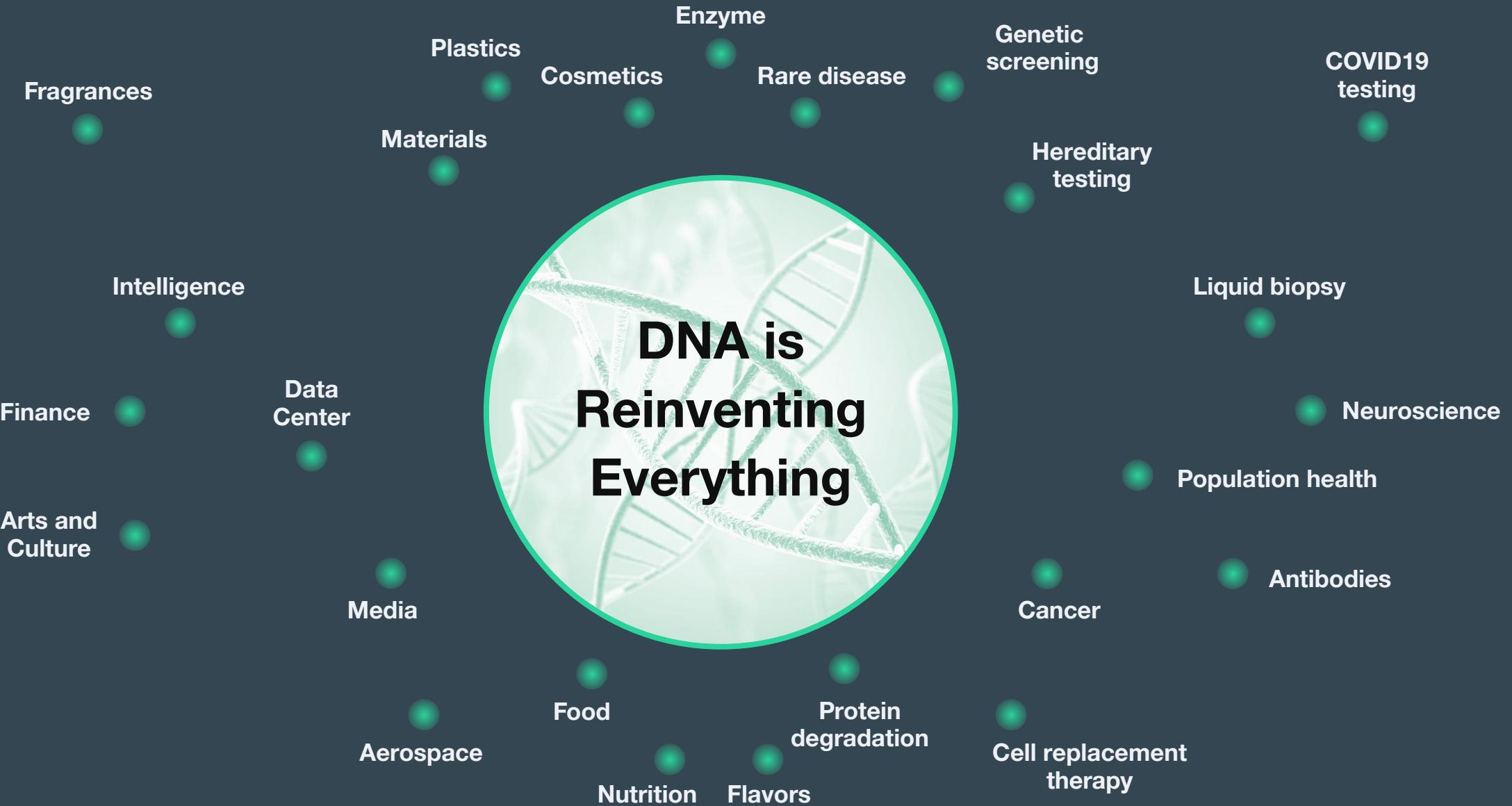




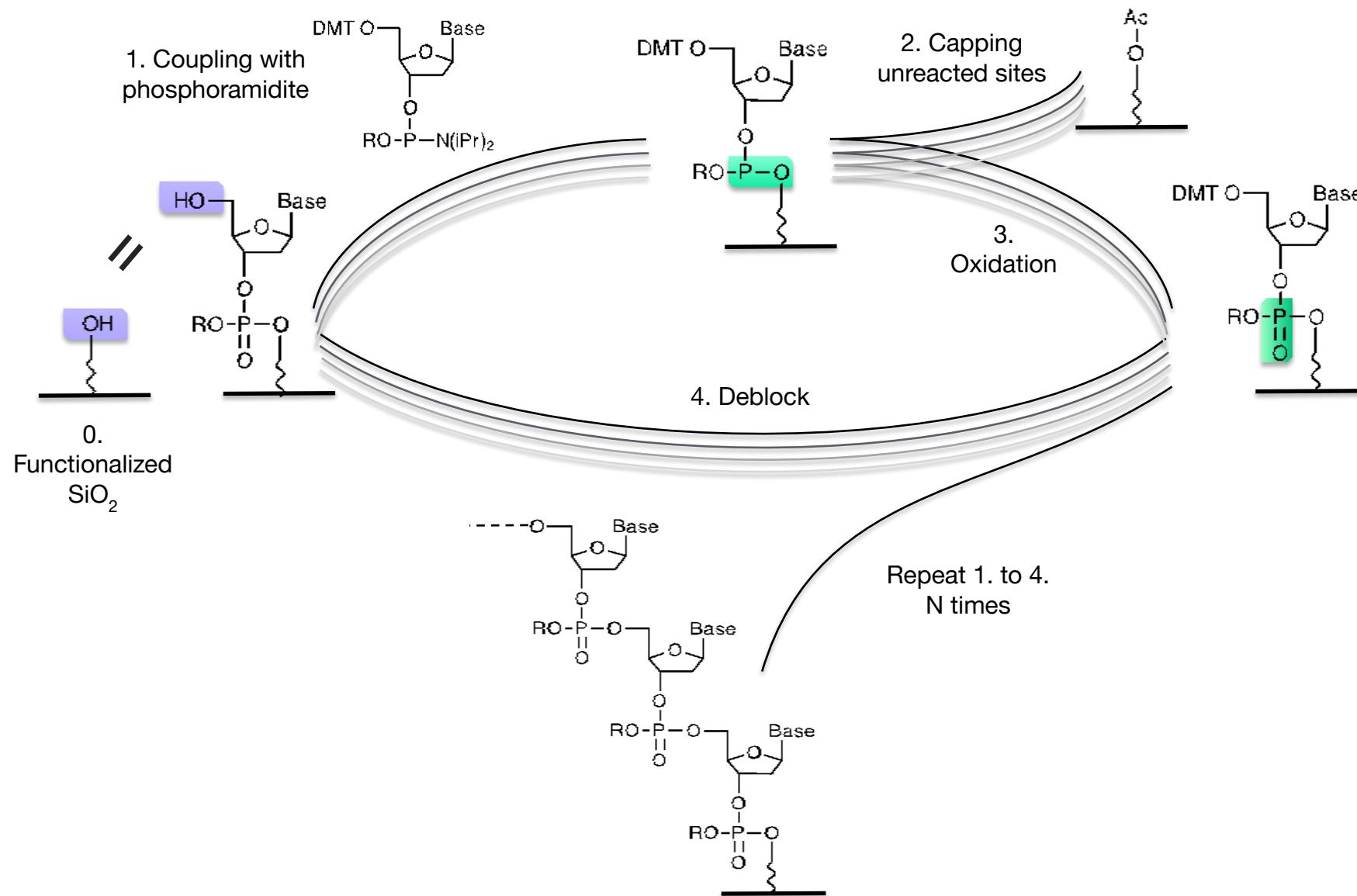
DNA Synthesis Development and Application:

“HOW TO GROW ALMOST ANYTHING”

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



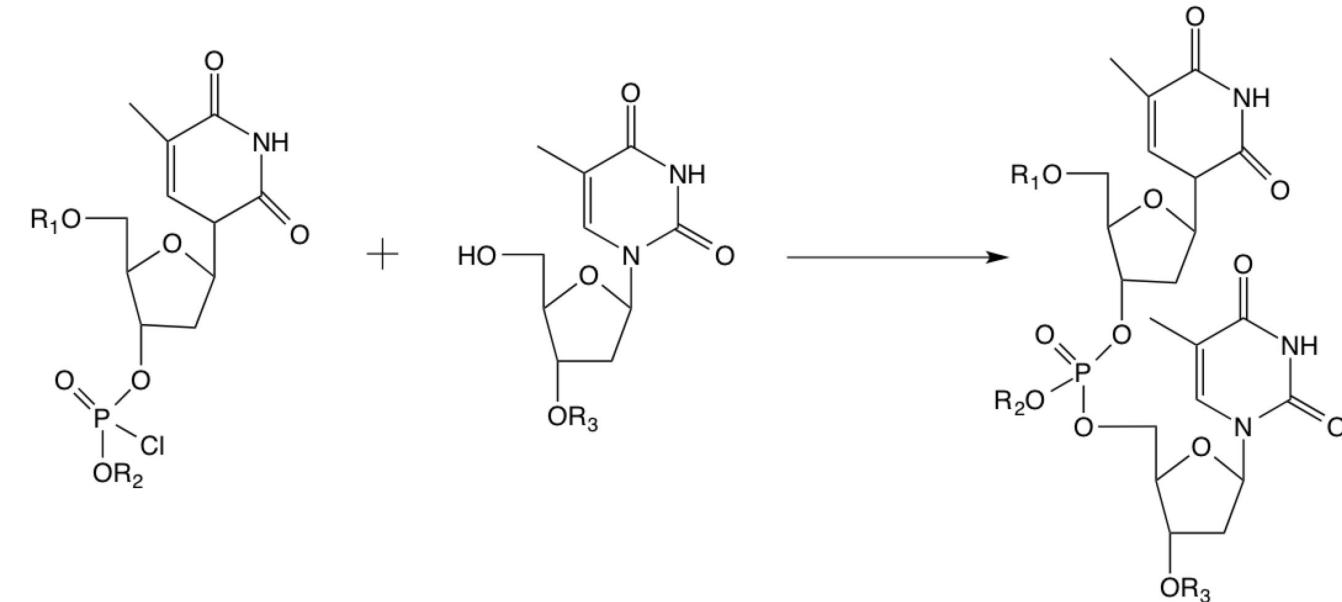
Oligonucleotide Synthesis



History of DNA Synthesis - Chemistry Development

1955

Synthesis of the first dinucleotide
by Michelson



1955

1960

1965

1970

1975

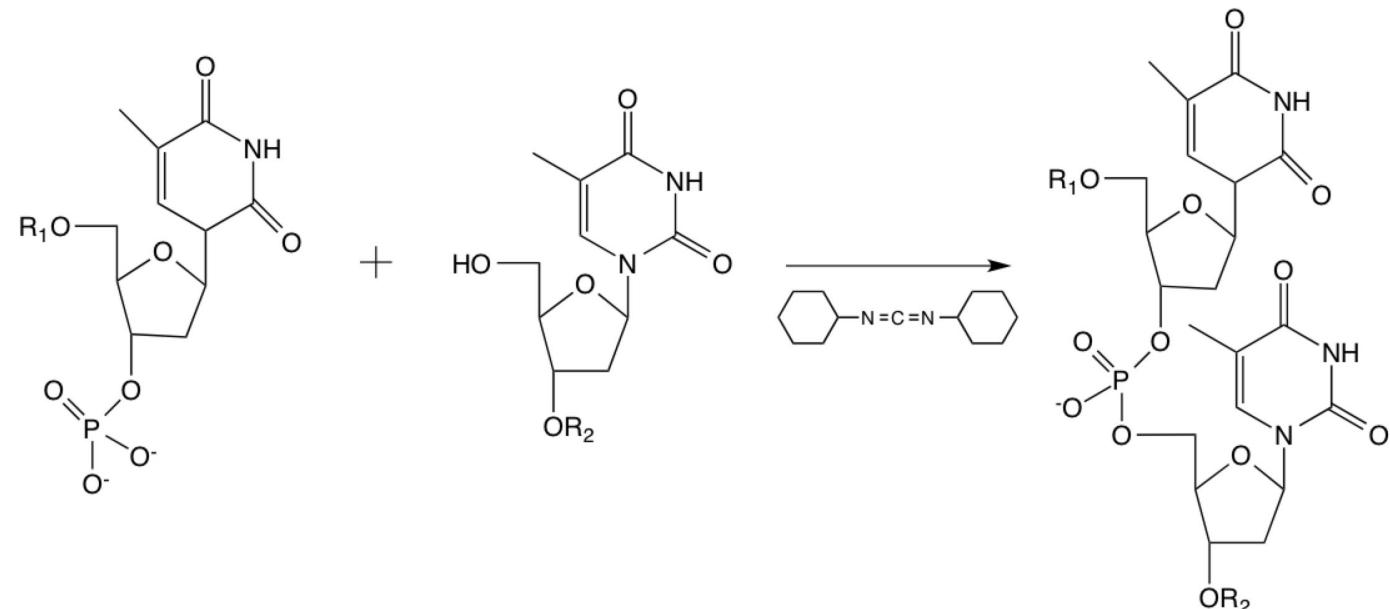
1980

1985

History of DNA Synthesis - Chemistry Development

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Synthesis of the first dinucleotide
by Michelson



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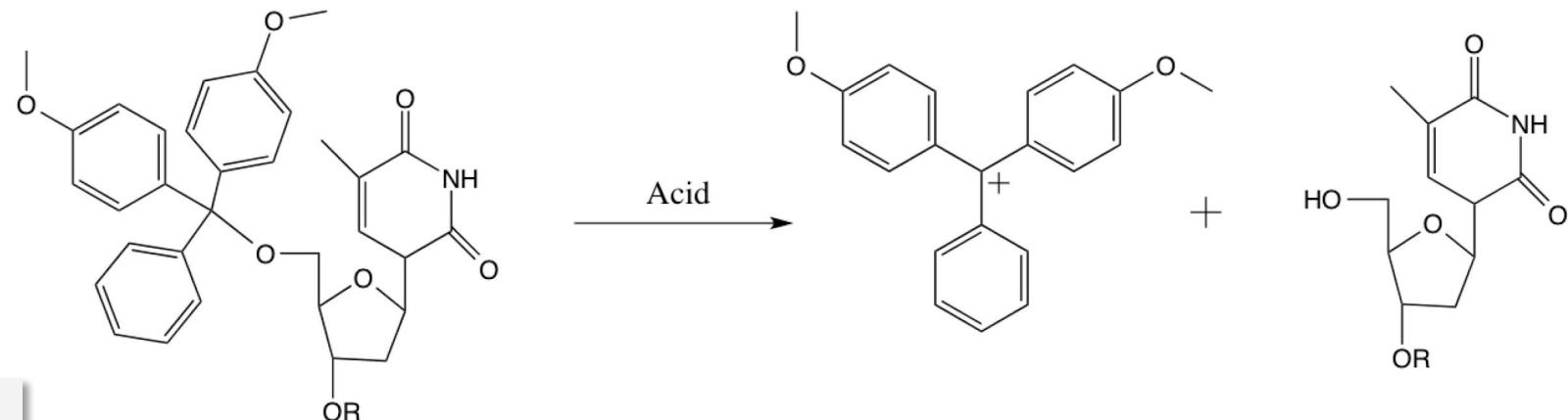
1956

Phosphodiester
method by Khorana

History of DNA Synthesis - Chemistry Development

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Synthesis of the first dinucleotide
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1961

On/Off protection scheme for
sequential synthesis by Khorana

1955

1960

1965

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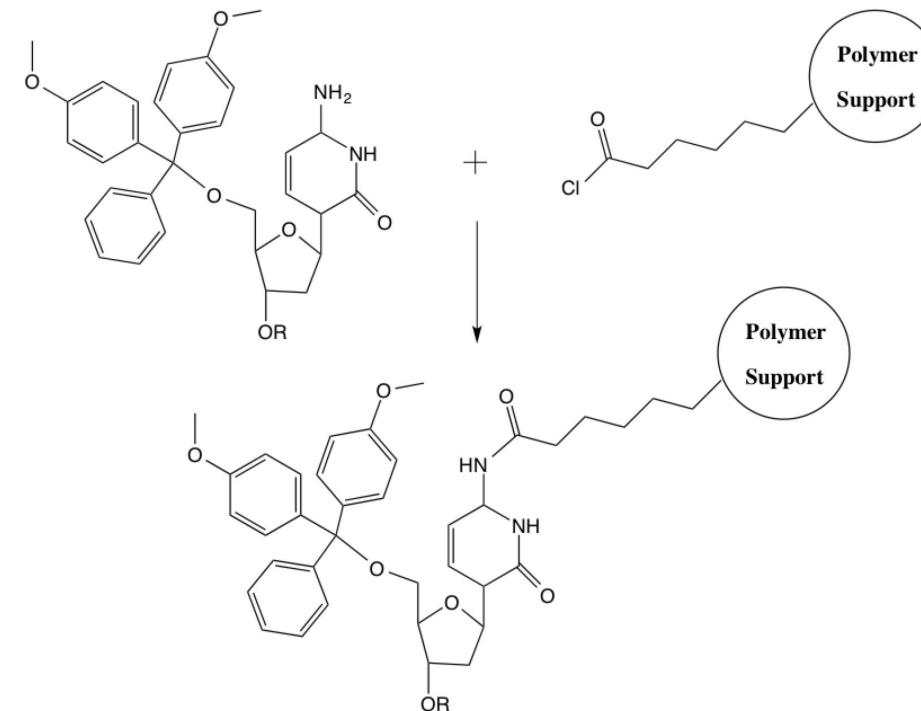
1965

1970

1975

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1985



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Phosphodiester method by Khorana

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Solid phase synthesis of oligos on organic polymer support by Letsinger

History of DNA Synthesis - Chemistry Development

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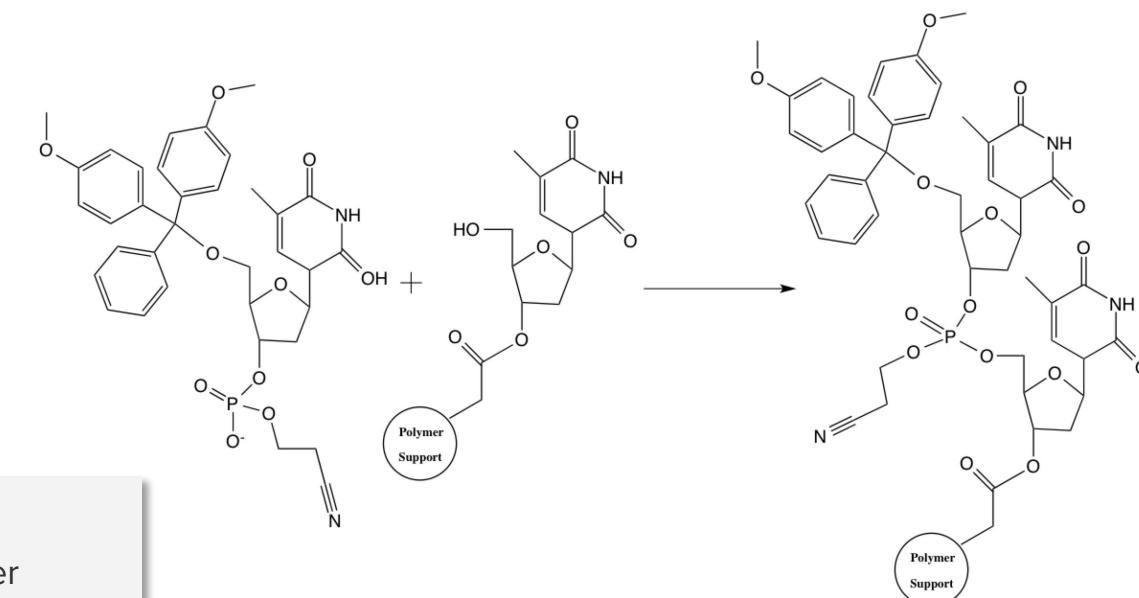
Phosphodiester
method by Khorana

1965

Solid phase synthesis of
oligos on organic polymer
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1969

Phosphotriester
method by Letsinger



History of DNA Synthesis - Chemistry Development

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Solid phase synthesis of
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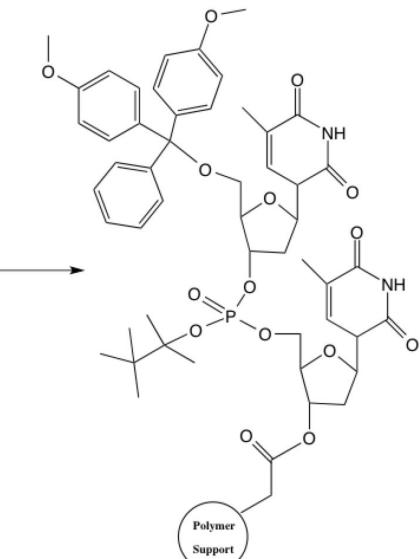
1969

Phosphotriester
method by Letsinger

1975

1976

Phosphitetriester
method by Letsinger



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

1955

1960

1965

1970

1975

1980

1985

1956

Phosphodiester method by Khorana

1965

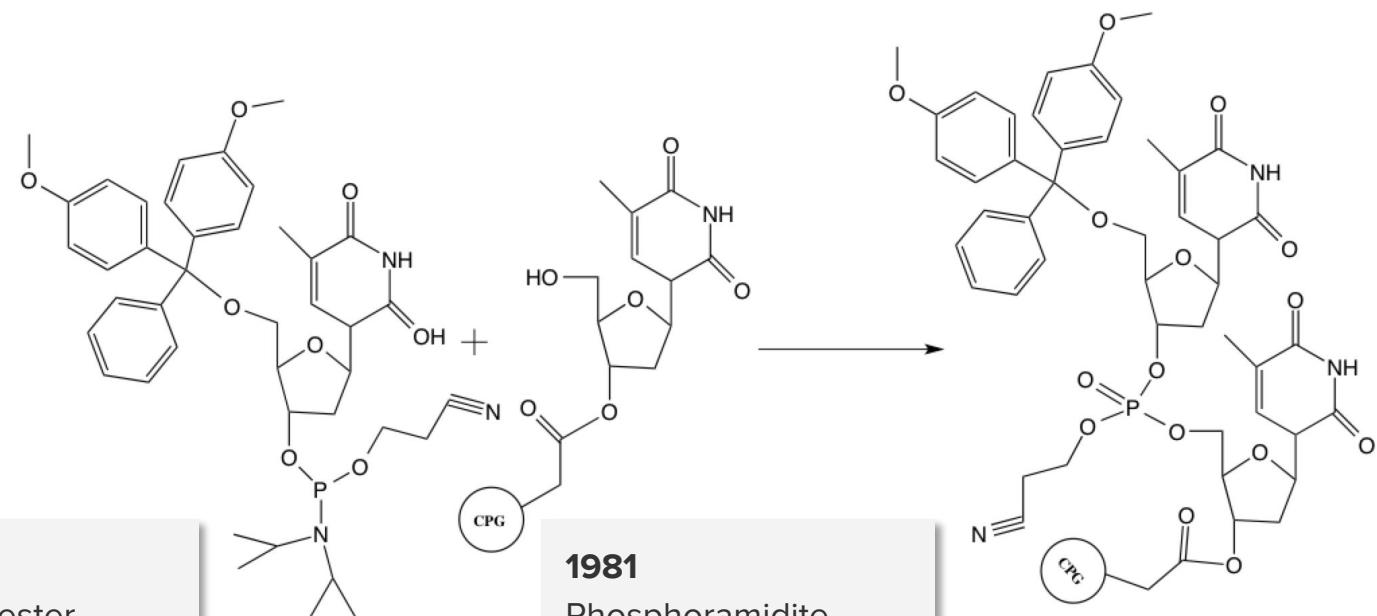
Solid phase synthesis of oligos on organic polymer support by Letsinger

1976

Phosphitetriester method by Letsinger

1981

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



History of DNA Synthesis - Engineering

1955

Synthesis of the first dinucleotide
by Michelson

1961

On/Off protection scheme for
sequential synthesis by Khorana

1969

Phosphotriester
method by Letsinger

1955

1960

1965

1970

1975

1980

1985

1956

Phosphodiester
method by Khorana

1965

Solid phase synthesis of
oligos on organic polymer
support by Letsinger



1983

First automated DNA
synthesizer by ABI

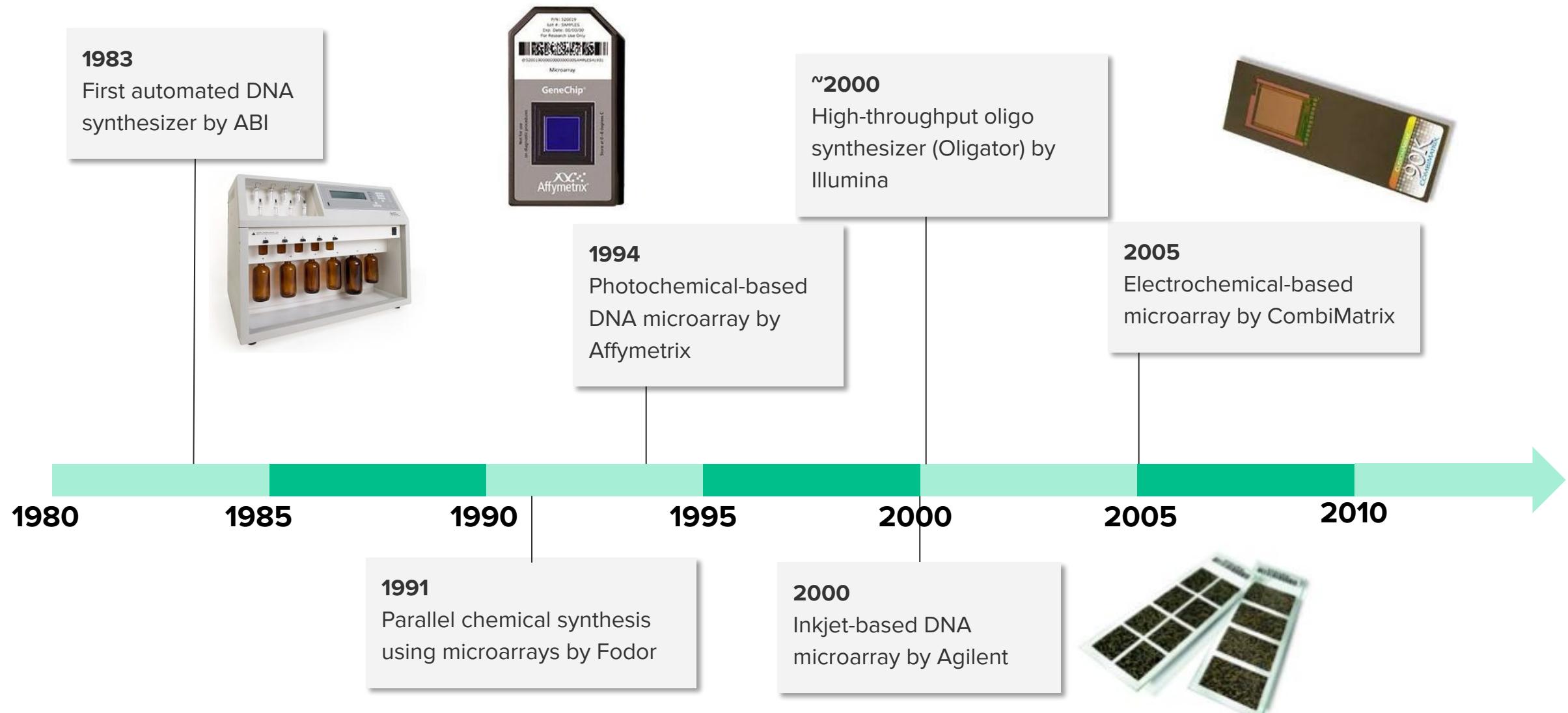
1976

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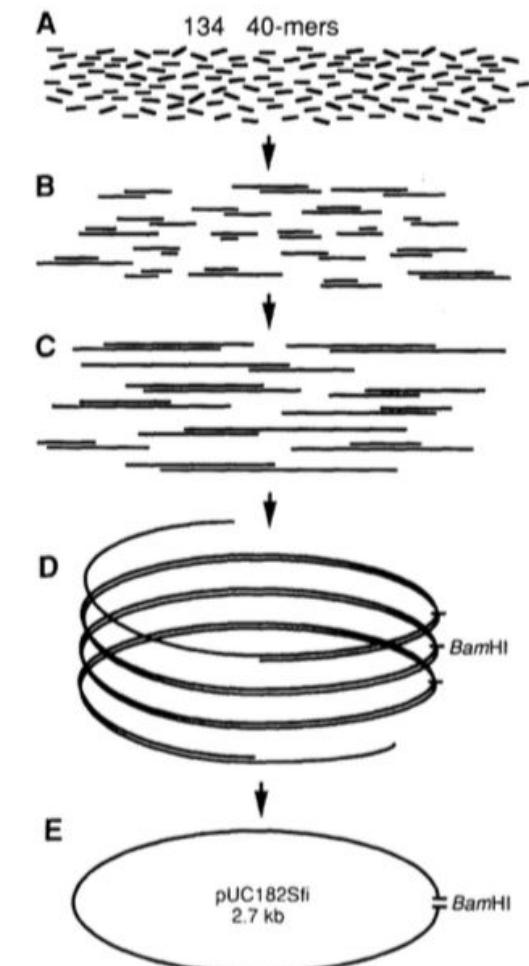
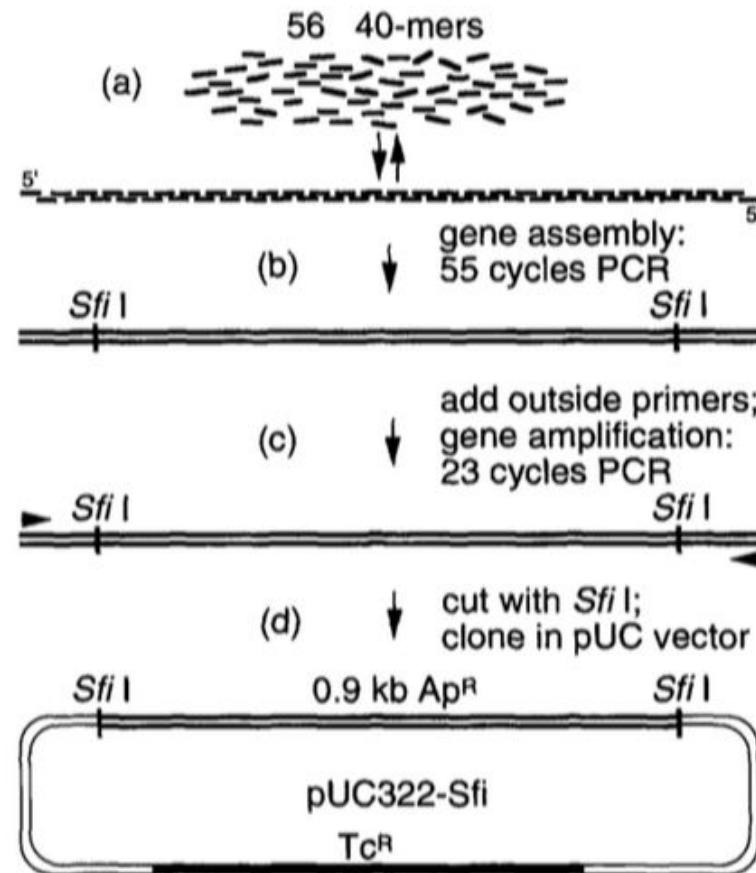
Solid phase synthesis of
oligos on inorganic support
(CPG) by Caruthers

