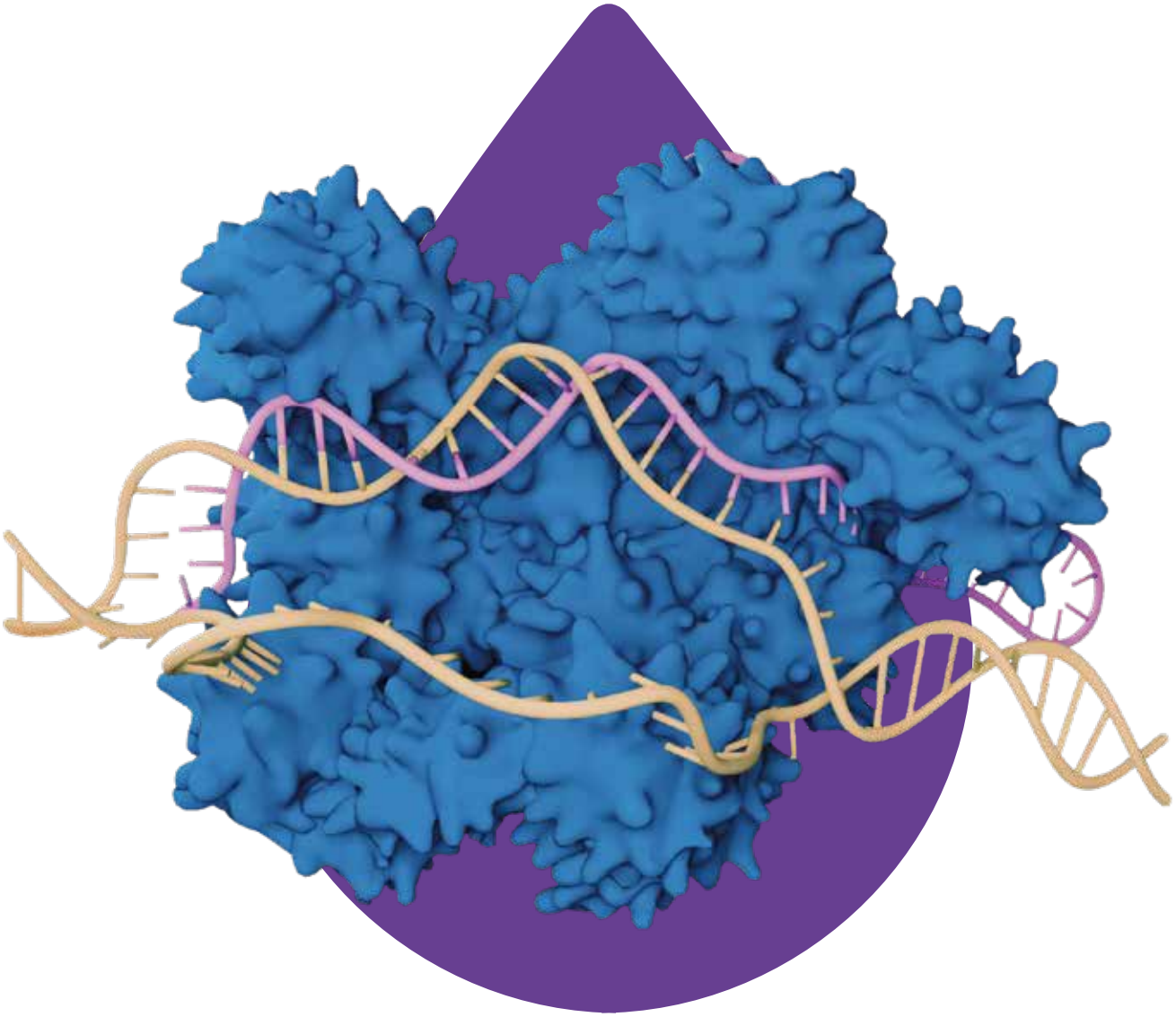
GenCRISPR™ Services

Make Genome Editing Easy



- CRISPR gRNA/Cas9 Plasmids
- Synthetic gRNAs & Cas9 Nucleases
- Single-Stranded DNA Synthesis
- Customized CRISPR Libraries
- Mammalian Cell Line Services
- Microbial Genome Editing

