

# Multiplexed Gene Fragments

Twist Bioscience's silicon-based DNA writing technology enables massively parallel production of high-quality oligos. Leveraging Twist's efficiencies of scale and excellence in DNA synthesis, Twist's Multiplexed Gene Fragments (MGF) offer a unique pooled format for up to hundreds of thousands of gene fragments, between 301 and 500 base pairs in length. This pooled format of fragments enables high-throughput screening applications such as prime editing, ultra-complex CRISPR-based functional screening, peptide and protein engineering, Ab discovery, and massively parallel reporter assays (MPRA), where cost per fragment can be a barrier to the desired number of variants.

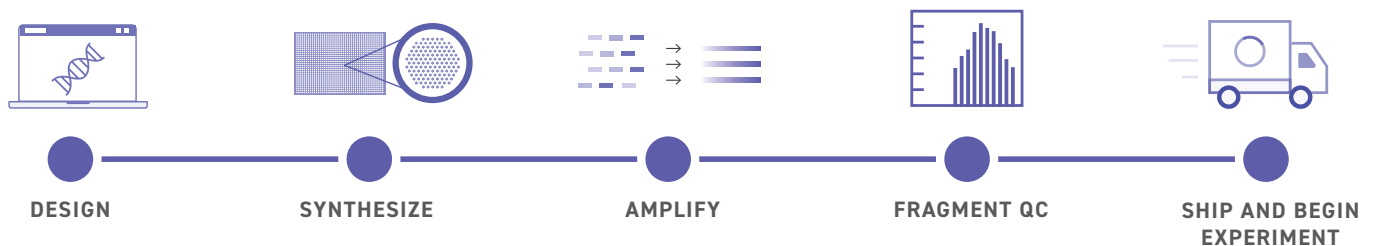
By offering an unlimited number of gene fragments at up to 500 base pairs in length, many high-diversity screening applications that were previously unfeasible or cost-prohibitive are now possible. For example, full nanobody (VHH) sequences can fit within a fragment, allowing researchers to design as many unique antibody sequences as desired without any limitations. In the case of larger antibodies, some researchers have stitched pools of MGFs to generate perfectly paired heavy/light chain combinations to screen *en masse* for antibody engineering<sup>1</sup>. To target multi-gene families or broad genomic regions for CRISPR experiments, several gRNA sequences can be designed within a single MGF to probe gene function in precise combinations.

## SPECIFICATIONS

- Double-stranded DNA
- Length: 301-500 bp
- Pool size: Starting at 1,000 sequences, with no maximum
- Delivery: lyophilized product pooled in a single tube
- Turnaround time: 8-12 business days

## KEY BENEFITS

- Large scale pooled fragment synthesis with customizable design options
- Lengths up to 500 bp for high-throughput screening
- Maximized pool quality with low error rates
- Even representation across fragment lengths and GC contents



<sup>1</sup>. <https://doi.org/10.1101/2024.03.14.585103>

## Easily integrated into a wide range of workflows

Twist’s Multiplexed Gene Fragments offer unprecedented design options to enable your research, unlocking design possibilities to allow for development of novel screening methods. What could you do with 10,000s to 1,000,000s of Multiplexed Gene Fragments?

### ULTRA COMPLEX CRISPR AND PAIRED-PRIME EDITING SCREENS

With 500 bp fragments, you can design more guides in a single sequence to:

- Greater number of tandem repeats (4-6) for more complex screening
- Knock out multiple genes with high targeting efficiency
- Reduce potential off-target effects due to poorly constructed guides
- Eliminate laborious and time-consuming construction of guide RNA components

### ANTIBODY AND NANOBODY DISCOVERY/ENGINEERING

Fit an entire variable region on a single fragment.

- Cover the entire VH region with up to 500 bp (460 bp for coding region) and enable researchers to create pools of Ab variants for HT Ab Engineering.
- Enable the complete and exact sequences designed and required for Ab discovery experimentation

### MPRA SCREENS

Expand the coding region and add genomic context for UTR/mRNA optimization screening.

