TWIST BIOSCIENCE

# SYNTHETIC DNA:

## THE FABRIC OF MODERN CANCER RESEARCH

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# 01 The Fading Enigma of Cancer

Now is a great time to be in the cancer research space. Recent technological and scientific advances have empowered researchers with a diverse array of tools, each of which helps us better understand this vexing disease. Next-Generation Sequencing (NGS) is exposing the nuanced genetic landscape of cancer, highlighting potential therapeutic and diagnostic strategies. CRISPR/Cas gene editing allows researchers to methodically annotate pathological gene networks while also enabling the creation of highly personalized cell and gene therapies.

Put simply, the enigma of cancer is slowly dissolving, washed away by modern technology. Central to this process is synthetic DNA—a basic material that is critical for NGS; it forms the basis of every CRISPR experiment and is foundational to the rise of Chimeric Antigen Receptor (CAR) and T-cell receptor therapeutics.

In the following sections, we explore the many ways that synthetic DNA is used in modern cancer research and therapeutic development. Along the way, we highlight products that are empowering researchers to overcome the frustration that has long characterized cancer research.

## Synthetic DNA is instrumental in:

**Discovery Research:** uncovering mechanisms of disease and building *in vitro* models

**Therapeutic Development:** generating novel treatments

**Detection:** identifying biomarkers for disease detection and characterization

# 02 Introduction: Synthesizing Life's Blueprints

Synthetic DNA is indispensable in modern molecular biology. Artificial genes can be synthesized and inserted into a bacterium's genome, transforming it into an antibody-producing biofactory. Alternatively, synthetic DNA can be used as the bait that captures specific DNA fragments from a sample, separating the DNA you want to sequence from the DNA you're not interested in. This simple step can improve the efficiency and depth of sequencing efforts.

The advent of modern DNA synthesis platforms is one of the many important advances of the last century that have collectively empowered researchers to explore the molecular world. Natural DNA consists of nucleotides organized into repeating units that form a chemical chain, with each nucleotide linked to another by the action of enzymes. Methods for creating synthetic DNA mimic this process through various means, with the most widely used and validated approach involving solid-phase phosphoramidite chemistry (Figure 1).

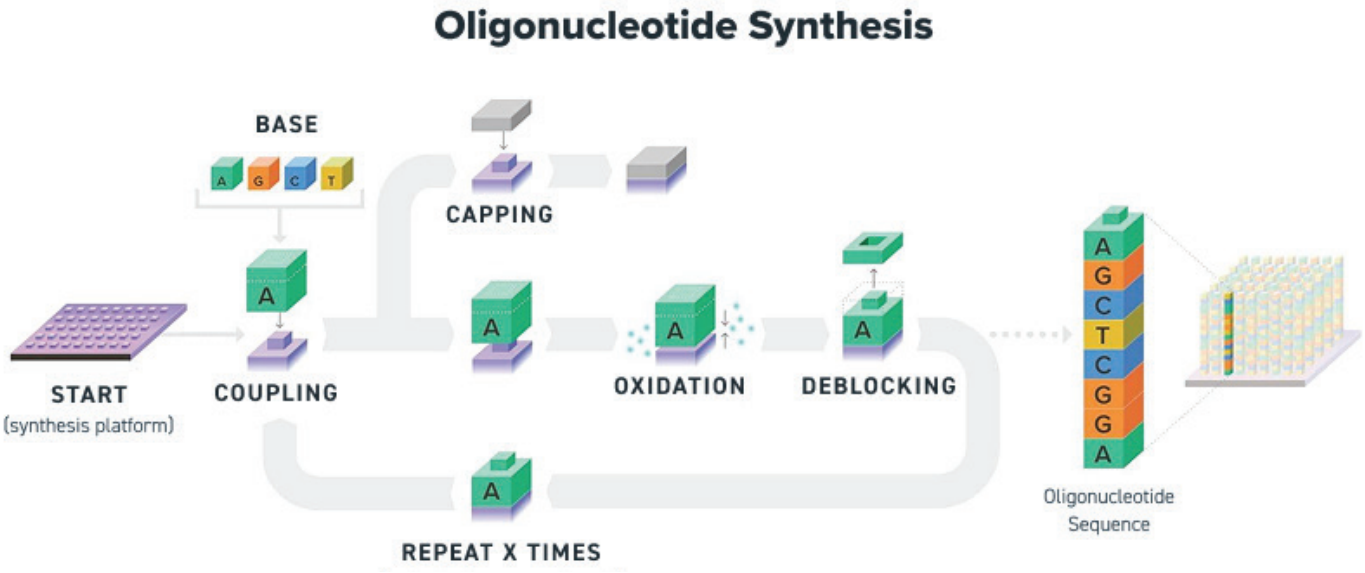



***Figure 1:*** *Twist's phosphoramidite reaction cycle.*

Exploring the differences between DNA synthesis platforms is beyond the scope of this ebook, but small nuances in their approaches can have a significant impact on their accuracy, uniformity, and scalability.

Twist has become a leading DNA synthesis company through its optimized silicon-based synthesis platform, capable of producing up to 9600 genes on a single silicon chip (Figure 2). With this technology, Twist is supporting researchers across the cancer continuum, from basic cancer biology to preclinical therapeutic development (Figure 3).