GenScript
Make Research Easy

GenScript GenCRISPR™ Services Overview

One-Stop Solution for Your Genome Editing Needs

Licensed from the Broad Institute and ERS Genomics

- ✓ CRISPR gRNA/Cas9 Plasmids
- ✓ Synthetic gRNAs & Cas9 Nucleases
- ✓ Single-Stranded DNA Synthesis
- ✓ Customized CRISPR Libraries
- ✓ Mammalian Cell Line Services
- ✓ Microbial Genome Editing

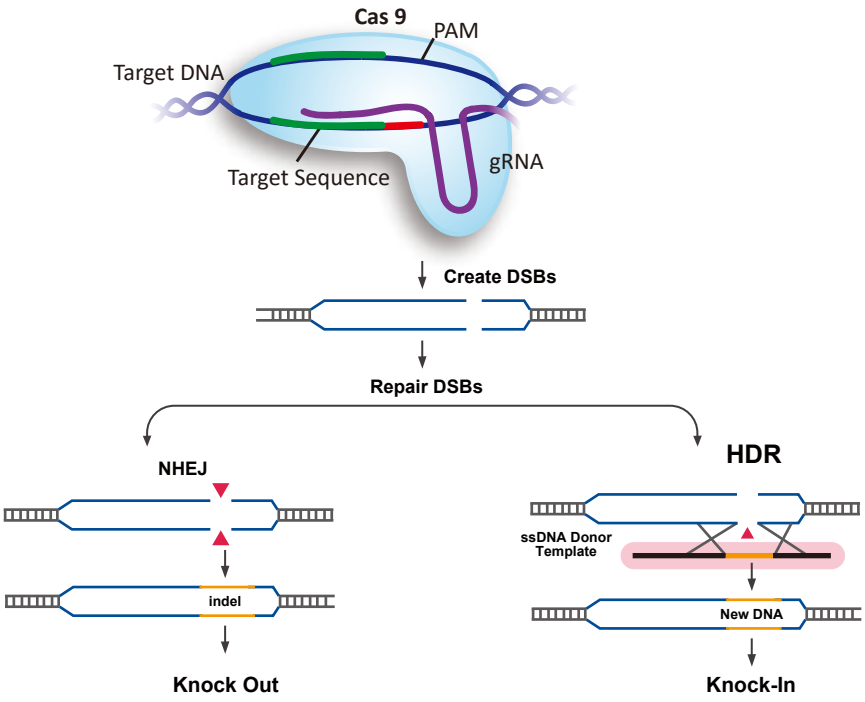
gene@genscript.com

1-732-885-9188

What is **CRISPR?**

Overview of CRISPR Genome Editing Mechanism

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the CRISPR-associated protein (Cas) were originally identified in the *Escherichia coli (E. coli)* genome, where they function as part of an RNA-based adaptive immune system. Researchers have adapted the CRISPR/Cas system to be used as a powerful tool for genetic modifications. Though only developed in 2013, CRISPR/Cas9 already become a popular genome editing tool, due to its simplicity, versatility, and specificity compared to prior techniques for genome modification.



The guide RNA (gRNA) recognizes specific regions on the host RNA and complexes with Cas9, which recognizes the protospacer adjacent motif (PAM) on the target and exerts its endonuclease function to cause double stranded breaks (DSBs). This triggers two mechanisms for repair: one is non-homologous end-joining (NHEJ), which introduces mutations in the DSB site. The other mechanism is homology directed repair (HDR) which enables the donor DNA information to be inserted at the break site.