### mRNA VACCINE DEVELOPMENT

Create designs encoding the entire region of interest to screen for expression levels, half-life, and enhanced RNA stability.

### HIGH THROUGHPUT SCREENING FOR AI/ML DESIGN

- High levels of uniformity and representation in Multiplexed Gene Fragments enables nearly 100% of the AI design, leading to improved selection
- No misrepresentation caused by stitching together 300mers, which would result in under-representation of a complete AI design

### ANY APPLICATION THAT REQUIRES POOLED FRAGMENTS WITH A CODING REGION OF UP TO 500 BP

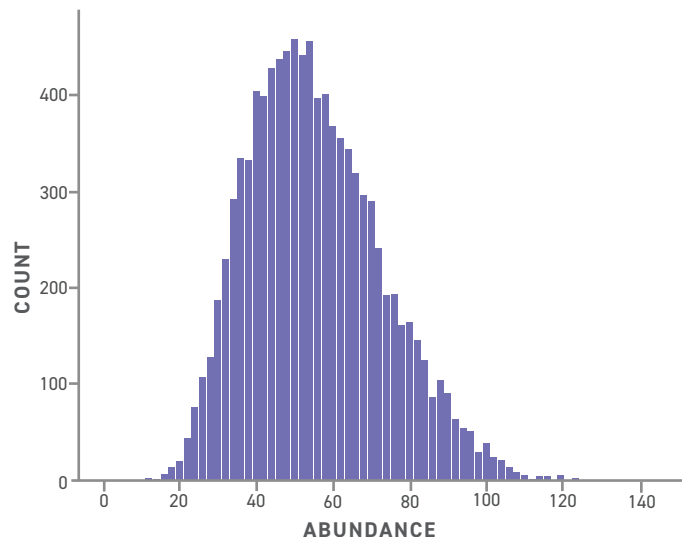
- Protein and enzyme engineering
- Pathway engineering
- Study of gene interactions

## High uniformity and low chimera rate for precise and efficient targeting

Regardless of length and GC content, Twist’s Multiplexed Gene Fragments generate hundreds of thousands of high-quality complex DNA fragments in parallel.

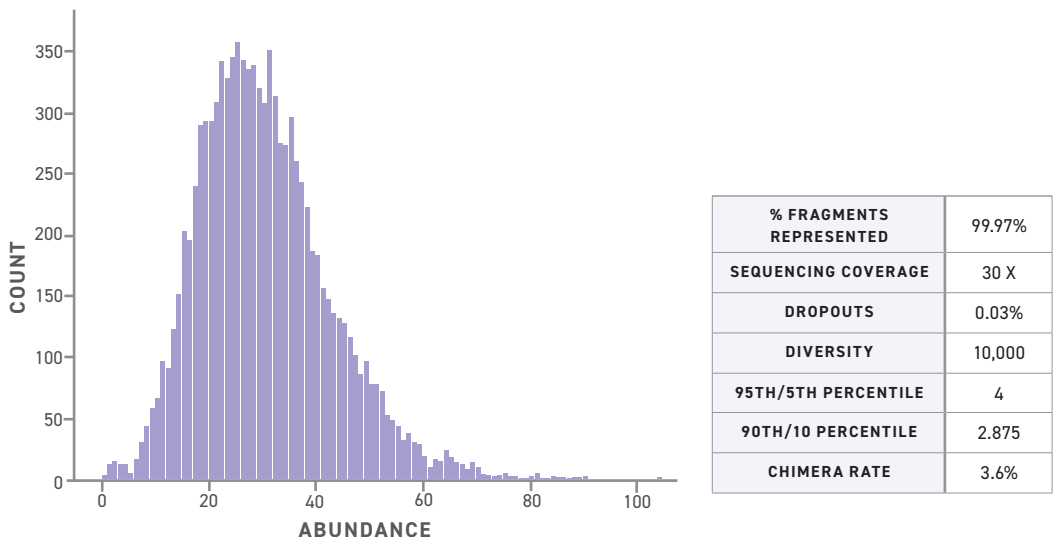
Twist’s Multiplexed Gene Fragment pools achieve comprehensive representation of every sequence ordered, to ensure precise control over variant construction for more targeted and rational screening.