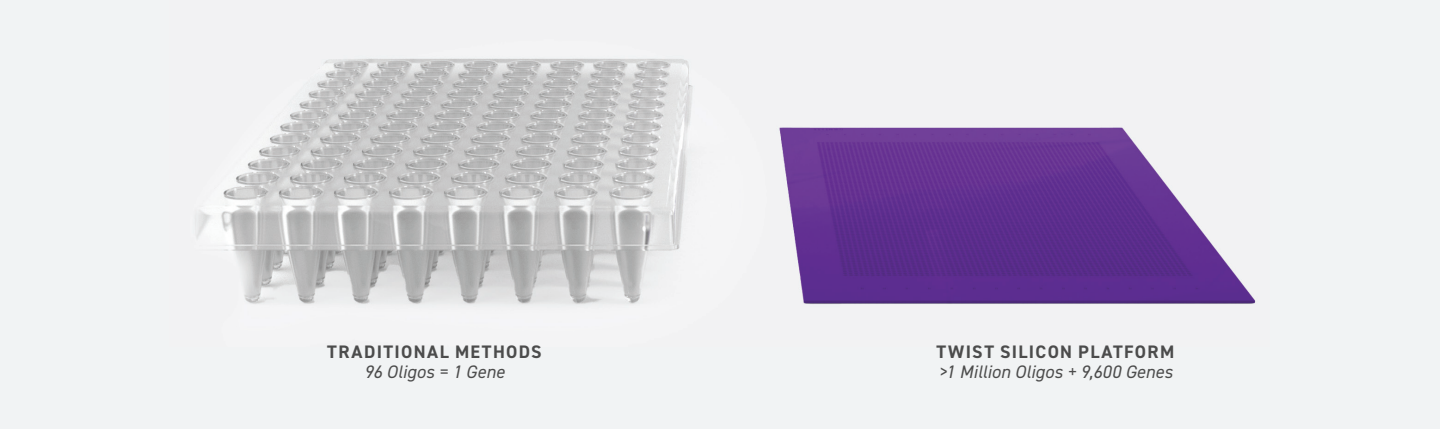



***Figure 2:*** *Twist's silicon chip greatly expands DNA synthesis capacity compared to traditional methods.*

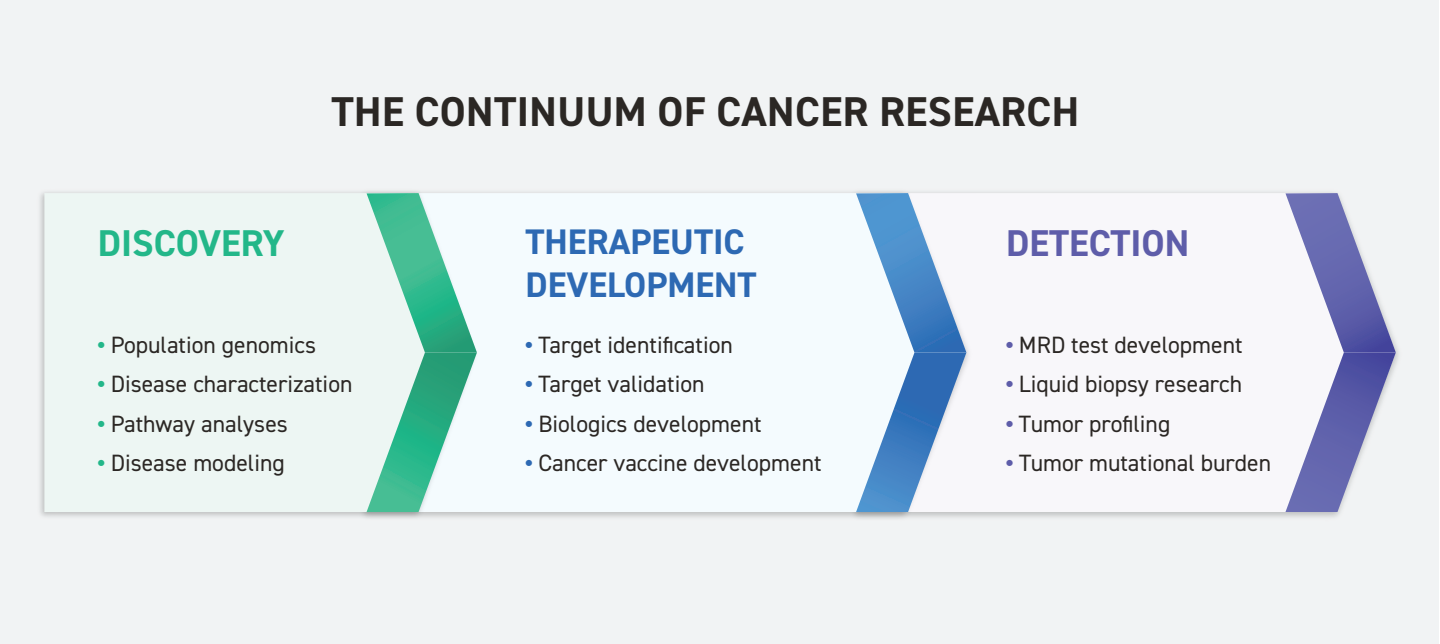



***Figure 3:*** *Twist provides solutions to empower researchers across the continuum of cancer research.*

Would you like to learn more about how Twist can help your research in the fight against cancer? **Watch this webinar about all the tools for the good fight.**

# 03 Target Enrichment: Capturing the Malignant Genetic Landscape

To better understand the genetic changes driving cancers, there may be no better technology than NGS.

NGS allows researchers to explore the genetic landscape of tumor cells, cataloging an ever-expanding range of mutations in DNA and recording aberrant RNA expression.

In an ideal world, researchers would be able to perform multimodal sequencing, wherein entire genomes and transcriptomes are sequenced in depth to ensure that no mutation goes undetected. However, the realities of sequencing make this impractical: Despite lowering costs, whole genome sequencing can still be prohibitively expensive for many laboratories (to say nothing of the challenges associated with data analysis and storage). The same holds true for unbiased whole transcriptome sequencing. Instead, researchers must often strike a balance between sequencing depth (which affords greater confidence in sequencing results), sequencing breadth (which determines the number of potential mutations that can be detected), and the number of samples that can be sequenced (Figure 4).



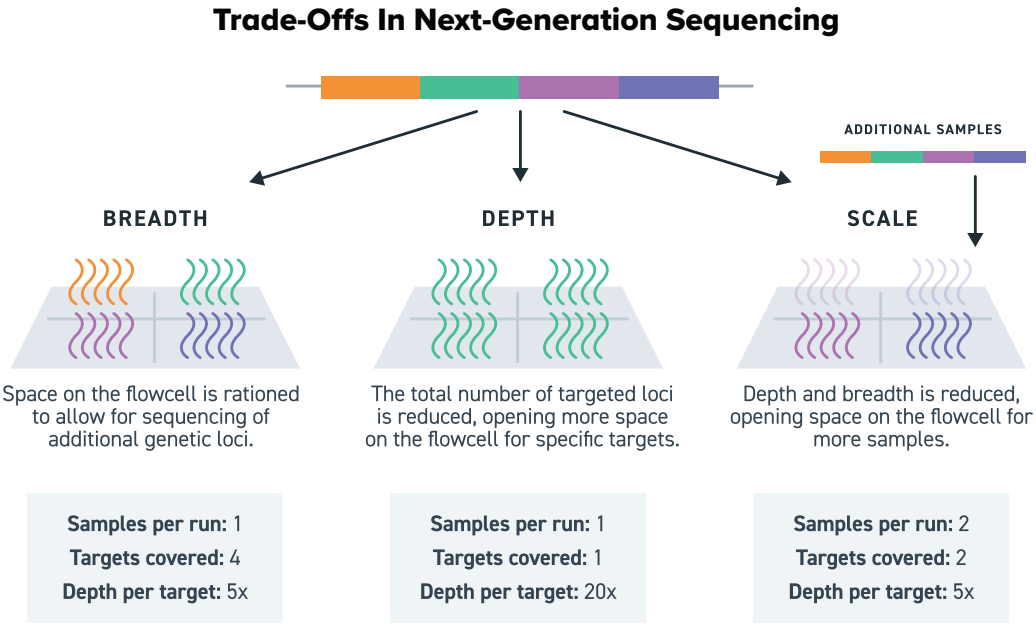



***Figure 4:*** *Traditional next-generation sequencing involves balancing trade-offs among sequencing breadth, depth, and scale.*

Such a balance is often found through the use of target capture panels. These panels use synthetic DNA probes that have been designed to bind with a limited pool of specific target DNA sequences. Isolating the probe-bound fragments allows superfluous DNA to be washed away, reducing the total number of fragments that need to be sequenced while ensuring that important regions remain covered [1].

This is important for cancer research because laboratorians are often working with samples containing a complex mixture of genetic material, or samples in which tumor DNA is vanishingly rare. In both cases, target enrichment makes it possible to focus sequencing resources sparingly, enabling deep and sensitive sequencing of the genetic material that most interests researchers.

## *Liquid Biopsy Research*

The potential for increased sequencing sensitivity is perhaps best emphasized by studies involving circulating tumor DNA (ctDNA). Following curative treatment, ctDNA may be as rare as 0.01% among the collection of cell-free DNA fragments captured by liquid biopsies [2]. Detecting these rare bits of DNA may be invaluable for the early detection of recurrent tumors, a primary goal of minimal residual disease testing. However, for this to become a reality, researchers need the ability to study ctDNA with greater sensitivity, meaning they need access to tools that enable the enrichment of ctDNA.

