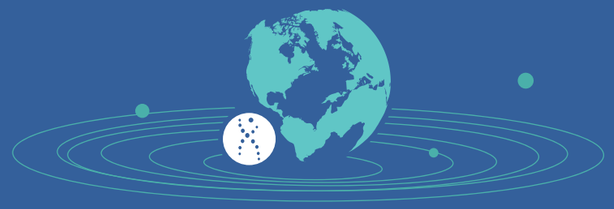
SERVICE BROCHURE

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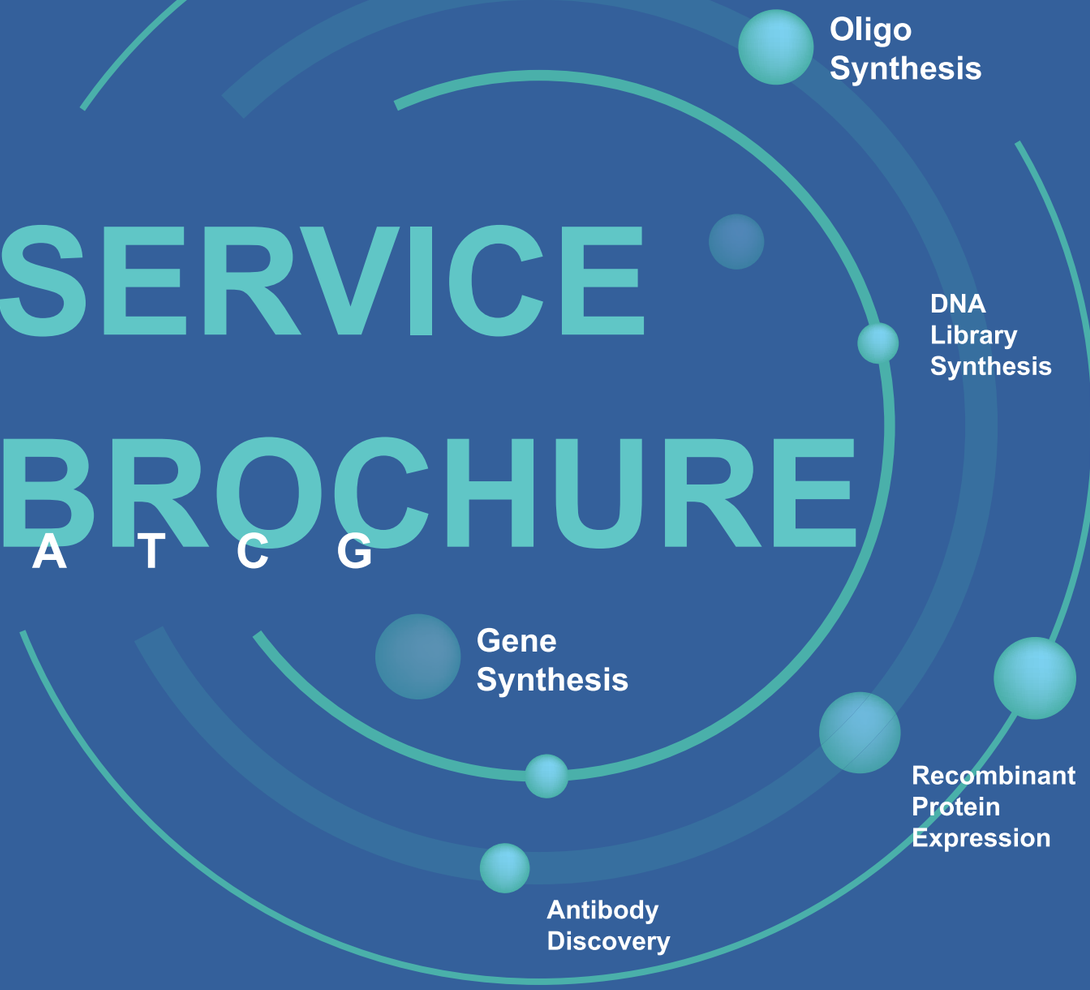
Find more information at *www.synbio-tech.com*

SYNBIO TECHNOLOGIES

# SERVICE BROCHURE

A T C G




**SCIENCE**
GENES FOR LIFE

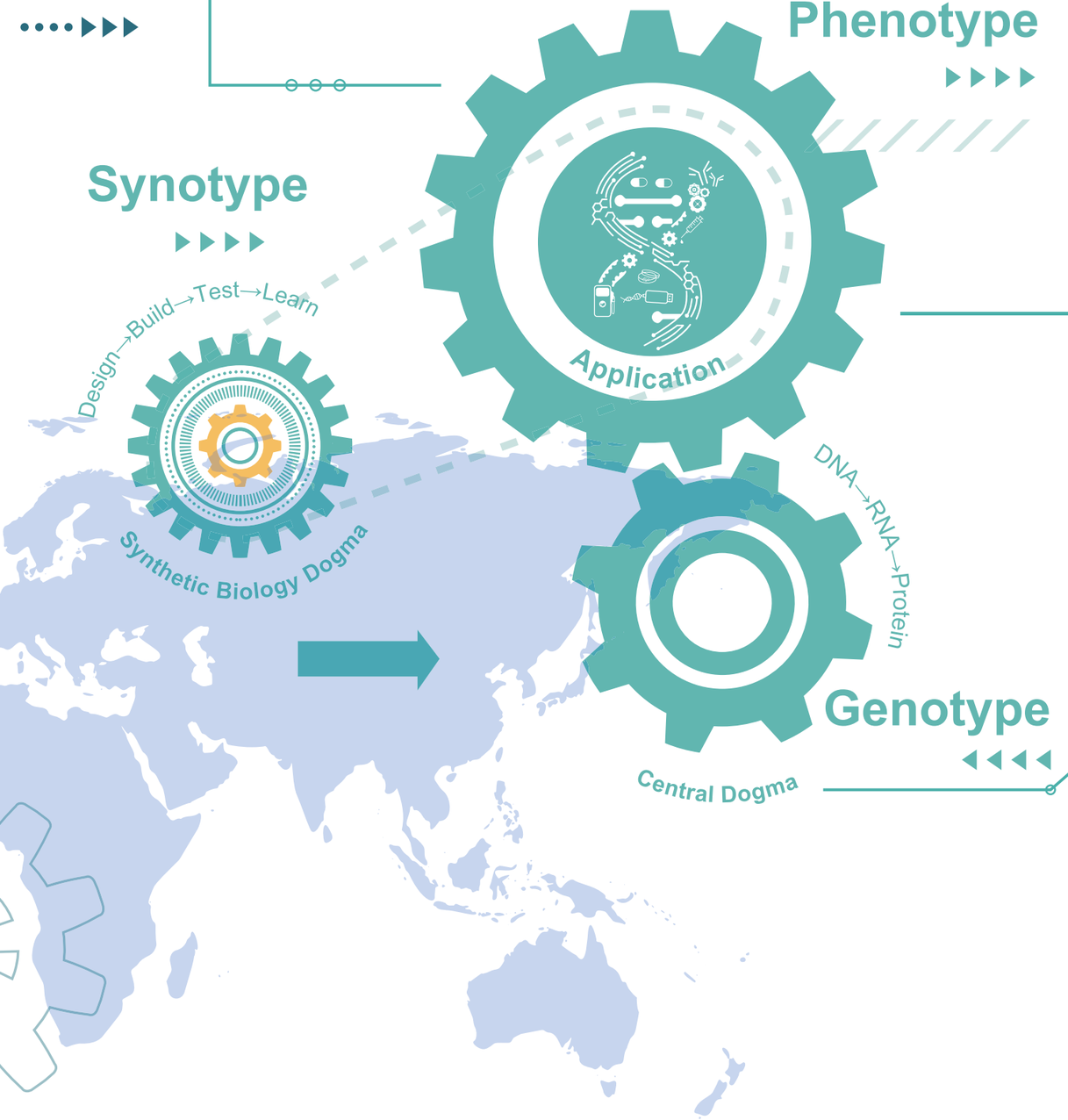
**QUALITY**
GENES FOR LIFE

**PEOPLE**
GENES FOR LIFE

THE DNA TECHNOLOGY COMPANY

# About Us

Founded in 2013 by a group of passionate synthetic biologists with decades of industry experience and a proven track record of delivering cost-effective DNA solutions, Synbio Technologies is incorporated with a single mission in mind: to empower scientific discoveries and drug innovations by providing the most advanced and cost-effective DNA technology platform. The company takes the lead in establishing an advanced biotechnology transformation and application “GPS” (Genotype-Phenotype-Synotype) platform. The innovative “GPS” Platform follows the biological central dogma, expands the genotype, phenotype, and synotype effectively to achieve the one-stop solution of XNA “design-construction-application”. With a full range of DNA reading (sequencing), DNA writing (synthesis), and DNA editing (engineering) capabilities, Synbio Technologies has delivered satisfactory solutions globally to researchers for various applications, including diagnostic DNA probes, precision medicine, protein production, antibody discovery, vaccine development, novel enzymes, molecular breeding, biofuel implication, etc.



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# Syno® C Oligo Synthesis

CHAPTER 1

# Syno® C Oligo Synthesis

## Oligo Synthesis

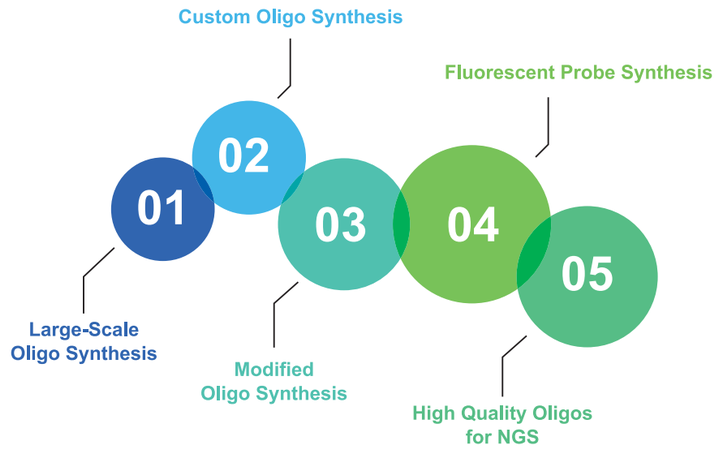
Synbio Technologies's oligo synthesis platform equips state-of-the-art synthesizers and leading professional teams experienced in oligo synthesis and modification technologies. This allows us to provide high-quality oligo products to scientific researchers and industrial customers around the world.

By leveraging our advanced and innovative manufacturing process, Synbio Technologies offers a wide range of oligo synthesis options including standard oligos, long oligos, ultra-high purity oligos, degenerate primers, aptamers, fluorescent probes, oligo pool synthesis, etc. In addition, we also provide high-throughput oligo synthesis to customers with large-scale oligo projects.

### Competitive Advantages

- ► Certified with the highest manufacturing standards (ISO9001 & ISO13485), large-scale sequencing that verifies the average mutation rate to be lower than 1/1000
- ► Various highly customizable options for purification, synthesis specifications, and delivery form.
- ► Qualified and available for multiple applications such as molecular diagnostics, NGS, functional genomics, genome engineering, drug development, protein/antibody function, etc.

### Oligo Synthesis Services



CHAPTER 1

# Syno® C Oligo Synthesis

## 1.1 Standard Oligo Synthesis

Synbio Technologies is always competent to offer tailor-made approaches to accomplish every oligo synthesis request.

### Competitive Advantages

**High Quality**
We can achieve extremely low mutation and error rates in accordance with stringent oligo synthesis quality control standards

**Highly-Customizable**
Flexible synthesis scales are available. Four alternative purification options include: DSL, HPLC, PAGE Plus, and PAGE

**Technical Support**
Professional teams experienced in oligo synthesis and various modifications offer support throughout the production progress

**Cost Effective**
Competitive prices and affordable services

### Purification Methods

| Purification Method | Characteristics | Purity | Applications |
|---|---|---|---|
| DSL | Effectively removes salt and meets most experimental needs | >75% | It is used for PCR cloning, site-directed mutagenesis, gene synthesis, Sanger sequencing, etc. |
| PAGE-Plus | Purification effect is better than DSL, especially suited for oligos longer than 59 nt and meets most experimental needs | >85% | Commonly used in sequencing, multiplex & quantitative PCR cloning, site-directed mutagenesis, gene synthesis, etc. |
| HPLC | Purification effect has high degree of automation and is good for modified oligos and effectively removing small fragments | >90% | Often used in modified oligos, probes, cloning, etc. |
| PAGE | Purification effect is good for removing small fragments and is generally more suitable for oligos of more than 59 nt or for high quality requirements | >95% | Commonly used in sequencing, gene synthesis, site-directed mutagenesis, PCR cloning, etc. |





**◆ Standard Oligos**

Synbio Technologies provides fast and convenient standard DNA oligo synthesis services with no design fee that are suitable for more than 95% of PCR projects. Large-scale and bulk custom DNA oligos can be ordered with the same high quality standards, competitive prices, and fast turnaround.

| | Purification Method | Synthesis Scale | 10 nm | 25 nm | 50 nmol | 100 nmol | 200 nmol | 1 μmol |
|---|---|---|---|---|---|---|---|---|
| 11-59nt | DSL | Final Yield | 1 OD | 2 OD | 5 OD | 10 OD | 15 OD | 50 OD |
| | PAGE Plus | Final Yield | – | – | 2 OD | 5 OD | 10 OD | 30 OD |
| | PAGE | Final Yield | – | – | – | 2 OD | 5 OD | 10 OD |
| | HPLC | Final Yield | – | – | – | 2 OD | 5 OD | 10 OD |
| 60-90nt | DSL | Final Yield | 1 OD | 2 OD | 5 OD | 10 OD | 15 OD | 50 OD |
| | PAGE Plus | Final Yield | – | – | 2 OD | 5 OD | 10 OD | 30 OD |
| | PAGE | Final Yield | – | – | – | 2 OD | 5 OD | 10 OD |
| | HPLC | Final Yield | – | – | – | 2 OD | 5 OD | 10 OD |

* Longer oligos, 96/384 plate batch oligos, or other large scale synthesis are available, please contact us at *quote@synbio-tech.com*.
* Please refer to the purification table for the final output corresponding to each synthesis scale.

**◆ Degenerate Primers**

Genetic codes have the tendency to degenerate, which lead to the use of degenerate primers in PCR experiments to exhibit specific genes. Synbio Technologies provides both the design and synthesis of these degenerate primers, as well as custom degenerate oligo pool synthesis upon request.

| Symbol | Base Type | Standard Ratio |
|---|---|---|
| R | A, G | 50% : 50% |
| Y | C, T | 50% : 50% |
| M | A, C | 50% : 50% |
| K | G, T | 50% : 50% |
| S | C, G | 50% : 50% |
| W | A, T | 50% : 50% |
| H | A, C, T | 33% : 33% : 33% |
| B | C, G, T | 33% : 33% : 33% |
| V | A, C, G | 33% : 33% : 33% |
| D | A, G, T | 33% : 33% : 33% |
| N | A, C, G, T | 25% : 25% : 25% : 25% |

* Non-standard base proportion should be declared clearly, for example, N (20%A: 30%C: 40%G: 10%T).

CHAPTER 1

# Syno® C Oligo Synthesis

◆ **Trimer Oligos**

Utilized for certain projects sensitive to codon bias. Synbio Technologies can significantly reduce the deviation inherent in the synthesis procedures and realize accurate proportional control. The basic principle is to introduce 20 amino acids into the specific sites of proteins by the chemically synthesized trimer phosphoramidites, reduce codon redundancy, avoid codon shift mutations, and stop codon insertion.

| Trimer Oligo Length | The Number of Trimer Sites | Purification Methods | Turnaround Time | Deliverables |
|---|---|---|---|---|
| ≤120 nt | ≤20 | PAGE/HPLC | 2-6 weeks | • Trimer oligos<br>• COA<br>• NGS analysis report (Optional) |

◆ **Long Oligos**

Synbio Technologies's oligo synthesis platform utilizes an optimized ultra-long chain design and synthesis program to meticulously monitor and control the synthesis process. This process allows us to generate long, high-quality oligos (90-129 nt) and even ultra-long oligos (130-150 nt) according to individual requests.

| Length (nt) | Quantity | Purification Methods | Turnaround Time (Business Days) |
|---|---|---|---|
| 90-109 | 1 OD | PAGE / Dual-PAGE | 5-6 |
| 110-129 | 1 OD | PAGE / Dual-PAGE | 5-7 |
| 130-150 | 0.5 OD | PAGE / Dual-PAGE | 5-8 |

Standard Deliverables

1. One tube lyophilized DNA (transparent or dark) or 96-well and 384-well plate
2. Certificate of analysis (COA) including sequencing information, OD, Tm, etc.





## 1.2 Oligo Modification & Labeling

Synbio Technologies provides comprehensive modification and labeling services to meet our customers' specified research requirements. Our stringent quality control guidelines ensure the synthesis of high-quality modified oligos and that they are subsequently delivered in a timely manner. We can satisfy various research needs in biology, diagnostics, personal medicine, drug discovery, etc.

### Competitive Advantages

**High Synthesis Efficiency**

Large-scale sequencing verifies that the average mutation rate will be lower than 1/1000

**Strict Quality Control**

Stringent ISO 9001- & ISO13485-certified processing for oligo synthesis, meticulously monitored with advanced traceability

**Comprehensive Modifications and Labeling**

Professional teams experienced in oligo synthesis and various modifications offer support throughout the production progress

### Service Specifications

◆ **General Modifications**

We provide a series of general modifications with high purity and competitive prices. We accomplish this by focusing on modification requests with special chemical classifications.

| Classifications | Modifications | Classifications | Modifications | Purifications |
|---|---|---|---|---|
| Modified Bases | dU | AMCA | 5' AMCA | HPLC/PAGE |
| | dI | | 3' AMCA | |
| | 5-Methyl dC | | dT-AMCA | |
| DBCO | 5' DBCO | Biotin | 5'Biotin | |
| | 3' DBCO | | 3'Biotin | |
| | dT-DBCO | | dT-Biotin | |
| Amino Linkers | 5'Amino C6 | Fluoro Bases | 2'-F-2'-dA | |
| | dT-Amino C6 | | 2'-F-2'-dC | |
| | 3'Amino C7 | | 2'-F-2'-dG | |
| | 5'Amino C12 | | 2'-F-2'-dU | |
| Digoxigenin | 5'Digoxigenin | Spacer | dSpacer | |
| | 3'Digoxigenin | | Spacer C3 | |
| | dT-Digoxigenin | | Spacer C9 | |
| Locked Nuleic Acid | LNA (A/G/C/U) | | Spacer C18 | |

# Syno® C Oligo Synthesis



| Classification | Modifications | Classification | Modifications | Purification |
|---|---|---|---|---|
| Phosphorylation | 5' PO4 | Hydrosulphonyl | 5' HS-C6 | HPLC/PAGE |
| | 3' PO4 | | 3' HS-C3 | |
| Phosphorothioates | SPO3 | – | – | |

* If you need other purification methods, please contact us at *quote@synbio-tech.com*.

**◆ Single Labeling**

Synbio Technologies provides multiple fluorescent groups at 3', 5', or other designated sites to comply with our customers' requests. These include FAM, HEX, ROX, BHQ, Texas Red, etc.

| Dye/Quenchers | 3' - | 5' - | Designated Sites | Purification |
|---|---|---|---|---|
| FAM | √ | √ | √ | HPLC/PAGE |
| TET | √ | √ | √ | |
| JOE | √ | √ | √ | |
| HEX | √ | √ | √ | |
| SIMA | √ | √ | √ | |
| TAMRA | √ | √ | √ | |
| ROX | √ | √ | √ | |
| Cy5 | √ | √ | √ | |
| Cy3 | √ | √ | √ | |
| Quasar670 | √ | √ | | |
| Quasar570 | √ | √ | | |
| DyLight547 | | √ | | |
| DyLight647 | | √ | | |
| Texas Red | | √ | | |
| BHQ-1 | √ | | | |
| BHQ-2 | √ | | | |
| BHQ-3 | √ | | | |
| Eclipse | √ | | | |
| DABCYL | √ | | | |

* If you need other purification methods, please contact us at *quote@synbio-tech.com*.



**◆ Dual/Multiple Labeling**

Aside from single fluorescent groups, Synbio Technologies also offers dual/multiple labeling services including TAMRA, BHQ, DABYCL, ELCISPE, etc. The most common dyes and quenchers are listed below:

| Dyes | Quenchers | Dyes | Quenchers | Purification |
|---|---|---|---|---|
| 5'6-FAM | 3'TAMRA | 5'HEX | 3'TAMRA | HPLC/PAGE |
| | 3'BHQ-1 | | 3'BHQ-1 | |
| | 3'Eclipse | | 3'BHQ-2 | |
| | 3'DABCYL | | 3'DABCYL | |
| 5'TAMRA | 3'Eclipse | 5'ROX | 3'Eclipse | |
| | 3'BHQ-1 | | 3'BHQ-1 | |
| | 3'BHQ-2 | | 3'BHQ-2 | |
| | 3'DABCYL | | 3'DABCYL | |
| 5'CY5 | 3'BHQ-1 | 5'CY3 | 3'BHQ-2 | |
| | 3'BHQ-2 | 5'Texas Red | 3'BHQ-2 | |
| | 3'BHQ-3 | 5'JOE | 3'Eclipse | |
| | 3'DABCYL | | 3'BHQ-1 | |
| 5'TET | 3'TAMRA | | 3'BHQ-2 | |
| | 3'BHQ-2 | | 3'DABCYL | |
| | 3'DABCYL | | 3'TAMRA | |

* If you need other purification methods, please contact us at *quote@synbio-tech.com*.

Standard Deliverables

1. One tube lyophilized DNA (transparent or dark) or 96-well and 384-well plate
2. Certificate of analysis (COA) including sequencing information, OD, Tm, etc.

## 1.3 High Quality Oligos for NGS

Next Generation Sequencing (NGS) is a high-throughput technology that enables the rapid sequencing of millions of DNA strands in parallel. With its ultra-high throughput capabilities, scalability, and speed, NGS has revolutionized genomics and biological sciences. With NGS, researchers can perform a variety of applications as well as study biological systems beyond the capacity of traditional sequencing technologies.

For most of the NGS platforms available on the market, there is a large demand for high quality oligos for successful sequencing. The quality of the oligos are crucial because any contamination can lead to strong background or splicing errors. These errors may seriously affect the overall sequencing results while also wasting valuable time and effort in the process. Synbio Technologies's Syno® C oligo synthesis platform allows us to avoid these potential errors while delivering custom, high-quality oligos for NGS applications.

### Competitive Advantages

**01 Industry-Leading Coupling Efficiency**
Reduce the dimer generation of synthetic oligos

**02 Exclusive Production Processes**
Standardized and stringent quality control ensures batch-to-batch consistency

**03 Low Levels of the Cross-Contamination**
Strict purification standards generate higher quality products, often with purity ≥90%

**04 Flexible Oligo Specifications**
Customizable formulation, mixing options, and documentation are available

### Custom High Quality Oligos for NGS Services

Our NGS-grade oligos are recommended for a variety of requests independent of the instrument or technology. Customized synthesis, modification, purification, and delivery are provided to meet all our customers' needs.

- Syno® Adapters
- Syno® Blocking Oligos
- Amplification Oligos
- Syno® Hybrid Capture Probes
- Syno® UMI
- Sequencing Oligos

| Specifications | Length (bp) | Turnaround Time (Business Days) | Deliverables |
|---|---|---|---|
| Indexed Adapters | 60-70 | 5-7 | Products in tube/plate/chip, Comprehensive QC report (HPLC, MS) |
| Capture Probes (DNA oligo – 120 nt biotin, Oligo pools, Multiplex PCR oligos) | 10-170 | Inquire | |
| Blocking Oligos | ~60 | 5-7 | |
| Other NGS oligos | 10-150 | 5-7 | |



## 1.4 Oligo Pool Synthesis

Many aspects of biological research require a large amount of primers, such as genetic library construction and target enrichment/capture in NGS. Conventional approaches to synthesizing hundreds of thousands of primers at a time are ineffective and time consuming. To circumvent this issue, Synbio Technologies provides a solution to high-throughput DNA synthesis with our industry-leading technology.

Synbio Technologies's Syno® HT synthesis platform can synthesize 890,000 oligos simultaneously on a semiconductor chip with single oligo lengths up to 300 nt. High synthesis efficiency can ensure high uniformity of the oligo pool, improving the efficiency and success rate of downstream high-throughput screening while perfectly matching customers' experimental needs and applications.

