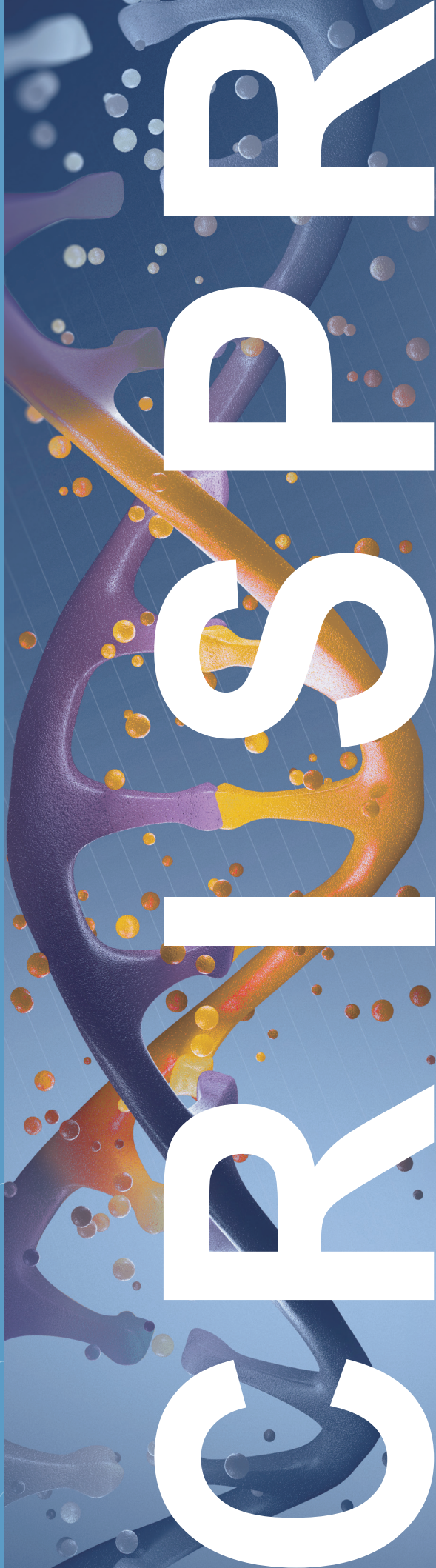
GenScript
Make Research Easy

Gene Editing Services

Comprehensive CRISPR Solutions from Screening to Clinical Trials

[2023-2024 Edition]



www.genscript.com

Make People and Nature Healthier Through Biotechnology

ABOUT US

GenScript Biotech Corporation (stock code: HK.1548) is a leading global provider of life science research, development and manufacturing services. Rooted in solid gene synthesis technology, GenScript has established four major platforms: life science service and product platform, biomedical contract development manufacture organization (CDMO) platform, cell therapy platform and industrial synthetic biological products platform.

Founded in 2002, GenScript established its R&D and manufacturing headquarters in Nanjing, China in 2004. In 2015, GenScript was listed on the Main Board of the Stock Exchange of Hong Kong, with legal entities in the United States, China, Hong Kong, Japan, Singapore, the Netherlands and Ireland. It operated business in over 100 countries and regions worldwide, providing quality, convenient and reliable services and products for more than 100,000 customers.

As of Jun 30th 2022, GenScript owned more than 5,500 employees worldwide, with over 38% of them holding a Ph.D. or master's degree. GenScript has a number of intellectual property rights, including more than 190 granted patents and more than 820 patent applications, as well as a high dense technical secrets. With its mission of "making people and nature healthier with biotechnology", GenScript is committed to be one of the most trusted biotechnology companies in the world. As of Jun 30th 2022, GenScript' s services and products have been cited in over 74,700 peer-reviewed international academic periodical articles.

HISTORY & MILESTONES OF GENSCRIPT GROUP

2002

Founded in
New Jersey, US

GenScript
Make Research Easy

2013

Established Bestzyme
(Industrial Synthetic Biology
Product Segment)

Bestzyme

2014

Founded Legend Biotech
(Cell Therapy Segment)

LEGEND BIOTECH

2015

GenScript was listed on HKEX
(stock code: HK.1548)

HKEX
香港交易所

2017

Legend Biotech and Janssen
entered into global strategic
collaboration on cilta-cel

LEGEND BIOTECH janssen

2020

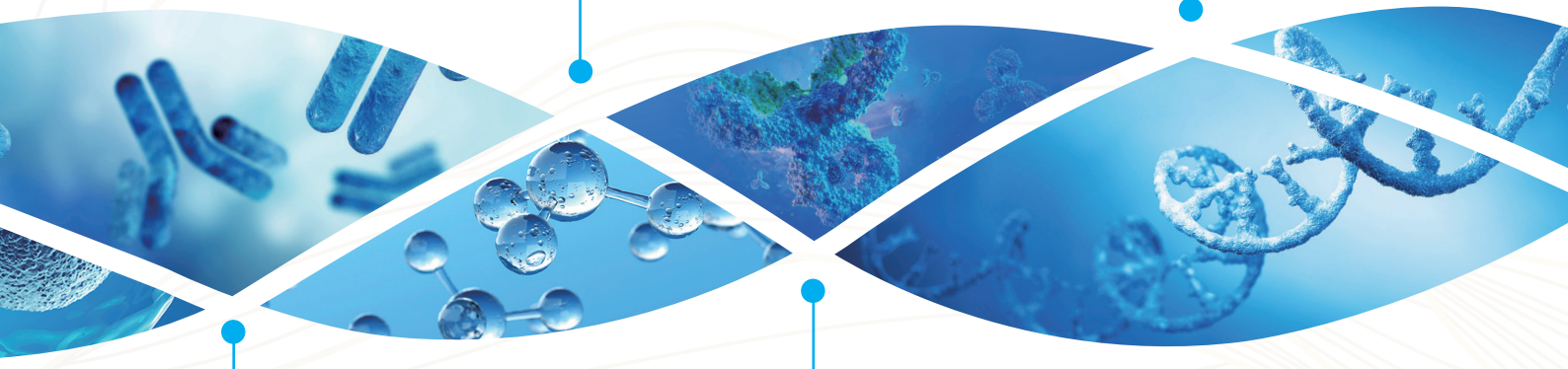
Legend Biotech was listed on Nasdaq
(NASDAQ: LEGN)
Launched GenScript ProBio

Nasdaq

2022

CARVYKTI® granted approval by
US FDA, EC and Japan MHLW

CARVYKTI™
(ciltacabtagene autoleucel) Suspension for IV Infusion



2018

Established Biologics
CDMO Segment
(GenScript ProBio)

GenScript PROBIO

2021

Group, ProBio and Legend Biotech
received funding of
$1 billion from Hillhouse Capital

HILLHOUSE

Content

CONTENTS

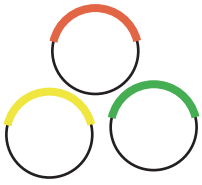
01

Introduction to Genome Editing Service

Technology Platform

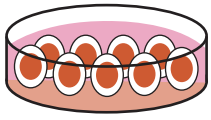
GenScript provides complete gene editing solutions, supporting target gene screening and sorting, plasmid/lentivirus/ RNP multiple delivery system experiments, and KO/KI cell line or animal model engineering.

Step1 Target gene screening



CRISPR gRNA Library

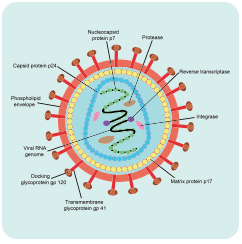
Step3 Drug development



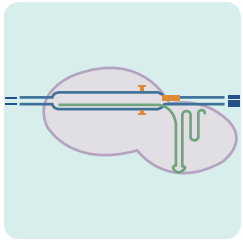
GenCRISPR™
Genome Editing Cell Lines

Step2 Genome editing model construction

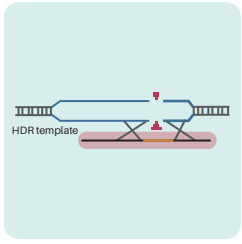
CRISPR gRNA plasmid



gRNA/Cas9 Lentivirus package

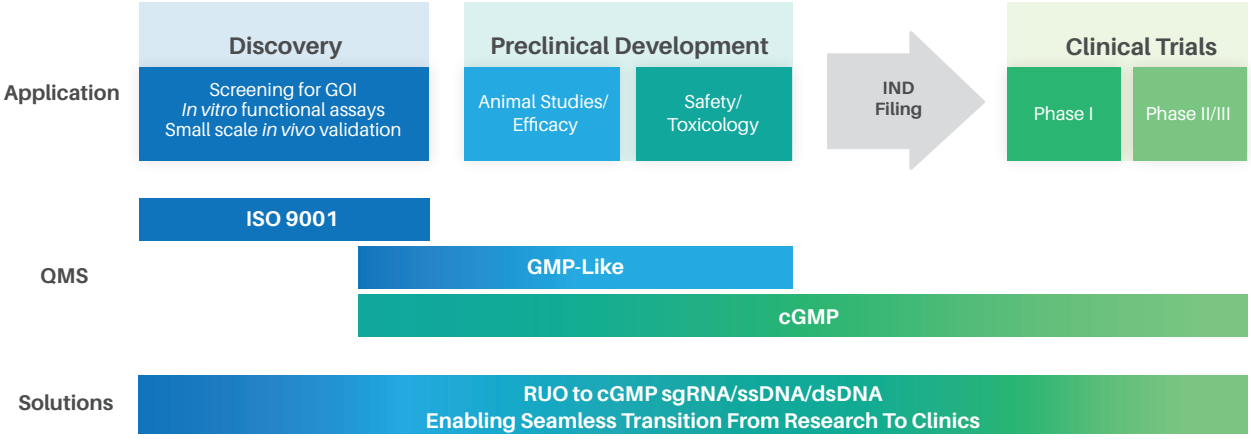


Chemical synthesis sgRNA[Hot]



HDR knock-in templates[Hot]

Phase-appropriate material and comprehensive QA/QC documentation for successful IND filing



Production Capacity

ISO 9001 Certified

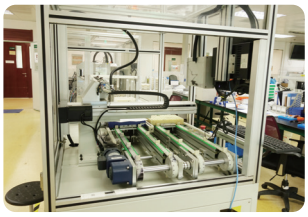
P2 Laboratory: complying with the new Biosecurity Act

Provincial demonstration intelligent workshop: high-throughput & efficiency

CRISPR nucleic acids production platform

Synthesis

Quality Control



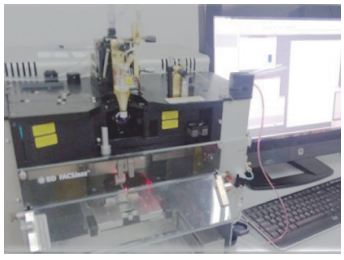
Automatic Labeling

Purification

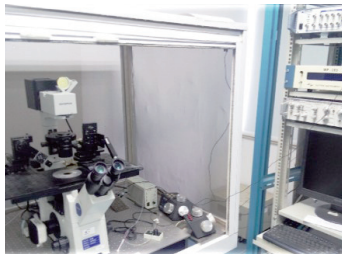
Automatic Weighting and Distributing

Lentivirus Packaging and Cell line Development Platform

BSL2 Level Biosafety Cabinet



Flow Cytometer



Fluorescence Microscope

Service Advantages

Diverse Technology Platforms

High-throughput & large scale platform form μg to g level deliverable

Support screening, validation to GMP request

Excellent Production Capacity

Chemical synthesis of sgRNA up to 180 nt

Expert in different sequence & high proportion modification

Strict Quality Control Standards

Customize QC indicators based on application Improve application success rate and stability

Professional Technical Support

Project management team led by Ph.Ds.

Timely solution of production and application problems

Please scan the QR code to visit CRISPR Service home page for more information

02

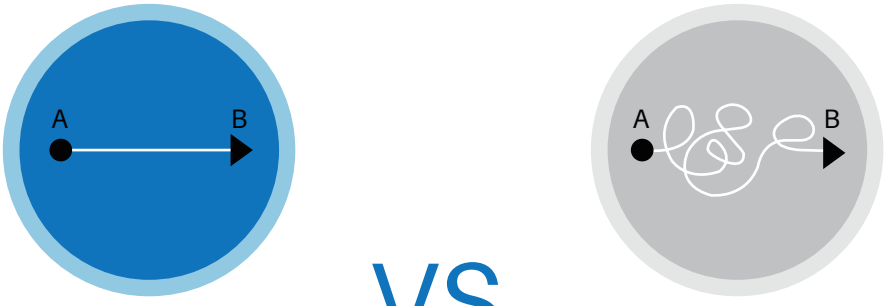
Genome Editing Reagent Service

Synthetic sgRNA

Traditional CRISPR editing techniques involve transfecting expression plasmids encoding guide RNA and/or Cas9 nuclease into host cells. However, combining Cas9 protein with synthesized sgRNA into ribonucleoprotein (RNP) prior to delivery can significantly simplify the experimental process, while improving editing efficiency, reducing off-target effects, and avoiding immune response.

GenScript offers sgRNA synthesis service with guaranteed sequence correctness, which is more efficient, less toxic and safer than plasmid based editing solutions, improving efficiency and saving time and labor costs for your project.

Service Features



Chemically synthesized sgRNA

- Ready to use and cost-effective
- Low cytotoxicity, good stability, high editing efficiency
- Accurate sequence with guaranteed deliver quantity
- High batch to batch consistency

***In vitro* transcribed sgRNA**

- 5-6 steps of synthesis work with high cost
- High cytotoxicity, poor stability, low editing efficiency
- Non-guaranteed success rate
- Large differences among batches

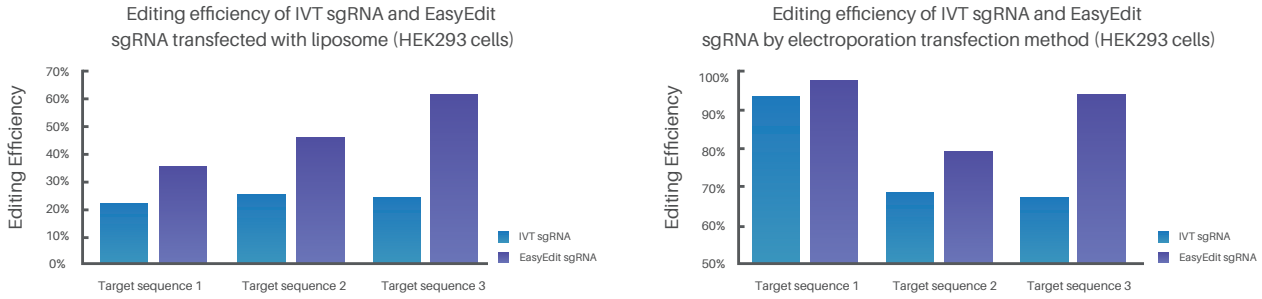
Service Details

Service Name	Length (nt)*	Specification (nmol)*	Delivery Time (Business Day)	Delivery standard
EasyEdit sgRNA	97-103	1.5-500 nmol	Form 6 days	Accurate sequence Guaranteed delivery
SafeEdit sgRNA	97-103	3-1,000 nmol	From 9 days	Accurate sequence HPLC purification

*GenScript provide GMP-Like & cGMP sgRNA to support preclinical development and clinical trials, please find the information in page 17-18.

