amplification



# Multiplexed Gene Fragments

Twist's Multiplexed Gene Fragments (MGF) offer a unique pooled format for up to hundreds of thousands of double-stranded DNA gene fragments, between 301 and 500 base pairs in length. This pooled format of fragments enables a wide array of high-throughput screening applications.



Twist will synthesize, amplify, and QC your product. Our QC process for Multiplexed Gene Fragments includes fragment analysis to ensure purity, and that >90% of your pool is at the desired length.

# Multiplexed Gene Fragment Specifications

## Key Features:

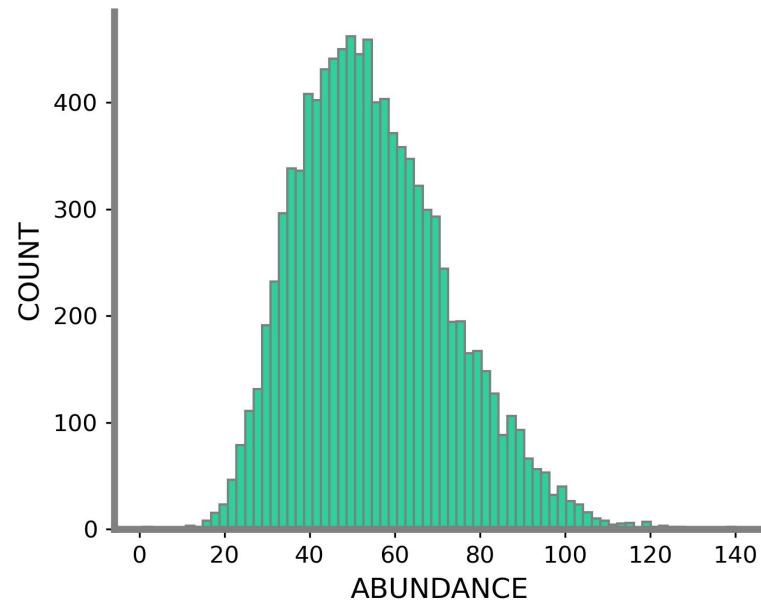
- **Length:**  
Unlimited scale at lengths that expand traditional applications and screens
  - Fit **entire antibody CDR regions** in each fragment sequence
  - Fit up to **4-6 tandem repeats** or **multiple guide RNA** for CRISPR screening
- **Low error rate:**  
Maximizes the quality of the fragment for sequence perfect full length pooled fragments
- **No complexity requirements:**  
Allows for highly complex sequences such as promoters, tandem repeats, homopolymers, and hairpins

<b>Yield</b>	Minimum of 200 ng amplified dsDNA
<b>Turnaround time</b>	8-12 business days
<b>Delivery Format</b>	Dried-down, dsDNA pooled in a tube
<b>Length</b>	301 - 500 bp
<b>Pool size</b>	<b>No limit to pool size</b> (Starts at 1,000 sequences, no maximum)
<b>Uniformity*</b>	<b>95th/5th = 3.0-3.5**</b> <b>90% of sequences are within ~3x of the mean</b> (pool type dependent)
<b>Quality Control (QC)</b>	Fragment analysis to ensure >90% of genes in a pool are the correct length*
<b>Error rate*</b>	1:2000 nt

\*Uniformity and error rate values are averages as calculated during product development. Product QC includes capillary electrophoresis and fluorescence-based DNA mass quantification (no sequencing QC). Results may vary depending on sequence composition.

For research Use Only. Not for Diagnostic Procedures.

# Quality and Scale without Compromise



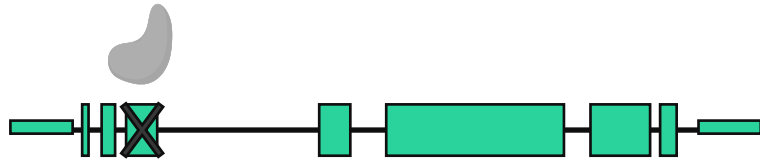
% Fragments represented	99.99%
Sequencing coverage	55X
Dropouts	0.01%
Diversity	10,000
95th/5th Percentile	3.03
90th/10 Percentile	2.35
Chimera Rate	5.5%

Twist's Multiplexed Gene Fragment pools achieve comprehensive representation of every sequence ordered, to ensure **precise control over variant construction** for more targeted and rational screening.

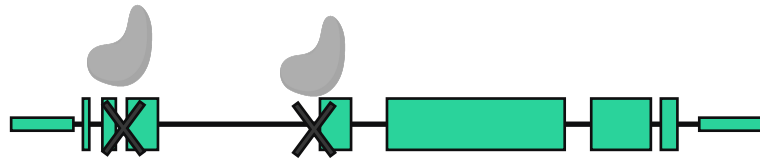
A pool of dual gRNA variants for an amplified Multiplexed Gene Fragment of 500 bp shows complete oligo representation with minimal dropouts.

# Benefits of Multiplexed Gene Fragments

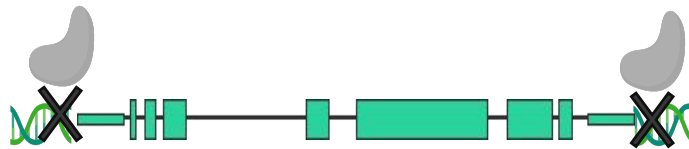
Normal knock-out



Dual knockout of the same gene: higher knock-out rate



Dual knockout outside gene to remove whole gene



## Maximize Targeting Efficiency & specificity

- Increases the probability of inducing a double stranded break at the intended loci which increases overall targeting efficiency and reduces off-target effects

## Induce larger deletions

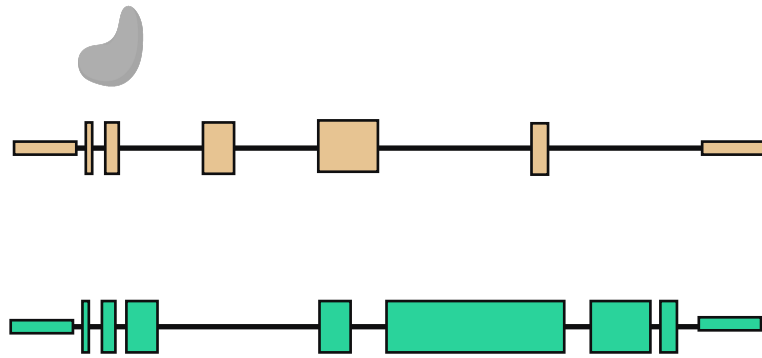
- Remove large regions of a gene or the entire gene for improved knockout efficiency

## Introduce combinatorial precise mutations

- Design paired pegRNAs to "write" precise mutations into the genome using combinatorial PRIME-editing

# Benefits of Multiplexed Gene Fragments

## Dual knockout two different genes



## Enable Multiplex Genome Editing

- Target multiple genes or regulatory elements within a cell or organism

## Enhanced Functional Studies

- Targeting of multiple gene loci enables the study of gene interactions
- Analysis of functional relationship between genes for a more comprehensive understanding of pathways
- Gain a better understanding of synthetic lethality where a perturbation of both genes can result in the loss of viability



# Multiplexed Gene Fragments Use Case | Precise Antibody Profiling at Scale

Enabling interrogation of unprecedented scale & throughput of completely defined CDR region in a single fragment (full VH or VL).



DNA Library

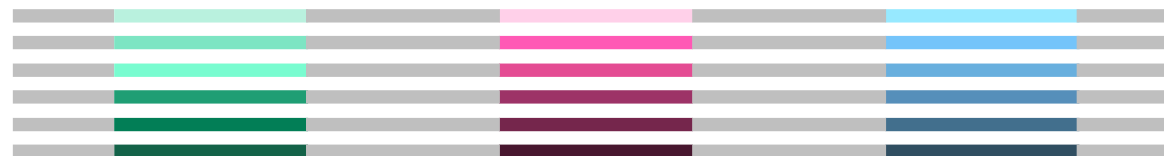


Randomized CDR Pairing

Partial variable regions  
(VH and VL) pieced together



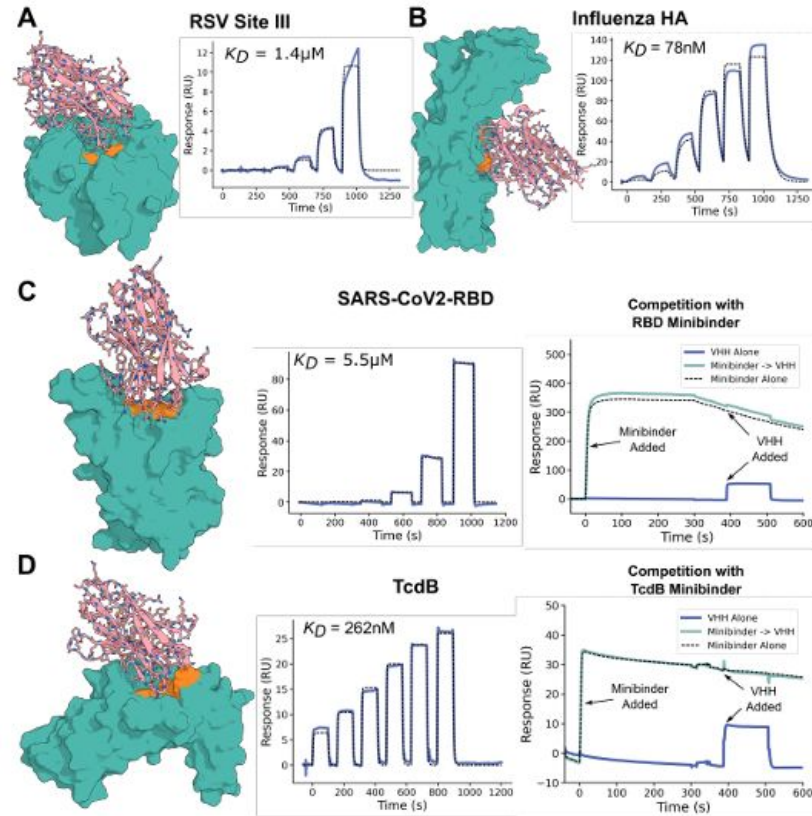
Multiplexed  
Gene Fragments



Precise CDRs Incorporation

Full-length variable  
regions (VH and VL)  
explicitly written

# Multiplexed Gene Fragments Case Study: de novo Design of Single Domain Antibodies



In each assay, 9000 designed VHHs were screened against disease targets with yeast surface display, before soluble expression of the top hits in *E. coli*.

DOI: <https://doi.org/10.1101/2024.03.14.585103>

## Goal: Rationally design and test novel Antibodies

- **Multiplexed Gene Fragments:**
  - Designed VHHs to a range of disease-relevant targets
  - ProteinMPNN was used to design the sequences of the CDR loops (but not the framework) in the context of the target. Designs were then filtered with the fine-tuned RoseTTAFold2 network
  - 9000 designs per target were screened by high-throughput yeast display
- Demonstrated that a fine-tuned RFdiffusion network is capable of designing *de novo* antibody variable heavy chains (VHH's) that bind user-specified epitopes, and then experimentally confirmed binders to disease-relevant epitopes

**Libraries**

# Twist Libraries: What Do We Offer?

## Large Scale Silicon-based DNA Synthesis



## Custom Designed Libraries



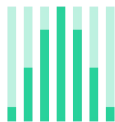





- ✓ Base-by-Base precision
- ✓ Codon usage control
- ✓ Precisely controlled combinatorial diversity
- ✓ Ratio-controlled amino acid distribution
- ✓ CDR length variation
- ✓ Avoid restriction sites and unwanted motifs
- ✓ Incorporate multiple germline scaffolds
- ✓ Library validation by Next Generation Sequencing



Our oligonucleotides are formatted into libraries by our team of leading DNA experts.

# Precision DNA Variant Library Synthesis

By using the highest quality raw materials and automation, our variant libraries provide:

 <p>Base-by base precision</p>	 <p>Codon usage control</p>	 <p>CDR length variation</p>	 <p>Avoid restriction sites and unwanted motifs</p>
 <p>Precisely controlled combinatorial diversity</p>	 <p>Ratio controlled amino acid distribution</p>	 <p>Multiple germline scaffolds</p>	 <p>Library validation by next generation sequencing</p>

# Which Library fits your Design?

## Site Saturation Variant Libraries

Diversify single amino acids across your sequence of interest.



**Example application:** Alanine scan your protein but pick every amino acid or codon.

## Combinatorial Variant Libraries

Introduce combinatorial diversity confined to domains of <99 bp along the sequence of interest.



**Example application:** Generate a phage display antibody discovery library with  $10^{10}$  unique sequences.

## Spread Out Low Diversity Libraries

Scatter combinatorial diversity across the gene of interest.



**Example application:** Co-mutate multiple target amino acids forming the active site of an enzyme to identify novel substrate specificity.

## Combinatorial Assembly Libraries

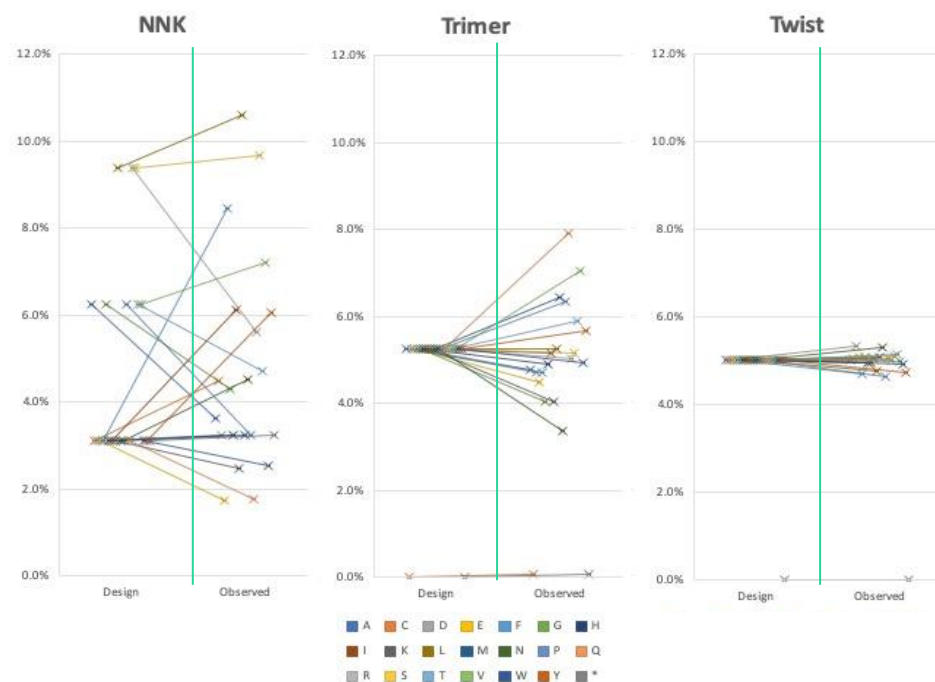
Shuffle explicitly designed cassettes up to 1 kb each.



**Example application:** Find the best TCR by shuffling alpha and beta chain pairs.



## Uneven nucleotide and codon incorporation of competing technologies **creates unwanted bias in library**



The observed amino acid frequency is less than  $\pm 1\%$



**Twist's** *in-silico* DNA synthesis platform empowers scientists to **precisely design** and **customize** variant libraries that enables a comprehensive interrogation and analysis of the variant space

## From **Needle** in a Haystack



- ✗ Random diversity
- ✗ Biased representation
- ✗ >99% inefficiency
- ✗ Lengthy optimization cycle
- ✗ Expensive process

## To **Stack** of Needles

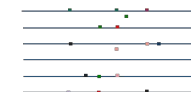


- ✓ Explicit
- ✓ Even representation
- ✓ Human repertoire based
- ✓ Fast
- ✓ Affordable

## Precise Introduction of Variants, Diversity that Enables Screening Efficiency



```
gt catctcAccc tActtg
gt catctcGGcc ttGttg
gt catctcCAcc tCAttg
gt catctctTcc tGTttg
```



### Gene Synthesis



### Single Site



### Multi-Site



### Stretch



### Multi-Domain





# Available Library Types

## Site (Saturation) Variant Libraries (SSVL)



- ✓ Change one position at a time to any number of desired codons
- ✓ Variation via precise single codon substitutions
- ✓ Introduce single site deletions

## Combinatorial Variant Libraries (CVL)



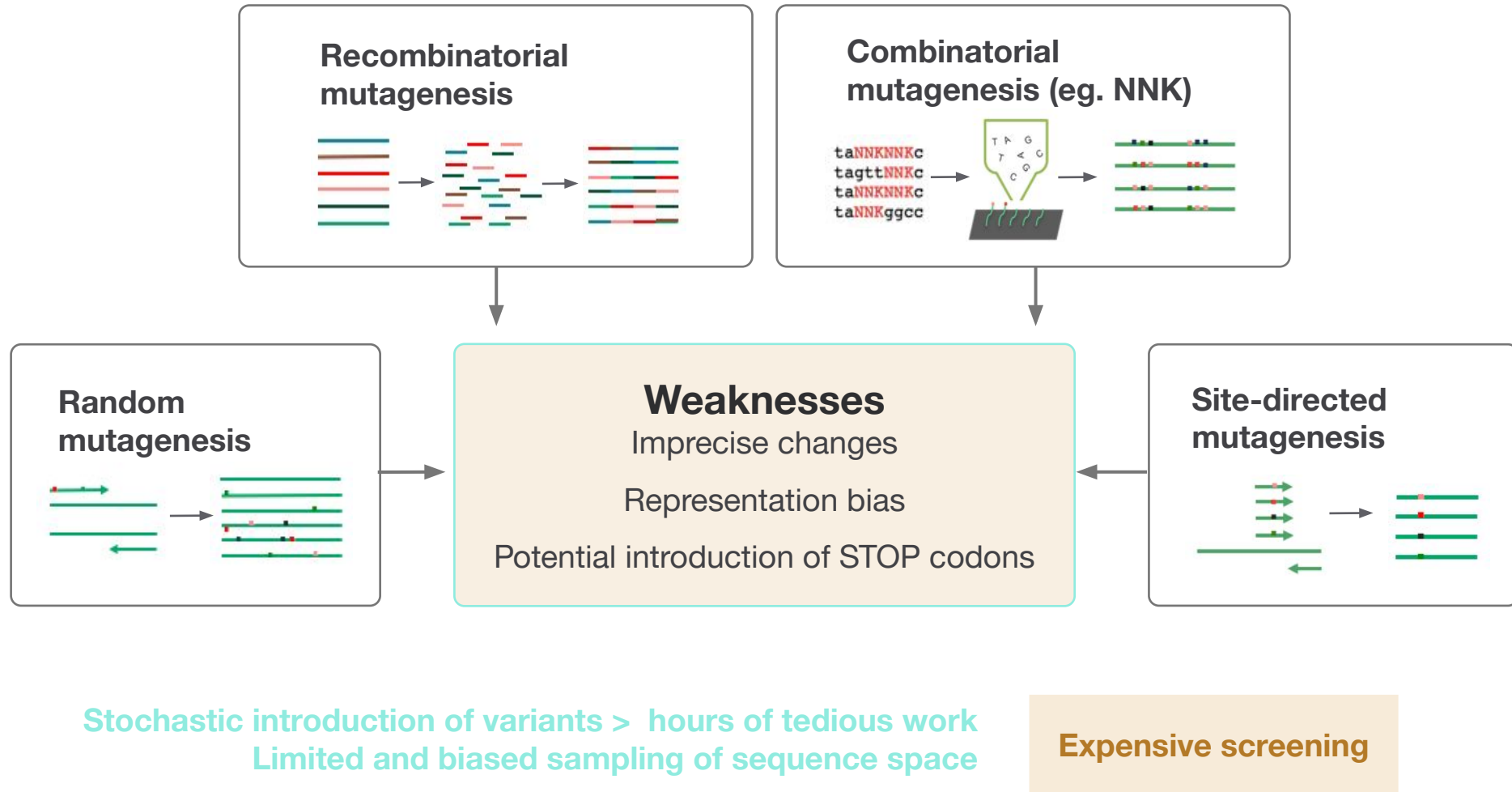
- ✓ Variants differ by precise codon substitutions combined in all positions in each domain
- ✓ Variants confined to one or more domains
- ✓ Define unique ratios of variants at each position, or define total number of changes from WT per domain
- ✓ Total diversity of up to  $10^{10}$  final variants

## Spread out Low Diversity (SOLD) Libraries



- ✓ A subtype of combinatorial variant library designed for enzyme evolution projects
- ✓ Variants differ by precise codon substitutions combined at single positions scattered along a WT sequence
- ✓ Completely synthetic design, no template required

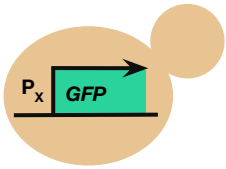
# Current Approaches Deliver but with Compromises



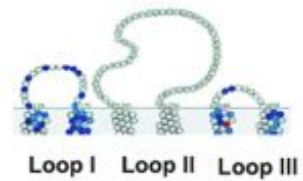
# Advancing Human Health – Pharma Development

## Case Study: Rational Design vs Random Mutagenesis

### 1 Assay Development



### 2 Construct mutagenesis library

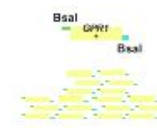


Saturation  
mutagenesis

T W I S T  
BIOSCIENCE

Random mutagenesis  
(epPCR)

VS.