### Competitive Advantages

**High Throughput**
Up to 890,000 sequences are synthesized in parallel at a single time

**High Accuracy**
Error rate as low as 1:2,000/ nt

**High Coverage**
100% coverage of the target sequences

**High Homogeneity**
More than 90% of oligonucleotides vary within 2.5 times

**Cost-effective**
Multiple streamlined services available at minimal cost

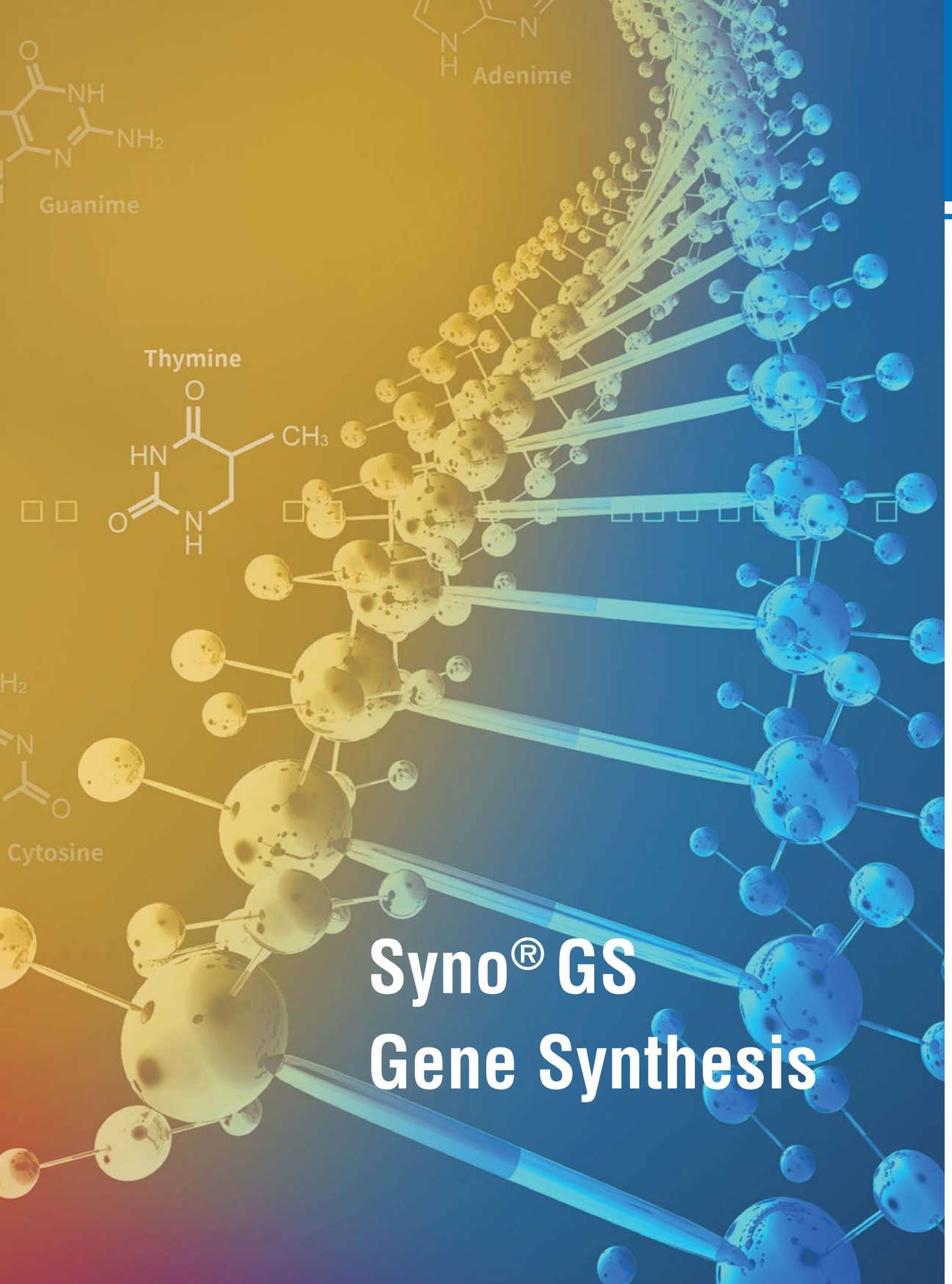
### Service Specifications

| Length (nt) | Turnaround Time (Business Days) |
|---|---|
| 20-120 | 10-15 |
| 121-150 | 10-15 |
| 151-200 | 10-15 |
| 201-250 | 15-20 |
| 251-300 | 20-25 |

### Multiple Downstream Applications

Synbio Technologies's oligo pool services offer our customers industry-leading high-throughput synthesis techniques as well as high-quality constructs. With the synthesized oligo pools, our customers can then streamline the various downstream applications within their research.

- Generate native DNA sequences
- Create *de novo* DNA sequences
- Build genes, chassis, operons, pathways, and genomes
- Build DNA variant libraries
- Improve the features of a protein
- Test all orthologs of a gene
- Optimize any antibody through affinity maturation
- sgRNA screening library
- shRNA library
- NGS hybridization capture probe library
- FISH probe




CHAPTER 2

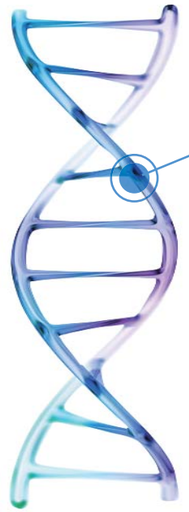
# Syno® GS Gene Synthesis

## Gene Synthesis

While Synbio Technologies offers many different services, our main focus is on DNA synthesis. With our professional research team and comprehensive Syno® systems, we can provide global customers with various types of DNA synthesis services, such as gene synthesis, gene fragment synthesis, pathway synthesis, small-genome synthesis, etc. We routinely synthesize over 3 million base pairs of DNA sequences each month for a wide range of applications including protein production, antibody discovery, genetically engineered vaccine, molecular breeding, biofuel, and many more.

- ►100% sequence accuracy guaranteed with success rate >99.5%
- ►AI-aided sequence analyses and proprietary NG™ codon optimization
- ►Industry's most competitive rate
- ►FREE to subclone into most commercial vectors
- ►FREE to deposit your vector for future use

**Gene Synthesis Services**



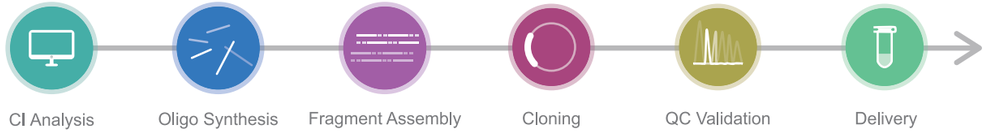
- Standard Gene Synthesis
- High Throughput Gene Synthesis
- NG™ Codon Optimization
- Vector Construction
- Small Genome Synthesis & Assembly
- Pathway Synthesis & Assembly
- Synstrands Gene Fragment Synthesis

CHAPTER 2

# Syno® GS Gene Synthesis

## 2.1 Standard Gene Synthesis

According to the DNA or amino acid sequences that customers provide us, our scientists will design the oligos and the procedure of fragment assembly and cloning within 30 minutes. By using our automated system that guarantees efficient and error-free synthesis, our customers will be provided with the highest quality output. Sanger sequencing will be conducted to verify 100% sequence accuracy.



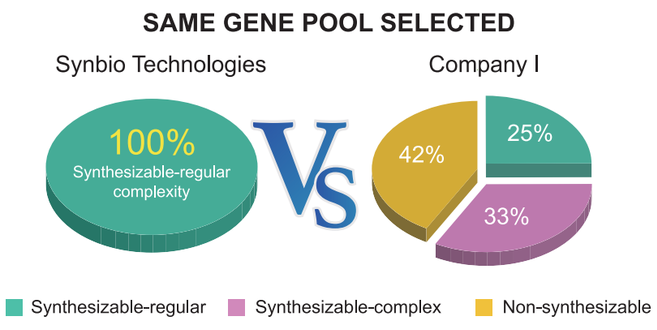

◆ **Service Specifications**

The following turnaround times only apply to non-complex sequences. Gene products will be cloned into our default pUC57-Amp or pUC57-Kan vectors with multiple cloning sites. We can also clone genes into customers' supplied vectors upon request.

| Gene Length | Turnaround Time (Business Days) | Standard Deliverables | Optional Services |
|---|---|---|---|
| < 250 bp | 5-10 | • 2-5 μg lyophilized plasmid DNA<br>• Sequencing chromatogram<br>• Certificate of analysis | • Free codon optimization<br>• Free vector deposit |
| 250-1,500 bp | 5-10 | | |
| 1,501-3,000 bp | 10-15 | | |
| 3,001-4,500 bp | 15-20 | | |
| 4,501-6,000 bp | 20-25 | | |
| > 6,000 bp | Quote | | |

* We offer discounts for bulk gene synthesis service. Please contact *quote@synbio-tech.com* for additional information.

Synbio Technologies independently developed a CI sequence difficulty index system, which can facilitate the accurate, fast, and high-quality synthesis of any sequence.






Find more information at
*www.synbio-tech.com*

## 2.2 Synstrands Gene/DNA Fragments

Synbio Technologies supplies our customers with double-stranded, sequence-verified Synstrands gene fragments in only a few business days. These fragments offer affordable and accessible construction or modification of genetic sequences. The various applications include antibody research and CRISPR-mediated gene/genome editing, being used as qPCR standards, etc. Synstrands gene fragments are available in two delivery forms: eppendorf tubes or plates.

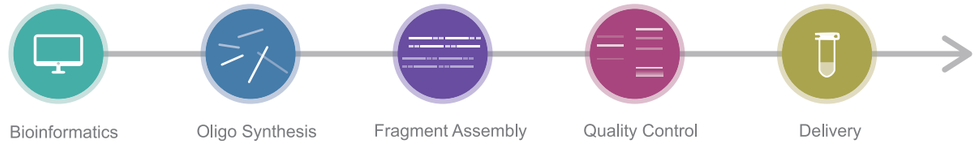
### Competitive Advantages

**Cost-Effective Approach**
Competitive prices without sacrificing the overall quality

**High Performance**
Compatible with all downstream clones

**High Efficiency**
Sanger sequencing verification, ultra-high success rate of downstream cloning

### Process of the Service




### Service Specifications

◆ **Normal Sequences**

| Length (bp) | Turnaround Time (Business Days) |
|---|---|
| <300 | 8-10 |
| 300-1,800 | 8-10 |
| 1,801-3,200 | 10-12 |

## 2.3 Pathway Synthesis & Assembly

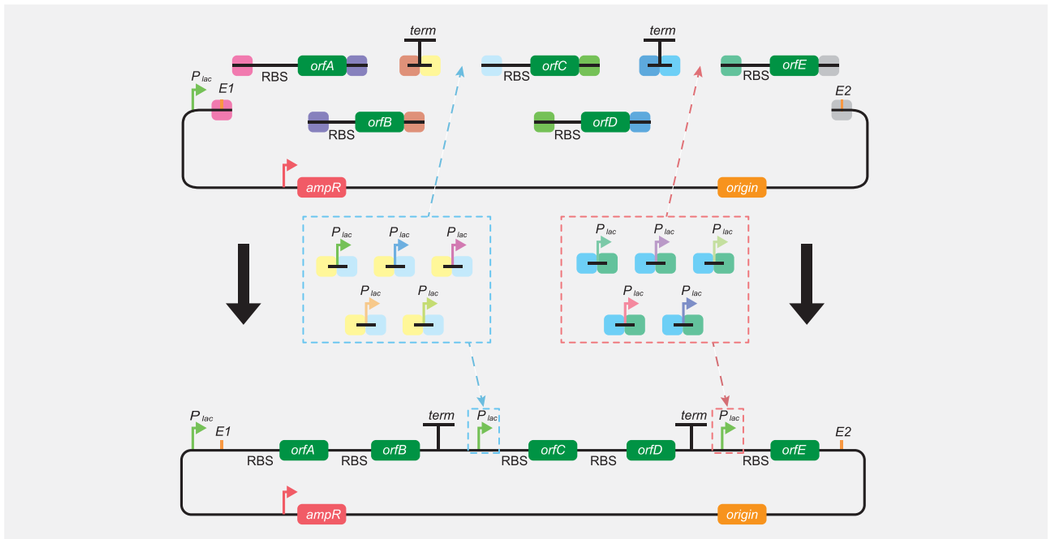
The synthesis and construction of metabolic pathways, as well as diverse DNA assembly technologies, play fundamental roles in microbiology, biochemistry, and many other relevant fields. With their rapid development, the price of large DNA fragment or plasmid synthesis is decreasing year by year. Based on our Syno® gene synthesis tools and excellent long DNA assembly capability, Synbio Technologies is confident in providing professional technical support for metabolic pathway synthesis and library assembly.

### Services Specifications

| Services | Details | Turnaround Time | Delivered Products |
|---|---|---|---|
| Metabolic Pathway Synthesis | Customized Metabolic Pathway Synthesis Services | <10 Kb, 4-8 weeks<br>>10 Kb, Inquiry | Plasmids with synthesized metabolic pathway and glycerol bacteria |
| Metabolic Pathway Library Assembly | Customized Metabolic Pathway Library Assembly Services | Varies depending on the pathway requested | Plasmid library after assembly and glycerol bacterial library, or monoclonal plasmids and glycerol bacteria |

### Case Study

The promoter library's diversity determines the final diversity of the constructed library. The theoretical library capacity is 100, with 100 times coverage, leading to the production of 10,000 clones. Our PCR test of the bacterial solution showed 78% positive and 100% accuracy.






## 2.4 Small Genome Synthesis & Assembly

The rapid development of synthetic biology has allowed for researchers to place greater emphasis on powerful concepts and techniques within the field itself. These techniques include the comprehensive analysis of complex pathways and the re-design of natural biological systems, such as Gibson Assembly. Currently, Gibson Assembly is the most popular mechanism for achieving directed cloning and *in vitro* multiple-segment assembly. However, Gibson Assembly is limited to plasmids around 10 Kb or less in length, which largely restricts its applications within certain aspects of synthetic biology.
Several researchers discovered that assembly in yeast has the potential to conveniently assemble large DNA segments with ease. This is because yeast can connect multiple DNA segments automatically without DNA polymerases and ligases. This important aspect of yeast made it possible to circumvent the length restriction associated with Gibson Assembly, and efficiently construct a plasmid over 10 kb in length. For this reason, yeast homologous recombination technologies are greatly beneficial for efficient and effective assembly of long DNA fragments and genomes.

### Competitive Advantages

**01**

Powerful gene synthesis platforms provide one-stop solutions for yeast assembly platforms such as gene synthesis, assembly, and sequencing

**02**

Synthesis and assembly of long DNA fragments with high fidelity and speed. Synbio Technologies can deliver single gene sequences up to 150 Kb, guaranteeing reliable assembly of long DNA fragments

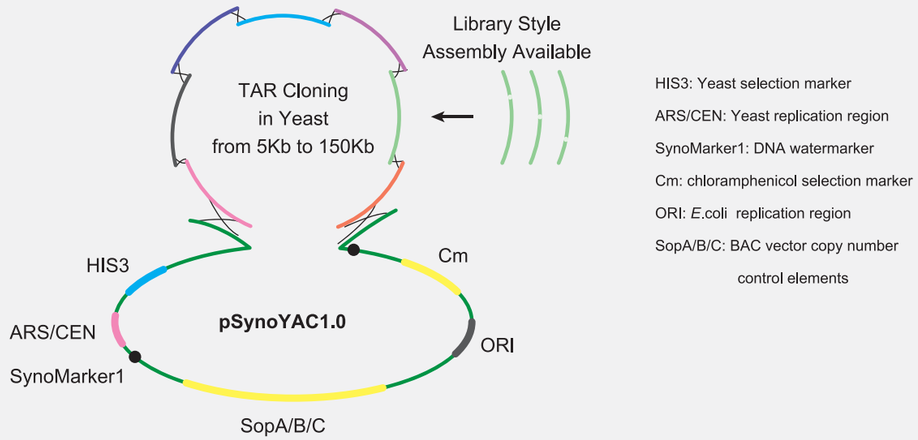
### Services Specifications

| Services | Details | Turnaround Time | Deliverables |
|---|---|---|---|
| Synthesis of gene clusters and small genomes | Customized service for metabolic pathway synthesis | <10 Kb, 4-8 weeks<br>>10 Kb, Inquiry | Synthethized gene clusters, plasmids withsmall genomes, and glycerol bacteria |

CHAPTER 2

# Syno® GS Gene Synthesis

By utilizing yeast homologous recombination technology, Synbio Technologies is able to provide one-stop services for long gene synthesis/assembly and gene cluster/small genome synthesis and assembly.




HIS3: Yeast selection marker
ARS/CEN: Yeast replication region
SynoMarker1: DNA watermarker
Cm: chloramphenicol selection marker
ORI: *E.*coli replication region
SopA/B/C: BAC vector copy number control elements

**Ultra DNA Assembly Technologies**

- Diagram of the synthesis and assembly platform of gene clusters and small genomes -



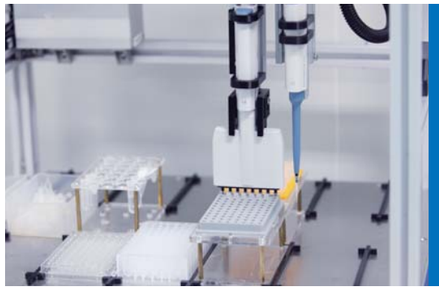
# DNA Library Synthesis

CHAPTER 3

# DNA Library Synthesis

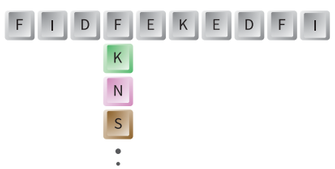
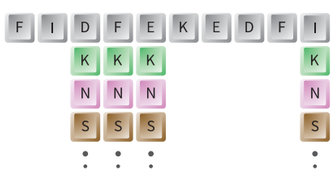
## DNA Library Synthesis

Synbio Technologies offers high quality DNA libraries of any size and complexity with an efficient time frame and at the industry's most competitive rate. Our synthetic DNA libraries are designed by an experienced bioinformatics team to facilitate various research applications, including functional genetics, directed evolution, protein engineering, antibody studies, etc.

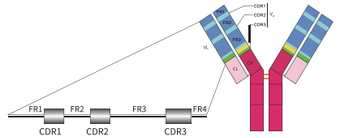
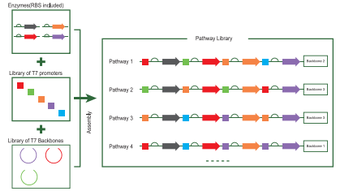
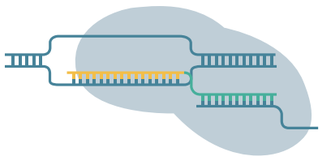


- ► Proprietary synthesis platforms guarantee the successfully synthesis of multiple libraries
- ► High-quality libraries can be accurately synthesized with capacity from $10^6$ to $10^9$, coverage rate>99%, uniformity index<10, NGS verification accuracy>95%
- ► Flexible deliverables: oligo pools, PCR fragments, pooled plasmids, plasmid arrays, etc

### Library Types

| Library Types | Descriptions | Schema |
|---|---|---|
| **Alanine Scan Library** | Research on amino acid residues critical to protein function, interaction, and shape by replacing an individual amino acid with alanine at every position. |  |
| **Site-Saturation Mutagenesis Library** | Mutate only one site at a time within a sequence, it can be substituted to all 20 possible amino acids or specified codons. |  |
| **Combinatorial Mutagenesis Library** | Mutate multiple sites within a sequence, it can be defined by the customer's desired amino acid types and ratios at each site. |  |



| Library Types | Descriptions | Schema |
|---|---|---|
| **Antibody Library** | Create a high diversity synthetic antibody library or improve existing antibody functionality such as specificity, immunogenicity, affinity, expression, and/or aggregation. |  |
| **Modular Substitution Library** | Build new genetic modules, regions, or pathways from DNA parts including regulatory elements and/or coding sequences. |  |
| **CRISPR Library** | Customized CRISPR library and human genome CRISPR knockout library meet the screening needs of different customers. |  |

CHAPTER 3

# DNA Library Synthesis

## 3.1 Precision Mutant Libraries

*In vitro* synthesis of precision mutant libraries are one of the common methods in molecular directed evolution. Researchers can change specific protein sites or DNA regulatory regions according to different purposes, while controlling the diversity of sequences and improving screening efficiency. With more than ten years of experience in precision mutant library synthesis, Synbio Technologies provides customers around the world with efficient and accurate precision mutant library synthesis services. We are committed to providing customers with efficient and cost-effective solutions for downstream research, such as protein characteristic improvement, protein directed evolution, antibody sequence optimization, affinity maturation, target discovery, etc.

### Competitive Advantages

- Accurate Sequence Control
- High Synthesis Efficiency
- Cost-Effective Solution
- Highly Customized

### Services Specifications

| Library Type | Complexity Analysis | Diversity Analysis by Sequencing | Turnaround Time (Business Days) | Deliverables |
|---|---|---|---|---|
| Site-Saturation Mutagenesis Library, eg.NNK Library | √ | √ | 20-40 | • 50-1000 μg plasmids<br>• COA files |
| Combinatorial Mutagenesis Library, eg Trimer Library | √ | √ | 20-40 | |





### Degenerate Mutation Library

Genetic codons have degeneracy, that is, an amino acid can be encoded by more than one triplet codon. Using the degeneracy of genetic code can not only maintain the diversity of amino acid sequences, but also reduce the redundancy of codons and improve screening efficiency.

### Competitive Advantages

**01**

Quick & efficient product delivery to promote downstream screening

**02**

The synthesis technology is stable and the quality control is strict

**03**

100% coverage of 20 amino acids fully ensures the diversity of the library

### Case Study

The NNK library is a classic degenerate mutation library. The NNK degenerate primers used contain 32 (4x4x2) codon combinations (N= A/C/G/T, K= G/T), covering all 20 amino acids, and can saturate amino acids at any site.

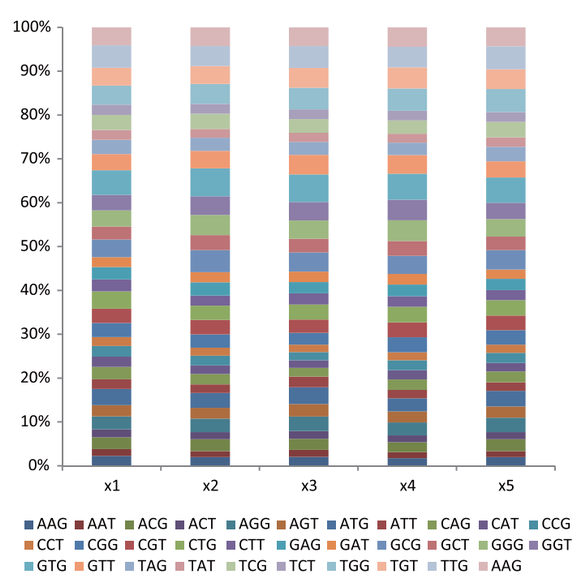



Fig.1 The proportion of each codon contained in the five NNK sites in the NNK library

CHAPTER 3

# DNA Library Synthesis



## Trimer library

Primers synthesized with a single nucleotide will inevitably produce redundant mutations and termination codons at the mutation site. The trimer phosphoramidites formed by connecting the three nucleotides in a fixed order can correspond to a single amino acid. It can not only introduce the mixture of all 20 amino acids at any position of the mutated sequence, but also avoids the problems of codon offset and the incorporation of termination codons.

Precise mutant libraries designed with the trimer mixture as the element, also known as trimer libraries, can ensure that the amino acids at the mutation site are composed according to the designed ratio to meet the amino acid diversity and codon preference of the specific site. Trimer libraries are an efficient solution for complex libraries and can further improve the accuracy of library screening.

### Competitive Advantages

**01**

Cost-effective solutions for complex libraries

**02**

Reduces difficulty of downstream screening

**03**

High synthesis efficiency and accurate sequence control

### Case Study 1

Trimer libraries can be used for site saturation mutation to distribute 20 amino acids in equal proportion, further reducing codon redundancy and removing termination codons in the library.

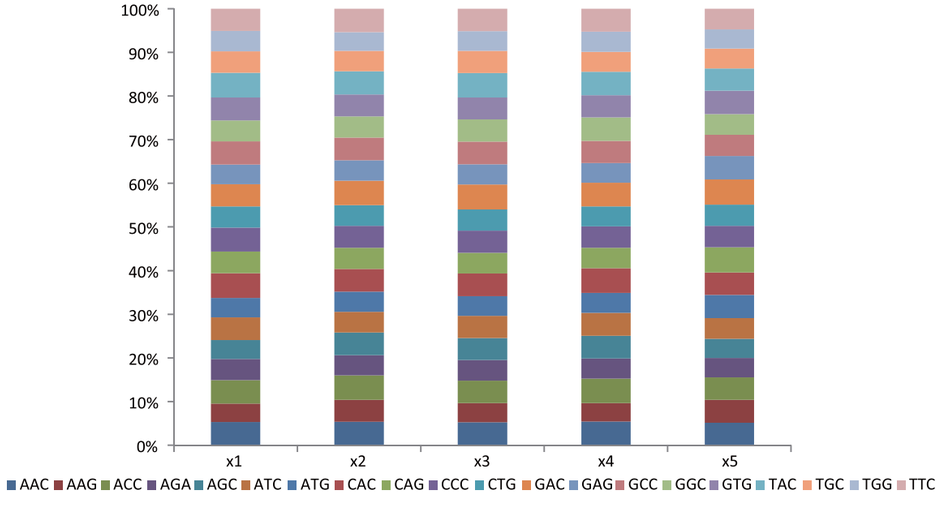



Fig.1 Trimer libraries are also a saturated mutation library with equal proportion distribution of each amino acid. The proportion of each codon contained in the five sites.



