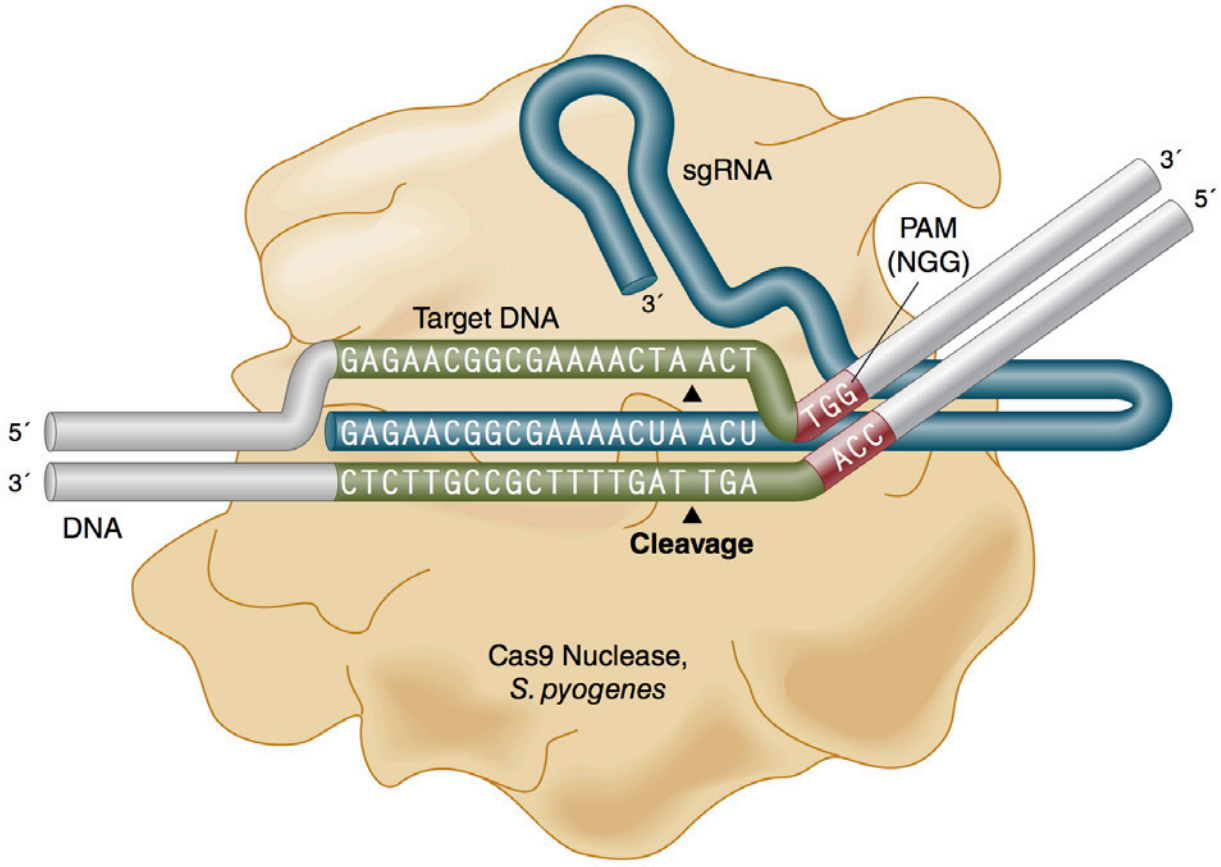
BioNordika
YOUR SCIENTIFIC SUPPLIER

Science where you are

Volume 3 Issue 1

GENOME EDITING

CRISPR/Cas9



Origene

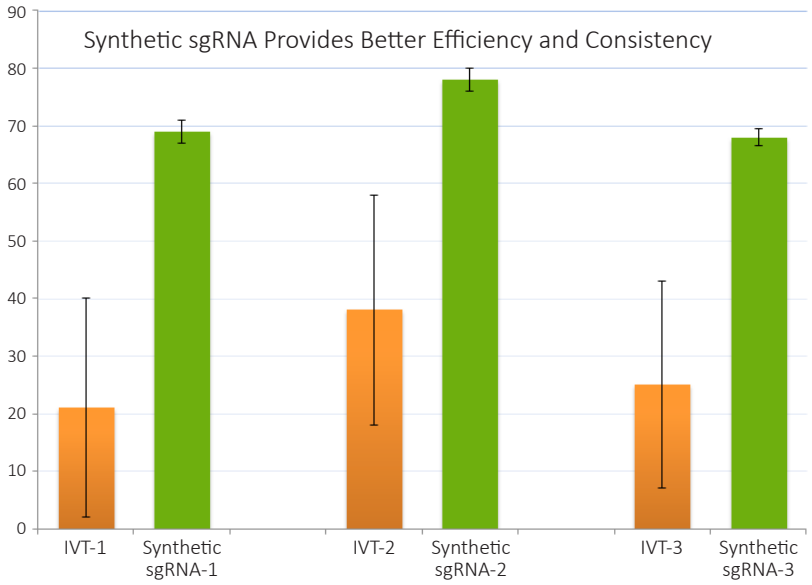
Your Solution for CRISPR/Cas9 and Genome Editing

- **Synthetic sgRNA (100mer)**
- **CRISPR/Cas9 Vectors**
- **AAVS transgene Insertion**
- **Gene Knockout Kits**
- **gRNA and Donor cloning**
- **Cas9 antibodies**

Synthetic Single Guide (sgRNA) for Efficient Genome Editing – Pure and Simple

Synthetic single guide has been recognized as the preferred way for highly efficient and accurate editing. The synthetic single gRNA is a pure 100-mer RNA oligo that contains the target gRNA sequence and the tracrRNA scaffold in a single entity.

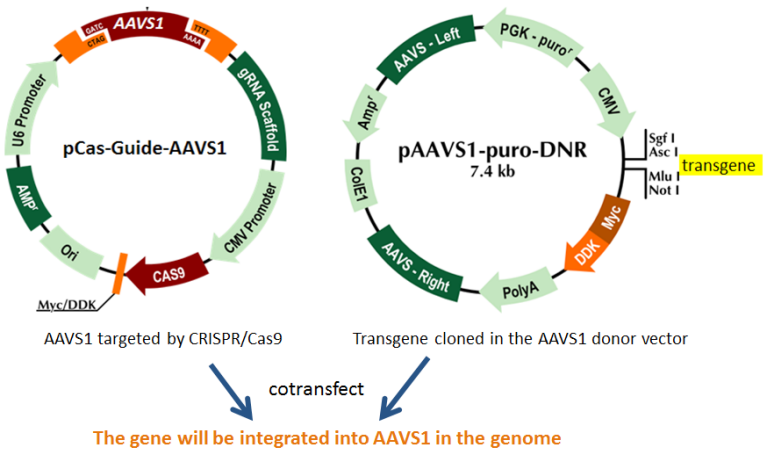
- **Simple workflow**
  - » Ready-to-use solution
  - » No in vitro transcription (as in IVT)
  - » No annealing/purification (as in the 2-oligo system)
- **Better in vivo stability**
  - » No worry for RNase carry-over from the source
- **100% DNA-free**
  - » No risk of integrating foreign DNA into cell line
- **Better efficiency**
  - » Up to 90% genome editing efficiency



The editing efficiency (solid bars) and consistency (error lines) of synthetic sgRNAs against in vitro transcribed (IVT) guide RNAs in HEK293T cells. The experiment was conducted by a third-party using three different gene targets and was replicated three times.

Targeted Transgene Insertion into the AAVS1 Locus

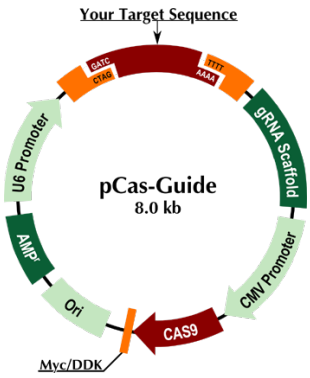
Adeno-associated virus integration site 1 (AAVS1) in human genome is a safe harbor for transgene integration. It is transcriptionally active and transgene expression from this site is robust and stable. CRISPR technology is ideal for targeted transgene insertion at the AAVS1 locus.