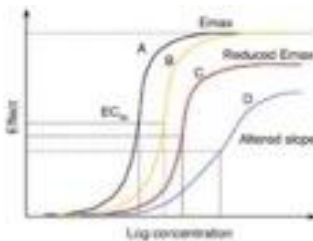
DNA Assembly Transformation Screening



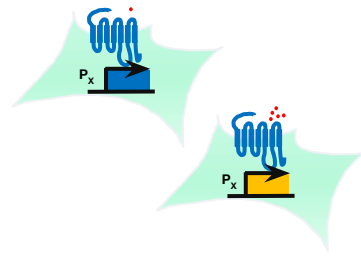
### CHARACTERIZATION

### 4 Pharmacological Characterization

Sequencing



### 5 Circuit integration



### Acknowledgements:

AstraZeneca

- David Oling
- Lina Lawenius
- Niklas Larsson
- Mark Wigglesworth

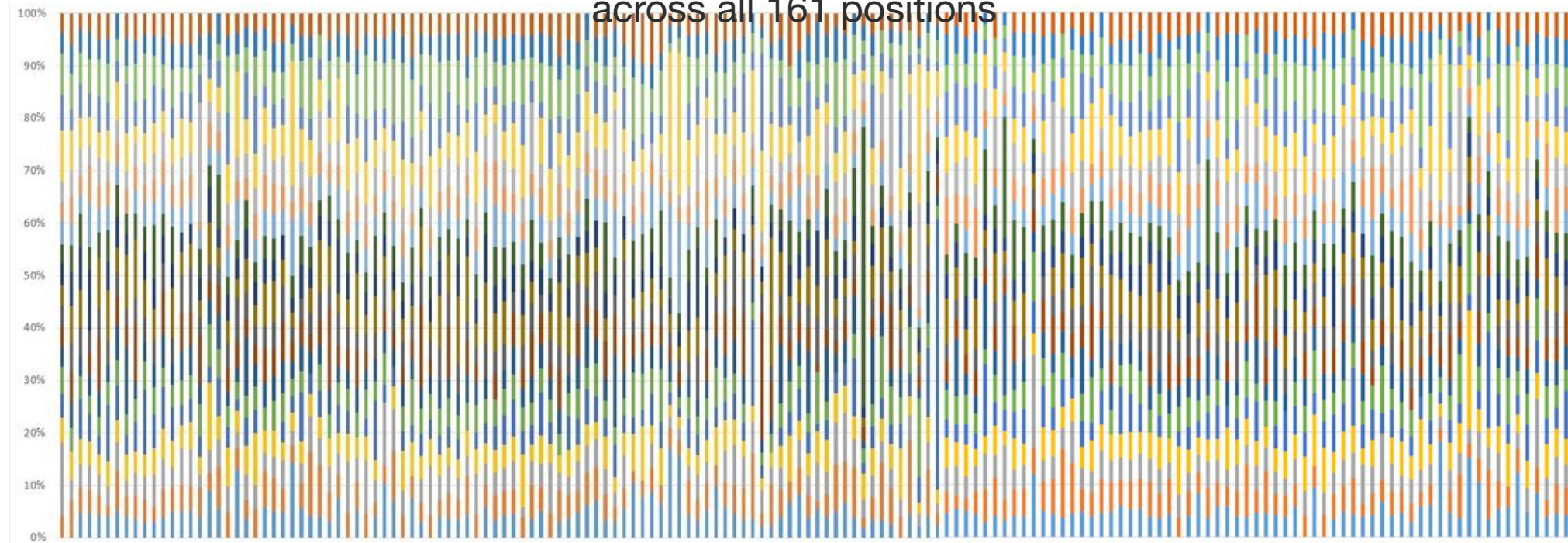
Imperial College  
London

- Tom Ellis
- William Shaw



# Twist Saturation Mutagenesis Library

Highly uniform representation of every  
mutant  
across all 161 positions





# Less Work, More Hits

	Twist Saturation Library	Error Prone PCR Library
Variants	All variants present	Unknown
Number of Hits	10/10 hits	2/10 (both present in Twist library)
Overall	<ul style="list-style-type: none"><li>+ <u>More hits</u></li><li>+ Pure, cloning ready DNA</li><li>+ Fast production time</li></ul>	<ul style="list-style-type: none"><li>- Less hits</li><li>- Unknown variants</li><li>- Large fraction of empty vectors</li></ul>



# AI-Guided Protein Design

# Power your AI Guided Protein Engineering with Twist DNA

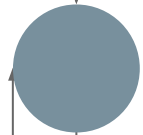
*Accurate gene synthesis in as few as 2 BD allows for rapid training iterations and expedited AI-guided design build test cycles.*

## Get to ground truth

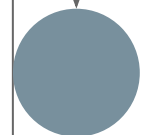


### Initial training dataset development

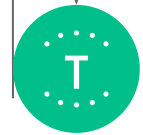
Genes, Multiplexed Gene Fragments, Site Saturation Variant Libraries and IgG offer flexible formats for building training datasets that match your screening capacity and capability.



### Functional assay



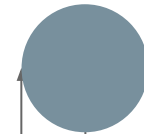
### Train model



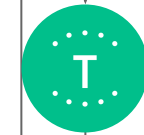
### Sequence Output

Expedite training iterations for model development with expedited synthesis. Accurate Gene Fragments, Cloned Genes and IgG shipped in days.

## Quickly navigate sequence space

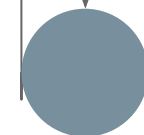


### Model guided Navigation of sequence space



### Generate sequence outputs

Get to proteins with optimal properties without long waits for synthesis. Gene Fragments, Cloned Genes or IgG shipped in days.



### Test Sequence outputs

# Generative Protein Design Guided Twist in vitro Library Production

*Efficient design and construction of target-specific libraries to improve candidate output & functional activity*

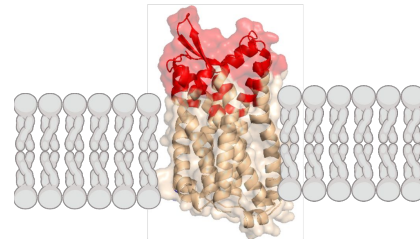
## In Vitro Discovery

Fully Human Library of Libraries  
Phage and Yeast Workflows  
Immune Libraries

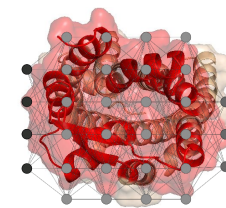


## Chemical and Conformational Evaluation of Existing Structure

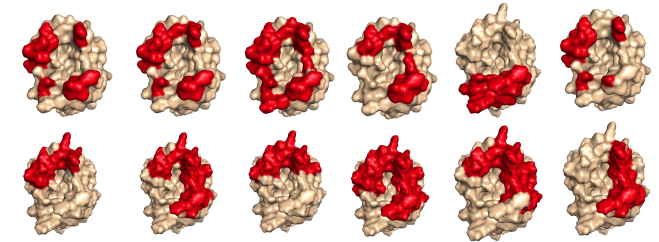
Solvent Exposed Region of **C5aR**



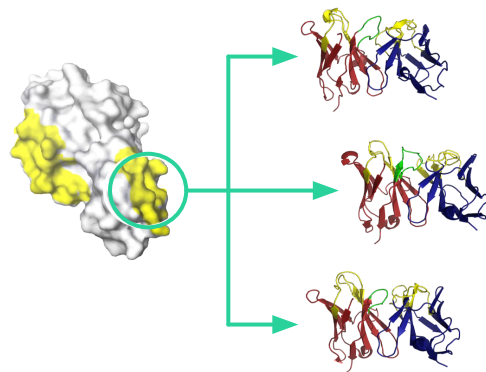
Probability Matrix  
Generated



Discrete “Druggable” Epitopes Identified

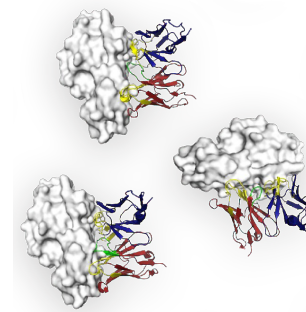


## Run ML Design Algorithm to predict binders



Millions Of Unique  
Candidate Antibody Structures

## Run Neural Network Model to Select Final Designs



Neural Network Models  
to Narrow Down Designs  
\*Proprietary IP

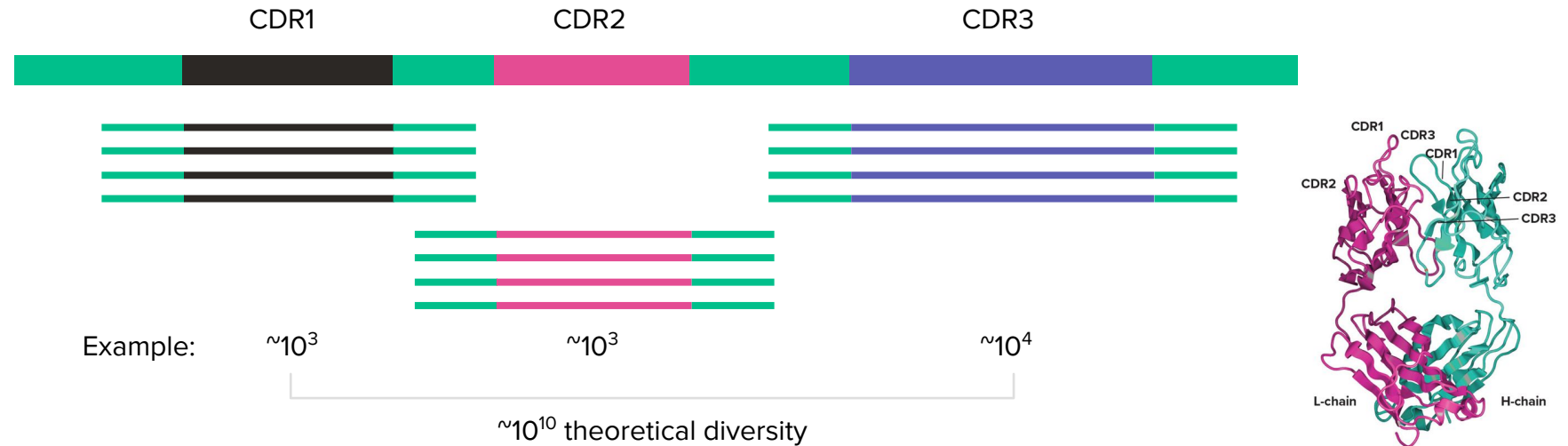
**~10,000 unique designs  
(3307 x HCDR1, 2221 x  
HCDR2, 6113 x HCDR3)  
selected to move forward  
into neighborhood  
diversification library**

# Generative Protein Design Guided Twist in vitro Library Production

*Efficient design and construction of target-specific libraries to improve candidate output & functional activity*

## In Vitro Discovery

Fully Human Library of Libraries  
Phage and Yeast Workflows  
Immune Libraries



### ● Oligo pools can be designed

To match the natural CDR repertoire.

### ● Liabilities can be removed

E.g., isomerization, cleavage, deamidation, glycosylation sites.

### ● Rational sampling

From desired sequence space.

### ● Accurate representation

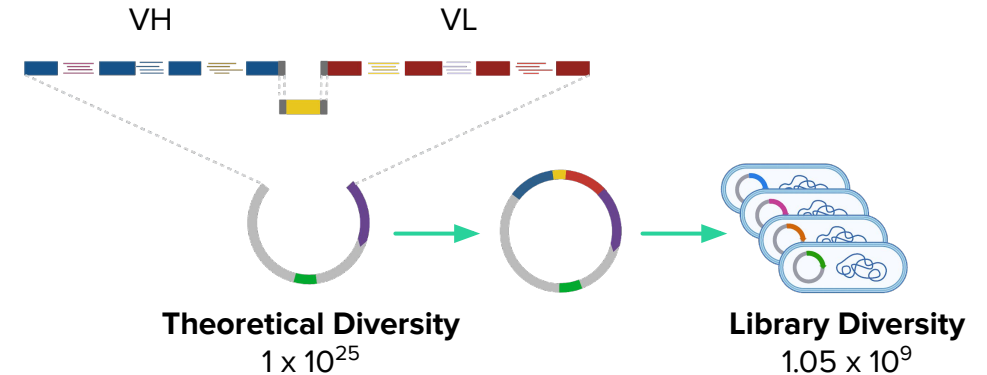
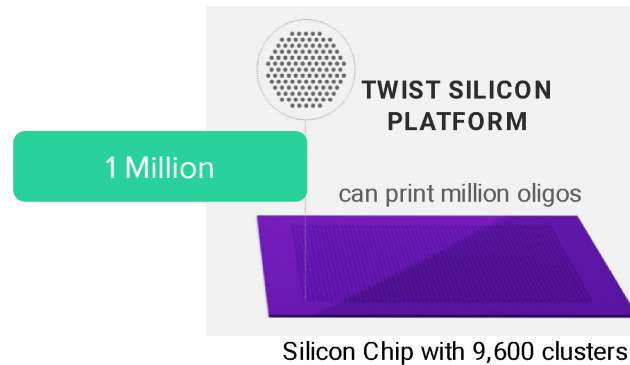
E.g., motif sequences can be explicitly encoded in oligos.

# Generative Protein Design Guided Twist in vitro Library Production

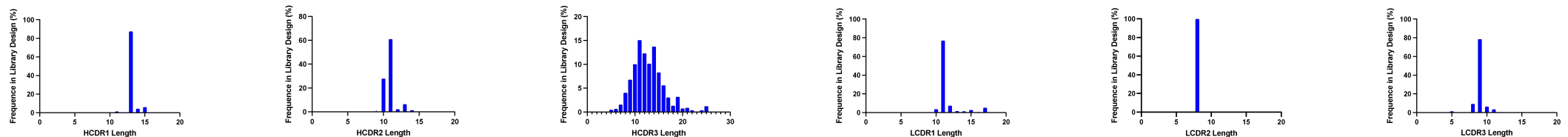
*CDR content from final designs used to generate C5aR specific human framework scFv library*

## In Vitro Discovery

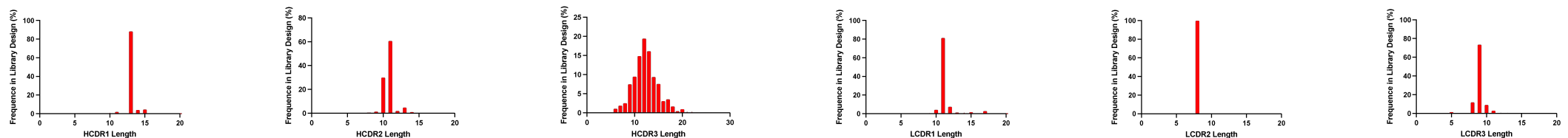
Fully Human Library of Libraries  
Phage and Yeast Workflows  
Immune Libraries



## Designed CDR Length Distribution Profile



## Observed CDR Length Distribution Profile



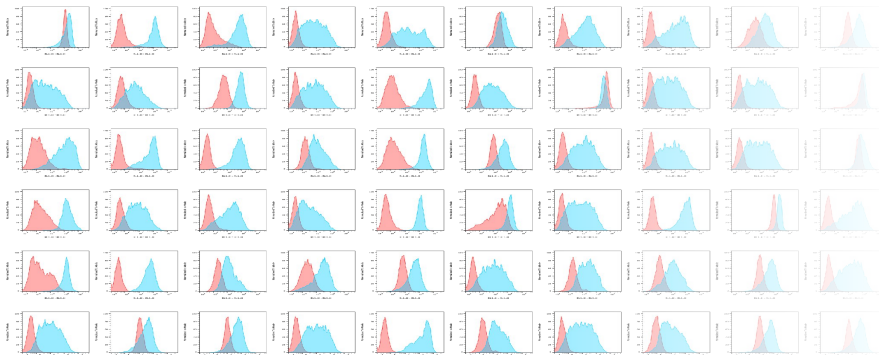
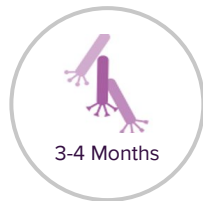


# Primary Screening Yields High Positive Hit Rate

*On-cell screening by flow cytometry and SPR affinity determination with C5aR-containing micelles*

## In Vitro Discovery

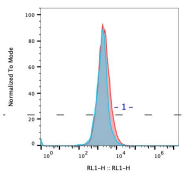
Fully Human Library of Libraries  
Phage and Yeast Workflows  
Immune Libraries



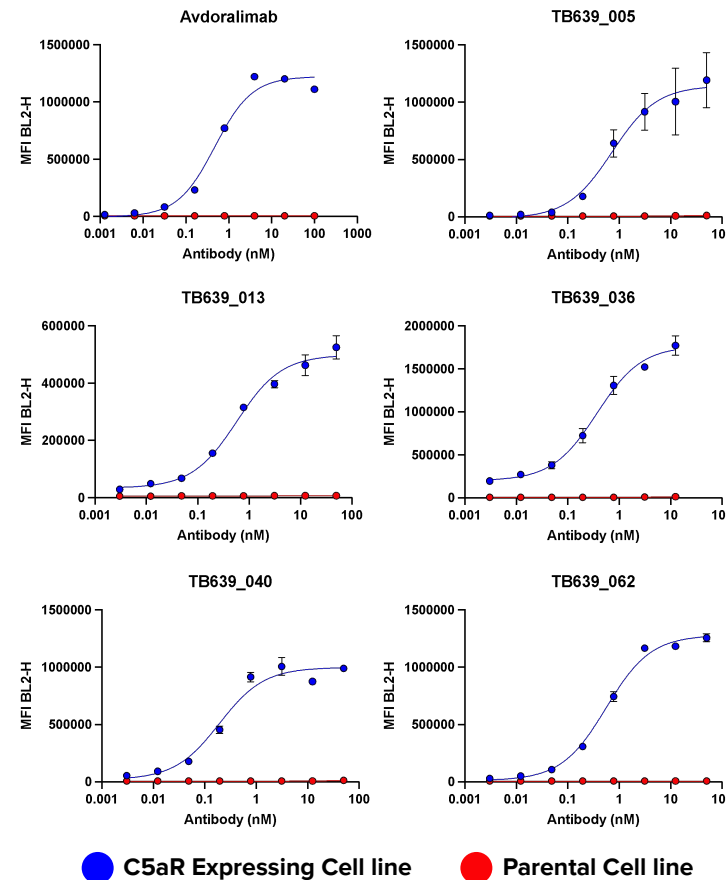
[Antibody] = 100 nM

Secondary Only Control

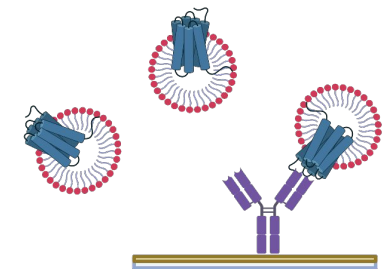
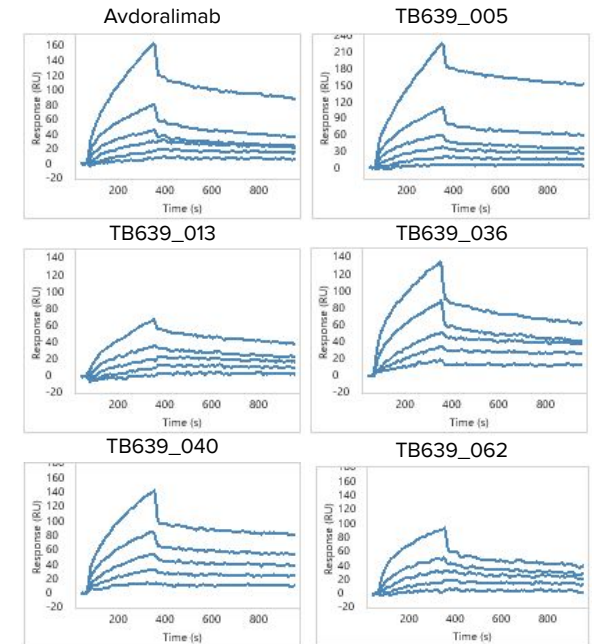
Parental Cell line  
C5aR Expressing Cell line



## Flow-Based Titration of Select Clones (on Attune Cytometer)



## Clones Bind C5aR-micelles by SPR



# Case Study: T4 DNA Ligase Engineering

## Challenges

Low input cfDNA for tests.  
Impacted conversion efficiencies.  
Buffer sensitivity of ligase.