### Case Study 2

Trimer libraries can also customize each site to be composed of different ratios of amino acids. Among the four Trimer sites, for example, the X1 Trimer site is A (80%) : G (3%) : R (3%) : S (3%): T (8%) : V (3%)

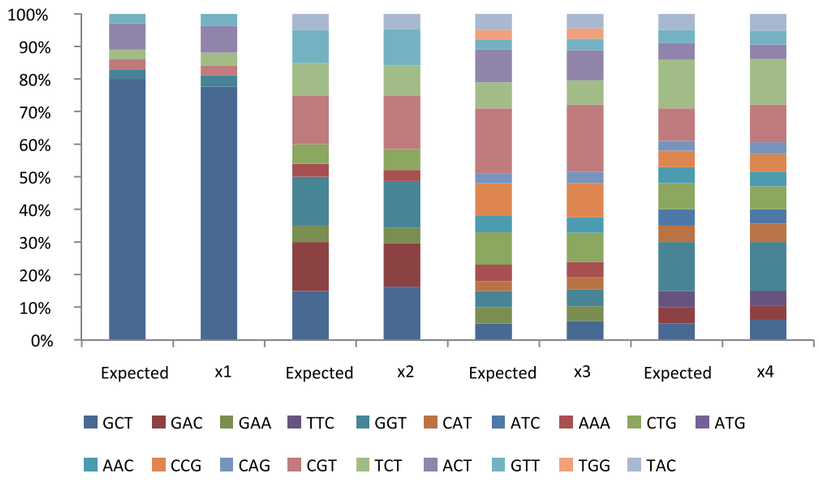



Fig.2 Types and proportions of custom amino acids

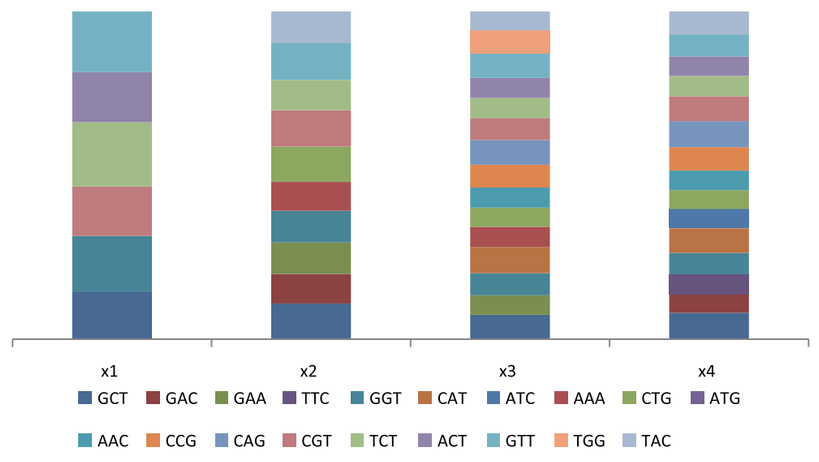



Fig.3 Ratio between actual and theoretical proportion of each amino acid

## 3.2 CRISPR sgRNA Library

CRISPR-Cas9 technology has become a powerful tool for forward genetic screening. Screening libraries of various functions can be constructed by CRISPR, and the genes related to this function are then identified through functional screening and enrichment, PCR amplification, and deep sequencing analysis.
Synbio Technologies can provide one-stop services such as sgRNA design, oligo pool synthesis, sgRNA library construction, NGS validation, virus packaging, and bioinformatics analysis. With our professional CRISPR-Cas9 sgRNA library system, we can construct sgRNA libraries in a fast and efficient manner.

### Competitive Advantages

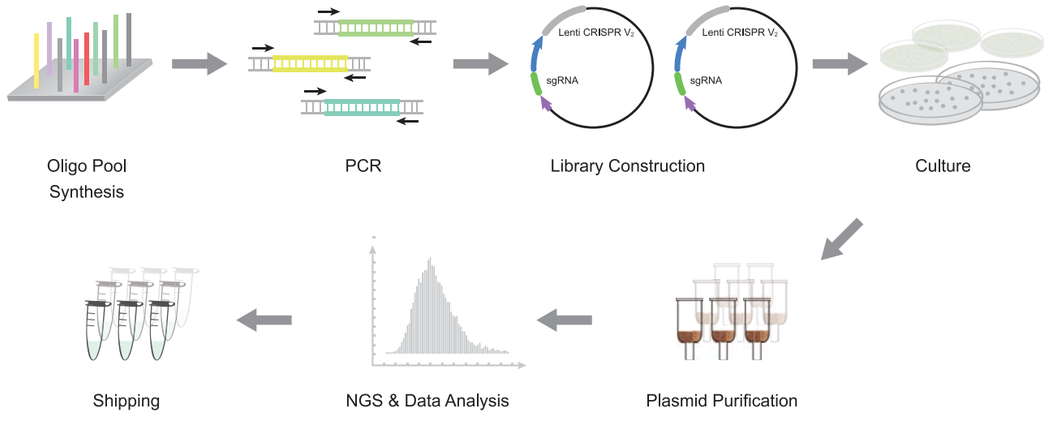
**01**
Advanced sgRNA Design
Design of sgRNA of dozens of species

**02**
High Efficiency
Designed sgRNA has a low off-target rate and shows strong editing efficiency

**03**
Proprietary DNA Synthesis Platform
Fast and efficient sgRNA library construction

**04**
One-Stop Solutions
Customized services ranging from sgRNA design, oligo pool synthesis, sgRNA library construction to lentivirus packaging and NGS sequencing

### CRISPR Cas9 sgRNA Library Process




### Service Specifications

| Service Steps | Services Specifications | Turnaround Time |
|---|---|---|
| sgRNA Design | sgRNA sequences were designed for specific genes.<br>Each gene will be designed with 3-6 sgRNAs. | 1-2 weeks |
| Oligo Pool Synthesis | All designed sgRNAs and negative controls will be synthesized on one chip | 3-4 weeks |
| sgRNA Library Construction | Cloning these sgRNAs on the target vector | 2-3 weeks |
| Plasmid Purification | Plasmid preparation to meet the shipping need | 1 week |
| Library Quality Evaluation | 1. Library coverage is greater than 100 times<br>2. Cloning accuracy rate is more than 80%<br>3. Library accuracy rate of NGS verification is greater than 90%<br>4. Library uniformity index is less than 10 | 2-3 weeks |
| Total | | 9-13 weeks |

## Syno® Human Genome CRISPR Knockout Libraries

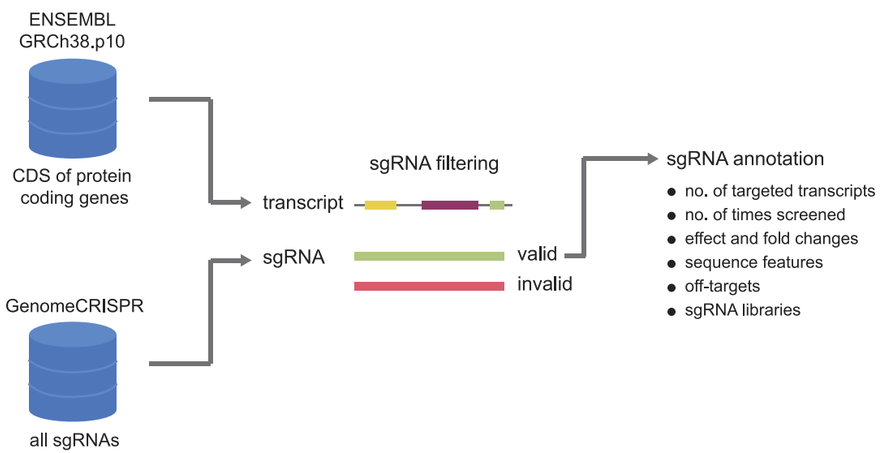
Synbio Technologies focuses on the research of CRISPR gene editing technology, continuously working to increase the number of services and products offered. Our Syno® Human Genome CRISPR Knockout Libraries select sgRNA sequences with a high score according to the phenotypes in the previously published CRISPR screening recorded in the Genome CRISPR database, based on the criteria of high target activity and low off-target efficiency combined with a variety of different design principles. According to recently published scoring rules, Syno® CRISPR libraries scored better than GeCKOv2 libraries and randomly selected published sgRNA samples.

Syno® Human Genome CRISPR Knockout Libraries consist of three independent sub libraries: A, B, and C. Each gene in Library A and B contains four sgRNA sequences for 18,913 and 18,334 protein coding genes respectively. Sub library A contains the best ranked sgRNA according to the design criteria to achieve high-quality screening under low library coverage. Sub Library B contains a second layer of sgRNA, which can be used to supplement sub library A when higher sgRNA coverage is required.

By integrating the standardized resources of large-scale CRISPR screening and multiple efficiency indicators, sub Library C evaluates and sorts more than 300,000 unique sgRNAs in the CRISPR library, and designs an optimized human minimum genome knockout library. For 18,761 genes, 2 optimized sgRNAs are designed for each gene, in total, there were 37,522 genes targeted and 200 non-targeted sgRNAs. The size of sub Library C is smaller than the currently published human genome CRISPR library. Although the sub Library C is small, it does not affect the identification of necessary genes. It is mostly used for CRISPR screening of cancer cell models under complex conditions.

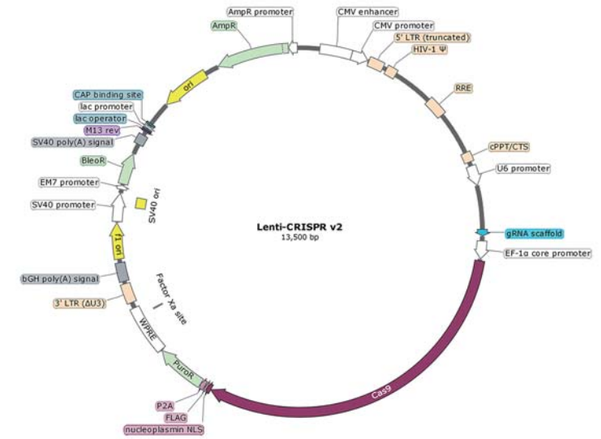
### Competitive Advantages

1. Accurate sgRNA Design: sgRNA sequences with high target activity and low off-target rate are designed by using effective sgRNA design models and deep learning algorithms.






2. Optimized Vector: LentiCRISPRv2 vector with Puro drug screening function can be used for lentivirus packaging and improve transfection efficiency.




3. Cost Saving: High editing efficiency CRISPR library reduces the difficulty of later screening and further reduces research cost.

4. Strict Quality Control: The coverage of the constructed library is > 99.9%, and the uniformity is < 10.

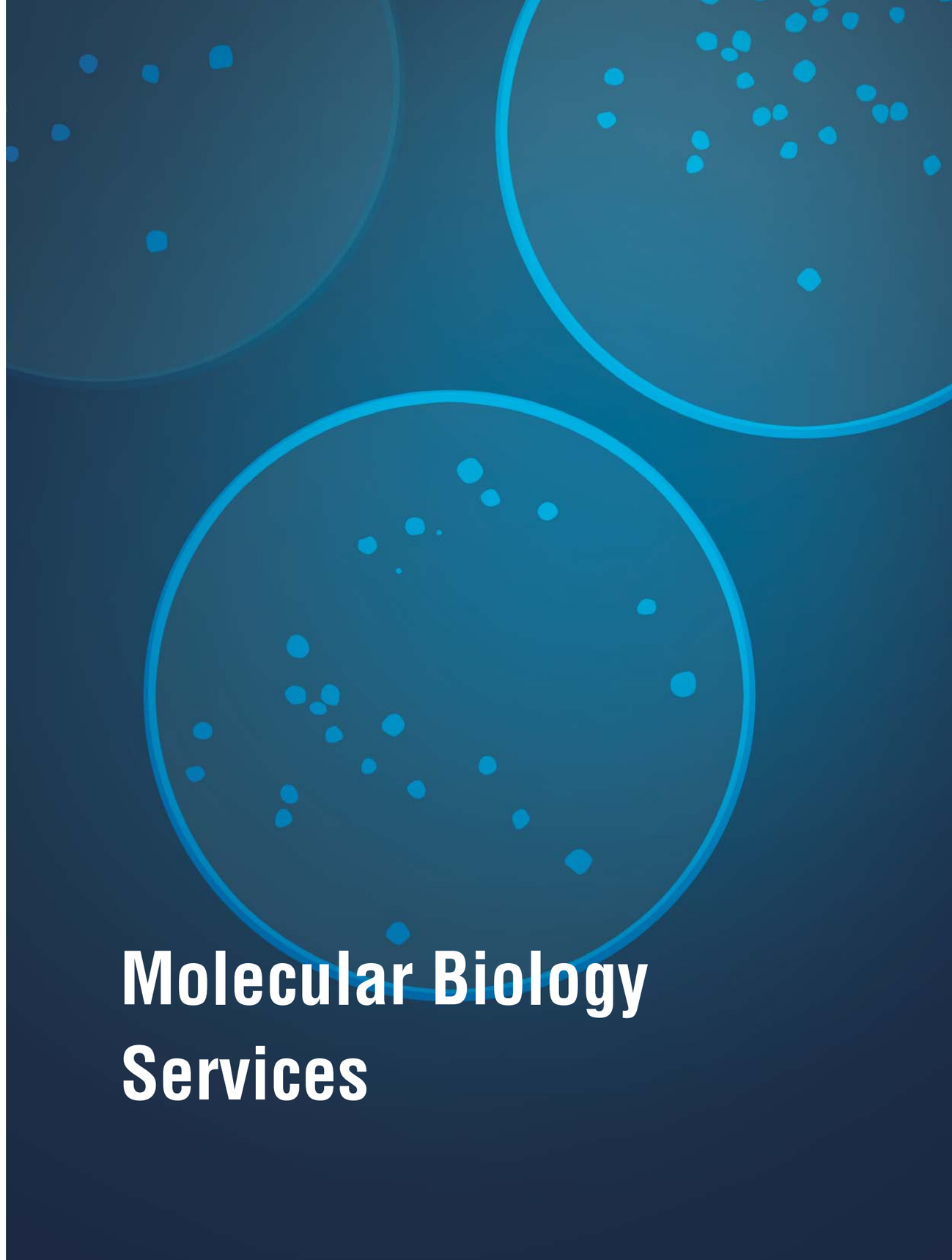
CHAPTER 3

# DNA Library Synthesis

## Service Specifications

| Catalog | Product Name | Sequence Information | Vector Information | Specifications |
|---|---|---|---|---|
| STCRI211101 | Syno® Human Genome CRISPR Knockout Libraries | Sub library A 18,913 genes are targeted, 74,552 sgRNA sequences and 435 control (non-targeted) sgRNA sequences are designed, with a total of 74,987 sgRNA sequences. | lentiCRISPR v2 (with Puro mark) | 200 μg |
| STCRI211102 | | Sub Library B 18,334 genes are targeted, 71,048 sgRNA sequences and 435 control (non-targeted) sgRNA sequences are designed, with a total of 71,483 sgRNA sequences. | lentiCRISPR v2 (with Puro mark) | 200 μg |
| STCRI211103 | | Sub Library C 18,761 genes are targeted, and 2 sgRNA sequences are designed for each gene, a total of 37,522 sgRNA sequences and 200 control (non targeted) sgRNA sequences. | lentiCRISPR v2 (with Puro mark) | 200 μg |





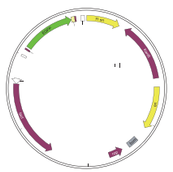
CHAPTER 4

# Molecular Biology Services

Molecular biology is the cornerstone of many life science research fields, such as biopharmaceutical and human biology. For years, molecular biology technologies have been applied to various fields of biological research and development including molecular mechanisms, drug interactions, targets and effects, etc.

Synbio Technologies provides a wide range of molecular biology services to meet our customers' specific requests and research needs. By relying on revolutionary breakthroughs in DNA synthesis and engineering technologies, our comprehensive services range from plasmid DNA preparation and site-directed mutagenesis to vector construction. These services have allowed Synbio Technologies to become one of the premier companies within the molecular biology technology industry.

Compared to the "do it yourself" process within molecular biology services, Synbio Technologies is interested in saving our customers from consuming too much time and energy in laborious operations with fluctuating, sometimes unreliable, results. Our molecular biology services provide our customers with cost-effective and efficient final products. Synbio Technologies can decrease your overall research time and save your lab on both cost and headaches. This process allows our customers to focus on various other research topics in your field of interest, while Synbio Technologies prepares your orders.

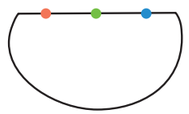


**Plasmid DNA Preparation**

- Fast delivery: starting at one day
- Low endotoxin level: <5 EU/mg
- Diversity of scale: microgram to gram
- ISO 9001 quality management

**PCR Cloning & Subcloning**

- 100% sequence accuracy
- Customizable service: no limits
- Save your time & budget
- Professional technical team



**Site-Directed Mutagenesis**

- Short TAT: starting at 5 business days
- Vast region: ≤40 bp region as one mutation
- Precise mutation: error-free
- Large-scale production, such as performing alanine scan library





## 4.1 Plasmid DNA Preparation

For years, Synbio Technologies has provided comprehensive plasmid DNA preparation services to our customers from research institutes in both biotechnology and biopharmaceutical industries.

Synbio Technologies conducts comprehensive quality control of our plasmid products to provide you with aseptic plasmids with no RNA pollution or genome pollution. All of our manufactured plasmids are free of animal-derived materials and can contain low levels of endotoxin (<100EU/mg, <30EU/mg, <5EU/mg, on request). According to our customers' different application needs, standard plasmid DNA preparation services are divided into two levels, the research level and the transfection level. The plasmid DNA preparation process is designed to meet our customers' diverse downstream applications such as transfection, antibody preparation, vaccines, gene therapy research, etc.

### Competitive Advantages

**01**
Express and Guaranteed Plasmid Preparation Services
USA-based manufacturing allows high quality products and fast delivery

**02**
Highly Customized
Microgram-to-gram-scale quantity can meet various customers' needs

**03**
Stable quality with low endotoxin level

**04**
Batch-to-batch consistency

### Experimental Procedure

CHAPTER 4

# Molecular Biology Services

### Service Specifications

| | Research Grade | Transfection Grade | TAT | Deliverables |
|---|---|---|---|---|
| Midi (100 μg) | Endotoxin: NA | Endotoxin: < 100EU/mg | 2-4 BDs | • Prepared plasmid DNA<br>• Certificate of analysis (COA) |
| Maxi (200-500 μg) | | Endotoxin: < 30EU/mg | 2-4 BDs | |
| Mega (1 -10 mg) | | Endotoxin: < 5EU/mg | 3-5 BDs | |
| Giga (>10 mg) | | | Starting from 5 BDs | |

This form is used for high-copy plasmids. Fore low-copy plasmids, please inquire *quote@synbio-tech.com*.

Please Note:
1. Sanger sequencing and restriction enzyme analysis are available per request.
2. If you wish to provide the plasmid template, you will need to prepare a sample over 1μg (diluted with $ddH_2O$ or TE buffer), colonies (fresh), or bacteria stored in glycerol.
3. Synbio Technologies also provides upstream services of plasmid preparation such as gene synthesis and subcloning into both commercial and custom vectors. This allows us to offer a unique approach to satisfy all our customers' requests.

### Quality Control

Synbio Technologies's plasmid preparation service focuses on quality. We have strict quality control which can increase quality, stability, and reduce batch difference to satisfy customers' high-quality needs.

| Quality Control | Standard Range | Measurement Technique |
|---|---|---|
| Appearance | Clear, transparent | Visual inspection |
| A260/280 Analysis | 1.80-2.00 | UV spectrophotometer |
| Concentration | Up to 5mg/ml, default 1±0.05 mg/ml | UV spectrophotometer |
| Supercoil Percentage Analysis | >95% | Density scan on agarose gel |
| RNA Residue Analysis | Not detectable | Visual inspection on agarose gel |
| Genomic DNA Analysis | Not detectable | Visual inspection on agarose gel |
| Sterility Assurance | No clone growth on LB plate >48 h | Incubate 0.1 mg of product on LB plate for >48 h |
| Restriction Enzyme Analysis (optional) | As expected, no other minor band | Incubate with enzyme and analyze on agarose gel |
| Sequence Verification (optional) | Correct | DNA sequencing |
| Endotoxin Analysis (optional) | <100EU/mg, <30EU/mg, <5EU/mg, on request | Endotoxin analysis assay |





## 4.2 PCR Cloning & Subcloning

PCR cloning and subcloning are two main approaches to amplifying DNA sequences. Synbio Technologies is confident in our ability to clone any target gene into any requested site of any vector on the basis of our Syno® Platform. The combination of the DNA synthesis tool and the cloning technology allows us to insert the requested genetic sequence into any customer-specific vector for cloning.

During PCR cloning, Synbio Technologies offers sequence verification before introducing target gene materials into selected vectors. This sequence verification allows us to verify the sequence to be 100% accurate to the customer's request before amplification. According to the model clone and sequence from customers, we will design the necessary primers, clone the PCR product of interest into a new vector, and finally verify it by Sanger sequencing. This allows us to tailor-make a system to supply our customers with the exact specifications necessary to fit their particular research interest.

### Competitive Advantages

**100% Sequence Accuracy**

We will quickly and accurately synthesize your requested sequence. The generated sequence will then be verified by Sanger sequencing to guarantee 100% sequence accuracy

**Customizable Subcloning**

Guarantee successful cloning at any site in any vector system of interest, both commercial and custom, with special amplified primer design

**Professional Technical Team**

Synbio Technologies's professional technical support offers you proactive communications on your project's progress

### Service Specifications

| Service Type | Fragment Length | Turnaround Time (Business Days) | Deliverables |
|---|---|---|---|
| PCR Cloning & Subcloning | <1 Kb | 5-10 | • 2-5 μg Lyophilized plasmid DNA<br>• Sequencing chromatogram<br>• Certificate of analysis (COA) |
| | 1-2 Kb | 5-10 | |
| | 2-3 Kb | 5-10 | |
| | >3 Kb | Inquire | |

* Note: The turnaround time applies to non-complex sequences.
Please contact *quote@synbio-tech.com* to quote for the complex sequences.

CHAPTER 4

# Molecular Biology Services

## 4.3 Site-Directed Mutagenesis Service

If the specific site on your gene or vector is not what you want or you need to carry out functional studies of genes or elements, you may need to do a point mutation on a specific site or multiple sites. Site-directed Mutagenesis is a very useful tool for gene research, which can efficiently change the character and characterization of the target protein. Generally, we introduce site-directed mutagenesis through PCR, which can include insertion, deletion, or point mutation. Site-directed Mutagenesis technology has been widely used when studying protein functional site structure, enzyme activity optimization, DNA component function or component interaction, and gene therapy.