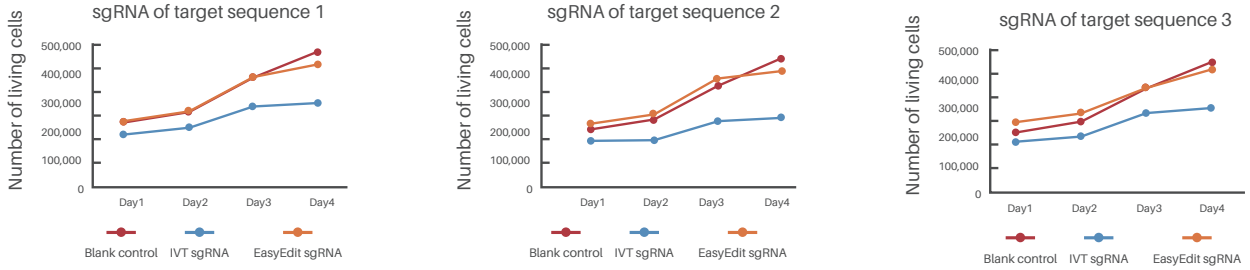
Cases

Case 1: High Editing Efficiency



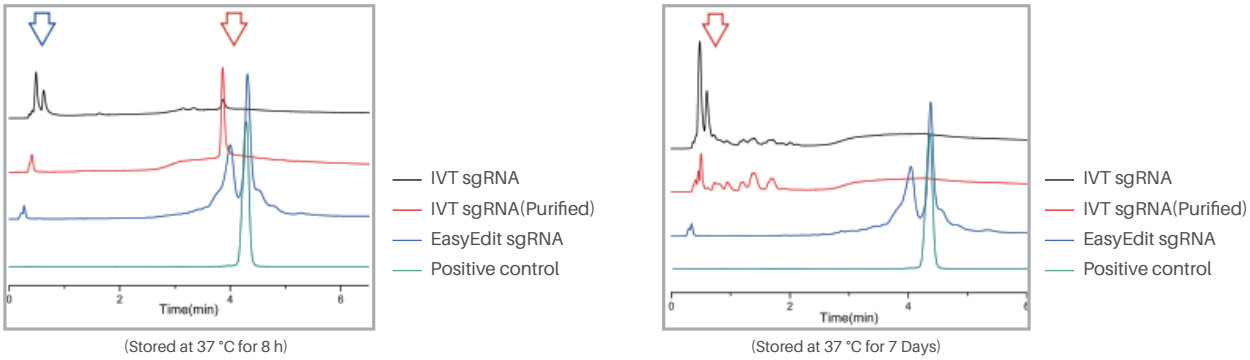
After liposome transfection and electroporation transfection, **the chemosynthetic EasyEdit sgRNA showed high editing efficiency, which was consistent with the literature reports**[1].

Case 2: Low Cytotoxicity



EasyEdit sgRNA cells showed lower cytotoxicity and less impact on host cell proliferation and cell activity, which was consistent with the literature reports[2]. IVT sgRNA has 5′ triphosphate modification, which can cause cellular immune response, resulting in cytotoxicity and interfere with subsequent experiments, while chemosynthetic EasyEdit sgRNA has no such interference factor.

Case 3: High Stability



(Stored at 37 °C for 8 h)

(Stored at 37 °C for 7 Days)

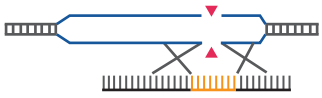
Chemosynthetic EasyEdit sgRNA is more stable than IVT sgRNA, which degrades during storage (red arrow) because IVT sgRNA cannot be modified, **whereas the chemically synthesized EasyEdit sgRNA can be modified to ensure that sgRNA is more stable in storage and experiments**. IVT sgRNA involves a multi-step biological reaction, and there are impurities (blue arrow) such as buffer and enzyme in the product, **while EasyEdit sgRNA is stable in the chemical synthesis process, and has better lot-to-lot stability than IVT sgRNA**, ensuring repeatability and consistency of results in subsequent experiments.

[1] Hendel, A. *et al.* Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. Nat.Biotechnol.33 (2015).
[2] Kim *et al.* CRISPR RNAs trigger innate immune responses in human cells. Genome Res. 28 (2018).

CRISPR HDR Knock-in Templates

One powerful application of CRISPR/Cas genome editing technology involves the precise insertion of DNA sequences via the homology-directed repair (HDR) pathway. While many factors may affect the efficacy of this approach, choosing the right HDR template is certainly an important one. GenScript now offers high quality, sequence verified HDR templates in both linear and circle format for maximizing the editing efficiency of your CRISPR experiments, and efficiently support non-viral cell therapy development .

GenExact™ ssDNA
Precise KI and low cytotoxicity



Application
- Ideal for T cell therapy
- Ideal for cell/animal model generation

Advantage
- **Minimal cytotoxicity**
- **Precise knock-in, minimized off-target effect**
- High purity and sequence verified

Specifications
- Insertion length: 150-5,000 nt
- μg to g scale
- Research to cGMP grade

GenWand™ dsDNA
Long gene knock-in in large scale

Application
- Ideal for long gene CRISPR KI
- Ideal for screening and scale up

Advantage
- **Covalently closed ends protection for better accuracy**
- **More suitable for scale up**
- High purity and sequence verified

Specifications
- Insertion length: 1-10 kb
- μg to g scale
- Research to cGMP grade

GenCircle™ dsDNA
Circular template with no resistance gene

Application
- Ideal replacement for plasmid HDR template
- Ideal for scale up and fast delievery

Advantage
- **KI efficiency increase by up to 30%**
- **429bp backbone, lower cytotoxicity & higher transfection efficiency**
- **No antibiotic resistance gene to avoid regulatory concern**
- High purity and sequence verified

Specifications
- Insertion length: 1-20 kb
- μg to g scale
- Research to cGMP grade

GenExact™ ssDNA Synthesis Service

Linear GenExact™ ssDNA offers the lowest toxicity solution for HDR mediated gene knock-in. Precise insertion of sequences up to 5kb makes GenExact™ ssDNA an ideal solution for T cell therapy and cell or animal model engineering.

Why GenExact™ ssDNA?

Cas9/sgRNA complex is cut at the target site, resulting in double-strand breaks

ssDNA as a repair template containing DNA fragments to be knocked in

Genes that obtained precise knock-in of new DNA fragments

DNA fragments is knocked-in by homologous recombination

- ☑ Low cytotoxicity
- ☑ Low off-target effect
- ☑ High knock-in efficiency
- ☑ Improved gene editing accuracy

Service Advantages

Low cytotoxicity and low off-target effect

Improve the efficiency and accuracy of target gene knock-in

Guarantee the production of cell therapy products

Accurate sequence guaranteed and high purity

Correct plasmid and Sanger sequencing of delivered product

Deep removal of impurities, with the purity up to 98%

Powerful synthesis capacity

Rich experience in synthesis of sequence synthesis

Optimized production technology that is conducive to scaling up the production

Support declarations from R&D to clinic

Support μg to mg scale delivery

Provide scientific research to cGMP level products

Service Details

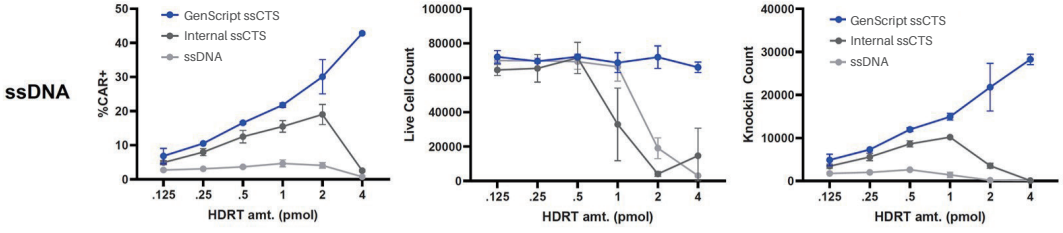
Service Type	Length (nt)	Quantity	Production time	Delivery form*	Price
GenExact™ ssDNA#	151-5,000	3μg to 100mg	From 22 days	Lyophilized powder (default) Liquid solution (customized)	For details, please consult us

If you need an antisense chain, the cycle remains unchanged. It will be more favorable to order the same sequence again.
*For other length and delivery information's or special difficulty orders needing assessment cycle, please email oligo@genscript.com or call 1-877-436-7274.
#GenScript provide GMP-Like & cGMP GenExact™ ssDNA to support preclinical development and clinical trials, please find the information in page 17-18.

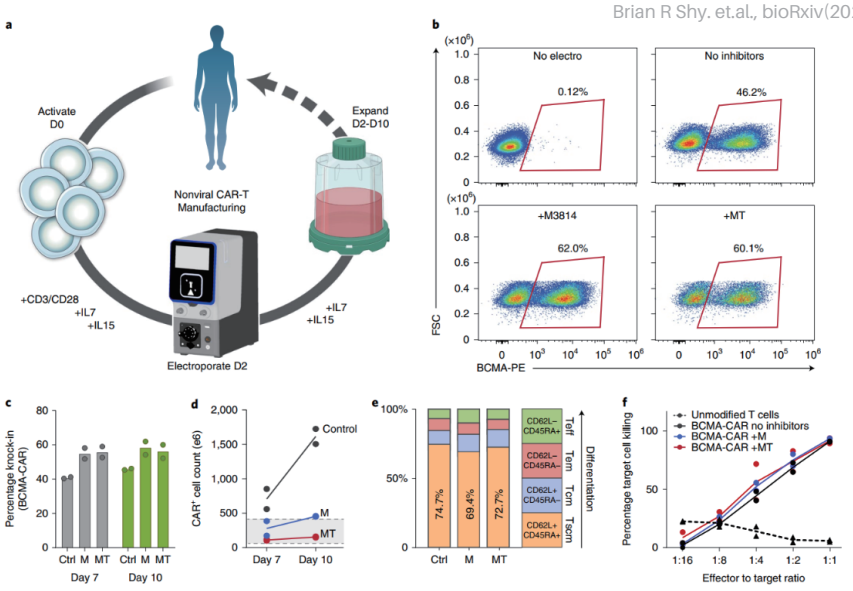
Cases

The experimental results of Marson Lab at University of California, San Francisco (UCSF) showed that:

- GenExact™ ssDNA provides **lower cytotoxicity and higher knock-in efficiency** than lab-synthesized knock-in templates
- GenExact™ ssDNA of the cGMP scale has a **knock-in efficiency of up to 46.2%** without the addition of enhancers
- Cells that have been knocked-in by GenExact™ ssDNA **can reach the patient's dosage (1.5×10⁹), and the prepared BCMA-CAR cells exhibit tumor-killing activity**



ssCTS: ssDNA with CTS (Cas9 target sequences)
Brian R Shy. et.al., bioRxiv(2021)



Brian R. Shy. Et.al. Nature Biotechnology (2022)

GenWand™ dsDNA Synthesis Service

The linear GenWand™ dsDNA double-ended covalently closed design offers improved protection and stability. GenScript's universal production vector and thermostable enzyme process allows for large-scale manufacturing of long insert sequences, without the use of animal-derived ingrediants.

Why covalently closed linear dsDNA?

Compared with PCR dsDNA GenWand™ dsDNA has the following advantages:

- ☑ Professional purification for removing impurities and low cytotoxicity
- ☑ Higher stability to avoid digestion and degradation of exonuclease
- ☑ Avoid introducing interference plasmid sequence
- ☑ Reduced non-homologous end connection and high knock-in efficiency
- ☑ Long gene knock-in and cost-effective scale-up production

Service Advantages

Long Gene Knock-in Template
With the synthetic length of 1-10 kb

Scale-up Process and Production
Delivery from μg-scale to g-scale

Strict Quality Control
Strictly control of impurities and endotoxin

Customized Quality Control
Support R&D, preclinical / clinical research

Service Details

Service Type	Length (nt)	Quantity	Turnaround Time	Delivery form*	Price
GenWand™ dsDNA#	1-10 kb	50 μg to g	From 3 weeks	Lyophilized powder (default) Liquid solution (customized)	For details, please consult us

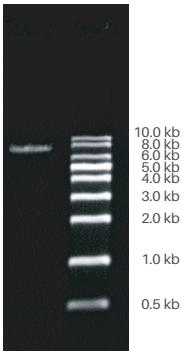
*For other length and delivery information's or special difficulty orders needing assessment cycle, please email oligo@genscript.com or call 1-877-436-7274.

#GenScript provide GMP-Like & cGMP GenWand™ dsDNA to support preclinical development and clinical trials, please find the information in page 17-18.

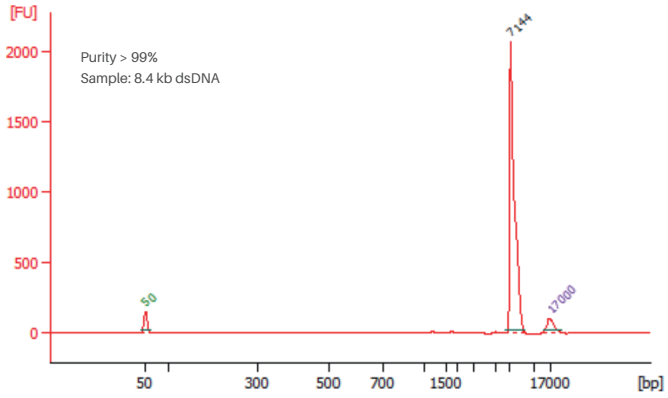
Cases

High purity to avoid unexpected interference

As shown in the case below, a single electrophoretic band and a purity of > 99% can be seen in the GenWand™ dsDNA product inspection.