Research Applications for CRISPR

- Gene Deletion, Insertion, and Correction
- Genome-Wide or Pathway-Focused Screening for Functional Genes
- Transcription Activation or Repression
- T Cell Engineering for CAR-T Cancer Therapy
- RNA Editing
- Generate Embryonic Stem Cell Models and Transgenic Animals
- Visualization or Epigenetic Editing of Genomic Loci

To accelerate your research, GenScript offers validated CRISPR products, services and resources to help you harness the power of CRISPR genome editing.

GenCRISPR™ gRNA/Cas9 Plasmids

GenScript licenses CRISPR technology from the Broad Institute of MIT and Harvard. Our offerings include the latest pre-validated plasmids developed by the CRISPR pioneering Feng Zhang laboratory.

Our Advantages

Never Miss a Deadline Again!
CRISPR plasmids in just 10 days

Use What Works!
Broad Institute pre-validated CRISPR plasmids

Guaranteed Quality
All plasmids are sequence validated

- **Price starting at only $99!**
- **Licensing partnership with the Feng Zhang Lab** at the Broad Institute of MIT and Harvard
- **Guide RNA design and optimization** using algorithms created at the Broad Institute
- **Comprehensive plasmid options**, including eSpCas9, SpCas9, SpCas9 Nickase, SaCas9, and Transcription Activation (SAM) plasmids
- **24/7 Ph.D. level** service representatives to assist with your CRISPR related questions

FREE Online gRNA Database and Design Tool

gRNA Database *www.genscript.com/gRNA-database.html*

GenScript is proud to offer free online access to our gRNA sequence design tool, developed by the Broad Institute of MIT and Harvard. Our gRNA design tool will identify single guide RNAs for use with wild-type *S. pyogenes* Cas9 for any DNA sequence you input.

gRNA Design Tool *www.genscript.com/gencrispr-grna-design-tool.html*

Interactive GenCRISPR gRNA Design Tool to make your guide RNA design and gene knockout experiments easier.

GenCRISPR™ gRNA/Cas9 Plasmids

Comprehensive Vector Systems

Depending on your specific research needs, GenScript offers gRNAs and a variety of Cas9s in either single or dual vector system. Available vector system includes plasmid (non-viral)), lentiviral, or AAV.

Available Cas9 Systems

Cas9 Name	Applications	Characteristics
SpCas9	Research Standard	• Create site-specific double strand breaks (DSBs) in the genome.
eSpCas9 (Enhanced Specificity SpCas9)	Less Off-Target Effects	• Mutation vs. SpCas9: K848A, K1003A, & R1060A; • Reduces off-target effects by up to 10-fold compared to wild-type SpCas9; • Maintaining robust on-target cleavage efficiency.
SpCas9 Nickase (Cas9n D10A)	Creating Single-Strand Nicks	• Contains a mutation allowing the endonuclease to create single-strand nicks, as opposed to DSBs. • Pairing two opposite facing gRNA sequences with SpCas9 nickase can efficiently prevent unwanted indels from forming.
SaCas9	Adeno-Associated Virus (AAV) Applications	• SaCas9 is approximately 1kb shorter than SpCas9, and offers additional flexibility around AAV packaging constraints.

Transcription Activation (SAM) Plasmids	Transcription Activation	• Enable transcriptional activation of both coding and non-coding genetic elements.
		• Includes three components:
		  ◦ A gRNA incorporating two MS2 RNA aptamers
		  ◦ A catalytically inactive dCas9-VP64 fusion protein
		  ◦ A MS2-P65-HSF1 activator fusion protein

CRISPR sgRNA Services

GenScript provides synthetic sgRNA with different grades and flexible delivery options to support your gene editing needs.

EasyEdit sgRNA - The easy yet effective one-step solution!