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

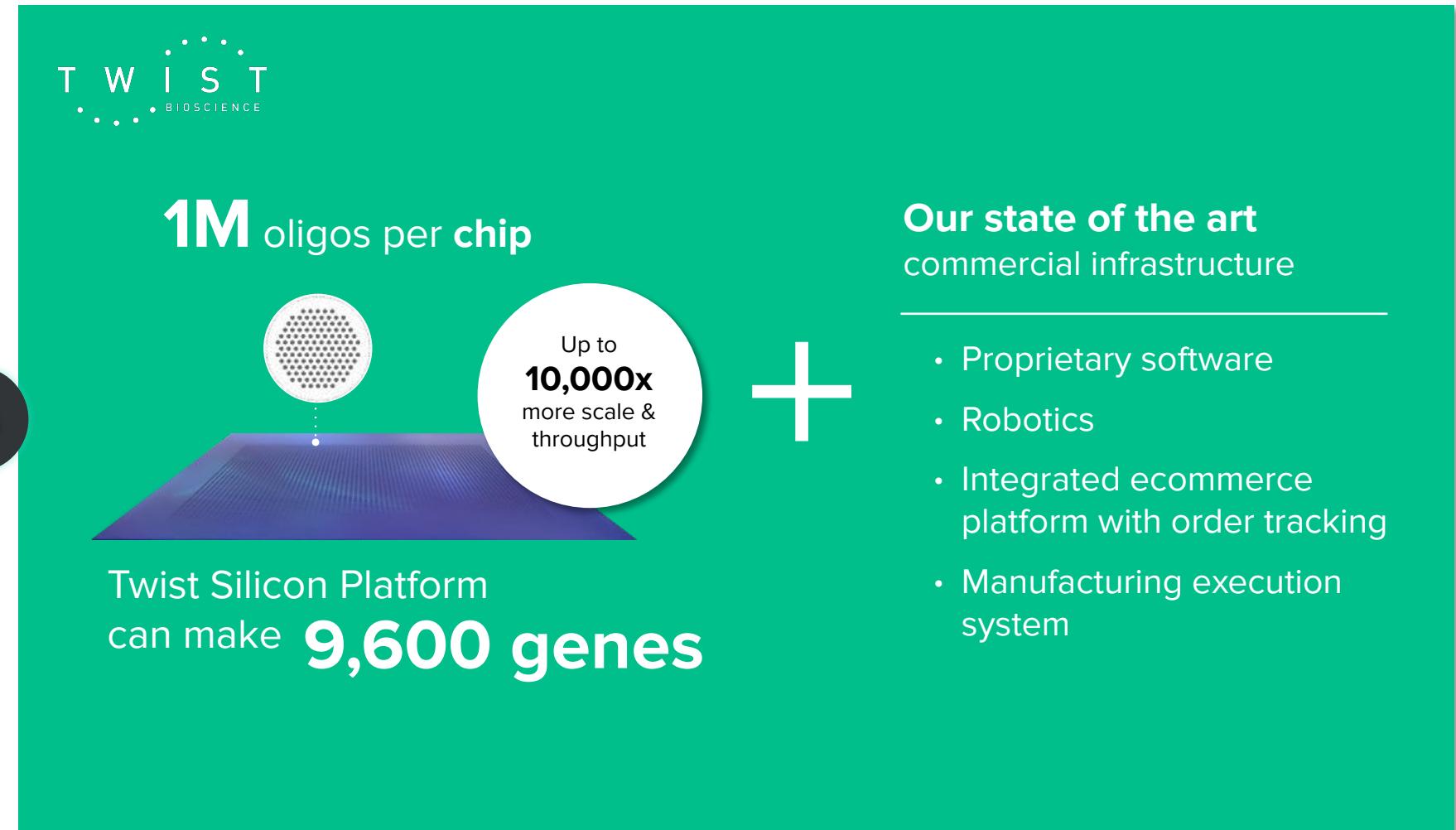
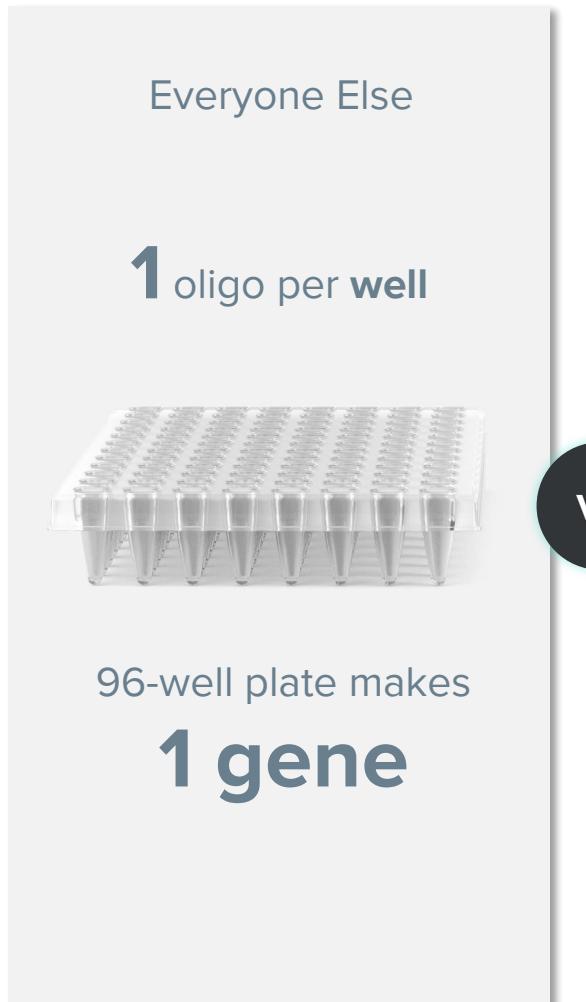
Precision Medicine



Data Storage

Preserving Heritage

Game-Changing Throughput, Quality & Affordability



We Continue Loading **MORE** On The **SAME** chip

Rapid Growth, Unmatched Scale, High Margin Expansion

MORE
Products

MORE
Customers

DNA on Silicon Platform

MORE
Applications

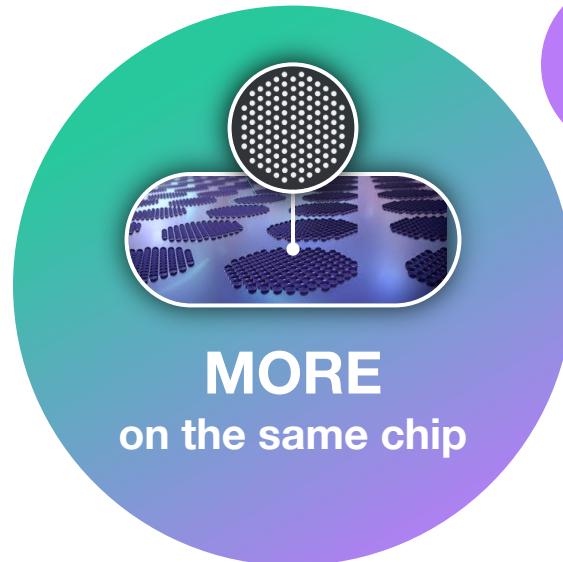
MORE
Markets



T W I S T
• •
BIOSCIENCE

Solutions That Work Together

2021



NGS APPLICATIONS

Synthetic
Controls
UMIs/Adapters
3000 UDI

- 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

Variant
Libraries
+ Multiplexed
Gene Frag / Gene
Pools

In Vitro
Discovery

In Vivo
Discovery
In Silico
Discovery

Gene
Fragments /
Long
GF

Gene
Pools

IgG
Proteins

Antibody
Characterization

Engineered
Enzymes

Clonal
Genes /
Express
Genes

Linearized IVT
Template

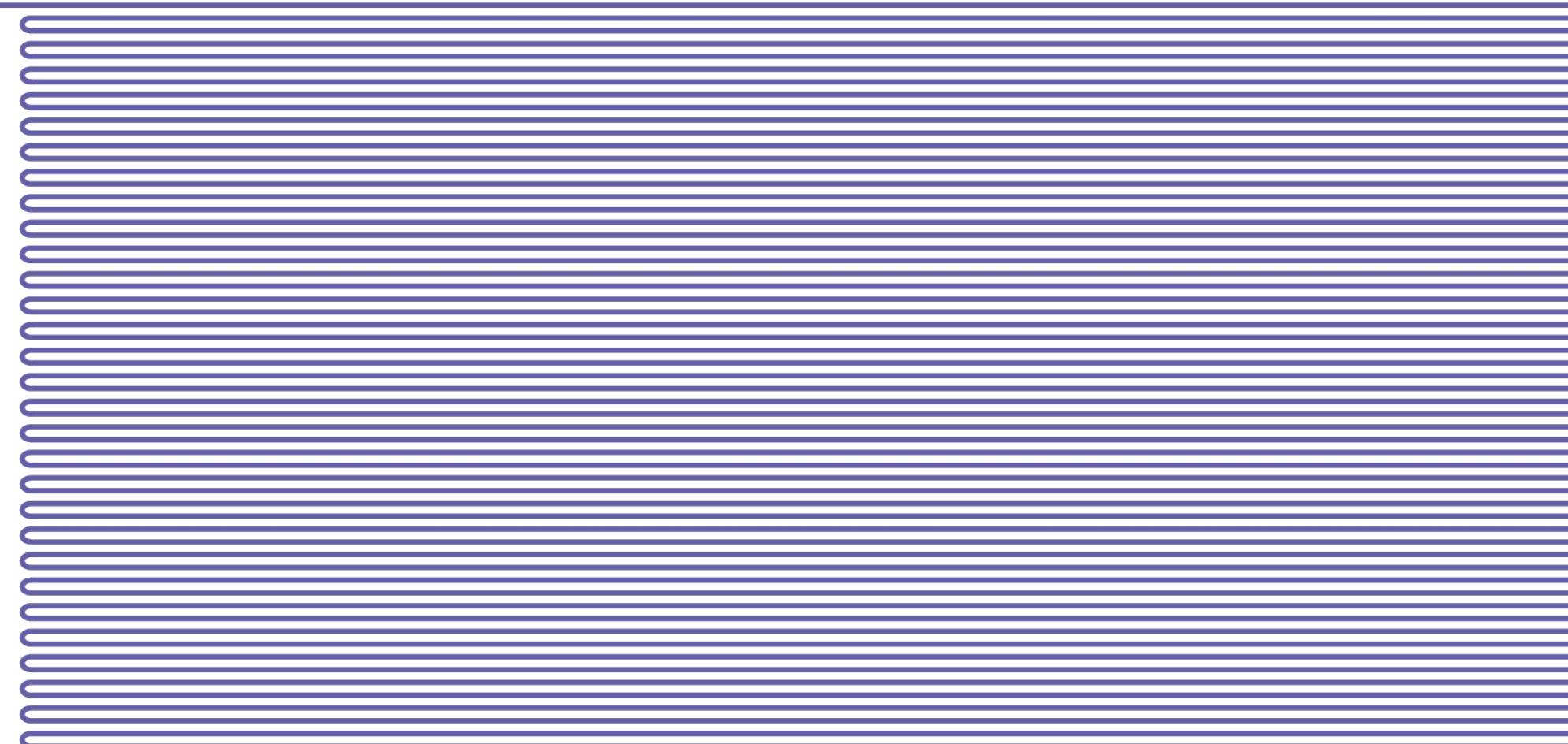
HT mRNA
(Early access)

AI Enabled Discovery

Manufacturing One Gene is Equivalent to Driving:



TWIST BIOSCIENCE
0.092 miles (0.15 km)



1 horizontal line = 1 mile driven

From the EPA Greenhouse Gas Equivalencies Calculator EPA 2024



STANDARD 96-WELL PLATE APPROACH

59 miles
(95 km)

Twist's Oligo CO₂e

(Specific to NGS TE Panels):

| Twist CO ₂ e | Industry Standard |
|------------------------------------|--|
| 180,000 kg CO₂e* | 470,000,000 kg CO₂e* |



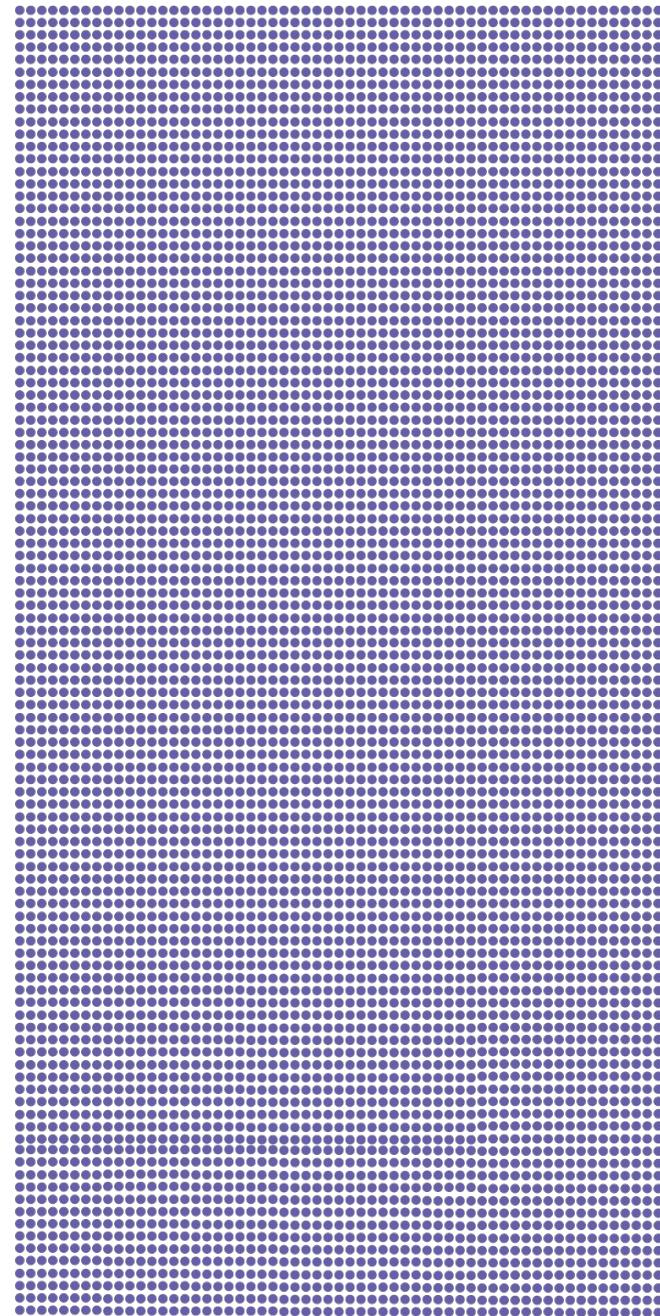
STANDARD 96-WELL PLATE APPROACH

**6,222 tanker trucks
worth of gasoline**



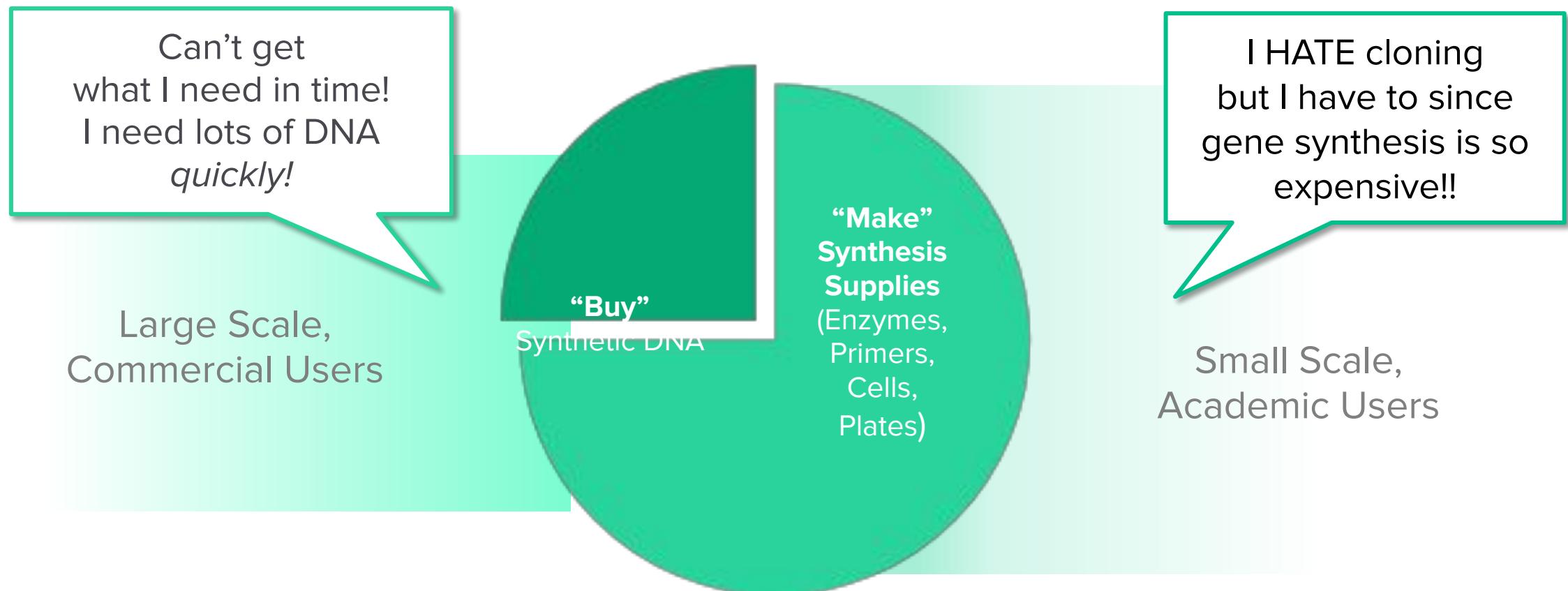
TWIST BIOSCIENCE

**2.4 tanker trucks
worth of gasoline**



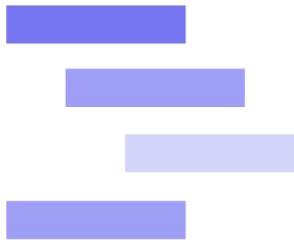
Genes and Clonal Genes

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.

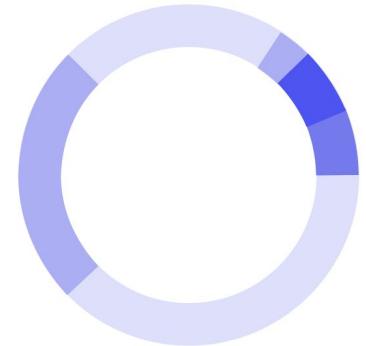


Genes

CLONAL GENES

0.3 to 5 kb

10 business days
Express Genes:
4-7 business days

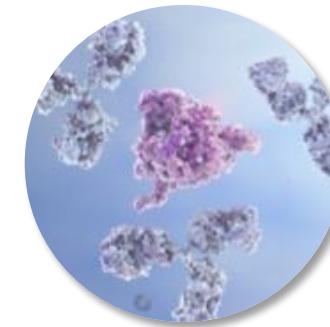


Clonal Genes

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

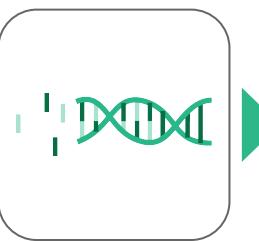


Upload your vector map and define your insertion site.

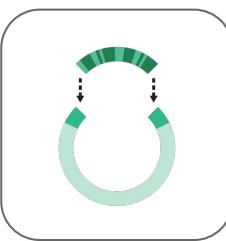


Ship us your vector.

----- TAT – 10 BUSINESS DAYS -----



We sequence and bank the vector for you.



We clone Twist Genes into your vector.

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



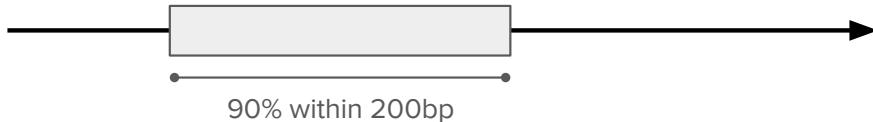
Long Repeats



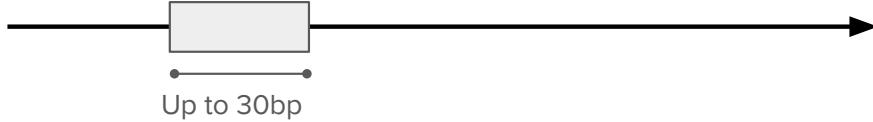
Inverted Repeats (hairpins)



High GC/AT content



Homopolymers



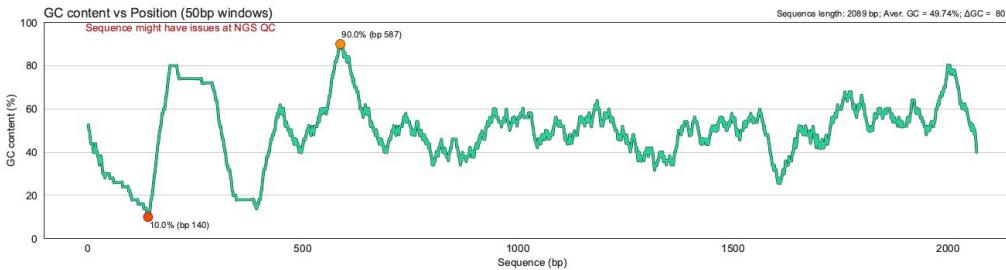
- Know immediately if we can or cannot make a sequence in our ordering portal
- Get sequences containing complexities within 10 business days
- Explore more sequence space: Design complex promoters, UTRs, long linkers, secondary structure

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

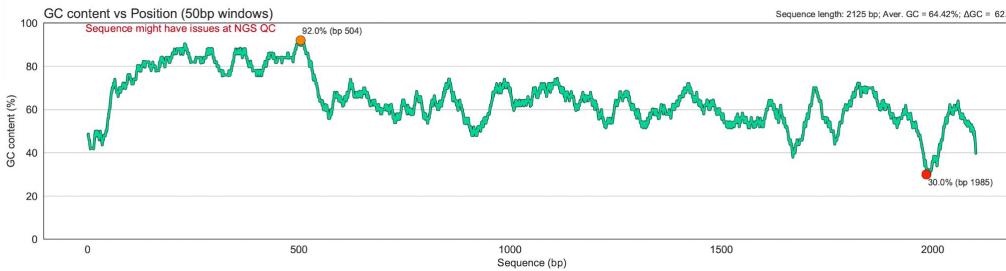
... and Genes up to 10 kbp!

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

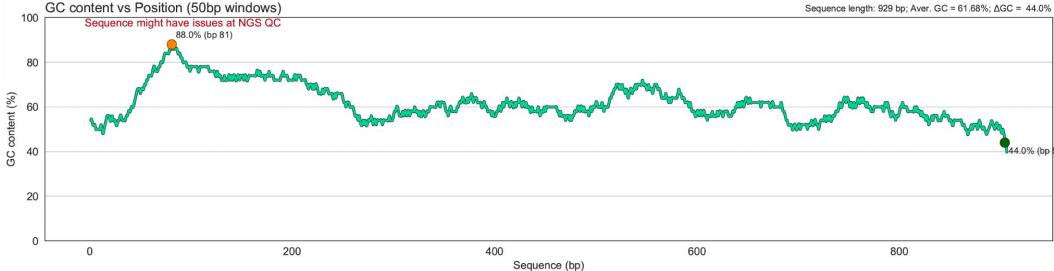
Extreme changes in GC content



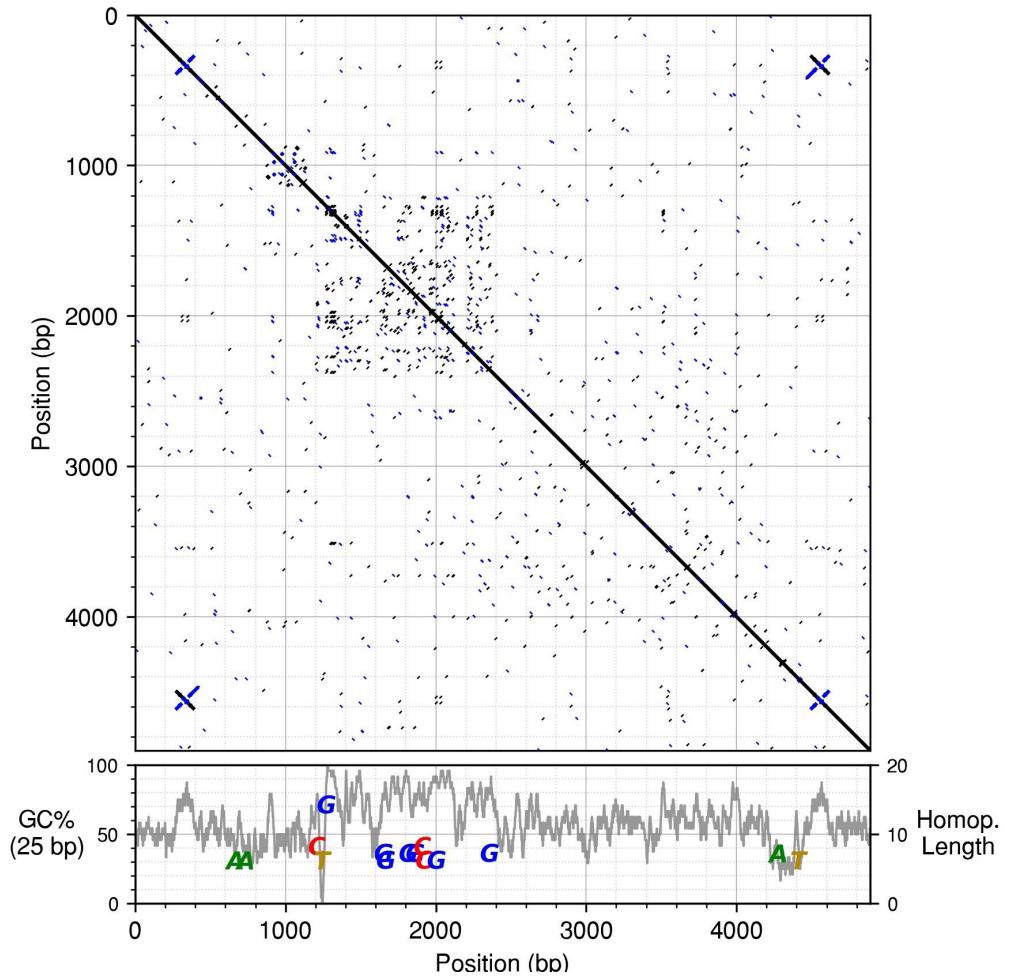
Regions of high average GC content



High average GC content

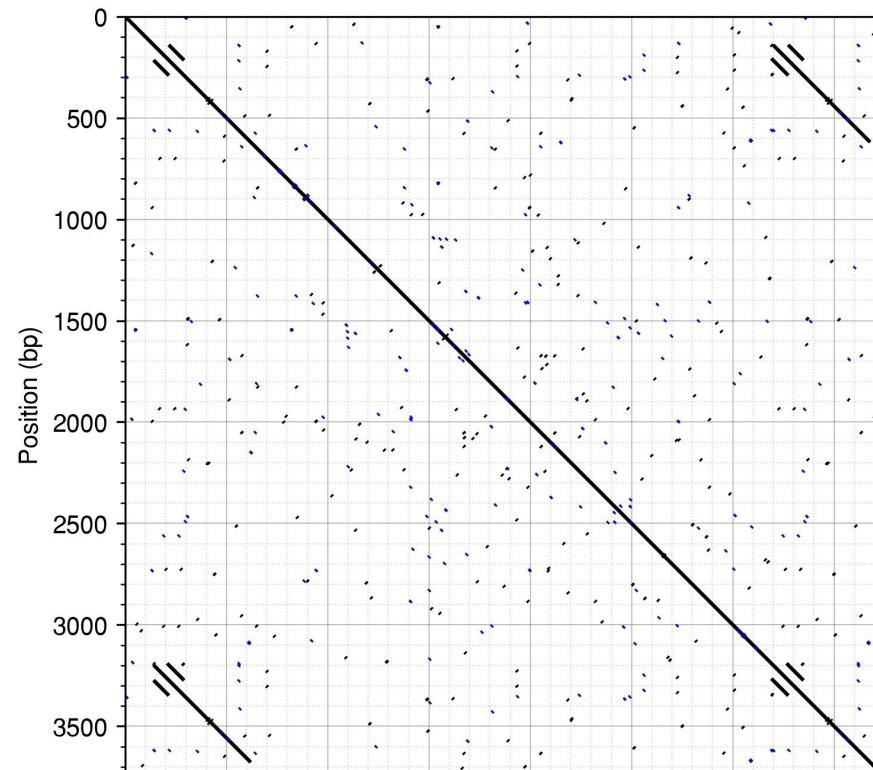


Hairpins and secondary structure

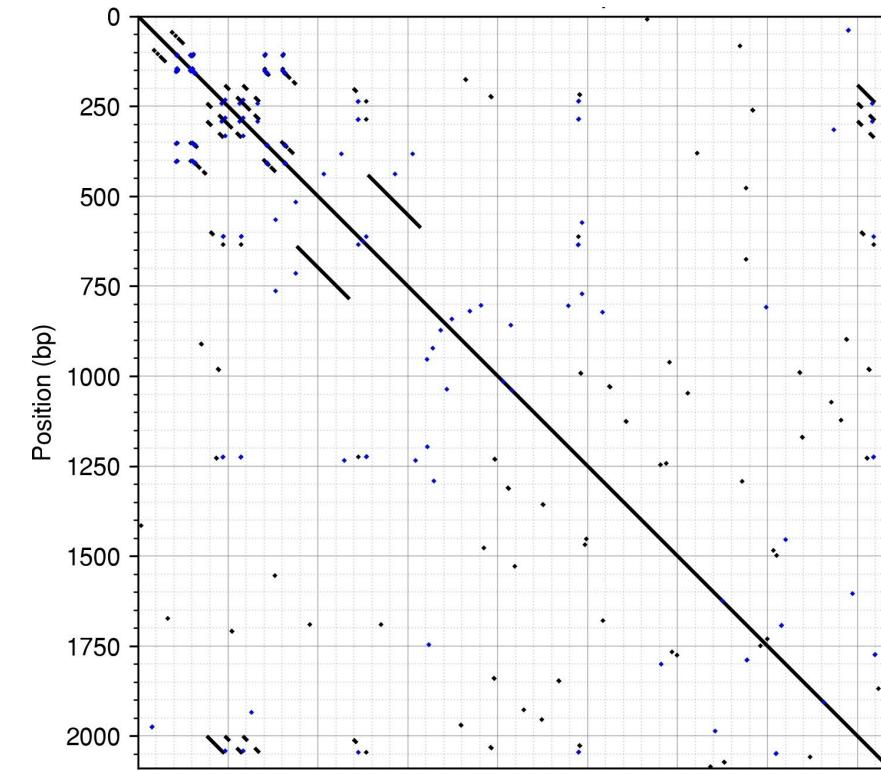


Twist Can Now Synthesize Otherwise “Impossible Genes” With The Following Complexities (Part 2):

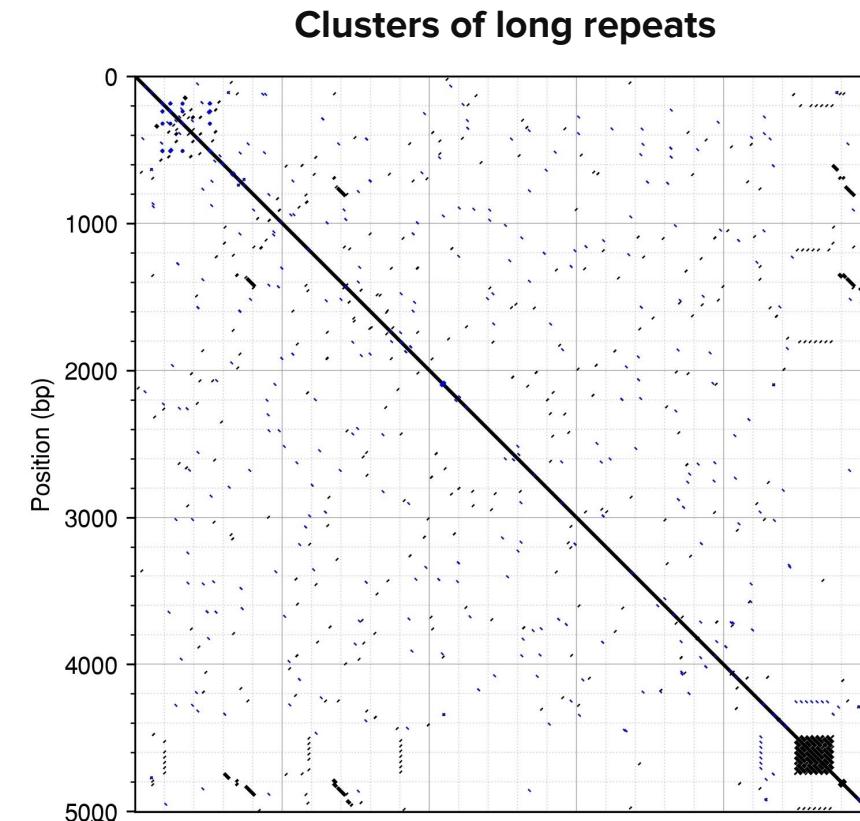
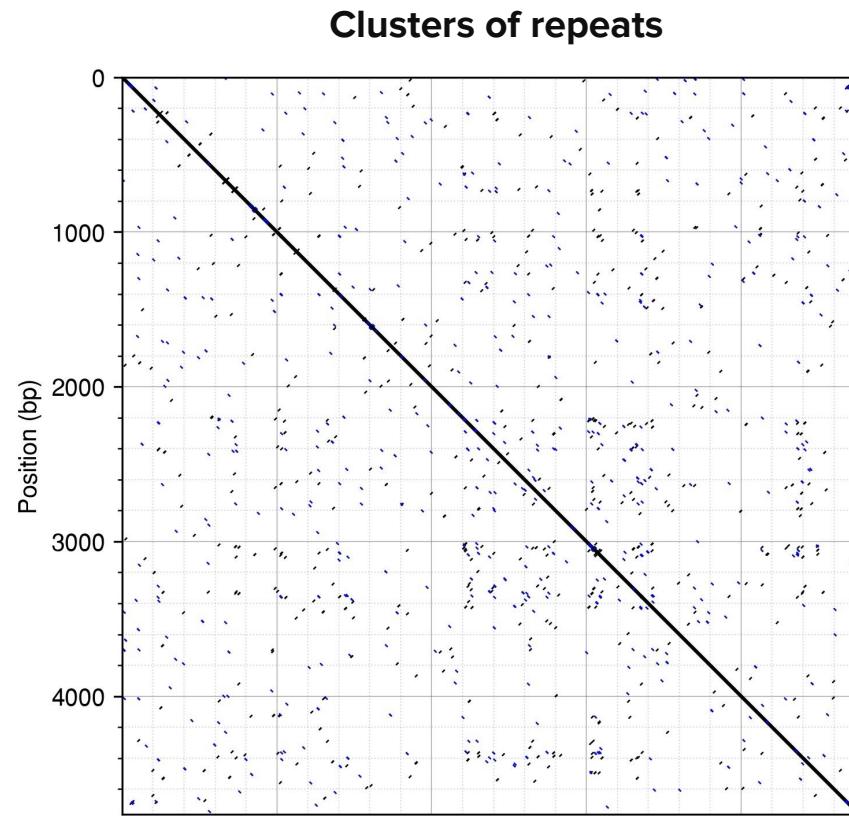
Long terminal repeats



Long internal repeats and tandem repeats



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



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.**

Twist Clonal Genes made on the HELIX2 Gene assembly platform make this possible

Ultra-long
oligo synthesis



Enzymatic
assembly



Long-read
sequencing

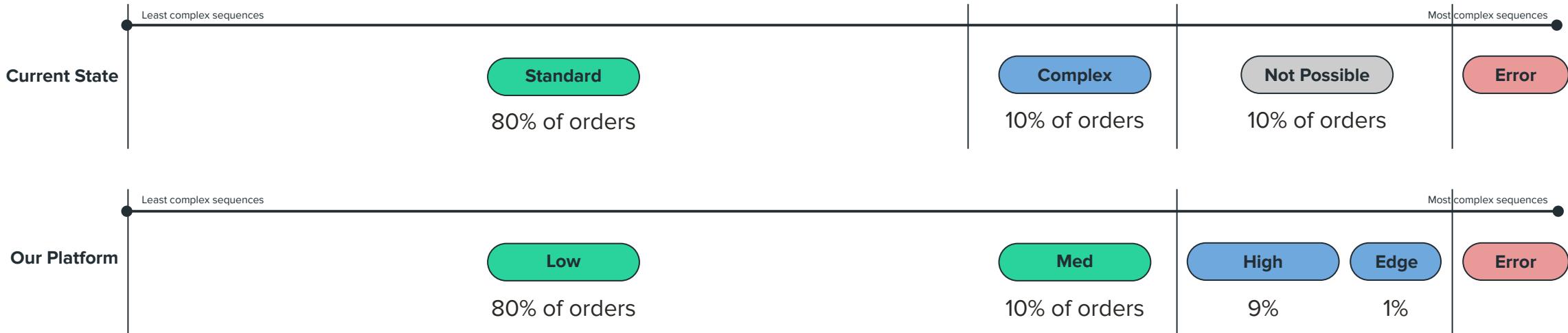


Massive
parallel scale



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 Huntington protein. GS Linkers in proteins.