### Competitive Advantages

**Short Turnaround Time**

Common sequences can be delivered to you within 5 business days

**Vast Operating Region**

All mutations found within a 40 bp region will be considered as one whole mutation; This helps our customers save on the overall cost of their project

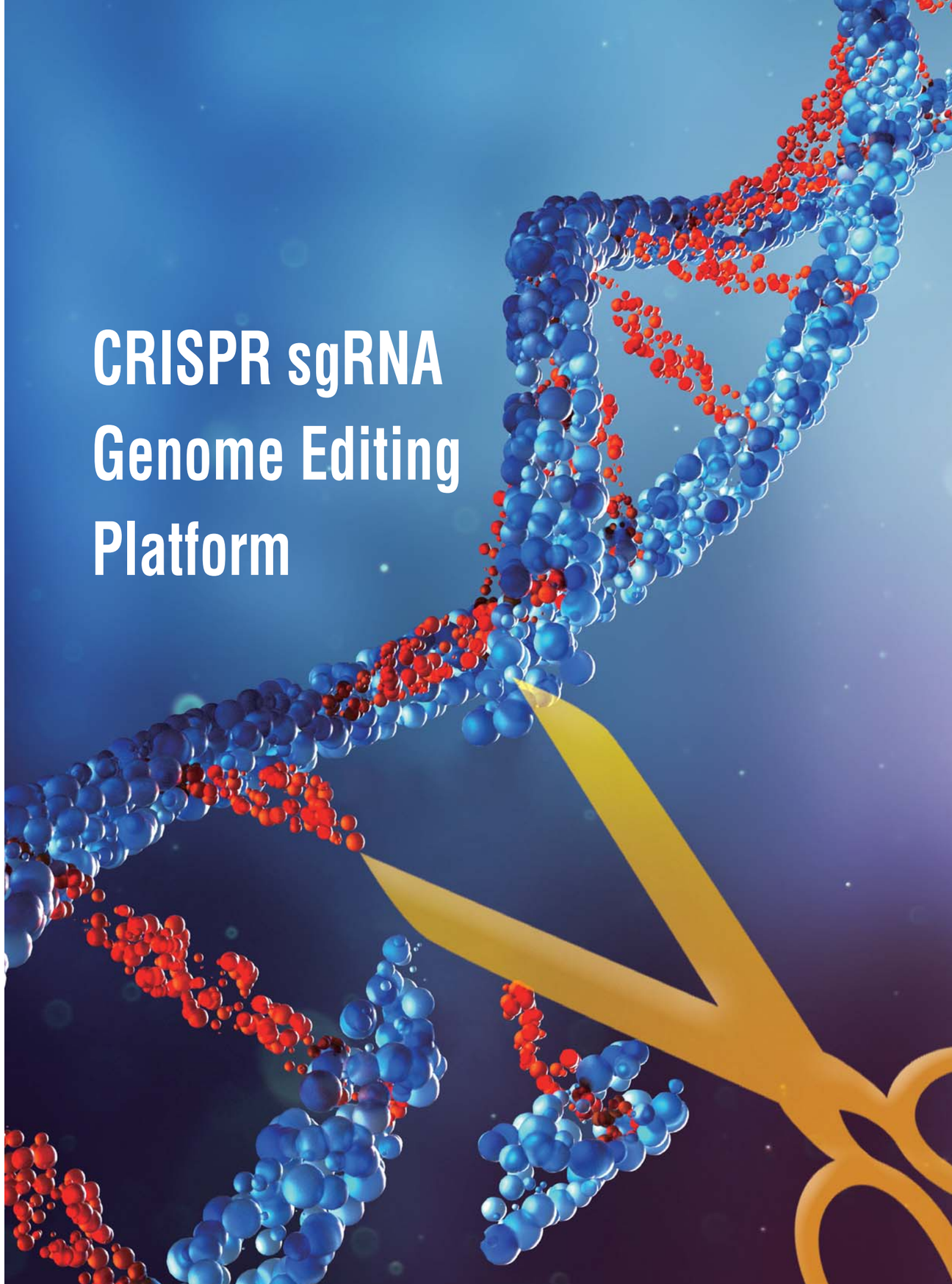
**One-stop Solutions**

We provide one-stop solution services, including: gene synthesis vector construction and protein expression and purification, which will certainly improve efficiency

### Service Specifications

| Cloned Fragment Length | Point Mutation Amounts | TAT (Business Days) | Deliverables |
|---|---|---|---|
| <2 Kb | 1 | 10 | • 2-5 μg lyophilized plasmid DNA<br>• Sequencing chromatogram<br>• Certificate of analysis (COA) |
| | 2 | 10 | |
| | 3 | 10 | |
| 2-3 Kb | 1 | 13 | |
| | 2 | 13 | |
| | 3 | 13 | |
| 3-5 Kb | 1 | 16 | |
| | 2 | 16 | |
| | 3 | 16 | |



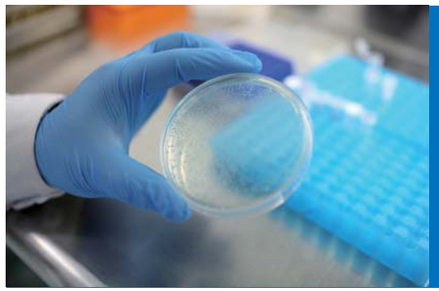


CHAPTER 5

# CRISPR sgRNA Genome Editing Platform

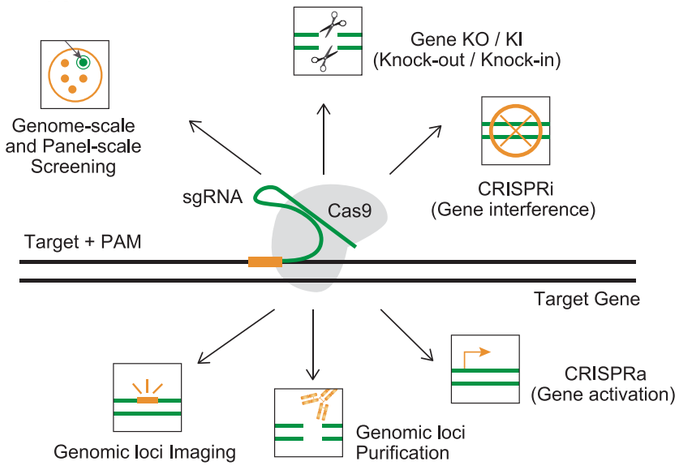
CRISPR-Cas9 is a major breakthrough after TALEN. The CRISPR-Cas9 system is a prokaryotic immune system driven by RNA that triggers resistance to foreign genetic elements and provides a form of acquired immunity. sgRNA serves as a guide to recognize and combine the target with DNA, allowing activated Cas9 nuclease to attach, cleave, and modify the PAM downstream target DNA. CRISPR-based genome editing technology has made it easier and faster than ever to alter specific DNA sequences in the genome or to perfom genome-wide functional screening tests to identify genes involved in a particular phenotype. CRISPR-Cas9 is considered the most promising tool in gene modification.
Synbio Technologies's CRISPR-Cas9 technology, combined with our patented synthesis and assembly platform, allows for efficient editing of genes/genomes.

## Competitive Advantages



- ► Professional team offers customized design of sgRNA for gene family or genome region, without gene sequence or species restrictions
- ► Comprehensive services, including ready-to-use sgRNA, sgRNA vector construction, sgRNA library construction services, etc.
- ► One-stop solution from design, construction, to gene editing

## CRISPR-Cas9 Applications

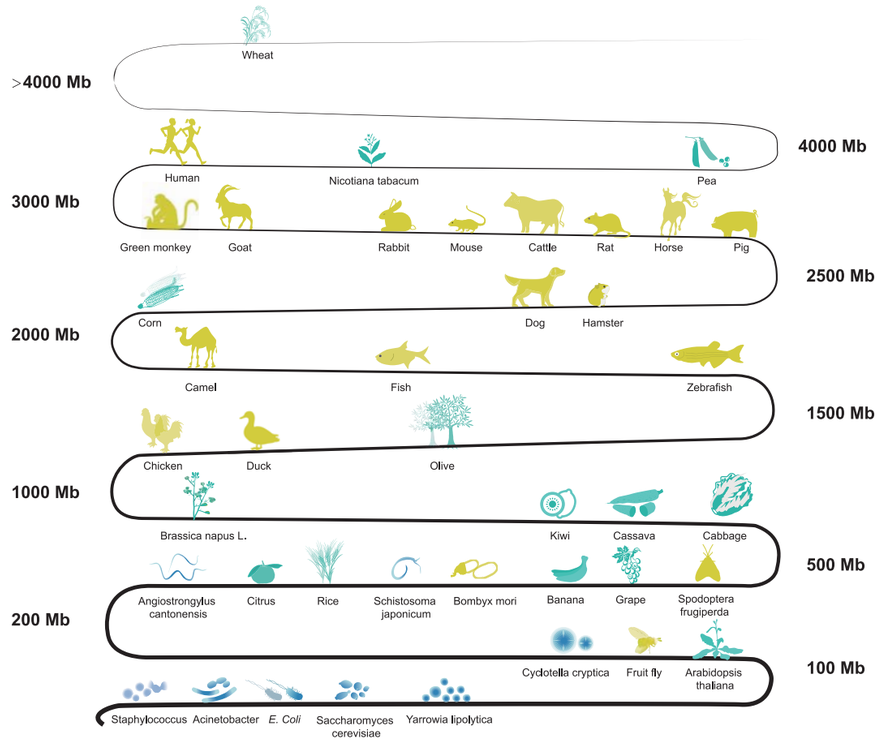





Find more information at
*www.synbio-tech.com*

## 5.1 CRISPR sgRNA Design Center

The sgRNA design center of Synbio Technologies provides services such as single sequence sgRNA design or whole-genome sgRNA library design, downstream verification, and stable cell line construction. We offer a one-stop solution for CRISPR-Cas9 projects to achieve high genome editing efficiency.




## Competitive Advantages

**Professional Design Center**
We provide single sgRNA, multiple sgRNA, and sgRNA library design services

**Experienced**
Well-designed sgRNA sequences with lowest off-target rate; Professional analysis is also provided to ensure the success of each project

**Applicable in Many Species**
Database covers sgRNA design for >20 different species; Specific sgRNA design is also available upon request for certain species

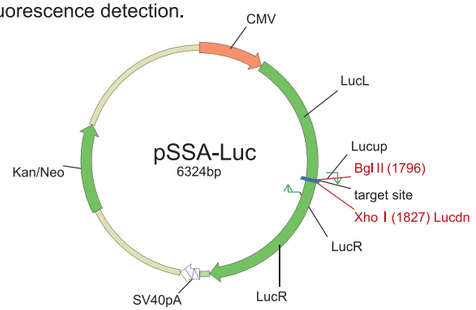
**Custom Vector Construction**
sgRNA can be designed in any vector that customers require

# 5.2 CRISPR System Activity Detection

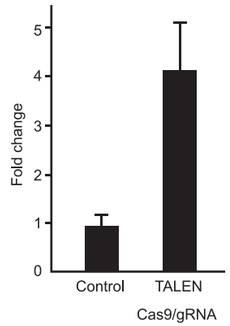
In the CRISPR-Cas9 system, different guide RNA (gRNA) can be designed based on the target gene. The DNA cutting efficiency of Cas9 can vary depending on the gRNA and target gene. It is therefore important to be able to accurately verify the activity of the CRISPR-Cas9 system in a given assay, in order to maximize the cleavage and gene knock-in/knock-out efficiency of CRISPR-Cas9. Synbio Technologies provides SSA activity assays, *in vitro* cleavage activity assays, and endogenous activity assays to detect gRNA activity.

## SSA Activity Assays

Single-strand annealing (SSA) assays can verify whether the target gRNA plasmid can mediate Cas9 to cut naked DNA. It is a common method to assess the activity of CRISPR-Cas9. The plasmid-based SSA assay contains two inactive luciferase-encoding fragments, which contain a stop codon and a segment of gRNA in the middle of the target sequence. If CRISPR-Cas9 can recognize and cleave the target site, the resultant double-stranded break can be repaired by the SSA mechanism, rescuing the now-active luciferase gene. Thus the SSA assay can be used to predict the cleavage activity of CRISPR-Cas9 by fluorescence detection.



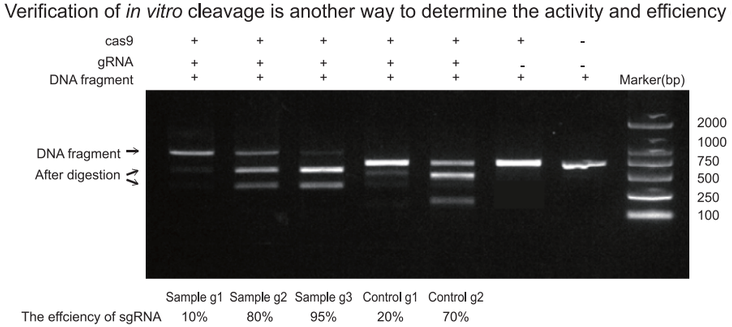

Construction of plasmid-based SSA




Results of SSA assays

## Verification of *in vitro* Cleavage Assay

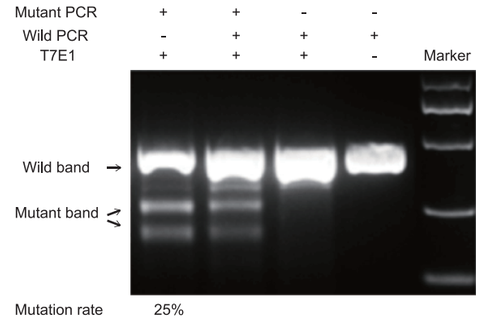
The target DNA sequence is digested with Cas9 *in vitro* to obtain two DNA fragments. Agarose gel electrophoresis allows analysis of gRNA-mediated DNA cleavage by comparing the yield of the smaller, digested fragments to the remaining undigested target DNA. Verification of *in vitro* cleavage is another way to determine the activity and efficiency of CRISPR-Cas9.






## Endogenous Activity Assay

Mismatch endonuclease can also be used to detect sgRNA activity. The suitable primers are first designed on both sides of the target, followed by the PCR amplification of the DNA containing potentially mismatched mutation sites. T7E1 endonuclease can recognize and cleave mismatched DNA heteroduplexes. The digested product is finally analyzed by agarose gel electrophoresis to estimate the ratio of the cleavage and un-cleaved bands, reflecting the activity of CRISPR-Cas9.




## Service Specifications

| Services | Details | TAT | Deliverables |
|---|---|---|---|
| SSA activity assay | • gRNA target primer design and synthesis<br>• pSSA-luc vector construction<br>• Cell transfection | Inquiry | Assay Report |
| *In vitro* cleavage activity assay | • *In vitro* transcription gRNA<br>• CRISPR-Cas9 *in vitro* cleavage reaction | | |
| Endogenous Activity Assay | • Genome extraction<br>• Target primer design<br>• T7E1 enzyme digestion | | |

## Comparison of different activity assay services

| Assay Services | Differentiation |
|---|---|
| SSA activity assay | Easy to design, simple to use, cost-effective |
| *In vitro* cleavage activity assay | Easy to use (only target design is needed), cell-free assay system, cost-effective |
| Endogenous activity assay | Fast turnaround time, accelerated validation process |

CHAPTER 5

# CRISPR sgRNA Genome Editing Platform

## 5.3 Ready-to-Use sgRNA Synthesis

CRISPR-Cas9 is one of the most convenient methods of gene editing technology, and has been widely used for editing genes for many different species. The traditional methods to deliver sgRNA into the cell are by plasmid transfection and lentiviral infection, which both have the risk of gene insertion and immune response. Currently, the novel way of directly transferring the ribonucleoprotein (RNP) formed by Cas9 and sgRNA into cells has the advantages of being safer and faster with a lower off-target effect and higher editing efficiency. This has gradually become a much more efficient way to use CRISPR/Cas9 technology.

To improve the efficiency of the CRISPR-Cas9 system, Synbio Technologies has developed ready-to-use sgRNA synthesis services. The ready-to-use sgRNA products can be directly transfected into cells or animals, avoiding the drawbacks of potentially non-degraded plasmids.

### Chemical Synthesis of CRISPR-Cas9 sgRNA

Synbio Technologies uses a chemical synthesis strategy to provide our sgRNA synthesis service with sequences and desired modifications that are 100% correct. The synthesized sgRNA has more stability, low toxicity, and high editing efficiency. It's an excellent choice for your gene editing experiments.

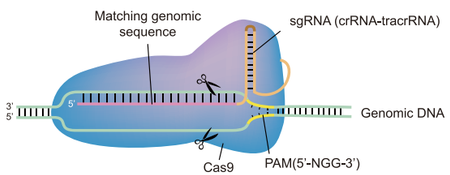



Figure 1. Cas9 complex with sgRNA editing the genomic DNA

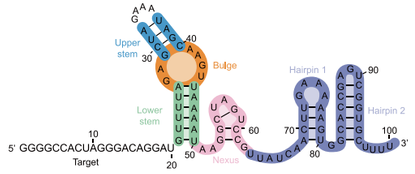



Figure 2. Secondary structure of the sgRNA's target and scaffold sequence

### Competitive Advantages

High Efficiency: The sgRNA is chemically modified, which will increase the stability and editing efficiency in vivo.

Good Stability: Unrivaled control of sgRNA specifications ensures batch-to-batch consistency and traceability.

Convenient: The sgRNA is ready-to-use and covers the sequence and function of both crRNA and tracrRNA, without annealing.

### Service Specifications

| Length (nt) | Order Size | Modification | Purification | Turnaround Time (Business Days) | Deliverables |
|---|---|---|---|---|---|
| 95-105 | 1-10 OD | 2'-OMe and Phosphorothioate | HPLC | 7-10 | 1. Lyophilized RNA<br>2. Certificate of Analysis (COA)<br>3. QC Report (MS/HPLC) |





### *In Vitro* Transcription of CRISPR-Cas9 sgRNA

Synbio Technologies can also provide one-stop services including sgRNA target design, DNA template synthesis of the sgRNA, sgRNA *in vitro* transcription, and sgRNA purification, providing customers with sgRNA products that can be transferred into cells directly. According to publications, *in vitro* transcription of sgRNA has successfully edited the genes of many different species including zebrafish, mouse, filamentous fungi, etc.

### Competitive Advantages

**One-Stop Solution**

Synbio Technologies provides integrated services from sgRNA target design to high purity ready-to-use sgRNA production

**Fast Turnaround Time**

In just 3 business days, Synbio Technologies will deliver up to 20 μg of customized ready-to-use sgRNA

**Convenience**

Ready-to-use sgRNA can be directly injected into animals or transfected into cells, improving the efficiency of gene editing experiments

### Work Flow

DNA template synthesis → *In vitro* transfection into sgRNA → DNA template degeneration and sgRNA purification → Ready-to-use sgRNA

### Service Specifications

| Services | Specifications | Deliverables |
|---|---|---|
| Ready-to-use sgRNA synthesis | sgRNA design DNA template synthesis<br>*In vitro* sgRNA transcription and purification | sgRNA<br>COA |
| Syno® negative control sgRNA | Negative control sgRNA *In vitro* sgRNA transcription and purification | Syno® negative control sgRNA1<br>Syno® negative control sgRNA2<br>Syno® negative control sgRNA3 |

## Case Study

Synbio Technologies has designed several sgRNAs to target several genes in the mouse genome and have performed *in vitro* transfection with these constructs. The experimental period was shortened to 2 days and the sgRNA amount was increased to 10-20 μg. This change could potentially result in a significant jump in efficiency for synthetic biology experiments utilizing CRISPR-Cas9.


Result*

1. Clone DNA template into pUC57. The sequencing result (Fig.1) coincided with the designed sequence.

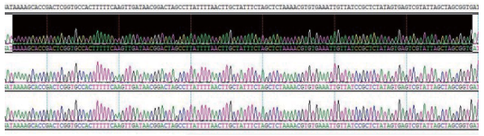


Fig.1 Comparison between blunt end ligation result and designed sequence

2. Agarose gel electrophoresis of sgRNA obtained by *in vitro* transcription. Clear bands shown in Fig.2.

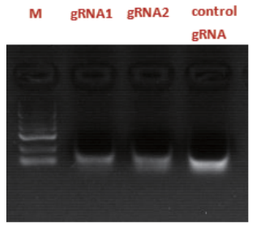



Fig.2 Agarose gel electrophoresis of sgRNA

3. sgRNA Verification: Transcribe sgRNA into cDNA, design sgRNA amplification primer, and obtain the complementary DNA sequence by PCR reaction. Clone DNA sequence into pUC57 vector. The sequencing result (Fig.3) showed the sgRNA sequence is correct.

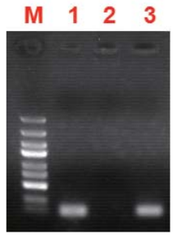



Fig.3 sgRNA sequence verification using agarose gel electrophoresis

*The template of Lane 1 is reverse transcribed cDNA, the template of Lane 2 is sgRNA digested by DNase I, and the template of Lane 3 is *in vitro* transcribed DNA.





# 5.4 CRISPR sgRNA Vector Construction

The CRISPR-Cas9 system is one of the most popular types of gene editing technologies and has been used in many research projects over the last few years. Due to its ease of use and strong applicability, CRISPR-Cas9 has been applied to biology fields including laboratory research, biopharmaceutical research, and genetic engineering of flora and fauna. This technology can also be applied to the construction of genetically modified animal models, construction of gene-regulated cell lines, breeding of plants, cancer research, and treatments of other serious diseases/screening of relevant drug targets. Synbio Technologies offers sgRNA plasmid construction services for several different animal and plant models. We guarantee fast and high-quality sgRNA plasmid construction, tailor-made to meet a large variety of requests and specifications set by our customers.

## Competitive Advantages

**Many Plasmid Options**

We can provide several validated plasmids for different species to help you best fulfill the requirements of your experiment

**High Success Rates**

Several target sites can be designed for different genes which enhance the success rate of plasmid construction

**Plasmid Validation Service**

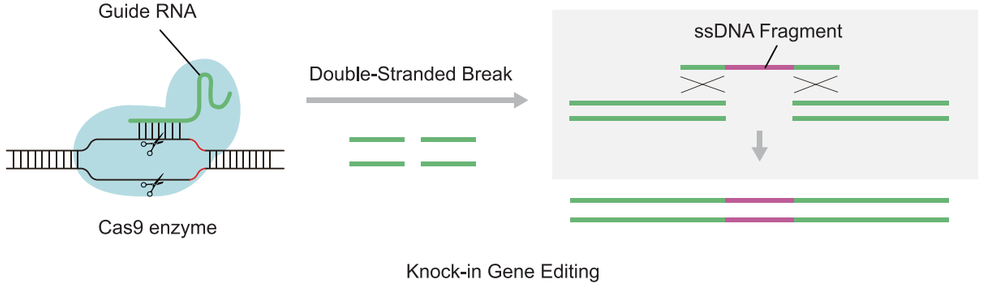
We also provide sgRNA activity validation services, which can enhance the success rate of experiments

## Service Specifications

| Processes | Details | |
|---|---|---|
| Confirm species and vector | Different species and different vectors | |
| Target site design | We usually design 3-5 target sites on common exon for different transcription products. The important domain should be damaged as far as possible which causes loss-of-function in the target gene. | |
| Synthesis | Should check the target site for single nucleotide polymorphisms (SNPs) before synthesis. (If there are SNPs, T7E1 enzyme cannot be used to detect mutations) | |
| Target cutting activity detection | SSA activity assays | *in vitro* cleavage activity assay |
| Endogenous active validation (Conditional choice) | If the target cell has a high transfection rate, like 293T cells, the genome can be extracted after 72 h. Afterwards, amplify target sequence, verify T7E1 enzyme digestion, and validate by sequencing | |

## 5.5 ssDNA Synthesis

ssDNA (single-stranded DNA) is usually used as a homologous recombination repair (homology directed repair, HDR) template to achieve site-specific repair and long fragment knock-in in gene editing. Synbio Technologies provides high-purity, 100% accurate long ssDNA synthesis services.




### Competitive Advantages

**Sequence Guarantee**
Sanger sequencing ensures 100% sequence accuracy

**Non-toxic**
The enzymatic approach ensures that ssDNA is non-toxic and has higher homology repair and knock-in efficiency

**Highly Customized**
Customised to produce the desired ssDNA for different application use

### Service Specification

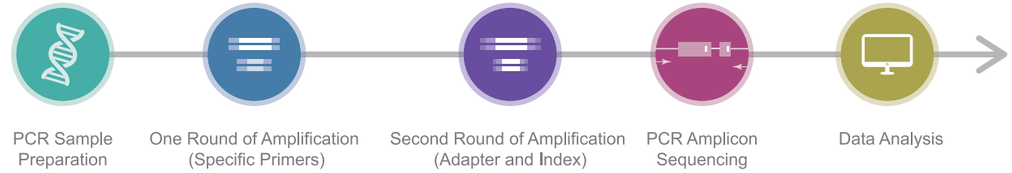
| Length (nt) | Order Size (μg) | Turnaround Time (Business Days) | Deliverables |
|---|---|---|---|
| 200-400 | 2-200 | Start from 10-15 | • Lyophilized ssDNA<br>• Sequencing chromatogram<br>• Certificate of analysis (COA) |
| 400-1,000 | | Start from 10-15 | |
| 1,000-2,000 | | Start from 10-15 | |
| 3,000-4,000 | | Start from 15-20 | |
| >4,000 | | Inquiry | |



## 5.6 CRISPR Sequencing

When CRISPR technology is used for gene editing, a large number of target sites or samples are generated, which is suitable for high-throughput sequencing. That is, primers are designed near the target gene region, PCR amplification is performed, and then the amplicons are sequenced. Unlike CRISPR sequencing, traditional Sanger sequencing cannot quickly and efficiently identify the editing efficiency of a large number of genes.
Synbio Technologies's amplification and sequencing technology can be used to detect CRISPR/Cas9 genome editing results and analyze off-target effects, helping researchers quickly locate gene mutation sites and study gene function.