A Multiplexed Gene Fragment pool of dual gRNA variants of 500 bp shows complete fragment representation with minimal dropouts (**Figure 1**) as does a pool of triple gRNA variants of 450 bp (**Figure 2**). Lastly, a Multiplexed Gene Fragment pool with variants containing GC content across a wide range including fragments with up to 80% GC shows high representation and minimal bias (**Figure 3**).

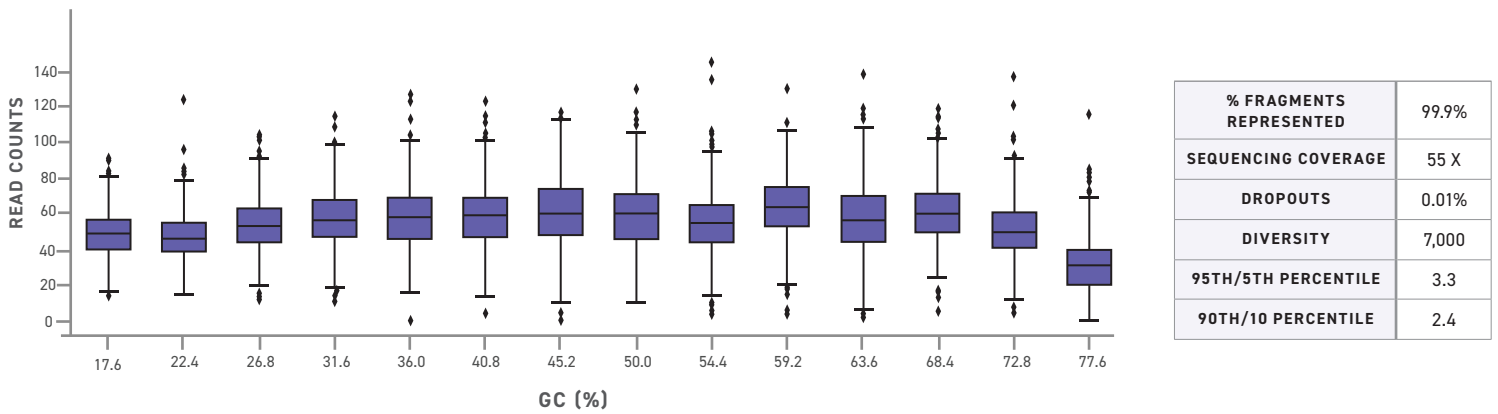


<b>% FRAGMENTS REPRESENTED</b>	99.99%
<b>SEQUENCING COVERAGE</b>	55 X
<b>DROPOUTS</b>	0.01%
<b>DIVERSITY</b>	10,000
<b>95TH/5TH PERCENTILE</b>	3.03
<b>90TH/10 PERCENTILE</b>	2.35
<b>CHIMERA RATE</b>	5.5%

**Figure 1.** Graph of read count of each of the mapped dual gRNA variants (X-axis) for a pool of Multiplexed Gene Fragments of 500 bp.



**Figure 2.** Graph of read count of each of the mapped triple gRNA variants (X-axis) for an amplified pool of Multiplexed Gene Fragments of 450 bp.



**Figure 3.** GC plots of an amplified pool of Multiplexed Gene Fragments of 500 bp length were created and show minimal bias among a wide range of GC content up until 77.6%. X-axis are categories of 14 groups of sequences with different GC content. Y-axis is the read count.



**Quality and scale come without compromise  
when you partner with 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.

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