Synthetic sgRNAs with modifications supporting easy and effective editing

- Option to deliver in 96-well plates supporting high throughput screening

Ready to use sgRNA
No *in vitro* transcription or annealing step needed

High KO efficiency
Even in hard-to-transfect cells

Flexible at scale
1 to 1000+ sgRNAs
2 to 100+ nmol

SafeEdit sgRNA - Safeguard your cells!

High purity sgRNAs with modifications ensuring minimum off-target & cytotoxicity

- More than 90% purity guaranteed with HPLC purification
- Ideal for primary cells and stem cell

Minimal impact for cell viability

Minimized off-target from truncated oligos

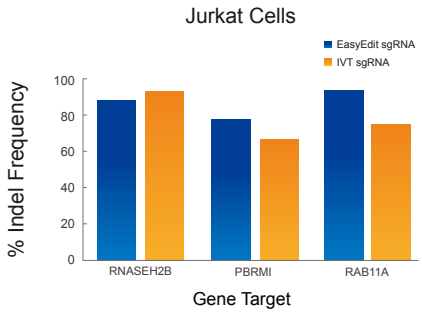
One stop shop from CRISRP machinery to knock-in templates

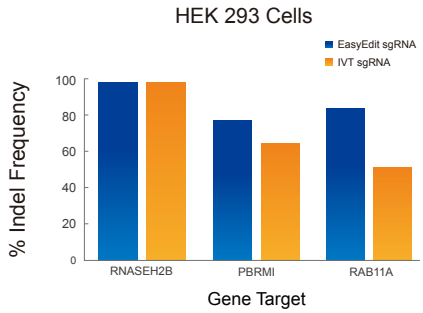
Add-on QC options to ensure sustained quality of your cells

- Mycoplasma testing (qPCR)
- Endotoxin testing (Kinetic LAL)
- Stability testing
- Cytotoxicity testing (co-culture with client specified cell line)

CRISPR sgRNA Services

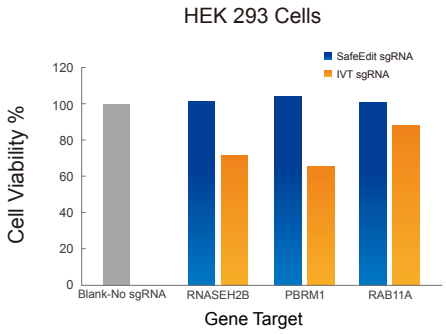
Why waste time on reagent preparation?
Use one piece sgRNA avoiding IVT steps or unstable annealing

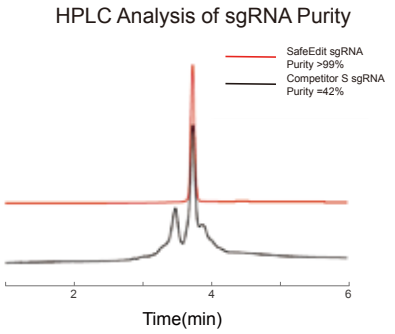




- Synthetic sgRNA provides much better editing efficiency compared to IVT sgRNA
- Did we mention that preparing IVT sgRNA from plasmid template takes ~4 hours in-house?

Choose HPLC purified SafeEdit sgRNA for minimal impact for cell viability and avoiding off-target resulting from truncated oligos in samples





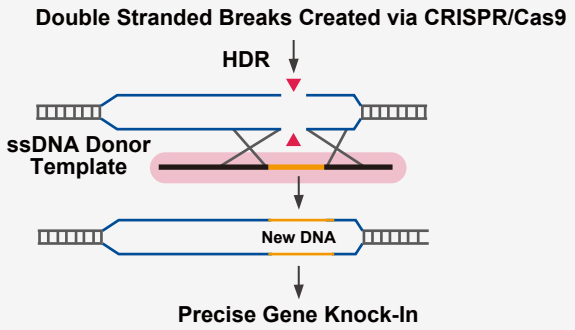
GenScript Pricing: Make gene editing easy and affordable!