### CRISPR Amplicon Sequencing Process




### Competitive Advantages

**High Coverage**
Each reaction can perform multiplex analysis of hundreds to thousands of amplicons

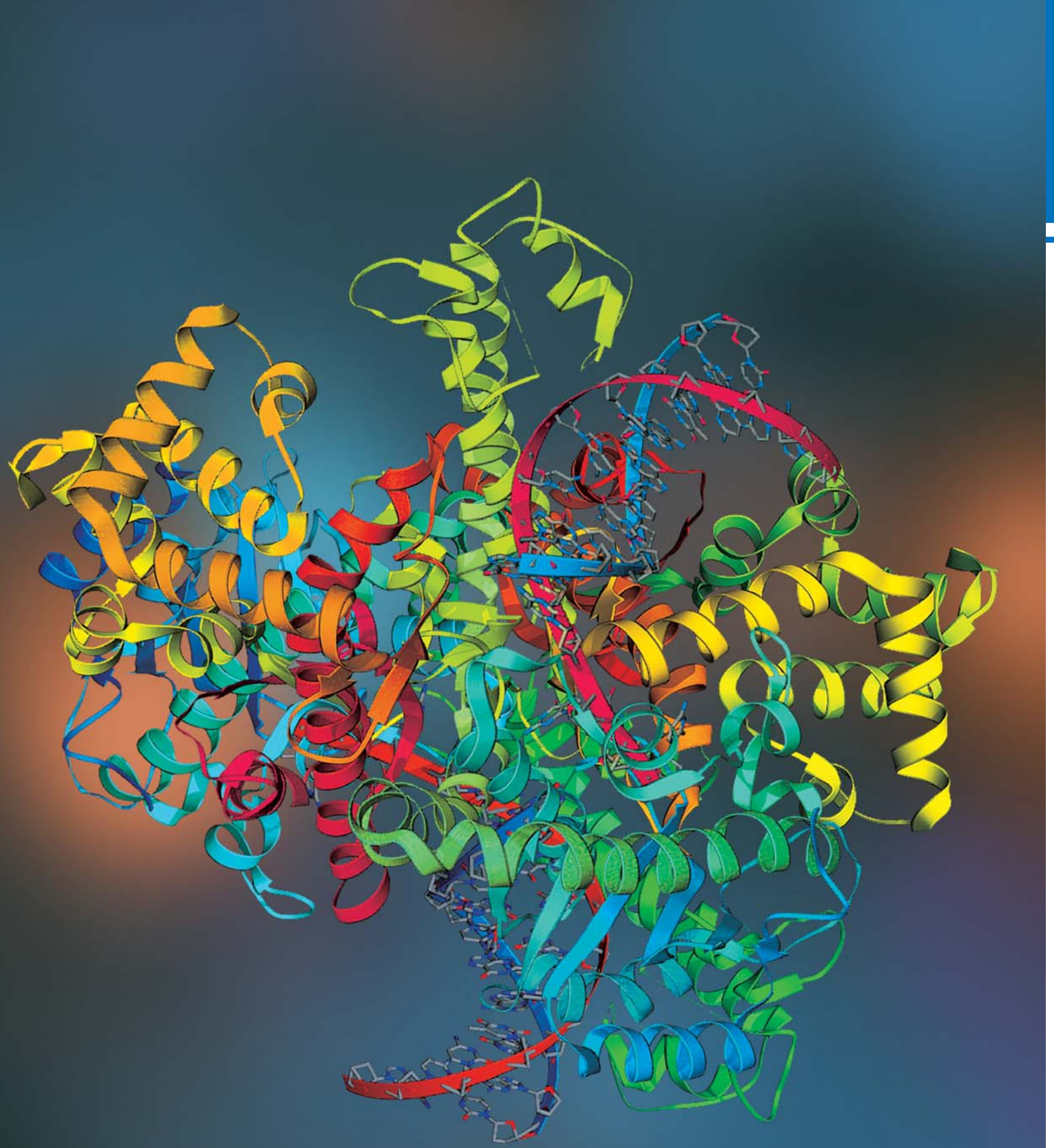
**High Flexibility**
It can be used for all kinds of mutation verification and screening genetic variation

**Highly Customized**
Analysis services can be customized

**Cost-effective**
Compared with whole-genome sequencing, it can reduce sequencing cost and turnaround time

### Service Specification

| Amplicon Size | Platform Configuration | Turnaround Time | Deliverables |
|---|---|---|---|
| 70-280 bp | Illumina 2×150 bp | 3-4 weeks | • Sequencing chromatogram<br>• Data analysis report |
| 280-480 bp | Illumina 2×250 bp | 4-5 weeks | |



# Protein Expression and Purification

CHAPTER 6

# Protein Expression and Purification

With our experienced research team and leading protein expression platform, Synbio Technologies provides our customers a variety of protein expression and purification platforms, including bacterial, yeast, insect, and mammalian expression systems. By utilizing our powerful gene synthesis platform, Synbio Technologies can generate high-purity recombinant proteins ranging from milligrams to grams. Combined with our patented NG™ Codon Optimization Technology, the protein expression level can be significantly improved. Simply submit your gene or protein sequence and your high-quality purified recombinant protein will arrive to your bench in as soon as 3 weeks.

## Competitive Advantages

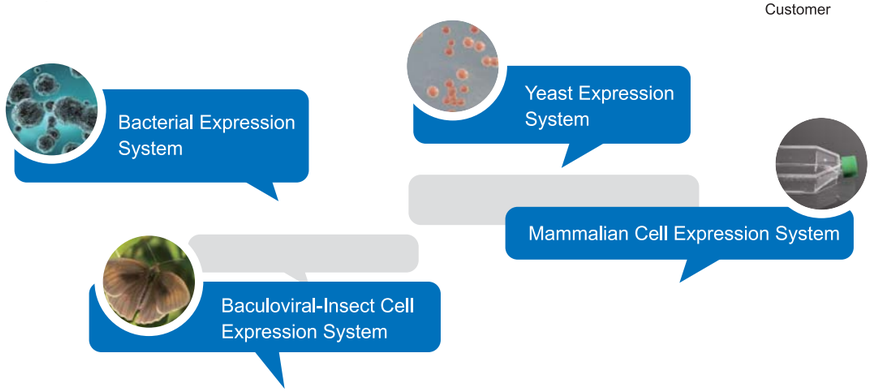
► **NG™ Codon Optimization Technology**
Increase the protein expression level in most host cells

► **Comprehensive Expression Systems**
Bacteria, yeast, baculovirus/insect cell, and mammalian cell expression systems, plus various vectors for each system

► **One-stop Services**
Streamlined process from gene synthesis, codon optimization, to protein expression and purification

► **Scalable Synthesis Capacity**
Provide your desired proteins from milligram to gram scale

## Service Procedure


**Protein Expression and Purification Services**

## 6.1 NG™ Codon Optimization Technology

Codon optimization is a method of utilizing preferred codons to increase overall sequence stability and resulting protein expression. This is accomplished by avoiding rare codons with low utilization, simplifying the mRNA's secondary structure, and adjusting the overall GC content. The increased commercialization of gene synthesis has greatly promoted the application and influence of codon optimization. Synbio Technologies's NG™ Codon Optimization Technology optimizes complex sequences resulting in a significantly higher protein expression level.

### Competitive Advantages

✓ Raise the protein expression level significantly

✓ Gene synthesis codon optimization is available in all hosts

Different organisms have their own preferred codons. For example, the prioritized termination codon of yeast and mammalian are UAA and UGA, respectively. Specific gene synthesis codon optimizations are applied for different organisms. Specific codon optimizations are provided in correllation to the hosts that clients provide.

✓ Excellent results even in the most difficult sequences

NG™ Codon optimization is capable of optimizing the most complex sequences such as hairpin sequences, highly repeated sequences, and extremely long sequences.

✓ Intellectual property (IP) protection

All of your IP-related rights are fully protected by Synbio Technologies. The DNA sequence and its related information are kept strictly confidential in the entire process. All materials and mid-products including synthesized oligos, genes, plasmids, and the cells will be destroyed in one month after delivery.

### Case Study


T: Test
C: Control
M: Marker

Target proteins were reproducibly expressed in *E. Coli* system after codon optimization by NG™ Codon Optimization Technology.



## 6.2 Guaranteed Bacterial Protein Expression

With years of experience in protein expression, Synbio Technologies has developed a cost-effective bacterial expression platform to deliver high-quality proteins to our customers. By using our self-developed NG™ codon optimization technology and mature expression system, the expression level in bacteria can be significantly improved.

We offer guaranteed services, starting with our proprietary codon optimization, followed by gene synthesis, vector construction, all the way to protein expression and purification to ensure the expression of the target protein. If we fail to give you your desired protein, there will be no charge.

You only need to provide us with the sequence of the target protein. Then, we can deliver the specified quantity and purity of the target protein within a minimum of 4 weeks, ensuring your downstream experiments.

### Competitive Advantages

**01**
Proprietary NG™ Codon Optimization Technology
Significantly improve protein expression level and solubility, with a success rate of up to 95% in the expression system of *E. coli*

**02**
Comprehensive Solutions
Provide one-stop solutions from gene synthesis to protein expression and purification

**03**
High Quality Guaranteed
There is no charge if we fail to deliver you desired proteins

**04**
Competitive Price and Fast Turnaround Time

### Service Specifications

| Services | Descriptions | Deliverables | TAT |
|---|---|---|---|
| Gene Synthesis | • Codon optimization and gene synthesis<br>• Subcloning into appropriate expression vector<br>• Various tags are available, such as His, SUMO,TRX,GST… | • 2-5 μg lyophilized plasmid DNA<br>• 1-5 mg protein products<br>• Purity options: 85%, 90%, 95%<br>• Endotoxin level options: ≤0.1 EU/μg<br>• Certificate of analysis | ≤2 weeks |
| Protein Expression Testing and Purification, Tag Removal (Optional) & Refolding (Optional) | • Transform plasmids into appropriate bacterial expression strain<br>• Protein expression evaluation | | ≤3 weeks |
| | • Scale up protein expression<br>• Purification to reach desired protein amounts and purity<br>• Tag removal and separation of tag-free protein (if requested)<br>• Refolding in case of insoluble protein (performed only if required) | | |
| QC & Delivery | • SDS-PAGE<br>• Western Blot (if requested)<br>• Bradford assay for quantitation<br>• Protein delivery | | ≤1 weeks |

* Suitable for proteins with molecular weight between 15-120 KD, excluding membrane proteins and toxic proteins.
* For gram level large-scale protein production, please contact quote@synbio-tech.com.

## 6.3 Yeast Protein Expression System

Prokaryotic expression is mature and easy to be operated, but when it comes to the proteins of eukaryotic organisms, error folding is a frequent occurrence since glycosylation and folding partners are involved. Fortunately, the expression system of *pichia pastorisis* is complementary to this situation to a certain extent. The *Pichia pastoris* expression system is a simple and economic system among eukaryotic systems. It has many advantages in protein post-processing, folding, and post-translational modification. Synbio Technologies has abundant resources in vectors and host cells, and can effectively improve protein yield and provide you high-quality and considerate yeast expression protein services.

### Service Specifications

| Services | Descriptions | Deliverables | Turnaround Time |
|---|---|---|---|
| Gene Synthesis (Optional) | • Codon optimization<br>• Gene synthesis<br>• Vector construction | • 2-5 μg lyophilized plasmid DNA<br>• Certificate of analysis (COA) | Starting from 10 BDs |
| Electrotransformation and Screening Positive Clones (PCR method) | Linearize the plasmid and transform into pichia pastoris cells to screen positive clones | Screening report | 2-3 weeks |
| Pilot Protein Expression | Three positive clones will be used to verify the expression, then perform expression analysis validation (WB) | Expression evaluation report | 4-6 weeks |
| Protein Expression and Purification | • 1 L or larger scale expression<br>• One or more purifications steps<br>• Purity will be detected by SDS-PAGE or Western blot (Optional) | • Purified protein products<br>• Certificate of analysis (COA) | 3-4 weeks |



## 6.4 Baculovirus-Insect Cell Expression System

The baculovirus insect cell system offers several unique advantages over the *E. coli* expression system, such as post-translational modifications, higher yield for secreted proteins, and improved solubility. It is appropriate for expressing proteins that maybe harmful to mammalian cells, such as kinases and toxic proteins. With years of experience in protein expression, Synbio Technologies possesses abundant experience in baculovirus insect cell protein expression, particularly the expression of toxic proteins, kinases, large proteins, membrane proteins, and intracellular proteins.

### Competitive Advantages

**01**

Proprietary NG™ Codon Optimization Technology
Significantly improves protein expression levels and success rate

**02**

Multiple Purification Strategies
Affinity chromatography, ion exchange, hydrophobic chromatography, gel filtration, etc., providing diversified choices of protein purities

**03**

Comprehensive Protein Identification Services
SDS-PAGE, Western Blot, HPLC, etc

### Service Specifications

| Services | Descriptions | Deliverables | Turnaround Time |
|---|---|---|---|
| Gene Synthesis (Optional) | • Codon optimization<br>• Gene synthesis<br>• Vector construction | • 2~5 μg lyophilized plasmid DNA<br>• Certificate of analysis (COA) | Starting from 10 BDs |
| Baculovirus preparation | • Generation of recombinant Bacmid DNA<br>• Generation of P1 stock, P2 stock, and determination of virus titer | Experiment report | 3-5 weeks |
| Pilot Protein Expression and Purification | • P2 generation virus infects SF9 cells<br>• Expression analysis validation (WB)<br>• Ni column affinity purification | • A small amount of protein<br>• Expression evaluation report | 2-4 weeks |
| Protein Expression and Purification | 1 L or larger scale expression | • Purified protein products<br>• Certificate of analysis (COA) | 1-2 weeks |

# 6.5 Mammalian Cell Expression System

Protein with post-translational modifications and appropriate protein folding are critical for many downstream applications. Synbio Technologies's mammalian expression platform offers a variety of services, including eukaryotic protein expression, recombinant antibody production, etc. Combined with our advanced protein purification facilities, we can deliver recombinant protein products with high purity ≥ 98% and endotoxin level less than 0.001 EU/ug. With our Avitag technology, we also offer a variety of biotinylated proteins and antibodies to facilitate your assay development.

## Competitive Advantages

**High Efficiency**

Various self-developed high expression vectors, meeting the different screening needs of customers

**High Purity and Quality**

Many of our antibody products have a purity higher than 95%, with comprehensive quality control

**Low Endotoxin Level**

Endotoxin level is less than 0.005 EU/μg

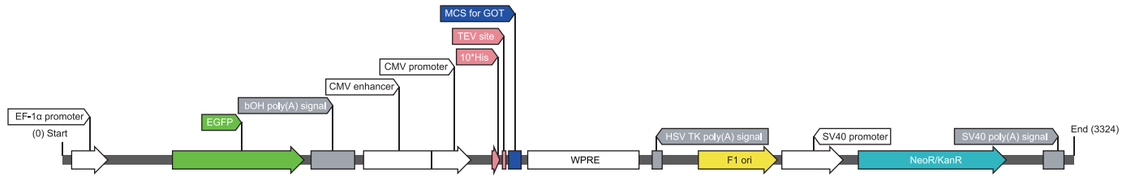
## Service Specifications

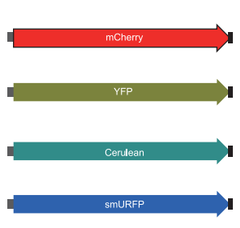
| Services | Descriptions | Deliverables | Turnaround Time |
|---|---|---|---|
| Gene Synthesis (Optional) | • Codon optimization<br>• Gene synthesis<br>• Vector construction | • 2-5 μg lyophilized plasmid DNA<br>• Certificate of analysis (COA) | Starting from 10 BDs |
| Pilot Protein Expression and Purification | • Transient transfection<br>• Pilot protein expression<br>• Expression analysis validation (WB) | Expression evaluation report | 2-3 weeks |
| Protein Expression and Purification | • 200 ml, 1 L or larger scale expression<br>• Purity Identification (PAGE) | • Purified protein products<br>• Certificate of analysis(COA) | 2-3 weeks |

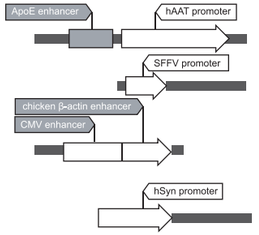


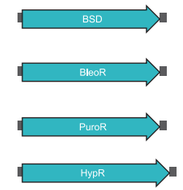
**Selection of Fluorescent Proteins, Promoters, and Selectable Markers**

Different promoters have different activation activities in different cells. For example, in hematopoietic stem cells, SFFV has much better activation activity than CMV. There are also differences in tissue-specific expression. AAT is highly expressed in liver tissues and weakly expressed in other tissues. hSyn is highly expressed in nerve cells and weakly expressed in other tissues. Synbio Technologies provides a variety of fluorescent proteins, promoters, and mammalian expression vectors with selectable markers to meet the needs of cell-specific screening.



## 6.6 Peptide Synthesis

With our experienced research team and leading synthesis platform, Synbio Technologies provides our customers with a full spectrum of peptide services ranging from custom peptide synthesis to peptide library synthesis. Our custom peptides and peptide libraries are available with flexible modification, conjugation, and isotopic labeling options. Up to date, our success rate of peptide synthesis has achieved more than 98%. The high quality peptide synthesis products provided by Synbio Technologies can be applied to drug development, protein functional analysis, monoclonal antibody preparation, antibody-antigen interaction, enzyme specificity research, etc.

### Competitive Advantages

**Modification Variety**
Hundreds of peptide modifications, including cyclic peptide synthesis, N/C terminal mdifcations, linker spacers, PEGylation, etc.

**Different Purity Levels**
From crude to >99% purity

**High-Quality Products**
Advanced peptide synthesis technology, complete MS, HPLC reports, and certificate of analysis

**Fast Turnaround Times**
Custom synthesis with on-time delivery and professional technical support

**Strictly Confidential**
One-to-one confidentiality agreement and time-saving products to accelerate your experiments

### Service Specifications

| Services | Turnaround Time | Deliverables |
|---|---|---|
| Custom Peptide Synthesis | 2-3 weeks,<br>1-2 weeks for urgent orders | • Peptide Products<br>• HPLC Reports<br>• MS Reports<br>• Certificate of Analysis |
| Peptide Modification | | |
| Peptide Library | | |

* Please contact *quote@synbio-tech.com* for more information.



### Types of Peptide Libraries

**Overlapping Peptide Library**

DRVYIHPFHLWIEG
DRVYIHPFHL
RVYIHPFHLWIE
VYIHPFHLWIE
VYIHPFHLW
VYIHPFHL

Individual peptides can be divided into several fragments that overlap. The resulting overlapping peptide libraries can then be used for processes including continuous and linear epitope mapping.

**Truncation Peptide Library**

DRVYIHPFHLWIEG
DRVYIHPFHLW
DRVYIHPFHL
DRVYIHPFH
DRVYIHPF
DRVYIHP

The library of truncated peptides can be used to predict the minimum length amino acid that could be used to achieve optimal epitope activity.

**Alanine Peptide Scanning Library**

DRVYIHPFHLWIEG
DRVYIHAFHLWIEG
DRVYIHPAHLWIEG
DRVYIHPFALWIEG
DRVYIHPFHAWIEG
DRVYIHPFHLAIEG

Each amino acid is substituted individually and systematically for alanine. This library enables quick determination of each individual amino acid's contribution to the peptide's functionality.

**Random Peptide Library**

DRVYIHPFHLWIEG
DRVYIHXXXLWIEG
DRVYIHXXHLWIEG
DRVYIHPFHXXXEG
DRVYIHPFXXWIEG
DRVYIHPXXLWIEG

Selected positions are substituted with all 20 natural amino acids simultaneously, which might increase peptide activity.

**Scrambled Peptide Library**

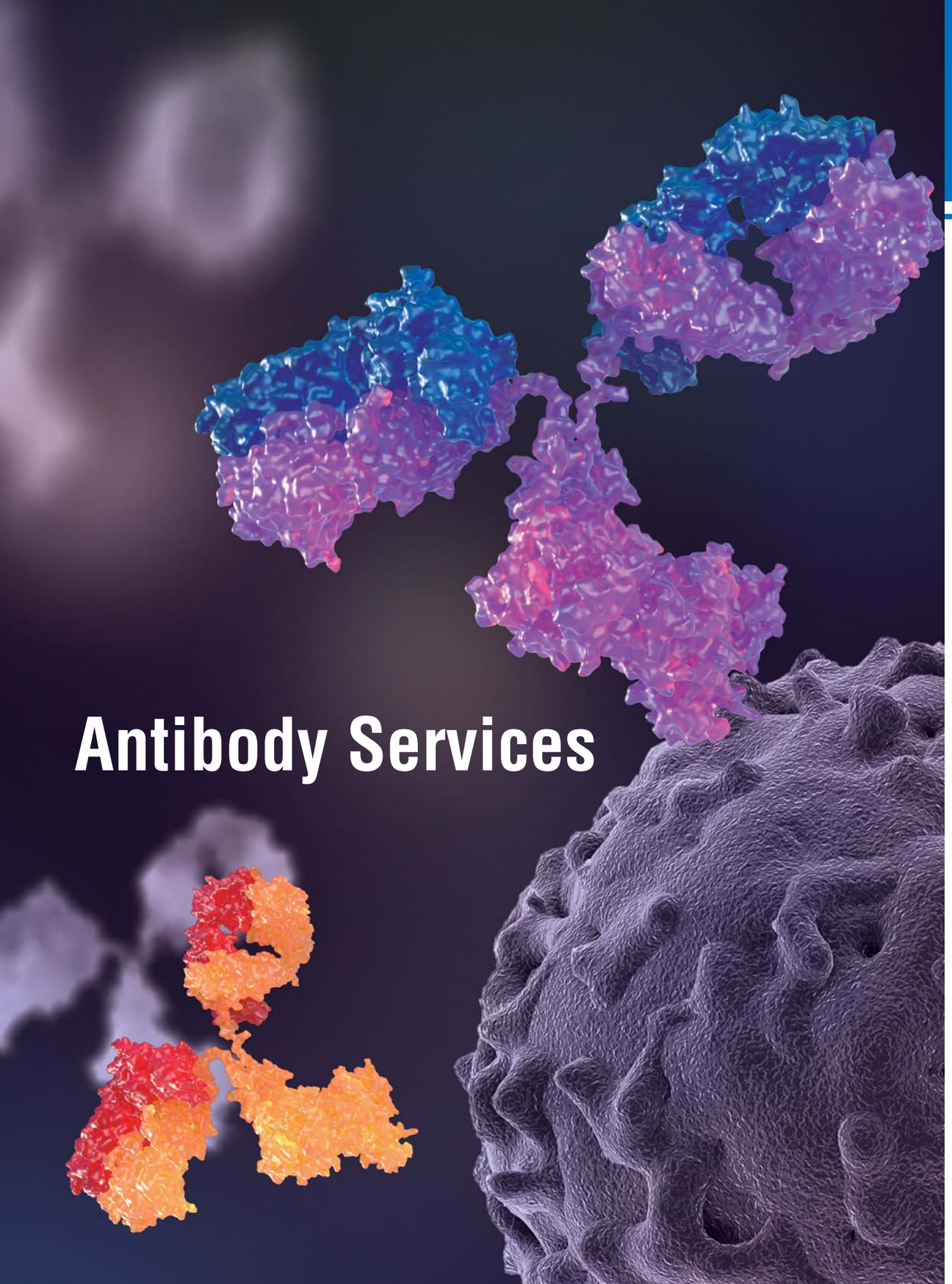
DRVYIHPFHLWIEG
DPVYIHRFGEWILH
DRGWIHPFHLYIEV
HLVPIHYFDRWIEG
RIVYDHPFHLWIEG
DHFYIHPFVRWIEG

Scrambled libraries have the highest variation of any peptide library. The resulting peptides are used generally as negative controls to show that a specific sequence is critical to the protein function or activity. It is also a random screening tool used to find new leads.

**Positional Peptide Library**

DRVYIHPFHLWIEG
DRVYIHAFHLWIEG
DRVYIHEFHLWIEG
DRVYIHGFHLWIEG
DRVYIHQFHLWIEG
DRVYIHSFHLWIEG

Selected sites or regions within a peptide sequence are replaced systematically with all other natural amino acids. This allows for amino acids that enhance peptide activity to be identified.