**CASE STUDY 1**

### Enriching For Sensitive ctDNA Detection

In a 2024 study published to Oncotarget by researchers at Personalis, a tumor-informed minimal residual disease assay (MRD) was reported to detect ctDNA in quantities as low as 1.67 parts per million (PPM), with a limit of detection at 3.45 PPM [3]. This ultra-sensitive assay was developed using custom target enrichment panels (synthesized by Twist) to capture fragments of DNA containing patient-specific mutations (identified through the sequencing of previous tumor biopsies). The sensitivity of this assay stands out in its field, as other such assays demonstrate a limit of detection that ranges from 80 to 2,500 PPM. This boost in sensitivity has the potential to greatly improve the speed at which tumor recurrence is detected.

While powerful tools, tumor-informed MRD assays present certain challenges to researchers. First, each patient will require a bespoke panel whose design is optimized for their unique tumor profile. Doing so requires expertise in probe design to ensure that key variants are captured with high specificity. Additionally, the sensitivity of such an assay also depends on uniform probe manufacturing to ensure that lowly represented variants are accounted for. Doing all of this at scale and on a fast timeline can be a significant challenge, which is why Twist's team of experts are here to help. Twist's silicon-based DNA synthesis platform can then be used to manufacture these custom, often complex, panels at scale with high precision and accuracy.

Read the primary paper here.

## *Methylation Detection*

A unique challenge facing some researchers is the need to carry out genome and epigenome sequencing in tandem. Capturing information about the methylome, for example, can provide valuable information about a ctDNA fragment's tissue of origin. However, most current methods for methylation sequencing must alter the target DNA sequence prior to target capture, converting unmethylated cytosine bases into uracil (and ultimately thymine). This greatly reduces the unique complexity of DNA fragments and can be challenging to anticipate when designing capture probes. Designing target capture for methylation sequencing thus requires careful consideration and expertise.

### CASE STUDY 2

#### Tapping the Epigenome for Non-Invasive Colorectal Cancer Detection Research

In 2023, researchers from the Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, reported a novel approach to colorectal cancer detection (CRC), one that uses target-enriched methylation sequencing. Having identified CRC-specific methylation patterns, the team of researchers designed a custom Twist target enrichment panel to enable the capture and assessment of 149 cfDNA methylation markers in blood samples. With the Twist Custom Methylation Panel, the team was able to better detect CRC relative to conventional blood tests, demonstrating an 85% sensitivity at a 90% specificity threshold. These results suggest that target enrichment for methylation sequencing can be a valuable approach to sensitive CRC detection [4].

Read the primary paper here.

## *The Importance of Uniformity*

Whether for ctDNA enrichment or methylation sequencing, the value of any target enrichment panel depends on the quality of the synthetic DNA used to build it. If capture probes contain errors, they're less likely to bind to their target sequence, which leads to reduced overall coverage. Similarly, a non-uniform target capture panel may have under-represented capture probes. The targets of these rare probes may be inadvertently filtered out during data analysis as they're overwhelmed by the much more numerous sequences targeted by highly-represented probes. In both cases, the end result may be inefficient sequencing, incomplete coverage, and loss of data—data that may have revealed the presence of a critical mutation. It is thus crucial to not only design panels well but to synthesize them with high uniformity and accuracy.

To this end, Twist has developed several target capture panels and associated tools that aid in cancer research:

| **Library Preparation Kits** | | | |
|---|---|---|---|
| Twist Library Preparation Enzymatic Fragmentation Kit 2.0 | Twist cfDNA Library Preparation Kit | Twist NGS Methylation Detection System | Twist RNA Library Preparation Kit |
| **Target Enrichment Panels** | | | |
| Twist Human Comprehensive Exome | Twist Exome 2.0 | Twist Alliance Clinical Research Exome | |
| Twist MRD Rapid 500 Panel | Twist Human Methylome Panel | Twist Alliance Pan-cancer Methylation Panel | |
| Twist RNA Exome | Twist Alliance CeGaT RNA Fusion Panel Kit | Twist Custom Panels | |
| **Other** | | | |
| Twist cfDNA Pan-Cancer Reference Standard v2 | Twist UMI Adapter System | Twist Methylated UMI Adapters | |

# 04 CRISPR: Tinkering With the Cancer Genome

Over the last decade, CRISPR/Cas has become an invaluable tool in molecular biology by enabling the precise editing of genomic DNA. Thanks to ongoing innovation, the different types of edits that can be made grow ever more diverse, from gene knockouts to epigenetic repression, single-base editing, gene activation, and combinatorial perturbation [5]. Together, the breadth of the CRISPR/Cas toolkit is such that researchers now have the ability to engineer advanced disease models, methodically interrogate gene function, and validate potential therapeutic strategies—all of which are proving advantageous for the study of cancer [6].

## *Improving Research Models*

Cancer research benefits greatly from advances in CRISPR/Cas. Preclinical cancer modeling often involves the use of immortalized cell lines, patient-derived cells, or animal models, each of which has significant shortcomings. CRISPR/Cas is enabling the construction of improved models by allowing researchers to modify healthy cells with mutations that are suspected factors in cancer development.

For example, Caeser et al. used CRISPR/Cas engineering to build a model of early-stage diffuse large B cell lymphoma (DLBCL) [7]. Several complex genetic variants had been identified in DLBCL, but little was known about how these variants contribute to disease development and progression. To change this, Caeser et al. performed a functional genomic screen in primary human germinal center B cells (the cell-type in which DLBCL is thought to originate). The screen proved highly informative, identifying cooperating genetic alterations that produced malignant cellular behavior. With this data, the team was able to fully transform healthy B cells into synthetically engineered DLBCL models, thereby creating a highly relevant model system for researchers to use while studying this disease.

## *Identifying Therapeutic Targets*

Functional genomic screening can similarly be used to identify potential therapeutic targets (Figure 5). Behan et al., for example, performed a genome-scale CRISPR screen (targeting >18,000 genes) using 324 human cell lines that represented 30 different cancer types [8]. Their goal was to identify high-priority targets worthy of therapeutic development. To perform this screen, they relied on gRNAs generated from a Twist-synthesized oligo pool. The screen was more than successful, identifying 628 unique priority targets, including 92 genes that appeared to be valuable therapeutic targets across a wide range of different cancer types.

## CASE STUDY 3

### Greater Scale and Dimensionality in CRISPR Screening

Recent advances have enabled researchers to begin CRISPR screening with high-dimensional phenotypic readouts. Rather than simply measuring cell survival in response to gene perturbation, methods like direct capture perturb-seq (dcPerturb-seq) can be used to assess complex phenotypes (such as alternative splicing dynamics, gene network expression changes, and more) with single-cell resolution.

This approach not only makes CRISPR screening more informative but also more efficient, as fewer cells are needed for robust results. For example, Replogle et al. leveraged dcPerturb-seq in a series of large-scale CRISPR screens [9–11]. In one such study, transcription-based phenotypes in over 2.5 million cells across nearly 10,000 genes were analyzed [10]. Their findings revealed new gene functions, insights into aneuploidy, and unexpected genetic regulation of mitochondrial genes, all achieved at lower costs and higher throughput.