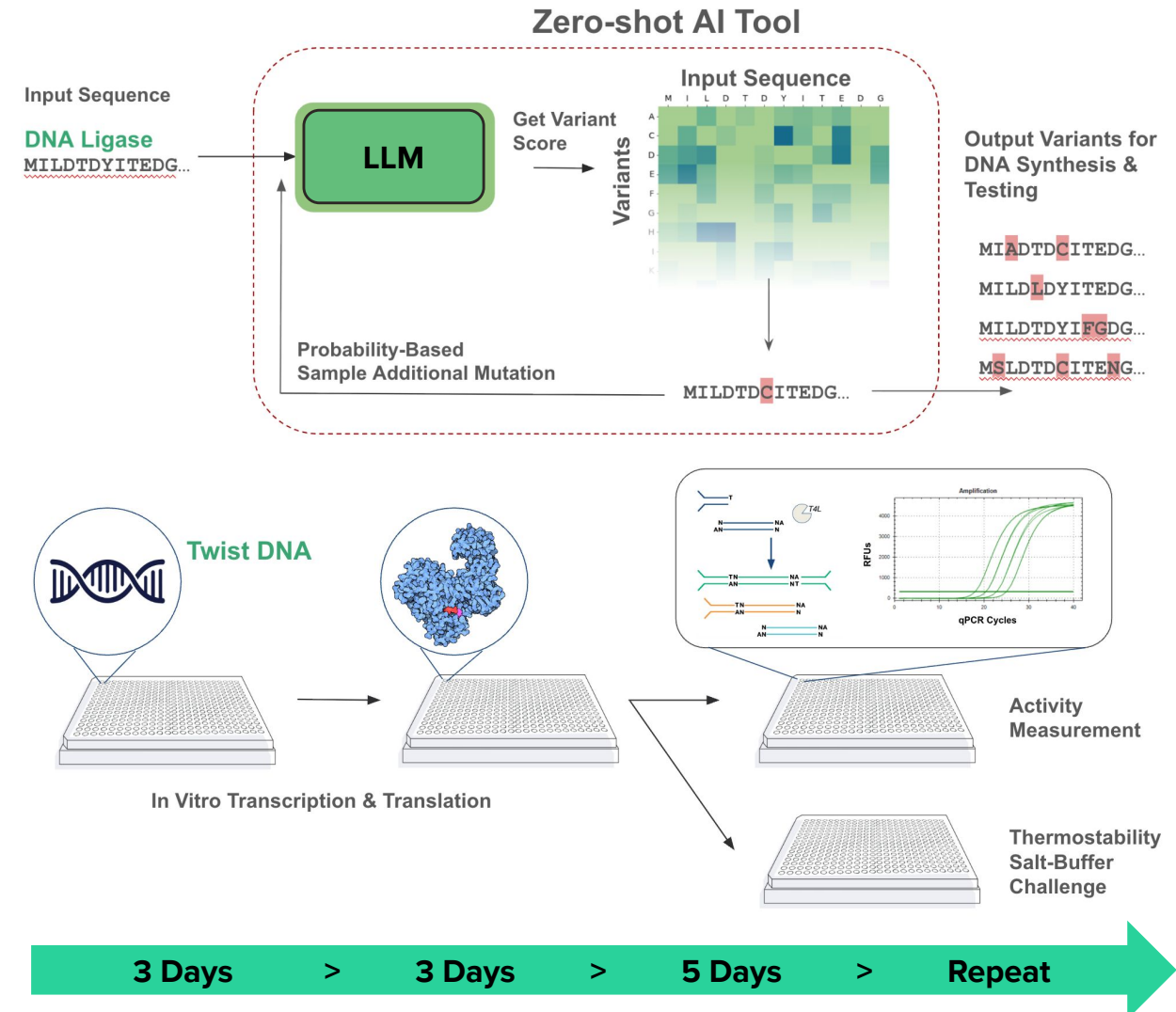
## Goal

Engineer a **Robust, best-in-class efficiency DNA ligase** that works with **Low input DNA**.

## Strategy

Utilize **LLM-based design tools** to introduce beneficial stability and activity mutations. Leveraged **Twist Adaptor-On DNA Fragments** with cell-free transcription and translation mix to produce screening scale enzymes. 13 rounds of High-throughput assays to screen for desirable properties.

Used **Twist Clonal DNA** for large scale purification and validation.



<https://www.twistbioscience.com/sites/default/files/resources/2024-07/Twist-Engineered%20T4%20DNA%20Ligase%20Technical%20Note.PDF>

# Case Study: Engineering Outcome

## Advantages

**Rapid DNA turnaround time** for weekly iterations.

**Adapter-on to protect** from cell-free exonucleases.

**Normalized DNA input** for immediate plug in & play.

**Low price point** for exhaustive sampling of epistatic mutations.

## Outcomes

Best-in-class conversion of low input DNA.

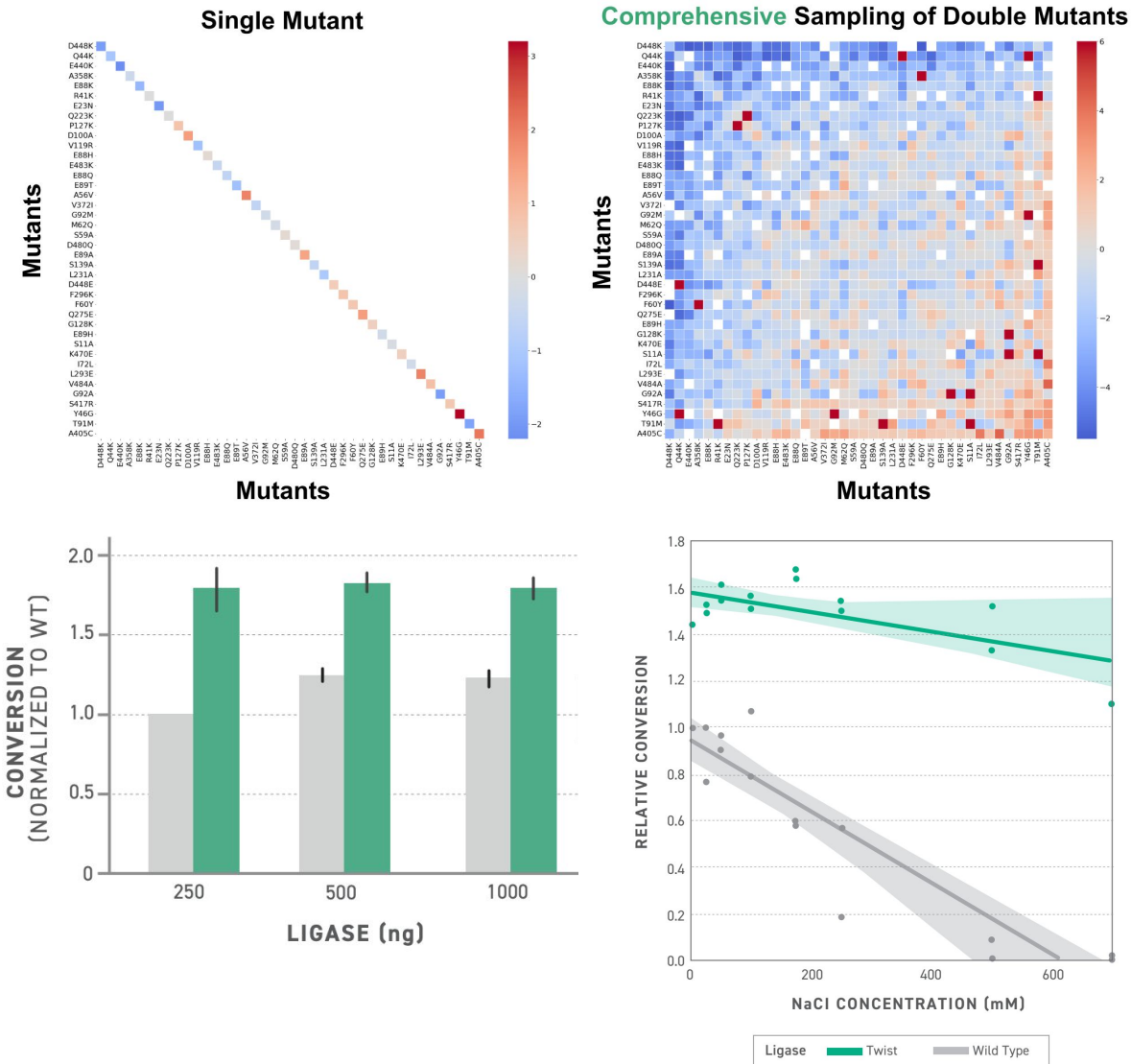
Insensitivity to salt content in buffer.

Lower DNA end motif ligation bias.

Independence from crowding agents (PEG).

**Twist leverages this DNA ligase in NGS Library Prep product portfolio.**

**Twist uses 10x lesser ligase internally for high conversion.**



WO2024123733A1

# Case Study: DNA Polymerase MasterMix

## Challenges

GC amplification Bias in complex libraries. Weak tolerance to GC enhancers in mastermix. Processivity & dissociation issues that causes polymerase fidelity/slippage.

## Goal

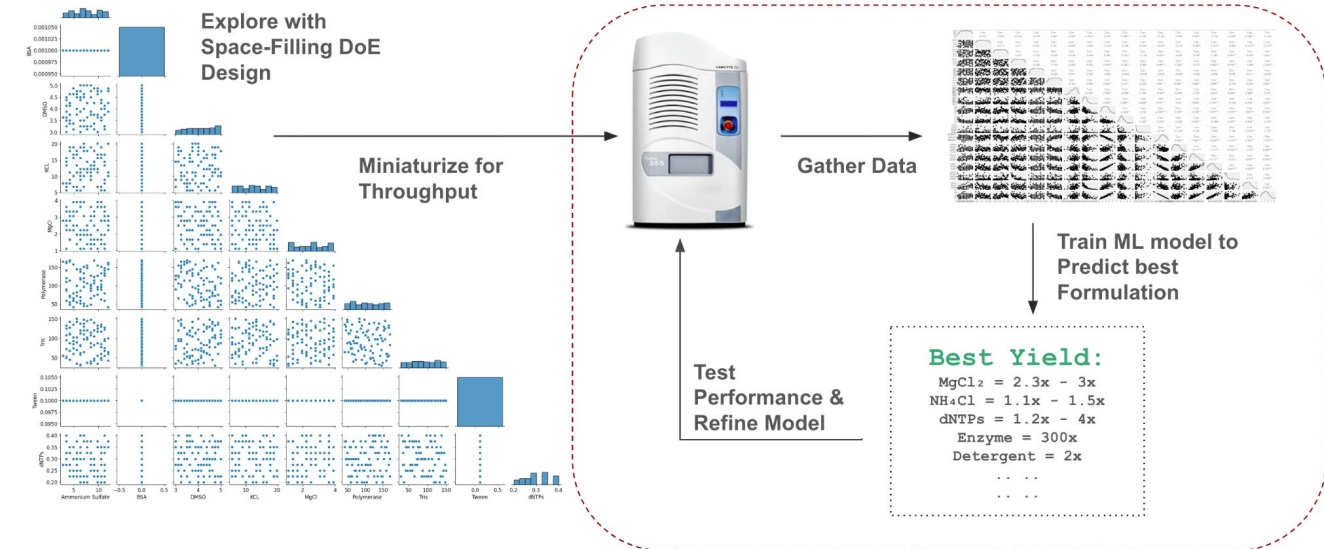
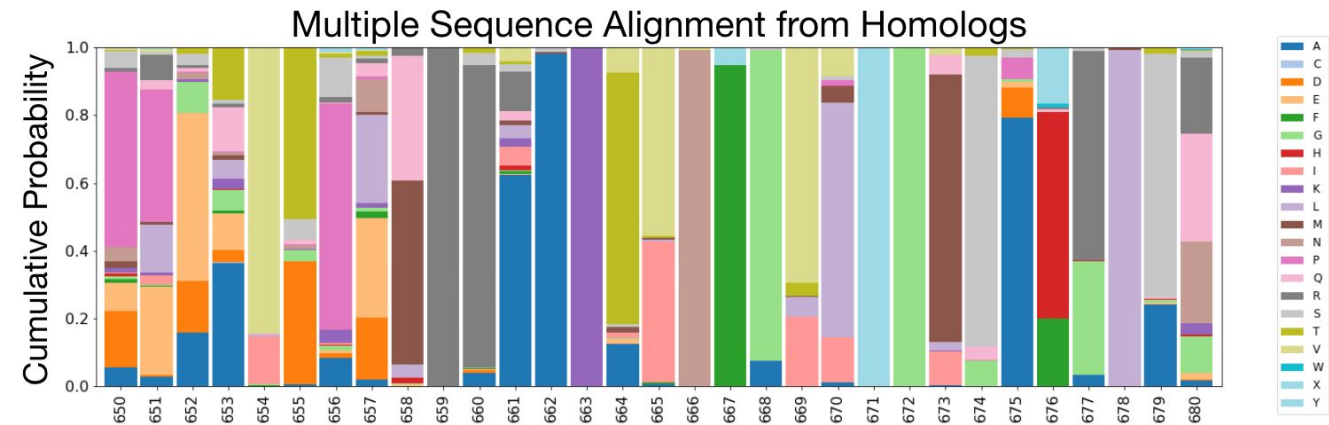
Engineer a **high-fidelity DNA polymerase** solution with a **high-performance buffer** for best-in-class uniformity.

## Enzyme Strategy

Utilize **LLM** and **Multiple Sequence Alignment (MSA)** Tools and **Structure Modeling** to sample mutations in functional hot-spots.

## Buffer Strategy

Utilize **space-filling Design of Experiments (DoE)** approaches to broadly sample and **supervised learning** to predict best buffer compositions.





# Case Study: MasterMix Engineering Outcome

## Twist Polymerase solution encompasses

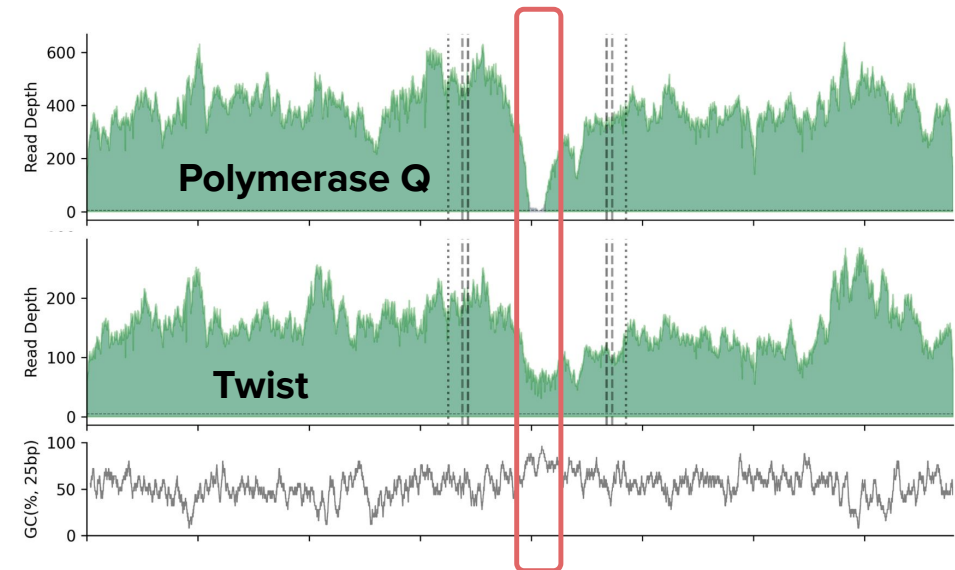
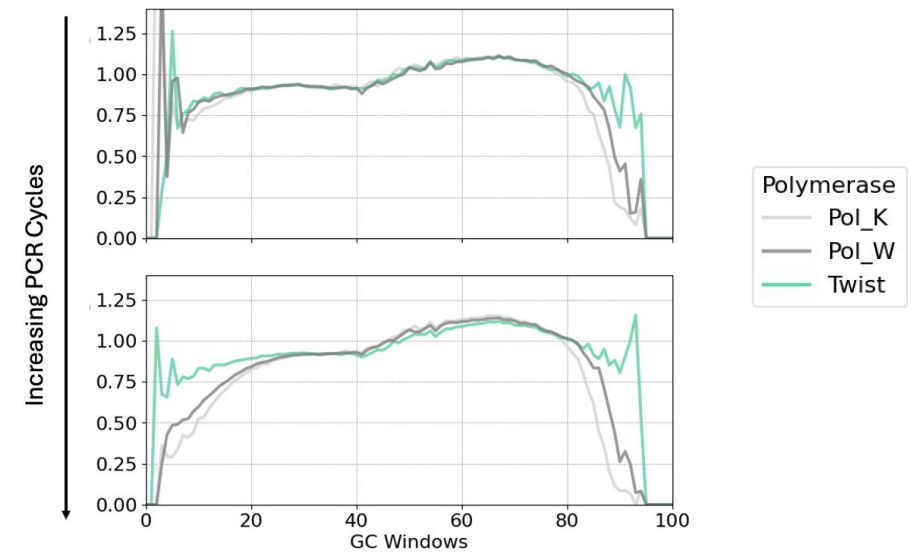
1. High-Fidelity Polymerase
2. Robust and High-Performance Buffer

Leveraged **Twist Express Clonal Genes** and **Twist Cloned Single Variant Libraries (SVL)** for *E.Coli* expression, purification and arrayed screening to identify winning mutations for improved activity and fidelity.

## Outcome

Best-in-class uniformity.  
High Fidelity with lesser C->T Incorporations.  
Improved errors from slippage on Homopolymers.

Twist leverages this DNA polymerase in sequencing confirmation of every gene that is delivered in Synbio Portfolio.



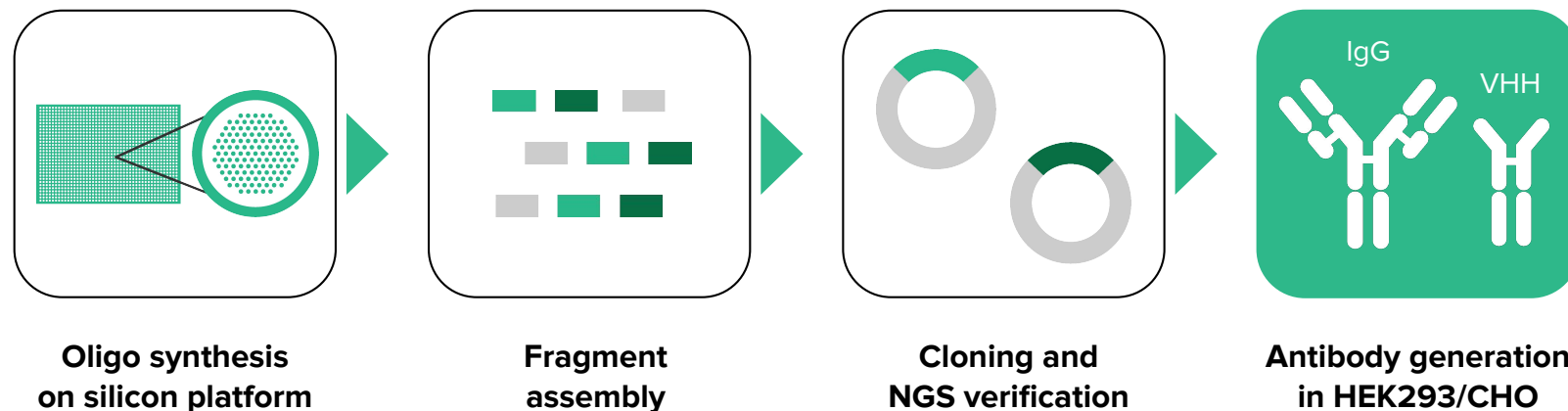
# Antibody and Characterization



# Twist Express Antibodies

Access unlimited recombinant expression.

**Twist Express Clonal Genes enable Twist Express Antibody generation in as few as 10 business days.**



## Product Specifications

### Formats

- Full-length human IgG (hIgG1, hIgG2, hIgG4)
- Single Domain VHH-Fc, VHH-His

### Turnaround Time

- HEK293: starting at 10 BD
- CHO: starting at 13 BD

### Average Yields\* (ProA Purified)

#### 293 Expression:

- 1 mL: ~180 µg (IgG) – 290 µg (VHH Fc)
- 8 mL: ~760 µg (IgG) – 1050 µg (VHH Fc)

#### CHO Expression:

- 1 mL: ~99 µg (IgG)
- 8 mL: ~690 µg (IgG)

\*Turnaround time starts at 10 – 15 business days for 1 mL antibody expressed in HEK293 and 13 – 18 business days for 1 mL antibody expressed in CHO. 8 mL expression volumes take an additional business day.

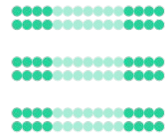
# Addressing the Challenges of Generating Hundreds to Thousands of Antibody Hits

Transitioning from antibody hits to functional candidates is slow and resource-intensive.



## Expression Capacity

- Bottlenecks with expressing 10s to 1000s of antibodies
- Limitation with purification methods
- Limitation with antibody QC and quality checks



## High Throughput Screening Infrastructure

- Narrowing down 1000s+ hit to 10s of potential candidates by screening for initial properties is time and resource intensive
- Expensive infrastructure and scientific expertise is required to quickly screen candidates for desirable characteristics like binding or off-target binding



## Developability Assessment Capabilities

- Developability data needs to be considered early in antibody discovery to de-risk lead antibodies from downstream manufacturability issues
- A comprehensive assessment that is scalable and customizable is critical to quickly down-select antibodies and select lead candidates

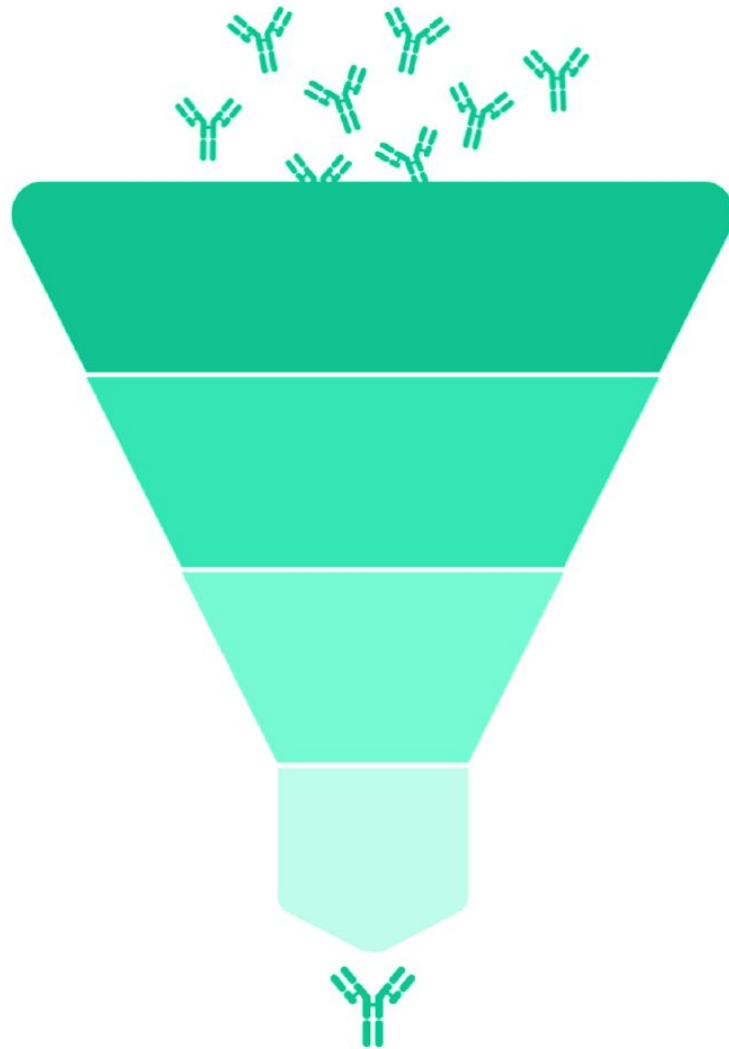


## Time to Next Review

- Squandering your one precious resource (time) on cloning, sequencing expressing, purifying and measuring various data points to update your next investment round

**With Twist, you get the data you need to complete your discovery program.**

# Twist's Approach to Enable AI/ML in Antibody Discovery



## Scalable and Sustainable

Whether you're looking for a one or thousands of antibodies or data points, Twist's infrastructure will match your needs without any wait time. Get consistent turnaround and performance at any volume.