- Targeted transgene insertion
- Robust and stable expression
- No random genetic interruption and insertational mutagenesis
- Genome-wide TrueORF collection serves as the source for transgenes
- Easy shuttling from TrueORF clones into AAVS1 donor vector (Precision Shuttling System)

CRISPR/Cas9 Vectors

- **All-in-One vectors:** Contain both gRNA and Cas9 expression (lenti option available).
- **T7 vectors:** For in vitro production of gRNA and Cas9 mRNA
- **Cas9 Nickase:** Decrease off-targeting



Knock-out CRISPR/Cas9 Genome Editing Kits

- Complete kit for gene knockout via CRISPR (targeted sites around the 5' end of the ORF)
- 2 guide RNA vectors in pCas-Guide to ensure an efficient cleavage
- Donor vector with predesigned homologous arms
- Knockin GFP-Puro for selection
- pCas-Guide-scramble is also provided as a negative control

transOMIC

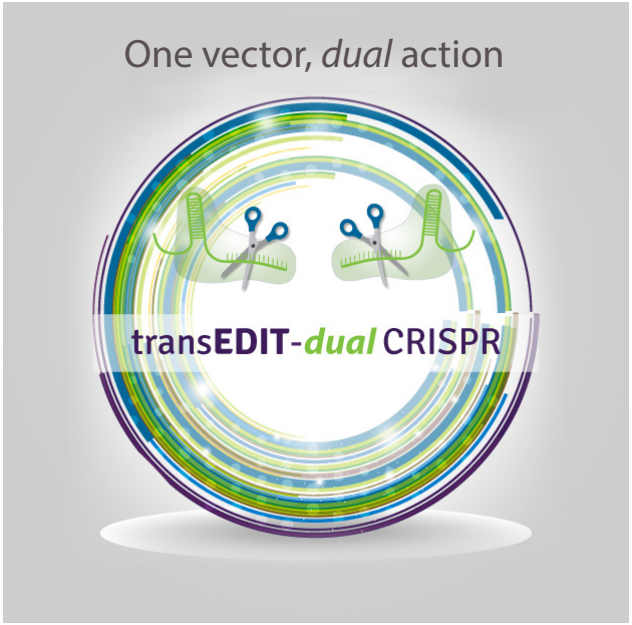
Optimized gRNA Designs and Versatile Expression Vectors

transOMIC technologies uses the most current design algorithms to provide gRNA designs with the highest ranking efficiency and lowest number of off target effects. A non-targeting negative control is also included.

transEDIT-dual CRISPR Arrayed Library

Individual, combinatorial and multiplexed gene knockout.

The new **transEDIT-*dual* CRISPR lentiviral library** was designed using the most comprehensive algorithm for gRNA design available. The ***CRoatan algorithm*** (Erard, Knott and Hannon) was developed by a random-forest-based gRNA prediction tool (trained on numerous exiting algorithms and comparative bench-marking) (Doench, *et al.*, Chari, *et al.*, Bae *et al.* and Shi *et al.*) to create novel and ***superior*** gRNA designs. By using the ***CRoatan algorithm***, two independent gRNAs were paired to optimize their dual activity to create deleterious mutations in coding genomic DNA.