Learn more about the benefits of dcPertrub-seq from Replogle in this webinar, Scalable and combinatorial single-cell CRISPR screens by direct guide RNA capture and targeted seq.

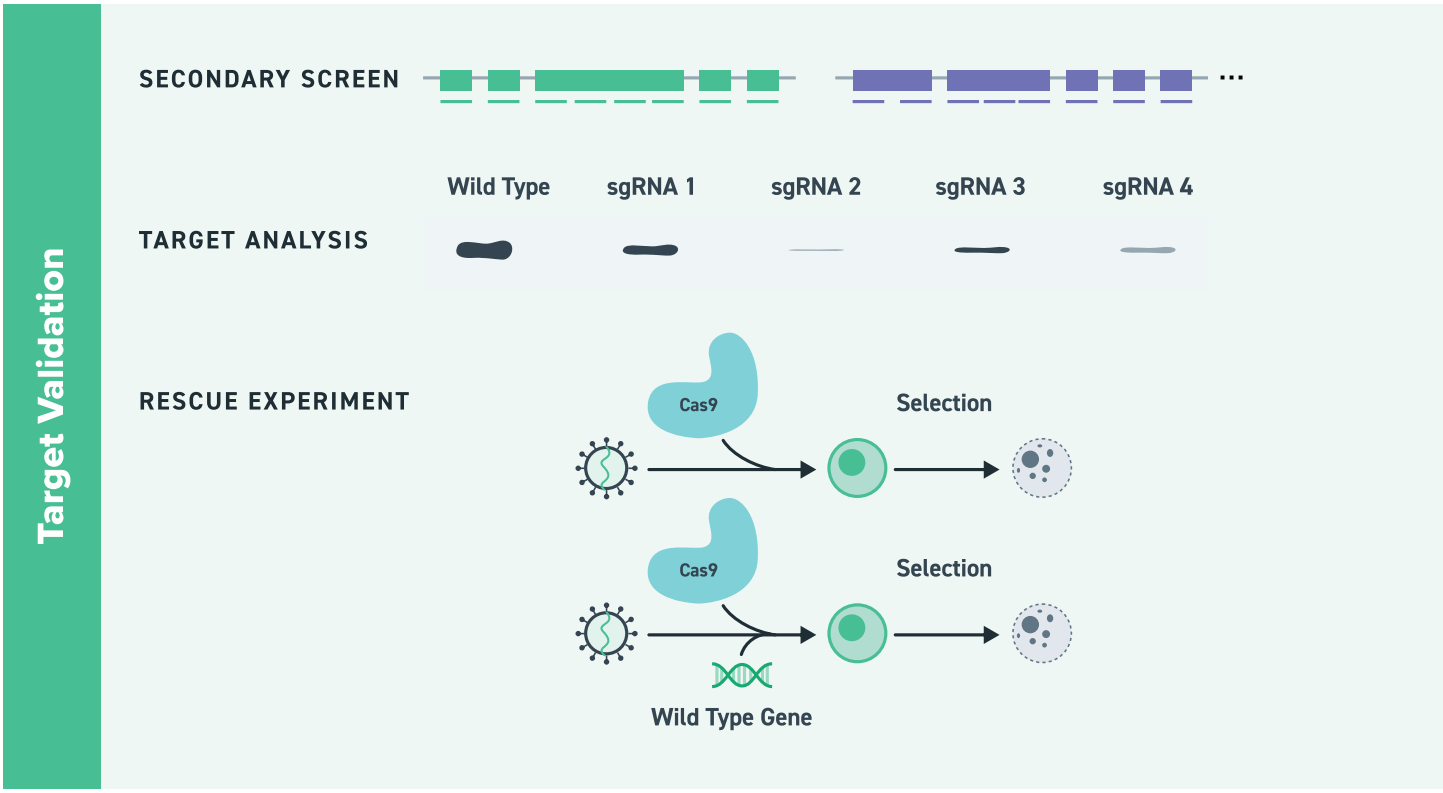



***Figure 5:*** *Synthetic DNA is a powerful tool for understanding the role of specific genes within human disease through screening, target analysis, and rescue experiments.*

## *Uniformity in Genetic Engineering*

These examples represent just a few of the many different ways that CRISPR/Cas can be used to advance our understanding of human disease. But, as with NGS, the value of any CRISPR/Cas engineering study is highly dependent on the quality of the CRISPR components used. CRISPR engineering requires the use of a synthetic RNA to guide the Cas enzyme's editing, leading it to specific genomic sequences. For most applications, this RNA is known simply as a guide RNA (gRNA). Here again, we see a repeated theme: The accuracy and uniformity of gRNA synthesis have a direct influence on the quality of CRISPR editing.

Mutations in synthesized oligos translate into mutations in the gRNA, which can impede nuclease activity, cause off-target editing, and reduce on-target editing.

Non-uniform gRNA synthesis is particularly important for large-scale screening (Figure 7) (as was done by Behan et al.). In these screens, 10s to 100s of thousands of gRNAs are needed to systematically perturb every gene of interest. If guides are non-uniformly synthesized, it can result in a substantial increase in experimental costs. This is because researchers will need to increase the number of cells screened to ensure that rare gRNAs are sufficiently represented in the final dataset. Costs mount as the number of cells needed increases—which can present practical challenges as well if using primary or difficult-to-culture cells—and as researchers compensate for underrepresented gRNAs by increasing the number of sequencing rounds. Therefore, improving the accuracy and uniformity of gRNA synthesis can substantially affect the accuracy, efficiency, and cost of CRISPR screening.

To this end, Twist offers high-quality oligonucleotide synthesis to support CRISPR/Cas studies, synthesizing gRNAs with supreme accuracy and uniformity (Figure 6).

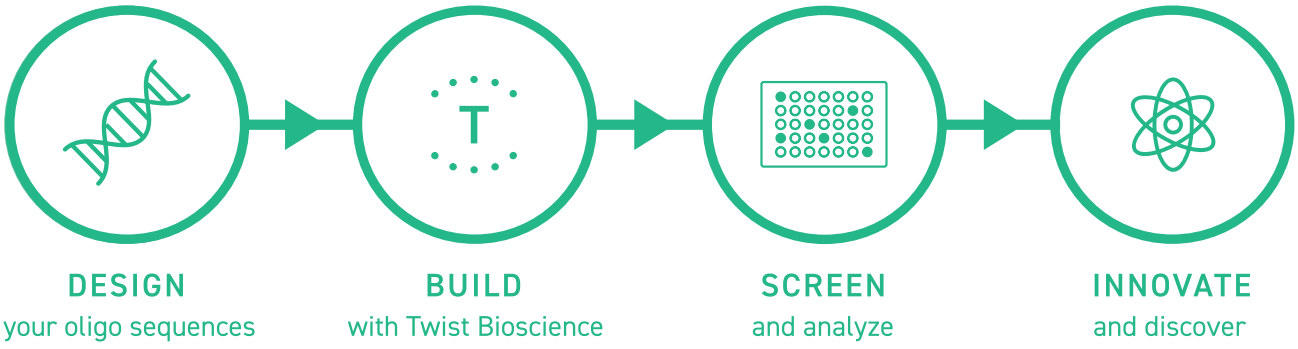



***Figure 6:*** *Twist helps teams efficiently conduct CRISPR screens with oligo sequences.*

Not all oligo pools are created equal—uniform libraries lead to more leads.
**Learn more about the importance of uniformity in this white paper.**