Long Repeats



Definitions: Regions ≥ 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)

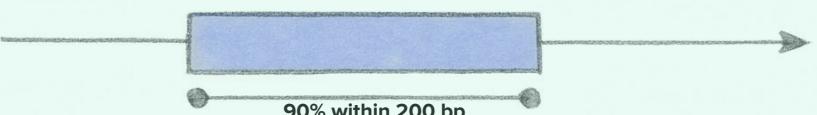


Definitions: Repeats complementary to each other (repeats forwards, then backwards), with up to 100% of homology between repeats.

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

Applications: functionalized exonic sequences, functional mRNA, DNA origami, effectors, promotors

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!



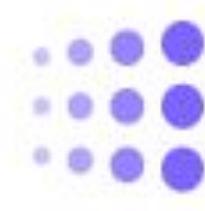
Your Genes, Your Way

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

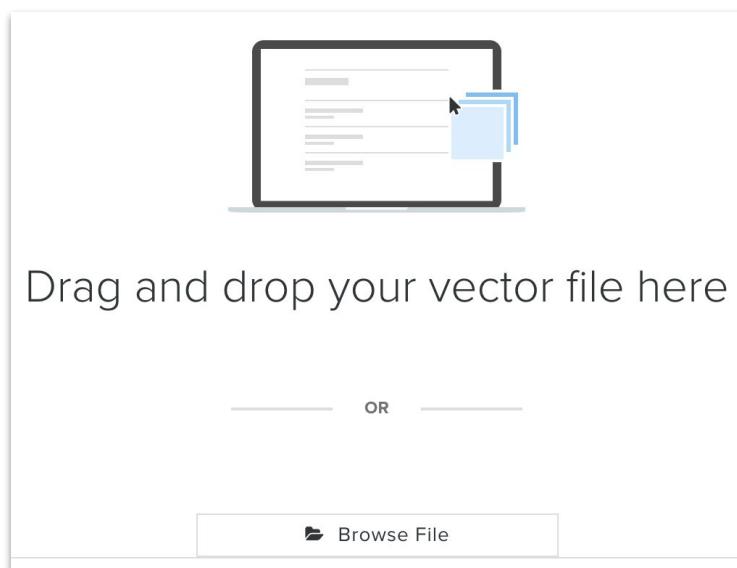
From one gene to a million we offer a scale of synthesis that is unmatched. Order as few or as many genes as you need.



Easy Online Custom Vector On-Boarding

1

Simply input your vector sequence

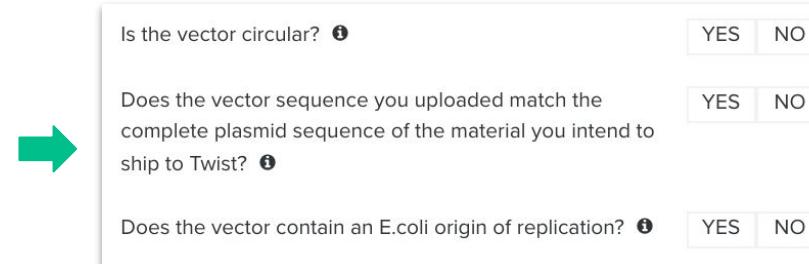


Drag and drop your vector file here

OR

2

Provide some information about the vector



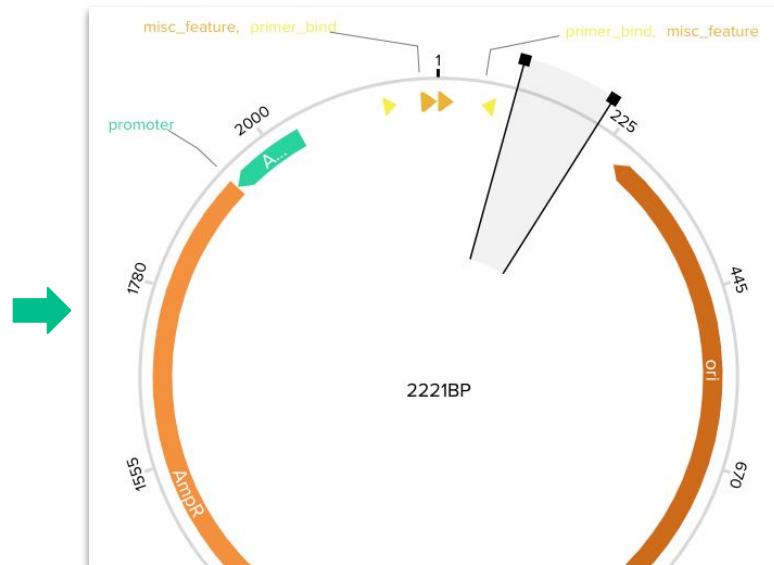
Is the vector circular? YES NO

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

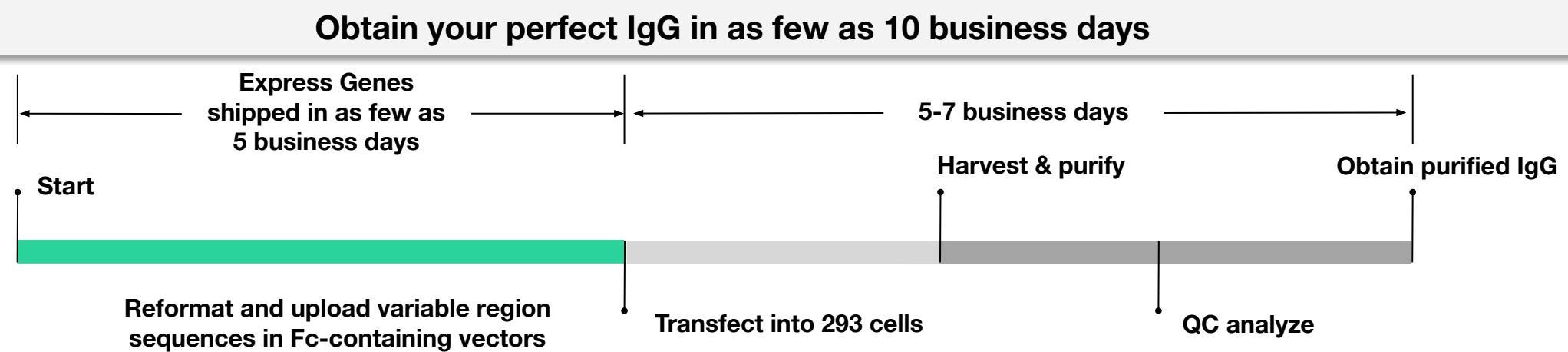
3

Select your insertion site

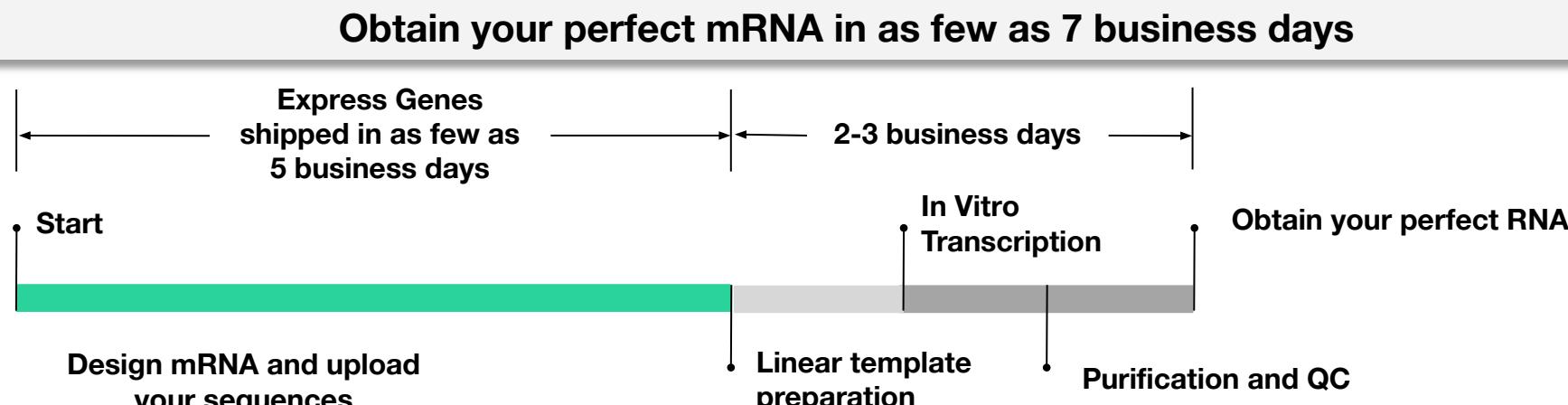


Twist Express Genes Service | Enabling Faster Applications

Antibody Expression

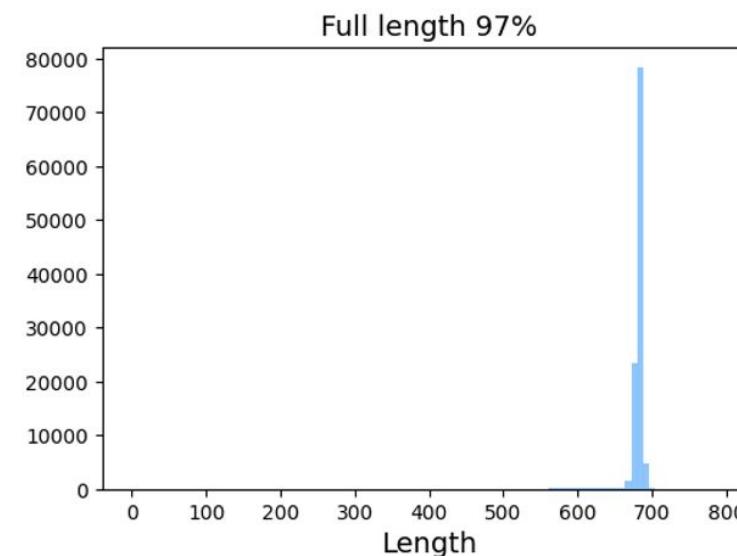
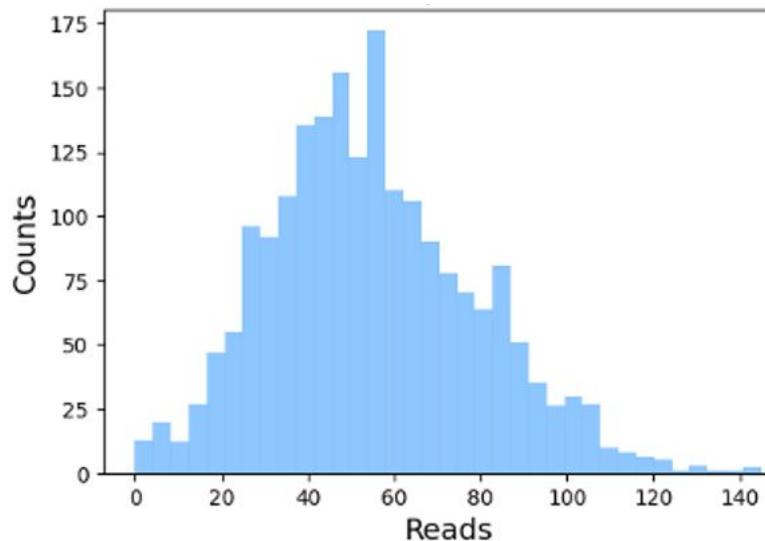
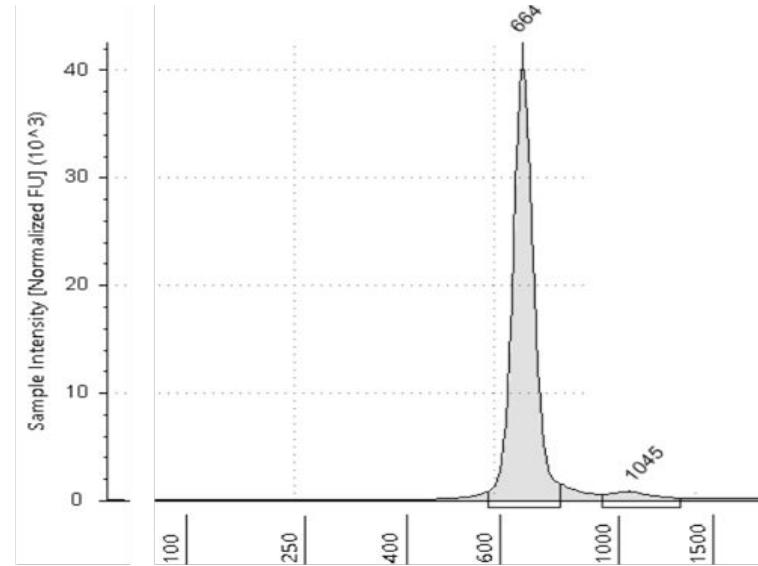


mRNA Production



Oligos and Multiplexed Gene Fragments

We Continue to Push the Boundaries of Oligo Synthesis

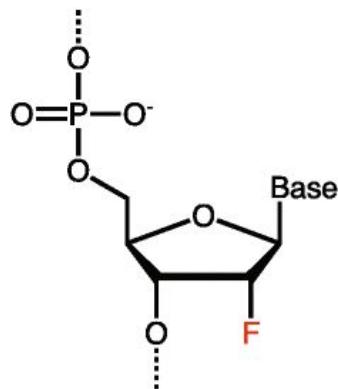


With enhanced chemistry, we demonstrated the direct synthesis of **700mer** for the first time:

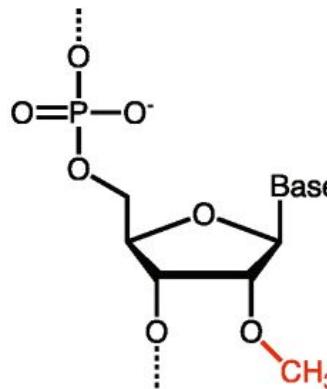
- Sharp and clean peak on fragment analysis
- 97% full length material per Nanopore sequencing analysis
- Uniform distribution among all oligos

We Now have Validated Chemistry With Modified Nucleotides

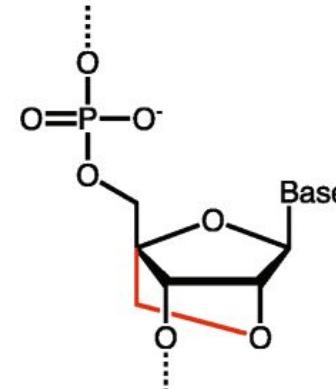
2'F RNA



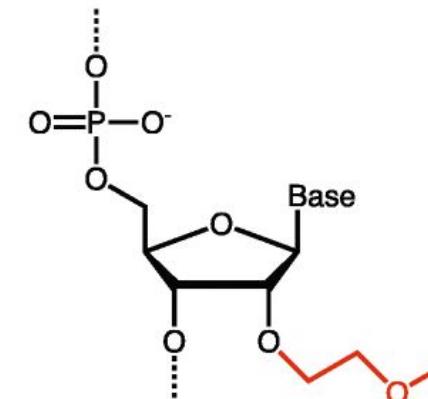
2'OMe RNA



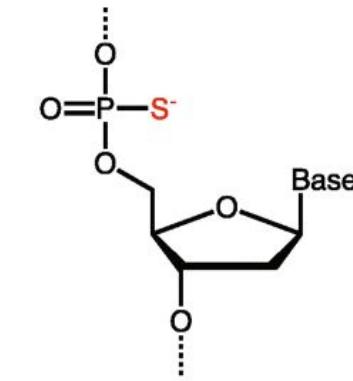
LNA



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

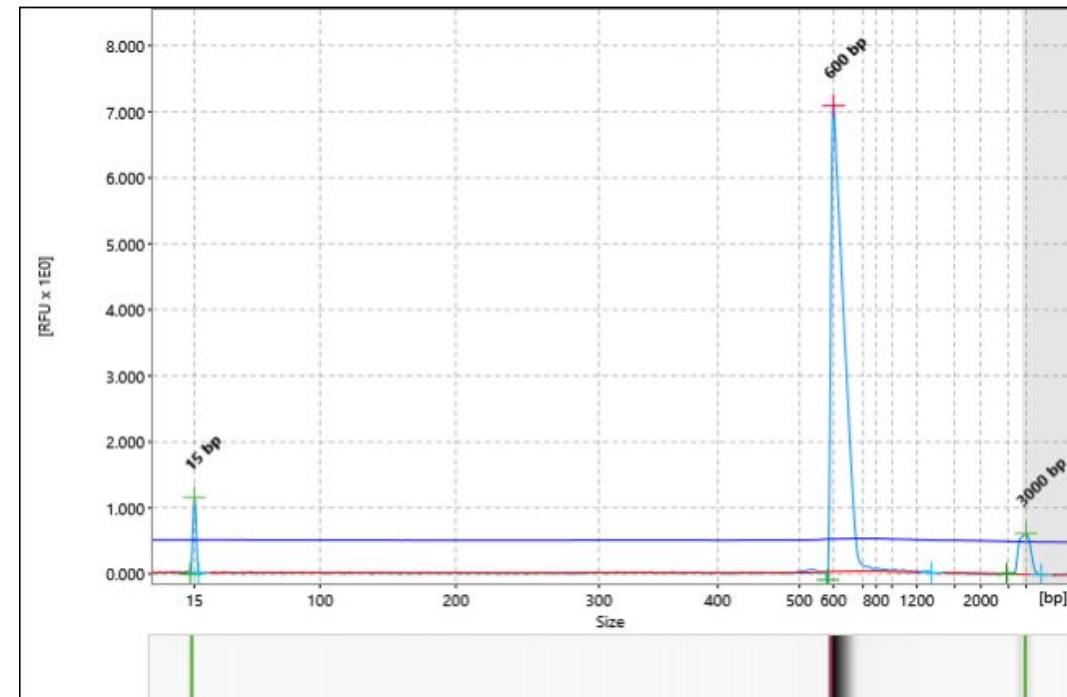
Sometimes The Limitation is PCR, Not Synthesis

- 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:

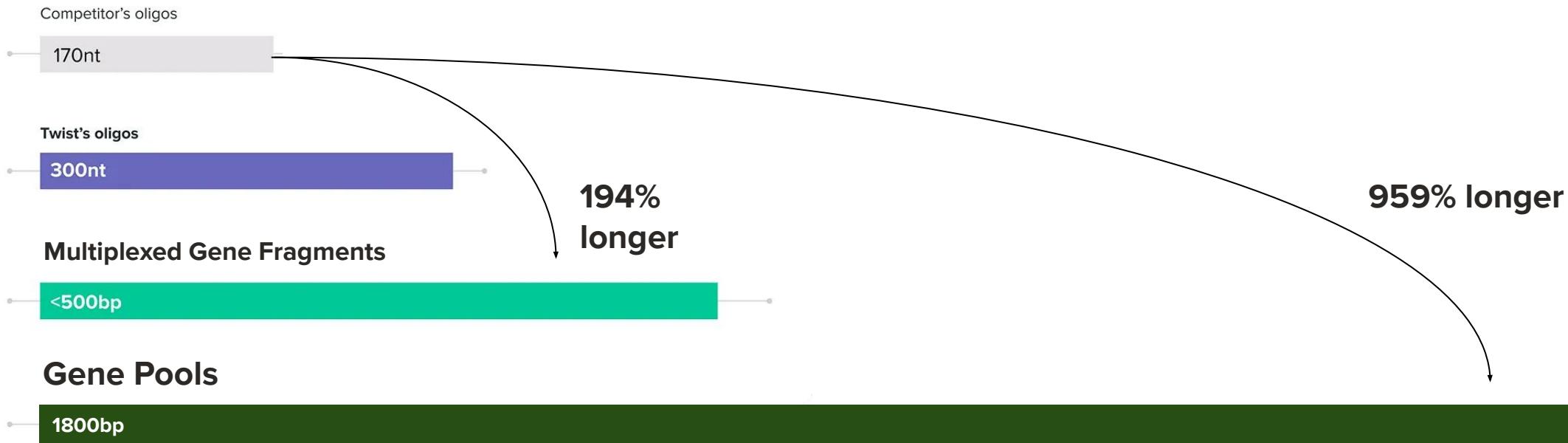
- Individually assembled gene fragments
- Pooled purification
- 1 in 3,000 bp error rate. Absolutely zero chimera artifacts
- Only Twist has the capacity to process tens of thousands assemblies



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**NNNNNN**NTGTCGTTAACTGTTGA

CGTT**N**CGCGCTCGTT**N**ATGTCGTTAACT**N**TTGA

The screenshot shows the 'OLIGO POOL PLATES' software interface. At the top, there's a navigation bar with a tree icon, the text 'OLIGO POOL PLATES', and 'Oligo draft'. Below the navigation bar, the text 'All Plates · Plate 1 of 4 < > View all plates' is displayed. The main area is titled 'Plate name' and shows a 384-well plate layout. The plate is divided into 8 columns (A-H) and 48 rows (1-48). Each cell in the grid contains a number representing the sequence count for that well. A legend at the top of the grid indicates sequence counts: 0 (white), 1-19 (light green), 20-39 (medium green), 40-59 (dark green), 60-79 (darker green), 80-99 (darkest green), and 100-121 (black). Below the grid are buttons for 'Download' and 'Import'. To the right of the plate layout, there's a 'DESIGN SEQUENCES' panel for 'Well name G13'. It shows the following data:

| Sequences | Longest | Shortest | Average |
|-----------|---------|----------|---------|
| 120 | 350 bp | 350 bp | 350 bp |

The 'Coding Sequence' section lists six sequences, each 350 bp long:

- AGCTGTCAGTACGTAGATGACGACATC...
- AGCTGTCAGTACGTAGATGACGACATC...
- AGCTGTCAGTACGTAGATGACGACATC...
- AGCTGTCAGTACGTAGATGACGACATC...
- AGCTGTCAGTACGTAGATGACGACATC...
- AGCTGTCAGTACGTAGATGACGACATC...

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.

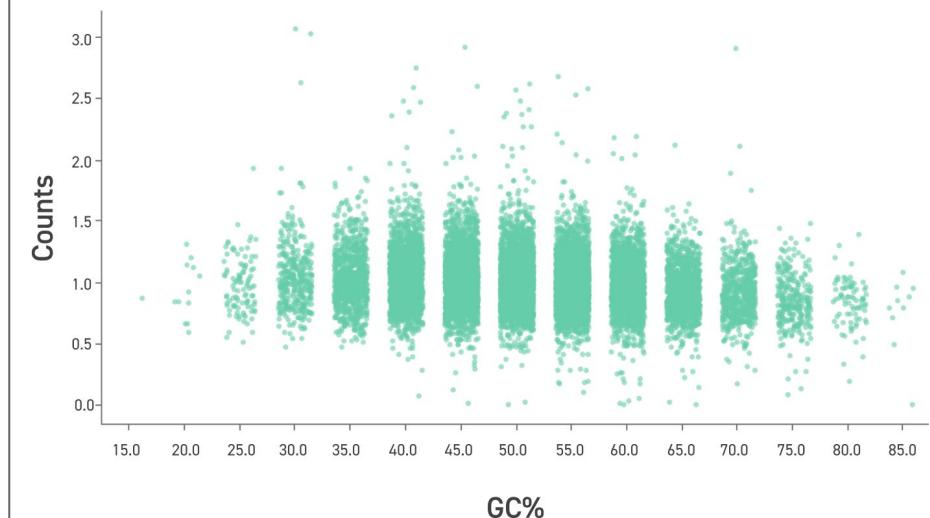
| | |
|-------------------------|--|
| Oligo Length | Up to 350 nt |
| Oligo Pool Size | No minimum, no maximum |
| Cloning Available | Yes |
| Oligo Pool Yield | >0.2 fmol average of each ssDNA oligo |
| Cloned Oligo Pool Yield | Starts at 50 µg total plasmid DNA |
| Uniformity | >90% oligos represented 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

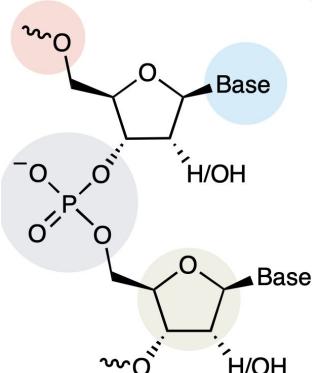
- Therapy to regulate gene transcription and protein expression
- Rapidly designed and produced, especially for newly identified targets

Small RNA

siRNA, ASO, aptamer

Among approved drugs

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



Translatable RNA

mRNA, circRNA, saRNA

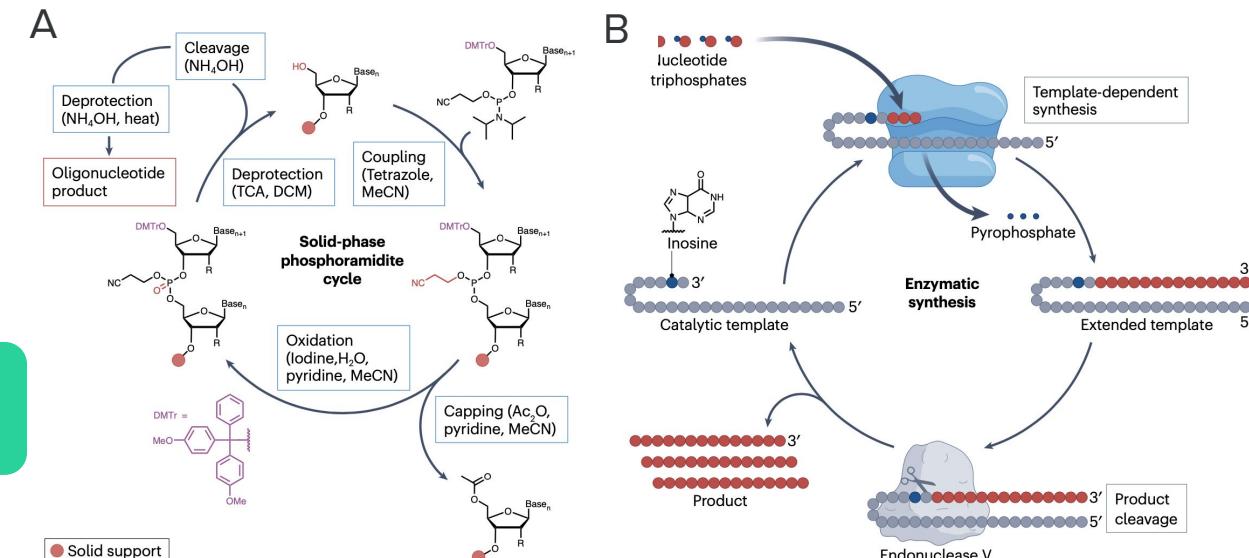
- Only COVID and RSV vaccine approved
- Personalized cancer vaccine

CRISPR gRNA

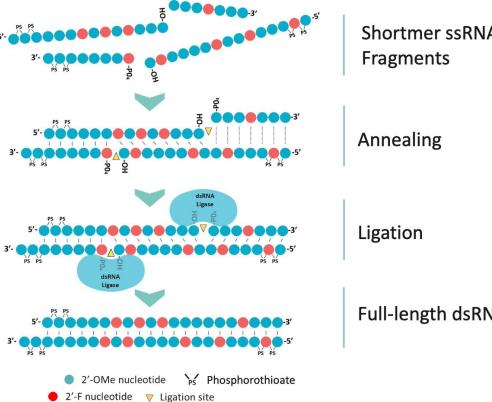
guide RNA

Modifications:

- Improve stability
- Increase binding affinity
- Decrease inflammatory response



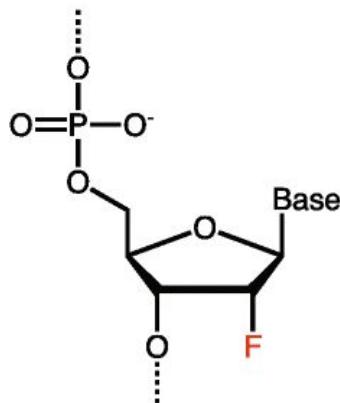
C Fragment Ligation for siRNA Synthesis



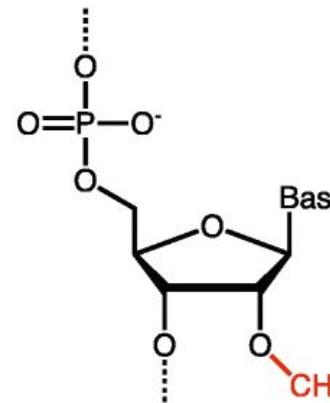
- Chemical synthesis on solid support
- Enzymatic synthesis via extension (TdT or KOD polymerase)
- Enzymatic synthesis via ligation (T4 DNA ligase)

Validated Chemistry with Modified Nucleotides

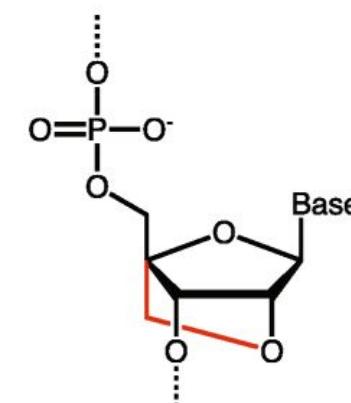
2'F RNA



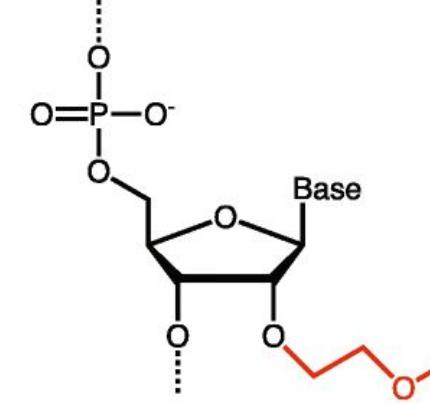
2'OMe RNA



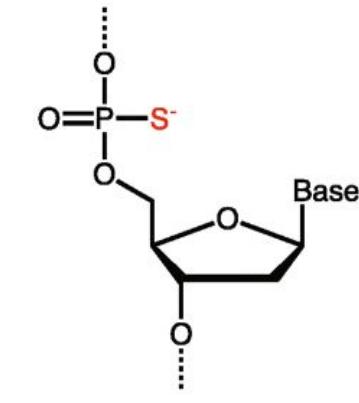
LNA



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.

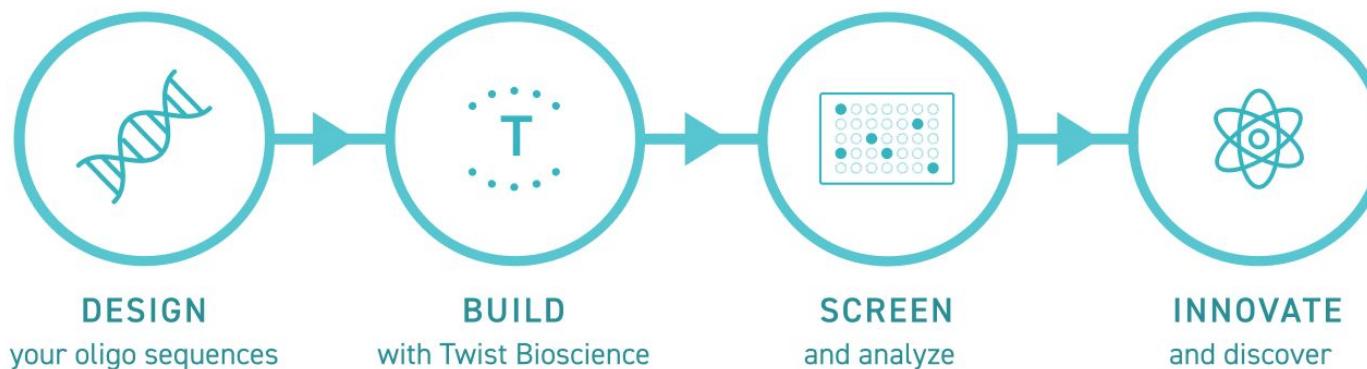
Enhanced Oligo Pool offering

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 |

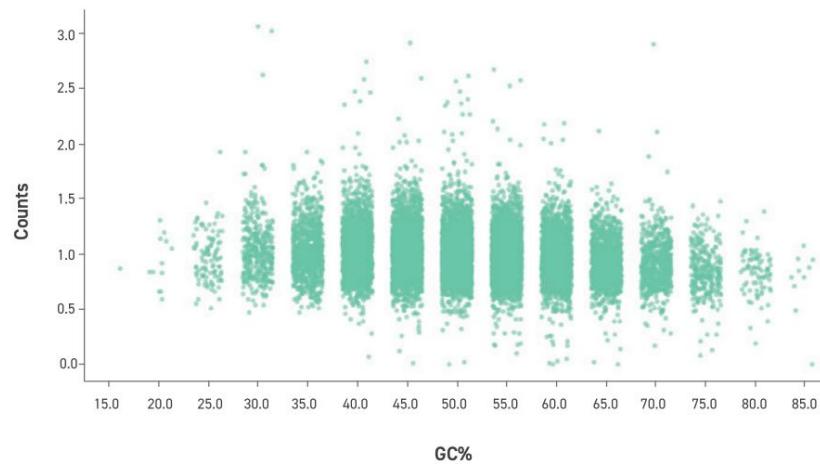


*Turnaround time for the standard Oligo Pool offering varies based on length and sequence complexity. Oligo Pools at 20-121 nucleotides in length range from 3-6 business days, 121-200 nucleotides in length Baseline turnaround time for Cloned Oligo Pools, which includes cloning and amplification, of up 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.