## Confidently Outsource Your Overflow

Twist serves as your external R&D engine, giving you access to precision and expertise without the need to invest in wet lab space or CapEx in internal equipment, lab space, or staffing.

## Scientific Expertise

Twist understands that data is the key to your workflow and algorithm. Work directly with Twist's protein scientists to design and troubleshoot projects from setup, screening optimization and complex data interpretation.

## One, End-to-End, Trusted Partner

Work with a single provider for your entire make-test workflow to ensure that your data is secure, reliable, and delivered with the same quality you expect from Twist. We are your partner at every step of antibody discovery and development.

# Why choose Twist for Antibody Production?



## Customization

Design your antibodies to incorporate custom constant regions for effector silencing or optimized linker regions to boost expression yields. We can be flexible to clone into a Twist antibody or your own custom expression vector.



## Speed and Efficiency

Our gene synthesis technology enables the rapid generation of sequence-perfect DNA, reducing the time required for antibody production, which can accelerate your development timeline.



## Scale-up Flexibility

Automated gene synthesis and high throughput production allow for easy scale-up of antibody production. Whether you need a small amount for initial experiments or a larger quantity for drug discovery projects, we meet your needs.



## Access to State-Of-the-Art Technology Sans the Price Tag

Partnering with a gene synthesis company provides you access to the latest developments, ensuring that you can benefit from cutting-edge technologies and methodologies.



## Expertise and Support

24/7 access to our team of experts who can support you throughout the antibody design and submission process.

# What does Twist Express Antibody Production Provide?

Antibodies produced and delivered through Twist’s high throughput **CHO** and **HEK293** expression systems generates tens to thousands of sequence-defined antibodies for rapid screening.

Our gene-to-protein workflow, starting with **Twist Express Genes**, removes production bottlenecks, shortens timelines, and delivers antibodies made entirely from your specified sequences.

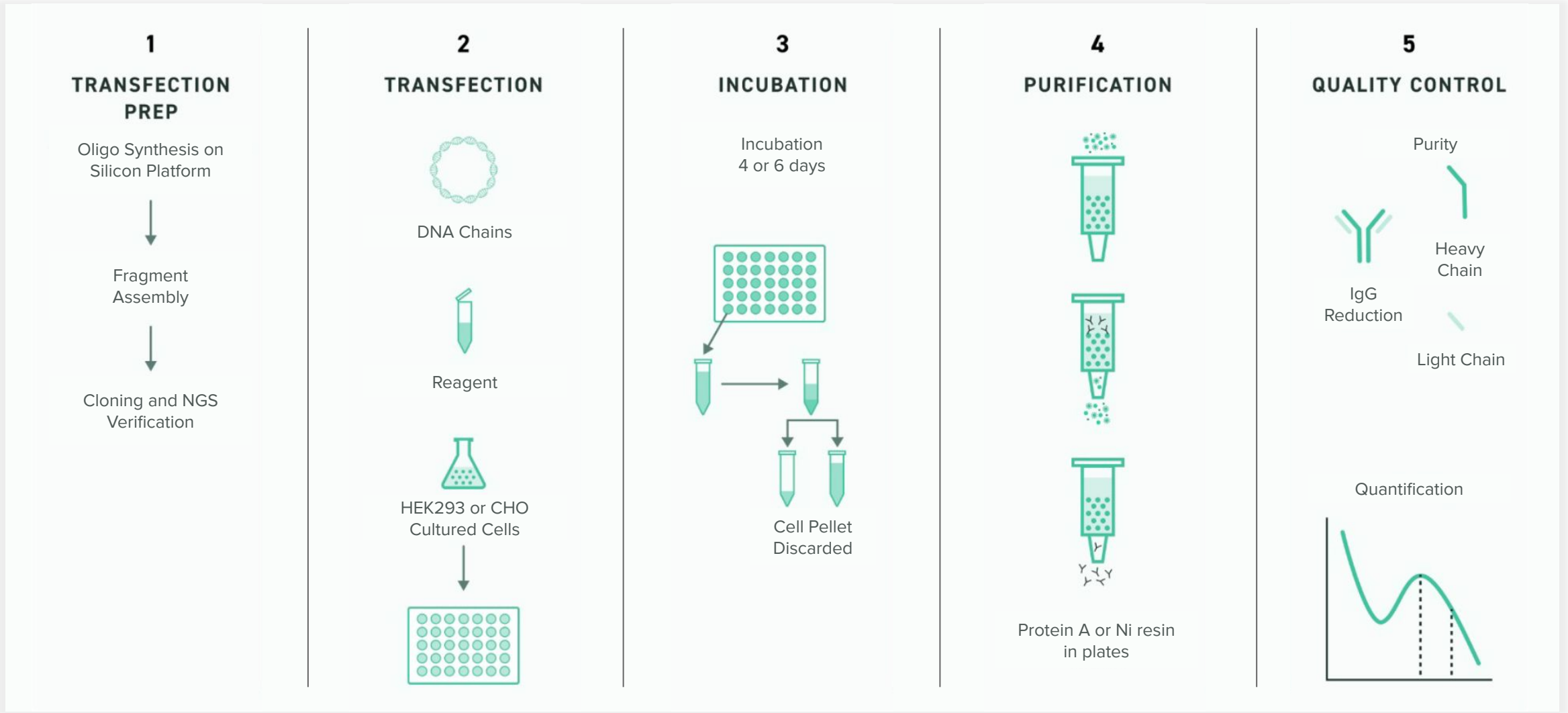
## HIGH-THROUGHPUT ANTIBODY PRODUCTION OVERVIEW



*\*Terms and Conditions: Turnaround time starts at 10 – 15 business days for 1 mL antibody expressed in HEK293 and 13 – 18 business days for 1 mL antibody expressed in CHO. 8 mL expression volumes take an additional business day.*

# You Design It, We Build It

Our antibody expression workflow and techniques.





# Antibody Expression Data in HEK293 or CHO cells

A curated panel of antibodies was subjected to the Twist Express Antibody production workflow on 1 and 8 mL scales at three independent time points over several weeks. Antibodies were expressed using either Thermo Fisher's Expi293™ or ExpiCHO expression.

## High reproducibility

Data not shown. Twist produces the same antibodies with low variability in expression levels.

## Low endotoxin levels

A chromogenic LAL assay showed nearly all purified samples had endotoxin levels below the detection threshold (<0.125 EU/mL).

## High quality and purity

QC confirmed antibodies were correctly sized and highly pure via CE-SDS/microfluidic electrophoresis.

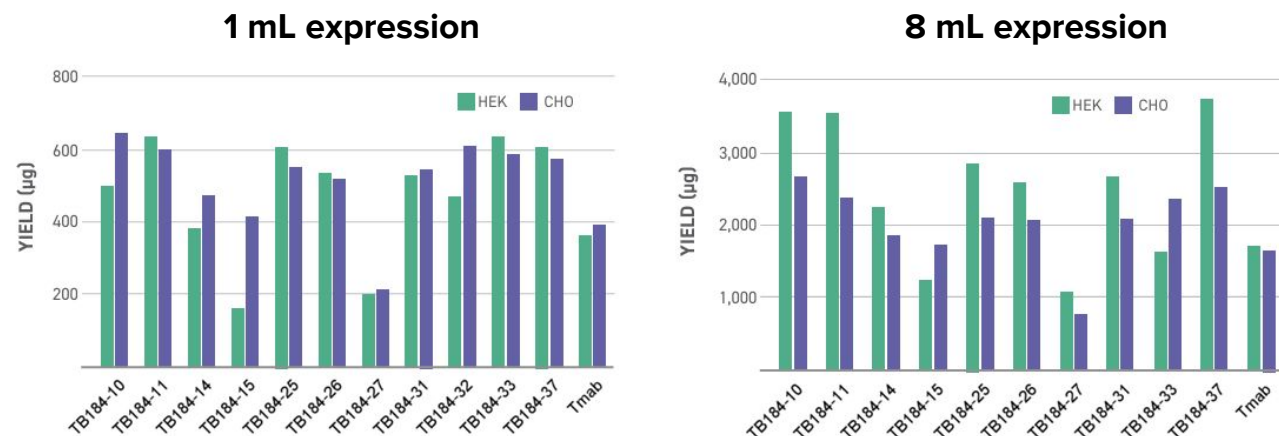
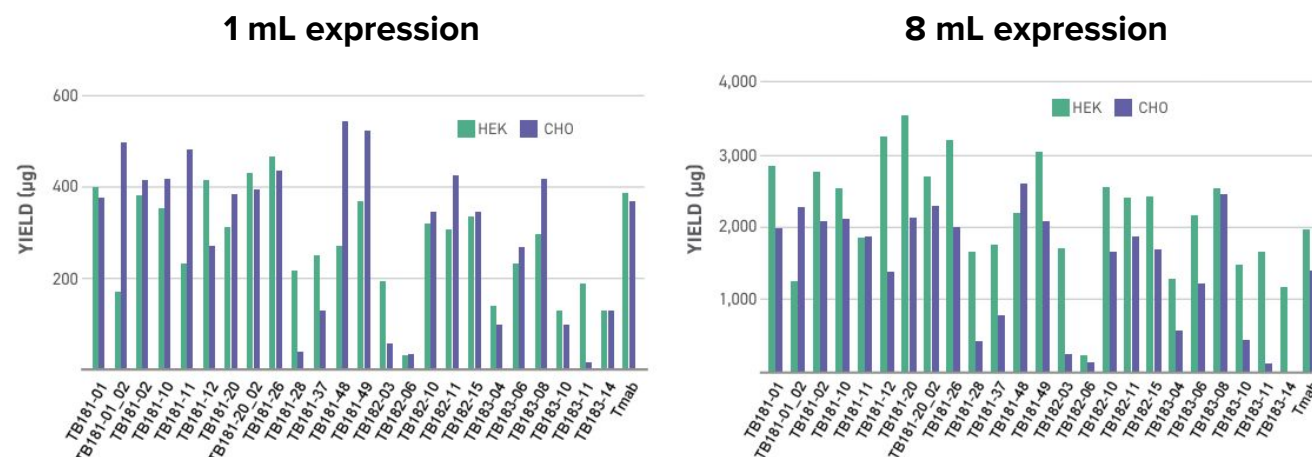


Figure 3. VHH-Fc yield comparison by scale and cell line.



# Our High Throughput Antibody Production Comprehensive Offering:

## ANTIBODY TYPE

VHH-Fc  
IgG

Twist offers two antibody types with built-in Fc regions in vector backbone: Single domain (VHH) proteins with isotype options IgG1 or IgG2, and IgG's with isotype options IgG1, IgG2, or IgG4.

## CELL LINE

CHO  
HEK

HEK cells transfect easily for replicability and is faster to better support discovery pipelines. CHO cells are preferred in the pharmaceutical industry for their robust history of therapeutic use and scalability.

## EXPRESSION SCALE

1 mL  
8 mL

Expression is offered in two scales. Yields vary and depend on several factors such as sequences and complexity. In a twelve month period, 72% of 1 mL expressions surpassed 100 ug and 95% of 8 mL expressions exceed 1 mg.

## PRODUCT TYPE

Purified  
Supernatant

An Fc region is required for purified orders. Purification occurs via Protein A chromatography with the final product eluted in Glycine or Citrate buffer. Supernatural products are 0.2 µm filtered.

## ELUTION BUFFER

Citrate neutralized with HEPES  
Glycine neutralized with Tris-HCl

Citrate and HEPES or Glycine and Tris-HCl combinations are available for purification. Citrate lacks primary amines which allows for future modifications to the product.

## BUFFER EXCHANGE

DPBS pH 7.4 or PBS pH 6  
Normalization 0.2 µm Filtration

Automated buffer exchange is executed upon request using DPBS pH 7.4 or PBS pH 6. Samples can also be normalized to 0.25, 0.50, or 0.75 mg/mL. Customers may also choose to add a 0.2 µm filtration step.

## CUSTOM REQUESTS

Endotoxin Testing  
Pooling  
Aliquoting

Additional customizations are available to accommodate order requests. Twist is committed to delivering products in the format that work best for customers and is constantly introducing new custom options.

# Appendix

# Synthetic Assay Controls

## Synthetic Viral RNA & DNA Controls



**Laboratories  
around the world  
need high quality tools**

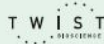
To address the unprecedented need for diagnostic testing to detect SARS-CoV-2 virus

- ✓ Positive controls are needed for verification and validation of diagnostic tests including both next-generation sequencing (NGS) and reverse transcription polymerase chain reaction (RT-PCR) assays.
- ✓ Powerful alternative to “live virus” controls - Synthetic controls created through gene synthesis broaden access across diverse strains while mitigating safety and security concerns.
- ✓ **Serve in a wide range of applications from diagnostic assay development to day-to-day testing**

# Synthetic Viral RNA Controls

At present, there are hundreds of **variants** of the SARS-CoV-2 virus in public repositories.

Leveraging our powerful silicon-base writing platform, Twist is able to manufacture and deliver customized synthetic controls based on any of these viral references within approximately 2 weeks.

SARS-CoV-2

## Twist Synthetic SARS-CoV-2 RNA Controls

**KEY HIGHLIGHTS**

- Fully synthetic RNA generated from Twist gene fragments
- Full viral genome coverage. NGS sequence verified
- Positive control for both RT-PCR and NGS-based assays


The recent Coronavirus pandemic has led to the unprecedented need for diagnostic tests for detecting the presence of the SARS-CoV-2 virus in a variety of sample types. To address this need, laboratories around the world need high quality tools to enable them in catering to this rapidly expanding requirement for testing.


Positive controls provide quality control measures for the verification and validation of diagnostic tests including both next-generation sequencing (NGS) and reverse transcription polymerase chain reaction (RT-PCR) assays.


Twist has created synthetic RNA controls for two variants of the SARS-CoV-2 virus: MT007544.1 and MN908947.3 (GenBank IDs).

These synthetic controls, consisting of six non-overlapping 5 kb fragments were generated from Twist Gene Fragments then transcribed into ssRNA. The Twist Synthetic SARS-CoV-2 RNA Controls provide coverage of greater than 99.9% of the bases of the viral genome. The RNA controls are supplied in 100 µL at a concentration of one million copies per microliter.

At present, there are over 100 variants of the SARS-CoV-2 virus available in public repositories. Leveraging our powerful silicon-base writing platform, Twist is able to manufacture and deliver customized synthetic controls based on any of these viral references within approximately two weeks. Customers purchasing synthetic controls are subject to Twist's leading biosecurity screening protocols and applicable laws and regulations.

**BIO SAFETY**  
LEVEL 1

**STORAGE TEMP**  
-70°C TO -90°C

**SPECIFICATION RANGE**  
1 X 10<sup>6</sup> COPIES/µL

Twist Synthetic SARS-CoV-2 RNA Controls are a component of the Twist portfolio of products.

**LEARN MORE**  
twistbioscience.com  
sales@twistbioscience.com

**ORDERING INFORMATION**

102019: Twist Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1)

102024: Twist Synthetic SARS-CoV-2 RNA Control 2 (MN908947.3)

These products are for research use only, and subject to additional use restrictions as set forth in Twist's Supply Terms and Conditions: [www.twistbioscience.com/supply-terms-and-conditions](https://www.twistbioscience.com/supply-terms-and-conditions)

DOC-001157 Rev.1



# Synthetic Viral RNA Controls - SARS-CoV-2

Twist has created 37 synthetic RNA controls for multiple variants of the SARS-CoV-2 virus:

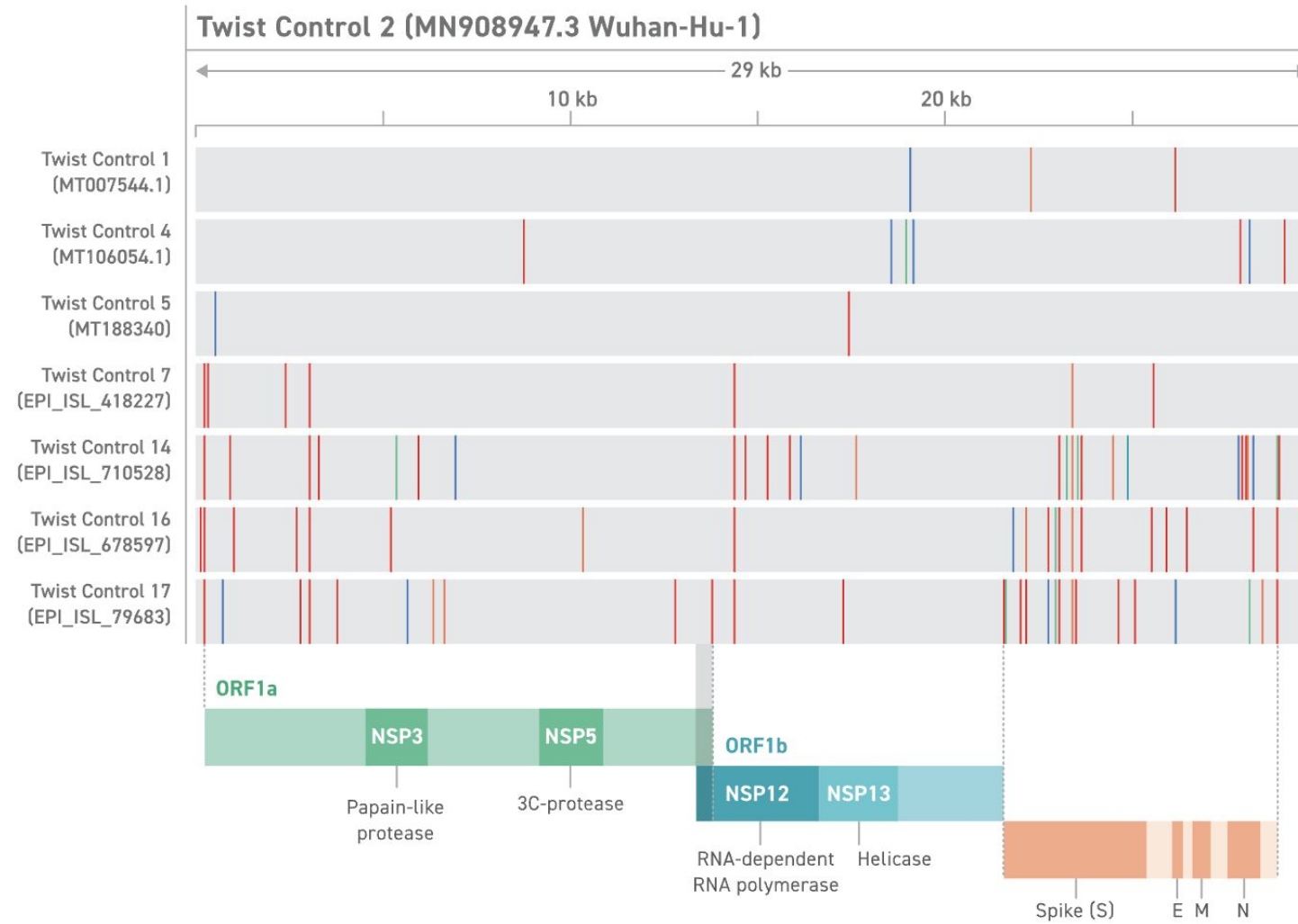
PART NUMBER	CONTROL	VOI/VOC	GENBANK/GISAID ID	GISAID NAME
102019	Control 1		MT007544.1	Australia/VIC01/2020
102024	Control 2*		MN908947.3	Wuhan-Hu-1
102860	Control 3		LC528232.1	Japan/Hu_DP_Kng_19-020/2020
102862	Control 4		MT106054.1	USA/TX1/2020
102917	Control 5		MT188340	USA/MN2-MDH2/2020
102918	Control 6		MT118835	USA/CA9/2020
102916	Controls 1 – 6		N/A	N/A
103087	Control 7		EPI_ISL_418227	France/HF2393/2020
103086	Controls 1 – 7		N/A	N/A
103511	Control 8		MT066176	Taiwan/NTU02/2020
103512	Control 9		MT152824	USA/WA2/2020
103513	Control 10		EPI_ISL_414648	USA/CA-PC101P/2020
103514	Control 11		EPI_ISL_417739	Iceland/5/2020
103515	Control 12		EPI_ISL_420244	England/SHEF-C05B2/2020
103533	Control 13		EPI_ISL_421184	Belgium/ULG-10004/2020
103907	Control 14* (B.1.1.7)	Alpha	EPI_ISL_710528	England/205041766/2020
103909	Control 15* (B.1.1.7)	Alpha	EPI_ISL_601443	England/MILK-9E05B3/2020
104043	Control 16 (B.1.351)	Beta	EPI_ISL_678597	South Africa/KRISP-EC-K005299/2020
104044	Control 17 (P.1)	Gamma	EPI_ISL_792683	Japan (Brazil)/IC-0564/2021
104338	Control 18 (B.1.617.1)	Kappa	EPI_ISL_1662307	India/CT-ILSGS00361/2021
104529	Control 19 (B.1.526)	Iota	EPI_ISL_1300881	USA/NY-MSHSPSP-PV24650/2020