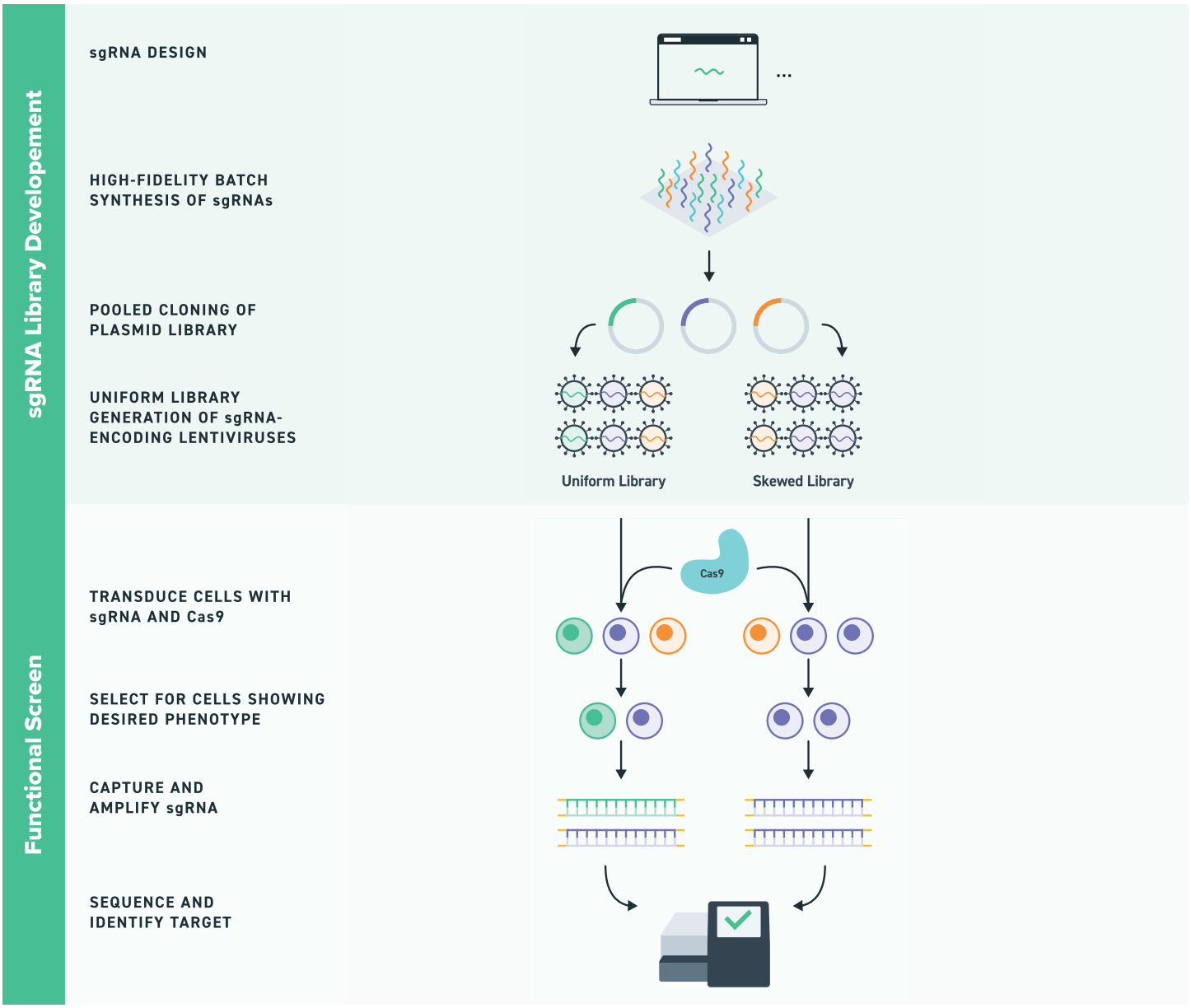



***Figure 7:*** *Example workflow of sgRNA synthesis to conduct a functional CRISPR screen and identify novel targets.*

# 05 Antibodies and CARs: Synthesizing a New Form of Hope

Advances in protein engineering and synthetic biology have given rise to new and powerful classes of anti-cancer therapeutics. Novel drug targets identified with NGS and validated through CRISPR screening can now be targeted by a wide armamentarium of therapeutics, from antibodies to T cells carrying chimeric antigen receptors (CARs).

## *Therapeutic Antibody Development*

The therapeutic value of antibodies has long been recognized. However, it is only in recent years that researchers have gained the ability to approach antibody discovery and development with the precision, rigor, and scale that's typical of small molecule research.

Nonetheless, the challenge before antibody developers is complex. These multimeric proteins typically consist of two heavy-chain and two light-chain peptides, each containing multiple distinct domains. Variations in amino acid composition, domain arrangement, and peptide combinations can all affect antibody pharmacology in diverse ways [12–14]. Therefore, the discovery and development process involves testing billions of antibody variants for desired properties, most often with variation concentrated in the hyper-variable complementarity determining regions (CDRs), which play a critical role in antigen recognition and affinity.

**CASE STUDY 4**

### Practical Limitations of Antibody Variant Testing

Consider a typical antibody which has approximately 60 CDR amino acids that are critical for target binding. To home in on the most clinically valuable combination of amino acids, a comprehensive approach would aim to test every potential amino acid combination in each CDR. Doing so would require synthesizing and testing more unique sequences than physically possible. Instead, most antibody libraries max out somewhere between $10^{10}$ and $10^{12}$ unique sequences. Researchers must therefore design their antibody libraries carefully in order to focus their efforts, sampling specific portions of the sequence space in search of an optimal antibody sequence—one that can itself be used to build and iterate towards a truly optimal antibody.

Read the primary paper here.

Developers can approach this challenge using hybridoma, B-cell screening, or display technologies such as phage and yeast.

*In vivo* platforms such as hybridoma and B-cell screening leverage the natural antibody diversity found in living organisms. The human body, for example, is believed to have at least $10^7$ unique antibody sequences in circulation at any given time [15, 16]. Antigen-binding immune cells will be isolated and, ultimately, their immunoglobulin domains sequenced using NGS technology. The resulting dataset will represent an enriched catalog of antibody variants that bind to the target antigen.

Twist's *in vitro* phage and yeast display platforms leverage synthetic library technology. Library designs may be inspired by natural repertoires, existing datasets (such as those produced by in vivo immunizations), or *in silico* modeling data. In any case, genes encoding putative antigen binding domains are synthesized and integrated into a phagemid, enabling antibody expression on the viral surface. Subsequent screening can isolate antigen-binding phage, whose unique antibody sequence is determined through NGS.

DNA synthesis technology is integral to antibody discovery and optimization, regardless of the initial technology used. For phage or yeast display, synthetic DNA enables researchers to design vast libraries of antibody variants for high-throughput screening, typically on the order of $10^{10}$ variants. Candidates identified with each technology will often require optimization, wherein individual and combinatorial variants are synthesized and screened for improved pharmacological and pharmacokinetic properties. Those antibodies identified through non-humanized *in vivo* workflows or, in particular, through the use of *in vitro* technologies, may need further optimization to reduce immunogenicity (a process known as humanization).

To this end, Twist has developed an extensive suite of tools to support researchers in their study of antibody development and discovery (Figure 8).