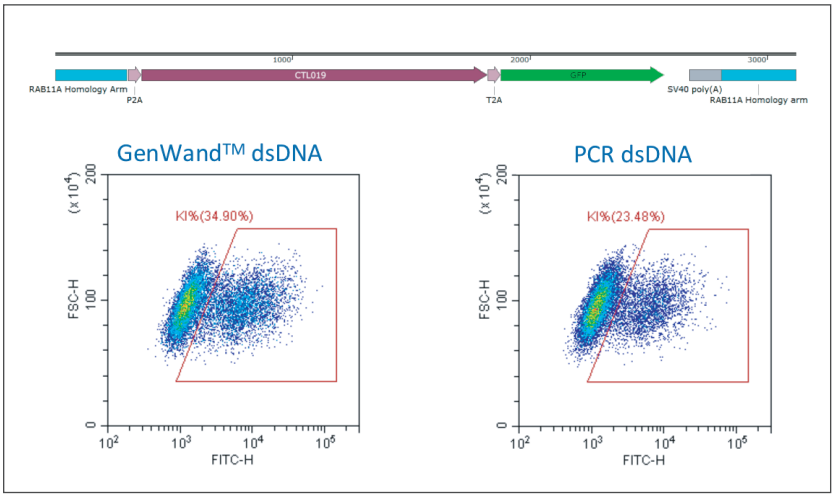


Lane 1: 8.4 kb dsDNA
Lane M: 10000dsDNA marker



High gene knock-in efficiency

The knock-in efficiency of the 2.5 kb sequence⁎ knocked into the RAB11A site of HEK293T cells was measured by the Neon® Transfection System with 2 μg of dsDNA template, and the gene knock-in efficiency of **GenWand™ dsDNA was higher than that of PCR dsDNA prepared in laboratory (34.90% vs 23.48%)**.

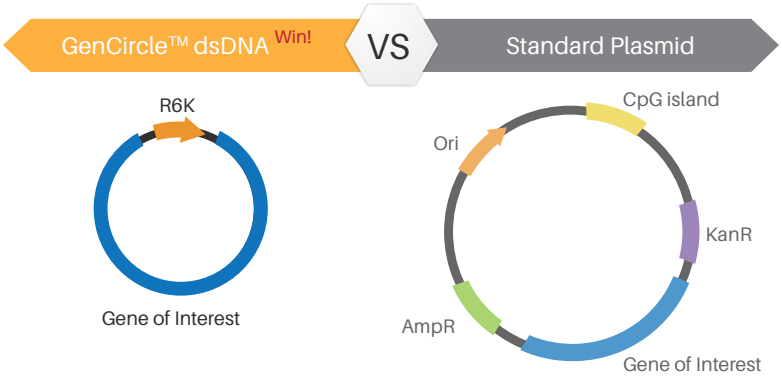


⁎ The length of knock-in sequence was 2.5 kb (excluding homologous arms)

GenCircle™ dsDNA Synthesis Service

- Micro vector with no antibiotic resistance gene & Only 429 bp backbone.
- A safe & efficient Knock-in template, transposon vector, viral packaging plasmid or non-viral GOI vector.

Advantages



Safer! No Antibiotic Resistance Gene

- No antibiotic resistance gene & antibiotic residual
- Prevent plasmid replication outside of the engineered production host strain

Smaller! High fidelity 429 bp backbone

- Higher gene expression from equivalent plasmid quantity
- Achieve higher transfection efficiency

Simpler! Lower Cytotoxicity & Immunogenicity

- Eliminate CpG islands and other bacterial origin sequences
- Increase stability for longer persistence

More Efficient! Increase KI/viral packaging/ GOI expression efficiency

- Increase knock-in efficiency by up to 30%
- Improve viral titer up to 3 folds
- Increase transcription levels up to 4 folds, and protein expression levels up to 39%

Applications Field

Cell Therapy

CRISPR KI template
Transposons vector

Gene Therapy

AAV/LV packaging plasmid
non-viral GOI vector
mRNA preparation template

Vaccine

Antigen expression vector for DNA vaccine
mRNA preparation template

Disease Model Gene Function Study

CRISPR KI template
Transposons vector

Service Details

Service	Grade	Insertion Length*	Quantity*	Format	TAT#	Application
GenCircle™ dsDNA	HighPure	1-20 kb	100 μg - g	Freeze-dried powder/ Solution liquid (Buffer is optional)	From 11 calendar days	• Transposons / Transposase vector • Viral packaging plasmid • GOI transcription & expression
	UltraPure					• CRISPR KI template

* For other insertion length & quantity request, please email oligo@genscript.com or call 1-877-436-7274.
# This TAT does not include gene synthesis turnaround time.

QC Item	QC Standard	HighPure	UltraPure
Sanger Sequencing	Correct sequence	√	√
Restriction Analysis	Conforming to reference pattern	√	√
Spectrophotometer	Delivery quantity guarantee	√	√
Nanodrop UV Value	A260/280 = 1.8-2.0	√	√
Electrophoresis	Non-detectable RNA/genome DNA residual	√	√
Homogeneity	≥ 90% Supercoiled	√	√
TAL Endotoxin Quantify	< 0.01 EU/μg	√	
	≤ 0.005 EU/μg		√
Bioburden Testing	No growth on agar plate after 48 hours	√	√
pH	8.0±0.5 (in TE buffer)		√
Residual E. coli DNA	Quantitative PCR ≤ 5%		√
Residual Host Protein	HCP ELISA ≤ 1%		√

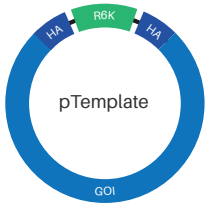
Application Case Studies

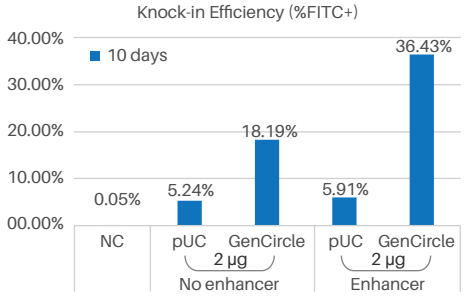
Case 1. GenCircle™ dsDNA as Knock-in template

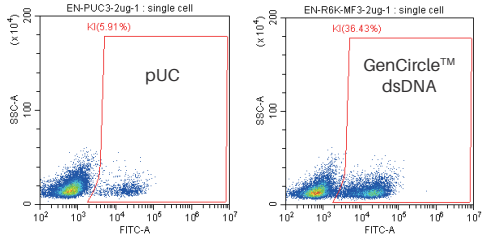
Result: CRISPR KI efficiency increased by up to **30%**, transposon integration efficiency increased by up to **15%**, compared to standard plasmid.

CRISPR KI template: 2.5kb insert (CD19 CAR+GFP) into TRAC locus of T cell via electrorotation

GenCircle™ dsDNA as CRISPR KI Template

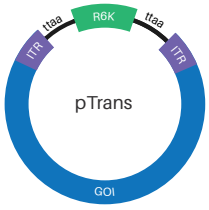


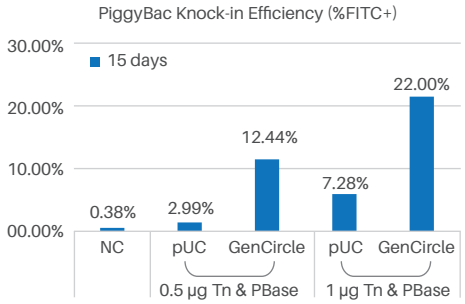


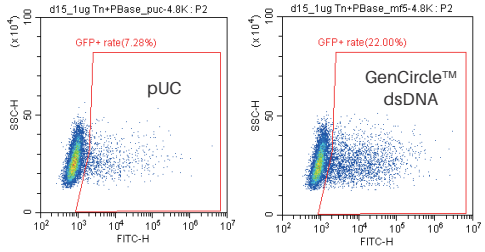


Transposon vector for KI: 4.8kb insert containing GFP into 293T cell via electrorotation

GenCircle™ dsDNA as Transposon Vector



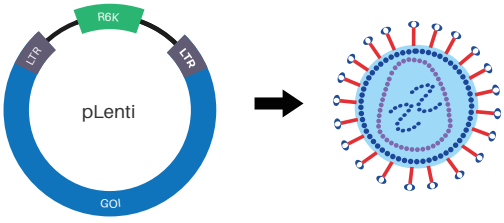




Case 2. GenCircle™ dsDNA as virus pacgking plasmid

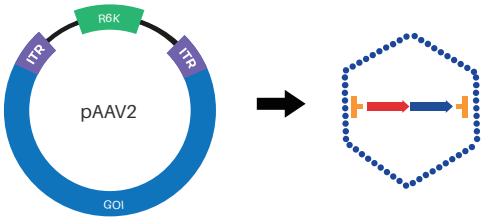
Result: Viral titer improve up to **3 folds** and avoid resistance gene compared to standard plasmid, with stable ITR.

GenCircle™ dsDNA as lentivirus packing plasmid



Name	Virus Titer (IFU/mL)	Antibiotic Resistance Gene
pRRL-PGK-EGFP (Regular Plasmid)	1.23E+8	Significant residue (Ct value<30)
pRRL-PGK-EGFP MF (GenCircle™ dsDNA)	3.81E+8	Undetectable (Ct≥35)

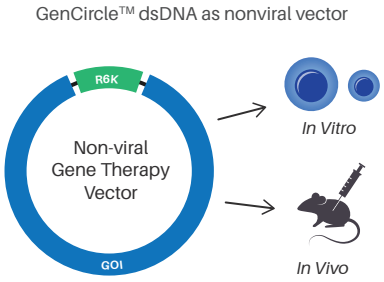
GenCircle™ dsDNA as AAV packing plasmid

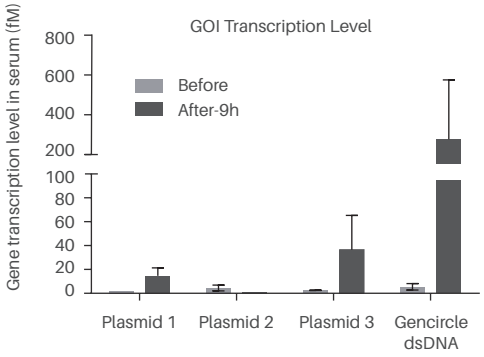


Name	Virus Titer (copies/mL)	Antibiotic Resistance Gene
CMV EGFP AAV2 (Regular Plasmid)	1.12E+12	2%
CMV EGFP MF (GenCircle™ dsDNA)	1.22E+12	Undetectable

Case 3. GenCircle™ dsDNA as GOI vector for transcription

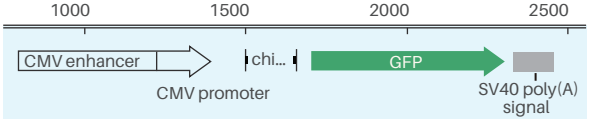
Result: RNA transcription level increased up to **4 folds**, with reduced immune response compared to standard plasmid.

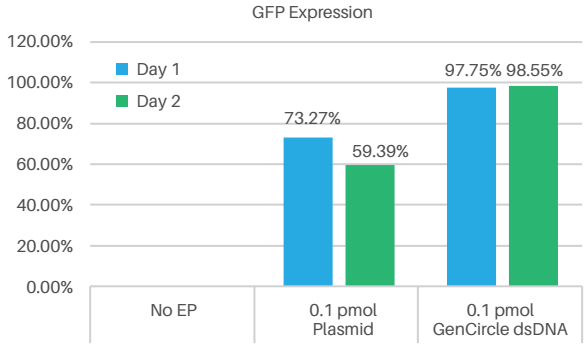




Case 4. GenCircle™ dsDNA as GOI vector for expression

Result: protein expression level increased by up to **39%**, with reduced immune response compared to standard plasmid.

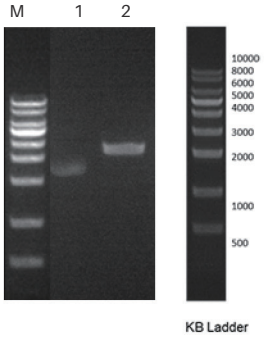




Successful Delivered Cases

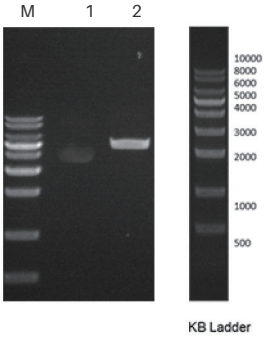
GenScript has successfully delivered multiple orders with a wide range of GOI lengths, including multiple complex or difficult-to-synthesize projects.

3.8 kb
(AAV transfer plasmid with ITRs, ITR stable)



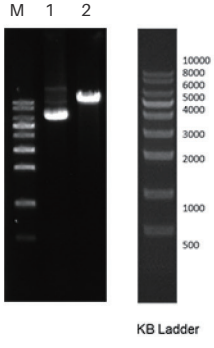
Lane M : KB Ladder Marker
Lane 1 : U33XXXXX90-1 plasmid
Lane 2 : U33XXXXX90-1 plasmid digested by EcoRV

5.1 kb



Lane M : KB Ladder Marker
Lane 1 : U72XXXXX90-1 plasmid
Lane 2 : U72XXXXX90-1 plasmid digested by AhdI

14 kb



Lane M : KB Ladder Marker
Lane 1 : plasmid
Lane 2 : plasmid digested by XbaI

CRISPR Non-viral CAR-T Knock-In Optimization Kits

GenScript is proud to offer CRISPR CAR-T knock-in optimization kits to efficiently optimize your CAR insertion process with pre-validated sequences, design and protocols. Save time on optimization by visually confirming the success of your next CAR-T knock-in experiment.

Knock-In Optimization Kit Includes

Synthetic SafeEdit sgRNA

- HPLC purified sgRNA targeting TRAC or Rab11a for CAR insertion in T cells

High Purity Cas9 Proteins

- Available in both wild type (Cas9) and enhanced specificity versions (eSpCas9)

HDR Templates

- GenExact™ ssDNA or/and GenWand™ closed-end dsDNA encoding GFP for easy KI detection
- Available with Cas9 Targeting Sequence (CTS) design*

Optimized protocol

- Comprehensive protocol from cell culture, KI experiment, to editing efficiency detection
- Trouble shooting FAQs

How to Build Your Kit?

1. Select TRAC or/and Rab11a as insertion site
2. Choose Cas9 or eSpCas9
3. Choose a single or/and double strand DNA template (ssDNA or/and dsDNA) encoding GFP
4. Select whether include the Cas9 Targeting Sequence (CTS) in HDR Templates for improved knock-in efficiency
5. Download and follow the knock-in optimization protocol and visualize your result
6. **Get your kit done! 1.5 nmol sgRNA + 200 µg Cas9 nuclease + 50 µg HDR Templates / per kit**

How does the Kit benefit your study?

Save 75% cost and delivery time compared to customized reagents

Accelerate your study by efficiently find out the optimized experiment conditions

* Shy, et al. Hybrid ssDNA repair templates enable high yield genome engineering in primary cells for disease modeling and cell therapy manufacturing. bioRxiv 2021.09.02.458799.

cGMP sgRNA & HDR Knock-in template

GenScript is proud to offer current Good Manufacturing Practice (cGMP) manufacturing of synthetic single guide RNA (sgRNA) and HDR template (ssDNA/dsDNA) supporting non-viral gene and cell engineering development.Our State-of-the-Art Production Facility operates dedicated cGMP production lines to reliably provide CRISPR sgRNA with the necessary documentation required for successful IND submission and clinical trials.