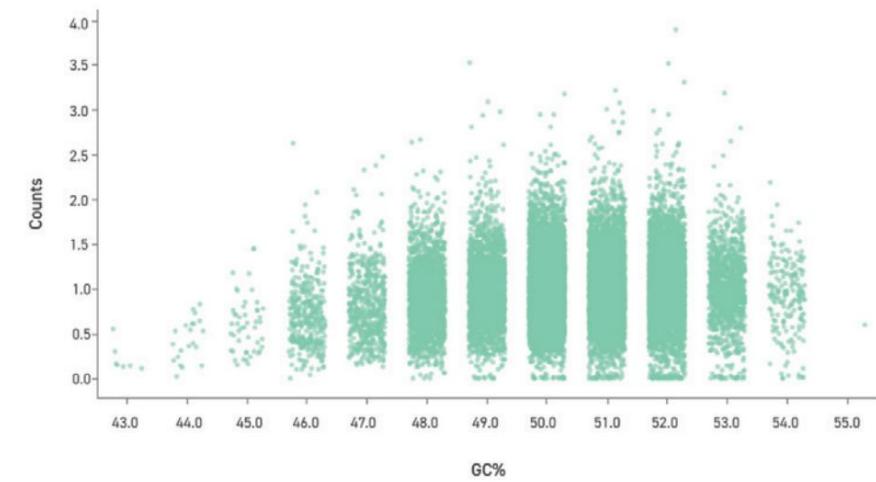
Cloned Oligo Pools

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



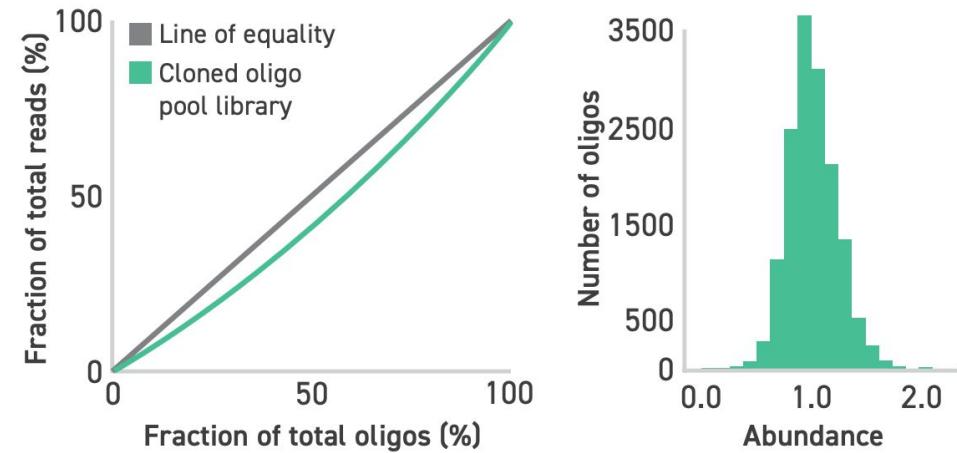
2B. Uniformity for Cloned Oligo Pools that are comprised of sequences of 300 nucleotides in length with high GC content. Percentile: 4.31, Dropouts: 77

Cloned Oligo Pools

Uniformity is maintained regardless of length

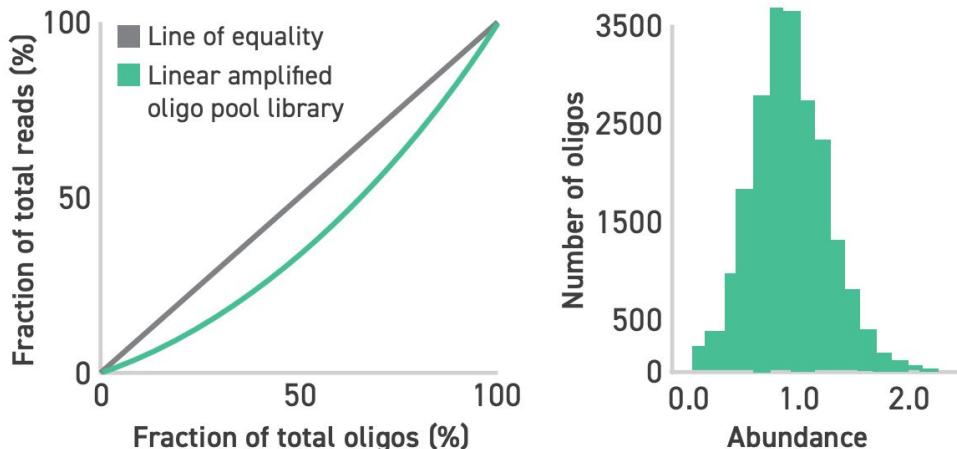
- Normalized read count of each of the mapped variants (X-axis) for a Cloned Oligo Pool of 141 nts (1A) and a Cloned Oligo Pool of 300 nts (1B)
- Abundance converging to 1 indicates that the Cloned Oligo Pools are distributed uniformly

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

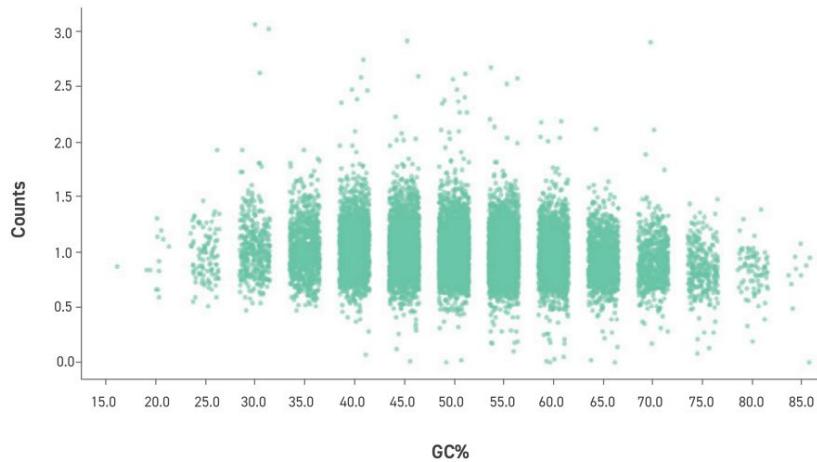


| | |
|------------------------------------|--------|
| % Oligos represented without error | 96% |
| Dropouts | 0.204% |
| Diversity | 21,554 |
| 95th/5th Percentile | 4.31 |
| 90th/10th Percentile | 2.92 |
| Full Length Percentage | 94.65% |
| Chimera Rate | 3.20% |

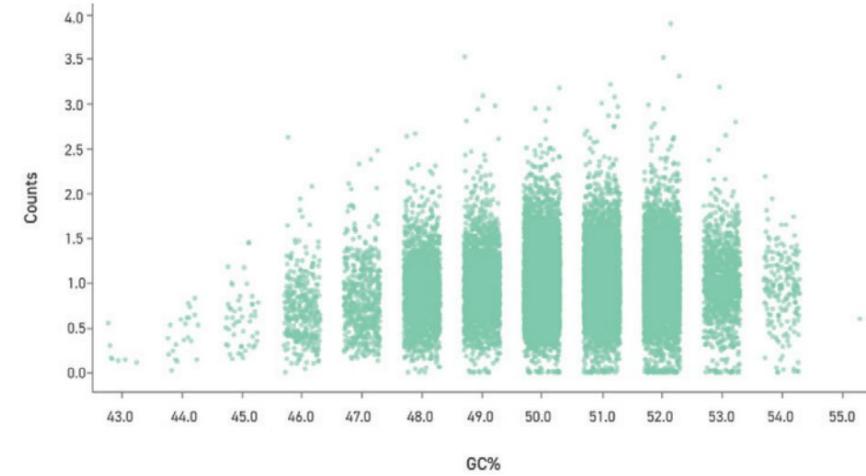
Cloned Oligo Pools

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.



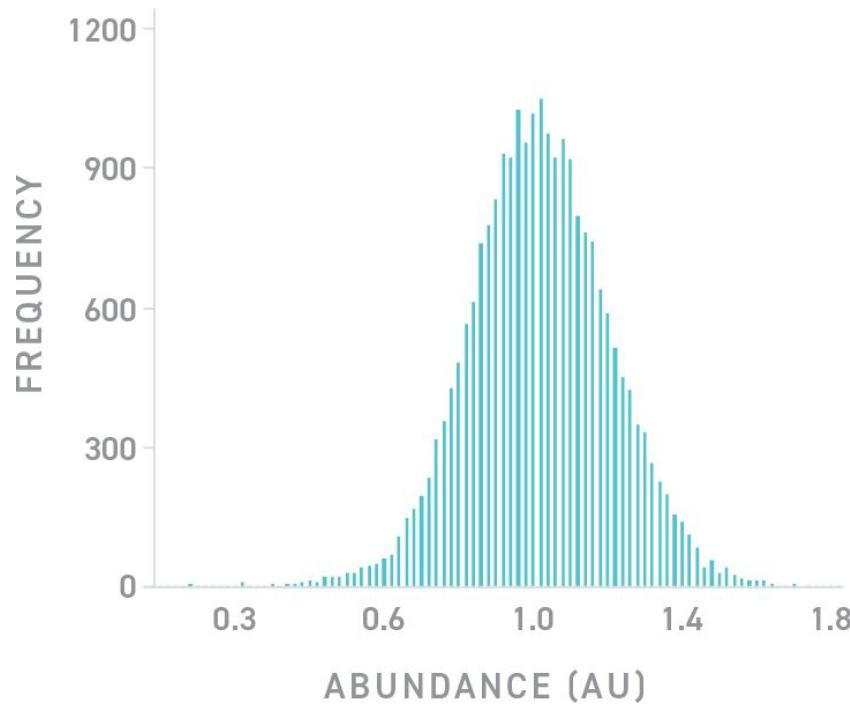
2A. Uniformity for Cloned Oligo Pools that are comprised of sequences of 141 nucleotides in length with high GC content. Percentile: 2.08, Dropouts: 1



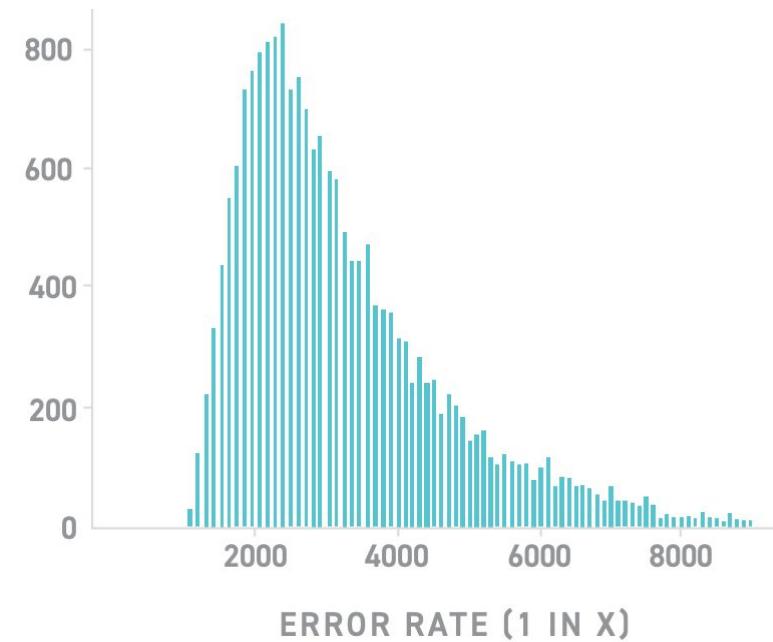
2B. Uniformity for Cloned Oligo Pools that are comprised of sequences of 300 nucleotides in length with high GC content. Percentile: 4.31, Dropouts: 77

Oligo Pools Performance Advantages

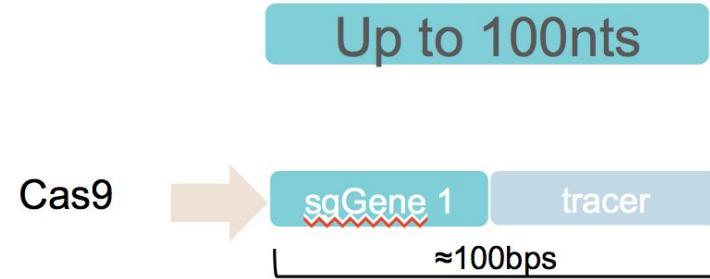
**High Uniformity with
100% Oligo Representation**



**High Quality
1:3000 Base Pair Error Rate**

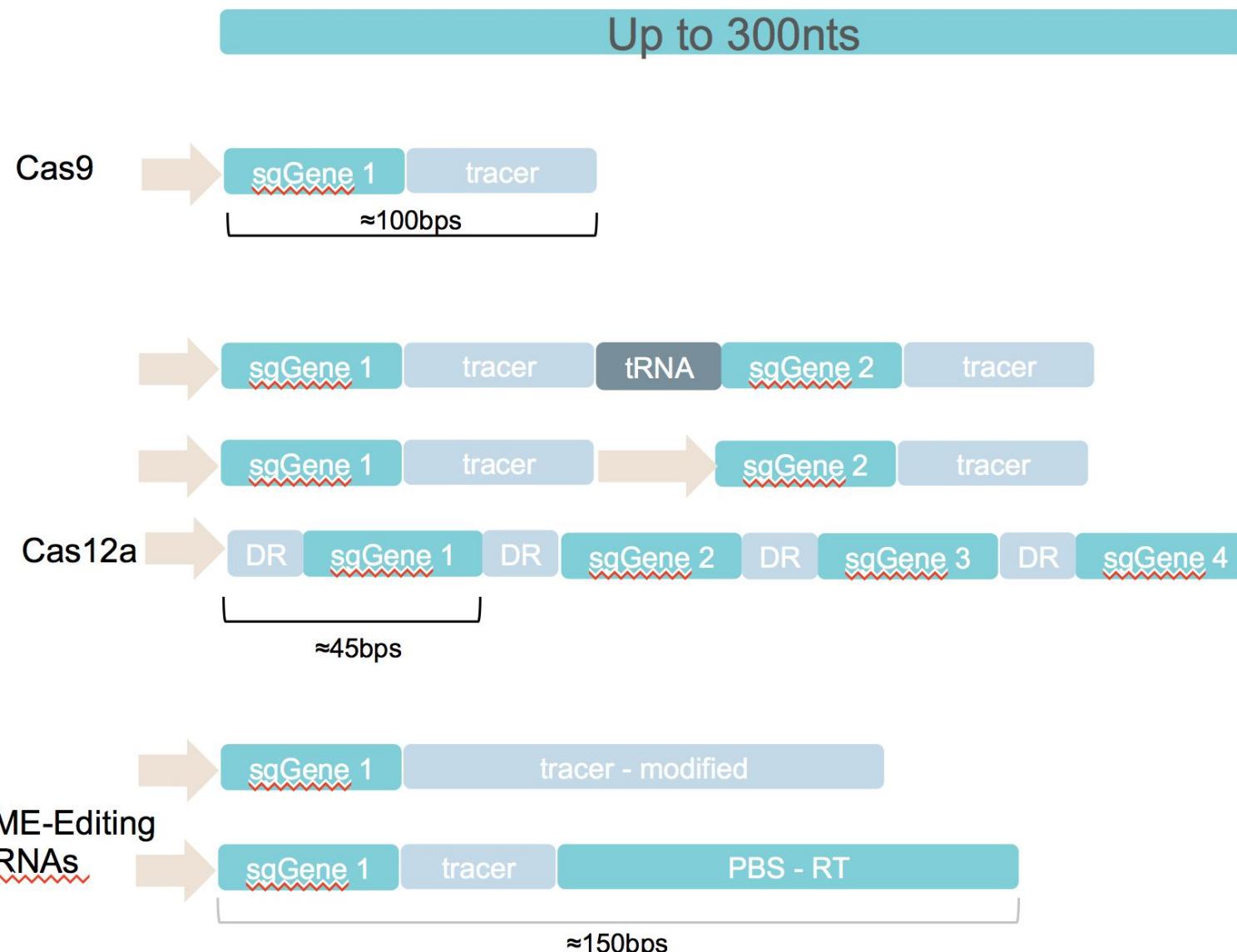


Longer Oligos Allow for Cutting Edge CRISPR Screens



- Standard CRISPR screens

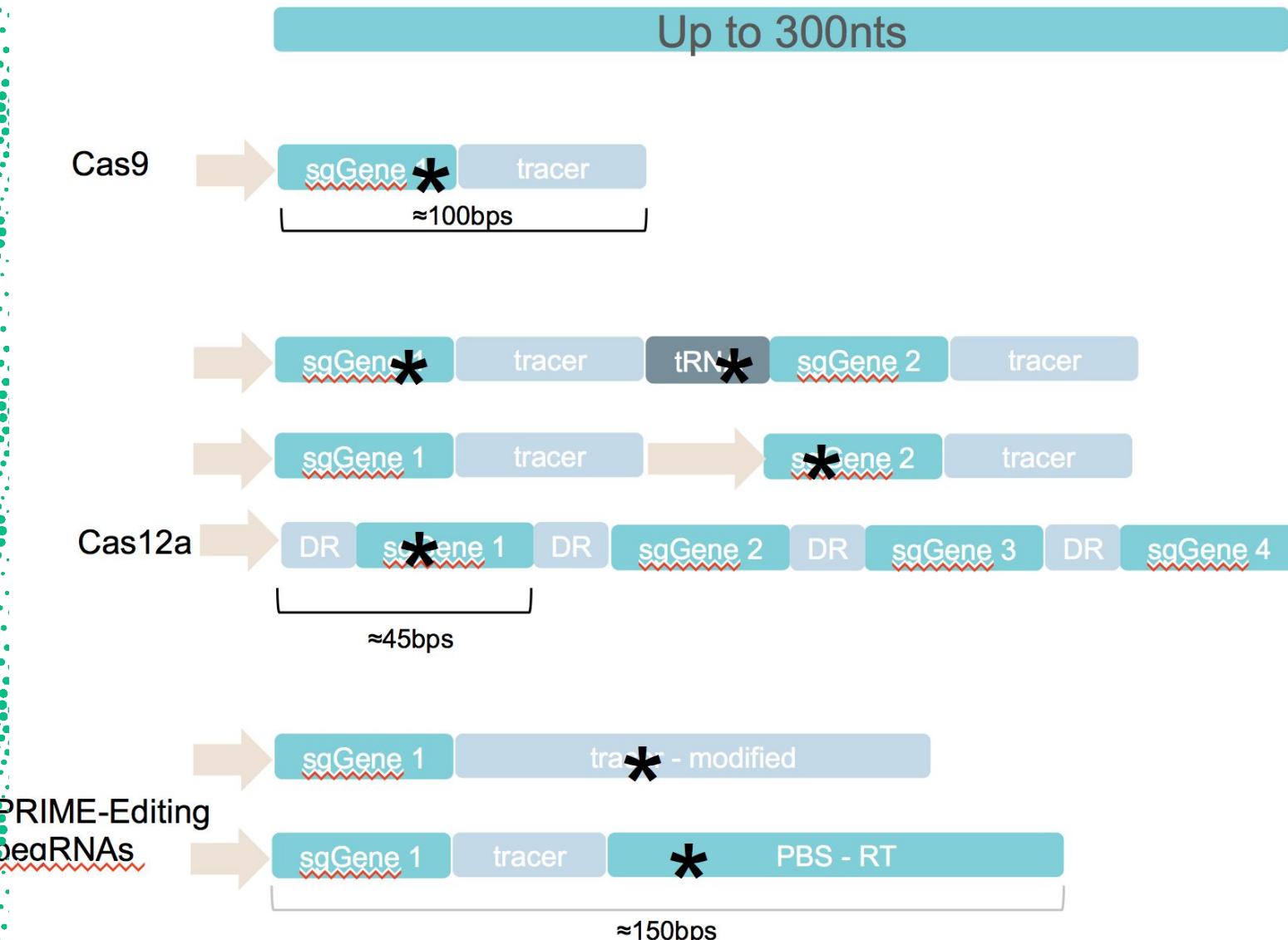
Longer Oligos Allow for Cutting Edge CRISPR Screens



Error Rate: 1:3,000 nt

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

Longer Oligos Allow for Cutting Edge CRISPR Screens



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



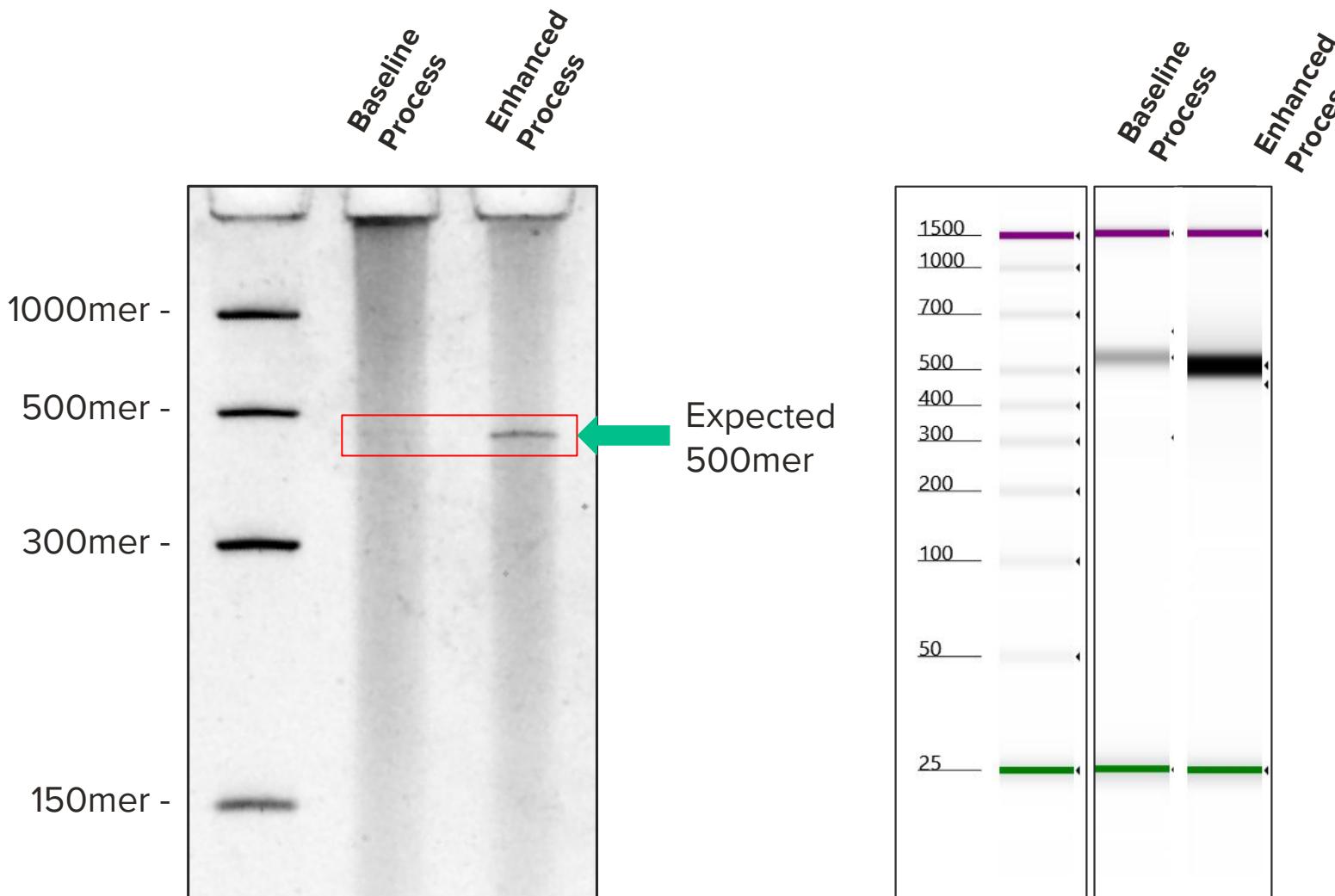
Ultra-complex CRISPR Screens



← 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

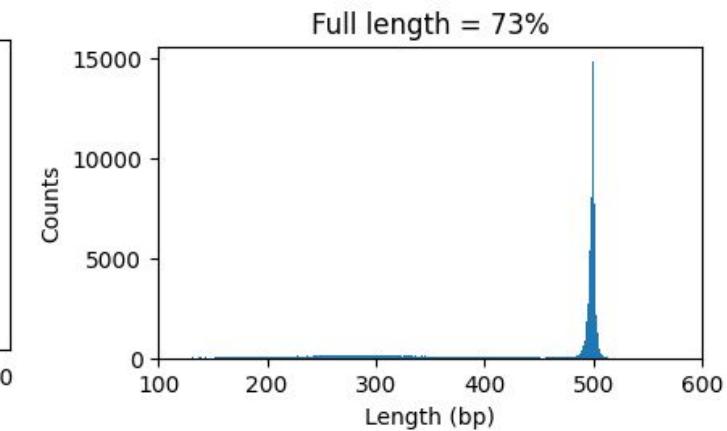
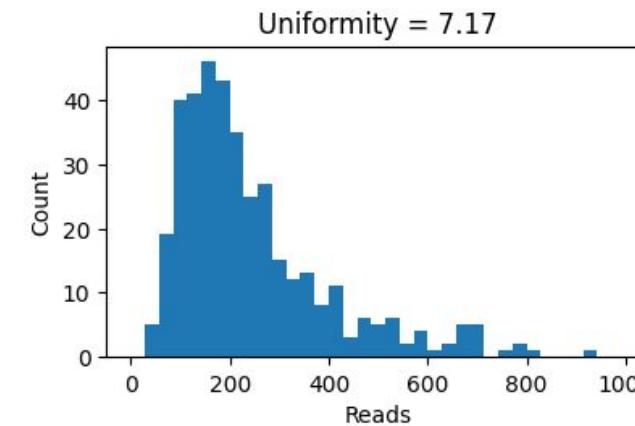
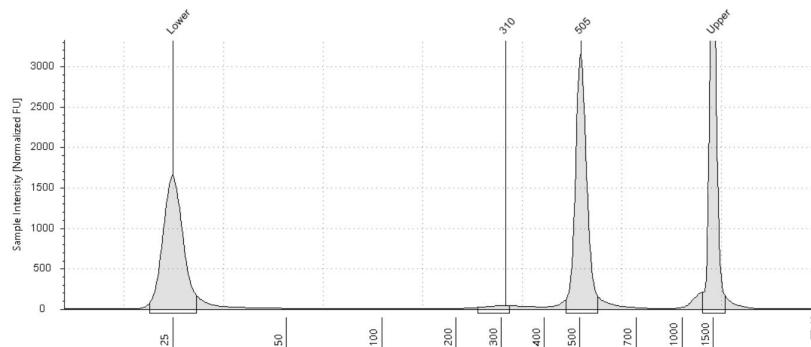


With enhanced chemistry:

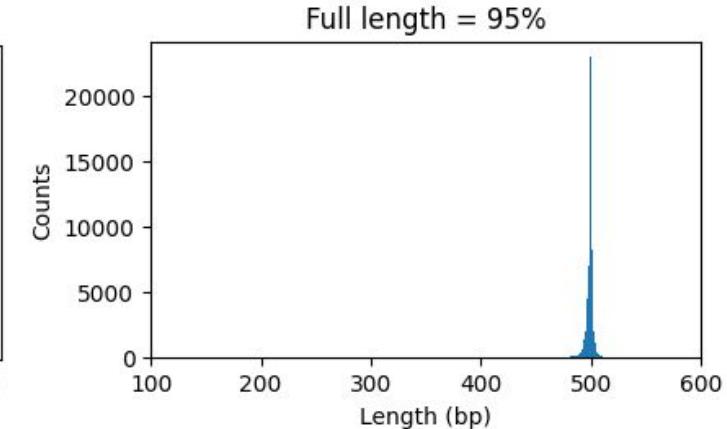
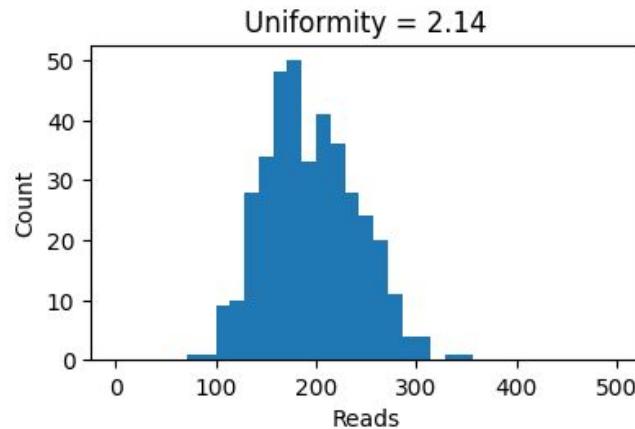
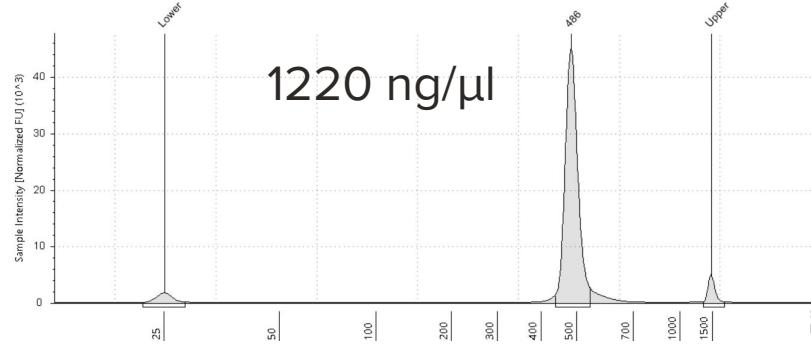
- Clear full length 500mer oligos on gel
- Maintaining 1 in 2,000 bp error rate
- >10 fold increase in PCR yield with better uniformity and more full length materials

We Have Made Continued Chemistry Improvements for Multiplexed Gene Fragments

Baseline Process:



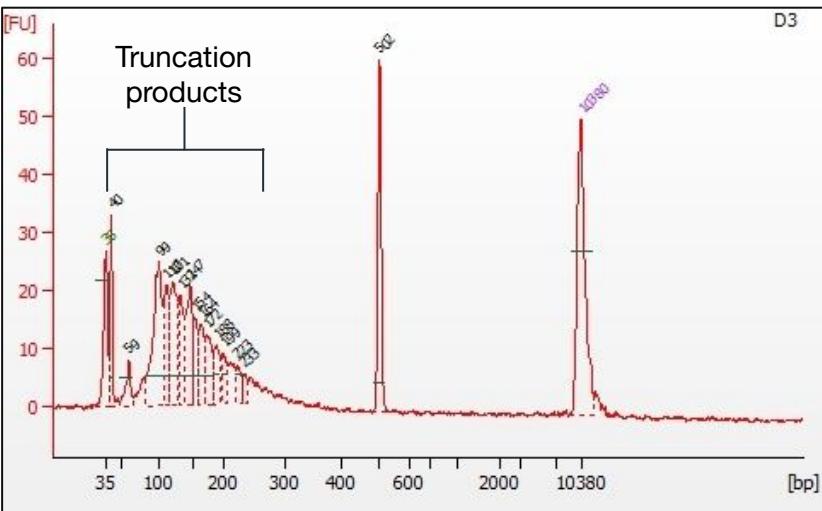
Enhanced Process:



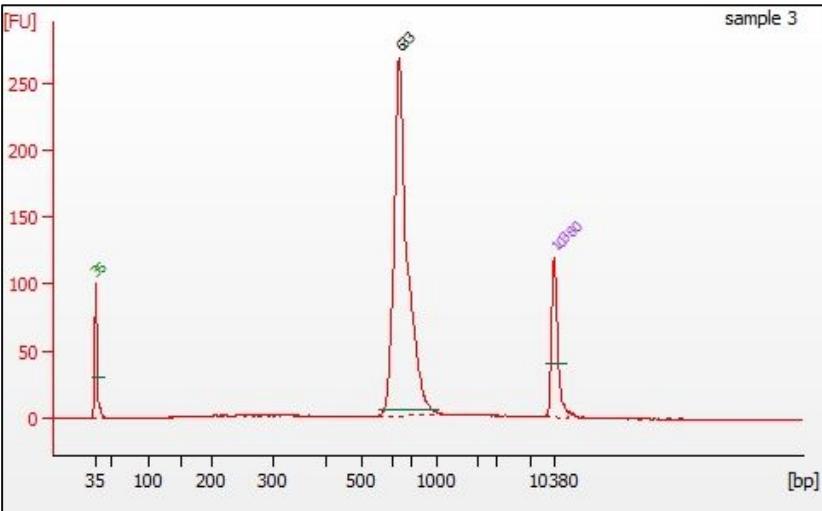
With enhanced chemistry, the PCR yield increased by >10 fold with better uniformity and more full length materials.