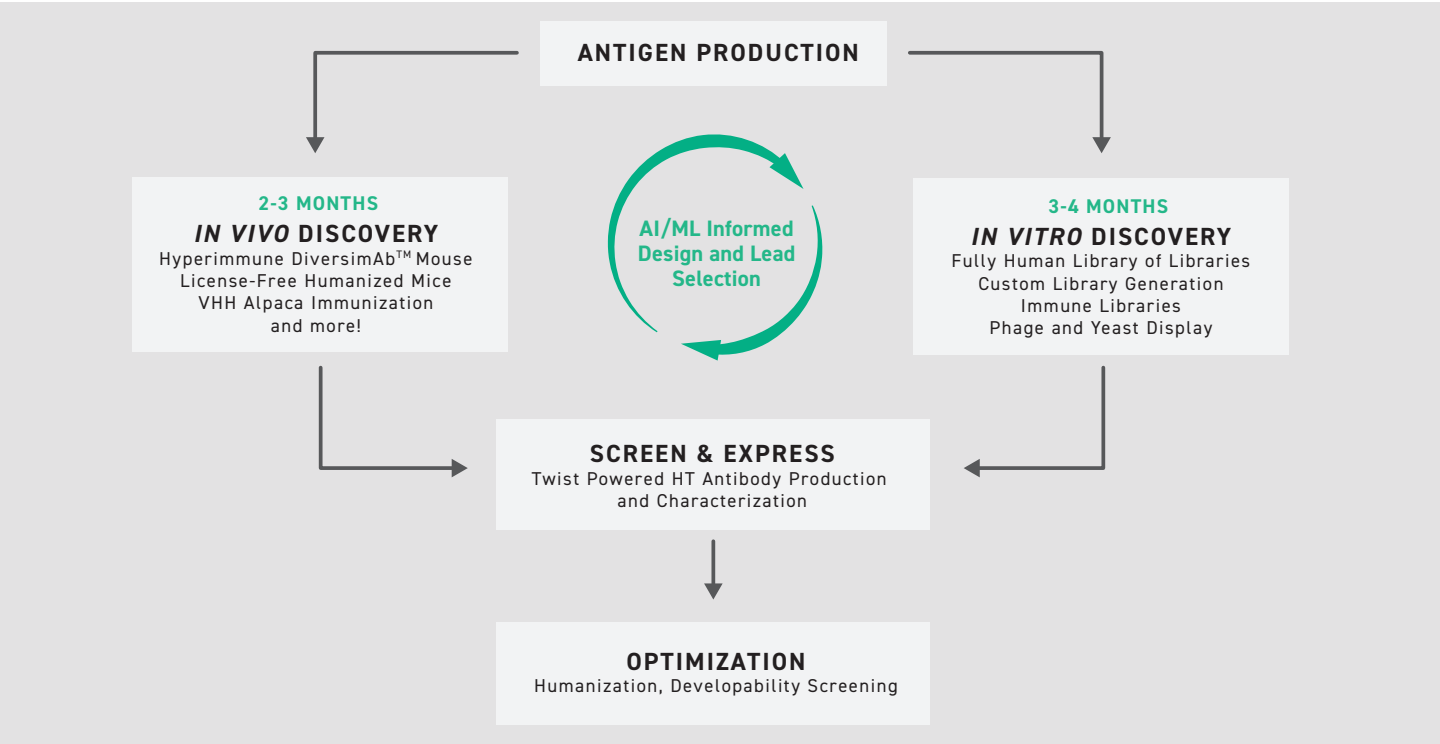



***Figure 8:*** *Twist has a robust antibody discovery and optimization pipeline.*

## Twist's Antibody Solutions

**Biopharma Solutions:** Among Twist's many offerings is a solution for antibody discovery that includes the use of hybridoma, single B-cell screening, phage-display, and machine learning technologies to enable rapid and effective screening for promising candidates.

**High-Throughput Antibody Production and Characterization:** Twist Bioscience's High-Throughput Antibody Production is a gene-to-protein workflow capable of reformatting tens to thousands of diverse antibody sequences as full-length IgGs and generating screening-scale amounts of recombinant antibody material for validation and testing. With the ability to precisely write thousands of genes per run, Twist's Express Antibodies enable immediate expansion of your antibody production pipeline. To complete the make-test cycle in any antibody development workflow, explore our portfolio of characterization and developability assays that can be complemented to your protein production projects. Simply upload the antibody sequences you need and let Twist do the rest.

**Library of Libraries:** Twist's pre-constructed, fully human, and validated phage display libraries are built using precision DNA writing technology, allowing the removal of motifs that can lead to downstream manufacturing liabilities, thus enriching the libraries for theoretically interesting antibody sequences devoid of conventional sequence-based developability liabilities. Twist Biopharma Solutions has designed multiple general, naïve, and target class-focused libraries with diversities ~$10^{10}$, giving you a multifaceted approach to your next discovery project. Select from libraries containing naïve human diversity, rationally designed with antibody structural considerations, or with diversity computationally tailored to high-value targets, including carbohydrates, ion channels, and GPCRs. They're available in a variety of VHH, Fab, and scFv frameworks, including common light chain options for the generation of bi- and multispecific therapeutic antibodies. These libraries can also be leveraged to develop monoclonal antibodies, multispecifics, and chimeric antigen receptors (CARs) for CAR T-cell therapy.

**Twist Antibody Optimization (TAO):** Twist's rapid, scalable DNA synthesis platform enables the rapid generation of defined synthetic libraries. Twist converts your parental antibody to the closest germline sequence and comprehensively explores the human mutational space with efficiency. Additionally, this system can be used for high-throughput conversion from scFv, Fab, or VHH formats to full-length IgG or VHH-Fc format. Twist's team of antibody experts leverage their collective 50 years of experience to streamline development with biochemical, biophysical, and functional screening of any TAO-derived antibodies.

| Hybridoma Workflow (3–5 months to sequence) | | | | | |
|---|---|---|---|---|---|
| 3–5 WEEKS (OPTIONAL) | 3–8 WEEKS | 4–6 WEEKS | 1–3 WEEKS | 2–3 WEEKS | 5–7 WEEKS |
| Antigen & Screening Tool Production | Mouse Immunization | Hybridoma Fusions & High-Throughput Screening | Advanced Screening | Hybridoma Sequencing | Recombinant Antibody Expression (+ downstream engineering/humanization) |

| B Cell Screening (2–5 months to sequence) | | | | |
|---|---|---|---|---|
| 3–5 WEEKS (OPTIONAL) | 3–16 WEEKS | 1 DAY | 2-3 WEEKS | 5–8 WEEKS |
| Antigen & Screening Tool Production | Mouse, Rabbit, or Alpaca Immunization | Beacon-Based B Cell Screening | B Cell Sequencing | Recombinant Antibody Expression (+downstream engineering/humanization) |

| Library of Libraries (2–5 months to sequence) | | | |
|---|---|---|---|
| 3–5 WEEKS (OPTIONAL) | 2–4 WEEKS | 2–3 WEEKS | 5–8 WEEKS |
| Antigen & Screening Tool Production | Phage & Yeast Display Panning (cell- and bead-based selections) | Antibody Sequencing (Sanger & NGS) | Recombinant Antibody Expression (+ downstream engineering) |

| Immune Libraries (2–5 months to sequence) | | | | | |
|---|---|---|---|---|---|
| 3–5 WEEKS (OPTIONAL) | 3–16 WEEKS | 4–6 WEEKS | 2–4 WEEKS | 2–3 WEEKS | 5–8 WEEKS |
| Antigen & Screening Tool Production | Mouse, Rabbit, or Alpaca Immunization | Immune Library Construction | Phage & Yeast Display Panning (cell- and bead-based selections) | Antibody Sequencing (Sanger & NGS) | Recombinant Antibody Expression (+ downstream engineering) |

## *Cell-Based Therapy Development*

A fast-growing sector of cancer research concerns the development of cellular therapies. These living therapeutics work by infusing an organism with enhanced immune cells, whether those are stem cells lacking pathogenic mutations or, in the case of CAR T-cells, differentiated cells with specific and potent pathogen-targeting abilities [17, 18].