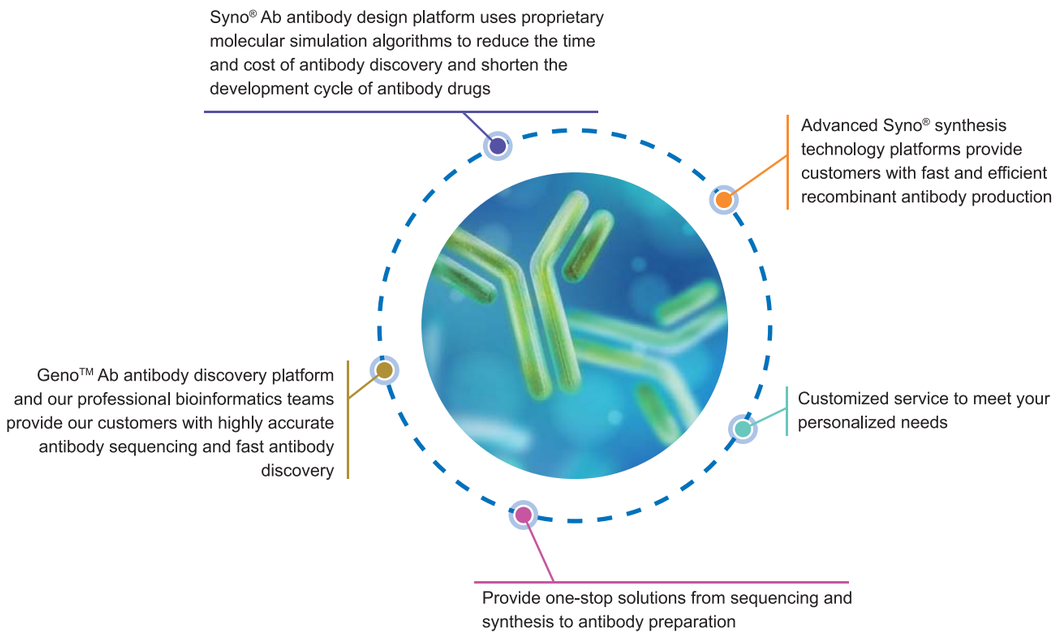



# CHAPTER 7
# Antibody Services

Antibody engineering refers to the modification and re-assembly of antibody genes through recombinant DNA and protein engineering technologies. The engineered genes are then further transfected into receptor cells and expressed accordingly. Additionally, antibody engineering also includes the reformatting of known antibodies via cell fusion and chemical modification. Both antibody engineering approaches yield new artificial antibody molecules that retain or newly add the specificity and biological activity of natural antibodies, or eliminate unrelated structures. For this reason, antibody engineering has great potential to further the capabilities of pharmaceutical research and development.

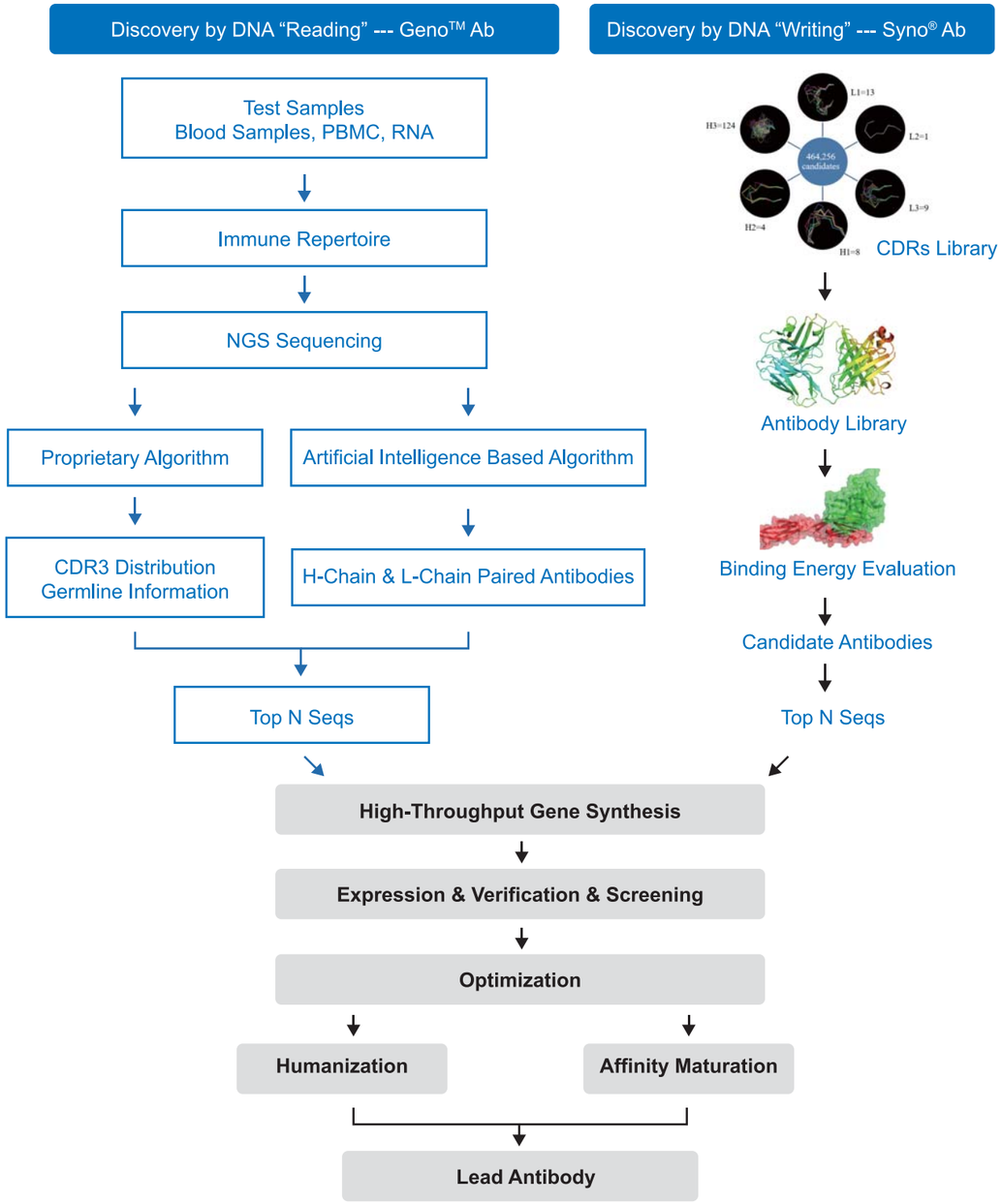
Synbio Technologies provides a broad range of DNA technologies to antibody services. Our technical platforms span all stages of antibody discovery by integrating “DNA Reading” (sequencing & discovery), “DNA Writing” (design & synthesis), “DNA Editing” (optimization & humanization). In addition, we can provide high-quality and cost-effective recombinant antibody products to support the development of biobased medicines in academia as well as in the biotechnology and pharmaceutical industries.

## Competitive Advantages



CHAPTER 7

# Antibody Services








## 7.1 Monoclonal/Hybridoma Antibody Sequencing

Obtaining the hybridoma monoclonal antibody (mAb) sequence is one of key requirements of recombinant antibody engineering. Monoclonal antibody sequencing includes hybridoma cell line sequencing and clonal B cell sequencing. Both of these kinds of cells can produce a single antibody against a unique antigen after screening. Synbio Technologies uses 5'RACE and Sanger sequencing technologies to provide both accurate and efficient hybridoma/clonal B cell sequencing services across a variety of species.

### Competitive Advantages

**01**

*De novo* sequencing of multi-species' (including mice, rats, Armenian hamsters, camels, rabbits, equines, bovines, canines, birds, etc.) monoclonal antibodies

**02**

Use of RACE technology obtains complete monoclonal antibody sequence information

**03**

Internally-developed custom primer sets and special reagents ensure optimal success rates and efficient turnaround time

**04**

Enhanced bioinformatics analysis removes redundant sequence information

**05**

Full traceability ensures batch-to-batch consistency throughout our customers' research development and manufacturing procedures

**06**

High-throughput and Cost-Effective
Whether it is a sample or hundreds of samples, we can do it all. The more samples that are provided, the lesser the cost

### Hybridoma Sequencing Process

mRNA isolated from hybridoma samples is subjected to RT-PCR with unique species-specific primer sets to amplify the target regions. Synbio Technologies's antibody variable region sequencing service has identified more than 10,000 hybridoma cell lines. More than 95% of the samples have been successfully sequenced with both the heavy chain and light chain identified. Even with technically difficult samples, Synbio Technologies's approach has demonstrated a high success rate in numerous cases. Synbio Technologies's services, coupled with our online data tracking and management system, provides customers with absolute confidence at every step.

CHAPTER 7

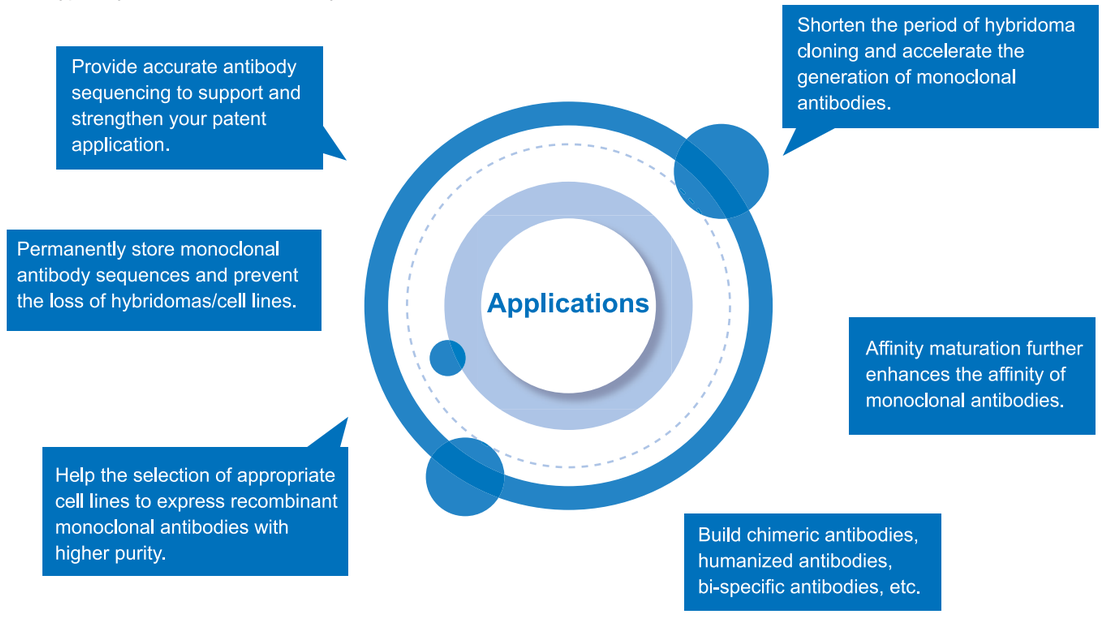
# Antibody Services

## Standard Hybridoma Sequencing Process


## Service Specifications

| Services | Descriptions | Turnaround Time | Deliverables |
|---|---|---|---|
| Standard mAb Sequencing | Standardized work flow for hybridoma sequencing | 2-3 weeks | • Raw sequence data (100% accuracy guarantee)<br>• Final sequence report with variable heavy chain and light chain sequences or full-length sequence and specific regions annotated<br>• Plasmid contains antibody coding sequence (upon your request) |
| Express mAb Sequencing | Rapidly identify antibody sequences from the target cell lines | 1 week | |

* For antibody sequencing services: customers will need to submit hybridoma cell samples (>1×10$^6$ cells) and indicate the subtype of your monoclonal antibody.








## 7.2 Immune Repertoire Sequencing

"Immune Repertoire" refers to the set of all functionally diversified B cells and T cells in the circulatory system of an individual at any given time. Immune repertoire sequencing (Immuno-Seq) targets T/B lymphocytes and utilizes 5' Rapid Amplification of cDNA Ends (RACE) Technology. 5' RACE technology amplifies complementarity-determining regions (CDRs) of B cell receptors (BCRs) or T cell receptors (TCRs). The CDRs are targeted because they control the diversity of the corresponding BCRs and TCRs. Combined with high-throughput sequencing technology, Immuno-Seq provides a comprehensive evaluation of immune system diversity.

Synbio Technologies provides our customers with a complete antibody repertoire sequencing system including immune repertoire sequencing, antibody CDR region sequencing, etc. Our Immuno-Seq System is designed to assist researchers in the observation and analysis of both T and B cells with unprecedented specificity. The specificity allows the Immuno-Seq technology to be applied to disease surveillance, antibody production, vaccine research, health examination, and other related areas.

## Competitive Advantages

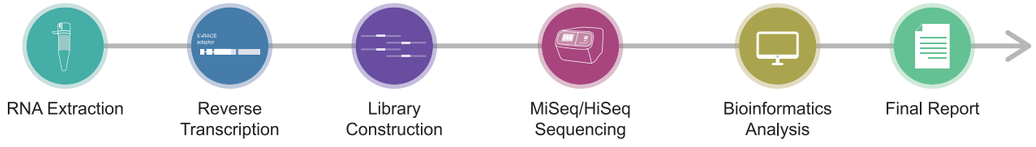
**01**
Robust RACE technology and specially-optimized PCR amplification systems designed to minimize the amplification bias of the immune repertoire genes

**02**
Diversified NGS analysis platform

**03**
Optimal success rates and fast turnaround time

**04**
Complete and advanced bioinformatics analysis designed to examine highly valuable information

## Standard IR-Seq Process



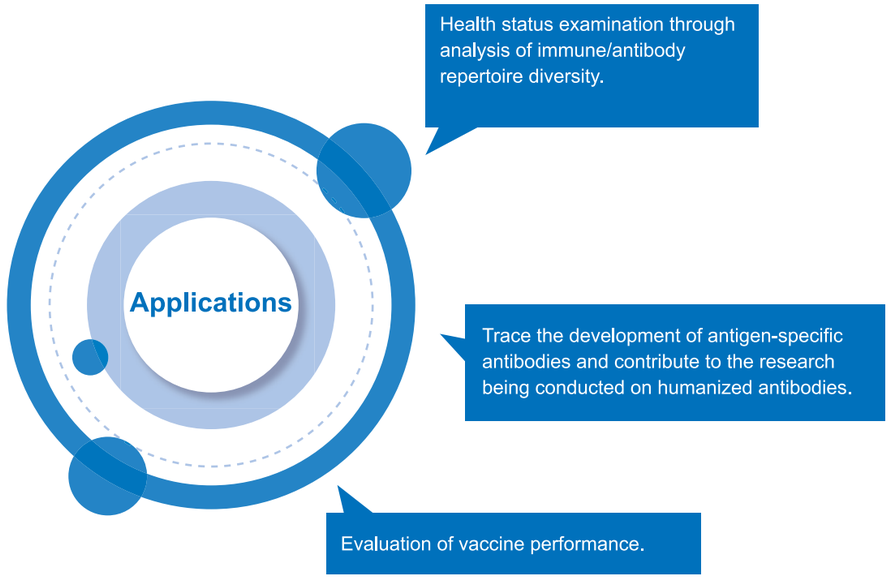
CHAPTER 7

# Antibody Services

## Service Specifications

| Service | Turnaround Time | Deliverables |
|---|---|---|
| Immune Repertoire Sequencing | 6-8 weeks | • NGS sequencing data<br>• Bioinformatics analysis report |

* For Immuno-Seq service: customers will need to submit one of the following: 1-2ml peripheral blood, splenocyte, selected immune cells, or extracted high quality RNA.






# CASE STUDY

**Synbio Technologies's immune repertoire sequencing and bioinformatics analysis:**

**1. Library Construction**

Synbio Technologies performed 5' RACE reverse transcription of the extracted RNA, then amplified and constructed the library.

**2. Library Quality Control**

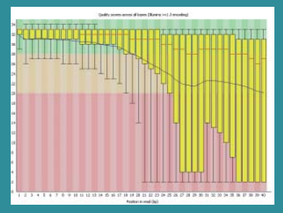
The Qubit 2.0 Fluorometer was used to perform preliminary quantification after the library construction. After dilution, insert size detection was performed on the library based on the Agilent 2100 bioanalyzer. Only when meeting expected requirements, the effective concentration of the library (>2nM) was accurately quantified by qPCR to ensure the quality of the library.

**3. Library Sequencing**

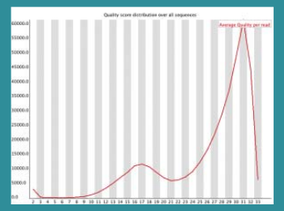
Different libraries were pooled and sequenced by Illumina Miseq/HiSeq according to the effective concentration and the required amount of target data. Four fluorescent labeled dNTPs, DNA polymerase, and adaptors were added to the flow cell for the amplification. When each sequence cluster extended and generated the complementary chain, the corresponding fluorescent signals were released and captured after each new dNTP was added. The optical signals were then transformed into sequencing peaks by professional software to obtain the sequence information of the fragments.

**4. Data Analysis**

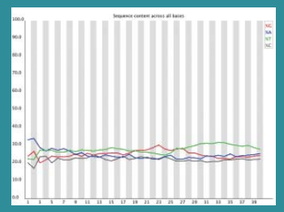
Quality assessment of sequencing data.



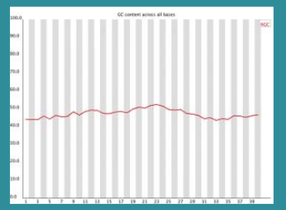
Per Base Sequence Quality



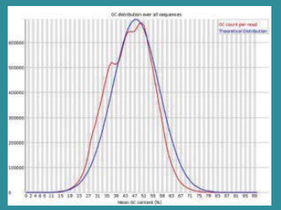
Per Sequence Quality Scores



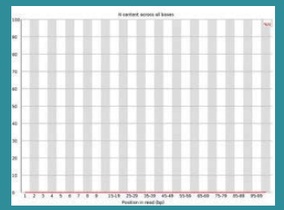
Per Base Sequence Content



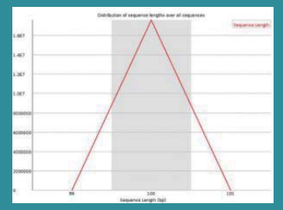
Per Base GC Content



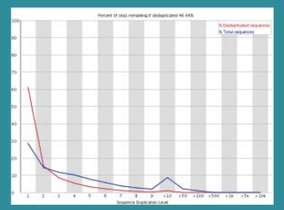
Per Sequence GC Content



Per Base N Content



Sequence Length Distribution reads



Duplicate Sequences

## 7.3 *De Novo* Antibody Design and Production

Biological drugs usually include genetic engineering drugs, antibody engineering drugs, blood product drugs, vaccines, etc. Among these, antibody drugs are one of the fastest growing therapeutic drugs for humans. This increase in research and development has proved to be effective for treatment of many important diseases such as cancer, chronic inflammatory diseases, cardiovascular diseases, immune system diseases, and infectious diseases. Antibody research and development also plays an important role in medicine and diagnostics.

Synbio Technologies's proprietary antibody platform utilizes molecular simulation technologies to provide *de novo* antibody design and preparation services. We can mimic the biological behavior of antigenic molecules at the molecular level. We analyze antibody structures as a starting point to solve a series of problems, such as the theoretical design of an antibody. The combination of molecular simulation technology and our experimental approach can help our customers effectively reduce the total cost of antibody R&D while shortening their R&D cycle.

### Competitive Advantages

**01**

Provides services such as *de novo* antibody design, antibody humanization, etc, to promote the development of "bio-better" molecules

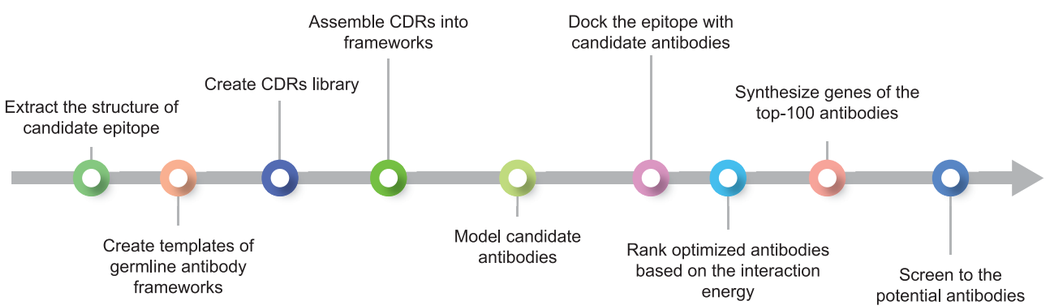
**02**

Proprietary molecular simulation algorithm effectively simulates antigene-antibody docking and screens for antibodies with the optimal affinity

**03**

Reduces the cost of antibody discovery by a factor of 100 and increases the yield by a factor of 1000 compared to the traditional method

### *De Novo* Antibody Discovery by Syno® Ab




### Service Specifications

| Service | Details | Turnaround Time | Deliverables |
|---|---|---|---|
| Syno® Ab Antibody Design and Production | • CDR construction<br>• Antibody modeling<br>• Epitope docking<br>• Screening optimization | 2-3 weeks | • Antibody sequence report<br>• Structure analysis of specific targets (antigens)<br>• Molecular simulation and molecular dynamics ordering<br>• Computational ordering of antibody library (human or specific species)<br>• Leads with the affinity greater than $10^{-9}$ M<br>• Antibody library or the synthesized leads antibodies |

* Customers can provide the antigen name or gene ID directly. If you have the antigen's crystal structure, please contact us directly.

## 7.4 Antibody Humanization Service

Immunogenicity or a Human Anti-Mouse Antibody (HAMA) response is what happens when antibodies derived from xenogeneic sources are administered to humans. This can potentially decrease the effectiveness of immune-treatment through rapid clearance of the therapeutic antibody or immune-conjugate. In some cases, the patient may even develop an allergic sensitivity and be at risk of anaphylactic shock upon any future treatment containing these "foreign" antibodies. Due to this potential risk of immunogenicity and HAMA response, the humanization of antibodies from xenogeneic sources to become safer and more reliable humanized antibody drugs is critical.

Synbio Technologies has developed an integrated antibody humanization and affinity maturation platform based on advanced phage/yeast display technologies and progressive computer modeling and simulation techniques. Collectively, we have combined methods from bioinformatics and structural biology to optimize our approach toward integrated antibody humanization. Our antibody humanization services include *in silico* CDR-grafting, computer-aided design and optimization, high-throughput screening of phage-display antibodies, and Biacore surface resonance analysis. With this approach, we are able to provide humanized antibodies with diminished immunogenicity, which have comparable or better specificity, bioactivity, thermos-stability, and productivity than parental antibodies. Our affinity maturation service assures optimal humanized antibody output with an increased binding affinity by at least five-fold.

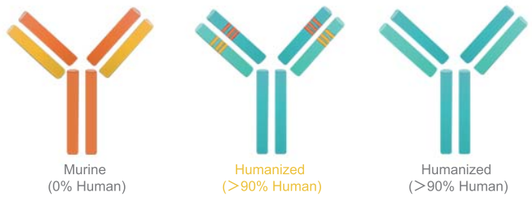
### Featured Advantages

✓ Extensive Experience: Our team has practical experience with humanized antibody design, screening, and production to ensure the success of our customers' projects

✓ *In Silico* Design: Effective and reliable antibody modeling and simulating from multiple antibody databases

✓ High-Throughput Screening: Phage/yeast display platform designed to enhance the valid antibody yield

✓ Guaranteed Affinity: After affinity maturation, the final construct's affinity is improved by at least five-fold




### Service Specification

| Details | Deliverables | Turnaround Time |
|---|---|---|
| • Chimeric antibody construction<br>• Antigen-antibody binding analysis | • Top 5 humanized antibody variants and corresponding DNA sequences.<br>• At least one variant with binding affinity comparable to parental antibody.<br>• Full report (PDF). | 4-5 months |
| • Antibody modeling library construction<br>• Phage/Yeast display antibody library construction | | |
| • High-throughput antibody screening | | |
| • Humanized antibody characterization and production | | |



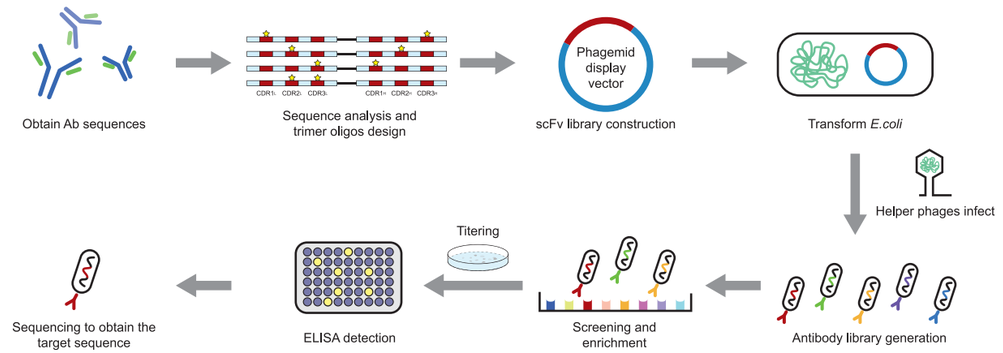
## 7.5 Antibody Affinity Maturation Service

The binding affinity of an antibody is one of the key parameters to determine the efficacy and dosage of an antibody drug. Currently, the methods for improving the binding affinity of antibodies mainly focus on site-mutation, CDR rearrangement, DNA recombination, etc. With our unique and effective affinity maturation technology, we can quickly spot the key amino acid sites in antibody domains. This, in turn, increases the binding affinity of the antibody, reaching at least nM level.