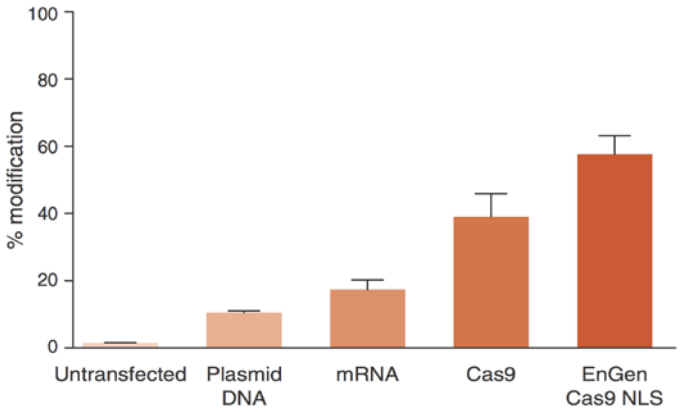


New England Biolabs – Tools for Genome Editing

New England Biolabs supports a broad variety of CRISPR/ Cas9 genome editing approaches. The EnGen® product line gives you tools for direct introduction of Cas9 ribonucleo-protein (RNP) to detection of edits using enzymatic mutation detection.

Genome engineering by direct introduction of active nuclease complexes with EnGen® Cas9 NLS

Cas9 is an RNA-guided endonuclease that catalyzes site-specific cleavage of double stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif). The PAM sequence, NGG, must follow the targeted region on the opposite strand of the DNA with respect to the region complementary sgRNA sequence. EnGen Cas9 NLS, *S. pyogenes* (#M0646T/M) contains nuclear localization sequences (NLS) on the N- and C- termini of the protein.

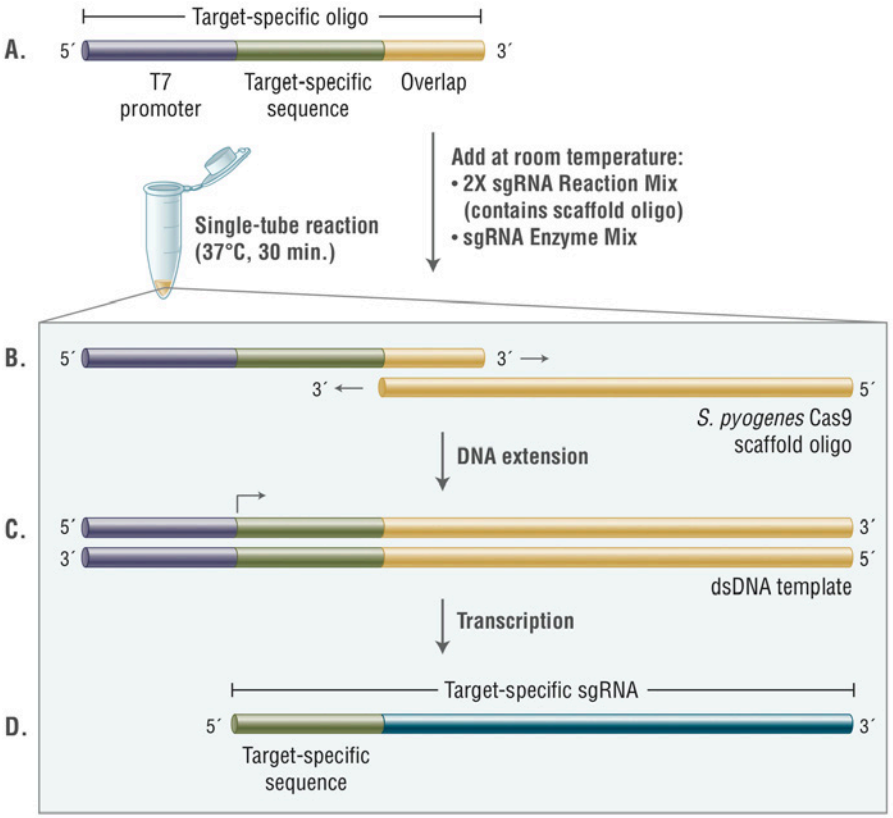


Cas9 and sgRNA targeting a human gene were delivered to HEK293 cells by transfection. Transfected plasmid DNA contained expression cassettes for 2x NLS (N- and C-terminal) Cas9 and sgRNA. Transfected mRNA was modified with pseudouridine and 5-methylcytosine and encoded 2x NLS (N-and C-terminal) Cas9. sgRNA was co-transfected with the mRNA. Cas9 RNPs were delivered in reverse transfections. Cas9 has no NLS in the protein sequence. EnGen Cas9 has N- and C-terminal NLSs. The efficiency of editing was determined using T7 Endonuclease I assay and is expressed as % modification.