PART NUMBER	CONTROL	VOI/VOC	GENBANK/GISAID ID	GISAID NAME
104530	Control 20 (B.1.427)		EPI_ISL_730092	USA/CA-ALSR-4704/2020
104531	Control 21 (B1.1.429)	Epsilon	EPI_ISL_672365	USA/CA-CZB-12943/2020
104532	Control 22 (B1.1.519)		EPI_ISL_933685	Mexico/CMX-InDRE_208/2021
104533	Control 23 (B.1.617.2)	Delta	EPI_ISL_1544014	India/MH-NCCS-P1162000182735/2021
104534	Control 24 (B.1.617.3)		EPI_ISL_1939891	India/MH-SEQ-221_S66_R1_001/2021
104538	Control 28 (AY.1)	Delta	EPI_ISL_2695467	Portugal/PT9543/2021
104539	Control 29 (AY.2)	Delta	EPI_ISL_2693246	USA/WA-CDC-UW21061750277/2021
105204	Control 48 (B.1.1.529/BA.1)	Omicron	EPI_ISL_6841980	Hong Kong/HKU-211129-001/2021
105345	Control 50 (B.1.1.529/BA.2)	Omicron	EPI_ISL_7190366	Australia/QLD2568/2021
105346	Control 51 (B.1.1.529/BA.2)	Omicron	EPI_ISL_7718520	England/MILK-2DF642C/2021
105865	Control 62 (BA.2.12.1)	Omicron	EPI_ISL_12248637.1	hCoV-19/Denmark/DCGC-493190/2022
105857	Control 63 (BA.2.12.1)	Omicron	EPI_ISL_12303256.1	hCoV-19/USA/NY-CDC-LC0579415/2022
106196	Control 64 (BA.5)	Omicron	EPI_ISL_12516495	hCoV-19/England/LSPA-3DC1269/2022
106197	Control 65 (BA.5)	Omicron	EPI_ISL_12620611	hCoV-19/USA/TN-ASC-210769476/2022
106198	Control 66 (BA.4)	Omicron	EPI_ISL_12454576	hCoV-19/USA/TX-HMH-M-96682/2022
106199	Control 67 (BA.4)	Omicron	EPI_ISL_12605687	hCoV-19/USA/CA-CDC-QDX36065390/202

106929	Control 70	EPI_ISL_14829147	hCoV-19/USA/MI-C
106930	Control 71	EPI_ISL_15381979	hCoV-19/Australia/

These Synthetic RNA controls serve as sequence diverse position controls mimicking diversity found globally

# Synthetic Viral RNA Controls - SARS-CoV-2



## SARS-CoV-2 UK Variant Timeline

12/20/2020

The prevalence of UK strain hits on the news

12/21/2020

Target sequences identified, designed,  
and sent for gene synthesis

12/29/2020

SARS-CoV-2 RNA made and QC-ed in bulk

01/05/2021

Control materials vialled, and QC-ed by  
NGS and dPCR

01/06/2021

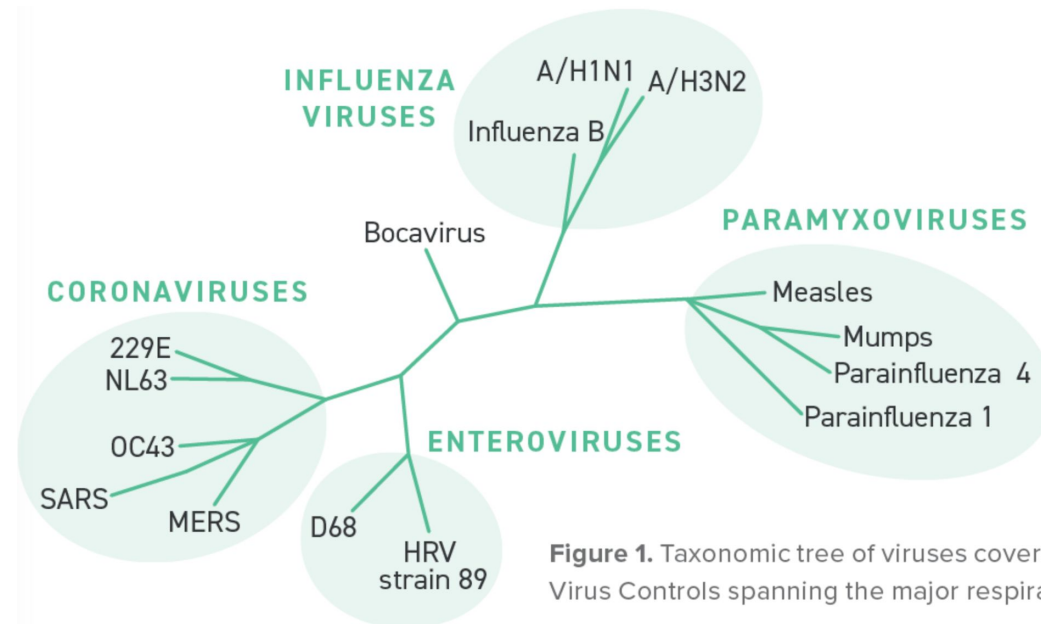
Available for early access customers

01/14/2021

Official product launch

# Synthetic Viral RNA & DNA Controls - Respiratory Viruses

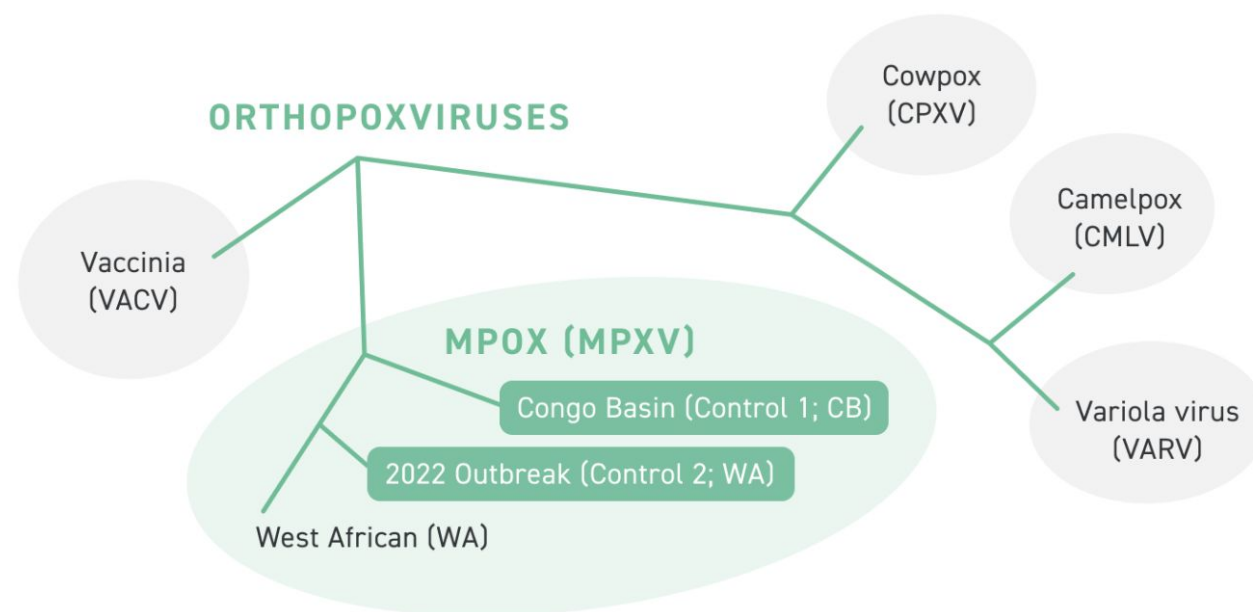
- Twist has created synthetic RNA & DNA controls corresponding to 15 important respiratory viruses.
- **The controls complement the Twist Respiratory Panel and the controls can be used together or individually as positive controls for NGS research.**
- **They are also excellent tools for SARS-CoV-2 researchers in the detection of respiratory viruses that may cause symptoms identical to COVID19 (qPCR or NGS)**
- Can be used as positive controls for qPCR assays in disease research or environmental testing



**Figure 1.** Taxonomic tree of viruses covered on the Twist Respiratory Virus Controls spanning the major respiratory viral clades.

# Synthetic Viral RNA Controls - Mpox Virus Controls

- Twist has created synthetic human Mpox Virus (hMPXV) Controls based on sequences from the Congo Basin (CB) or West African (WA) clades of hMPXV (formerly known as Monkeypox)
- **Support the design of custom assays targeting regions of the genome relevant to orthopoxvirus research**
- Can be used for both amplicon and capture-based detection methods
- **Validated and are compatible with CDC recommended real-time PCR testing procedures for Mpox detection**



Part No.	Name	Database/Accession	Virus Type	Length (bases)
106056	Twist Synthetic hMPXV Control 1 (CB)	GISAID / EPI_ISL_13056236	dsDNA	164678 bp
106059	Twist Synthetic hMPXV Control 2 (WA)	NCBI / ON585037	dsDNA	166798 bp

**NGS**



# Twist Sequencing Workflow: Unmatched Value Across Platforms

*Twist's tools are compatible with all major long and short read sequencing platforms. From focused SNP panels to whole-genome prep, Twist supports your research needs.*



## Library Preparation Kits

- Simplified, streamlined workflows
- Optimized for DNA, RNA, and methylation
- Reduced hands-on time and cost

## Target Enrichment Solutions

- Custom panels, exomes, methylome
- High uniformity, fewer off-target reads
- Precision-driven design with silicon-based synthesis

## Cross-Platform Compatibility

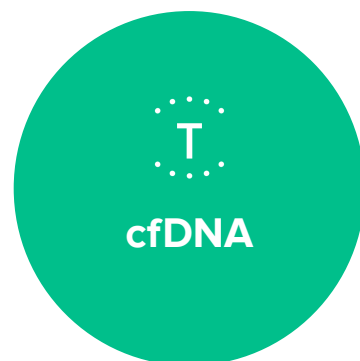
- Validated with Illumina, Element Biosciences, PacBio, and other leading sequencers
- Flexible solutions that can support research & clinical applications

# Twist Controls: Confidence in Every Sequencing Run

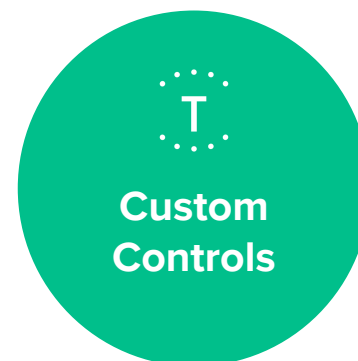
## Quality Assurance at Every Step



Fully synthetic RNA and DNA controls engineered from Twist gene fragments, all NGS sequence-verified.



Designed for **assay validation, QC, and monitoring** in both research and clinical workflows.



**Compatible** with Twist library preparation and target enrichment panels for seamless integration.

Engineered from Twist's high throughput platform, and sequence verified by NGS, our fully synthetic controls offer a consistent, flexible and scalable solution.

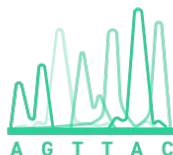
With Twist Controls, you can trust every result—achieving reproducible, reliable sequencing from start to finish.

# Twist Library Preparation: Application-Driven and Enzyme-Engineered Solutions



## Twist Enzyme Engineering

Twist's enzymes are innovatively engineered to drive superior performance via yield, uniformity and fidelity.



## Application Driven

Whether you're working with DNA or RNA, focusing on targeted panels or whole genomes, or conducting AgBio or liquid biopsy research, Twist's solutions are designed to support key applications.

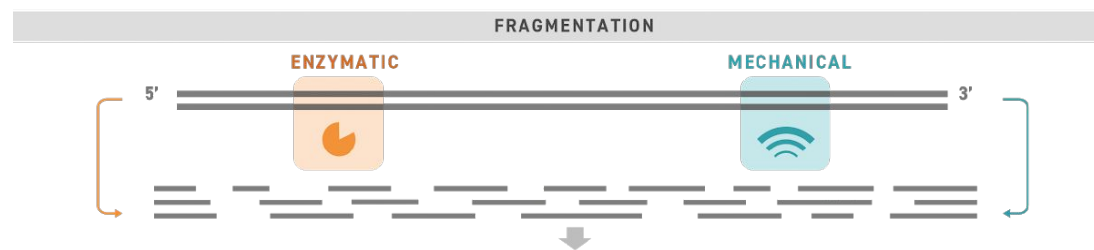


## Custom Solutions for Your End Goal

Leverage our suite of library prep solutions to ensure your inputs are as innovative as your outputs.

# Twist Library Preparation: Streamlined Workflows for Accurate Sequencing

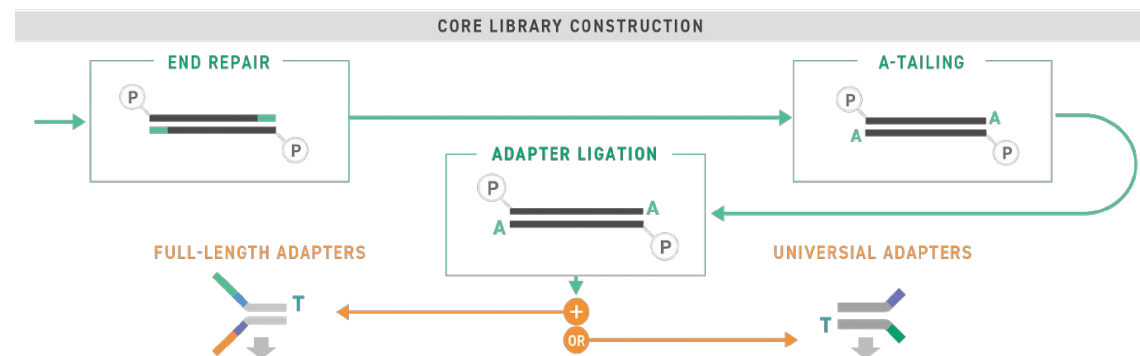
Our library preparation kits are built around Twist's engineered enzymes, which ensure high fidelity and efficient conversion.



**Higher Conversion Efficiency** → More sequencing-ready molecules from same input.

**Superior Coverage Uniformity** → Lower sequencing costs per sample, fewer wasted reads.

Twist's adapter compatibility streamlines workflows, allowing seamless transitions across genomics, epigenomics, and transcriptomics, as well as varying levels of throughput and depth.



**Flexibility in Your Workflow** → Twist Universal Adapters, offering compatibility with indexed primers or a PCR-free workflow using Twist Full-Length Adapters.

Offering scalability with auto-normalization and high-plexing for ultra-high throughput without requiring workflow overhaul.

# Specialized NGS workflows for any application

*Simplified solutions. Higher sensitivity. Suited for your needs.*



## Library Preparation Kits

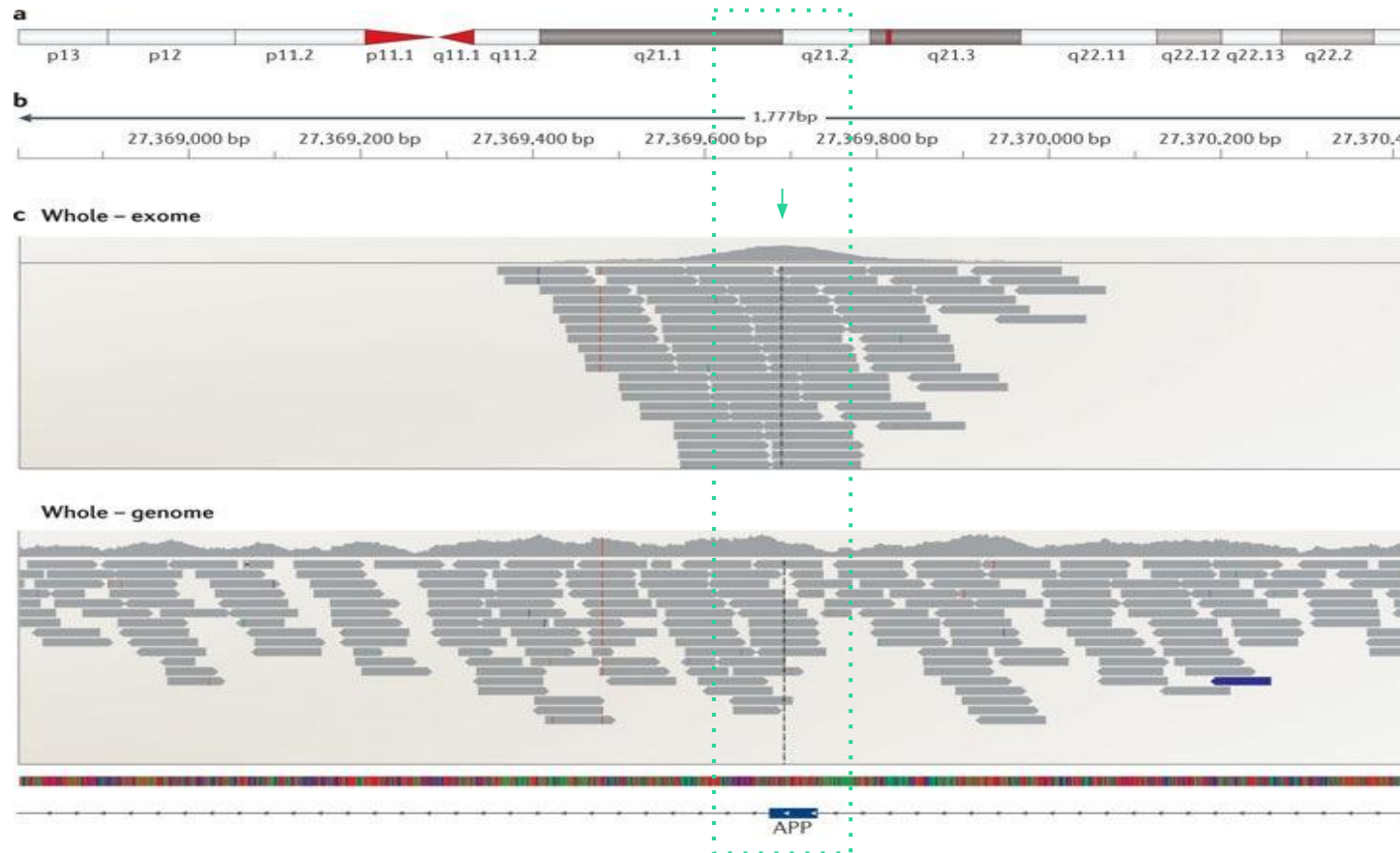
## Adapter Systems

<b>Infectious, Rare, or Mendelian diseases</b>	Enzymatic Fragmentation Kit 2.0 Mechanical Fragmentation Kit Human Sample ID	Universal Adapter System Full Length UDI Adapter System
<b>Early cancer detection / Minimal residual disease</b>	cfDNA Library Preparation Kit	UMI Adapter System
<b>Epigenomics</b>	Methylation Detection Kit	Methylated UMI Adapter System
<b>Agrigenomics / Population genomics</b>	FlexPrep UHT Library Preparation Kit	Universal Adapter System
<b>Transcriptomics</b>	RNA-Seq Library Preparation Kit <ul style="list-style-type: none"><li>• Whole transcriptome</li><li>• Target enrichment</li></ul>	Universal Adapter System UMI Adapter System
<b>WGS</b>	Flexprep UHT Library Preparation Kit	Full Length Adapter System

*Twist products are for research use only. The products presented here are not intended for the diagnosis, prevention, or treatment of a disease or condition. Twist Bioscience assumes no liability regarding use of the product for applications in which it is not intended. The results are specific to the institution to which they were obtained. The results presented are customer-specific and should not be interpreted as indicative of performance across all institutions.*