Partner with GenScript to accelerate your therapeutic pipeline from early-phase research to clinical applications.

State-of-the-Art Production Facility

- cGMP manufacturing of 30mg up to gram quantities per batch
- Compliant with FDA/EMA/PMDA/NMPA regulations

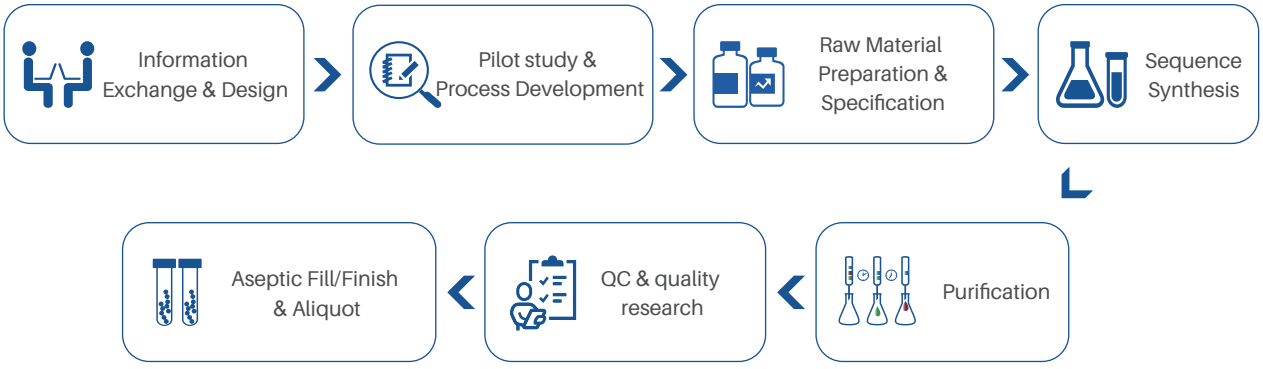
Comprehensive QA/QC Documentation

- 10+ QC options supporting global IND filing needs
- US Type II DMF and/or CMC filing support

Trusted Partner from Bench to Clinic

- Successfully delivered 100+ cGMP batches
- 6 IND approvals

cGMP Production Workflow



Experienced team successful support

100+ cGMP batches product

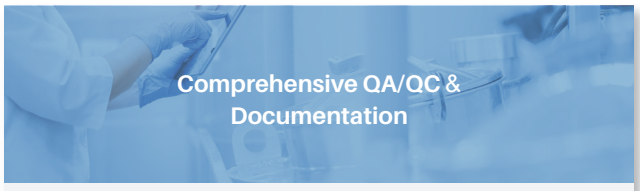
30 IND filling projects

6 IND approval

GenScript is further expanding cGMP sgRNA & HDR template production capacity to reduce the wait time for our partners. GenScript's new Zhenjiang, China 400,000 sq.ft. cGMP facility has been put into production.

Why GenScript?

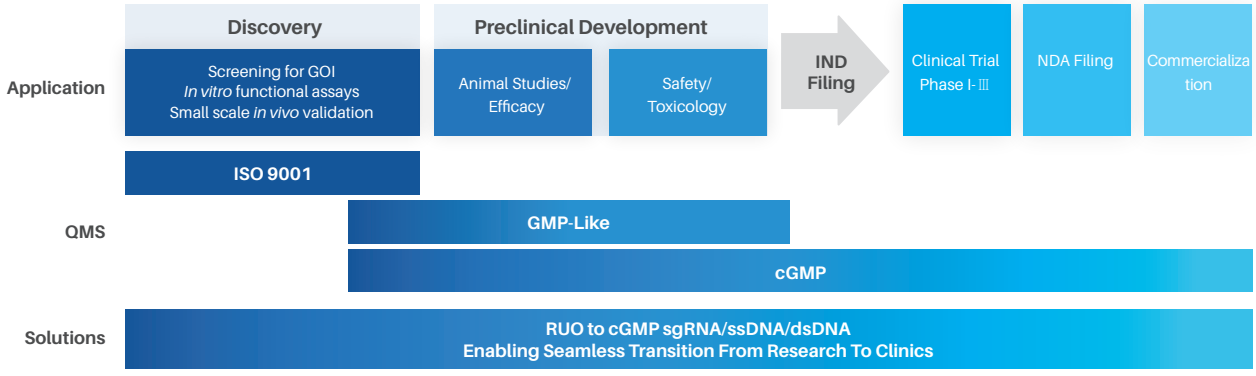
- cGMP production environment
- Clean suite with class A isolator in a class C background
- Sterile filtration and aseptic fill finish
- Process development and optimization
- CQA/CPP confirmed
- Flexible batch scale from 30 mg-1 g
- Sufficient capacity! No need to wait for cGMP scheduling



- Manufacturing Summary Report, TSE/BSE Statement
- Batch Records based on Master Batch Record and Change Control System
- Identity, purity, sterility, etc. clearly defined
- cGMP-compliant material and supplier management
- Validated QC testing procedures
- Long-term stability testing under various solutions and storage conditions
- Established QMS for training, process deviations, and CAPA

One Stop Solution Support Gene and Cell Therapy Development

Phase-appropriate material and comprehensive QA/QC documentation for successful IND filing



Successful Cases

Bioheng Biotech Co., Ltd.
cGMP sgRNA support the first UCAR-T IND approval in China

BRL Medicine Inc.
cGMP sgRNA support the first nonviral CAR-T IND approval globally

Marson Lab
ssDNA achieve up to 60% KI efficiency for CAR-T generation at a GMP-compatible scale

T-Maximum Biotech
cGMP sgRNA supported their MT207 CAR-T cell therapy's FDA-certified orphan drug designation (ODD)

Contact US

Welcome to consult us about cGMP reagents to support your GCT program at any stage. Professional technical support manger is at your service.

Email: oligo@genscript.com
Tel: 1-877-436-7274

CRISPR gRNA Library

CRISPR gRNA library is an important tool for high-throughput screening of target genes, which applies the high efficiency and specificity of CRISPR gene editing technology to knock out expressed genes in the genome or activate gene expression. CRISPR gRNA library construction service applies oligo pool synthesis technology to effectively reduce the cost and save the time of gene target screening.

Library Construction Workflow

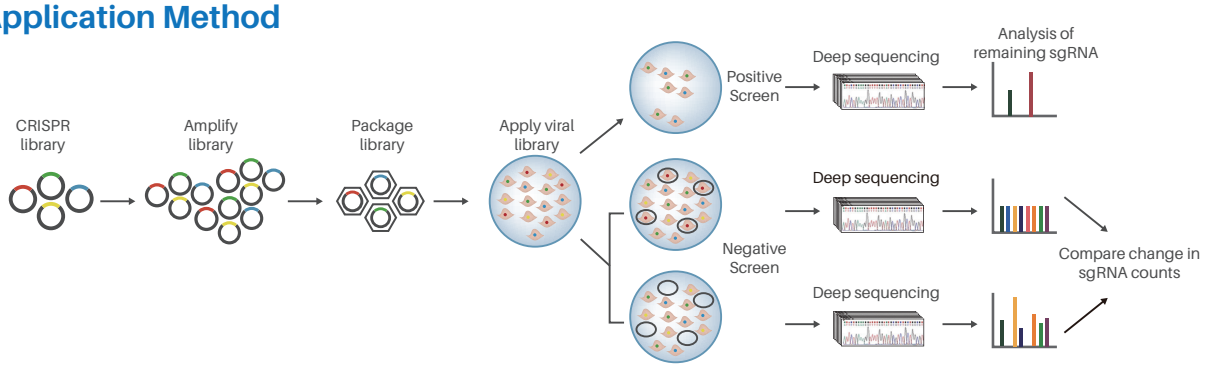
Service Features

- High coverage - NGS sequencing shows that full coverage could be achieved
- Excellent homogeneity (90%/10%), Better than the industry level
- Provided transfection grade plasmids with endotoxin < 0.005 EU/μg

Service Details

Service Type		Content	Number of gRNAs
Genome-wide gene knockout gRNA library (GeCKO gRNA library)		Human and mouse genome-wide gene knockout	3 or 6 gRNAs/genes
CRISPR transcriptional activation gRNA library (SAM gRNA library)		Transcriptional activation in human and mouse genome-wide	3 gRNAs/genes
Pathway-focused gRNA library		Pathway or disease-related gene knockout	Depending on the pathway
Customize gRNA library	Array	gRNA plasmids were synthesized and cloned separately	Tens to hundreds
	Pool	Different gRNA plasmids were mixed together	Up to one hundred thousand

Application Method



Application Method of CRISPR gRNA Library

GeCKO gRNA Library

GenScript provides CRISPR gRNA Library (GeCKO v2.0) introduced from Zhang Feng Laboratory of Broad Research Institute. It is patented and can knock out any gene and miRNA in the genome of human or mouse, which has been widely used in primary human or mouse cells, stem cells, cancer cells and various stable cell lines.

6 different gRNA sequences are designed for each gene in the GeCKO v2.0 library, which is divided into library A and library B. Each library contains 3 different gRNA sequences for each gene and 1,000 non-targeted control gRNAs. Library A also contains gRNAs targeting miRNAs (each miRNA has 4 targeted gRNAs). In addition, GeCKO v2.0 library has upgraded lentiviral vectors for transduction, which can enrich viruses more efficiently and improve the transfection efficiency of viruses in primary cells.

To ensure complete knockout for each gene, both library A and library B can be purchased for mixed use. If cell samples and follow-up high-throughput screening capacity are limited, it is recommended that only library A be used for sorting.

Library Details

Library Type	Sequence Information	Vector	Specification
Human Library A	**65,383 sequences:** • 19,050 genes were targeted, and each gene had 3 targeted gRNAs • 1,864 miRNAs were targeted, and each gene had 4 targeted gRNAs • 1,000 control (non-targeted) gRNAs	pLentiCRISPR v2 pLentiGuide-Puro pLentiCas9-Blast	25/100/200 μg
Human Library B	**58,028 sequences:** • 19,050 genes were targeted, and each gene had 3 targeted gRNAs • 1,000 control (non-targeted) gRNAs	pLentiCRISPR v2 pLentiGuide-Puro pLentiCas9-Blast	
Mouse Library A	**67,405 sequences:** • 20,611 genes were targeted, and each gene had 3 targeted gRNAs • 1,175 miRNAs were targeted, and each gene had 4 targeted gRNAs • 1,000 control (non-targeted) gRNAs	pLentiCRISPR v2 pLentiGuide-Puro pLentiCas9-Blast	
Mouse Library B	**62,804 sequences:** • 20,611 genes were targeted, and each gene had 3 targeted gRNAs • 1,000 control (non-targeted) gRNAs	pLentiCRISPR v2 pLentiGuide-Puro pLentiCas9-Blast	

Delivery standard

- Transfection grade libraries were delivered with 25 μg as a unit, libraries with 100 μg and 200 μg were delivered with 4 × 25 μg or 8 × 25 μg as a unit, respectively.
- We provide statistical summaries of project reports and NGS validation and COA files, as well as complete, usable NGS raw data if you require.

Application Method

1) Each gRNA in GeCKO v2.0 library was cloned into the optimized lentivirus vector, which could form a high titer virus after co-transfection with Cas9 vector in 293 cells. Finally, the gRNA sequence could be efficiently transcribed in the target cells;
2) GeCKO v2.0 libraries with completed virus packaging should be infected with target cells at a low MOI to ensure that no more than 1 gRNA enters a single cell;
3) After transfection, a round of NGS deep sequencing should be performed before sorting to evaluate the expression of gRNA in cell pools.
4) After the sorting, the second round of NGS deep sequencing was conducted, and the data were analyzed to determine the gRNA sequences loss or enrichment in the sorting process, so as to deduce the target genes.

CRISPR transcriptional activation gRNA library (SAM sgRNA library)

The dCas9 protein lacking cleavage activity was fused with the transcriptional activation element and equipped with the gRNA sequence targeting the upstream regulatory region of the gene to form the transcriptional activation complex, which can precisely target the upstream transcriptional activation region of genes and effectively activate gene expression.

SAM sgRNA library, a gRNA library targeting the whole gene wide, combines with dCas9 and transcriptional activation protein to up-regulate the whole gene expression level, which can be applied to high-throughput and rapid gain-of-function screening.

CRISPR/Cas9 SAM complex consists of three parts: gRNA with two MS2 RNA aptamers, MS2-P65-HSF1 activation accessory protein and dCas9-VP64 fusion protein

Library Details

Library Type	Sequence Information	Vector	Specification
Human SAM Library	**70,290 sequences:** • Targeted 23,430 genes • Each gene had 3 targeted sequences	pLenti sgRNA(MS2)_zeo pLenti dCas9-VP64_Blast pLenti MS2-P65-HSF1-Hygro	25/100/200 μg
		pLenti sgRNA(MS2)_puro pLenti dCas9-VP64_Blast pLenti MS2-P65-HSF1-Hygro	
Mouse SAM Library	**69,225 sequences:** • Targeted 23,439 genes • Each gene had 3 targeted sequences • 491 control (non-targeted) gRNAs	pLenti sgRNA(MS2)_puro pLenti dCas9-VP64_Blast pLenti MS2-P65-HSF1-Hygro	

Delivery standard

- Transfection grade libraries were delivered with 25 μg as a unit, libraries with 100 μg and 200 μg were delivered with 4 × 25 μg or 8 × 25 μg as a unit, respectively.
- We provide statistical summaries of project reports and NGS validation and COA files, as well as complete, usable NGS raw data if you require.

Application Method

1) SAM libraries with virus packaging should be transfected with target cells at a low MOI to ensure that no more than 1 gRNA enters a single cell;
2) After transfection, a round of NGS deep sequencing should be performed before sorting to evaluate the expression of gRNA in cell pools.
3) After the sorting, the second round of NGS deep sequencing was conducted, and the data were analyzed to determine the gRNA sequences loss or enrichment in the sorting process, so as to deduce the target genes.

Pathway-focused gRNA Library

Pathway-focused gRNA library is an ideal tool for targeted screening for specific pathways. Applied with gene targets identified by the Drug Gene Interaction Database, the library can be designed to sort for pathway or disease-related genes. All gRNA sequences were pre-designed and verified by the Broad Research Institute and can be customized to be cloned into lentiviral vectors.