Pushing the Boundaries of a 500 Nucleotide Synthesis

Recipe A

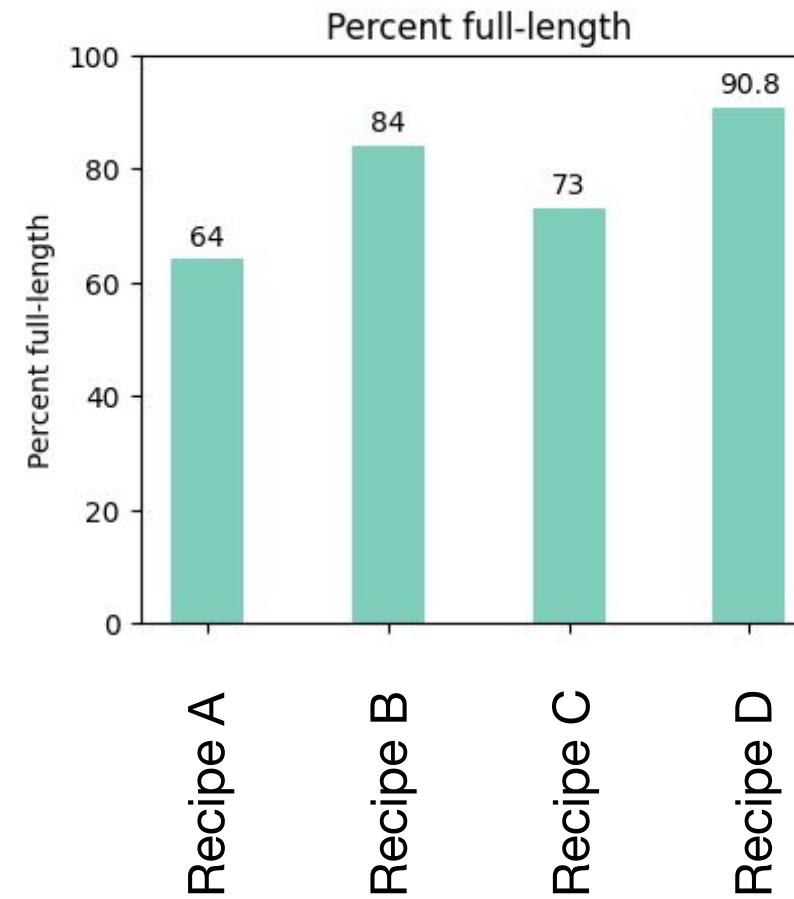


Recipe D



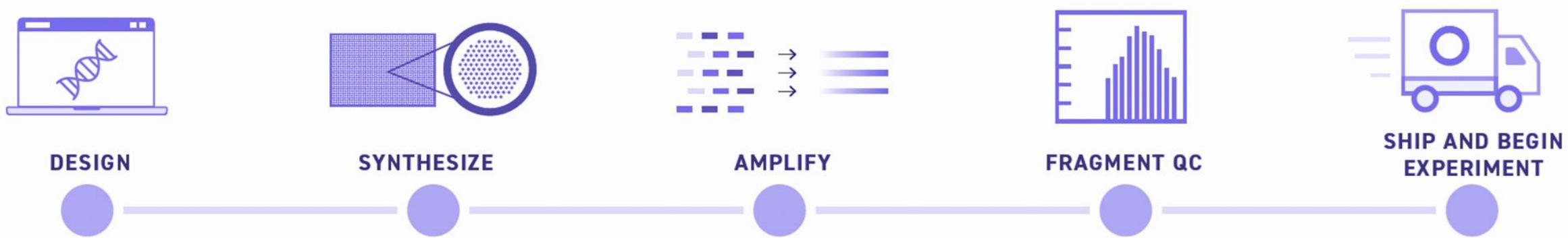
Post-amplification traces

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

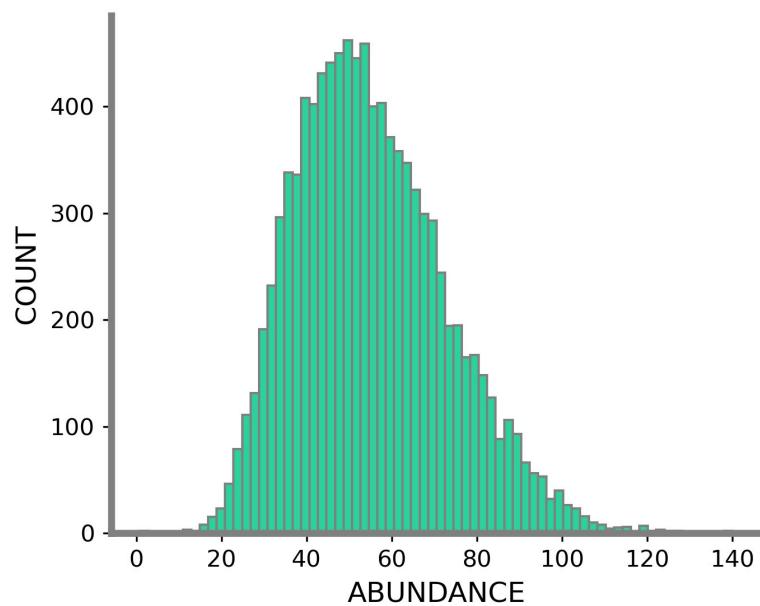
- **No complexity requirements:**

Allows for highly complex sequences such as promoters, tandem repeats, homopolymers, and hairpins

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

Quality and Scale without Compromise



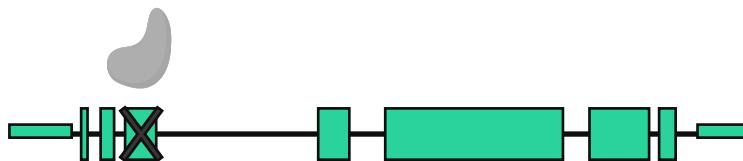
| | |
|-------------------------|--------|
| % Fragments represented | 99.99% |
| Sequencing coverage | 55X |
| Dropouts | 0.01% |
| Diversity | 10,000 |
| 95th/5th Percentile | 3.03 |
| 90th/10 Percentile | 2.35 |
| Chimera Rate | 5.5% |

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.

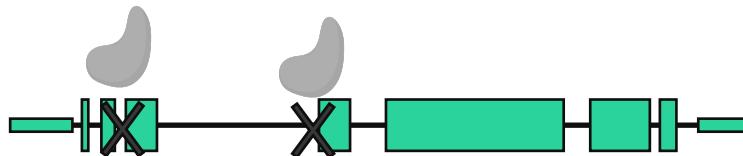
A pool of dual gRNA variants for an amplified Multiplexed Gene Fragment of 500 bp shows complete oligo representation with minimal dropouts.

Benefits of Multiplexed Gene Fragments

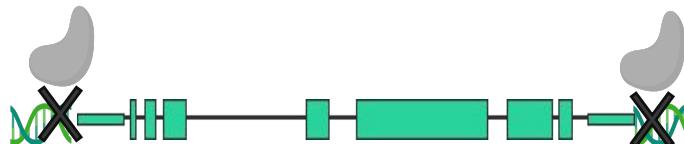
Normal knock-out



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

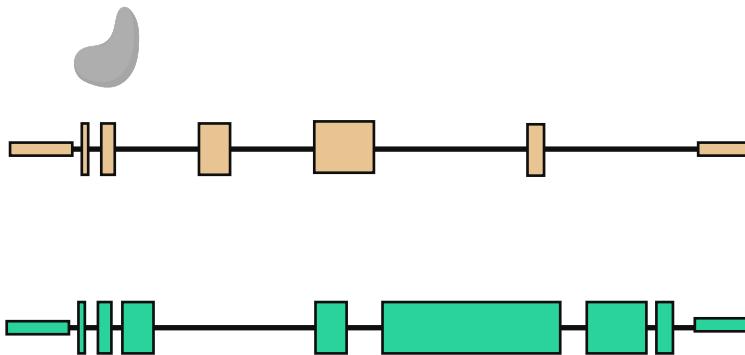
- Remove large regions of a gene or the entire gene for improved knockout efficiency

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

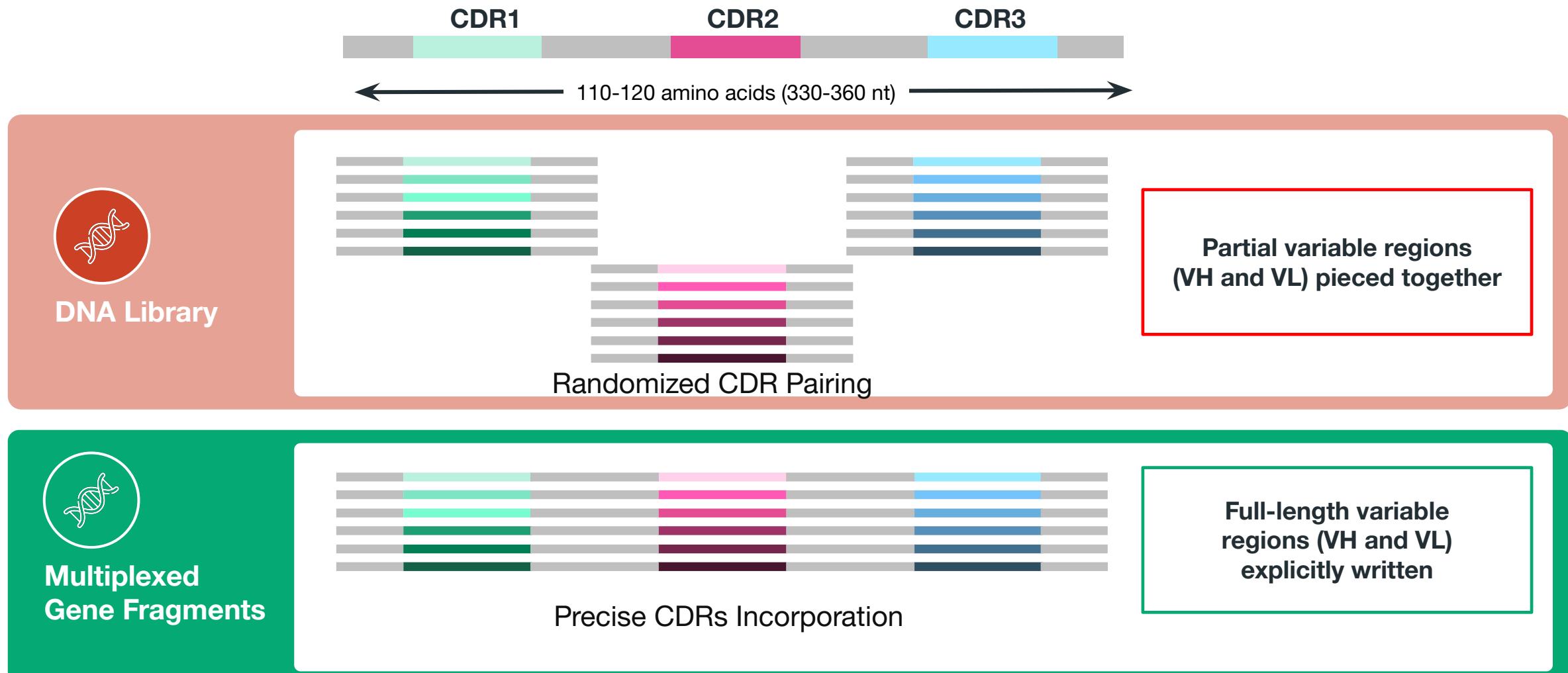
- Target multiple genes or regulatory elements within a cell or organism

Enhanced Functional Studies

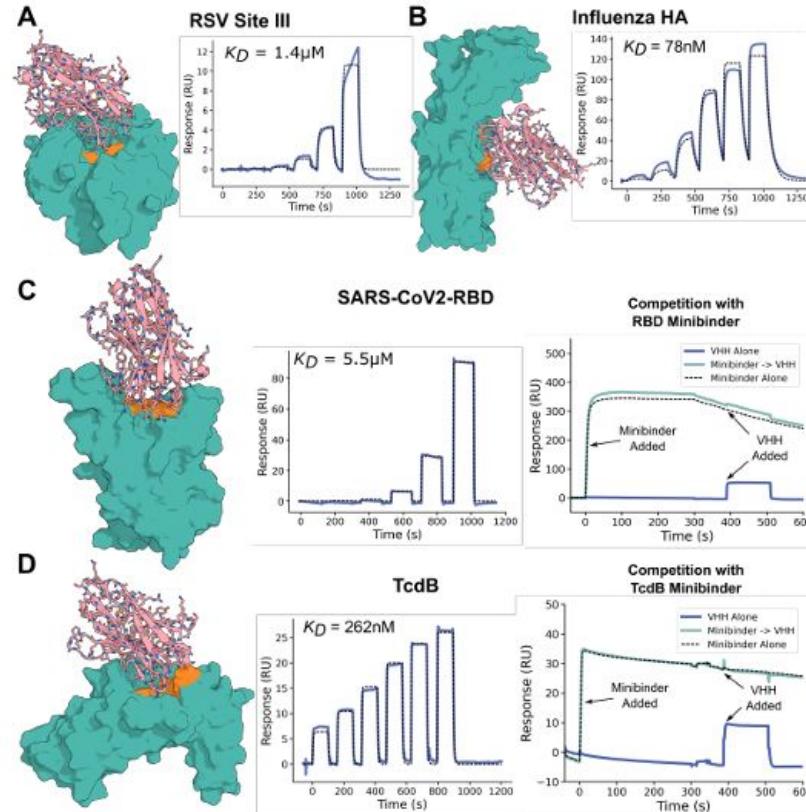
- Targeting of multiple gene loci enables the study of gene interactions
- Analysis of functional relationship between genes for a more comprehensive understanding of pathways
- Gain a better understanding of synthetic lethality where a perturbation of both genes can result in the loss of viability

Multiplexed Gene Fragments Use Case I Precise Antibody Profiling at Scale

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



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

Precision DNA Variant Library Synthesis



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

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



Base-by base precision



Codon usage control



CDR length variation



Avoid restriction sites and unwanted motifs



Precisely controlled combinatorial diversity



Ratio controlled amino acid distribution



Multiple germline scaffolds



Library validation by next generation sequencing

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.

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 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.

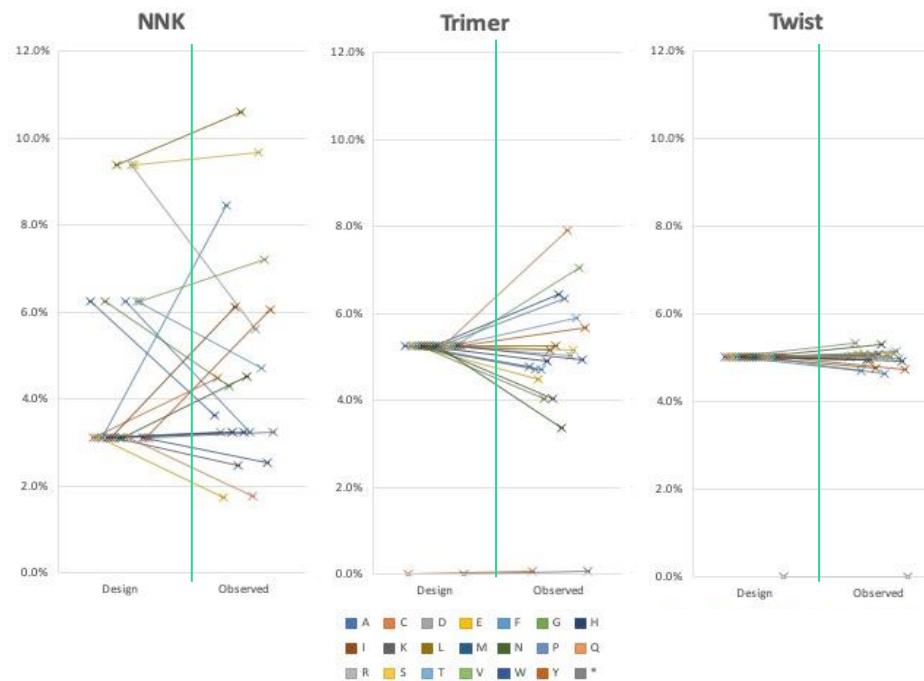
Combinatorial Assembly Libraries

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

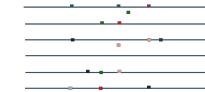


- ✓ Explicit
- ✓ Even representation
- ✓ Human repertoire based
- ✓ Fast
- ✓ Affordable

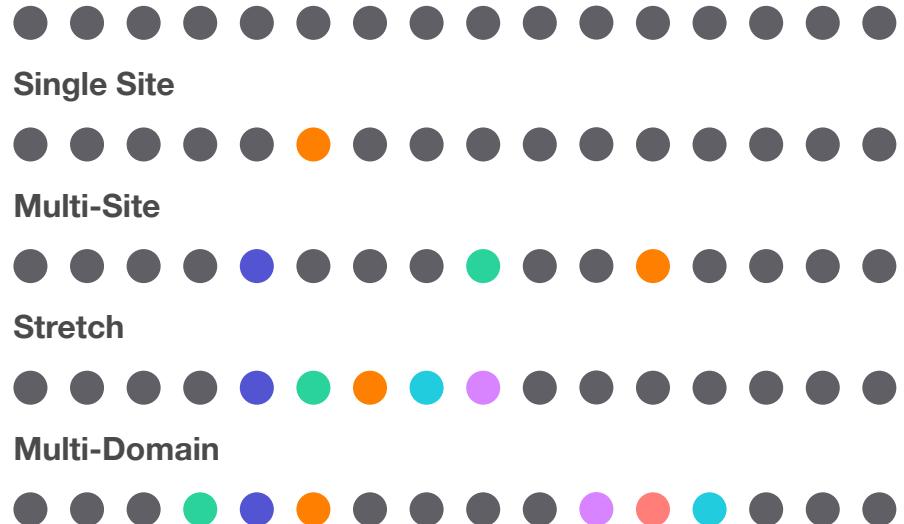
Precise Introduction of Variants, Diversity that Enables Screening Efficiency



gt cacttc**A**ccc **t**Acttg
gt cacttc**G**Gcc **t**t**G**ttg
gt cacttc**C**acc **t**C**A**ttg
gt cacttc**T**cc **t****G**ttg



Gene Synthesis



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)



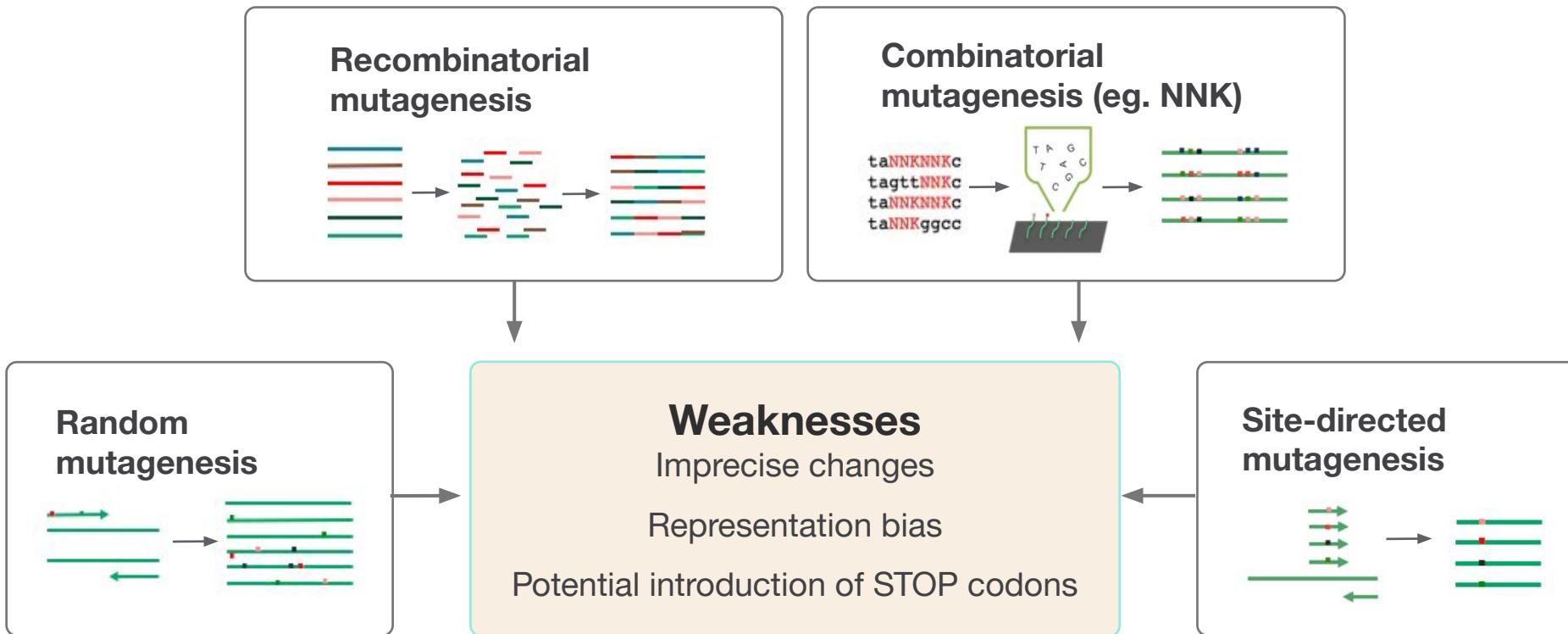
- ✓ Variants differ by precise codon substitutions combined in all positions in each domain
- ✓ Variants confined to one or more domains
- ✓ Define unique ratios of variants at each position, or define total number of changes from WT per domain
- ✓ Total diversity of up to 10^{10} final variants

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

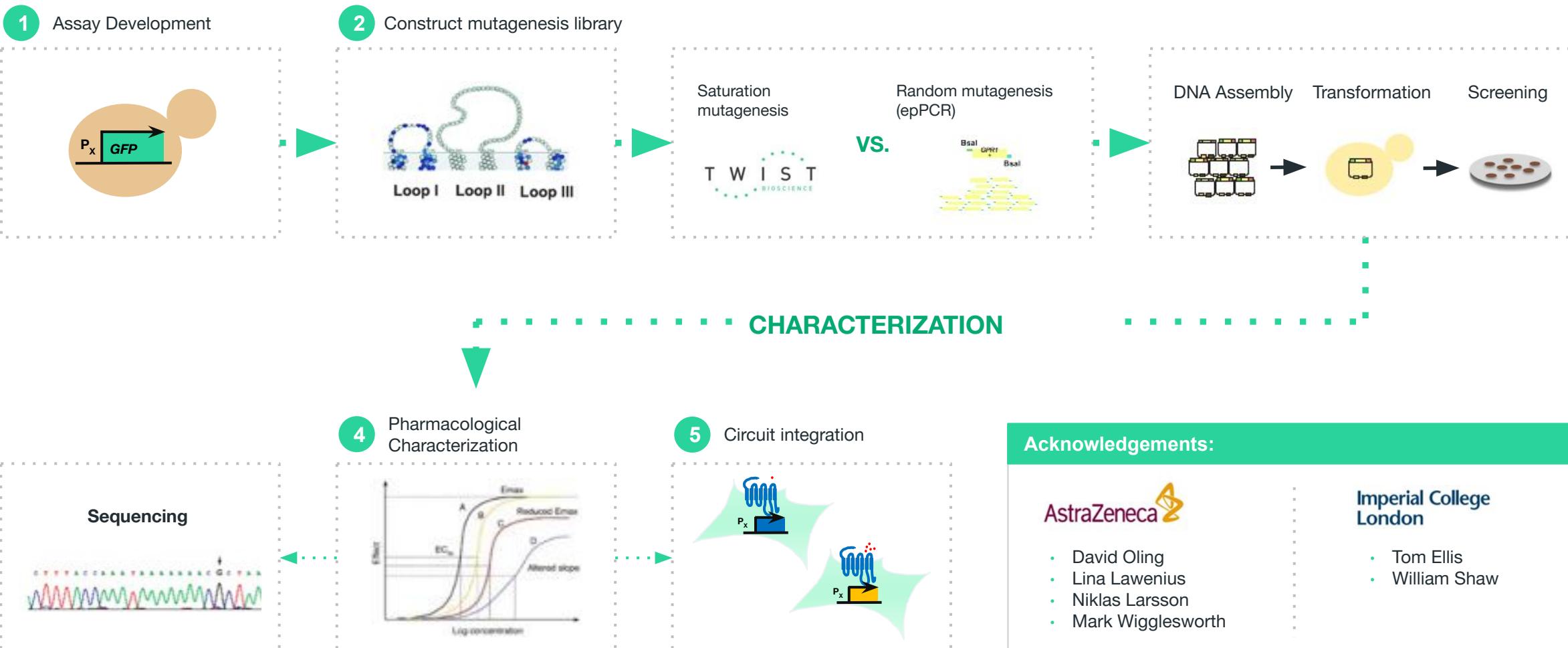


Stochastic introduction of variants > hours of tedious work
Limited and biased sampling of sequence space

Expensive screening

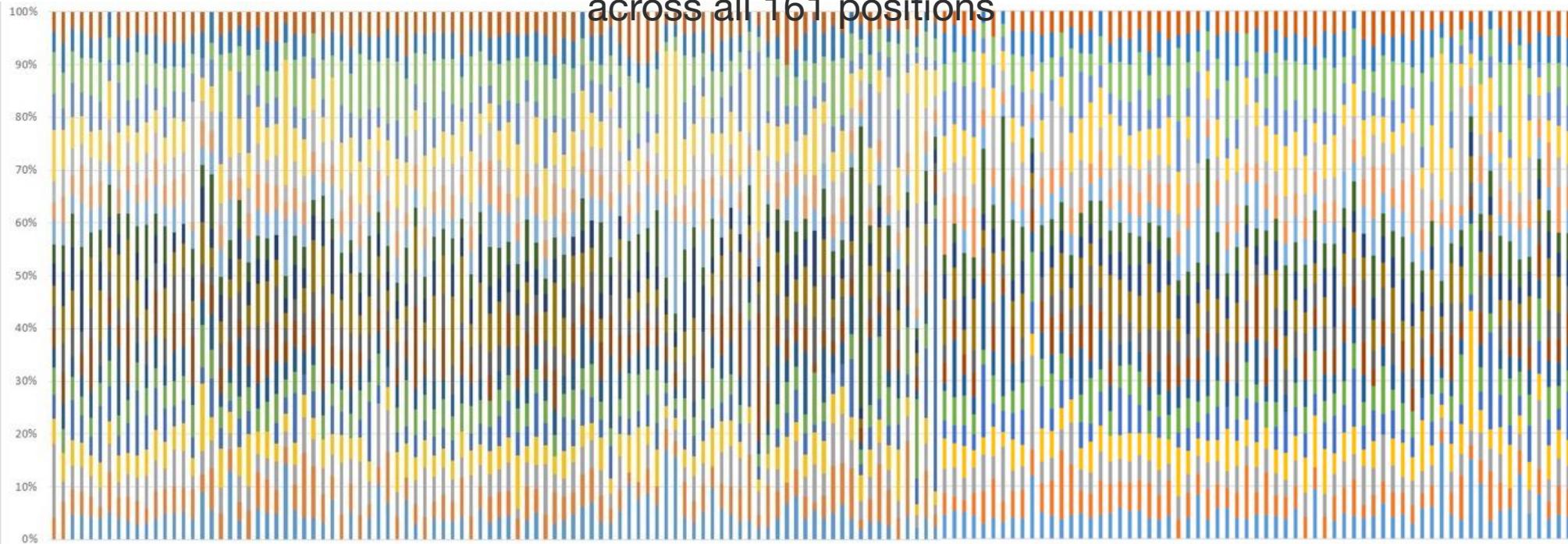
Advancing Human Health – Pharma Development

Case Study: Rational Design vs Random Mutagenesis



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">+ <u>More hits</u>+ Pure, cloning ready DNA+ Fast production time | <ul style="list-style-type: none">- Less hits- Unknown variants- Large fraction of empty vectors |



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.

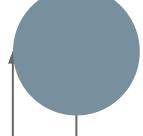
Get to ground truth



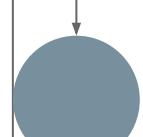
Initial training dataset development

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

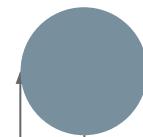


Sequence Output

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



Quickly navigate sequence space



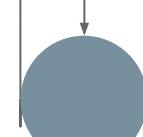
Model guided Navigation of sequence space



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

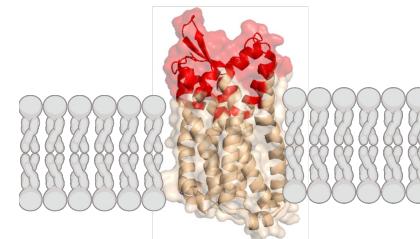
Fully Human Library of Libraries
Phage and Yeast Workflows
Immune Libraries



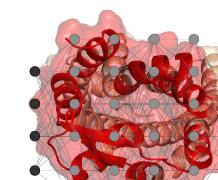
3-4 Months

Chemical and Conformational Evaluation of Existing Structure

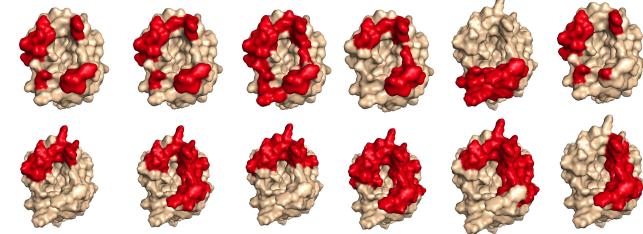
Solvent Exposed Region of C5aR



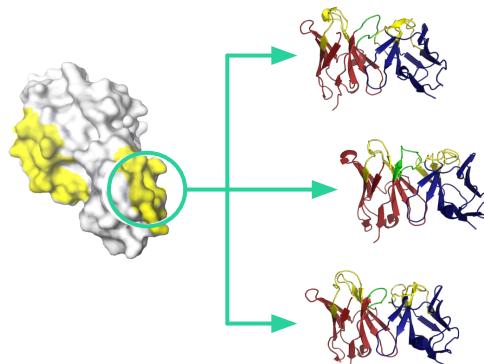
Probability Matrix Generated



Discrete “Druggable” Epitopes Identified

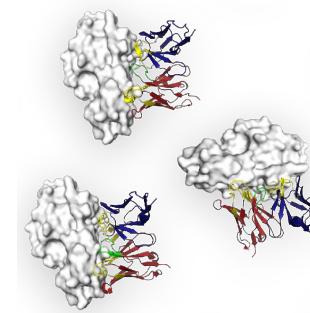


Run ML Design Algorithm to predict binders



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

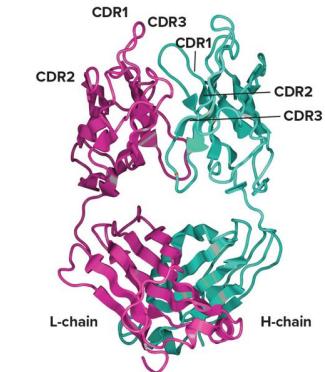
Fully Human Library of Libraries

Phage and Yeast Workflows

Immune Libraries



Diagram illustrating the diversity of CDR1, CDR2, and CDR3 regions. CDR1 and CDR2 each have 3 diversity segments (green, black, green) with a total length of approximately 10^3 . CDR3 has 3 diversity segments (green, purple, green) with a total length of approximately 10^4 . The total theoretical diversity is approximately 10^{10} .



- 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

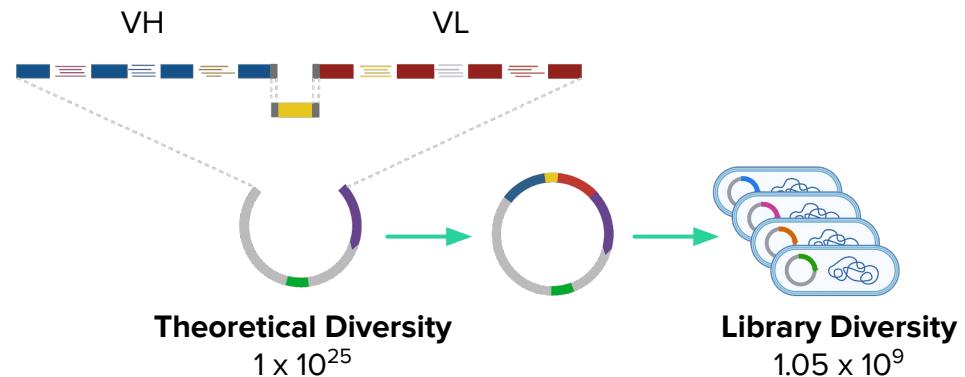
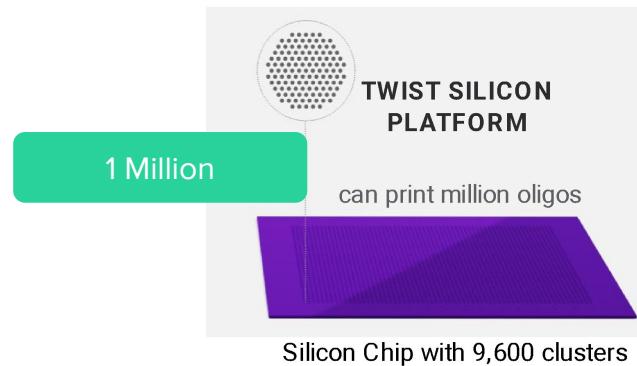
Fully Human Library of Libraries

Phage and Yeast Workflows

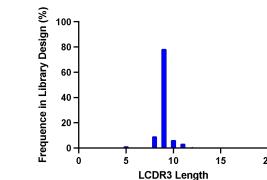
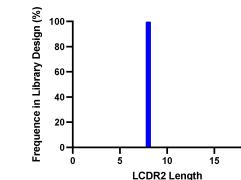
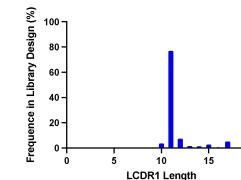
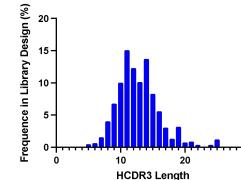
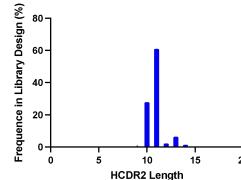
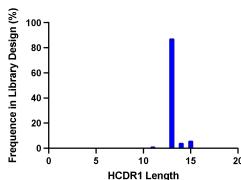
Immune Libraries



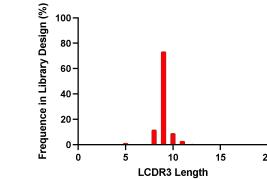
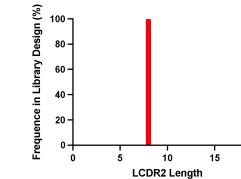
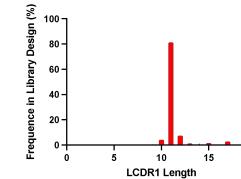
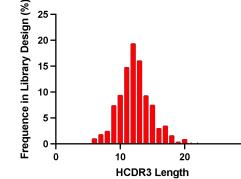
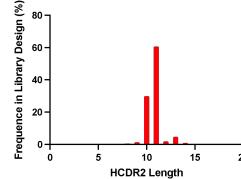
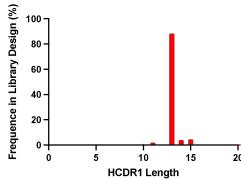
3-4 Months



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

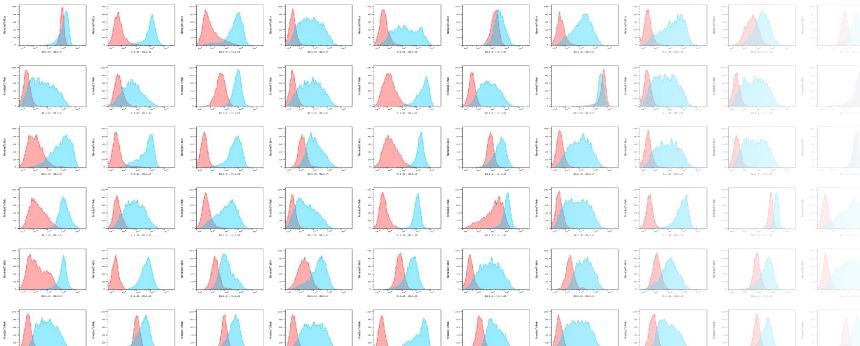
In Vitro Discovery

Fully Human Library of Libraries
Phage and Yeast Workflows

Immune Libraries



3-4 Months

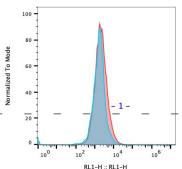


[Antibody] = 100 nM

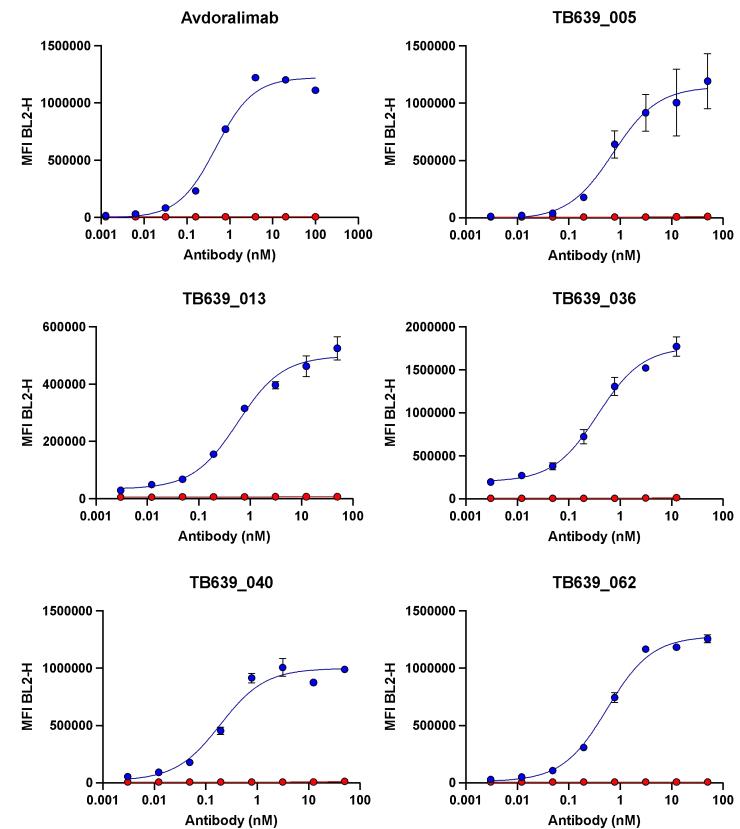
Parental Cell line

Secondary Only Control

C5aR Expressing Cell line



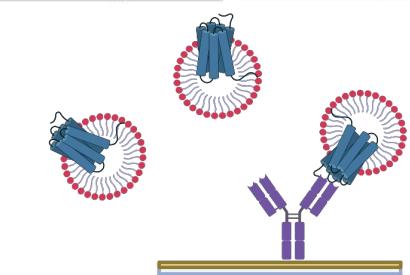
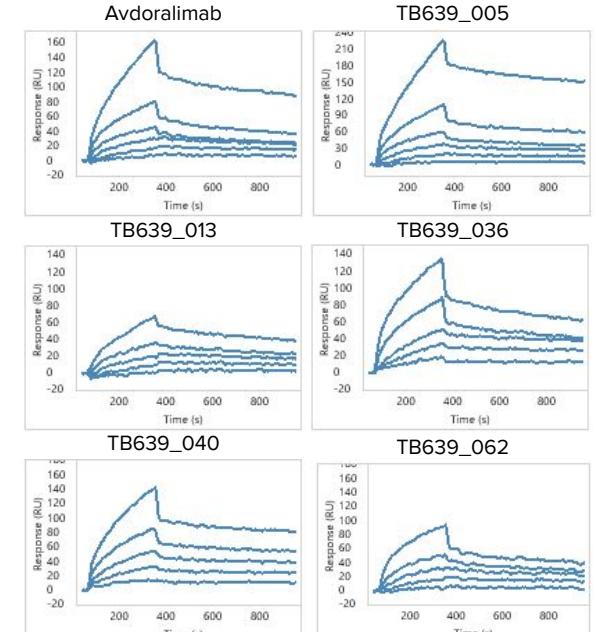
Flow-Based Titration of Select Clones (on Attune Cytometer)



● C5aR Expressing Cell line

● Parental Cell line

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.