# Key Benefits of Target Enrichment

## Increased Sensitivity



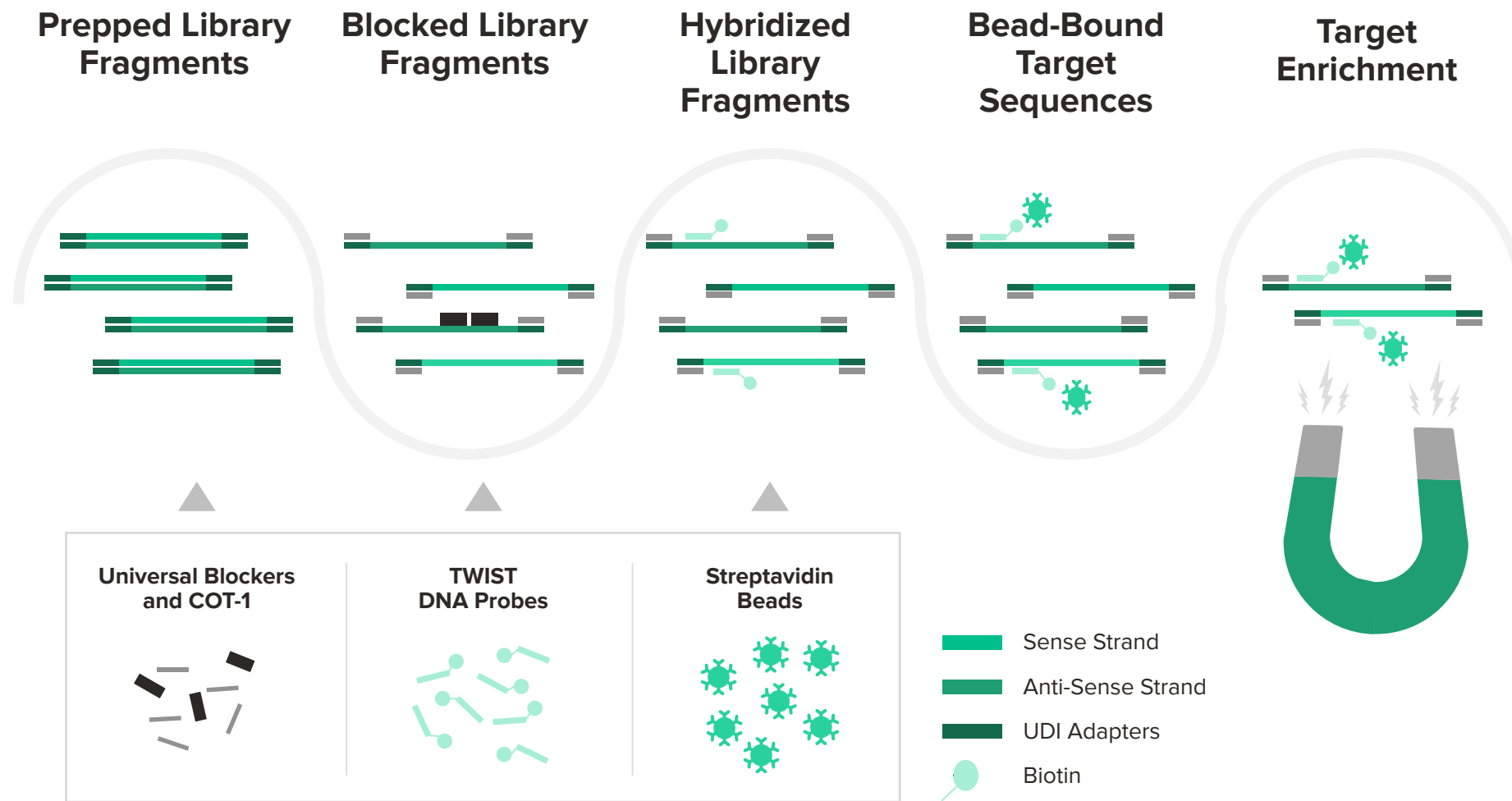
- Exome: 30x (5Gb)
- WGS: 30x (90Gb)

**Whole Exome  
Sequencing >20 Reads in  
Variant Locus**

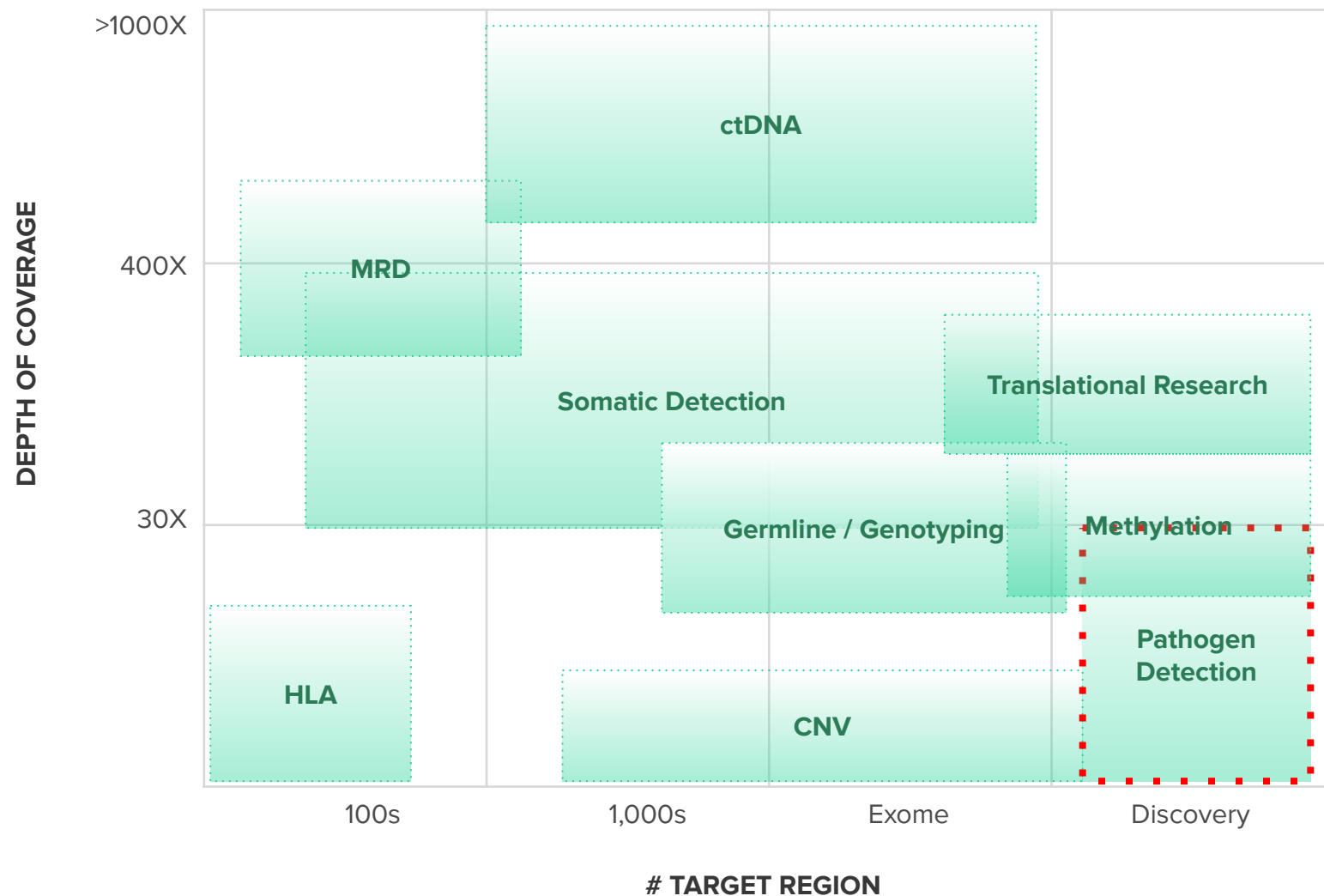
**Whole Genome  
Sequencing ~9 Reads in  
Variant Locus**



# Target Enrichment

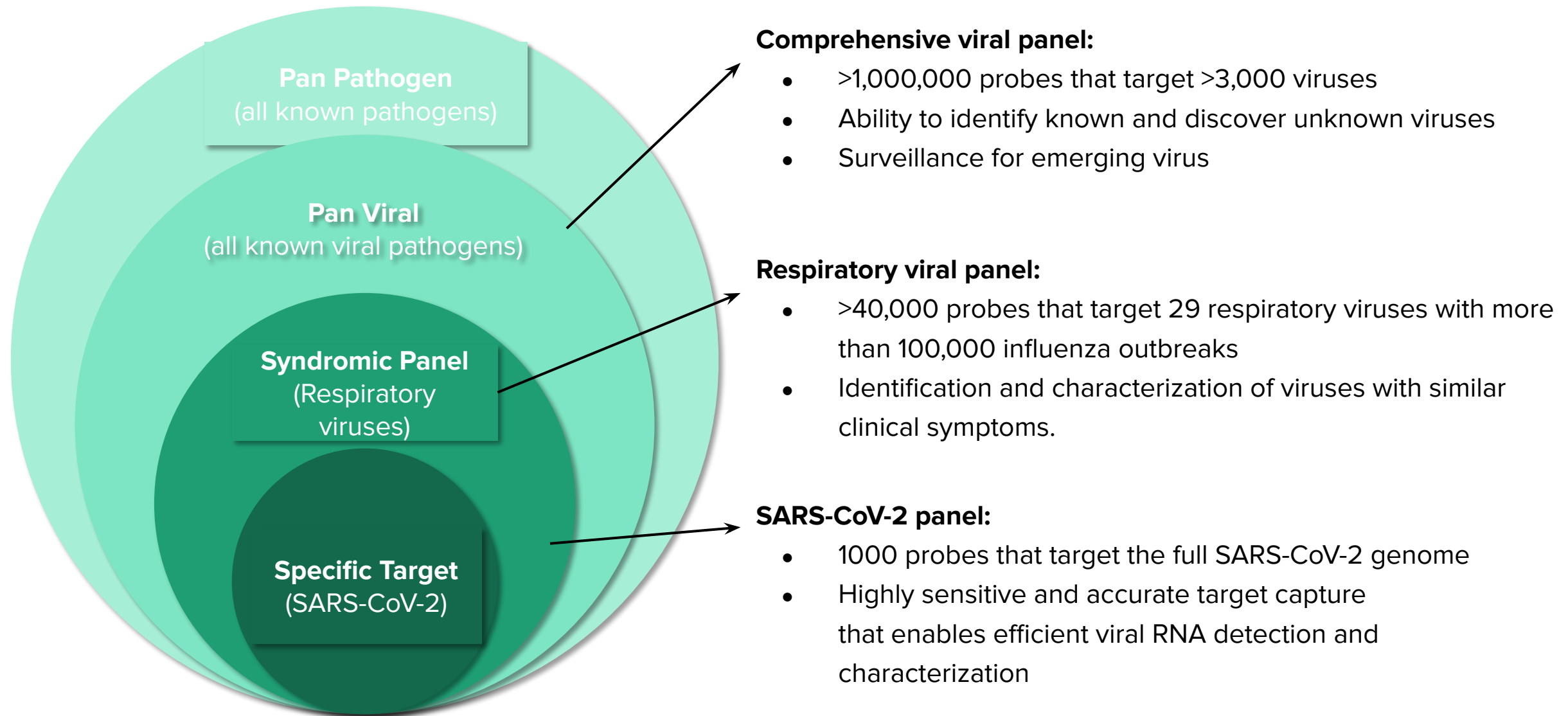


# Various Applications of Target Enrichment

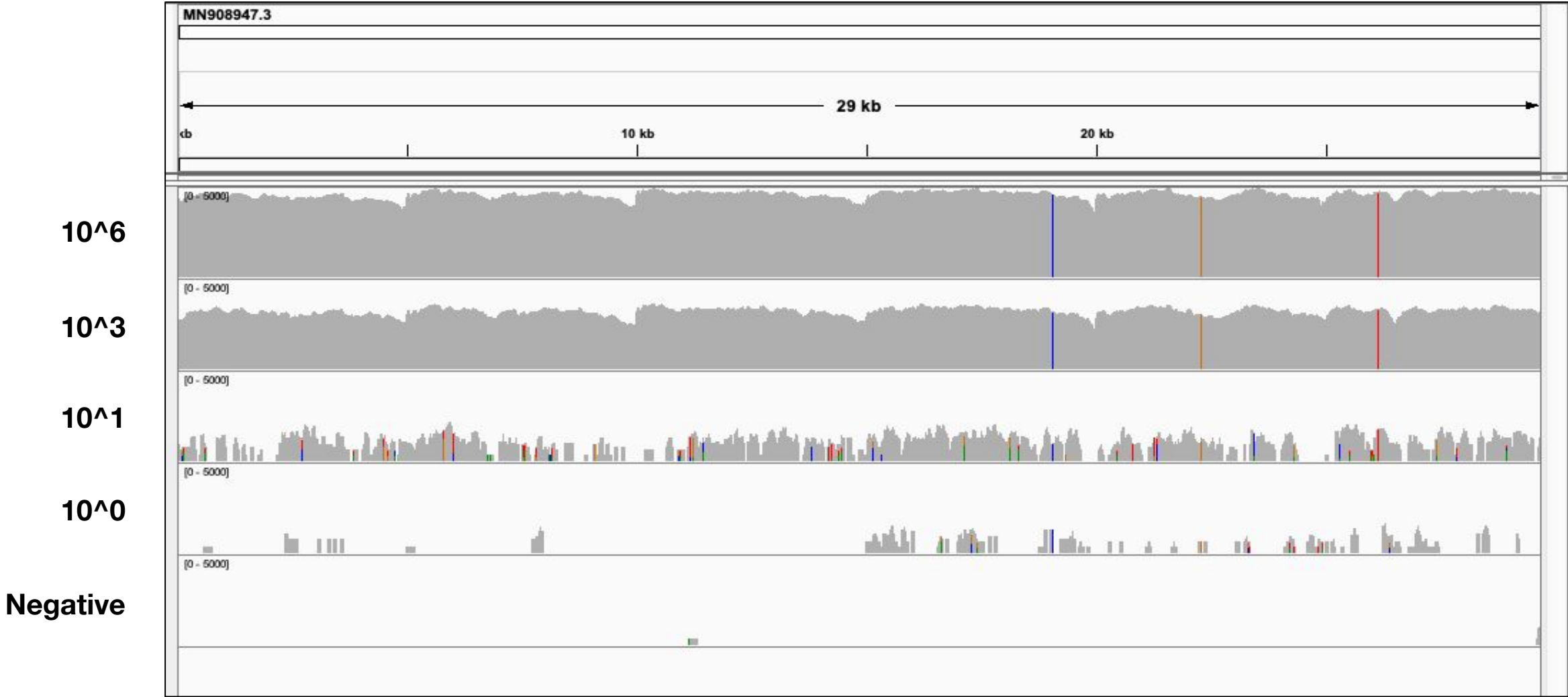


- Basic and Translational Cancer Research
- Clinical Oncology
- Microbiology and Infectious Disease
- Rare Disease
- Common and Complex Disease
- Drug Discovery

# Target Enrichment Based Pathogen Detection

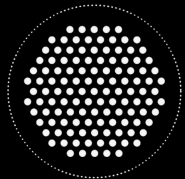


# SARS-CoV-2 IGV Coverage – Viral Copies vs. Genome Position



# Quality starts at the chip

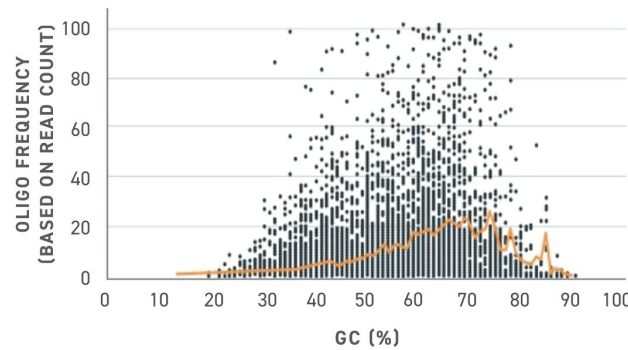
Large scale, quality oligo synthesis is the cornerstone of our portfolio.



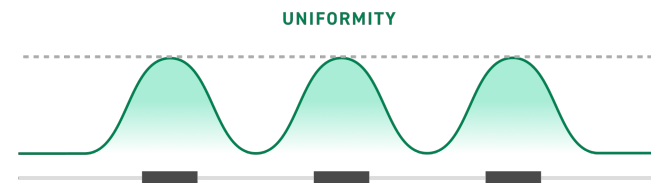
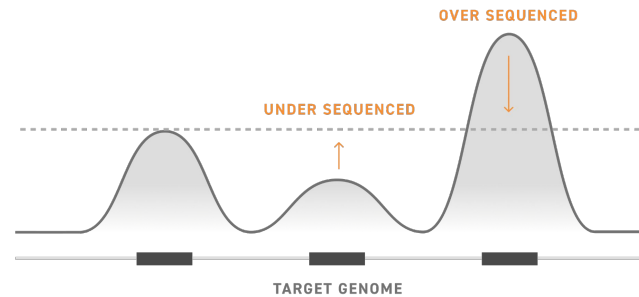
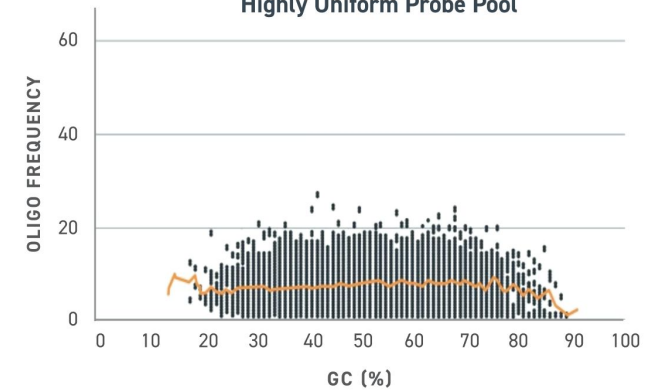
Our silicon platform enables the precision synthesis of millions of probes.

Our panels start at the writer with intelligent design strategy, get boosted on the writer, and then are uniformly amplified to create high quality panels.

Standard Amplification Method:  
Biased Probe Presentation



Twist Amplification Method:  
Highly Uniform Probe Pool



With Twist, you get flexibility without compromise: validated fixed panels to move fast, and custom panels to make your research uniquely yours.

You can design custom panels for DNA, RNA, and methylation to focus on the specific genes and targets most critical to your research—offering precision when fixed panels don't cover your needs.

# Fixed Panels with the Ability to Customize

## Fixed Panels: Off-the-Shelf Solutions

- ✓ Ready-to-use, validated panels for oncology, inherited disease, microbiome, and more.
- ✓ Optimized for high uniformity, sensitivity, and coverage.
- ✓ Accelerates research with proven designs and immediate availability.

## Custom Panels: Tailored to Your Research

- ✓ Fully customizable probe design for any genomic region.
- ✓ Fast, flexible design turnaround with Twist's silicon-based synthesis.
- ✓ Scalable for small studies to large population genomics programs.

### DNA

*For targeted precision*

### RNA

*For efficient expression profiling*

### Methylation

*For sensitive and accurate epigenetic insights*



# Custom Panels

Twist offers a customized panel design process tailored to your unique workflow.

The design will be refined through multiple iterations until it meets your specific needs and delivers optimal performance.

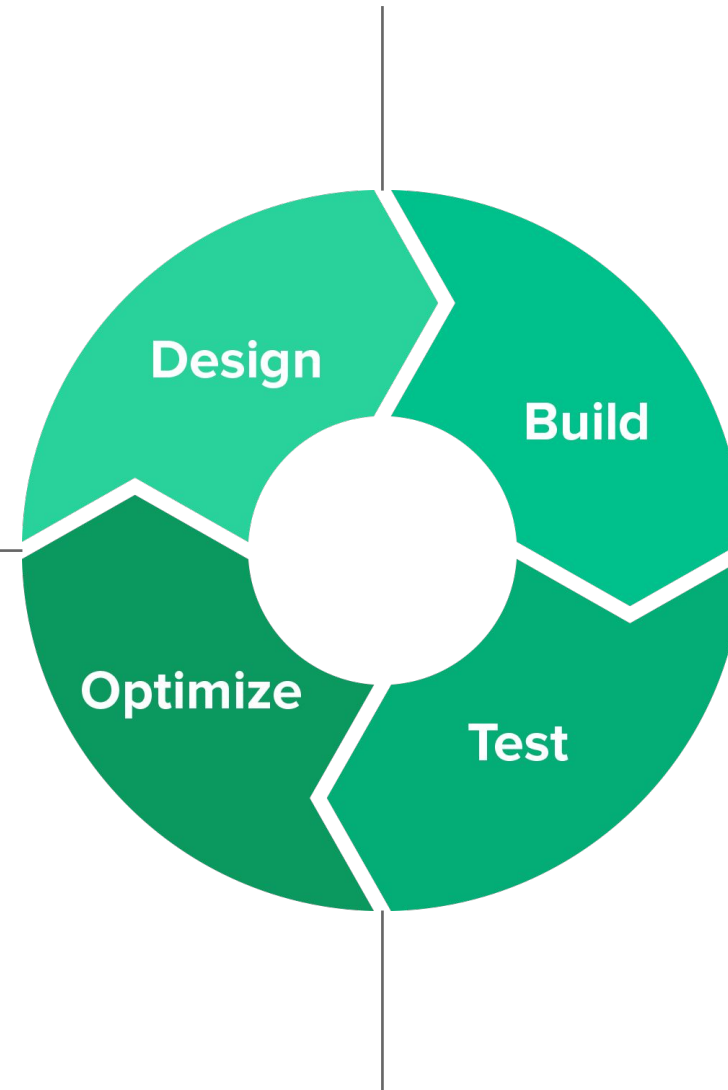
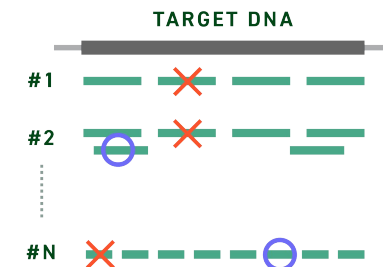
## Design

Driven by Twist's Intelligent Design Strategy.



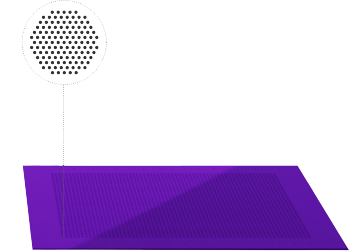
## Optimize

Probe selection for optimal performance.



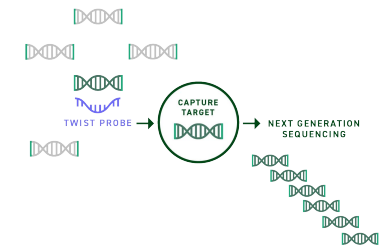
## Build

Reliable Manufacturing and QC.