### Competitive Advantages

| 01 | 02 | 03 | 04 |
|---|---|---|---|
| Effective random mutation through DNA-shuffling | Mature phage display platform assures high-quality affinity maturation services | Precise affinity evaluation | Competitive pricing |

### Phage-Display Screening Process




### Service Specification

| Details | Deliverables | Turnaround Time |
|---|---|---|
| DNA Shuffling | 1. Antibody sequence and sequencing report<br>2. Top 1-3 of the optimized antibodies<br>3. Full analysis report (PDF) | 6-7 months |
| Library Construction and Screening | | |
| Antibody Production and Characterization | | |

CHAPTER 7

# Antibody Services



## 7.6 Recombinant Antibody Production

With the development of antibody engineering technology, various engineered antibodies have been designed for cancer diagnosis and immunotherapy. Based on years of research experience and leading professional technologies in the field of antibody engineering, Synbio Technologies can provide a series of recombinant antibody preparation services, including single chain antibodies (scFv), antigen binding fragments (Fab), bispecific antibodies, etc.

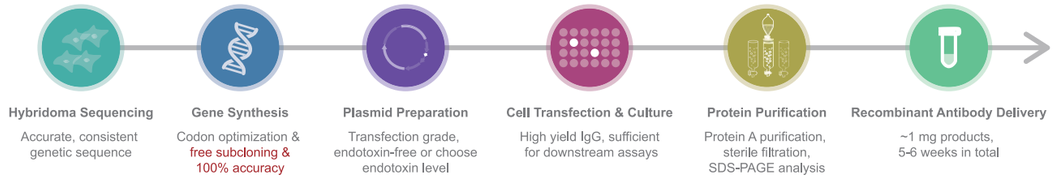
### Competitive Advantages

**One-Stop Solution**
Provide one-stop solutions from hybridoma sequencing to recombinant antibody production

**High Expression**
Independently developed a variety of high expression vectors

**High Efficiency**
10,000+ successful projects delivered and project success rate >95%

### Recombinant Antibody Production Process




### Service Specifications

| Services | Service Details | Deliverables |
|---|---|---|
| Antibody Discovery | Hybridoma sequencing or immune repertoire sequencing (optional) | Antibody gene sequencing report |
| Gene Synthesis | • Gene synthesis and vector construction: IgG, Fab, scFv, VHH, BsAb, etc<br>• Plasmid preparation | 2-5 μg lyophilized plasmid |
| Recombinant Antibody Expression and Purification | • Cell transfection & culture<br>• 30 ml, 100 ml, 200 ml or larger scale recombinant antibody expression<br>• Antibody affinity purification and purity detection (SDS-PAGE, SEC-HPLC), purity> 95%<br>• Endotoxin: <1 EU/mg | • Antibody products<br>• QC report |



## 7.7 Monoclonal Antibody Preparation

Hybridoma technology features the effective usage of innate functions of both immune and cancer cells to generate monoclonal antibodies (mAbs). Resulting hybridoma cell lines are produced to combat specific antigens of interest. Such technology has forged remarkable new approaches toward therapeutic mAb discovery, as well as disease diagnosis and prevention. Mice are the main host animals for monoclonal antibody production. Synbio technologies can provide customers with a full range of customized monoclonal antibody production services, including immunization, hybridoma development, antibody preparation, antibody function screening analysis, etc.

### Competitive Advantages

**01 Proprietary Immunization Technology**
Compared to traditional immunogen design, our Universal Antigen™ can induce antibodies to recognize epitopes containing less than 5 amino acids

**02 One-Stop Solution**
Streamlined strategy from immunization to hybridoma generation and characterization

**03 Reliable High-Throughput Screening Assays**
Optimized immunization and high-throughput screening for affinity measurements, epitope binning, isotyping, etc

**04 Comprehensive Functional Assays**
Extensive experience in antibody affinity ranking, affinity measurement, cross-reactivity, and other *in vitro* biological analysis

### Service Specifications

| Milestones | Details | Deliverables | Turnaround Time |
|---|---|---|---|
| Animal Immunization | • Blood collection before immunization<br>• Immunize 4 times<br>• Final blood collection | • Experimental report (including antiserum titer test results)<br>• The residual antigen | 6-8 weeks |
| Cell Fusion & Screening | • Cell fusion<br>• Subcloning and cell amplification<br>• Cell screening<br>• Clone selection and freezing | • Experimental reports (including screening data) | 5-6 weeks |
| Antibody Purification | • One hybridoma cell culture<br>• Antigen affinity purification or Protein A/G affinity purification<br>• Antibody purity test (SDS-PAGE)<br>• Antibody titer detection (ELISA, titer > 1:100000) | • 1-3 mg monoclonal antibody products, titer >$1*10^5$<br>• Full experimental report | 4-5 weeks |

# 7.8 Polyclonal Antibody Preparation

Polyclonal antibodies are a mixture of antibodies that recognize different epitopes of a specific antigen and can be produced by collecting antiserums after artificially immunizing the host. Because it can recognize multiple epitopes, it has a higher affinity and sensitivity than monoclonal antibodies. Polyclonal antibodies are also more tolerant to small antigen changes. With rich experience in antigen design and mature antigen/antibody preparation technology, Synbio Technologies can provide one-stop customized services from antigen design, gene synthesis, antigen preparation, animal immunity, to high throughput antibody production. In addition, some host animals (such as mice, rabbits, goats, etc.) and various kinds of antigens (such as peptide antigens, protein antigens, small molecule antigens, etc.) are available. Our technical team will evaluate your requirements and tailor your project to meet your needs. Ultimately, we can deliver high-quality polyclonal antibodies for your scientific research.

## Competitive Advantages

**High Titer**
The titer of ELISA will be greater than 1:100000. For protein antigens, the delivered polyclonal antibodies will be positive for Western Blot

**Customized Services**
Provide exclusive experimental scheme for each customer to meet your personalized requirements

**One-Stop Services**
We can provide one-stop services from gene synthesis, antigen preparation, to high-quality antibody production

**Confidentiality**
As your reliable partner, we will maintain a high degree of confidentiality for your sequences and products

**Professional Communication**
We will maintain frequent progress updates and issue communications throughout the entire project



## Service Specifications

| Milestones | Details | Deliverables | Turnaround Time |
|---|---|---|---|
| Antigen Preparation | Antigen provided by the customer:<br>1. The peptide 10 mg<br>2. Recombinant protein 5-8 mg, more than 85% purity<br>3. Small molecular antigens | The experimental report of antigen preparation | 2-4 weeks |
| | Synthesized or prepared by Synbio Technologies:<br>1. Design and synthesize polypeptide, coupling with KLH/BSA/OVA<br>2. Codon optimization, gene synthesis, recombinant protein expression and purification | | |
| Animal Immunization | Immunize 3-4 times | / | 7-8 weeks |
| Antibody Purification | Antigen affinity purification or Protein A/G affinity purification | 0.5-10 mg antibody products, purity>80% | 1-2 weeks |
| Quality Control | • Antibody purity test (SDS-PAGE)<br>• Specific detection of antigen and antibody (Western Blot analysis)<br>• Antibody titer detection (ELISA, titer > 1:100000) | Experimental report | 1-2 weeks |

Find more information at
*www.synbio-tech.com*

# 7.9 Single Domain Antibody (sdAb) Service

Complex structures and high production costs associated with monoclonal antibodies (mAbs) have limited their achievable clinical applications. For this reason, scientists focusing on antibody drug discovery have shifted their interests toward recombinant antibody fragments.

Recombinant antibodies, such as single domain antibodies (sdAb), are a new generation of engineered antibody fragment that consists of a single monomeric variable antibody domain. sdAbs are derived from camelid heavy chain antibodies which lack the light chain and CH domain of the heavy chain in the conventional Fab region. Therefore, sdAbs are smaller, more soluble, and have higher stability than conventional antibodies. sdAbs also feature higher permeability, lower immunogenicity, while being both simple and cost-effective. These advantages have made it possible for sdAbs to have a broader range of applications within the biotechnology, pharmaceutical, and immuno-therapeutic industries. Based on Synbio Technologies's extensive expertise in sdAb and advanced sdAb discovery technology, we can provide our customers with a comprehensive one-stop solution from antigen production to specific recombinant sdAb expression.

## Competitive Advantages

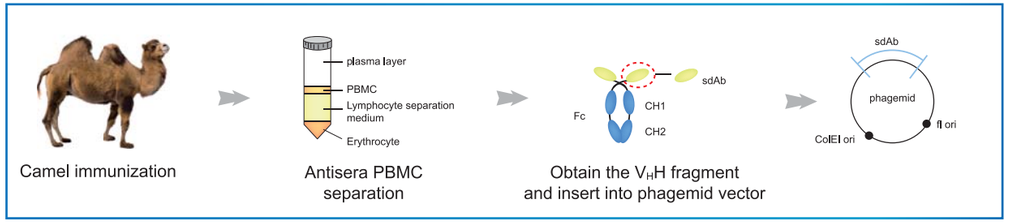
- 01 Experienced antibody production team
- 02 Proprietary Universal Antigen™ immunization technology
- 03 Robust phage-display technology platform
- 04 Advanced yeast-display technology platform (Large library capacity: ~ $10^9$)
- 05 Cost-effective and milestone payment plan

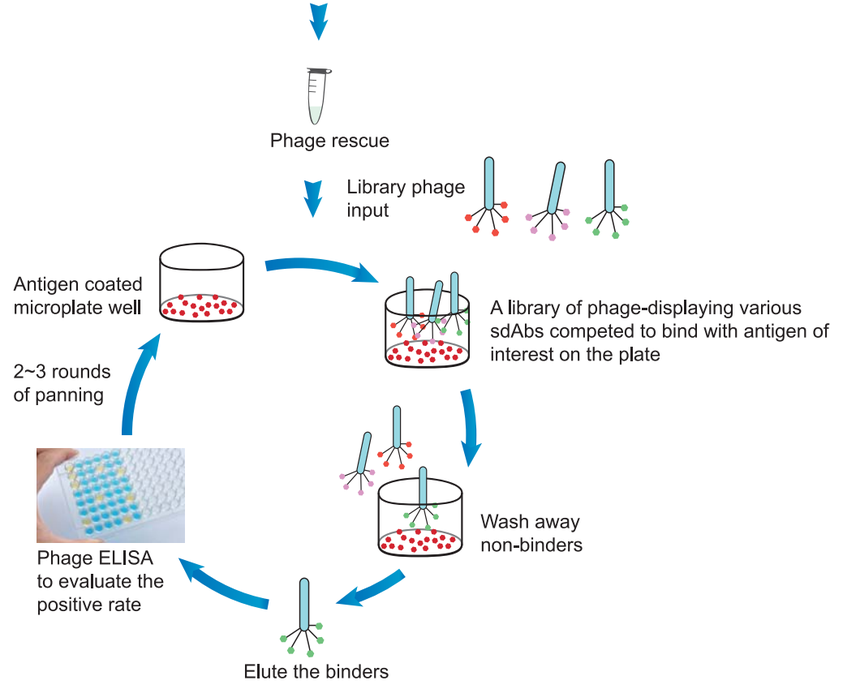
## Service Specification

| Milestones | Deliverables | Turnaround Time |
|---|---|---|
| Camel immunization (eg. dromedaries, camels, llamas, and alpacas) | 1. 100-500 μg sdAb products<br>2. Experimental report | 10-11 months |
| PBMC isolation & immune library construction | | |
| Screening of phage-displayed library | | |
| sdAb expression by eukaryotic or prokaryotic expression system | | |
| Functional assay for sdAb | | |



## Standard Process




# CHAPTER 8
# RNA Synthesis

RNA has been widely used in gene function analysis and novel therapeutic research/development, along with various other biological research fields. As the interest in RNA research has become more popular, the needs for RNA synthesis increase greatly.

Synbio Technologies has decades of experience in RNA research and development, our scientists can use both a chemical synthesis approach and IVT synthesis strategy of RNA to meet the unique requirements of every client. Synbio Technologies's RNA synthesis services include RNA oligo synthesis with various modifications and delivery specifications available, functional siRNA synthesis, miRNA synthesis, and *in vitro* transcription RNA synthesis. All our synthesized RNA sequences go through a standardized manufacturing process and undergo strict QC review. We promise to provide the most economical price to meet scientists' diversified needs without sacrificing on overall quality.

## Competitive Advantages

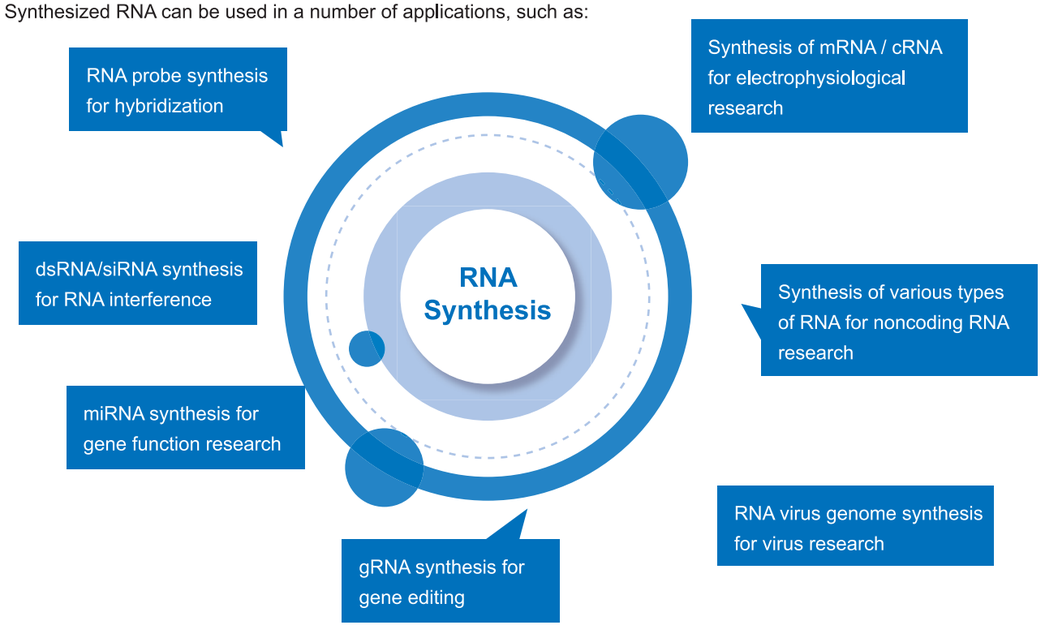
**01**
Excellent quality and stability

**02**
Flexible synthesis scales and various modification types to meet your customized requirements

**03**
Competitive prices and short turnaround time

## Applications

Synthesized RNA can be used in a number of applications, such as:



CHAPTER 8

# RNA Synthesis

Find more information at
*www.synbio-tech.com*

## 8.1 Custom RNA Oligos

When the fragment of mRNA you need is small, between 5 bases to 120 bases, we recommend using a chemical synthesis strategy, which is a quick and more economical method to produce a high yield of chemically synthesized RNA for various applications.

Synbio Technologies provides high-quality and diverse chemosynthetic RNAs, including customizable single/double stranded RNA oligos, modified RNA oligos, stable siRNA, miRNA, long RNA, etc. Chemically synthesized RNAs can be synthesized to contain nearly any sequence and chemical modification desired, with up to gram-quantity yields possible. All of our RNA products' quality will be confirmed by MS and HPLC purity tests to ensure the high-quality output.

### Competitive Advantages

**01** Various types of customizable RNA synthesis services capable of providing RNA fragments up to gram-scale

**02** Strict purification procedures of each batch of synthetic products by HPLC in an RNase-free environment, avoiding RNase contamination throughout the process, and a free HPLC detection report is provided

**03** Professional technical team provides you with comprehensive RNA solutions

**04** Commitment to keeping the details of the project strictly confidential, protecting your intellectual property rights

### Service Specifications

| Single-stranded RNA | | | | |
|---|---|---|---|---|
| Length | Order Size | Purification | Turnaround Time (Business Days) | Deliverables |
| 5-65 bases (General Sequences) | 2-10 OD | HPLC | 5-7 | 1. Lyophilized RNA<br>2. Certificate of Analysis (COA)<br>3. QC Report (MS/HPLC) |
| | 25-100 OD | HPLC | 6-8 | |
| | ≥250 OD | HPLC | Inquiry | |
| 65-120 bases | Inquiry | HPLC | Inquiry | |
| Double-stranded RNA | | | | |
| Each single strand is purified by HPLC and then annealed | | | | |



## 8.2 RNA Modifications

The modification of an RNA sequence can help the RNA to be delivered to the cell while increasing the stability of the RNA sequence, avoiding degradation by nuclease, such as phosphorothioate, 2'-OMe, and 2'-F modifications. The advantage of fluorescent labeling in RNA is to let the synthesized RNA be observed or located by fluorescence microscopy and confocal laser microscopy, while also using it as a nucleic acid detection tool to perform virus detection, disease diagnosis, and early tumor screening.

Synbio Technologies provides extensive modification options for synthesized RNA, making it convenient for customers to choose flexible and diverse modifications and fluorescent labeling according to the function of synthesized RNA sequences.

### RNA Modifications and Fluorescent Labels

| RNA Modifications | | RNA Fluorescence Labels/ Quenchers Groups | Dual/Multiple Labels |
|---|---|---|---|
| Modified Bases | • 2′-O-Methyl<br>• 2′ Fluoro<br>• m6A | • 5' Cy3<br>• 3' Cy3<br>• 5' Cy5<br>• 3' Cy5<br>• 5' FAM<br>• 3' FAM<br>• 5' ROX<br>• 3' ROX<br>• 5'HEX<br>• 5'TAMRA<br>• 5'TET<br>• 3'BHQ-1<br>• 3'DABCYL<br>• 3'TAMRA | • 5' FAM-3' BHQ1<br>• 5' HEX-3' BHQ1<br>• 5' TET-3' BHQ-1<br>• 5' FAM-3' MGB<br>• 5' FAM-3' TAMRA<br>• 5' HEX-3' TAMRA<br>• 5' TET-3' TAMRA<br>• 5' HEX-3' MGB<br>• 5' TET-3' MGB<br>• 5' FAM-3' DABCYL<br>• 5' HEX-3' DABCYL<br>• 5' Phos-3' FAM<br>• 5'TAMRA-3'Phos<br>• 5'C6-Biotin-3'Cy5 |
| Phosphorothioates | • P-S | | |
| Phosphorylation | • 5'- PHO<br>• 3'- PHO | | |
| Amino Linkers | • 5'-NH2 C6<br>• 3'-NH2 C7 | | |
| Thiol | • 5'-SH C6<br>• 3'-SH C3 | | |
| Biotin | • 5′ Biotin | | |
| Digoxigenin | • 5′ DIG<br>• 3′ DIG | | |

## 8.3 siRNA Synthesis

RNA interference (RNAi) using small interfering RNA (siRNA) is the simplest and most efficient way to knock down gene expression to study protein function in a wide range of cell types. It is also the best method for clinical treatment and drug development of RNAi.

Synbio Technologies provides double-stranded ordinary siRNA as well as modified or fluorescence-labeled siRNA. Available chemical modifications include phosphate backbone modifications, ribose modifications, and base modifications, which can increase the stability and duration of siRNA in vivo, or enhance base-base interactions to facilitate mRNA targeting.

### Service Specifications

| Products | Order Size (OD) | Purifications | Turnaround Time (Business Days) |
|---|---|---|---|
| NC FAM siRNA | 1-2,500 | HPLC/OPC | Starting from 3 business days |
| NC siRNA | | | |
| siRNA | | | |
| Modified or fluorescence labeled siRNA (2-OMe, Thiol, Chol, Biotin, FAM, Cy5, Cy3, etc.) | Inquiry | | Inquiry |

*For more information about chemical modification and fluorescent labeling, please contact us at *quote@synbio-tech.com*.

### Guaranteed siRNA Service

Synbio Technologies also provides a guaranteed siRNA service. The three designed and delivered siRNA ensure at least one siRNA can effectively inhibit the expression of the target gene, and the inhibition efficiency will be more than 70% (The transfection efficiency will be at least 90%).

| Products | Order Size (OD) | Purifications | Turnaround Time (Business Days) |
|---|---|---|---|
| Three siRNAs | 2 | HPLC/OPC | 6-9 |
| NC FAM siRNA | 1 | | |
| NC siRNA | 1 | | |
| Positive siRNA control | 1 | | |



## 8.4 miRNA Synthesis

miRNAs are single-stranded, small, non-coding RNA of 22 nucleotides that play important roles as endogenous gene regulators by mediating translation repression or promoting degradation of target mRNA. While the normal expression and function of miRNAs are vital for physiological processes, aberrant expression of miRNAs has been proved to be closely related to the occurrence of various cancers. Perturbation of endogeneous miRNAs help to study the function of specific miRNAs and hold the potential for therapeutics.

Synbio Technologies offers a wide range of high-quality miRNA synthesis products, including miRNA mimics/inhibitors, miRNA agomirs/antagomirs and miRNA negative controls, etc., to support your miRNA functional research.

### Service Specifications

| Products | Order Size (OD) | Purifications | Turnaround Time (Business Days) |
|---|---|---|---|
| NC FAM miRNA mimics | 1-2,500 | HPLC/OPC | Starting from 3 business days |
| NC miRNA mimics | | | |
| miRNA mimics | | | |
| NC FAM miRNA inhibitors | | | |
| NC miRNA inhibitors | | | |
| miRNA inhibitors | | | |
| miRNA agomirs | | | |
| miRNA antagomirs | | | |

## 8.5 RNA *In Vitro* Transcription Service

Another strategy of RNA synthesis is by *in vitro* transcription, especially suitable for long RNA synthesis. *In vitro* transcription takes linear DNA sequences as a template, using T7, T3, or SP6 RNA polymerase to synthesize RNA from the DNA sequence. Synbio Technologies provides highly efficient and cost-effective *in vitro* transcription RNA synthesis services. You can provide us with the DNA templates, such as the plasmid and PCR product, or we can help to do the *de novo* gene synthesis of the target DNA sequences. In our design, we synthesize the T7 promoter before the target sequence, which is used to initiate the transcription reaction during the RNA synthesis process. For mRNA transcription, Synbio Technologies provides optional mRNA modifications, including cap structures, poly(A) tails, and various modified nucleosides.