Whereas therapeutic antibodies and small molecules are ephemeral in the body, cell therapies developed by Twist clients have the potential to help patients by providing lasting protection against recurrent malignancies.

Among the many different cell therapies, CAR T-cell technology has seen prominent success against intractable cancers. Researchers looking to develop CAR T-cell therapies have many challenges ahead of them, but these can be made easier if they have access to high-quality oligonucleotide synthesis platforms.

### Tools For Developing CARs

T cells are a type of lymphocyte whose activation is not dependent on antibodies. Instead, T cells rely on a unique cell surface protein—the T-cell receptor (TCR)—which recognizes major histocompatibility complex (MHC) proteins displaying antigenic (Figure 9) peptides. Once bound, T cells can direct adaptive immune cells against specific pathogens and thus have significant potential in therapeutic applications [19, 20]. By imbuing T cells with a form of synthetic, modified TCR (known as a chimeric antigen receptor, or CAR), researchers can target T cells against cancer cells.

The development of safe and effective CARs requires careful and extensive optimization to ensure safety, specificity, and efficacy. Fortunately, many of the same technologies used for antibody development can be useful. Antibody phage display systems, for example, enable large-scale and high high-throughput screening of potential antigen binding domains. Once identified as specific for the target antigen, DNA coding for binding domains can be synthesized and assembled into a CAR gene.

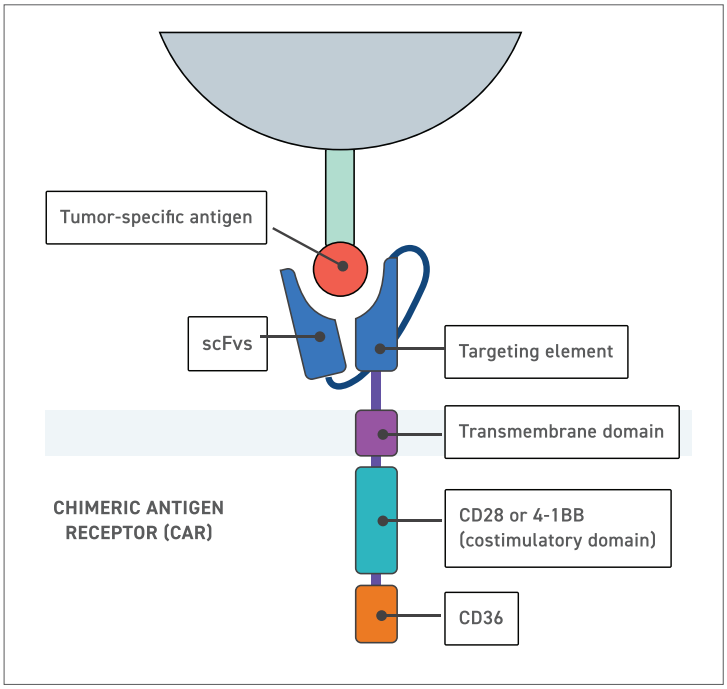



***Figure 9:*** *Structure of a CAR T-cell.*

As with antibodies, producing a clinically viable CAR requires extensive optimization due in part to the highly context-dependent nature of CAR signaling. Both CAR-antigen binding kinetics and the resulting cellular response can be significantly altered by transmembrane and intracellular CAR components. Therefore, researchers will need the ability to test variations in antigen binding domains, hinge and transmembrane domains, and costimulatory factor combinations. This will necessarily involve the generation and expression of combinatorial variant CAR libraries using large-scale DNA synthesis [21, 22].

CAR and antibody development is just the tip of the biologics iceberg. There are many similar forms of protein-based therapeutics, the development of which is aided by the use of combinatorial variant libraries and optimization of antibody-like proteins. This includes TCR engineering, antibody-drug conjugates, and CAR-NK cells, among many others. In each application, the same basic principles hold true: Discovery and optimization of promising therapeutics are made easier and more efficient when researchers have access to high-quality oligonucleotide synthesis platforms.

## Cell Development Libraries

**Combinatorial Variant Libraries:** Twist's massively parallel silicon-based DNA synthesis platform produces highly uniform and accurate oligos, with 90% of oligos represented within a <2.5x relative abundance range. Using this platform, Twist can fabricate highly diverse gene mutant libraries with excellent variant representation and highly specific user-defined composition with no unwanted bias or motifs. Twist library technology enables a comprehensive interrogation of the variant sequence space.

**Site Saturation Variant Libraries:** Protein engineering screens using single-site variant libraries allow researchers to explore a protein's sequence space and investigate the relationship between sequence and protein structure and function. Twist Site Saturation Variant Library construction leverages massively parallel oligonucleotide synthesis using Twist's proprietary silicon-based DNA synthesis platform. These libraries offer researchers complete control over codon usage (all 64 codons available) and allows for screening of 1 to 20 different amino acids at each position.

**Spread Out Low Diversity Libraries:** Spread-Out Low Diversity (SOLD) libraries provide a quick and precise tool for mapping protein sequences to explore the complex relationship between a protein and its environment. SOLD Libraries are a time- and money-saving tool for researchers who want to efficiently investigate combinatorial possibilities. A library of precise combinatorial variants with uniform amino acid distribution or ratio, balanced codon usage, and the ability to avoid unwanted restriction sites provides the ultimate tool to explore the variant space. All SOLD Libraries are NGS-verified, cloning-ready, and created using Twist's patented silicon-based synthesis platform, ensuring low error rates.