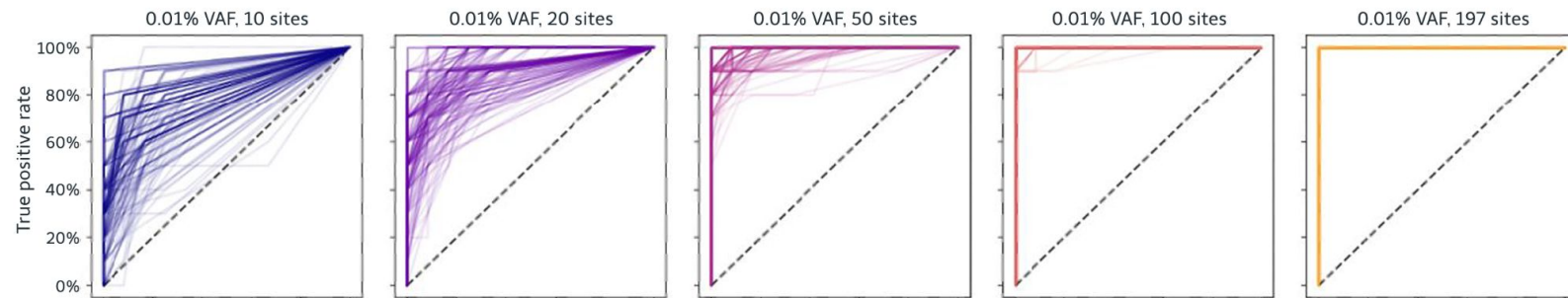


## Test

Probe Target Verification. Probes that allow you to design at your depth.



# Why Use a Custom Panel From Twist



## Proprietary Panel Design + Optimized UMI Workflow

Higher accuracy, lower off-target rates, and enhanced confidence in every result.

- Twist panels show exceptional performance across low variant allele frequencies (VAFs)

- **Twist NGS Methylation Detection System**  
**Compatible:** incorporate cancer-relevant methylation in MRD testing

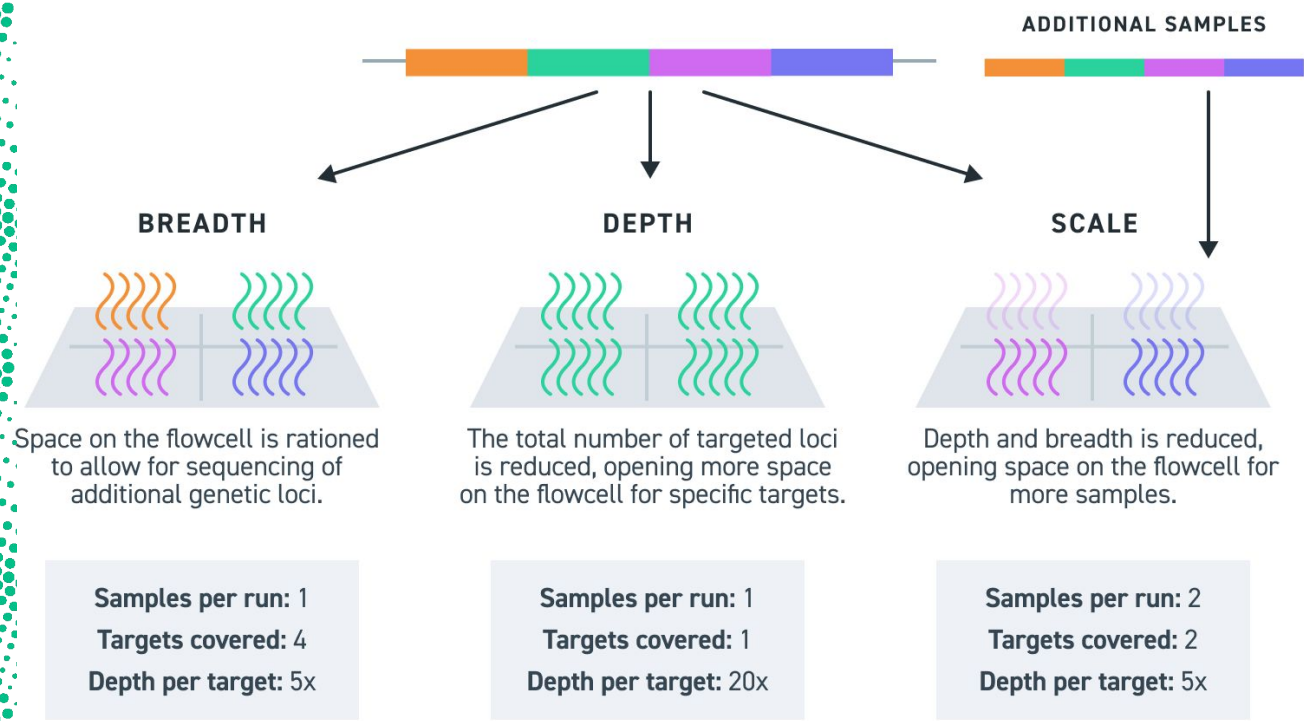
## High Sensitivity

ROC analysis indicates that Twist panels accurately and reliably detect **VAFs as low as 0.01%** by monitoring more variants.

**MRD**

# Balancing NGS Parameters for Effective MRD Testing

## Tradeoffs in Next Generation Sequencing



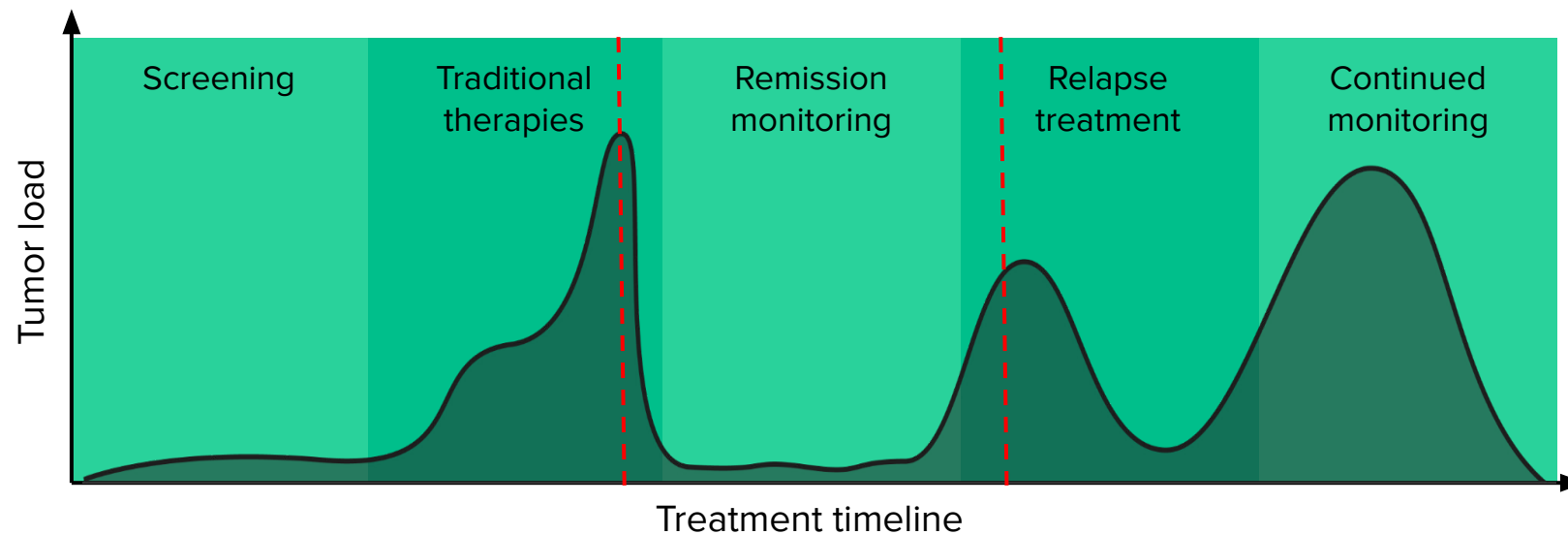
# Twist NGS & Target Enrichment Solutions

*Whole Genome Sequencing for biomarker and unbiased discovery, Target Enrichment for deep sequencing of selected targets.*

- **Ideal balance** for MRD testing
- **Deep and sensitive** sequencing
- **Low abundance NA capture** effective for liquid biopsy research
- Fixed panels detect many **variants at once (100+)**

Whole Genome	PCR-based NGS	Hybrid Capture NGS
+++breadth -depth -scalability -cost -turnaround	-breadth +depth -scalability +cost -turnaround	-breadth +depth +scalability +cost +turnaround time

# MRD in The Cancer Management Journey



- **Clinical Vigilance**  
Monitors therapeutic response, detects relapse, and assesses recurrence risks.
- **Liquid Biopsy Research Advancement**  
Tracks cancer through less invasive means to improve patient comfort and adherence.

- **Precision Oncology**  
Provides personalized treatment decisions and relapse risk stratification.
- **Drug Development Optimization**  
Serves as surrogate endpoints and improving clinical trial patient selection.

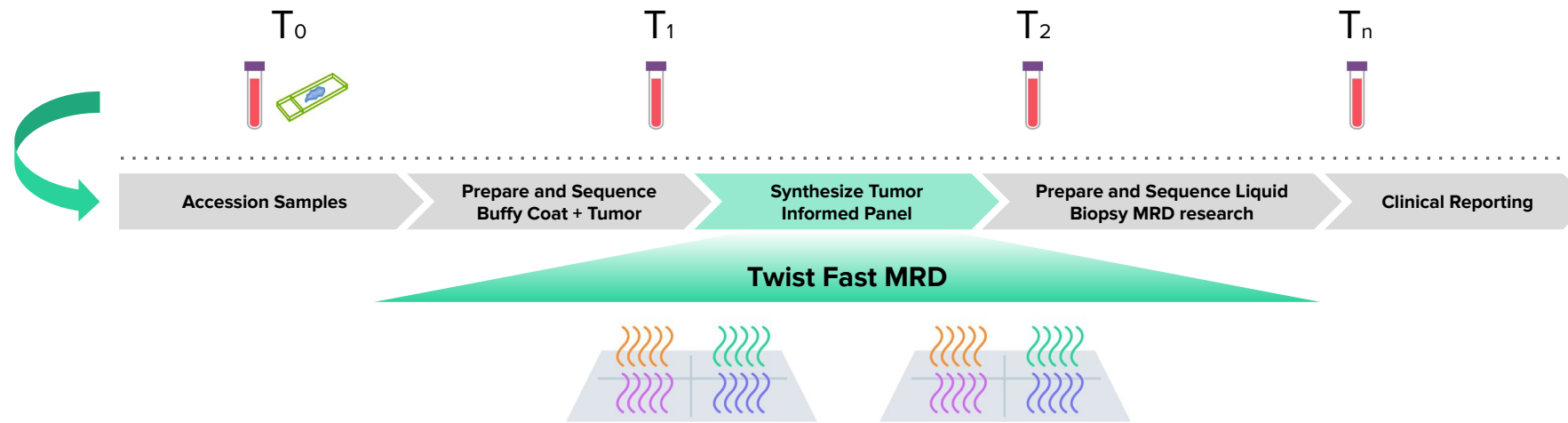
## What is MRD?

**Minimal Residual Disease (MRD)** is the presence of a small number of malignant cancer cells that remain in a patient after intervention.

## Why Next Generation Sequencing (NGS)?

NGS enables highly specific detection of cancer-derived DNA at the individual patient level, while leveraging advanced technologies that deliver the ultra-sensitive performance.

# NGS Utilization Challenges in MRD Testing



## ● Low Variant Allele Frequencies

Sequencing depths prohibitively expensive for unbiased NGS.

## ● Molecular Diversity Loss

Specificity at the level needed to monitor MRD depends on sequencing every molecule.

## ● Multiple Sample Types

Testing of multiple different sample types requires robust and dynamic workflows.

## ● Difficult and Expensive Testing

Personalized testing poses wet and dry lab logistical challenges.

## ● Data Interpretation

Analyzing complex sequencing datasets requires expertise in bioinformatics.

## ● Clinical Validation

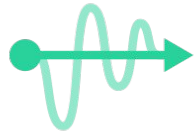
Navigating the regulatory market for personalized tests can prove arduous.

Better testing requires...

Excellent sensitivity, balanced with specificity, personalization, and accessibility.



# The Twist Advantage: How We Support Your MRD Development



## Ease of Use

- End-to-end workflow reagents
- Sequencing platform agnostic enablement
- API support for smooth ordering
- Painless panel updates



## Low Cost

- Library prep workflow reduces reagent use and hands-on labor
- High efficiency with low/degraded input, minimizes failed preps costly to liquid biopsy research



## Scalability

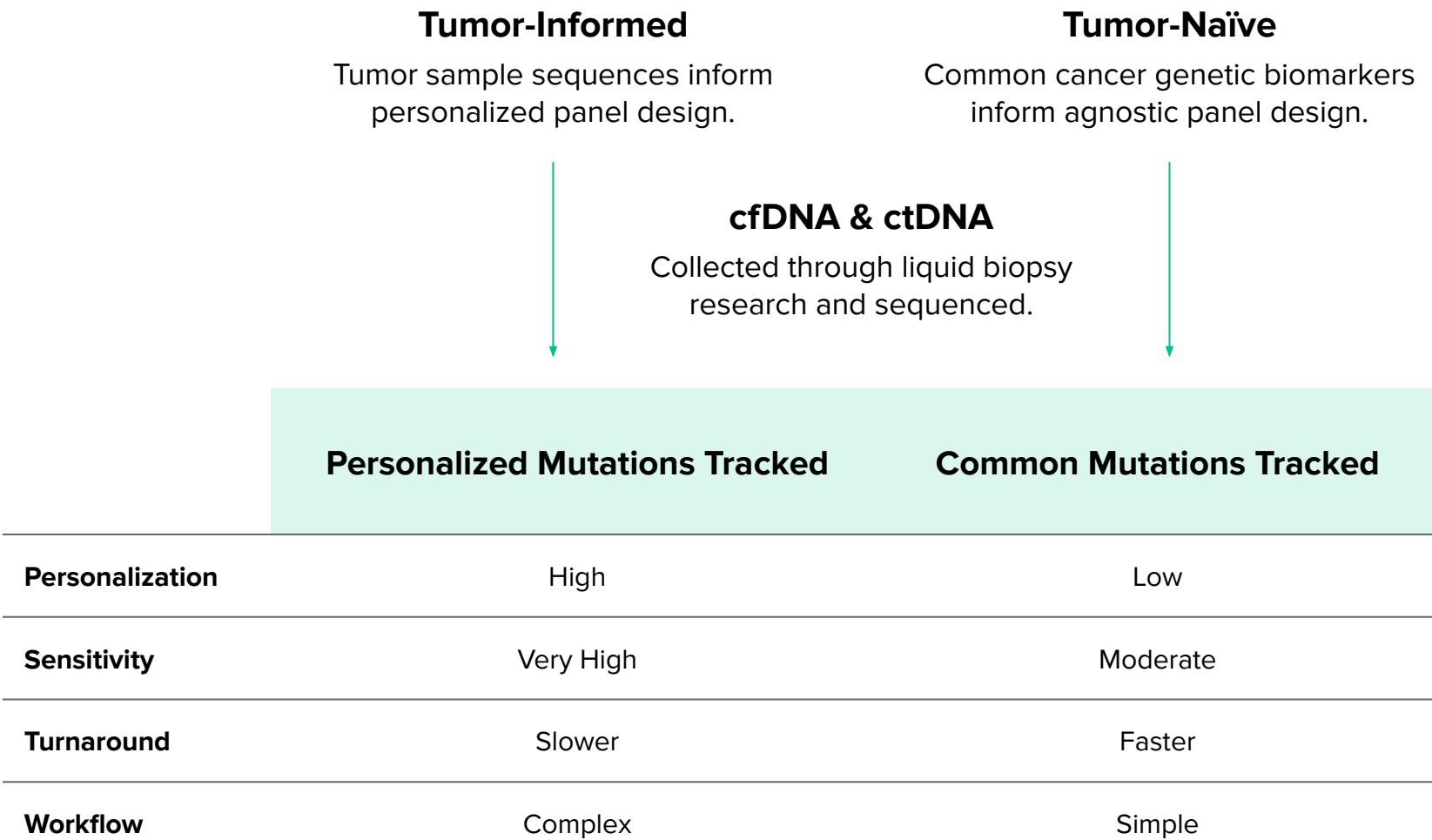
- Streamlined manufacturing & QC process
- High print capacity
- Automation friendly deliverables



## Expert Support

- Dedicated customer success and support teams
- Panel design and bioinformatics consultations
- Twist Applications Lab & ProLab service

# Measuring MRD: Tumor-Informed vs. Tumor-Naïve Approaches



## Twist Support

Tailored NGS offerings and workflows to suit your MRD approach.

### Tumor-Informed

- cfDNA Library Prep
- UMIs
- MRD 500 Panels
- cfDNA Standards

### Tumor-Naïve

- WGS
- WES & Custom Panels
- Methylation Detection

# NGS Technologies in MRD Detection

## Profile

## MRD Assays

### ● Whole Genome (WGS) & Exome (WES)

Sequence more complete genomic information, supporting target discovery & assay development (not used directly).

### ● Amplicon-Based Assays

Use amplicon strategies to improve detection; currently dominate the landscape of clinically approved MRD testing.

### ● Hybrid-Capture Target Enrichment

Uses hybridization to purify and enrich target sequences, significantly enhancing sensitivity.

## Technology Selection

Not all NGS approaches are the same.

To improve detection sensitivity, MRD assay developers must decide between **amplicon-based** and **hybrid-capture** workflows.

# Why Target Enrichment is Ideal for MRD

## Advantages of Hybrid-Capture NGS for MRD Testing

- **Higher Sensitivity & Low Input Performance**

Ultra-low VAF detection and low input deep sequencing.

- **Unlimited Target Range & Scalability (>100 targets)**

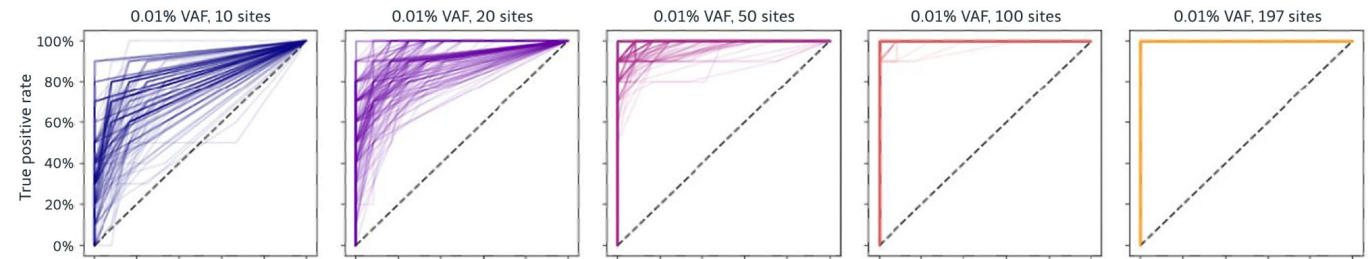
Sensitively detects many more variant targets at once.

- **Uniformity of Coverage**

More efficient sequencing saves costs by reducing runs.

- **Mitigates Clonal Hematopoiesis Interference**

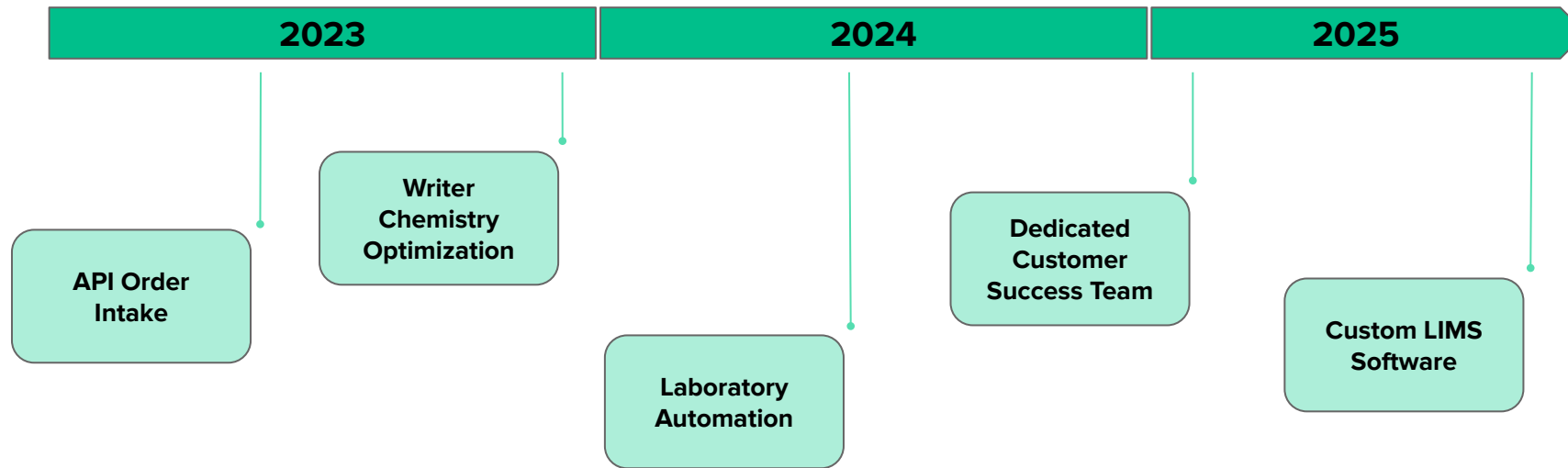
Fewer false positives than amplicon-based approaches.



### Proprietary Panel Design + Optimized UMI Workflow

Higher accuracy, lower off-target rates, and enhanced confidence in every result.