### Competitive Advantages

**Sequence Optimization**

Improve mRNA translation efficiency through codon optimization

**Fully Customized**

5 'cap, 3' polyA tail, and various nucleoside modification are available to enhance the stability and translation efficiency of mRNA and reduce its immunogenicity

**High Performance**

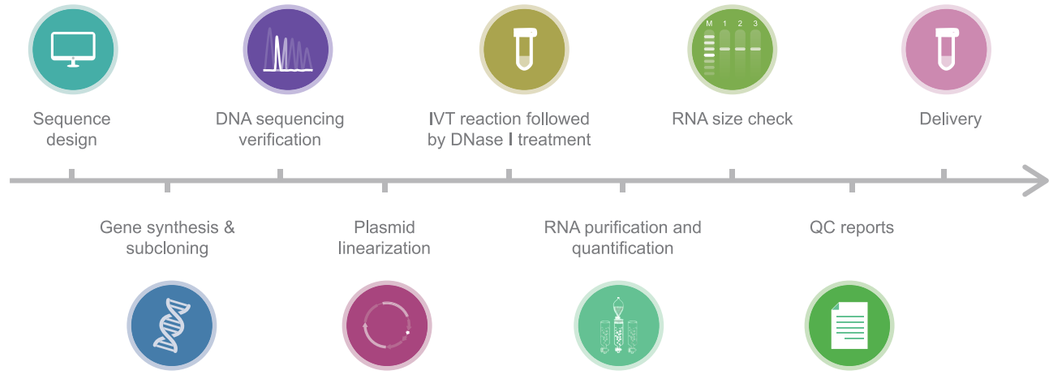
Standard experimental procedures, professional staff, and abundant expertise offer the most optimized and tailored solutions

**One-Stop Service**

Provide one-stop services from DNA template synthesis to RNA *in vitro* transcription

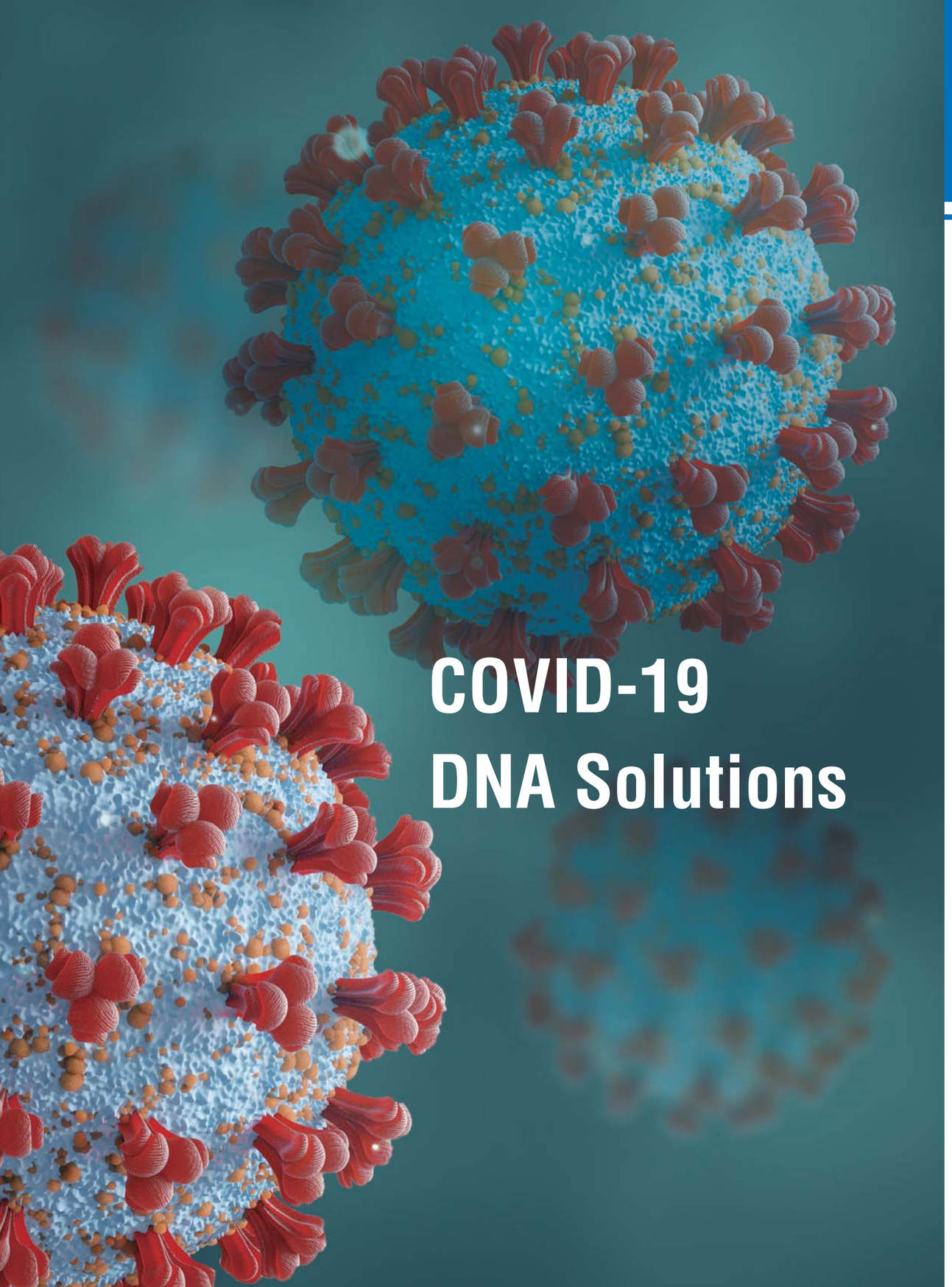


### RNA *In Vitro* Transcription Process




### Service Specifications

| Service Types | Details | | Purification Methods | Length | Turnaround Time | Deliverables |
|---|---|---|---|---|---|---|
| General RNA Synthesis | No UTR region, cap structure, poly(A) tail and nucleoside modification | | • Column Purification<br>• LiCl Purification<br>• Phenol/Chloroform Extraction | <9 kb | 1-2 weeks | • Liquid RNA products<br>• COA files |
| mRNA Synthesis | Cap1 analogs + transcription 120 poly(A) tail using template | N1-Me-Pseudo UTP<br>Ψ-UTP<br>5m-CTP<br>AF488/Cy3/Cy5 | | | 1-2 weeks | |
| | Cap1 analogs + post-transcription with poly(A) tail | | | | | |
| | Enzymatic capped + transcription 120 poly(A) tail using template | | | | | |
| | Enzymatic capped + post-transcription with poly(A) tail | | | | | |




# CHAPTER 9
# COVID-19 DNA Solutions

Since the outbreak of COVID-19, Synbio Technologies has set up a special coronavirus emergency project team, working against time to produce virus-related probes, genes, and protein products. We are currently providing comprehensive COVID-19 DNA solutions and working with our clients and partners from hospitals, scientific research institutes, and enterprises to fight the disease.

### ► Diagnostic Strategy

*Probe/Primer Synthesis for Virus Detection*

Synbio Technologies has prepared a series of primers and probes for COVID-19 detection in batches according to WHO and the CDC's latest official documents. We provide a free trial package and guarantee the supply of a million copies of nucleic acid diagnostic probes daily to speed up disease detection and research.

### ►Therapeutic Strategy

*From Gene Synthesis to Drug Discovery*

Our exclusive CI system can facilitate the accurate, fast, and high-quality gene synthesis of any sequence. Syno® Ab is our *de novo* antibody design & production platform based on the combination of DNA manufacturing with computer-simulated antibody molecule design. Syno® Ab helps researchers effectively reduce costs and time on designated target design, neutralizing antibody generation, and drug discovery.

### ►Preventive Strategy

*Vaccine Development and Production*

Syno® GPS is our advanced biotechnology transformation and application platform. We effectively serve the development and production of genetically engineered vaccines, nucleic acid vaccines, and therapeutic vaccines. Currently, we have completed the synthesis of all structural and non-structural protein genes / fragments of COVID-19. This facilitates the acquisition of viral recombinant proteins and vaccine development research.

Synbio Technologies has been using our efforts to realize the company's vision of "Genes for Life". We hope that, through our one-stop DNA solutions to COVID-19 and powerful synthetic technology platforms, we can accelerate virus research and help benefit each patient's life as soon as possible.

CHAPTER 9

# COVID-19 DNA Solutions

Find more information at
*www.synbio-tech.com*

## 9.1 Probe Synthesis for COVID-19 Detection

In early January 2020, Synbio Technologies designed and synthesized detection probes for COVID-19 based on our independently developed Syno® qPCR primer/probe design and synthesis platform. In less than two weeks, we completed the synthesis of detection reagent materials, such as amplification primers, probes, and quality controls.

### Competitive Advantages

**01**

**Speed Guarantee**
All COVID-19 related orders will be given priority to green channel production, with 24/7 professional online support

**02**

**Quality Assurance**
ISO 13485 standards of manufacturing, high-quality raw materials, zero cross-contamination, and perfect track records

**03**

**Production Capacity Guarantee**
Experienced synthesis experts, first-in-class instruments, more than one million probe kit materials supplied per day

### Probe/Primer Synthesis for Virus Detection

According to the official website documents of WHO and the CDC, Synbio Technologies has prepared a series of primers and probe materials for COVID-19 detection in batches. We can provide a free trial package and guarantee the supply of a million copies of nucleic acid diagnostic probes daily to speed up disease detection and research.

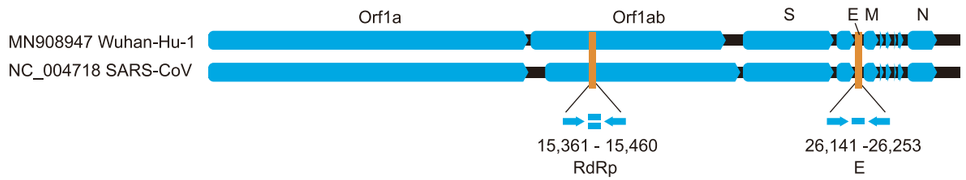



**Figure 1. Relative positions of amplicon targets on SARS-CoV an 2019-nCoV genome.**

ORF: Open reading frame

RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.



### ► SARS-CoV-2 Real Time RT-PCR Detection Assay

To help facilitate COVID-19 research and contribute to fight the virus, Synbio Technologies has manufactured a detection assay based on the CDC's Real-Time RT-PCR protocol for detection of the 2019 novel coronavirus. This assay has successfully detected SARS-CoV-2 in positive control samples N gene and human RNAse P (RNP) gene to assess the specimen quality. It should be used for research use only (RUO).

### Probe Assay Specifications

**Box #1:** Primers and Probes

| Reagent Label | Part # | Description | Quantity /Tube | Reactions /Tube |
|---|---|---|---|---|
| SARS-CoV-2_N1<br>SARS-CoV-2_N2<br>SARS-CoV-2_N3 | RV202001<br>RV202002<br>RV202003 | SARS-CoV-2_N1/N2/N3 Combined Primer/Probe Mix | 0.56 nmol | 24 |
| RP | RV202004 | Human RNase P Forward Primer/Probe Mix | 0.56 nmol | 24 |

**Box #2:** Positive Control

| Reagent Label | Part # | Description | Quantity | Note |
|---|---|---|---|---|
| RNAPC | RV202005 | SARS-CoV-2 Positive Control (RNAPC) | 1 tube | Providing (24) 5 µL test reactions |

### Features

✓ Based on CDC protocol, ISO 13485 standards of manufacturing reagents.
✓ TaqMan™ Real-Time RT-PCR assay primers and FAM probes for higher specificity.
✓ Targeting three targeted sequences of N gene in GenBank sequences NC_045512.2.
✓ Positive control included already, no extra preparation required.

### STATEMENTS

- This assay is for Research Use Only (RUO) and has not been tested on clinical samples.
- This assay is designed based on CDC protocol and public SARS-CoV-2 genome sequences.
- Different sample extraction methods, sample quality, operation parameters, data processing, and other un-tested factors might impact this assay's performance.

## ► Primer/Probe Set 01

| Name | | Description | Oligonucleotide Sequence (5'>3') | Length | 5'mod | 3'mod |
|---|---|---|---|---|---|---|
| RdRP gene | 1 | Primer RdRP_SARSr-F2 | GTGARATGGTCATGTGTGGCGG | 22 | | |
| | 2 | Primer RdRP_SARSr-R1 | CARATGTTAAASACACTATTAGCATA | 26 | | |
| | 3 | Probe RdRP_SARSr-P2 | CAGGTGGAACCTCATCAGGAGATGC | 25 | FAM | BHQ-1 |
| | 4 | Probe RdRP_SARSr-P1 | CCAGGTGGWACRTCATCMGGTGATGC | 26 | FAM | BHQ-1 |
| E gene | 5 | Primer E_Sarbeco_F1 | ACAGGTACGTTAATAGTTAATAGCGT | 26 | | |
| | 6 | Primer E_Sarbeco_R2 | ATATTGCAGCAGTACGCACACA | 22 | | |
| | 7 | Probe E_Sarbeco_P1 | ACACTAGCCATCCTTACTGCGCTTCG | 26 | FAM | BHQ-1 |
| N gene | 8 | N_Sarbeco_F1 | CACATTGGCACCCGCAATC | 19 | | |
| | 9 | N_Sarbeco_R1 | GAGGAACGAGAAGAGGCTTG | 20 | | |
| | 10 | N_Sarbeco_P1 | ACTTCCTCAAGGAACAACATTGCCA | 25 | FAM | BHQ-1 |

## ► Primer/Probe Set 02

| Name | | Description | Oligonucleotide Sequence (5'>3') | Length | 5'mod | 3'mod |
|---|---|---|---|---|---|---|
| Target 1 (ORF1ab) | 11 | Forward Primer 1ab | CCCTGTGGGTTTTACACTTAA | 21 | | |
| | 12 | Reverse Primer 1ab | ACGATTGTGCATCAGCTGA | 19 | | |
| | 13 | Probe 1ab | CCGTCTGCGGTATGTGGAAAGGTTATGG | 28 | FAM | BHQ-1 |
| Target 2 (N) | 14 | Forward Primer N | GGGGAACTTCTCCTGCTAGAAT | 22 | | |
| | 15 | Reverse Primer N | CAGACATTTTGCTCTCAAGCTG | 22 | | |
| | 16 | Probe N | TTGCTGCTGCTTGACAGATT | 20 | FAM | TAMRA |



## ► Primer/Probe Set 03

| Name | | Description | Oligonucleotide Sequence (5'>3') | Length | 5'mod | 3'mod |
|---|---|---|---|---|---|---|
| RP-F | 17 | RNAse P Forward Primer | AGATTTGGACCTGCGAGCG | 19 | | |
| RP-R | 18 | RNAse P Reverse Primer | GAGCGGCTGTCTCCACAAGT | 20 | | |
| RP-P | 19 | RNAse P Probe | TTCTGACCTGAAGGCTCTGCGCG | 23 | FAM | BHQ-1 |
| 2019-nCoV_N1-F | 20 | 2019-nCoV_N1 Forward Primer | GACCCCAAAATCAGCGAAAT | 20 | | |
| 2019-nCoV_N1-R | 21 | 2019-nCoV_N1 Reverse Primer | TCTGGTTACTGCCAGTTGAATCTG | 24 | | |
| 2019-nCoV_N1-P | 22 | 2019-nCoV_N1 Probe | ACCCCGCATTACGTTTGGTGGACC | 24 | FAM | BHQ-1 |
| 2019-nCoV_N2-F | 23 | 2019-nCoV_N2 Forward Primer | TTACAAACATTGGCCGCAAA | 20 | | |
| 2019-nCoV_N2-R | 24 | 2019-nCoV_N2 Reverse Primer | GCGCGACATTCCGAAGAA | 18 | | |
| 2019-nCoV_N2-P | 25 | 2019-nCoV_N2 Probe | ACAATTTGCCCCCAGCGCTTCAG | 23 | FAM | BHQ-1 |
| 2019-nCoV_N3-F | 26 | 2019-nCoV_N3 Forward Primer | GGGAGCCTTGAATACACCAAAA | 22 | | |
| 2019-nCoV_N3-R | 27 | 2019-nCoV_N3 Reverse Primer | TGTAGCACGATTGCAGCATTG | 21 | | |
| 2019-nCoV_N3-P | 28 | 2019-nCoV_N3 Probe | AYCACATTGGCACCCGCAATCCTG | 24 | FAM | BHQ-1 |

## ► Set 04 Control

Synbio Technologies has obtained the following viral RNA through *in vitro* transcription and prepared positive controls, which helps to control reverse transcription efficiency, achieve standard unification, reduce false negative rates, make test results more reliable, and greatly improve the accuracy of kit detection.

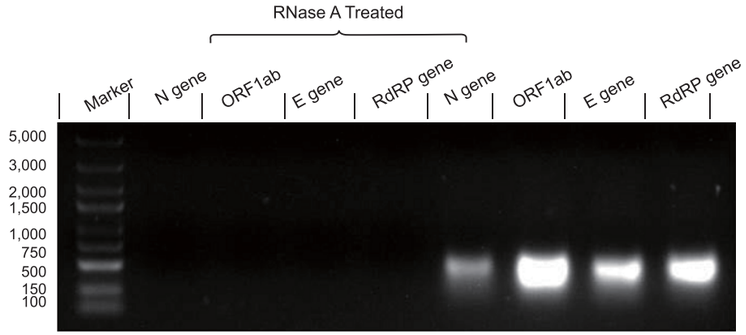
| Name | | Description | Transcription Sequence |
|---|---|---|---|
| RdRP gen | 29 | RdRP standards | GTGAAATGGTCATGTGTGGCGGTTCACTATATGTTAAACCAGG<br>TGGAACCTCATCA<br>GGAGATGCCACAACTGCTTATGCTAATAGTGTTTTTAACATTTG |
| E gene | 30 | E gene standards | ACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTT<br>CGTGGTA<br>TTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGT<br>GTGCGTACTGCTGCAATAT |

CHAPTER 9

# COVID-19 DNA Solutions

| Name | Description | | Transcription Sequence |
|---|---|---|---|
| Target 1 (ORF1ab) | 31 | ORF1ab standards | CCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGC GGTATGTGGAAAGGTTAT GGCTGTAGTTGTGATCAACTCCGCGAACCCATGCTTCAGTCA GCTGATGCACAATCGT |
| Target 2 (N) | 32 | N standards | GGGGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATG CTGCTCTTGCTTTGCTGC TGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTG |

| Name | Description | | Transcription Sequence |
|---|---|---|---|
| Endogenous control | 33 | RNase P | AGATTTGGACCTGCGAGCGGGTTCTGACCTGAAGGCTCTGCG CGGACTTGTGGAGACAGCCGCTC |




* The above figure shows the sample map of COVID-19 gene RNA extracted by Synbio Technologies (N-gene part, ORF1ab part, E-gene part, RdRP gene part).

Sequences above were collected from WHO, US CDC, and China CDC official website documents. Synbio Technologies's related products have been added with RNase P, which meets the double detection standard and can greatly reduce the "false negative" phenomenon in the nucleic acid detection process and improve the detection accuracy.





## 9.2 Gene Synthesis & Protein Expression for COVID-19

Synbio Technologies's Syno® DNA synthesis platform provides fast gene and fragment synthesis, high-throughput synthesis, etc. The independently developed CI sequence difficulty index system and NG™ Codon Optimization Technology can facilitate the accurate, fast, and high-quality synthesis of any sequence.
Early in the pandemic, we completed the synthesis of all structural and non-structural protein genes or gene fragments of COVID-19. This has facilitated the acquisition of viral recombinant proteins and the study of their protein structure biology. In addition, when the new COVID-19 variant Omicron was discovered, we responded quickly and successfully synthesized Omicron's wild-type Spike protein gene and codon-optimized Spike protein gene. We are committed to providing strong support for downstream vaccine development and neutralizing antibody research.

### Competitive Advantages

**Speed Guarantee**
All COVID-19 related orders will be given production priority with 24/7 professional online support

**Production Capacity**
Our patented NG™ Codon Optimization Technology optimizes genes at no extra cost to you, and we also have multiple protein expression systems available alongside customized production

**Product Supply**
Omicron wild type Spike protein gene and optimized Spike protein gene are available in stock

### Omicron Product Specifications

| Products | Amount | Availability |
|---|---|---|
| Wild Type Spike Protein Gene | 10 μg | In Stock |
| Optimized Spike Protein Gene | 10 μg | In Stock |

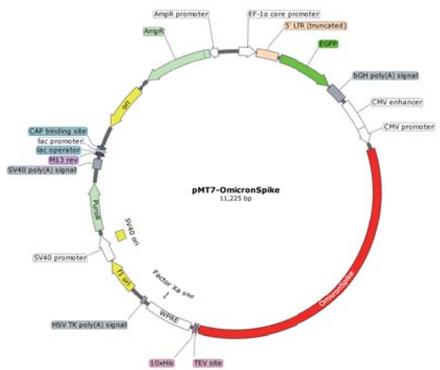



Fig.1 Wild type Omicron Spike protein gene

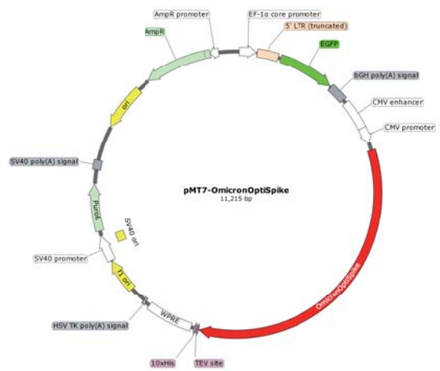



Fig.2 Optimized Omicron Spike protein gene

# DNA Data Storage

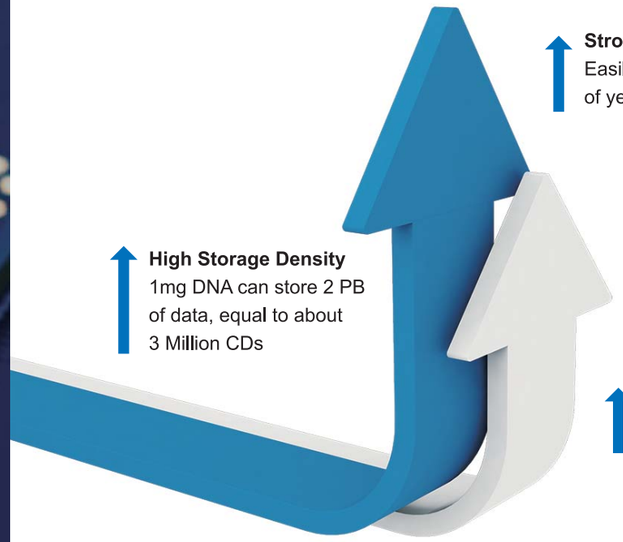
CHAPTER 10

# DNA Data Storage

DNA storage technology (using DNA as a data carrier) is a future-oriented technology with epoch-making significance. It uses the four bases of DNA (“A”, “G”, “T”, “C”) arranged in a specific order to form a code for information. As a technology that uses synthetic DNA as a storage medium, it can store text, images, audio and video, and then read subsequently read the data.

Scientists at Synbio Technologies have mastered the next-generation synthetic biology technology platform that could dramatically reduce the manufacturing cost of DNA synthesis. We have developed DNA Studio™ to enable bidirectional transcoding between "A, T, C, G" and digital information. Then, according to the code, the DNA sequences are rapidly and accurately synthesized on a chip in large quantities. We sincerely invite you to join in the development of DNA digital storage systems!

## Competitive Advantages



**Strong Resistance**
Easily preserved for over thousands of years in a natural environment

**Low Maintenance**
Do not need to maintain constantly

**High Storage Density**
1mg DNA can store 2 PB of data, equal to about 3 Million CDs

**Improvement of DNA Synthesis Technology**
Provides low cost, high-throughput synthetic DNA

**Living Medium**
Stored in organisms to achieve "soft storage" of information

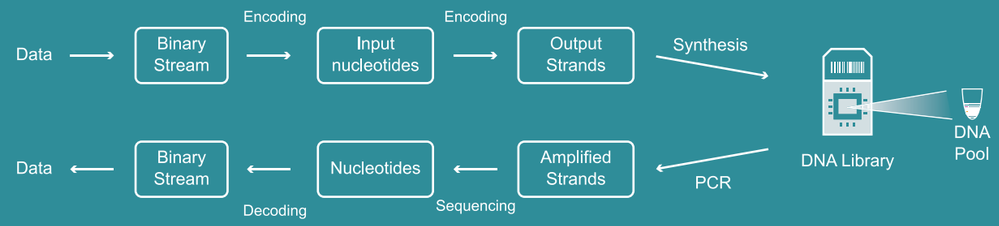
**Environmental Protection**
Carbon storage materials benefit "green initiatives"

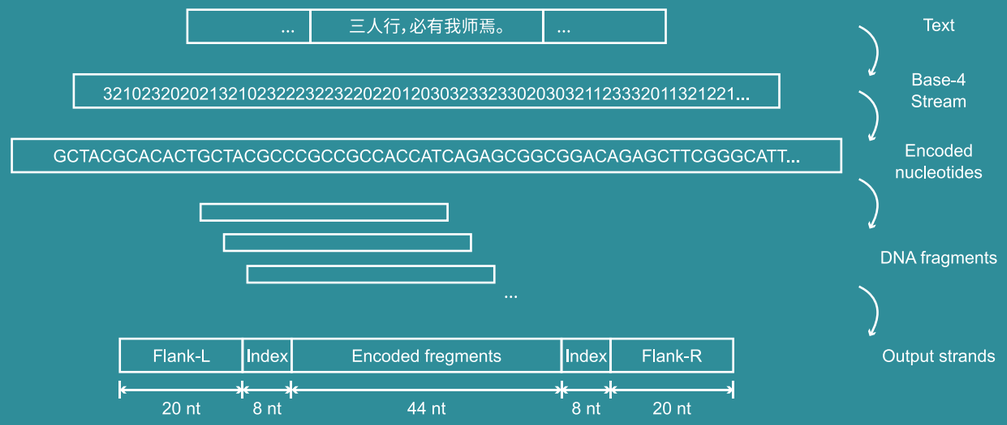
CHAPTER 10

# DNA Data Storage

## DNA Data Storage
## **Case Study**

Synthesized and stored in DNA via our powerful Syno® HT DNA synthesis platform, the Analects of Confucius was transformed into As, Ts, Cs, and Gs using our invented code. With DNA Studio™, the recovery rate after sequencing decoding was 99.9%. This is an example of hard storage, which we published in 2017.




Our R&D department transformed a Chinese poem "The Analects of Confucius" into yeast.

The process is: store-transfer-read-decode. This is a completely soft storage. This poem was copied 10 billion times via this fast and economic method to become the most printed work in the world.

Find more information at
*www.synbio-tech.com*

## 10.1 Your IP, Our DNA

Synbio Technologies has launched the world's first DNA Solution to protect our customers' proprietary information by encrypting and synthesizing it within a sequence of DNA. To accomplish this, Synbio Technologies utilizes our patented DNA Studio™ which accurately synthesizes a tailor-made sequence that acts as an exclusive DNA "security code". By utilizing our Syno® Synthesis Platforms, this unique sequence, or "security code", can be synthesized and placed into any position of the DNA. This allows the security information to be synthesized and stored as a biological "watermark".

### How it Works


1. Based on our developed DNA Studio™, Synbio Technologies can provide the requested DNA sequence at any position in the vector as a "security code".
2. With the encryption mechanisms and the Syno® Synthesis Platforms, cryptographic sequences can be synthesized into any section of the requested DNA sequence.
3. The "security code" can also be constructed to any vector requested by the customer.
4. The interpretation process can be easily completed by sequencing and analysis.

**DNA Studio™ IP Protection Solution**

**ALLOWS YOU TO**

Provide customized **IP protection** for your embedded "security code".

Make your DNA construction and synthesis **"smart"**.