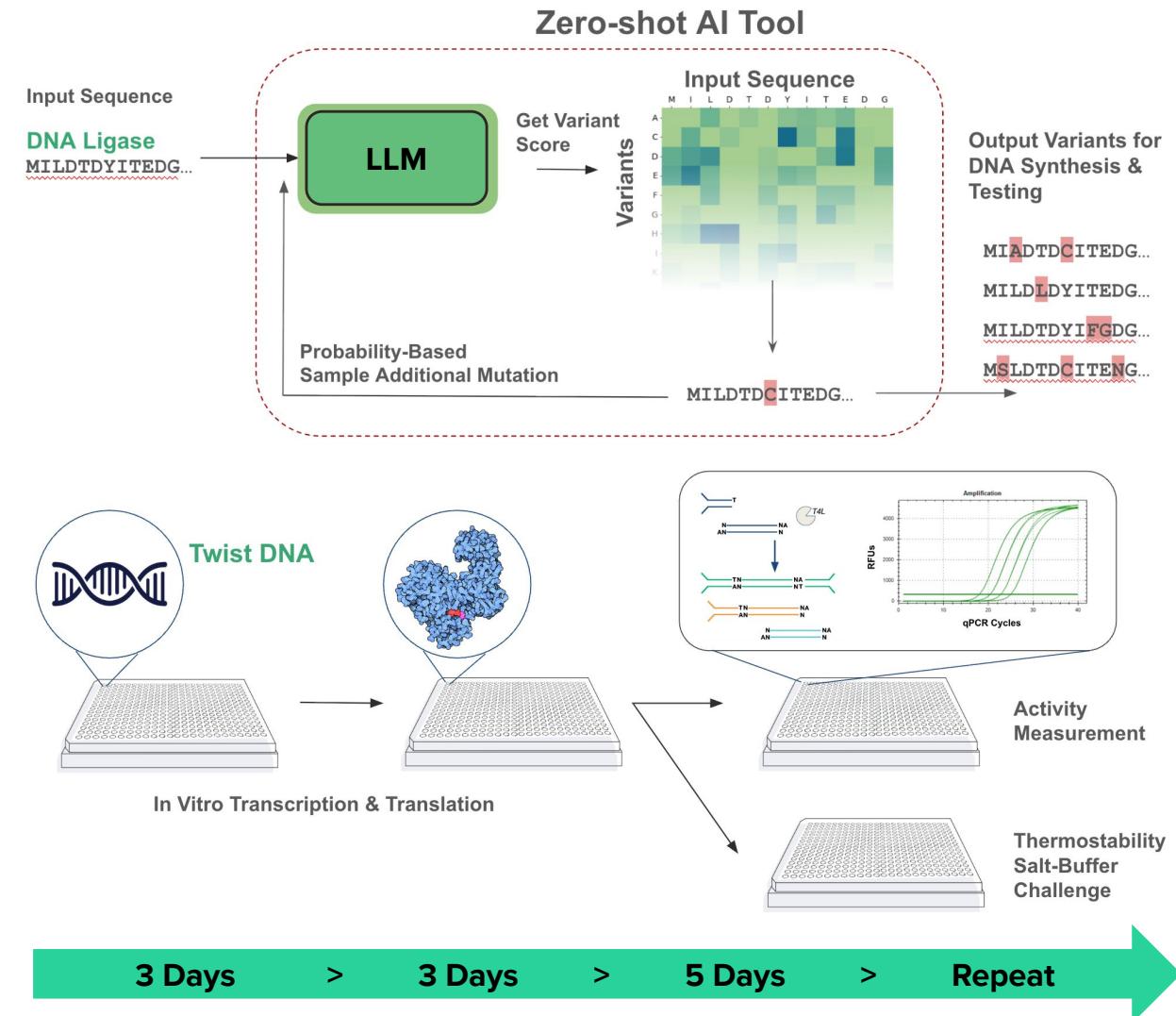
Goal

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

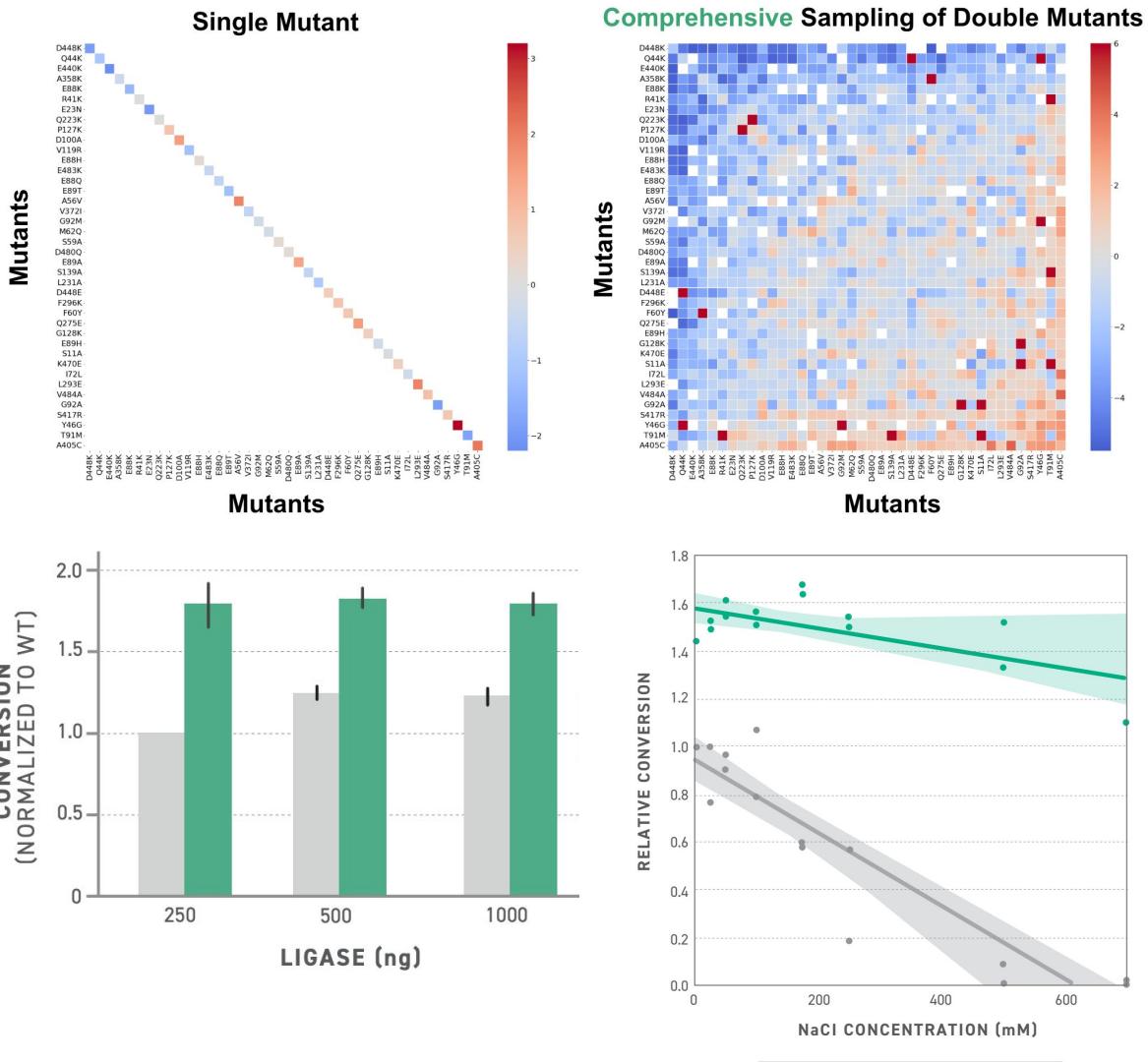
Advantages

- 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.



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

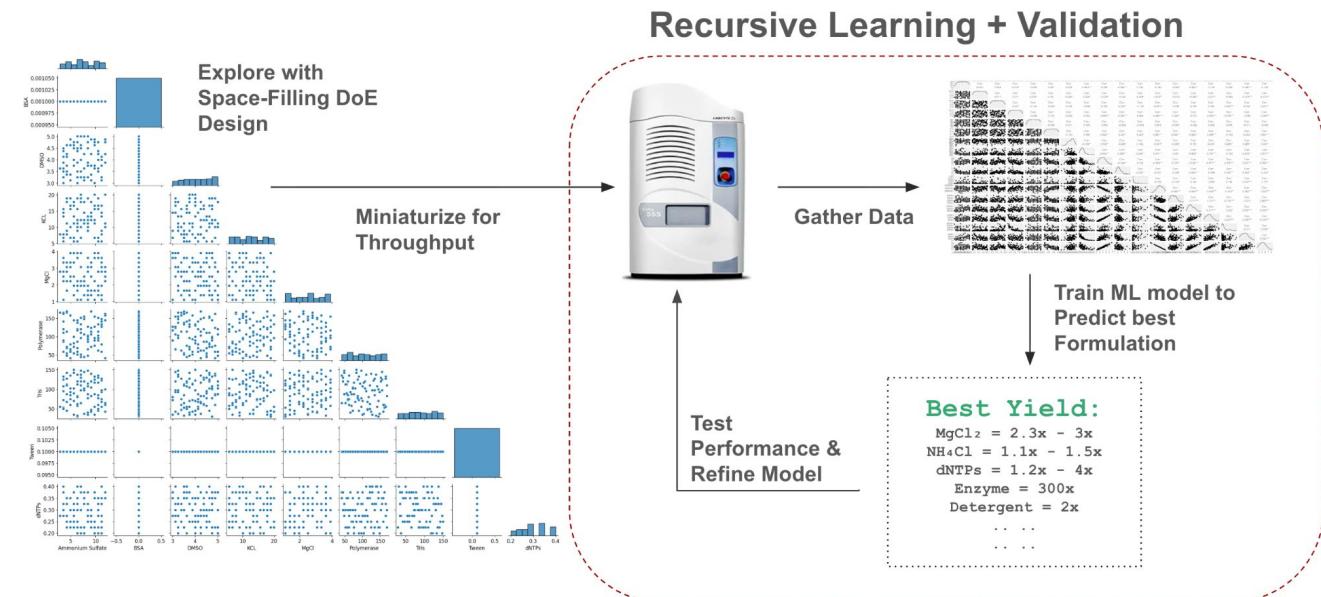
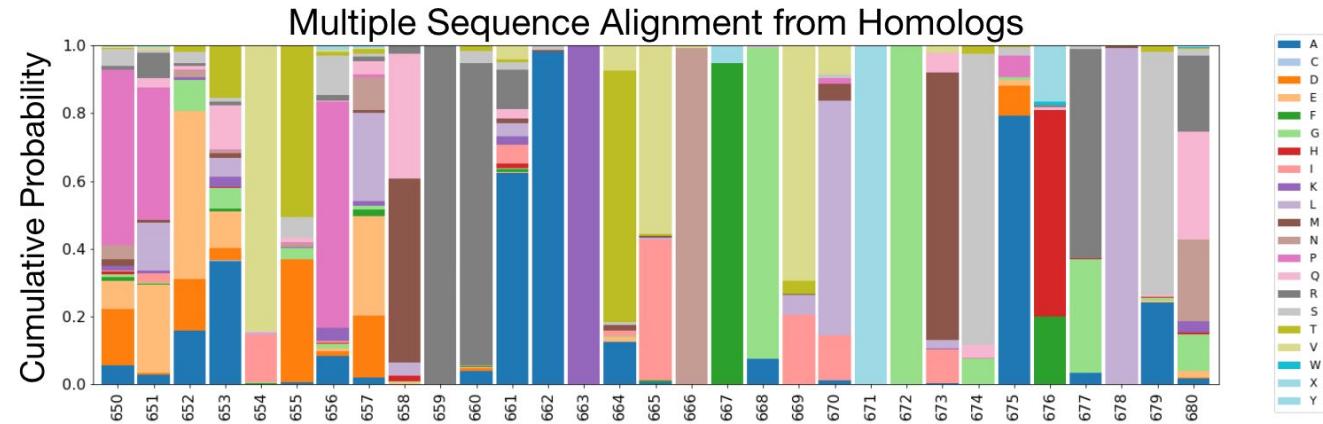
Engineer a **high-fidelity DNA polymerase** solution with a **high-performance buffer** for best-in-class uniformity.

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

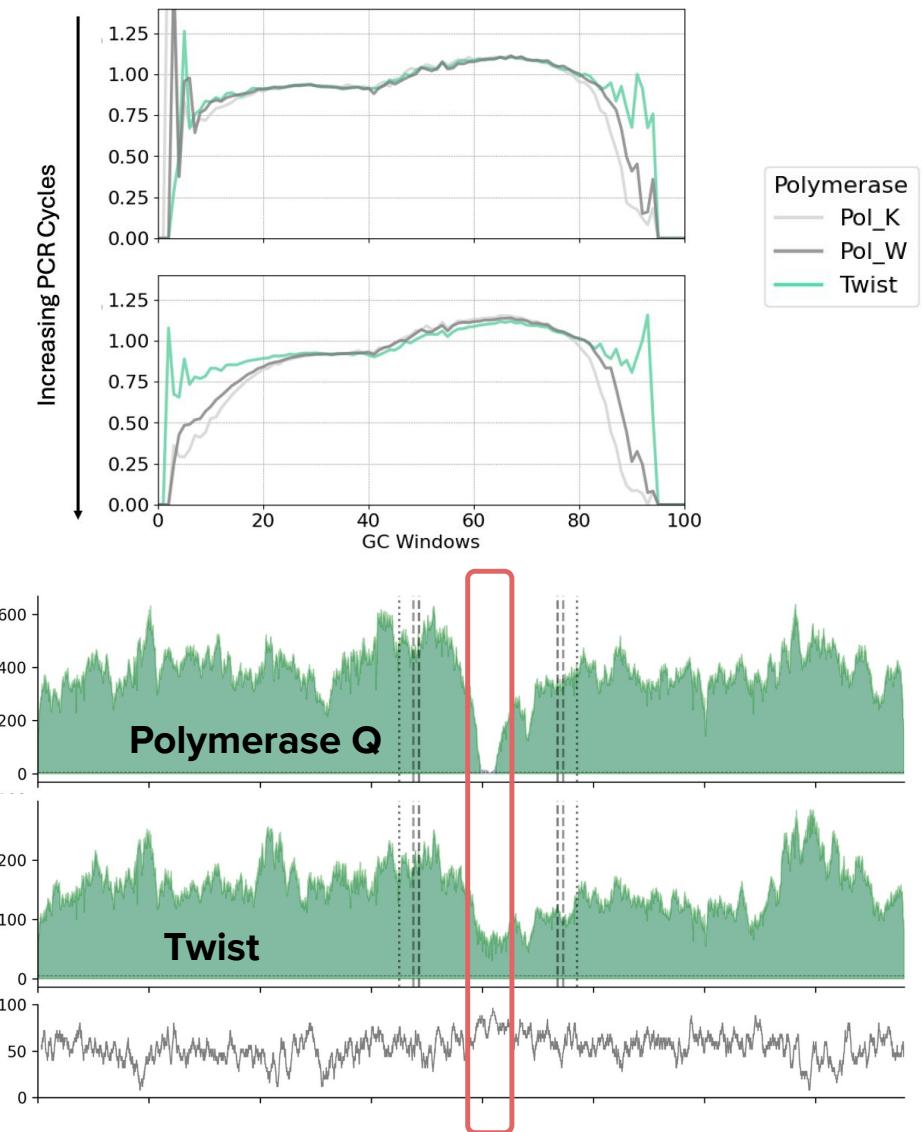
1. High-Fidelity Polymerase
2. Robust and High-Performance Buffer

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.

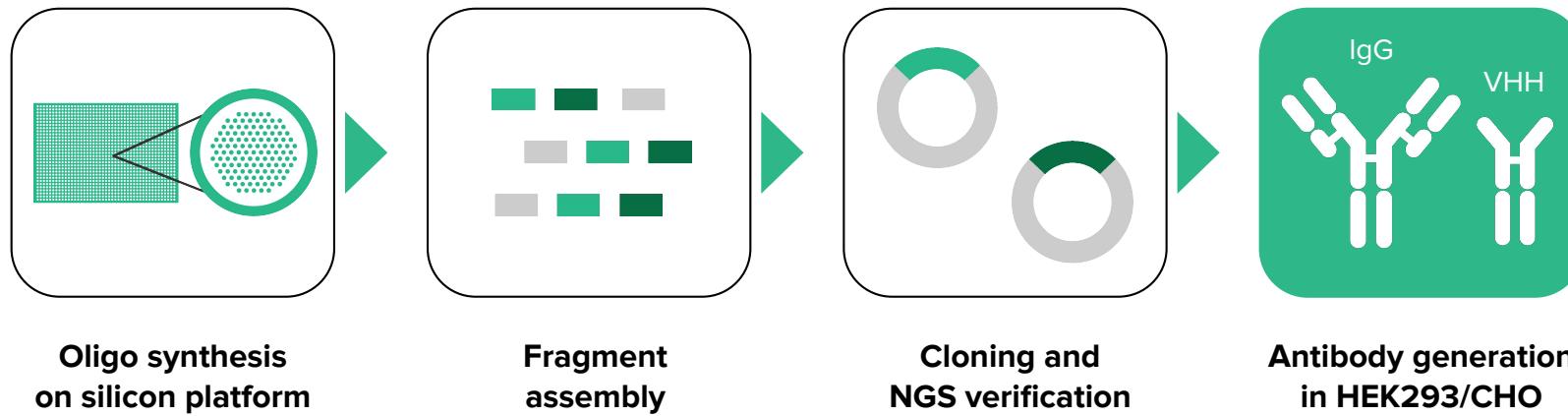


Antibody and Characterization

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.

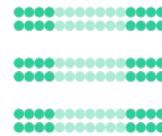
Addressing the Challenges of Generating Hundreds to Thousands of Antibody Hits

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

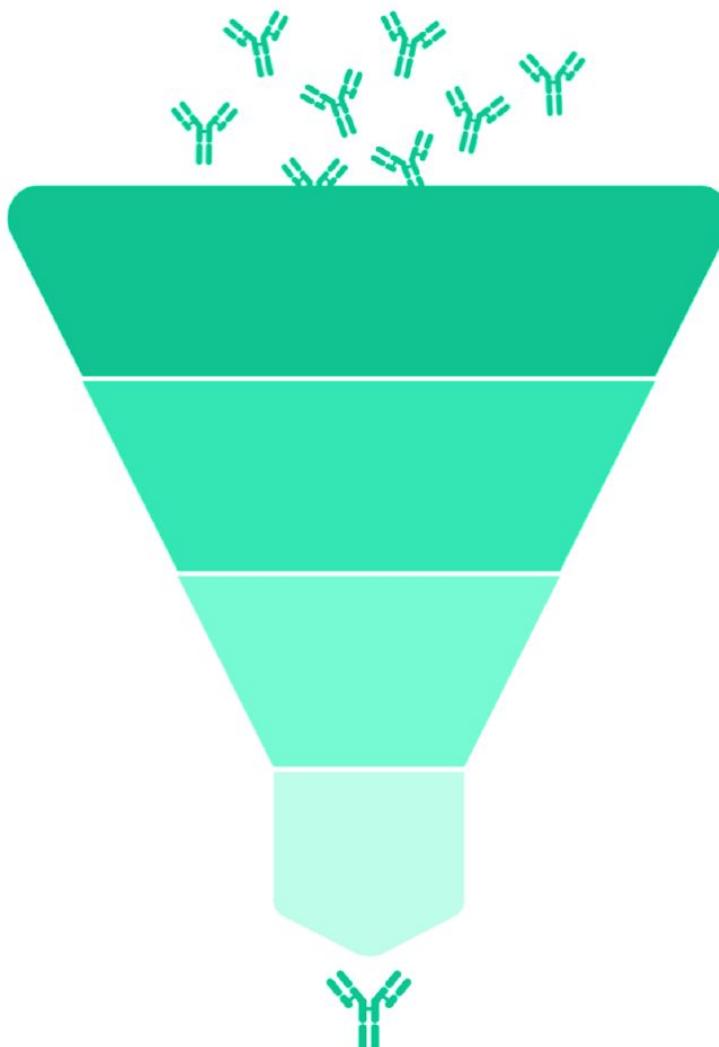


Time to Next Review

- 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.

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.

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.

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.

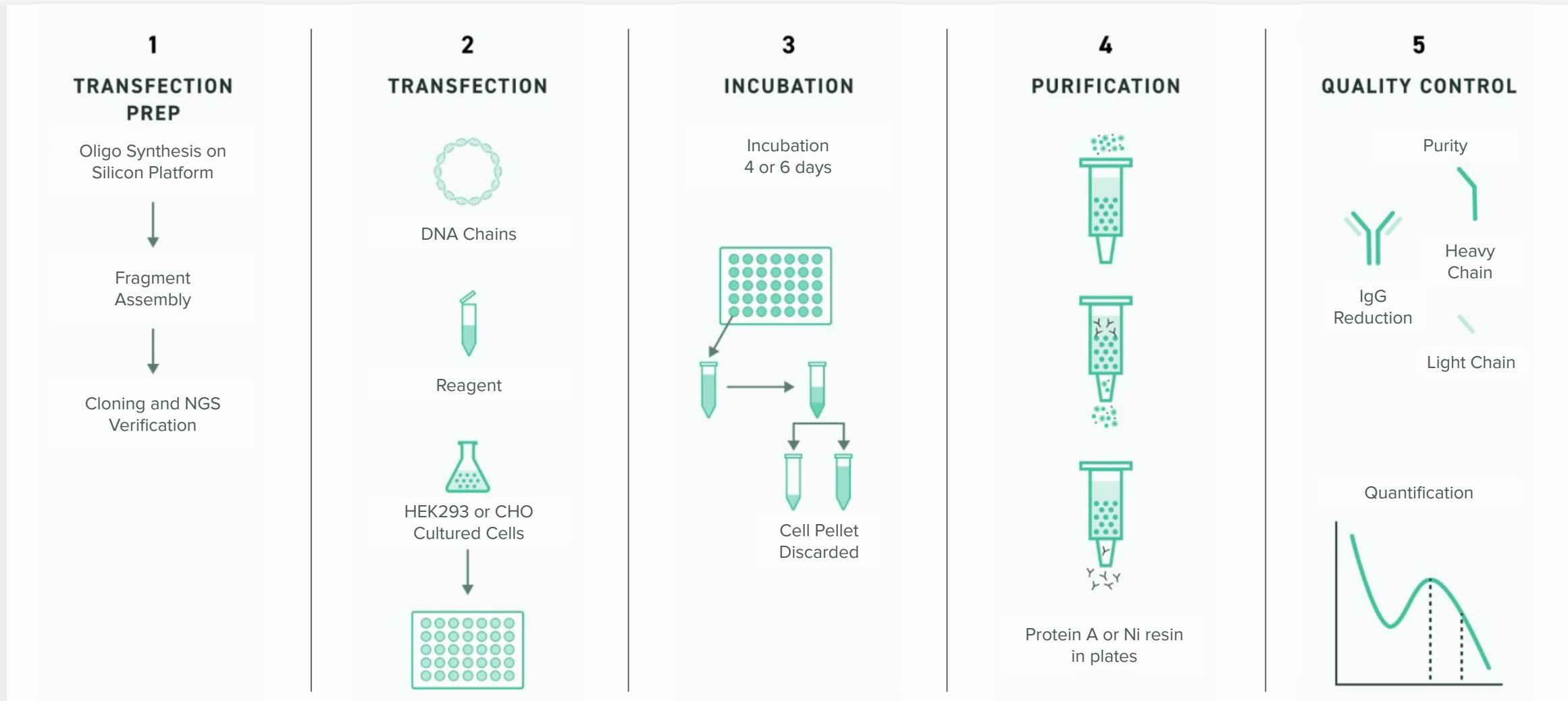
HIGH-THROUGHPUT ANTIBODY PRODUCTION OVERVIEW



*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.

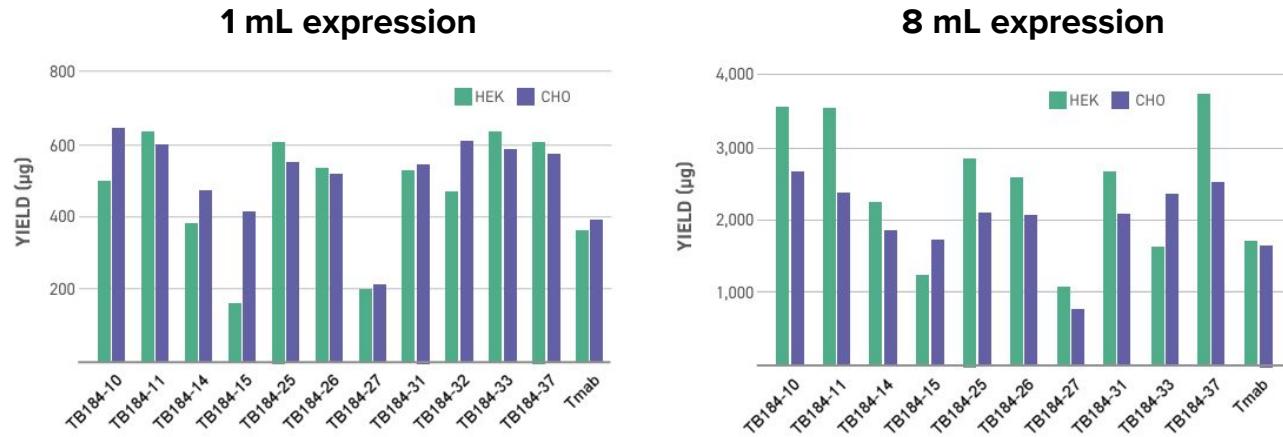
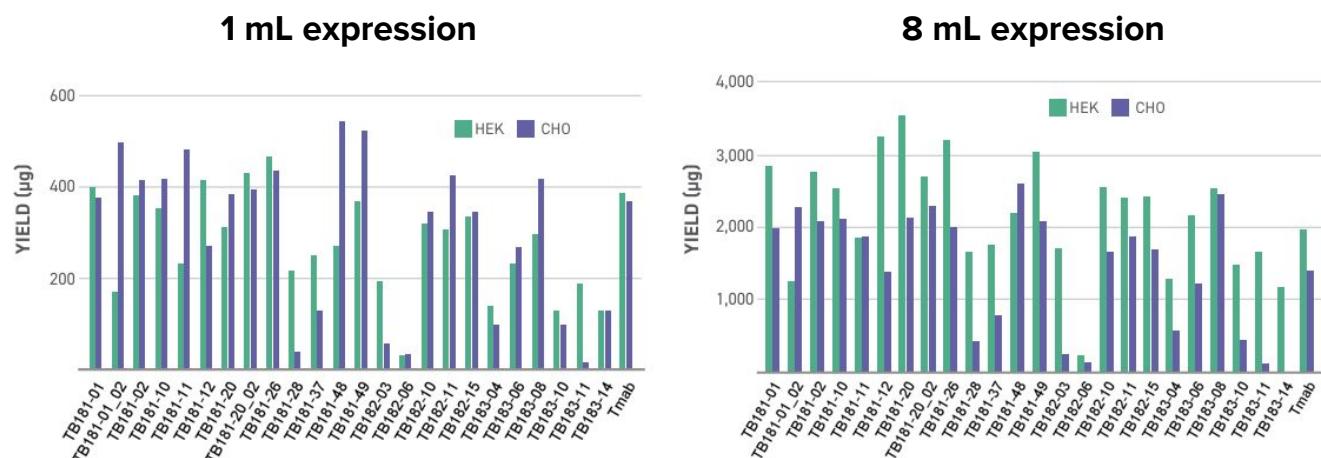


Figure 3. VHH-Fc yield comparison by scale and cell line.

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.

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. Supernatant products are 0.2 μ m 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 μ m 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 μ m 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.

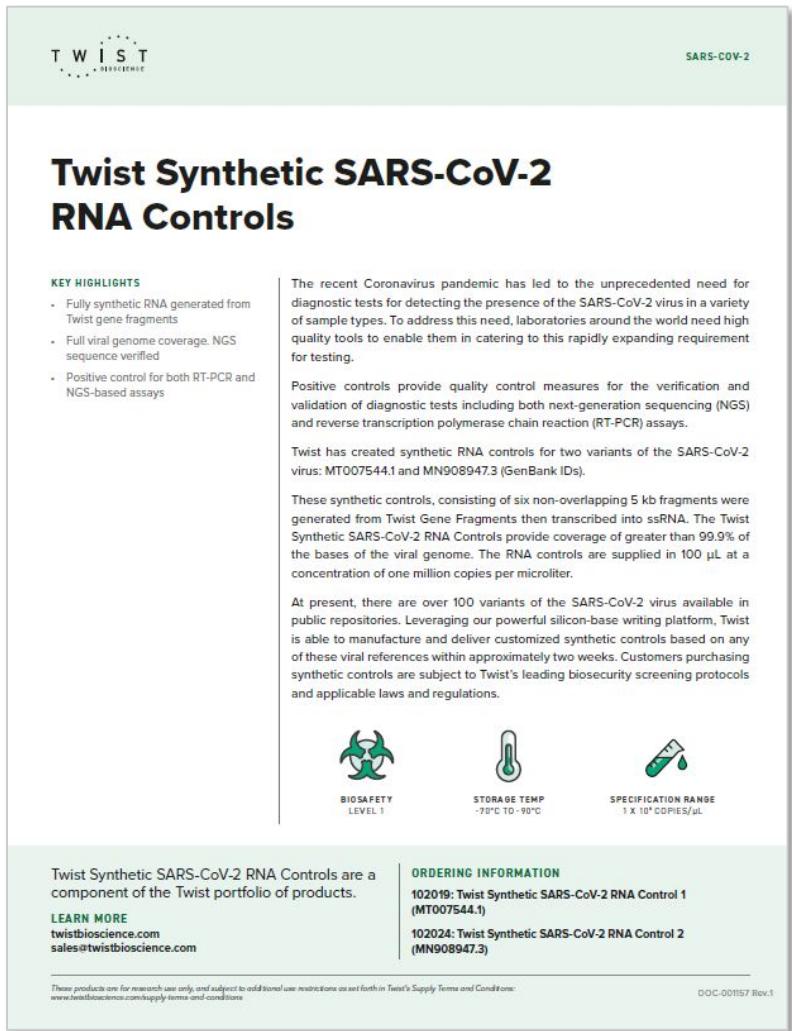


Powerful alternative to “live virus” controls - Synthetic controls created through gene synthesis broaden access across diverse strains while mitigating safety and security concerns.