Library Details

Pathway	Number of Genes	Number of gRNAs
ABC Transporter	100	298
Adult Stem Cells	58	174
Angiogenesis	92	276
Apoptosis	68	204
cAMP & calcium Signaling Pathway	91	271
Cell Cycle	84	252
Cytochrome P450	57	171
Drug Resistance	348	1,044
GPCR	280	840
Hormone Activity	108	324
Ion Channels (Potassium)	67	201
Tumor Metastasis	60	180
Nuclear Hormone Receptors	117	351
Oncogenes	289	867
Tumor Suppressor	720	2,155
WNT Pathway	92	276

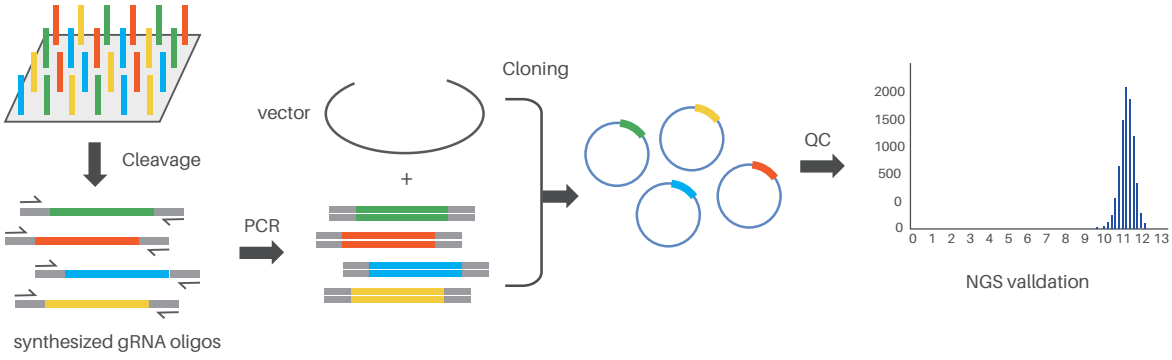
Pathway	Number of Genes	Number of gRNAs
Acute myeloid leukemia	45	135
Adipogenesis	126	378
Alzheimer's disease	94	281
B cell receptor signaling pathway	58	174
Bladder cancer	37	111
Cardiovascular Disease	91	273
Chemokine signaling pathway	134	402
Chronic myeloid leukemia	57	171
Colorectal cancer	54	162
Diabetes	44	132
Drug Metabolism	34	102
Drug transporters	84	252
ES Cell Differentiation	334	1,002
Extracellular Matrix & Adhesion	84	252
Growth Factor	161	483
Histone Modification	259	776

Please scan the QR code to visit CRISPR gRNA Library Service home page for more information

Customize gRNA library

GenScript has a high-throughput oligonucleotide synthesis platform based on semiconductor microarray technology to accurately synthesize single stranded oligonucleotides. After PCR amplification, double stranded gRNA is cloned into the selected vector to create a sorting library, which is combined with efficient oligo pool to achieve higher virus titers.

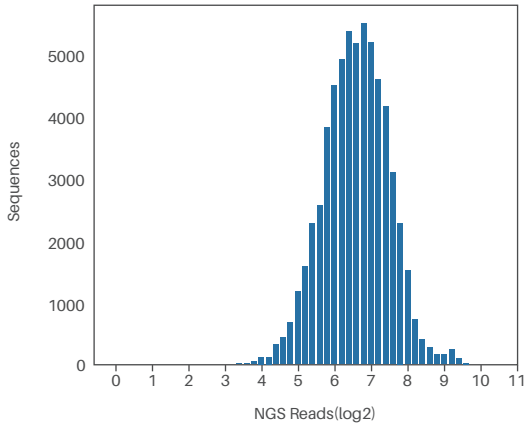
A fully customized gRNA library with complete coverage and homogeneous distribution is provided for CRISPR gene knockout, CRISPRa and CRISPRi.



Service Features

- Coverage of NGS validation > 99%
- Various cloning vectors: dual vector or all-in-one CRISPR vector approved by Zhang Feng Laboratory or other designated vectors
- Delivery plasmids can be used directly for virus packaging with flexible delivery (from μg to mg scale)
- No sequence restriction, short delivery cycle within 4 weeks

Case: The coverage and homogeneity of gRNA library were verified by NGS



10% effective read length	44
90% effective read length	206
Homogeneity (90%/10%)	4.68
Mean sequencing depth	117
Maximum depth sequencing	1,068
Theoretical gRNA diversity	62,804
Actual gRNA diversity	62,804

A gRNA library with a theoretical diversity of 62,804 was tested by NGS, and the results showed that the gRNA library completely contained gRNA sequences covering all designs. In addition, the homogeneity (90%/10% ratio) was less than 5, indicating that the required gRNA was homogeneously distributed in the library to ensure screening efficiency.

*For gRNA library service, please send an email to gene@genscript.com or call 1-877-436-7274, and our professional technical support team will be at your service.

GenCRISPR™ gRNA/Cas9 Plasmid Construction

The CRISPR reagents provided by GenScript are collected from Zhang Feng Laboratory. The CRISPR products and services provided by GenScript are licensed by Broad Institute in the United States. These include:

- sgRNA sequences that have been designed and verified by MIT
- Multiple vector types including binary vector and all-in-one are available, and resistance screening or methods can be selected as needed
- Empty vector verified by MIT
- Various Cas9: eSpCas9, SpCas9, SpCas9 Nickase, SaCas9 and SAM

Service Features

Specific sgRNA Sequence

sgRNA sequence verified by MIT
Ensure the specificity and efficiency of genome editing

Genome-wide Database

Human and Mouse gRNA database
Support for SpCas9 and SAM applications

Professional Design Tool

12 species optional
On-target/off-target scoring

On-line Ordering

Customized sequence
Vector and vector label optional

Service Details

Customized Cas9 plasmids		Expression System	Sorting label
eSpCas9 plasmids		Plasmid Lentiviral	Amp, Puro Amp, GFP
SpCas9 plasmids		Plasmid Lentiviral AAV	Amp Amp, Puro Amp, Neo Amp, GFP
SpCas9 Nickase plasmids		Plasmid Lentiviral	Amp Amp, Puro Amp, GFP
SaCas9 plasmids		AAV	Amp
SAM plasmids (transcriptional activation plasmids)	SAM gRNA plasmids	Plasmid Lentiviral	Amp Amp, Zeo
	SAM dCas9-VP64 plasmids	Lentiviral	Amp, Blast Amp, GFP
	SAM MS2-P65-HSF1 plasmids	Lentiviral	Amp, GFP Amp, Hygro

To meet your scientific research needs, GenScript provides empty vector service with vector sequences that have been verified by MIT. The vectors contain a 17 bp-1.8 kb expressible ligand in place of a customized sgRNA sequence, which can be modified according to your needs.

Service Items	Expression System	Sorting label
eSpCas9 and SpCas9 Broad Plasmid Collection	Plasmid Lentiviral	Amp, Puro Amp, GFP
SpCas9 Nickase Broad Plasmid Collection	Plasmid	Amp Amp, Puro Amp, GFP
SaCas9 Broad Plasmid Collection	AAV	Amp

CRISPR/Cas9 Related Vector Specification

Category	Vector	Expression Type	Delivery Type	Vector Type	Sorting Label
eSpCas9 Plasmids	eSpCas9-2A- GFP (PX458)	eSpCas9 & gRNA	Plasmid	All-in-one Vector	AmpR EGFP
eSpCas9 Plasmids	eSpCas9-2A- Puro (PX459) V2.0	eSpCas9 & gRNA	Plasmid	All-in-one Vector	AmpR PuroR
eSpCas9 Plasmids	eSpCas9-LentiCRISPR v2	eSpCas9 & gRNA	Lentiviral	All-in-one Vector	AmpR PuroR
SpCas9 Plasmids	pSpCas9 BB-2A-GFP PX458	SpCas9 & gRNA	Plasmid	All-in-one Vector	AmpR EGFP
SpCas9 Plasmids	pSpCas9 BB-2A-Puro (PX459) v2.0	SpCas9 & gRNA	Plasmid	All-in-one Vector	AmpR PuroR
SpCas9 Plasmids	pLentiCRISPR v2	SpCas9 & gRNA	Lentiviral	All-in-one Vector	AmpR PuroR
SpCas9 Plasmids SpCas9 Nickase Plasmids	pLentiGuide-Puro	gRNA Only	Lentiviral	Dual Vector	AmpR PuroR
SpCas9 Plasmids	pLentiCas9-Blast	SpCas9 Only	Lentiviral	Dual Vector	AmpR BsdR BleoR
SpCas9 Plasmids	pLentiCas9-EGFP	SpCas9 Only	Lentiviral	Dual Vector	AmpR EGFP
SpCas9 Plasmids	pGS-gRNA	gRNA Only	Plasmid	Dual Vector	AmpR
SpCas9 Plasmids	pGS-gRNA-Neo	gRNA Only	Plasmid	Dual Vector	AmpR NeoR
SpCas9 Plasmids	pSpCas9 PX165	SpCas9 Only	Plasmid	Dual Vector	AmpR
SpCas9 Plasmids	pAAV_SpGuide acceptor (PX552)	gRNA Only	AAV	Dual Vector	AmpR EGFP
SpCas9 Plasmids	pAAV-SpCas9 PX551	SpCas9 Only	AAV	Dual Vector	AmpR
SpCas9 Nickase Plasmids	pSpCas9n BB PX460	SpCas9 Nickase & gRNA	Plasmid	All-in-one Vector	AmpR
SpCas9 Nickase Plasmids	pSpCas9n BB-2A-GFP PX461	SpCas9 Nickase & gRNA	Plasmid	All-in-one Vector	AmpR EGFP
SpCas9 Nickase Plasmids	pSpCas9n BB-2A-Puro (PX462) V2.0	SpCas9 Nickase & gRNA	Plasmid	All-in-one Vector	AmpR PuroR
SpCas9 Nickase Plasmids	pLentiCas9n-Blast	SpCas9 Nickase Only	Lentiviral	Dual Vector	AmpR BsdR BleoR
SaCas9 Plasmids	pX601_AAV	SaCas9 & gRNA	AAV	All-in-one Vector	AmpR

Category	Vector	Expression Type	Delivery Type	Vector Type	Sorting Label
Transcription Activation (SAM)	pSgRNA(MS2)	gRNA Only	Plasmid	SAM Multi Vector	AmpR
Transcription Activation (SAM)	pLenti_sgRNA(MS2)_zeo	gRNA Only	Lentiviral	SAM Multi Vector	AmpR ZeoR BeloR
Transcription Activation (SAM)	pLenti_dCas9-VP64_Blast	Cas9 Activator	Lentiviral	SAM Multi Vector	AmpR BlastR BleoR
Transcription Activation (SAM)	pLenti_dCas9-VP64_GFP	Cas9 Activator	Lentiviral	SAM Multi Vector	AmpR EGFP BleoR
Transcription Activation (SAM)	pLenti_MS2-P65-HSF1_Hygro	Activator Adapter	Lentiviral	Multi Vector	AmpR HygroR BleoR
Transcription Activation (SAM)	pLenti_MS2-P65-HSF1_GFP	Activator Adapter	Lentiviral	Multi Vector	AmpR EGFP BleoR

CRISPR gRNA Database

GenScript provides over 20,000 plentiCRISPR v2 plasmids containing gRNA sequences, and all sgRNA sequences have been verified by MIT. You can find the relevant gRNA by searching for Gene Name, Gene Symbol, or Gene ID in the database. You can access the database through the following website or by scanning the QR code.

https://www.genscript.com/gRNA-database.html

gRNA/Cas9 Lentivirus Packaging Service

Lentiviruses, a type of retrovirus, are one of the most popular tools for gene transduction. Lentivirus can introduce target genes into some difficult-to-transfect cells, such as primary cells, stem cells, or non-dividing cells, thus greatly improving the efficiency of target gene transduction. Lentiviral vectors also have significant advantages in transient transfection and stable strain sorting. GenScript offers a lentiviral packaging service with flexible titers and specifications and delivery time as fast as 2 weeks.

Service Features

Standard and Professional Technology Platform

Traceable and authorized cell line materials
Third-generation lentivirus vector: serum-free suspension system

Strict Quality Control

Test standard that higher than the industry level
ELISA/FACS/qPCR/resistance sorting

Cost effective

Save 20% on getting the same amount of virus

Fast Delivery Cycle

Quick turnaround time of 2 weeks, faster than the industry average delivery time

Service Details

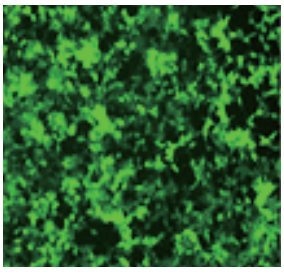
Titer*	Specification	Cycle	QC
>10^7 IFU/ml	1 ml	2-3 weeks	p24 ELISA detection Lonza 180 test FACS/qPCR/resistance sorting (customized QC)
	2 ml		
>10^8 IFU/ml	1 ml		
	2 ml		
>10^9 IFU/ml	0.1 ml		
	0.2 ml		

- Customers provide sequences for gene synthesis at an additional charge.
- In the lentivirus package project delivered by GenScript, the longest package gene fragment reached 6.6 kb and achieved a high titer level.

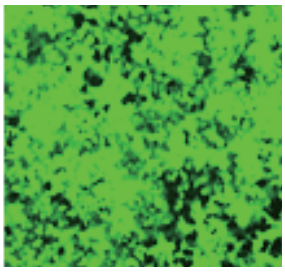
*For other titers or specifications informations, please send an email to protein@genscript.com.cn or call 400-025-8686 ext. 5821, and our professional technical support team will be at your service.