Quantity	EasyEdit sgRNA	SafeEdit sgRNA
2 nmol	$99	$199
4 nmol	$149	$319
10 nmol	$259	$579
50 nmol	$799	$1,699
100 nmol	$1,199	$2,499

- Option to deliver in 96-well plates supporting high throughput screening
- Email oligo@genscript.com to quote for other quantities or our CRISPR KI template offering

Single-Stranded DNA Synthesis

Single-stranded DNA (ssDNA or ssODN) is proven to be the best CRISPR homology directed repair (HDR) template for creating gene knock-in, with high editing efficiency and reduced off-target integration. GenScript now offers high quality, sequence verified ssDNA for maximizing the editing efficiency of your CRISPR experiments.



Why Use ssDNA as CRISPR Gene Knock-In HDR Templates?

- High editing efficiency
- Lower cytotoxicity
- Reduced off-target integration
- Increased editing accuracy
- Ideal for editing primary cells & stem cells
- Ideal for developing transgenic animal models

Our Advantages

- Sequence verification by sanger sequencing the final ssDNA product
- No harsh chemicals, enzymatic approach for ensuring non-detectable levels of dsDNA and minimum DNA base damage
- Up to 100 ug delivery quantity allowing for flexible study design
- Free life-time gene template storage supporting faster and more cost-effective re-orders
- Expertise & 16+ Years experience in synthesizing difficult genes as ssDNA templates

Length (Nucleotides)	Quantity	Price	Delivery Time (Business Days)
151-500	3 ug	$400	15-18
	5 ug	$550	
	10 ug	$800	
	20 ug	$1300	
	>20 ug	Inquiry	
501-4000	3 ug	$0.8/nt	18-23
	5 ug	$1/nt	
	10 ug	$1.3/nt	
	20 ug	$1.9/nt	
	>20 ug	Inquiry	
4001-5000	Inquiry	Inquiry	Inquiry

Have questions about ssDNA synthesis service? Email: oligo@genscript.com

Customized CRISPR gRNA Libraries

Pooled CRISPR gRNA libraries are ideal for high-throughput screening of important molecular targets. These libraries leverage the efficiency and specificity of CRISPR gene editing to either knock-out gene expression or transcriptionally activate genes in the genome. GenScript offers a variety of catalog gRNA library options, all of which contain sequences pre-validated by the Broad Institute, as well as high-fidelity fully customizable CRISPR gRNA libraries.

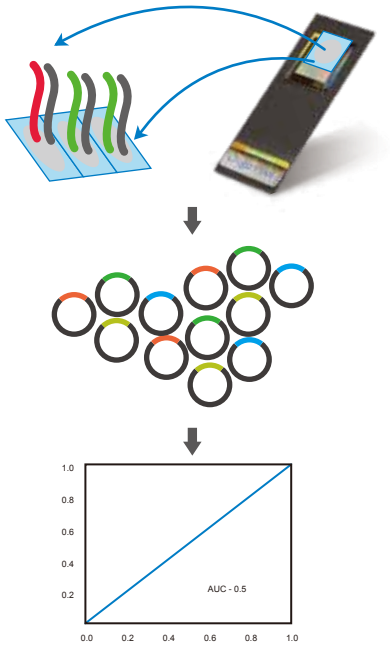
NEW! High-Fidelity Customized CRISPR gRNA Libraries

Build upon our proprietary **Semiconductor-Based Electrochemical DNA Synthesis Platform**, GenScript now offers fully customized, ready-to-package CRISPR gRNA libraries to help maximize your screening efficiency.

Our Advantages

- No sequence restriction
- Top-notch library converge & uniform distribution
- Next-generation sequencing report upon request
- Deliver up to 100 μg of DNA per library
- Up to 90,000 sgRNAs per library

Library Construction Process



Design and synthesize sgRNA oligonucleotides

sgRNA oligonucleotides are synthesized using our proprietary semiconductor-based electrochemical DNA synthesis platform.