Serve in a wide range of applications from diagnostic assay development to day-to-day testing

Synthetic Viral RNA Controls



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.

BIOSAFETY LEVEL 1

STORAGE TEMP -70°C TO -90°C

SPECIFICATION RANGE 1 X 10⁶ COPIES/μL

ORDERING INFORMATION

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

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

LEARN MORE
twistbioscience.com
sales@twistbioscience.com

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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.

Synthetic Viral RNA Controls - SARS-CoV-2

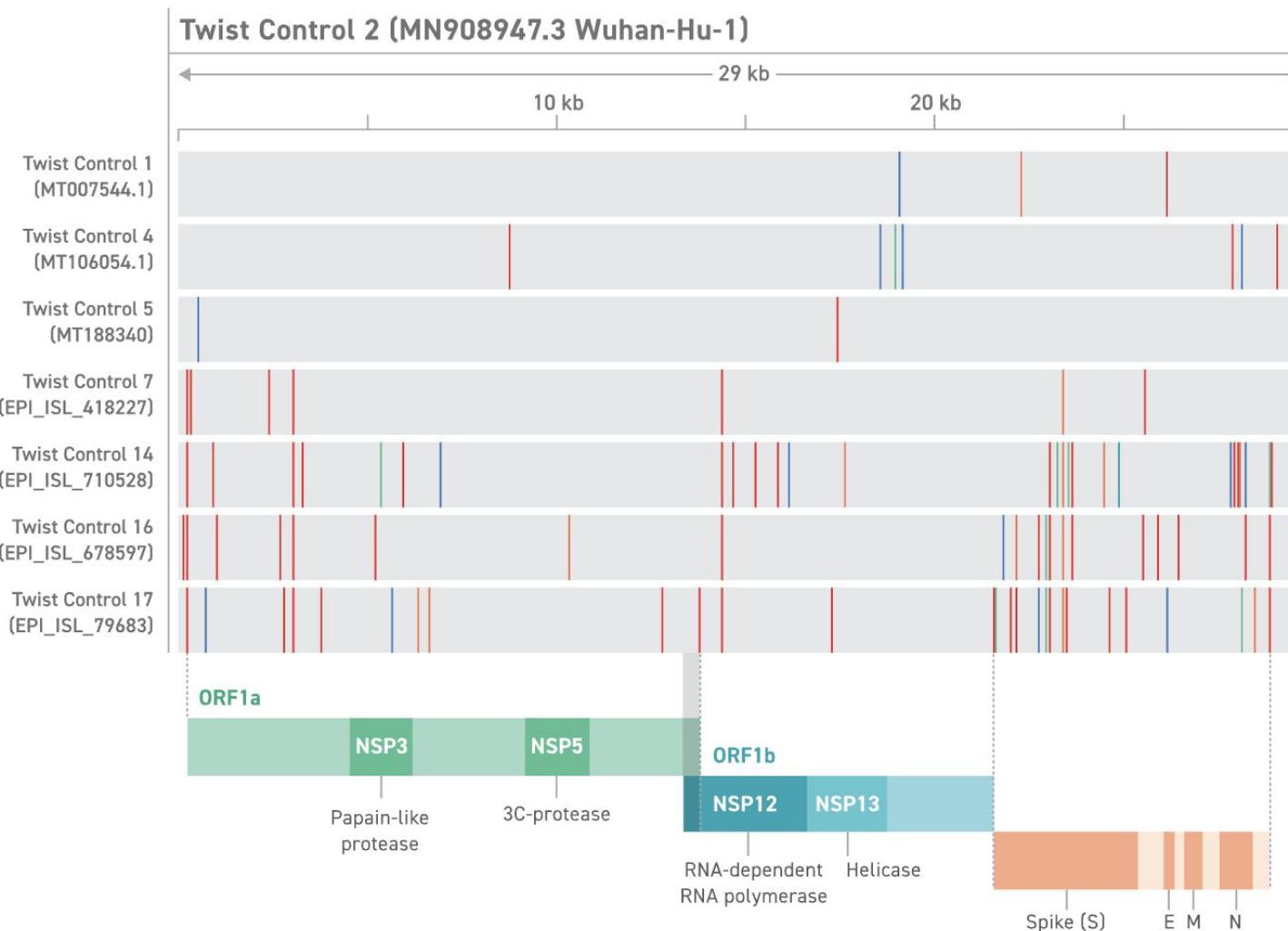
Twist has created 37 synthetic RNA controls for multiple variants of the SARS-CoV-2 virus:

| 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 (B.1.429) | Epsilon | EPI_ISL_672365 | USA/CA-CZB-12943/2020 |
| 104532 | Control 22 (B.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.1.529/BA.1) | Omicron | EPI_ISL_6841980 | Hong Kong/HKU-211129-001/2021 |
| 105345 | Control 50 (B.1.529/BA.2) | Omicron | EPI_ISL_7190366 | Australia/QLD2568/2021 |
| 105346 | Control 51 (B.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

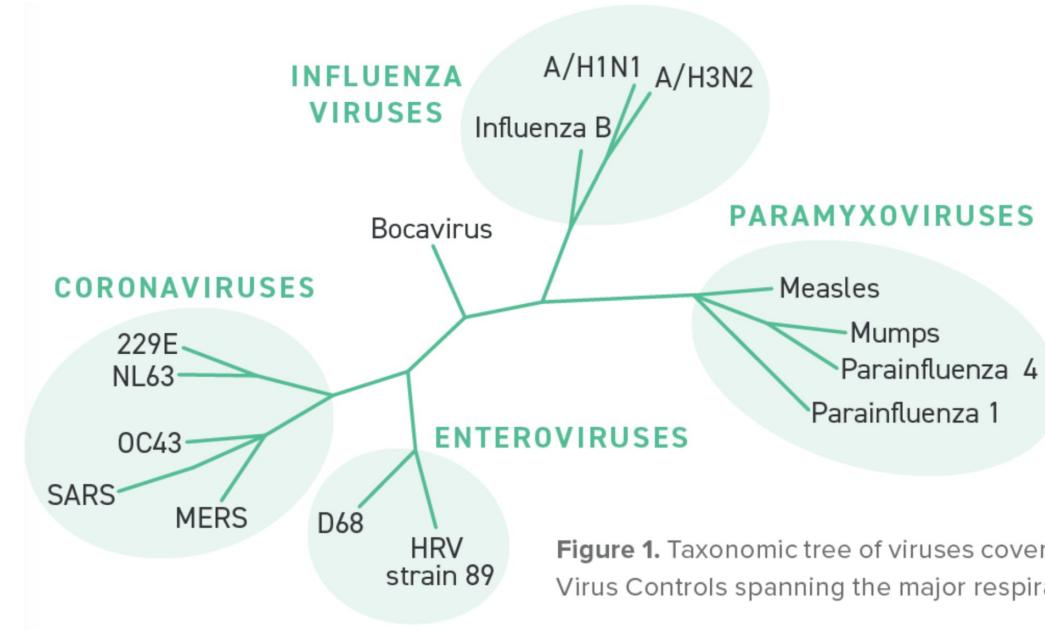
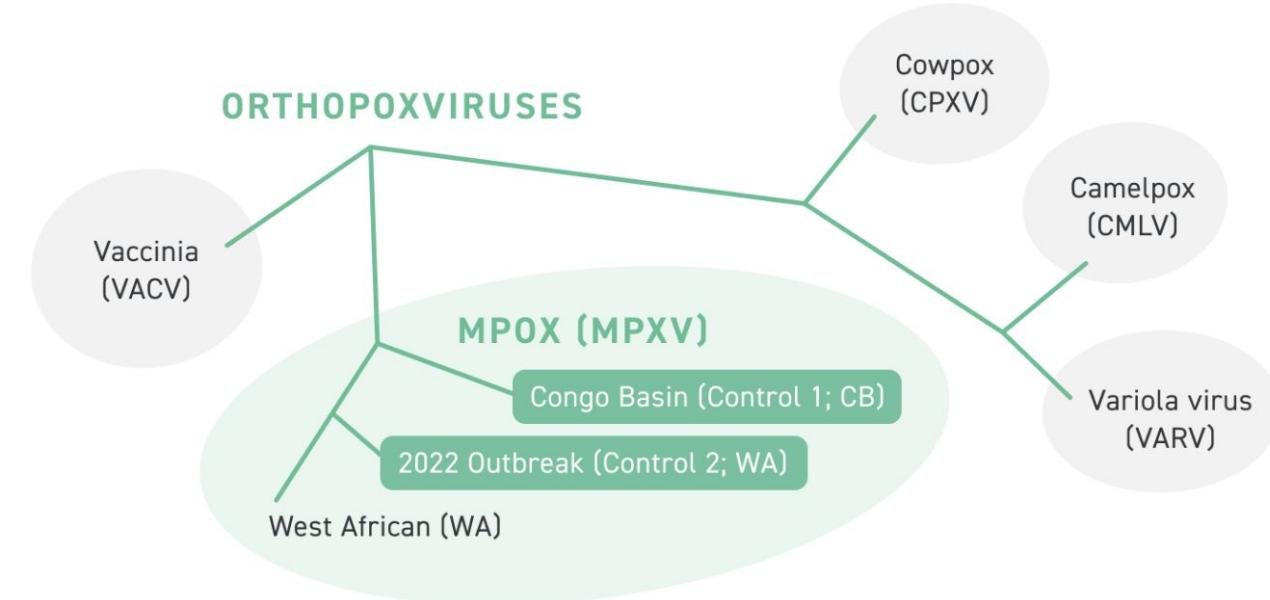


Figure 1. Taxonomic tree of viruses covered on the Twist Respiratory Virus Controls spanning the major respiratory viral clades.

Synthetic Viral RNA Controls - Mpoxy Virus Controls

- Twist has created synthetic human Mpoxy 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 Mpoxy 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

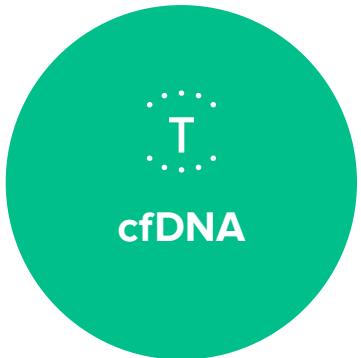
Twist Controls: Confidence in Every Sequencing Run

Quality Assurance at Every Step



Viral

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



cfDNA

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



Custom Controls

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.

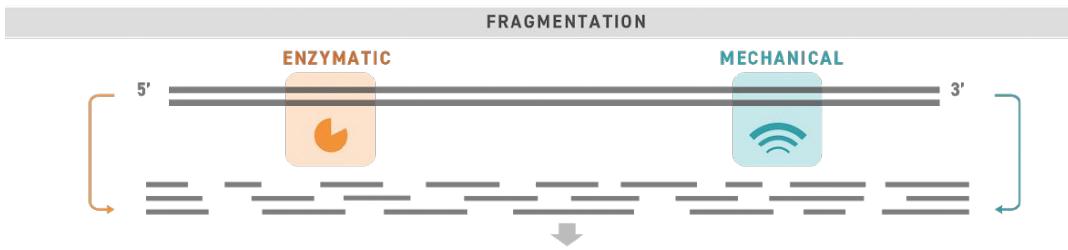


Custom Solutions for Your End Goal

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

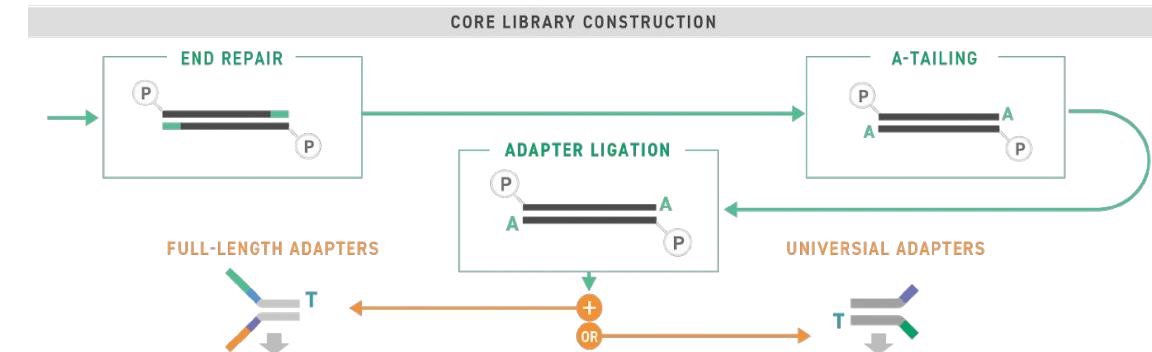
Our library preparation kits are built around Twist's engineered enzymes, which ensure high fidelity and efficient conversion.



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.

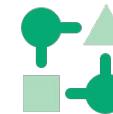


Flexibility in Your Workflow → Twist Universal Adapters, offering compatibility with indexed primers or a PCR-free workflow using Twist Full-Length Adapters.

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.

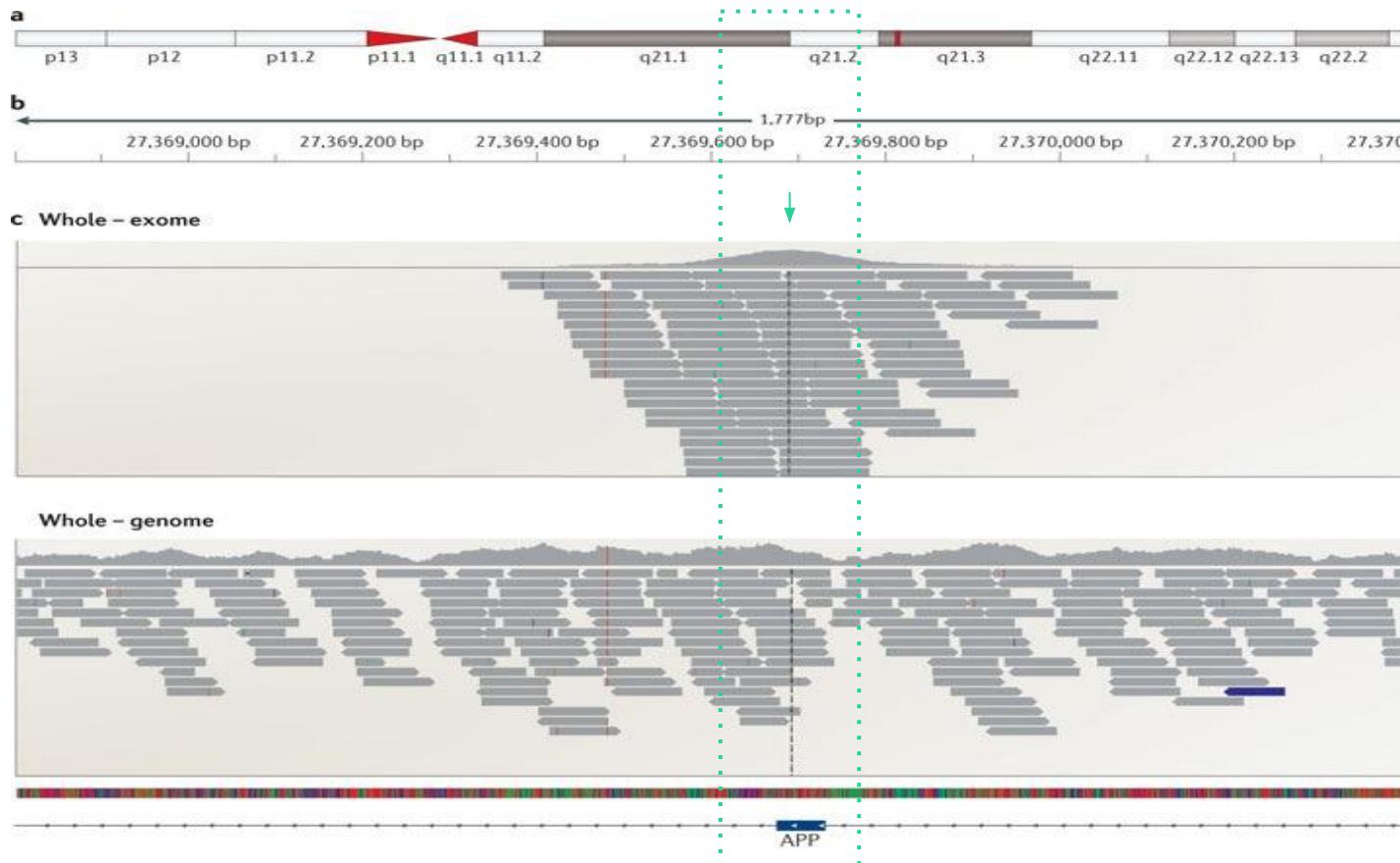


| | Library Preparation Kits | Adapter Systems |
|--|--|--|
| Infectious, Rare, or Mendelian diseases | Enzymatic Fragmentation Kit 2.0 Mechanical Fragmentation Kit Human Sample ID | Universal Adapter System Full Length UDI Adapter System |
| Early cancer detection / Minimal residual disease | cfDNA Library Preparation Kit | UMI Adapter System |
| Epigenomics | Methylation Detection Kit | Methylated UMI Adapter System |
| Agrigenomics / Population genomics | FlexPrep UHT Library Preparation Kit | Universal Adapter System |
| Transcriptomics | RNA-Seq Library Preparation Kit • Whole transcriptome • Target enrichment | Universal Adapter System UMI Adapter System |
| WGS | 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

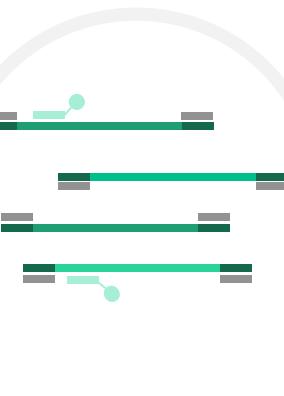
Prepped Library Fragments



Blocked Library Fragments



Hybridized Library Fragments



Bead-Bound Target Sequences



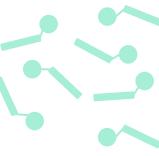
Target Enrichment



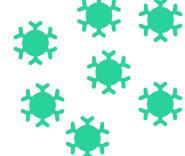
Universal Blockers and COT-1



TWIST DNA Probes

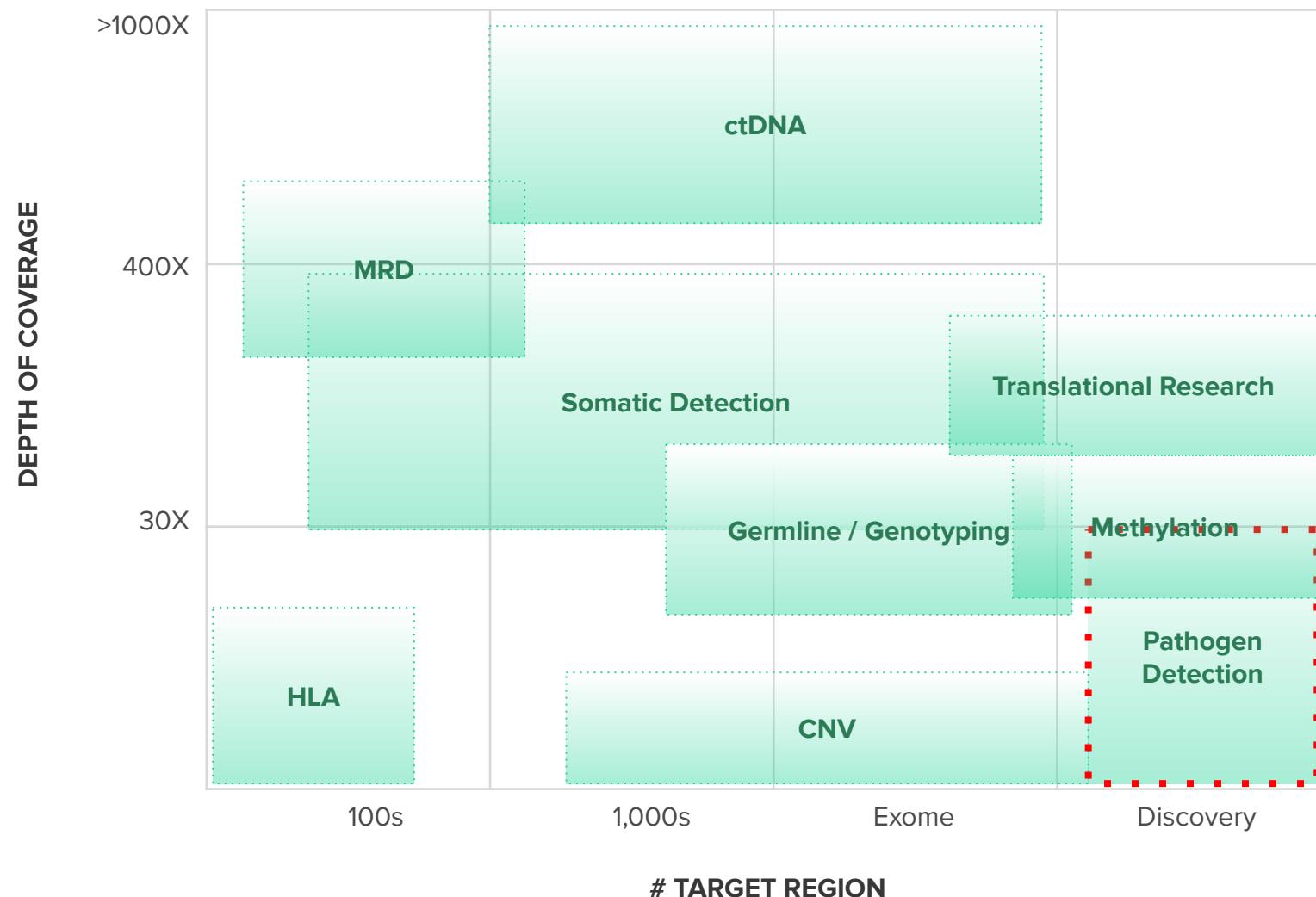


Streptavidin Beads



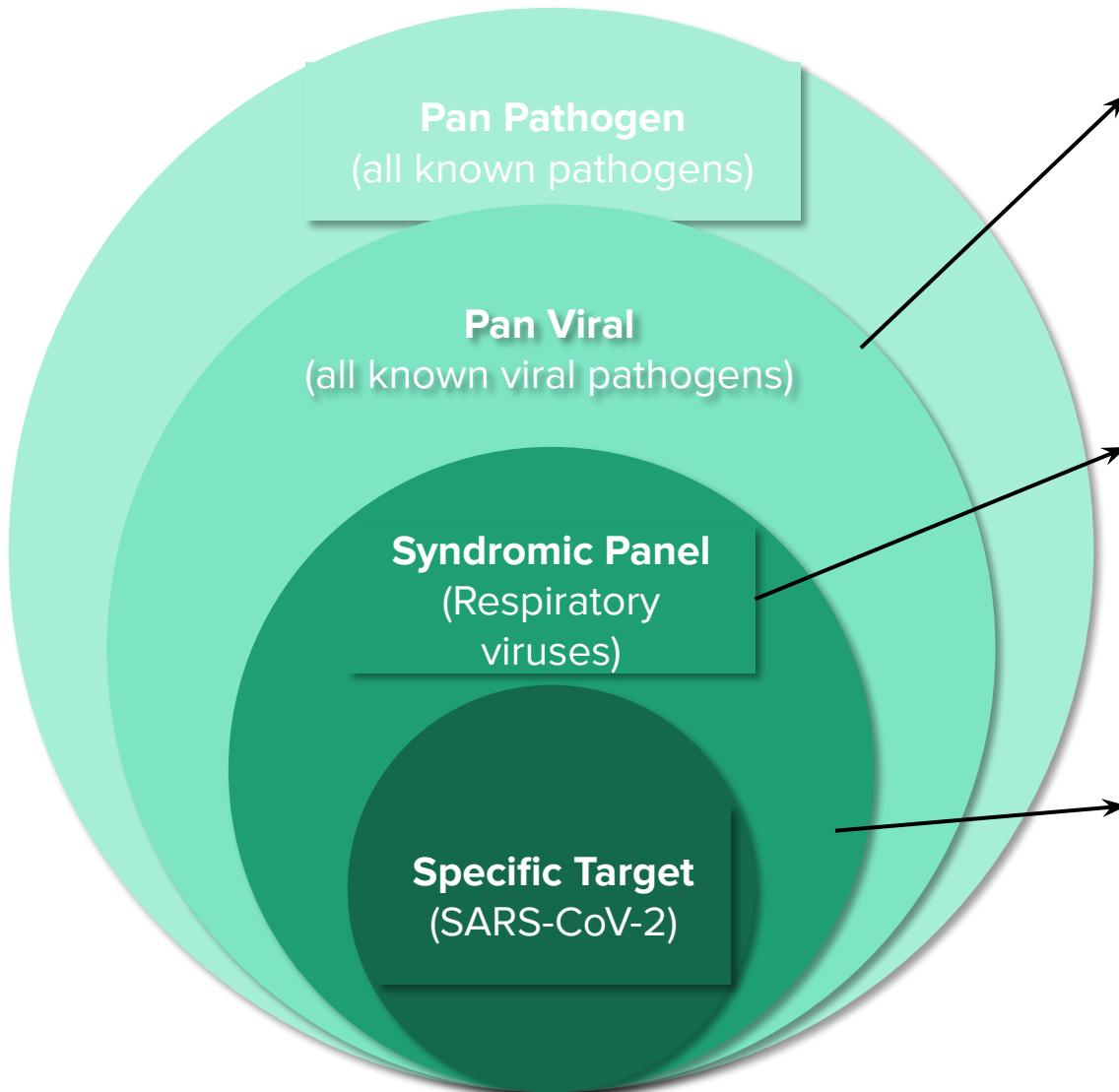
- Sense Strand
- Anti-Sense Strand
- UDI Adapters
- Biotin

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



Comprehensive viral panel:

- >1,000,000 probes that target >3,000 viruses
- Ability to identify known and discover unknown viruses
- Surveillance for emerging virus

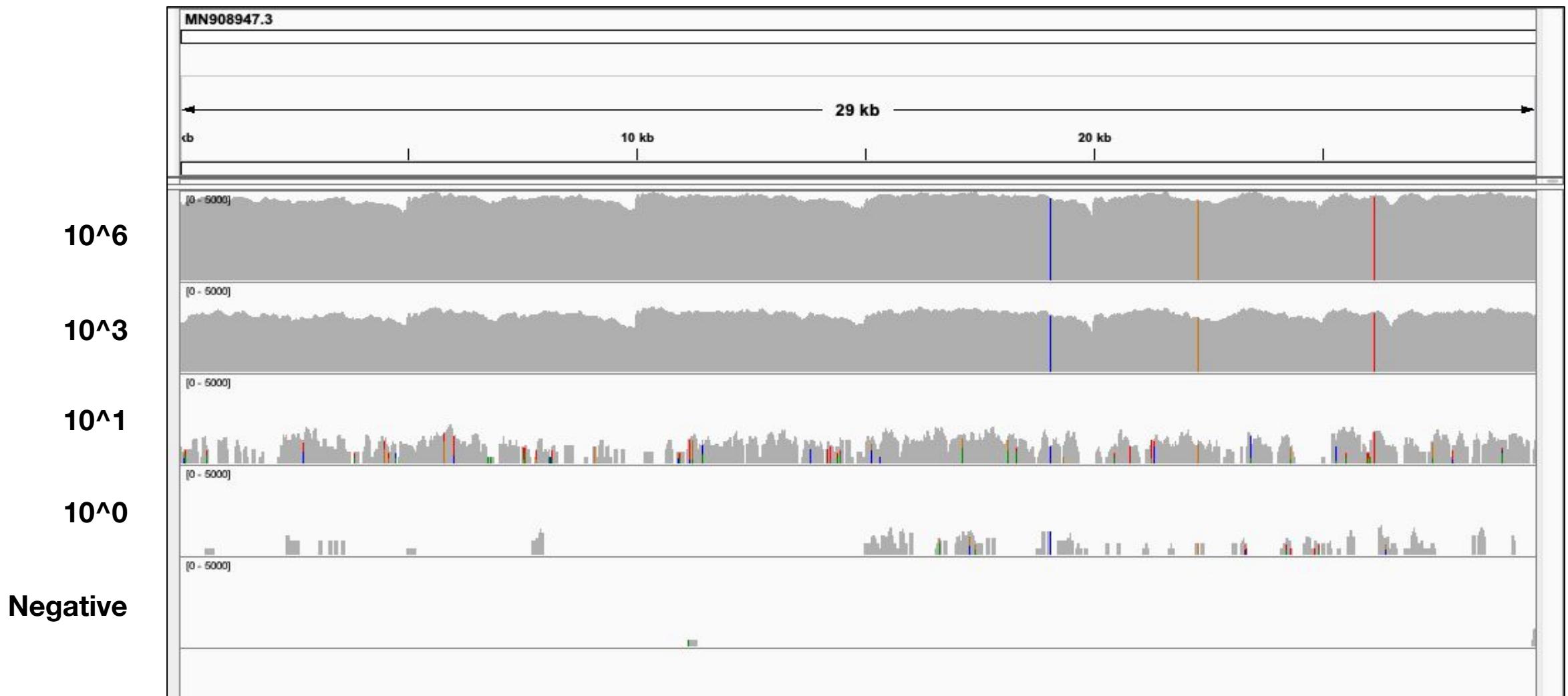
Respiratory viral panel:

- >40,000 probes that target 29 respiratory viruses with more than 100,000 influenza outbreaks
- Identification and characterization of viruses with similar clinical symptoms.

SARS-CoV-2 panel:

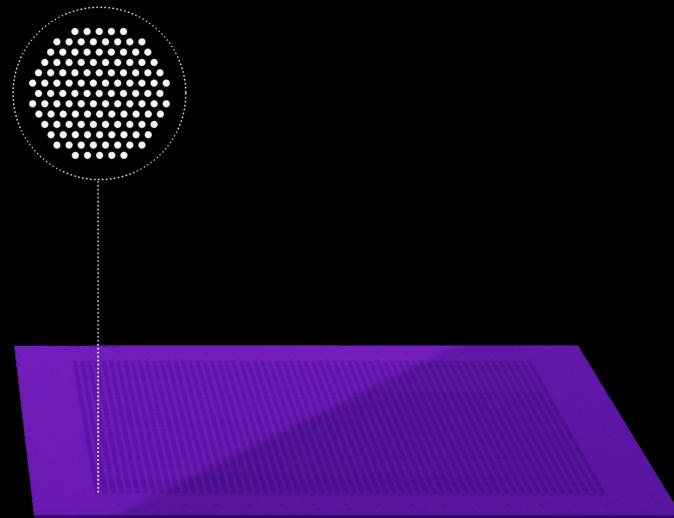
- 1000 probes that target the full SARS-CoV-2 genome
- Highly sensitive and accurate target capture that enables efficient viral RNA detection and characterization

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



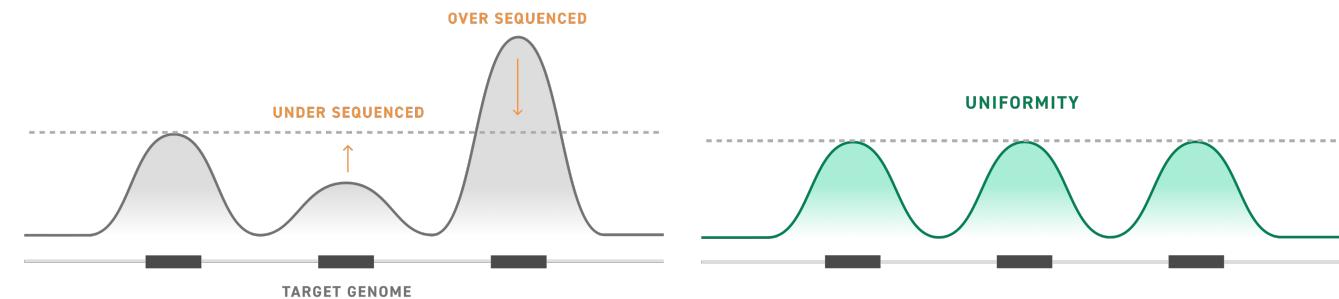
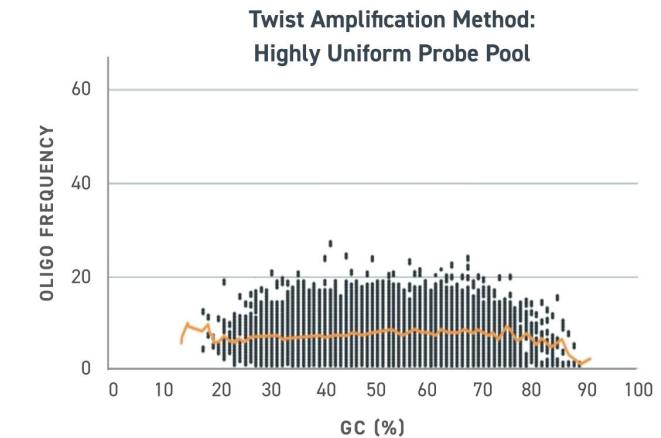
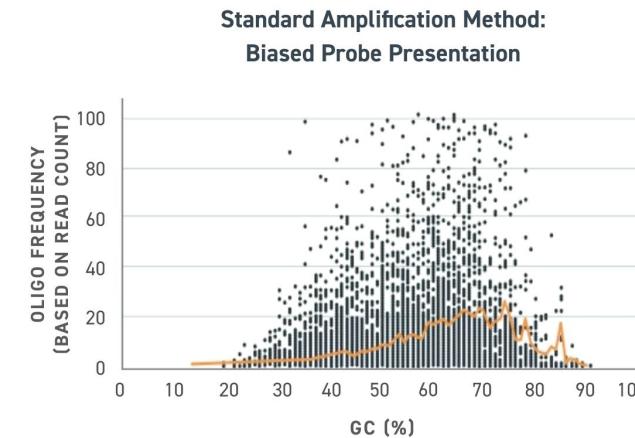
Quality starts at the chip

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.



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.

DNA

For targeted precision

RNA

For efficient expression profiling

Methylation

For sensitive and accurate epigenetic insights

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.