Cases

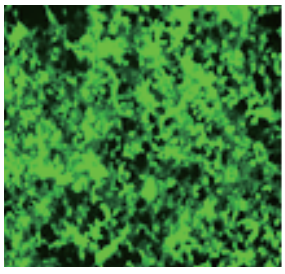
Case 1: Lentivirus infection experiment



Second-generation virus system - GenScript



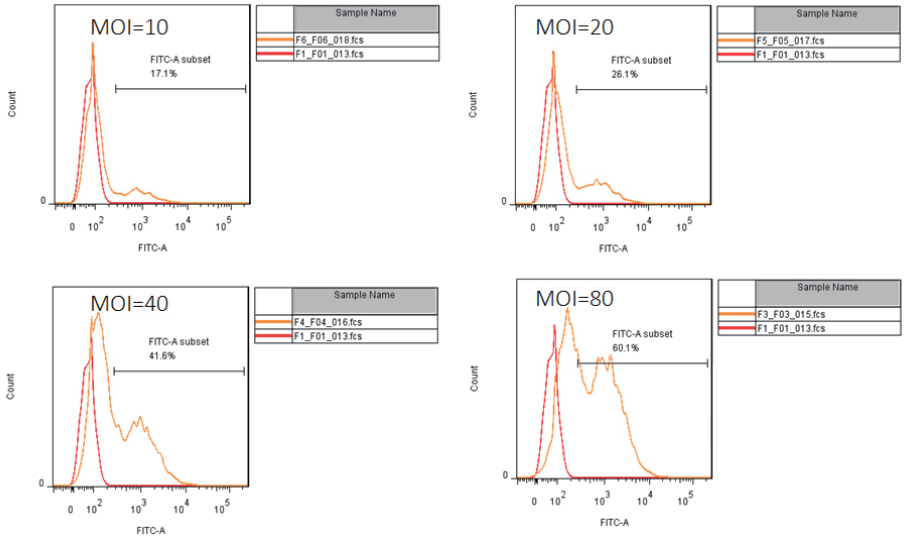
Third-generation virus system - GenScript



Third-generation virus system - other companies

EGFP lentivirus was packaged with the second-generation virus packaging system of GenScript, the optimized third-generation virus packaging system of GenScript and packaging system of other companies, respectively. After the determination of titers, they were adjusted to the same MOI to infect the same amount of 293T cells, and fluorescent photographs were taken 72 hours after infection. **The upgraded GenScript third-generation system had higher target protein expression.**

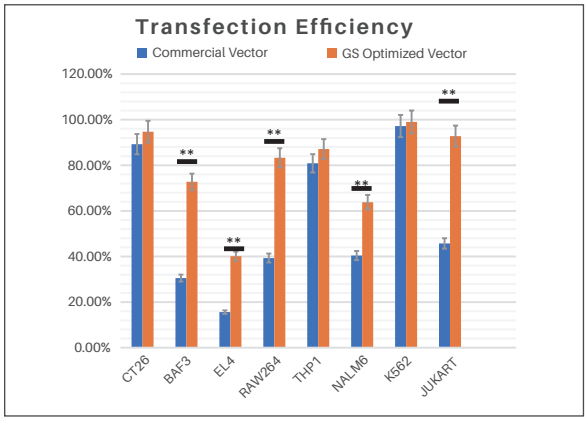
Case 2: GFP virus infection Jurkat MOI testing



MOI	GFP%	Infection time
10	17.1%	72 h
20	26.1%	72 h
40	41.6%	72 h
80	60.1%	72 h

Jurkat cell is a type of T lymphocyte that is difficult to transfect. Jurkat cells were infected with GFP virus packaged with **GenScript third-generation plasmid system and tested to achieve an infection rate of 60.1% at MOI=80**.

Case 3: High Transfection efficiency of optimized GLV3 vector



Cell Line	Name	MOI
CT26	Mouse colony	100
BAF3	Mouse B cell	
EL4	Mouse T lymphoma	
RAW 264.7	Monocyte/macrophage-like cells	
Jurkat	Human acute T cell leukemia	
K562	myelogenous leukemia cell line	
NALM6	B cell precursor leukemia cell	
THP-1	Acute monocytic leukemia	

MOI: Multiplicity of infection

Case 4: Gene carrying length testing of GenScript lentivirus packaging system

Gene name	Target gene length (kb)	Titer (x10^8 IFU/ml)
Gene 1	3.8	3.43
Gene 2	4.7	3.59
Gene 3	5.5	1.85
Gene 4	6.6	2.61

In general, target genes longer than 3,000 bp are difficult to reach high titer levels and even impossible to package. In the lentivirus package project delivered by GenScript, the longest package gene fragment reached 6.6 kb and achieved a high titer level. GenScript can guarantee a titer of 10^7 IFU/ml for conventional genes (moderate GC content, no complex repeats, no cell death and the length less than 3 kb).

Please scan the QR code to visit gRNA/Cas9 Lentivirus Packaging Service home page for more information

03

Genome Editing Model Construction Service

GenCRISPR™ Genome Editing Cell Lines

CRISPR/Cas9 technology was used to knock-out genes and knock-in the target sequence, resulting in gene editing cell lines that can be stably passaged. In the field of basic research, it can be used for target gene/protein tracing and functional research; in the field of cell/gene therapy and drug development, it can be used in immunotherapy target discovery, drug screening, disease biology/signaling pathway research and others.

Advantages of GenScript

GenScript provides a variety of CRISPR gene editing cell lines. Relying on the advantages of **chemically synthesized long single-stranded gRNA,** we can adopt **RNP delivery system** with high editing efficiency and low toxicity, and support multiple delivery systems of lentivirus and plasmid to provide you with gene-editing cell line services specifying target genes, editing regions and cells.

High Quality Delivery

Cell lines/pools verified by sequencing

A variety of validation, optimization, off-target analysis services

Compliance Qualification

P2 Laboratory: complying with the new Biosecurity Act

Broad/ERS authorization: Providing CRISPR services in compliance

Rich Experience

Over 1,000 cell lines of delivery experience worldwide

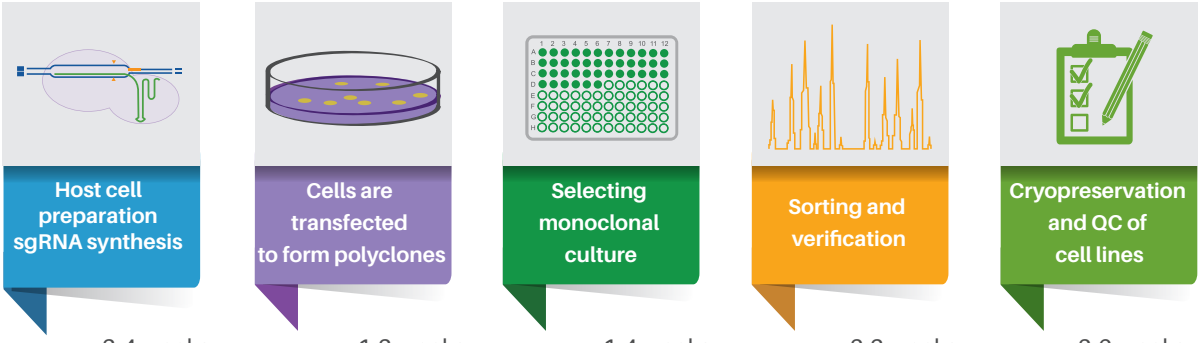
Excellent handling ability of difficult projects

Professional Technical Service

More efficient project communication and promotion

Timely answering questions by Ph.D. project manager

Genome Editing Cell Line Construction Process



- Host cell detection
- Promoter verification
- 3 sgRNAs designed for each gene

- Transfection/virus /RNP delivery: Multiple options to optimize transfection efficiency
- Cell pool testing: sequencing to confirm successful knock-out or knock-in

- FACS/Resistance screening enriched positive clones
- Select monoclonal

- 100+ monoclonal sequencing validation to confirm successful knock-out or knock-in of the target sequence

- Cryopreservation of cells
- Cell viability detection
- Mycoplasma detection
- Sequencing to confirm allelic gene knock-out in cell lines or target sequence knock-in

Service Details

Service Type	Service Details	Cell Line Options	Deliverables**	Delivery Tme
EZ knock-out cell line service	Single-gene knock-out stable cell line	90 + common cell lines suitable for transfection (including A549, CHO-K1, HT-29, MDA-MB-231, 4T1, A20, HCT116, MCF7, MDCK, U937, and RPMI 8866)	• 2 full-allelic knockout cell lines verified by sequencing • 1 negative knock-out cell pool control	8-15 weeks
Customized knock-out cell line service	Single-gene or multi-gene knock-out stable cell line	Any cancer cell line	• 1-2 full-allelic knockout cell lines verified by sequencing • 1 negative knock-out cell pool control	12-19 weeks
Full-length gene knockout cell line servic[New]	Whole gene fragments knock-out stable cell line	50 + common cell lines suitable for transfection (including A549, CHO-K1, HEK293, HEK293T, HT-29, MDA-MB-231, 4T1, A20, HCT116, and MCF7)	• 1 full-allelic knockout cell lines verified by sequencing • 1 negative knock-out cell pool control	12-20 weeks
Knock-out cell pool service	Single-gene knock-out cell pool	Any cell line	• Cell pool with knock-out clones verified by sequencing	5-11 weeks
Customized knock-in cell line service	Insert or modify genes at any genomic location	Any cancer cell line	• 1 knock-in cell lines verified by sequencing • 1 unmodified control cell	14-24 weeks
Customized screening and off-target effect detection[New]	Genome-wide libraries or customized libraries were used to screen target genes and iGUIDE was used to detect potential off-target effects	For any cells can be cultured, please consult us.	• Analysis report	For details, please consult us

*Cell lines are generally supplied by the customer, or for an additional fee if provided by GenScript.
**Congruent gene modification cell lines were verified at mRNA or protein levels as required. The project will be updated once every two weeks.

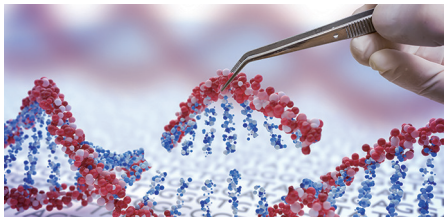
Other customized services

Additional Services	Details
Reverse transcription PCR (RT-PCR)	RT-PCR sequencing was performed to verify that the knock-out clones inserted deletion markers on CDS at the mRNA level
Western blot	Knock-out clones were verified by WB, and effective antibodies that bind specifically to target proteins in host cells were required
FACS analysis	Knock-out clones were verified by FACS, and effective antibodies that bind specifically to target proteins in host cells were required
Promoter activity analysis	The analysis of promoter (Cbh/CMV/EFS) activity was performed in host cells to optimize the cutting efficiency of gRNA-Cas9
Optimization of transfection method	The analysis of transfection method of difficult-to-transfect cells was performed to optimize the cutting efficiency of gRNA-Cas9
Off-target analysis	Multiple potential off-target sites were sequenced to identify knock-out clones and analyze off-target sites at genome level
Additional monoclonal	An another sequenced monoclone was selected from the cell pool and sent to the customer

*For GenCRISPR™ gene editing cell line services, please send an email to protein@genscript.com or call 1-877-436-7274, and our professional technical support team will be at your service.

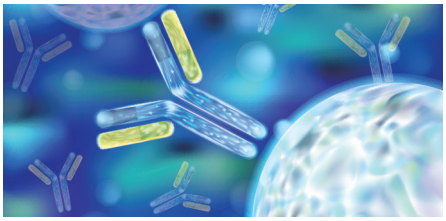
Full-length Gene Knock-out Cell Line Application Field

GenCRISPR™ delivers full-length knock-out cell lines with **long fragments of up to 200 kb in multiple copies**. More than 20 full-length gene knock-out projects have been successfully delivered worldwide, especially for the following applications to eliminate potential interference factors.



Unknown genome or DNA sequence

Non-coding RNAs, novel subtypes/functions of known genes

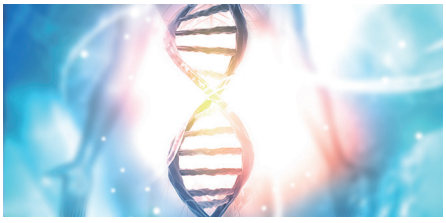


Difficult to obtain high specificity antibodies

Nonspecific binding still exists after routine gene knock-out

Special requirements from regulators

Negative control cell lines for diagnostic kits

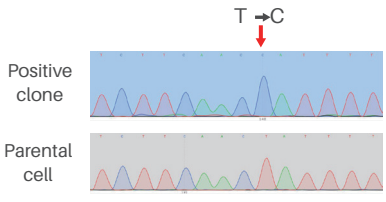


Core signal pathway, important data research

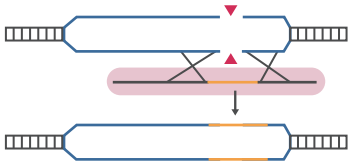
Full-length gene knockout eliminates possible interference factors

Gene Knock-in Cell Line Application Field

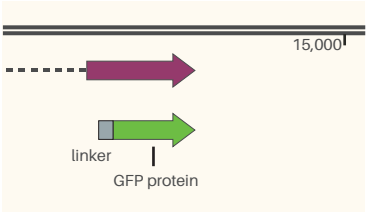
GenCRISPR™ technology provides congruent gene knock-in cell lines verified by sequencing with high editing efficiency and more efficient and rapid delivery. To date, we have successfully delivered dozens of projects, including SHSY5Y, hTERT-RPE1, KM12, HeLa, HCT116, Caco2, C2C12, U2OS, Min6, HepG2, and HEK293, providing support for the following applications.



SNP Research



Precise Gene Overexpression



Tracer Gene Labeling

Cases

Case 1: Knock-out Cell Line Service

Experimental Objective

GenCRISPR™ technology was used to knock-out GS alleles in DG44 cells to construct stable cell lines. QC was used to detect the knock-out effect of target genes at DNA and functional levels.

Experimental Result

sgRNA cutting efficiency: The cutting efficiency of sgRNA designed by GenScript is greater than 80% (Fig.1)

Sequencing verification: The sequencing of selected monoclones proved that frameshift mutations occurred (Fig.2)

Functional testing: The target gene knock-out cells lost corresponding functions and could not grow in the medium without glutamine (Fig.3)

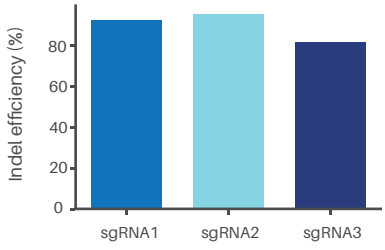


Fig.1 sgRNA knock-out efficiency

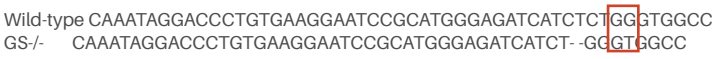


Fig.2 Frameshift mutation occurred by knock-out GS gene monoclonal sequencing

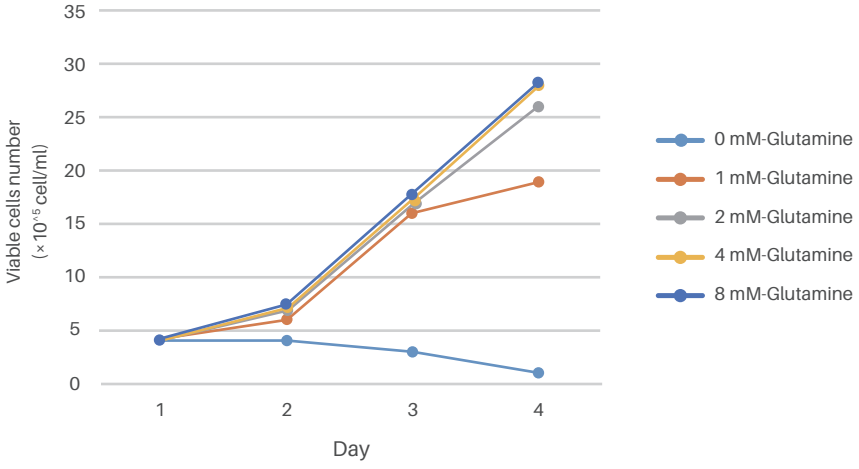


Fig.3 Growth trend of DG44 cells with GS gene knock-out

Case 2: Full-length gene knock-out cell line service

Experimental Objective

Conventional knockout induces gene knock-out by introducing frame-shift mutation, but in some special cases the target protein is still partially expressed and can be detected by some specific antibodies.
The vast majority of antibodies on the market have not been specifically verified, and many antibodies still detect the target protein bands in conventional knocked-out cells (Fig.4). The use of full-length knockout eliminates the concern of having target proteins remaining and shortens the research cycle.