Cloning into your vector of choice

Cloning into either a standard lentiviral vector from GenScript or customized vector of your choice.

Quality assurance

CRISPR library distribution is assessed using next-generation sequencing (NGS).

Have questions about customized CRISPR libraries? Email: gene@genscript.com

Mammalian Cell Line Services

GenScript offers GenCRISPR™ mammalian cell line services to produce genetically modified cells using any mammalian cell line and targeting any gene.

Our Advantages

One Stop Solution
From design to functional assays

Track Record
Hundreds of stable cell lines delivered

Fast Delivery
Starting from 10 weeks for standard cell lines

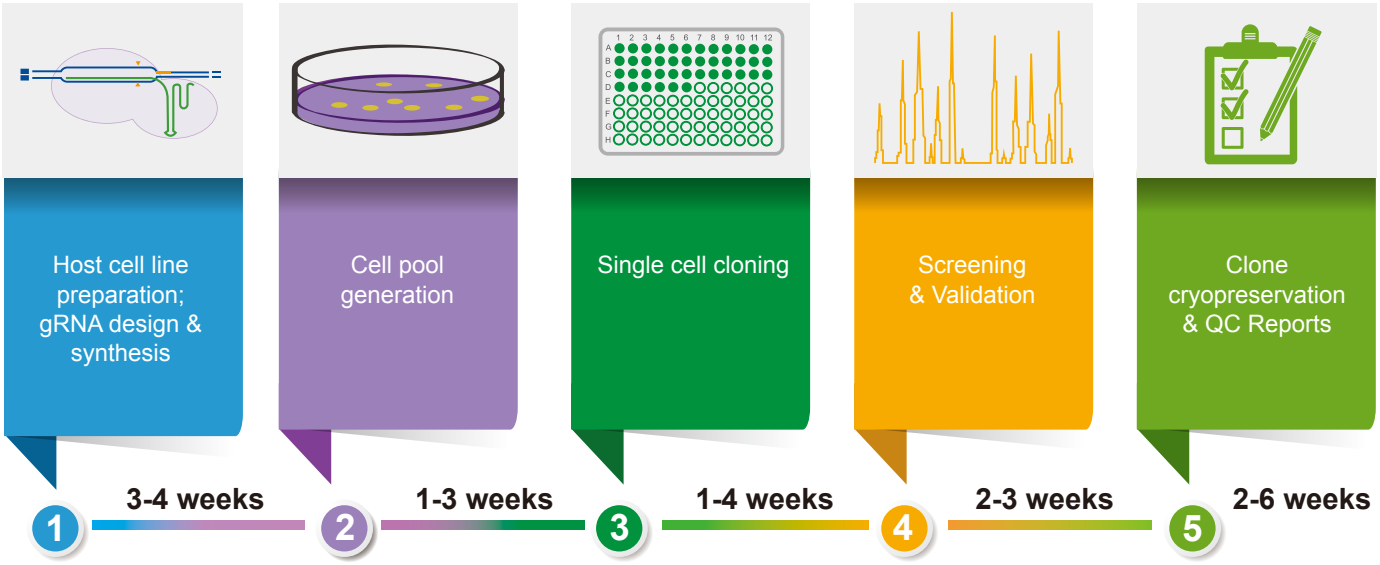
- Extensive experience from gRNA design, transfection to single clone generation
- Versatile CRISPR/Cas9 delivery format (vector, lentivirus or RNP)
- Expertise with various gene transfection methods (nucleofection, electroporation, chemical, or lentivirus)
- Ph.D level project manager providing technical consulting and biweekly project updates