Rapid Generation of sgRNA

EnGen® sgRNA Synthesis Kit, *S. pyogenes*

The EnGen sgRNA Synthesis Kit (#E3322S), *S. pyogenes* combines a Cas9-specific Scaffold Oligo that is partially complementary to the target-specific oligos designed by the user. The two oligos anneal at the overlapping region and are filled in by the DNA polymerase, creating a double-stranded DNA (dsDNA) template for transcription. Synthesis of the dsDNA template and transcription of RNA occur in a single reaction, resulting in the generation of a functional sgRNA.

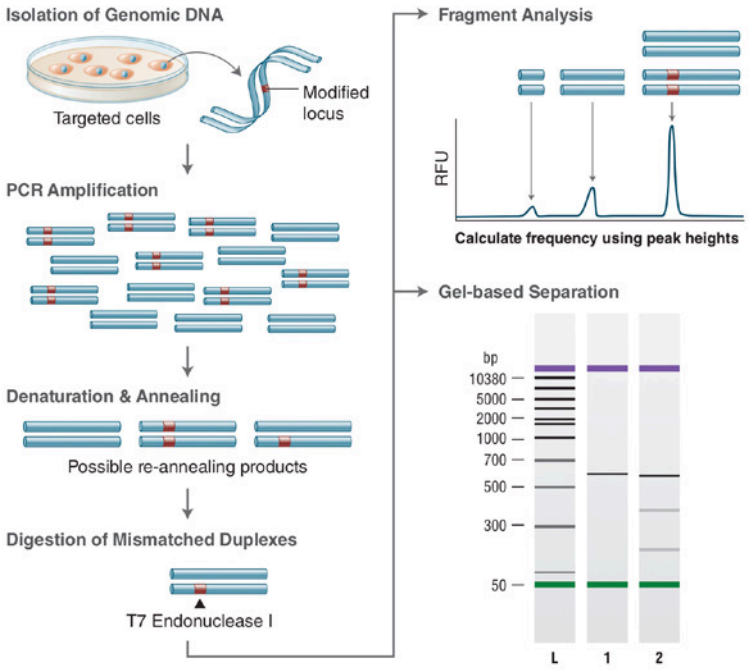


EnGen sgRNA Synthesis Kit Overview

Evaluating Targeting Efficiency with the EnGen® Mutation Detection Kit

The EnGen Mutation Detection Kit (#E3321S) provides reagents for detection of on-target genome editing events.

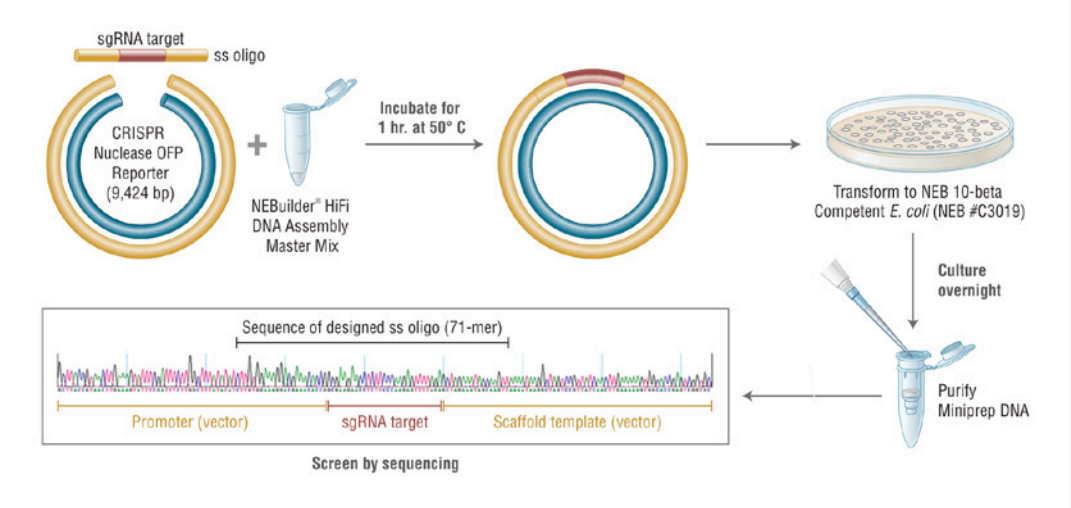
Genomic DNA is amplified with primers bracketing the modified locus. PCR products are then denatured and re-annealed yielding three classes of possible structures. Duplexes containing a mismatch greater than one base are digested by T7 Endonuclease I. The DNA is then electrophoretically separated and fragment analysis is used to estimate targeting efficiency.