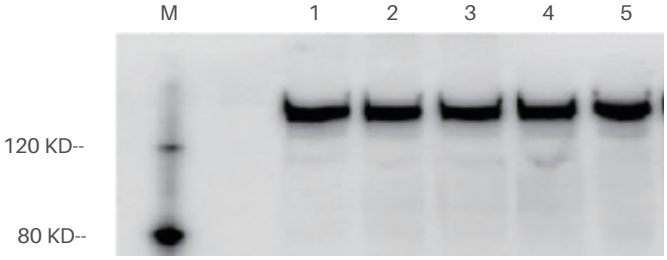


Fig.4 Knock-out cell line samples of supplier A for antibody detection
(1-4 are samples after conventional knock-out of the target sequence, and 5 is wild-type control)

Experimental Result

Experiment 1. PCR and sequencing: PCR amplification was performed on two monoclones of genotype A*+B* (lane 1), genotype A (lane 2) and genotype B (lane 3) samples (primer design scheme is shown in Fig.5). The whole target gene fragments of the three samples were knocked out (Fig. 6A.B) and the residues were reconnected (Fig.6C). Sanger sequencing was performed to confirm the accuracy of the results.

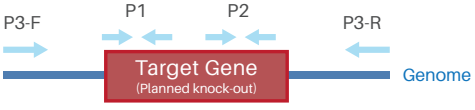


Fig.5 Design scheme for verifying full-length gene knock-out effect by PCR amplification

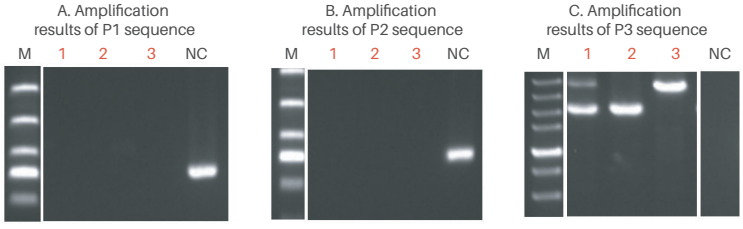


Fig.6 Results for verifying full-length gene knock-out by PCR amplification
(In A-C, lane 1: genotype A + B, lane 2: genotype A, lane 3: genotype B)

*Genotype A does not contain screening labels, while genotype B contains screening labels, both of which meet the delivery criteria

Experiment 2. Western Blot: The target protein marked by the red arrow was knocked out. Since full-length knockout cell line service was used, it could be determined that the green arrow was not a target protein that is degraded or incompletely expressed, but another protein with significantly increased expression in the knock-out cell line (Fig.7). Therefore, the interference was excluded.

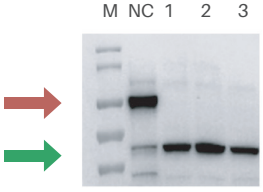
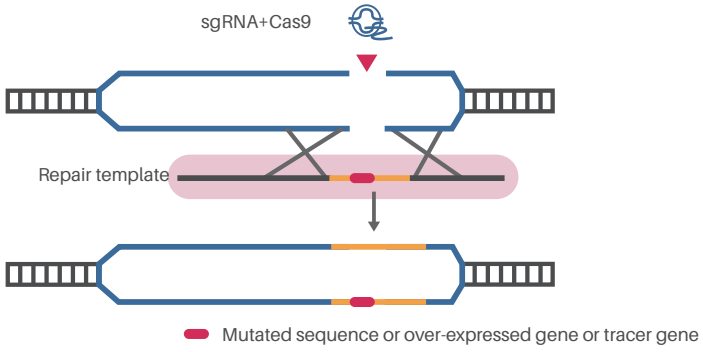


Fig.7 Verification of full-length gene knockout effect by Western Blot (1-3 are samples of full-length gene knock-out cells)

Case 3: Knock-in Cell Line Service

Cell lines constructed by CRISPR gene knock-in were used for point mutation, over-expression and tracer genes processes.



▶ Experiment 1

⩥ Objective: Point mutation study

The sequence containing point mutation was knocked into the target site to construct cell lines by gene knock-in technology, and the changes and mechanism caused by point mutation can be studied in physiological state.

⩥ Experimental Result

Sanger sequencing: Gene knock-in was used to complete point mutation, and successful point mutation was verified by sequencing (Fig.8).

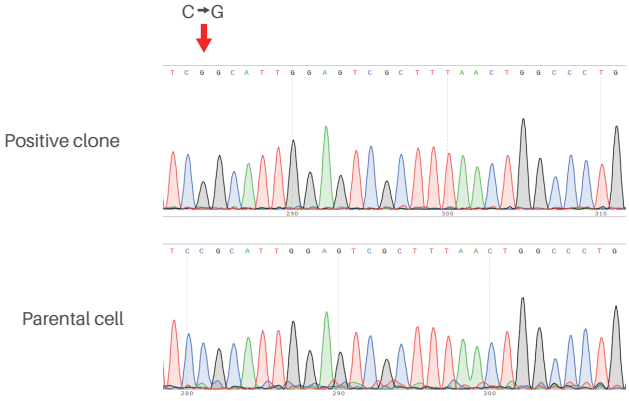


Fig.8 Sequencing results of positive clone of point mutation

Experiment 2

Objective: Over-expression study

The protein expression levels of 293 cell lines constructed by different over-expression methods were compared.

Experimental Result

Protein expression levels: S2 and S3 were the protein expression levels of cell line samples constructed by KI method, which were significantly higher than that of S1 samples transfected by plasmids (Fig.9).

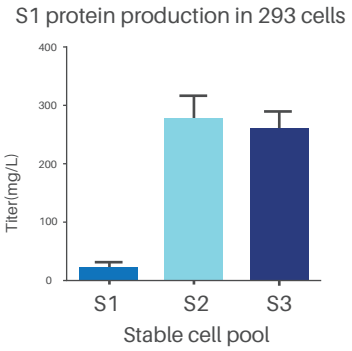


Fig.9 Protein expression levels of different over-expression schemes (S1: plasmid transfection, S2, S3: KI cell line)

Experiment 3

Objective: Tracer gene study

GenCRISPR™ technology is used to knock the target gene (GFP) into specific endogenous genes and construct fusion proteins to trace specific endogenous genes.

Experimental Result

Flow cytometry (FACS): Significant GFP expression could be detected in positive clones by detecting the protein expression in negative control cells, knock-in cell pools, and monoclones (Fig.10).

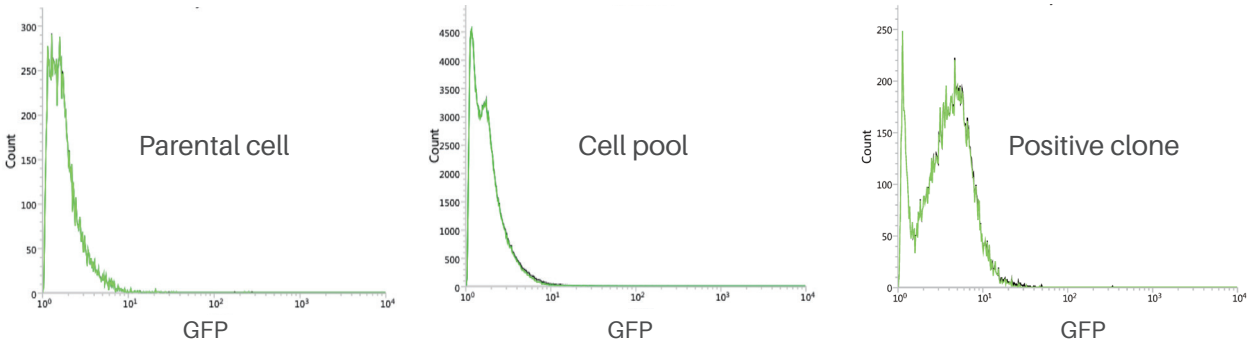


Fig.10 Expression of knock-in tracer gene GFP detected by FACS

Case 5: Sorting target loci using gRNA library

Experimental Objective

CRISPR gene editing was performed on host cells through gRNA libraries targeting different loci, and different target genes were screened out under different pressure conditions. Example: It is necessary to screen the genes that cause host cells to be sensitive to 6-thioguanine. The wild type fails to grow with 6-thioguanine addition; after knock-out of the target gene, it is resistant to 6-thioguanine, and it can grow normally with 6-thioguanine addition (Fig.12).

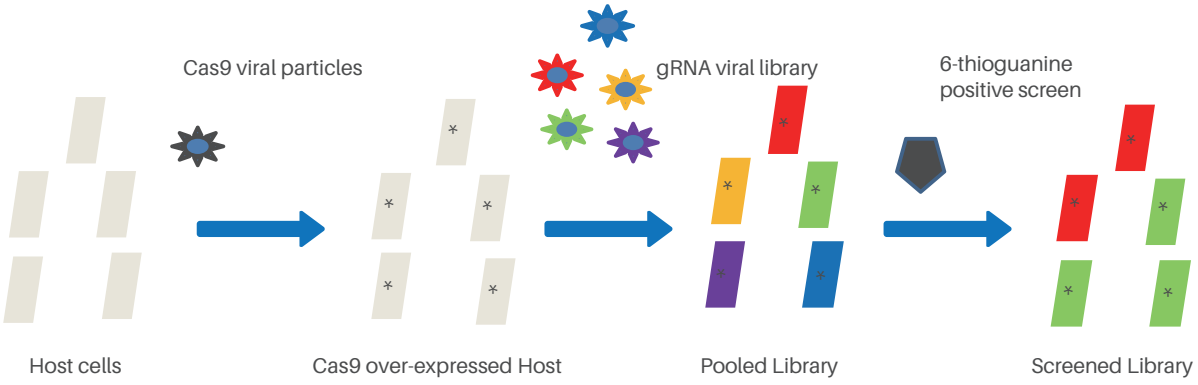


Fig.11 Process of sorting target gene using gRNA library

Experimental Result

Screening results: gRNA library was used for gene editing, 6-thioguanine was added for pressure sorting, and target genes HPRT1 and NUDT5 were sorted after the comparison and evaluation of NGS sequencing (Fig.13).

After the target genes HPRT1 and NUDT5 were knocked out, the ratio of knock-out cells to total cells was increased by adding 6-thioguanine (Fig.14), suggesting that the target genes were related to the sensitivity of 6-thioguanine, and host cells could grow in 6-thioguanine after knocking out.

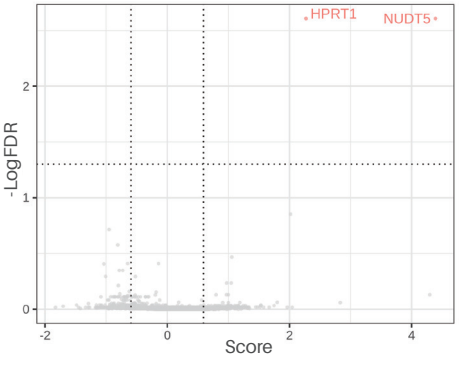


Fig.12 False discovery rate (FDR) and score of gRNA library sorting results (candidate genes marked)

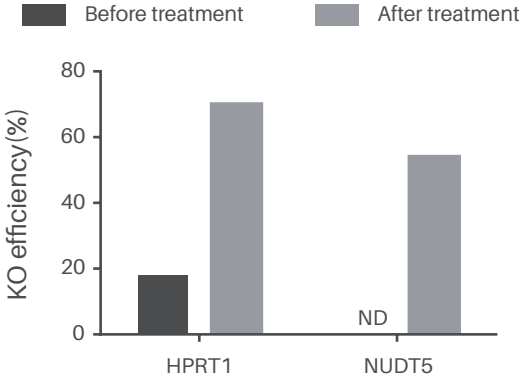
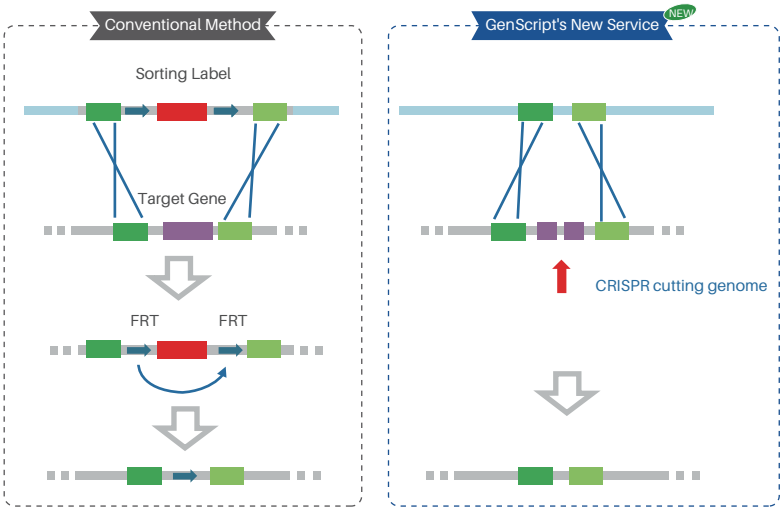


Fig.13 Enrichment of gene knock-out cells under pressure

GenCRISPR™ Microbial Genome Modification

GenScript introduces microbial genome modification services for gene knock-in, knock-out and replacement of bacterial (*E.coli*), saccharomyces cerevisiae, etc. For *E.coli*, GenScript has developed a novel λRed-CRISPR/Cas technique that combines traditional λRed recombination with CRISPR/Cas9 to achieve traceless target gene editing.



Service Features

- Traceless editing
- Easy sorting: No need for selective labeling
- Accurate to base
- Multiplex genome-editing: capable of knocking out up to three genes simultaneously

Service Details

Service Items	Provided by customers	Delivery standard	Delivery time
E.coli gene knock-out service	• Strains and information to be modified • Name or sequence of a knock-out gene	QC report on glycerobacteria of recombinant strain	At least 4-5 weeks
E.coli gene knock-in and replacement services	• Strains and information to be modified • Name or sequence of a gene to be knocked in⁎ • Insertion site/replacement sequence		

⁎GenScript can synthesize the gene sequence to be knocked in for you

We really appreciate the original plasmid of microbial genome modification provided by Prof. Yang Sheng and Dr. Jiang Yu of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (reference: Multigene editing in the Escherichia coli genome via the CRISPR-Cas9 system, *Appl Environ Microbiol* 2015 Apr; 81 (7): 2506-2514).

This plasmid has been modified by GenScript's R&D team to meet the needs of microbial genome editing services.

⁎For GenCRISPR™ microbial genome modification service, please send an email to oligo@genscript.com or call 1-877-436-7274, and our professional technical support team will be at your service.