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.

Custom Panels

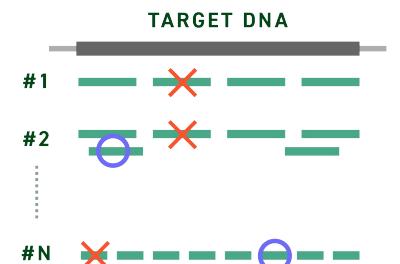
Design

Driven by Twist's Intelligent Design Strategy.



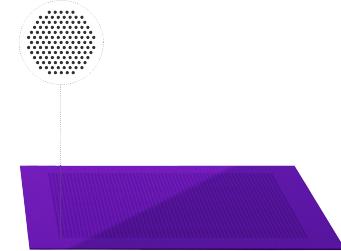
Optimize

Probe selection for optimal performance.



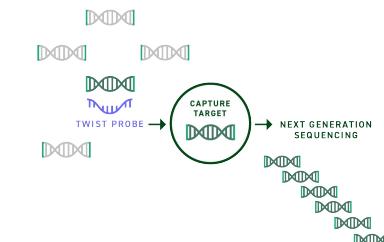
Build

Reliable Manufacturing and QC.

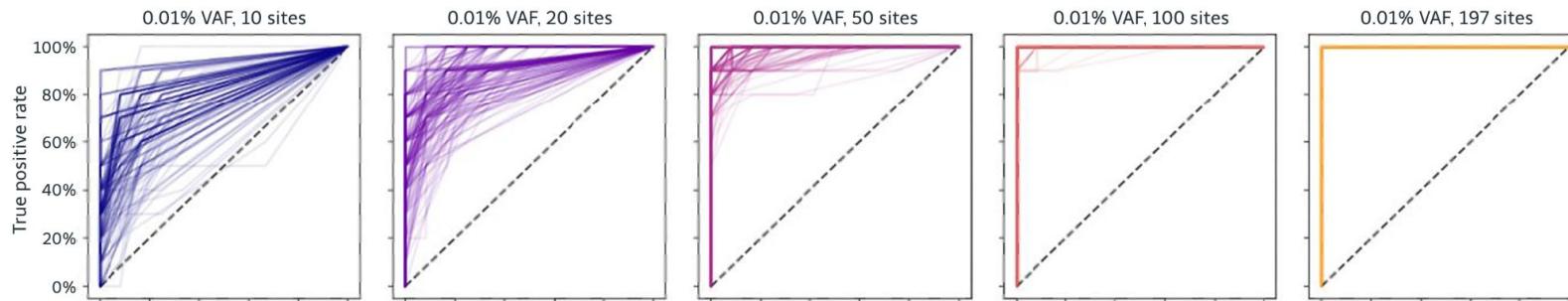


Test

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

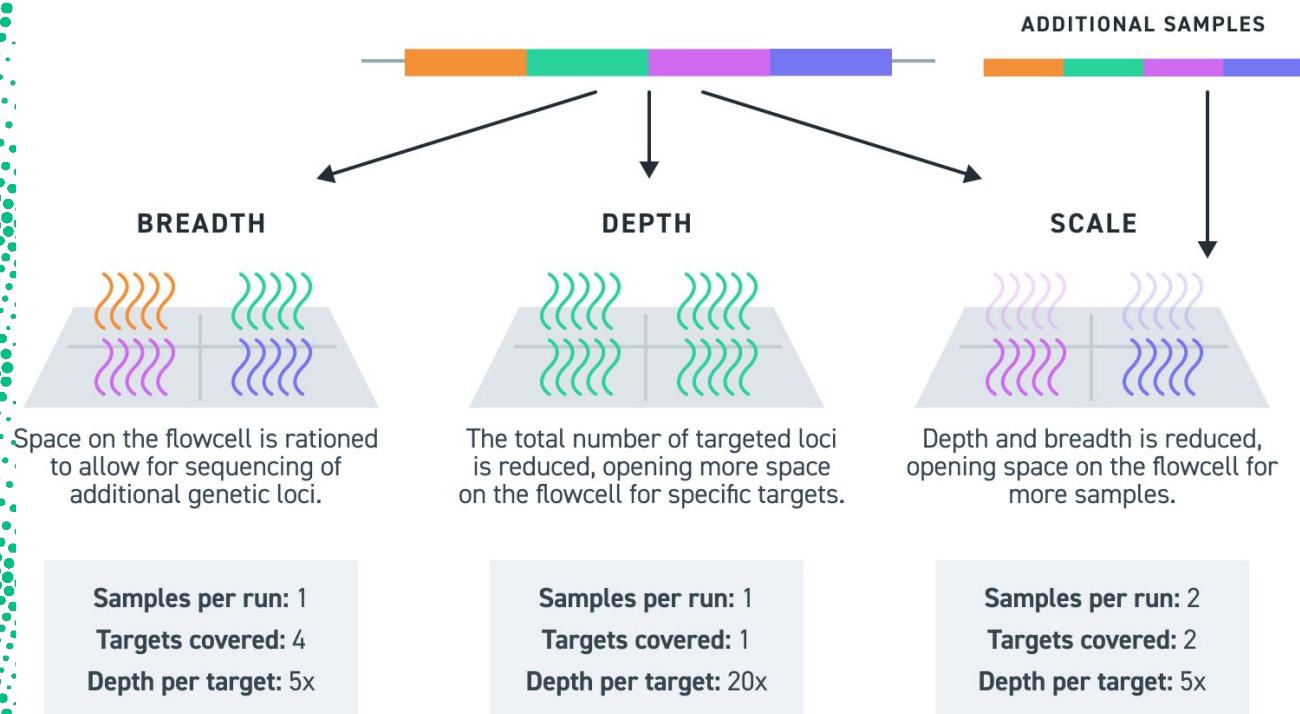
High Sensitivity

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



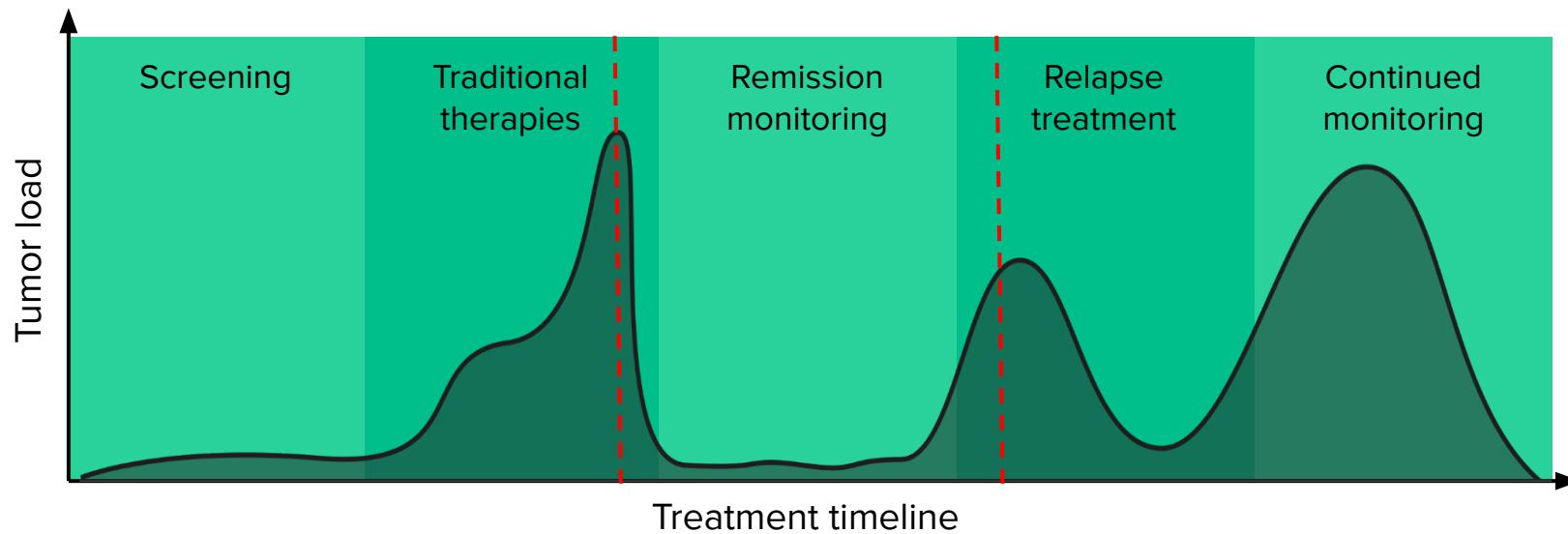
Low abundance NA capture effective for liquid biopsy research



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.

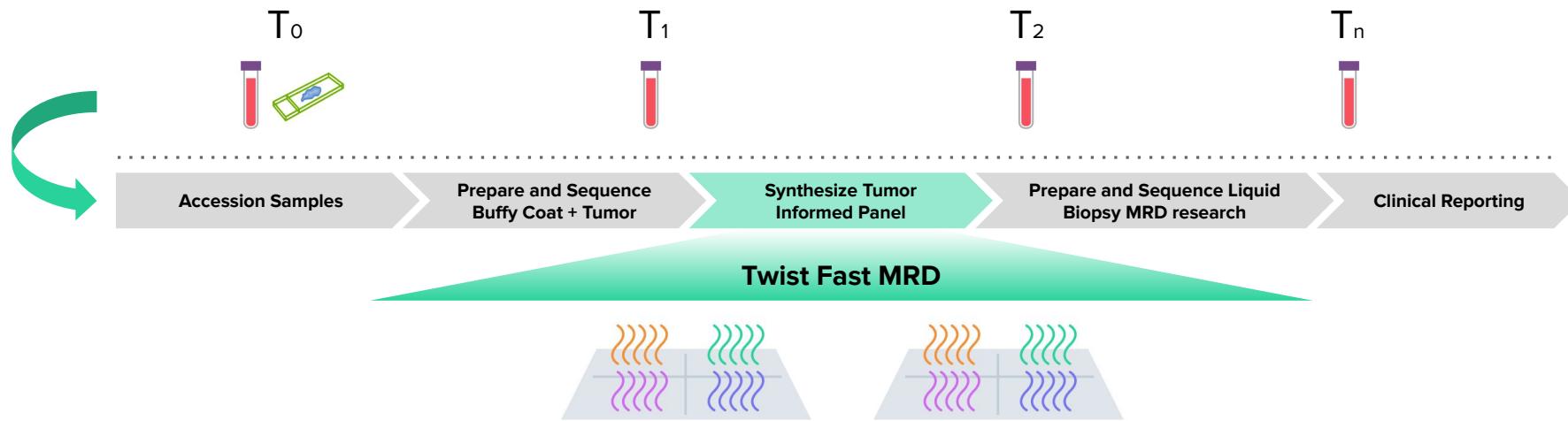
What is MRD?

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.

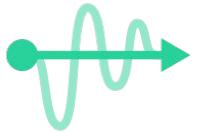
Clinical Validation

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

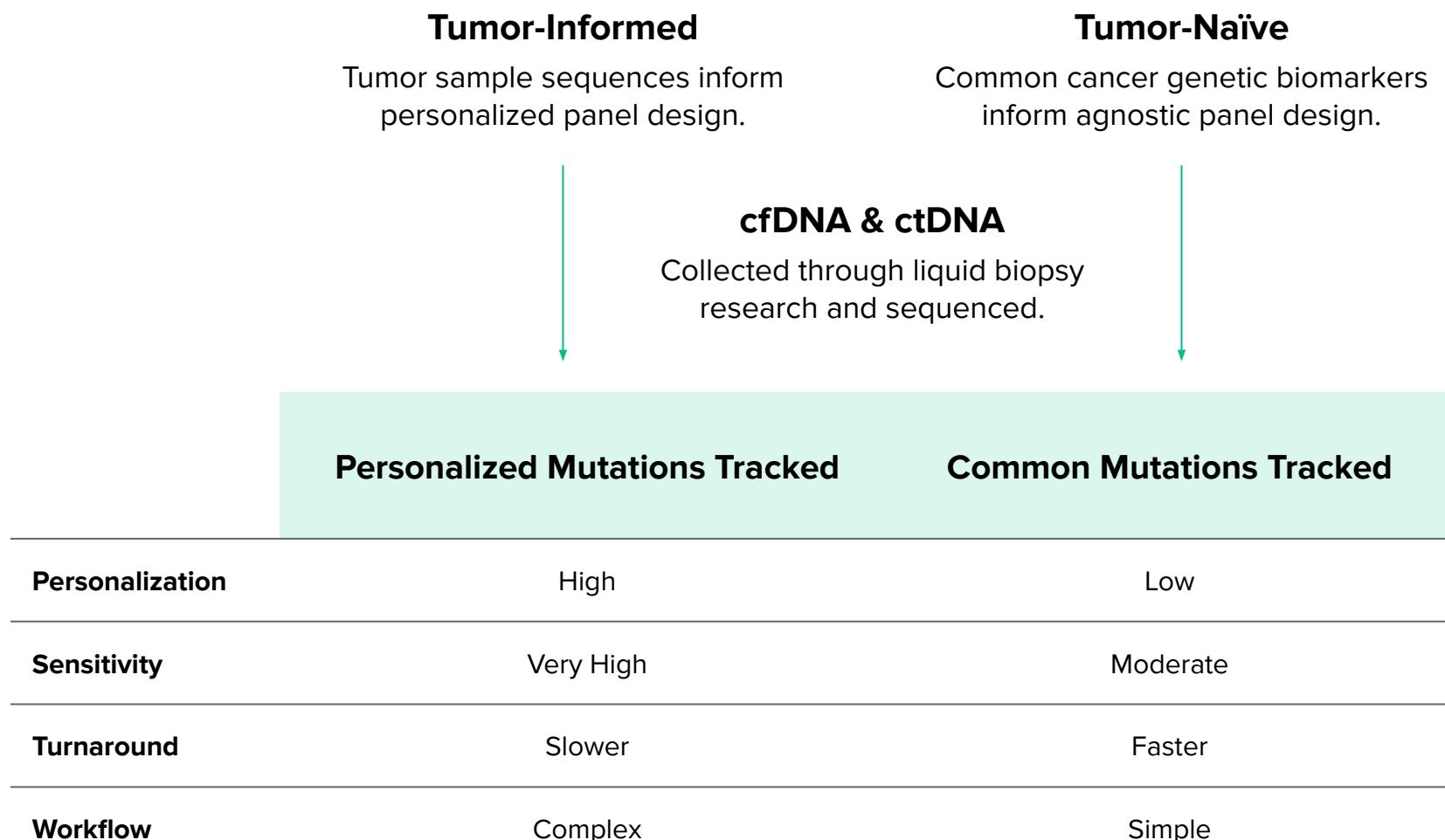
- Streamlined manufacturing & QC process
- High print capacity
- Automation friendly deliverables



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)

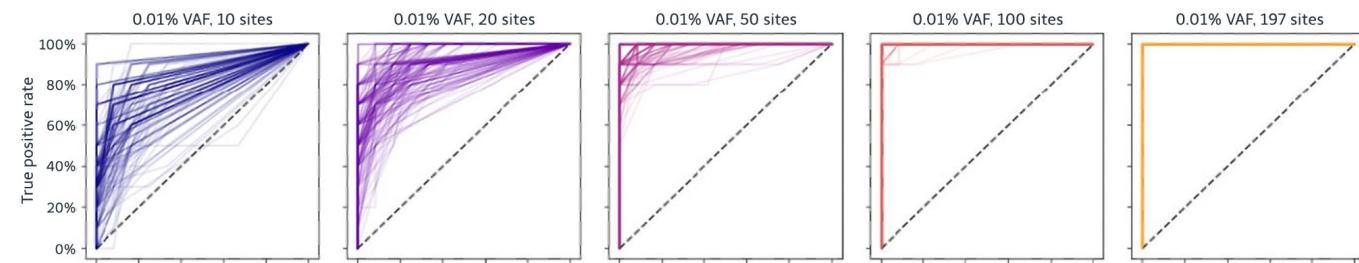
Sensitively detects many more variant targets at once.

Uniformity of Coverage

More efficient sequencing saves costs by reducing runs.

Mitigates Clonal Hematopoiesis Interference

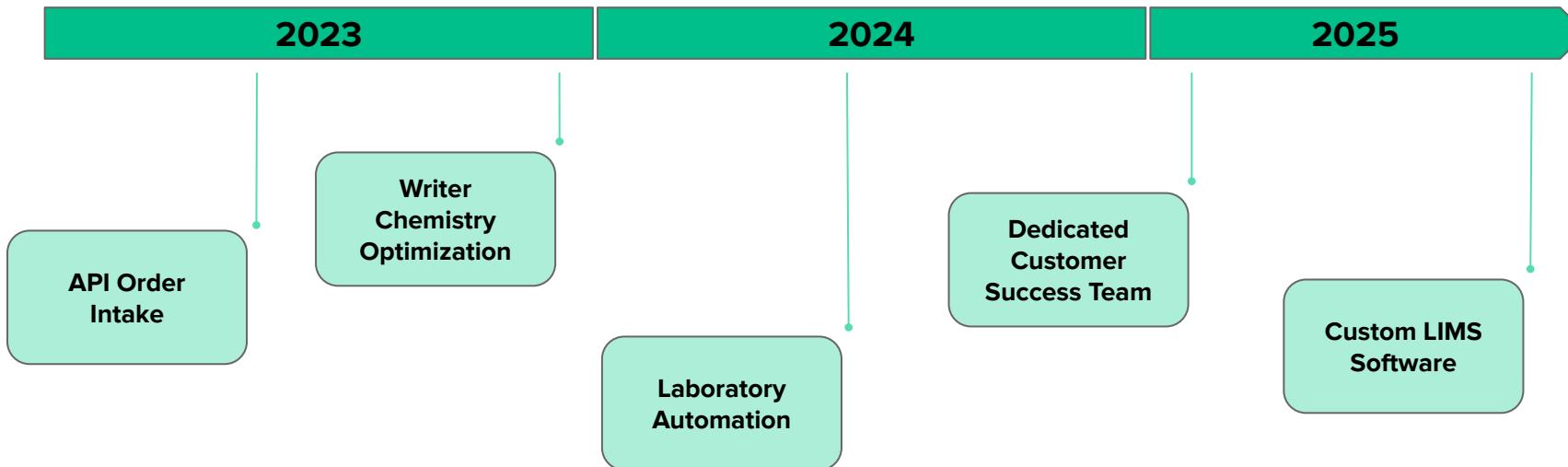
Fewer false positives than amplicon-based approaches.



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.

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.

Reference: Northcott et al., 2024, Oncotarget, 15, 200-218, <https://doi.org/10.18632/oncotarget.28565>

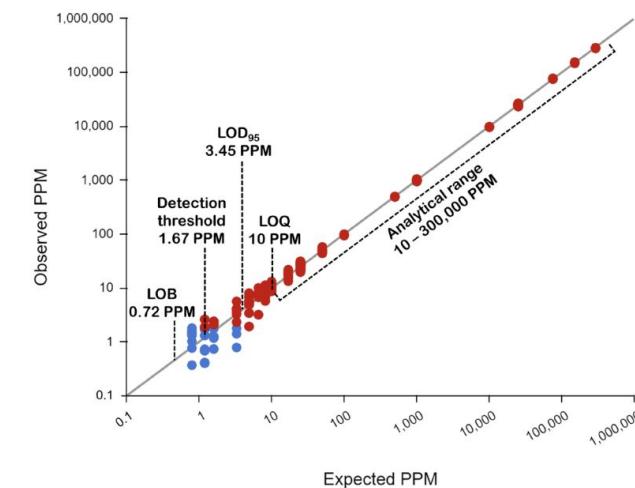
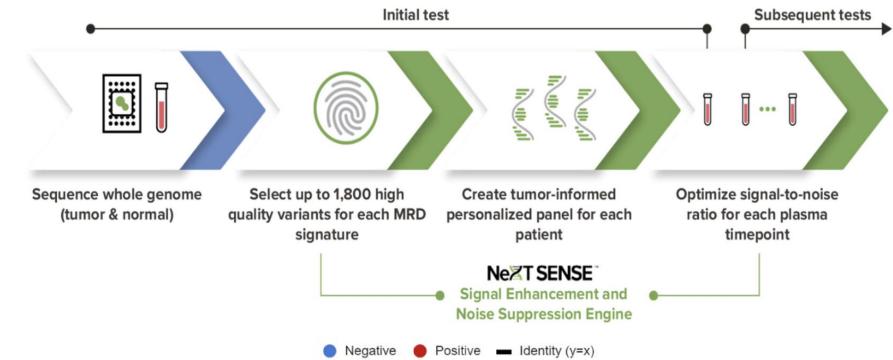
*Results from case studies are not predictive of results in other cases. Results in other cases may vary.

www.oncotarget.com

Oncotarget, 2024, Vol. 15, pp: 200-218

Research Paper

Analytical validation of NeXT Personal®, an ultra-sensitive personalized circulating tumor DNA assay



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](#)

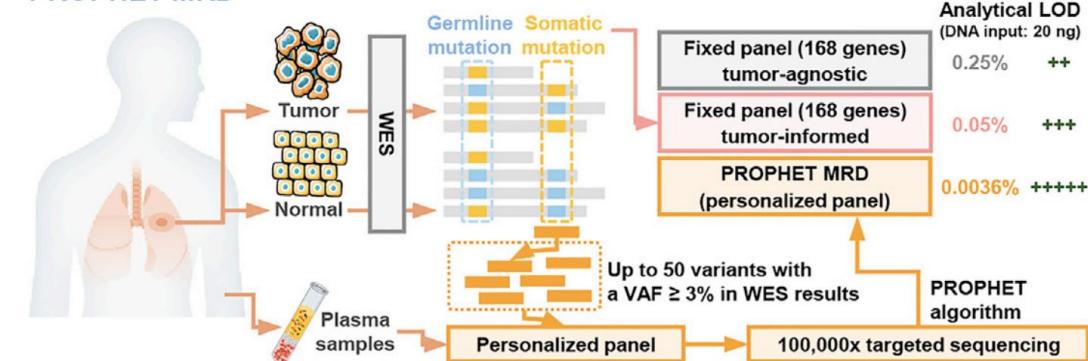
Highlight

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

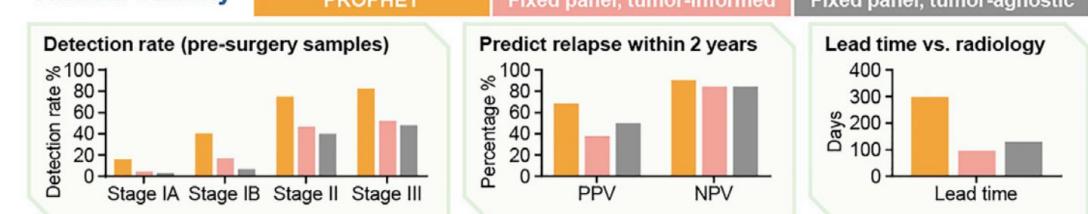
Cancer Cell

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

PROPHET MRD



Clinical validity



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

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

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

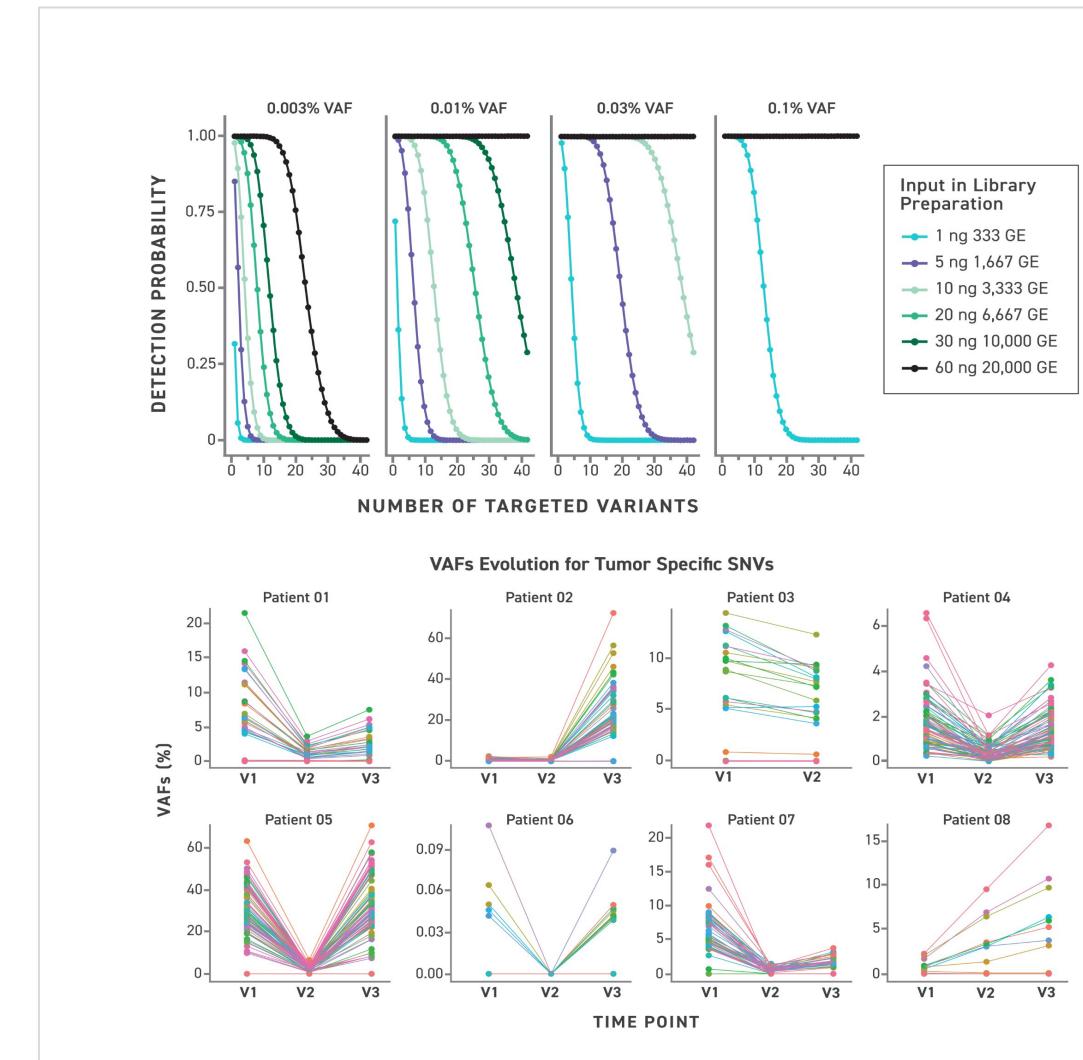
Established LoD of 0.003% using Twist Pancancer Reference standard.

- 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

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

Goals

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

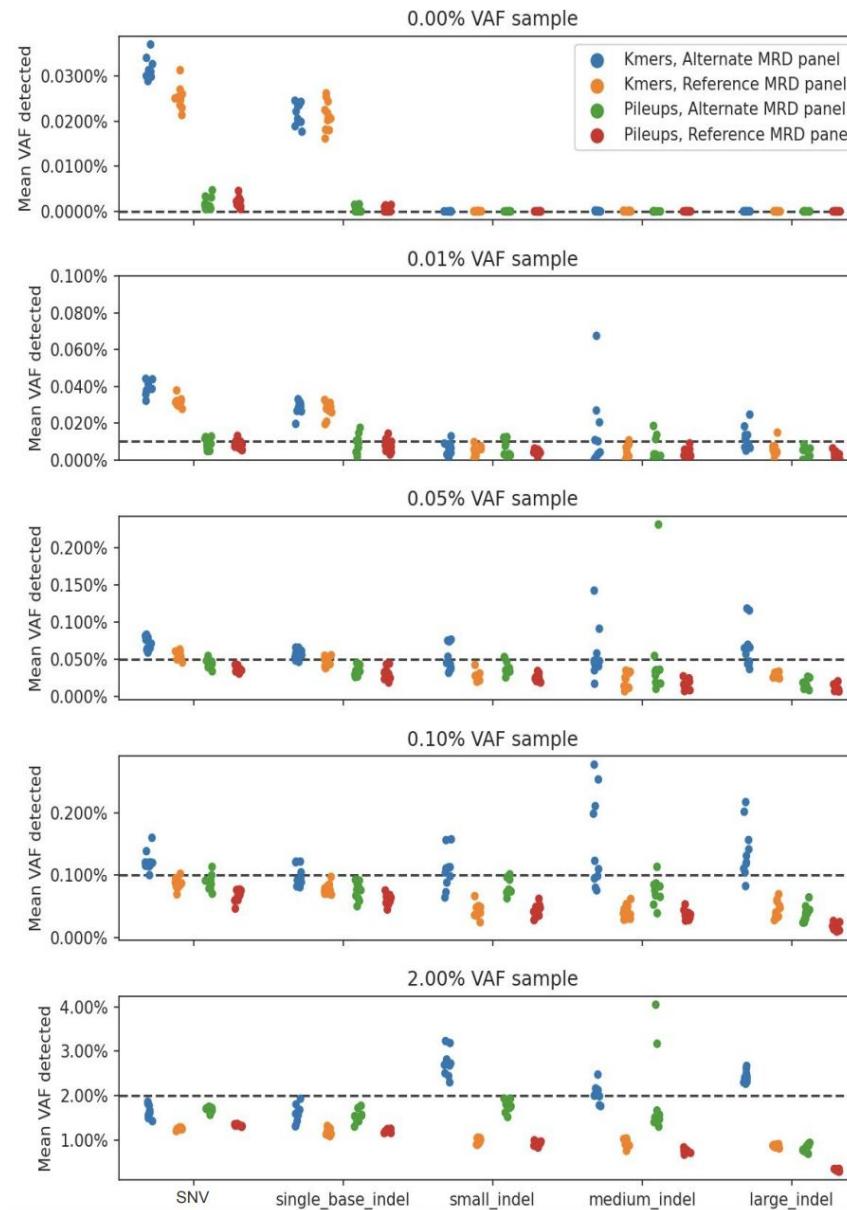


Twist MRD Rapid 500 Panels

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

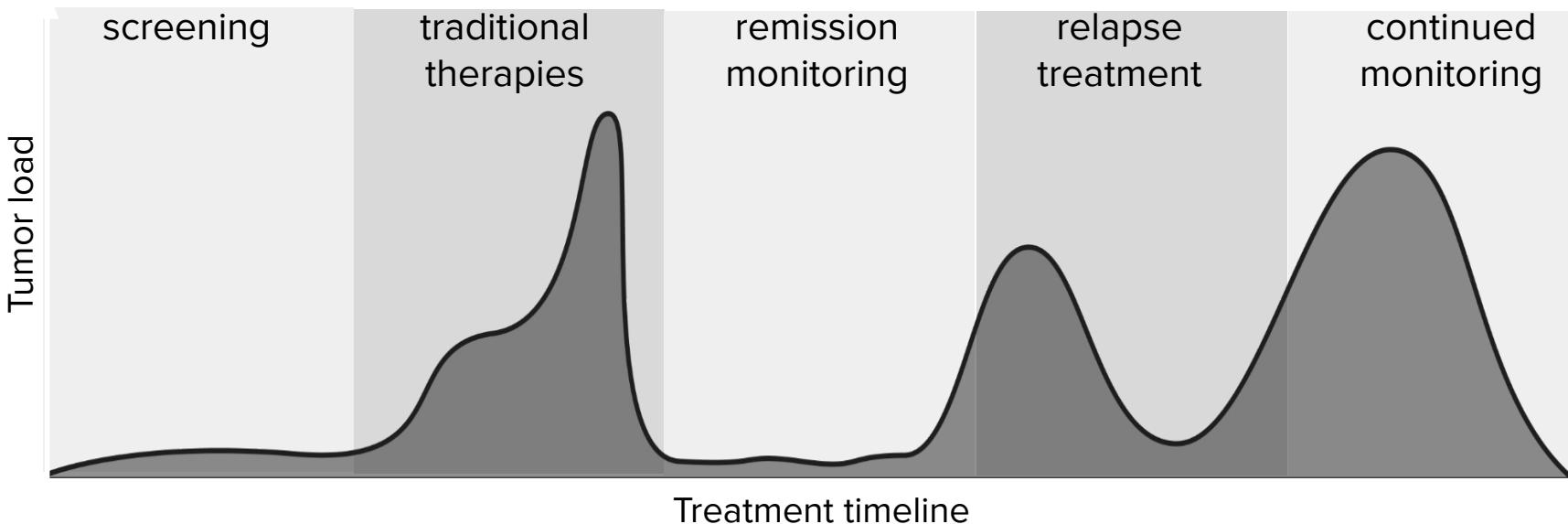
*Twist MRD 500 Panels based on internal data available as of 2025. This timeframe refers to the typical processing and handling time within our facilities before your order is handed over to the shipping carrier. Actual delivery times will vary depending on your location, the chosen shipping method,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



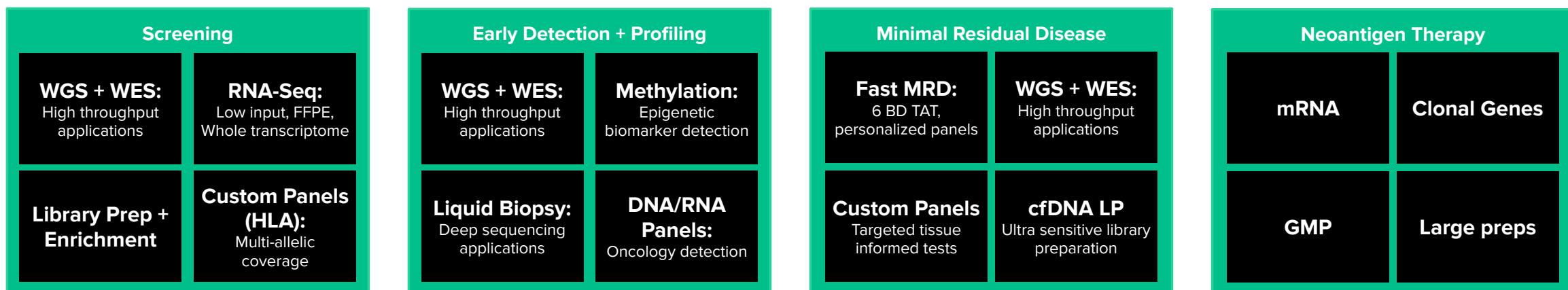
Sensitive Variant Detection

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



Powering the Genomics Revolution

Thank you!