**TCR Libraries:** Engineered T-Cell receptor (TCR) therapy is a type of cell therapy that leverages engineered T-cell receptors to target tumor-specific antigens. TCR repertoire sequencing can be done via single cell sequencing or bulk sequencing. Each has its own advantages. Bulk sequencing enables you to sample more of the sequence space, but information about the alpha-beta TCR pairing is lost. Single-cell sequencing enables you to capture information on the alpha-beta chain pairing and receptor composition. However, single-cell sequencing has a much lower throughput than bulk sequencing. Twist offers TCR libraries that complement both strategies:

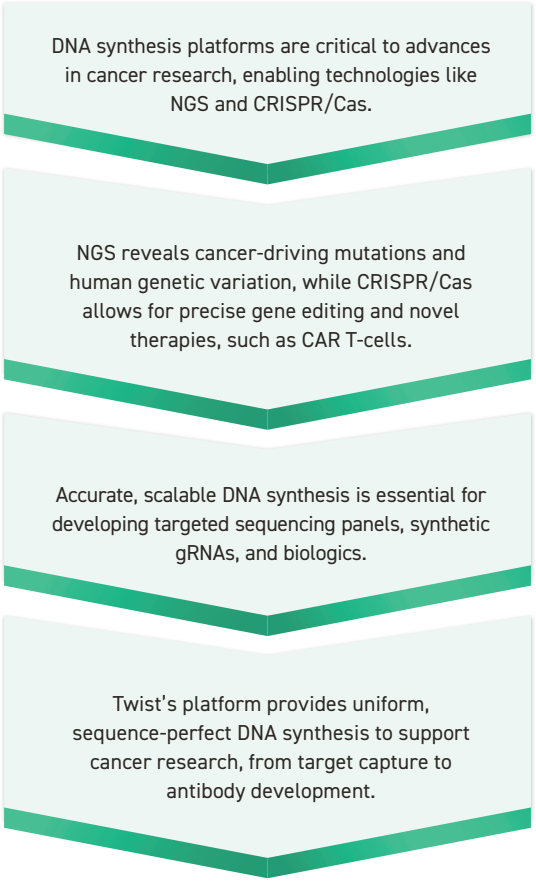
- A paired pooled TCR library enables you to retain the original alpha-beta chain pairing to maximize hit validation and replicate the TCR repertoire. This is a good complement to single-cell sequencing, where the alpha-beta TCR pairing is known.
- A combinatorial TCR library enables you to shuffle alpha and beta chain pairs to create additional diversity and explore novel combinations beyond the identified repertoire. This is a good complement to bulk sequencing, where the alpha-beta TCR pairing is unknown.

# 06 DNA Synthesis Across the Cancer Research Continuum

Despite decades of research, cancer remains an enigmatic disease. Fortunately, advances in recent decades have helped to demystify the nature of malignant cells. From among these critical advances, the rise of DNA synthesis platforms stands out as particularly transformative. Indeed, custom oligonucleotide synthesis is foundational to many of the technologies and discoveries that define modern molecular biology.

NGS technology, for example, has blown the doors open on the malignant genetic landscape, highlighting the presence of cancer-defining mutations and emphasizing the vast scale of human genetic variation. The consequences of this variation can be meticulously studied, and reproduced in advanced disease models, thanks to the evolution of CRISPR/Cas gene editing technology. Not only can the validation of novel targets inspire therapeutic development, but CRISPR/Cas enables the engineering of entirely novel therapeutic modalities, such as T cells armed with synthetic CARs.

## Synthetic DNA Across the Cancer Research Continuum




Each of these applications relies on the use of DNA synthesis technology. Whether it's RNA sequencing, DNA sequencing, or methylation sequencing, researchers using NGS to study cancer genetics benefit greatly from targeted sequencing panels, which allow for limited sequencing resources to be focused where they're needed most. Modern CRISPR/Cas editing is made possible through the use of synthetic gRNAs that dictate where editing should occur. Finally, the development of CARs and other biologics requires the synthesis of the protein's component parts and the large-scale, iterative process of designing, building, and testing different peptide combinations.

Stated simply, having access to accurate, uniform, and scalable DNA synthesis is proving invaluable across the cancer research continuum. This is why Twist has developed a sprawling catalog of products to support this important work, from custom target capture panels for the study of minimal residual disease testing to antibody development services. Twist's sequence-perfect, highly uniform, and scalable DNA synthesis platform empowers scientists to overcome the challenges of cancer research, ultimately with the hope of seeing a future where cancer treatment is trivial.

Whether you have a question about sales, customer support, media, or investor relations, our team is ready to answer all your questions.

CONTACT US

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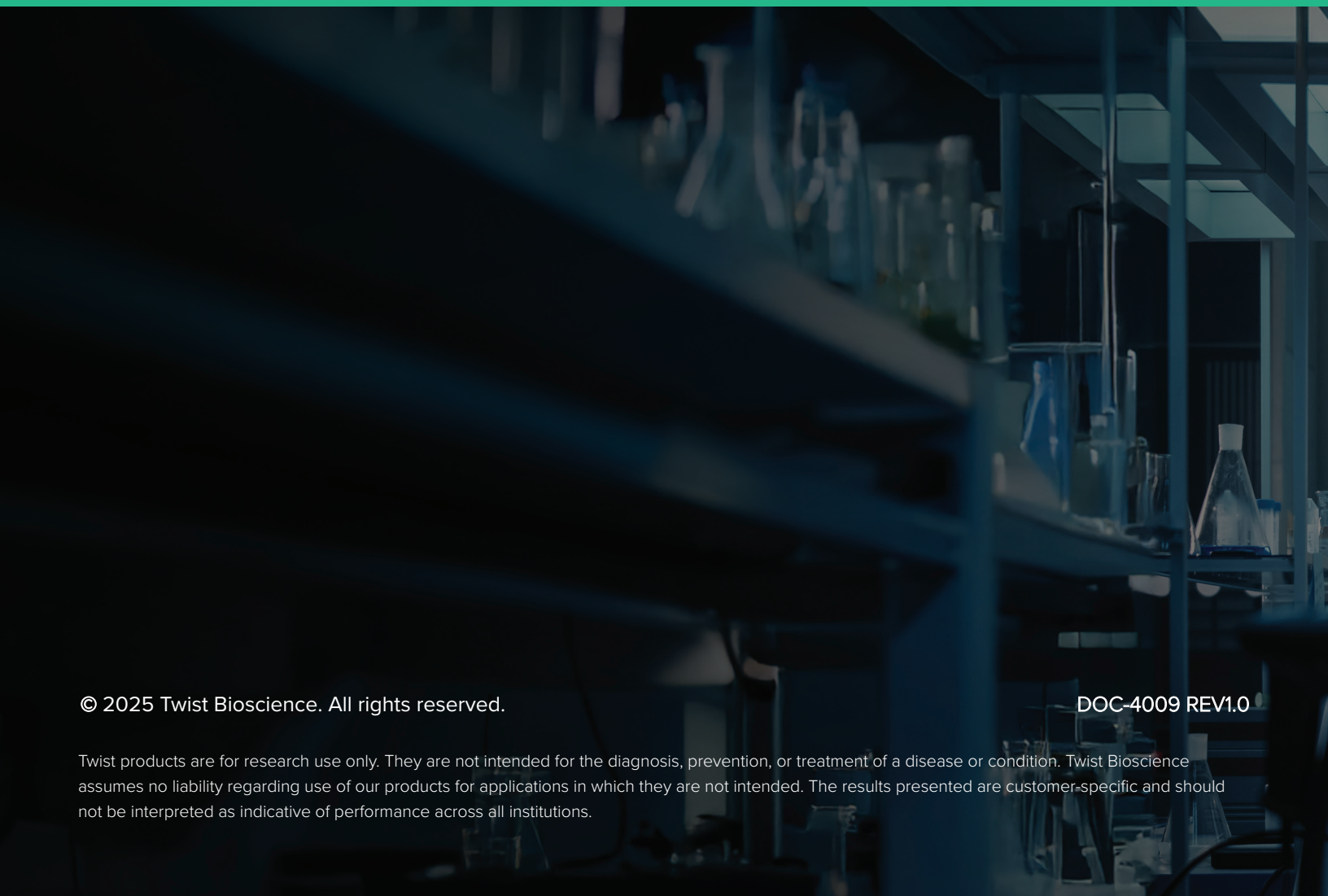



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