04

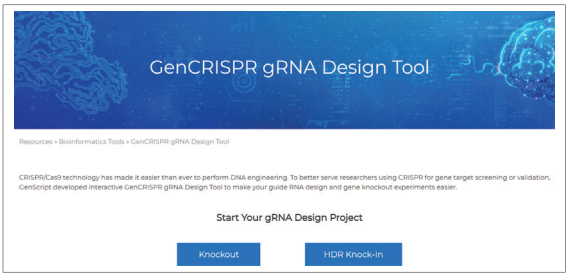
Resource Center

Bioinformatics Tools

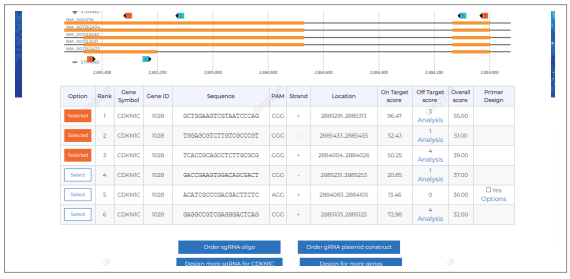
CRISPR Design Tool for sgRNA & HDR-template

www.genscript.com/gencrispr-grna-design-tool.html

Enter the target Gene ID or Gene Symbol to quickly complete the gRNA & HDR-template design online. Based on the algorithm developed and verified by published authoritative literatures, the evaluation parameters such as gRNA localization, on-target /off-target score and comprehensive ranking are provided to help you select the appropriate gRNA sequence.



Input interface

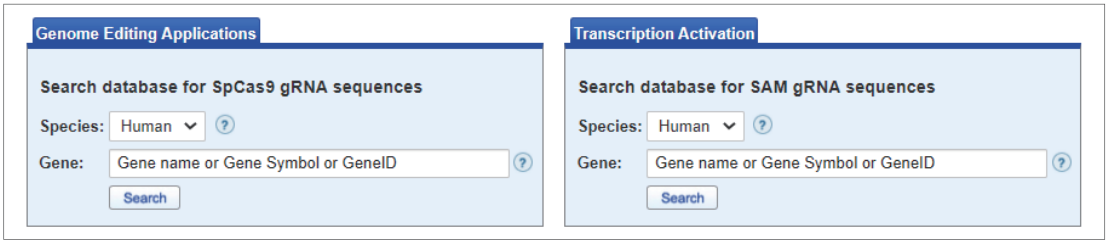


Output result

Genome-wide sgRNA Database

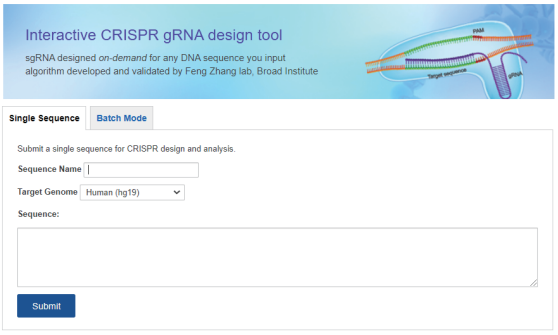
https://www.genscript.com/gRNA-database.html

An on-line genome-wide gRNA database for Human and Mouse can be applied to search for gRNA targeting any gene and support the selection of downstream CRISPR gene editing or CRISPR transcriptional activation applications.



CRISPR gRNA Design Tool (enter sequence)

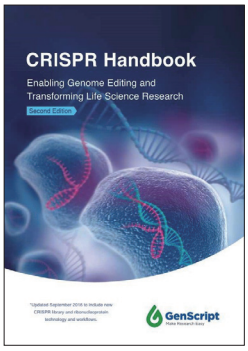
Change to https://www.genscript.com/tools/gRNA-design-tool



The target sequence is entered, the gRNA is designed online for any customized sequence, and 12 commonly used species are available. Galgorithms developed and validated by Zhang Feng Laboratory are applied to support your personalized research needs.

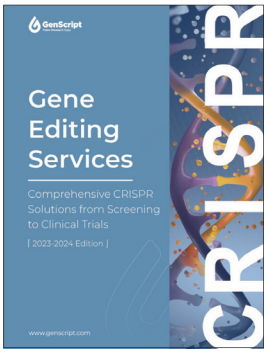
Technical Guidance Materials

From gene editing introduction and application to experimental details, GenScript provides you with comprehensive knowledge and support in various forms, such as paper version, electronic version and lecture video, to help you obtain valuable information more quickly and efficiently.



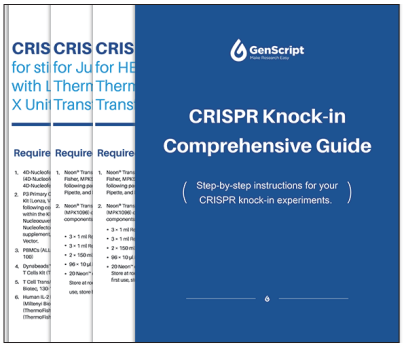
CRISPR Technical Manual

Principle and experimental process of gene editing technology
Downstream application and case analysis



Genome Editing Service Manual

Key points of CRISPR experimental reagent selection
One-stop CRISPR customized service offered by GenScript

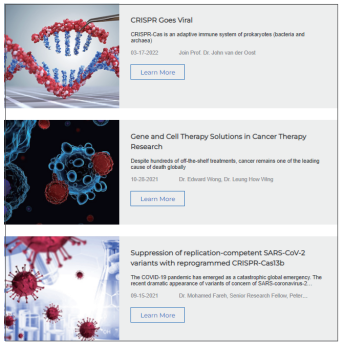


CRISPR KO/KI Protocol

HEK293T&293 / Jurkat / T cell
Thermo Fisher / Lonza / MaxCyte Electroporation Platform

Case Study

Increase editing efficiency and reduce off-target rates in GCT & animal mode generation



CRISPR Webinar

Classical literature and real-time analysis of hot topics
Concise interpretation of gene editing knowledge

Welcome to visit GenScript website - Genome Editing Resource Center for the above information:
(**https://www.genscript.com/crispr-services.html#resources**)
Or scan the QR code on the left

Articles Published by Customers

In terms of services and products, GenScript have been cited by more than 1,300 international famous academic journals such as Cell, Science and PNAS. GenScript's gene editing services and products have been used by a number of world-renowned institutions to publish their scientific achievements, demonstrating once again GenScript's ability to help scientists "Make Research Easy" in the industry.

The following are some scientific articles selected

Title: Genome-edited powdery mildew resistance in wheat without growth penalties

Journal: *Nature* IF: 43.07

Doi: 10.1038/s41586-022-04395-9

Title: High-yield genome engineering in primary cells using a hybrid ssDNA repair template and small-molecule cocktails

Journal: *Nat Biotechnol* IF: 41.667

Doi: 10.1038/s41587-022-01418-8

Title: Re-engineering the adenine deaminase TadA-8e for efficient and specific CRISPR-based cytosine base editing

Journal: *Nat Biotechnol* IF: 41.667

Doi: 10.1038/s41587-022-01532-7

Title: A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat.

Journal: *Nat Genet* IF: 27.959

Doi: 10.1038/s41588-019-0425-8

Title: Transfer of cGAMP into bystander cells via LRRC8 volume-regulated anion channels augments STING-mediated interferon responses and anti-viral immunity

Journal: *Immunity* IF: 22.845

Doi: 10.1016/j.immuni.2020.03.016

Title: Myristoleic acid produced by enterococci reduces obesity through brown adipose tissue activation

Journal: *Gut* IF: 17.943

Doi: 10.1136/gutjnl-2019-319114

Title: Fast and sensitive detection of SARS-CoV-2 RNA using suboptimal protospacer adjacent motifs for Cas12a

Journal: *Nat Biomed Eng* IF: 17.135

Doi: 10.1038/s41551-022-00861-x

Title: The oncomicropeptide APPLE promotes hematopoietic malignancy by enhancing translation initiation

Journal: *Mol Cell* IF: 14.714

Doi: 10.1016/j.molcel.2021.08.033

Title: γ-6-Phosphogluconolactone, a Byproduct of the Oxidative Pentose Phosphate Pathway, Contributes to AMPK Activation through Inhibition of PP2A.

Journal: *Mol Cell* IF: 14.714

Doi: 10.1016/j.molcel.2019.09.007

Title: High-Throughput and Efficient Intracellular Delivery Method via a Vibration-Assisted Nanoneedle/Microfluidic Composite System

Journal: *ACS Nano* IF: 13.942

Doi: 10.1021/acsnano.2c07852

Title: Identification of human CD4+ T cell populations with distinct antitumor activity

Journal: *SCIENCE ADVANCES* IF: 12.804

Doi: 10.1126/sciadv.aba7443

Title: Programmable CRISPR-Cas9 microneedle patch for long-term capture and real-time monitoring of universal cell-free DNA

Journal: *Nat Commun* IF: 12.124

Doi: 10.1038/s41467-022-31740-3

Title: SpG and SpRY variants expand the CRISPR toolbox for genome editing in zebrafish

Journal: *Nat Commun* IF: 12.124

Doi: 10.1038/s41467-022-31034-8

Title: Lupus enhancer risk variant causes dysregulation of IRF8 through cooperative lncRNA and DNA methylation machinery

Journal: *Nat Commun* IF: 12.124

Doi: 10.1038/s41467-022-29514-y

Title: VviPLATZ1 is a major factor that controls female flower morphology determination in grapevine

Journal: *Nat Commun* IF: 12.124

Doi: 10.1038/s41467-021-27259-8

Title: Aspartate aminotransferase Rv3722c governs aspartate-dependent nitrogen metabolism in Mycobacterium tuberculosis

Journal: *Nature Communications* IF: 11.878

Doi: 10.1038/s41467-020-15876-8

Title: dbGuide: a database of functionally validated guide RNAs for genome editing in human and mouse cells

Journal: *Nucleic Acids Research IF: 11.147*

Doi: 10.1093/nar/gkaa848

Title: A programmable omnipotent Argonaute nuclease from mesophilic bacteria Kurthia massiliensis

Journal: *Nucleic Acids Res IF: 10.162*

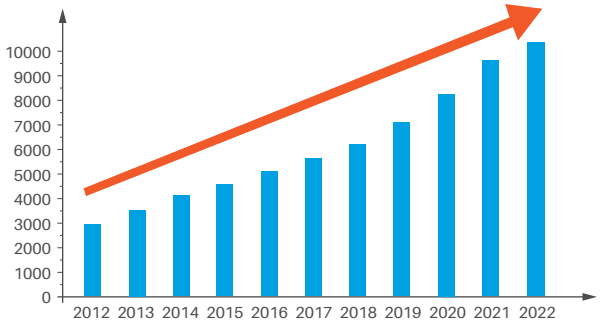
Doi: 10.1093/nar/gkaa1278

Title: Effector gene silencing mediated by histone methylation underpins host adaptation in an oomycete plant pathogen.

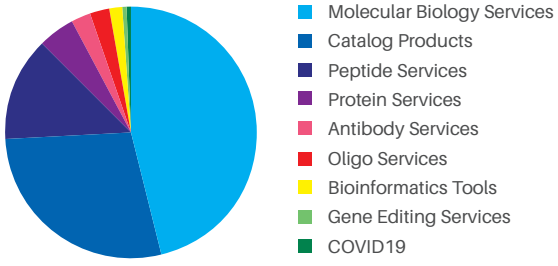
Journal: *Nucleic Acids Res IF: 10.162*

Doi: 10.1093/nar/gkz1160

In terms of services and products, GenScript have been cited nearly 10,000 times by more than 1300 journals of biomedicine such as *Cell, Nature, Science and PNAS*.
Please visit the customer's published literature at
https://www.genscript.com.cn/reference_peer-reviewed_literature.html.



Number of Literatures in GenScript from2010 to 2022



Proportion Distribution of GenScript Products and Services in the Literature

The growth of GenScript is inseparable from the support of our customers. Our value is fully reflected by the publication of papers and academic achievements. In order to thank our customers for their continuous love to GenScript, and more importantly, to thank the front-line researchers for their contribution to the progress of life science for all mankind, GenScript has launched an award-winning activity for publishing articles.

Customer Feedback

"GenScript's CRISPR service was a life saver for my research. We were totally stuck on a specific point mutation in our E.coli strain, and GenScript was able to step in and get it done. Updates are provided through every step of the process and they've been a pleasure to deal with."

— *Chad Johnston, Ph.D., Banting Postdoctoral Fellow in the Collins Lab at MIT.*

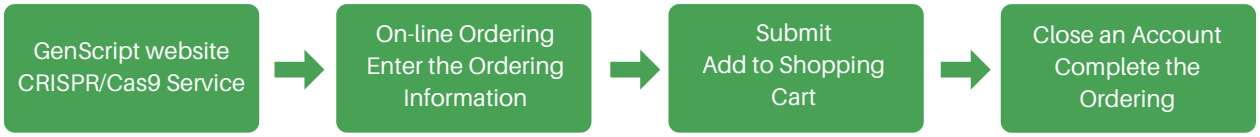
"Long ssDNA sequences are difficult to produce in the lab, especially at the high concentrations necessary for gene editing experiments, we were able to successfully integrate large DNA sequences into primary human T cells using GenScript's long ssDNA product."

— *Dr. Theodore Roth from the Marson Lab at University of California San Francisco.*

05

Ordering Guide and Contact Information

Order Method



On-line Ordering: Log in on-line ordering system, fill in and submit the ordering information, add to your shopping cart, close an account, and finally complete the ordering. **SgRNA, ssDNA and CRISPR plasmids can be ordered on-line**.

Ordering by Customer Service: Submit the inquiry form containing order information (can be downloaded at the bottom of the Service page on the official website of GenScript) and send it to oligo@genscript.com, and our professional technical support team will be at your service.

Order Query

How to query?

1. Log in to your GenScript account
2. Click Account Name - User Center
3. Click "My Order/Query" in the taskbar on the left of the page
4. Select "All Types of Orders" in the Order Type
5. Click the order number to enter the "Order Details" page to view the order progress. For delayed or difficult orders, please email us for consultation and confirmation. We will reply and follow up as soon as possible.

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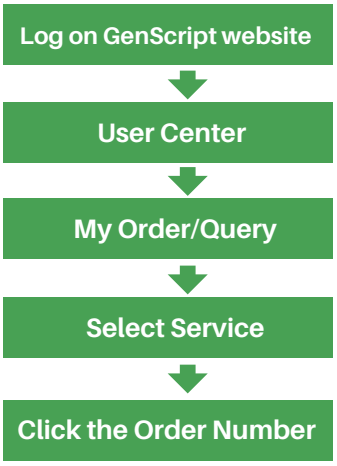

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GenScript is committed to supporting customer needs and to bringing cutting-edge technologies to labs globally

Explore more service & application information!

www.genscript.com

1-877-436-7274

gene@genscript.com

860 Centennial Ave. Piscataway NJ 08854