# MRD Panel Production Supporting Precision Medicine



## Supporting You with the Twist Advantage:

- Design and bioinformatic consultation
- Dedicated customer success and support teams
- Complete workflow reagents
- Rapid manufacturing & QC process
- API support for order intake, streamlined manufacturing, print capacity, and automation friendly deliverables

**8X**

Panel Capacity

**4X**

Efficiency

**0**

Time and effort  
to order

# MRD Panel Solutions

	Twist MRD Rapid 500	Twist MRD 10K	Twist Custom Panels
Turnaround Time	6 Business Days	5-10 Business Days*	14-21 Business Days
Order Input	Target Bed File	Probe Sequences via touchless API	Target Bed File Probe Sequences Genelists
Delivery Method	2D barcoded Matrix Tubes	2D barcoded Matrix Tubes or 2 mL Screw Cap Tubes	2 mL Screw Cap Tubes
Quality Control	qPCR probe pool confirmation	NGS probe confirmation or Probe pool optical density reading	NGS probe confirmation
Panel Size (probe count)	50 – 500	50 - 10,000	50 – unlimited
Reactions	12 reactions	12 - 120 reactions	Choice from 12 — unlimited
Price	\$600 ASP	Variable	Variable



Automation friendly order submission and deliverables



Reactions and volume tailored to your offering



Reliable turnaround time



# Case Study: Validation of Ultra Sensitive ctDNA Target Capture Assay for Tracking Solid Tumors

Current approaches to MRD assays often target only 50 variants or less, and suffer from limits of detection unable to detect early recurrence.

## ● Goal

Researchers aimed to develop a tumor-informed ctDNA assay that maintains high sensitivity and specificity while expanding the number of variants up to 1,800.

## ● Results

NeXT Personal ctDNA assay resulted in an **LOD of 3.45 ppm**, **excellent quantitative linearity**, and 100% accurate in 328 control samples.

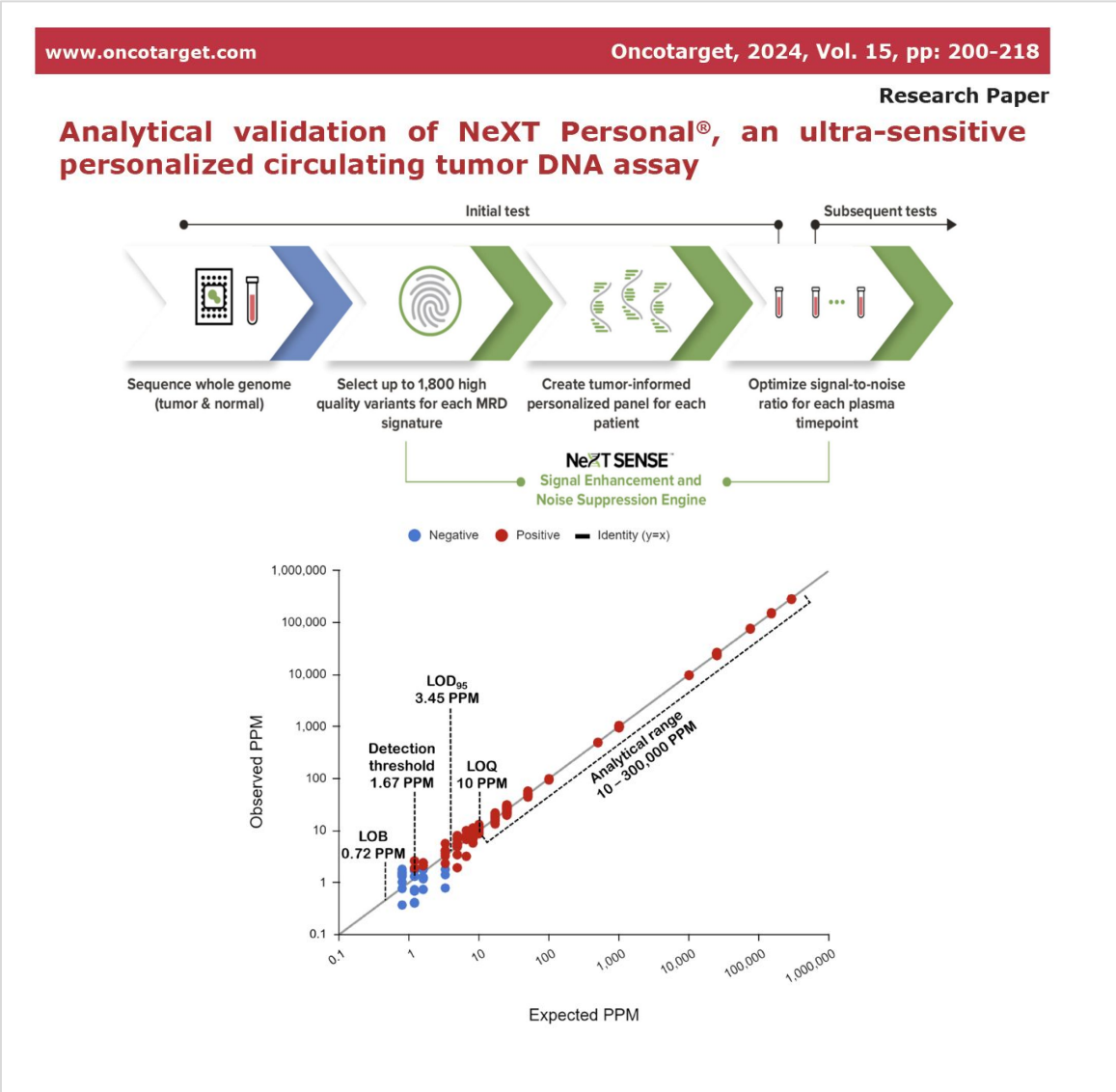
Reference: Northcott et al., 2024, Oncotarget, 15, 200-218, <https://doi.org/10.18632/oncotarget.28565>  
\*Results from case studies are not predictive of results in other cases. Results in other cases may vary.

## ● Twist Products

- [Custom Panel](#)
- [Fast Hybridization and Wash Kit](#)

## ● Highlight

**Twist hybrid capture products** allowed researchers to support a **large increase in assay multiplexing**, powering **excellent sensitivity and specificity** across 9 tumor types.



# Case Study: Whole Exome Sequencing Supports Identification of Personalized ctDNA Biomarkers for NSCLC Detection

Molecular residual disease (MRD) assay sensitivity limitations can obscure early detection of NSCLC recurrence.

## Goal

Researchers aimed to improve MRD detection sensitivity using a **personalized tumor-informed** approach (PROPHET) designed from **whole exome sequencing (WES)** data.

## Results

PROPHET MRD assay notably **improves sensitivity, detecting cancer 170 days earlier** than the next best fixed panel assay.

## Twist Products

- [Human Core Exome Panel](#)
- [Fast Hybridization and Wash Kit](#)
- [Mechanical Fragmentation Library Preparation Kit](#)
- [Universal Blocker](#)

## Highlight

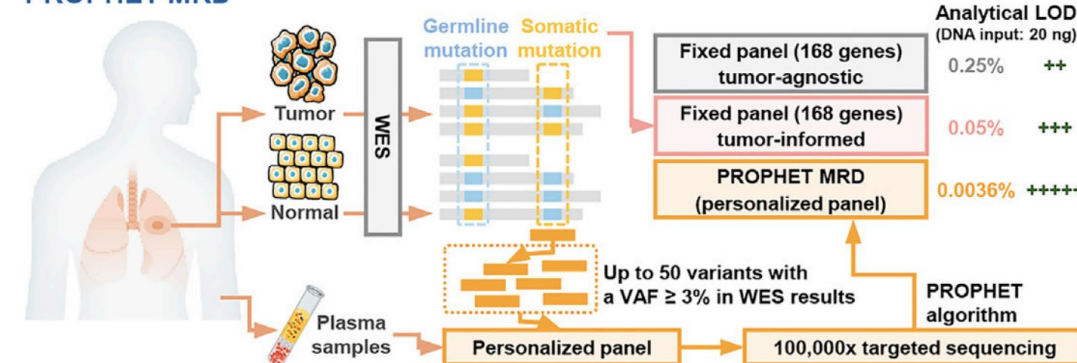
Data Collected using **Twist's Core Exome Panel** allowed researchers to inform the **selection of 50 patient-specific variant biomarkers** driving PROPHET MRD.

## Cancer Cell

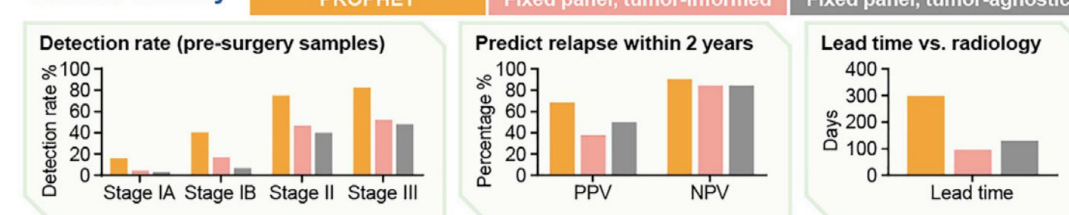
**Individualized tumor-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer**

Article

### PROPHET MRD



### Clinical validity



Reference: Chen et al., 2023, Cancer Cell, 41, 1749-1762, <https://doi.org/10.1016/j.ccell.2023.08.010>

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# Case Study: IntegraGen-Liquid Biopsy Research Solution for Highly Sensitive MRD Detection using Twist Tools

## Challenges

Being able to **detect extremely low occurrences of Minimal Residual Disease (MRD)** in liquid biopsy is critical to informing treatment strategies, predicting relapse risk and improving patient outcomes.

## Method

- Twist cfDNA library preparation kit with UMIs
- Twist Exome used with Integragen Mercury software to select tumor variants
- 2 MRD Panels created and tested with Twist Pancancer Reference Standards
- 24 samples taken from 8 patients at 3 time points V1: Before Treatment V2: 4 Weeks after Treatment V3: After Treatment and Relapse

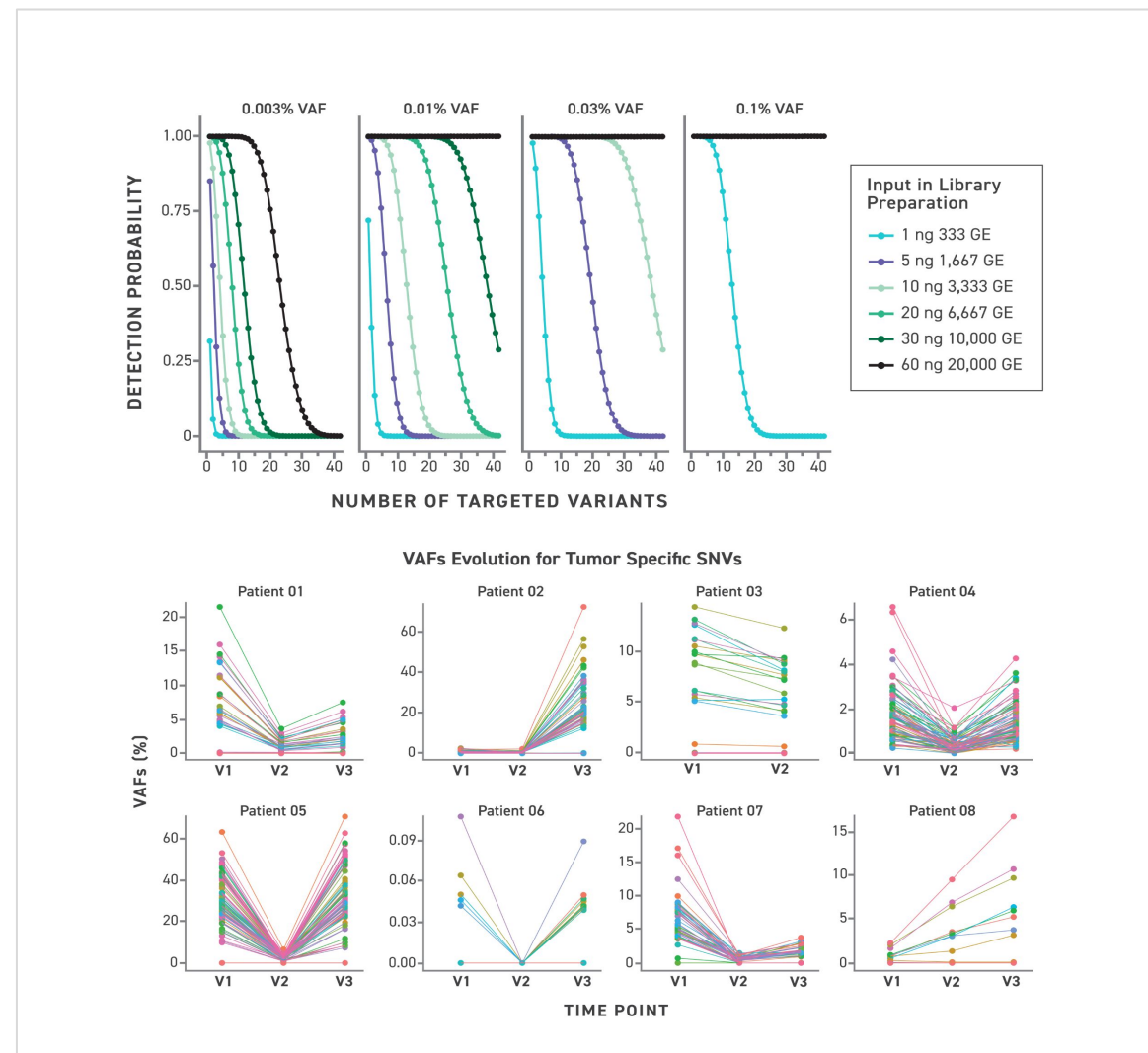
## Results

**Established LoD of 0.003% using Twist Pancancer Reference standard.**

- Validation on clinical samples **confirmed ability to reliably detect tumor variants** in all tested patients at follow up times
- Highly **customizable and sensitive assay** now implemented by IntegraGen under name OncoFollow

## Goals

Develop an efficient MRD workflow that is able to **detect extremely low variant allele frequencies.**



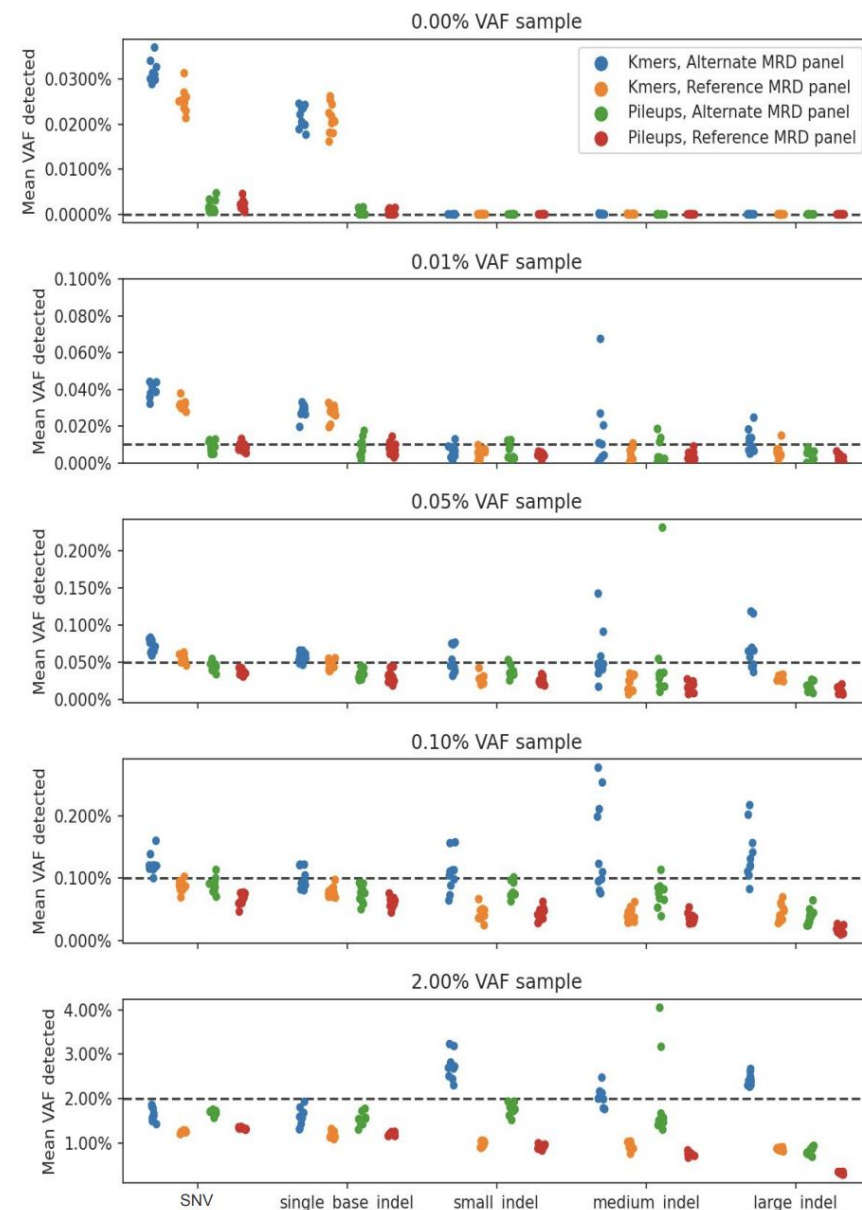
*\*Results from case studies are not predictive of results in other cases. Results in other cases may vary.*

# Twist MRD Rapid 500 Panels

*Fully personalized MRD Panels in 6 days\* from design to ship.*

- With up to **500 targets**, MRD Rapid 500 Panels offer better coverage with less variation than equivalent amplicon methods
- **Captures variants of interest** without losing critical information like fragment size and start/stop positions
- Offered at an **industry-leading price point**, retiring the trade-off between content and cost

\*Twist MRD 500 Panels based on internal data available as of 2025. This timeframe refers to the typical processing and handling time within our facilities before your order is handed over to the shipping carrier. Actual delivery times will vary depending on your location, the chosen shipping method, and the carrier's handling procedures.

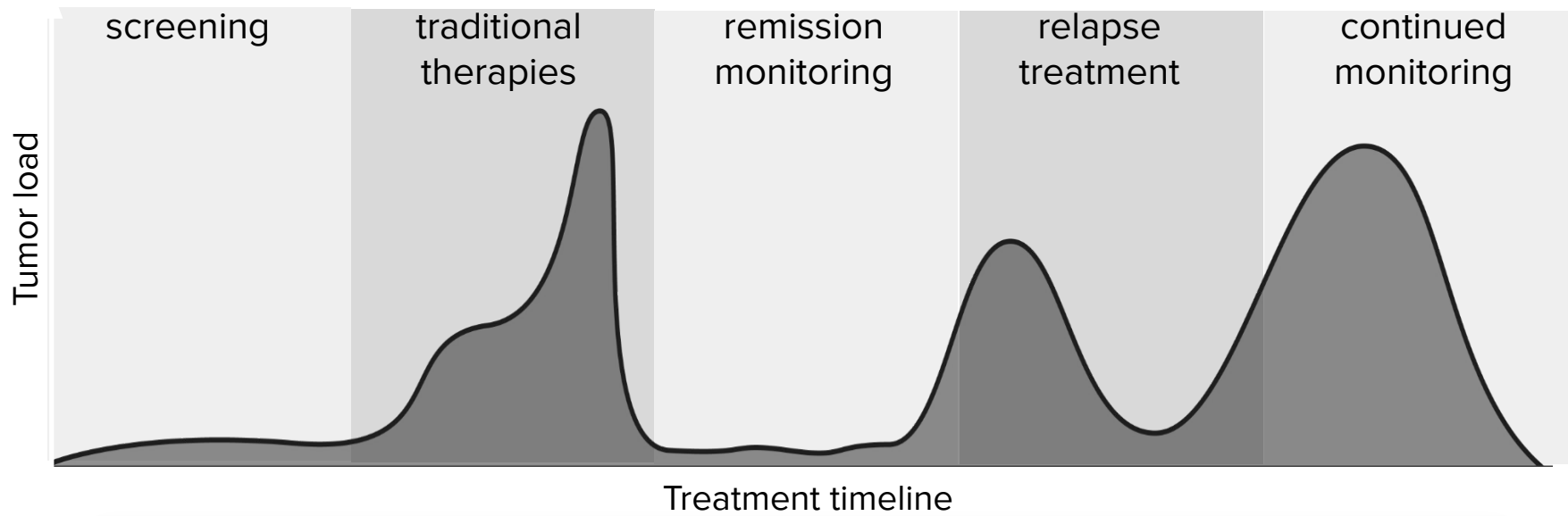


## Sensitive Variant Detection

Targeting alternative alleles with MRD Rapid 500 panels sensitively detects mutations at low variant allele frequencies (VAF), especially when combined with K-mers based searches (blue).



# NGS Solutions Across the Oncology Journey



## End-to-end cancer care solutions

Screening		Early Detection + Profiling		Minimal Residual Disease		Neoantigen Therapy	
<b>WGS + WES:</b> High throughput applications	<b>RNA-Seq:</b> Low input, FFPE, Whole transcriptome	<b>WGS + WES:</b> High throughput applications	<b>Methylation:</b> Epigenetic biomarker detection	<b>Fast MRD:</b> 6 BD TAT, personalized panels	<b>WGS + WES:</b> High throughput applications	<b>mRNA</b>	<b>Clonal Genes</b>
<b>Library Prep + Enrichment</b>	<b>Custom Panels (HLA):</b> Multi-allelic coverage	<b>Liquid Biopsy:</b> Deep sequencing applications	<b>DNA/RNA Panels:</b> Oncology detection	<b>Custom Panels</b> Targeted tissue informed tests	<b>cfDNA LP</b> Ultra sensitive library preparation	<b>GMP</b>	<b>Large preps</b>



# Powering the Genomics Revolution

Thank you!