Service Name	Service Details	Cell Line Options *	Deliverables	Price	Delivery Time
EZ Knockout Cell Line Service	• Single gene knockout cell line • Transfection-based	Available for 120+ popular transfection-suitable cell lines, including A549, CHO-K1, HEK293, HEK293T, HT-29, MDA-MB-231, 4T1, A20, HCT116, MCF7, MDCK, U937, RPMI 8866, etc.	• Two full-allelic knockout cell lines validated by sequencing • One negative knockout cell line as control	Starting from ~~$10,000~~ $7,000	10-19 weeks depending on project complexity and cell line difficulty
Customized Knockout Cell Line Service	• Single or multiplex genes editing knockout cell line • Transfection-based or Lentivirus-based or Ribonucleoprotein-based	Any cancer cell line	• 1-2 full-allelic knockout cell line(s) validated by sequencing • One negative knockout cell line as a control	Starting from ~~$21,500~~ $15,000	16-24 weeks depending on project complexity and cell line difficulty
Knockout Cell Pool Service	• Single gene knockout stable cell pool • Lentivirus-based	Any cell line	• Stable cell pool containing knockout clones validated by sequencing	Starting from ~~$6,800~~ $4,800	5-11 weeks depending on cell line difficulty

* It is preferred that customers provide their own cell lines, despite available cell lines can be purchased by GenScript for an additional fee. Please note that we currently do not provide genome editing service for primary cells, stem cells or iPS cells.

Mammalian Cell Line Services

CRISPR Cell Line Workflow



Add-On Services	Description
Reverse transcription (RT)-PCR	Validate the knockout clones to carry the INDELs on CDS at mRNA level by sequencing the RT-PCR product.
Western blot	Validate the knockout clones by western blot. A validated antibody in the host cells with specific binding to target protein (RNAi or knockout) must be provided.
FACS analysis	Validate the knockout clones by FACS analysis. A validated antibody in the host cells with specific binding to target protein (RNAi or knockout) must be provided.
Promoter activity survey	Survey the promoter activity (Cbh/CMV/EFS) in the host cells to maximize gRNA-Cas9 cleavage efficiency.
Transfection optimization	Survey the transfection methods for the hard-to-transfect cells to maximize gRNA-Cas9 cleavage efficiency.
Off-target analysis	Characterize one knockout clone by sequencing the top 10 of potential off-target sites.
One additional clone	Sequence additional single clones to identify one additional knockout clone. QC and deliver one additional knockout clone.

Have questions about CRISPR cell line services? Email: bioprocess@genscript.com

Microbial Genome Editing Services

GenScript is pleased to introduce the microbial genome editing service for knock-in, knock-out or gene replacement in ***bacteria (E. coli).***

Our Advantages

- Proprietary λ Red-CRISPR/Cas9 system that leverages the efficiency of traditional λRed recombineering with the ease of CRISPR/Cas for seamless genome editing
- Precise down to the base pair
- Multigene editing: can knock-out up to 3 genes simultaneously
- Easy selection: no selectable marker is required

Services	*E. coli* Knock-out	*E. coli* Knock-in
gRNA Design	Our expert scientists will optimize your guide RNA sequence for maximized efficiency and minimal off-target effects using design algorithms created at the Broad Institute of MIT and Harvard.	
Materials Needed from Clients	• Host strain • Target gene name • Target gene sequence, if the whole genome sequence is unavailable	
Multiplex Editing	Up to 3 targets	Single knock-in/replacement
Deliverables	Engineered strain in glycerol stock	
Quality Control	• Sequence Chromatogram • Quality Assurance Certificate • Quantitative RT-PCR Validation (Optional) • Western Blot Validation (Optional)	
Delivery Time	Starting from 4 weeks	
Price	Starting from $4,000	Starting from $5,400

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.

Have questions about CRISPR microbial genome editing? Email: microbe@genscript.com

Technical Resources Available **at GenScript**

- ✓ gRNA Design Tools
- ✓ gRNA Databases
- ✓ CRISPR Gene Editing Handbook
- ✓ CRISPR Ribonucleoprotein User Manual
- ✓ Free Webinars
- ✓ Case Studies
- ✓ CRISPR FAQs

Learn More About
CRISPR Services and Resources

www.genscript.com/CRISPR.html

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