sgRNA Template Construction for CRISPR/Cas9 Genome Editing

NEBuilder HiFi DNA Assembly

NEBuilder HiFi DNA Assembly Master Mix (#E2621S/L/X) allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. This method can be used to assemble single-stranded oligonucleotides containing your sgRNA target sequence into a vector of choice. The reaction includes three enzymes that work together in the same buffer.



Design an ssDNA oligo containing the target sequence (19–21 bases) of sgRNA flanked by 25 bases of sequence at both ends complementary to the linearized vector. Mix the single-stranded oligo, linearized vector and NEBuilder HiFi DNA Assembly Master Mix and incubate for 1 hour at 50° C.

Q5 Site-Directed Mutagenesis Kit

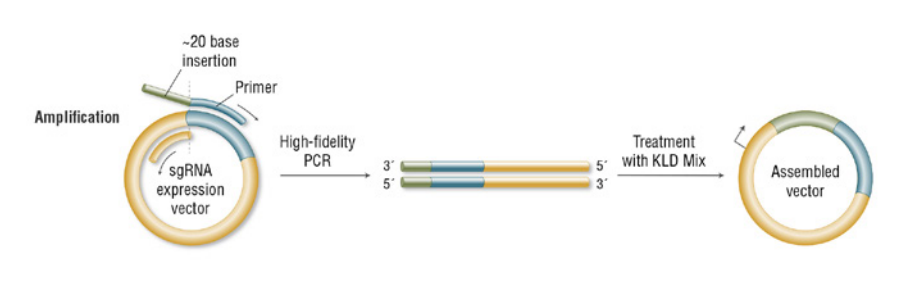
The Q5® Site-Directed Mutagenesis Kit (#E0554S) enables rapid, site-specific mutagenesis in less than 2 hours. Use with custom primers to create insertions, deletions and substitutions in a wide variety of plasmids. After PCR, the amplified material is added directly to a unique Kinase-Ligase-DpnI (KLD) enzyme mix for rapid (5 minutes), room temperature circularization and template removal.

HiScribe™ T7 Quick High Yield RNA Synthesis Kit

The HiScribe T7 Quick High Yield RNA Synthesis Kit (#E2050S) is designed for quick set-up and production of large amounts of RNA in vitro. Linearized plasmid DNA, PCR products or synthetic DNA oligonucleotides can be used as templates for in vitro transcription with the HiScribe T7 Quick High Yield RNA Synthesis Kit, provided they contain the T7 promoter upstream of the sequence to be transcribed.

HiScribe™ SP6 RNA Synthesis Kit

The HiScribe SP6 RNA Synthesis Kit (#E2070S) is designed for in vitro transcription of RNA using SP6 RNA Polymerase. Functional sgRNA generated will be ready for transfection or in vitro testing.



Workflow Q5 Site-Directed Mutagenesis Kit

Eurogentec

Custom Oligos for your Genome Editing Experiments

Use in combination with EnGen® sgRNA Synthesis Kit, NEBuilder HiFi DNA Assembly Mix and Q5 Site-Directed Mutagenisis kit from New England Biolabs to generate templates for sgRNA!

Eurogentec offers oligonucleotides with a large choice of chemistries, modifications, specifications and purifications. More than 300 modifications and several purity levels are available.

Specification			
Length:	From 5 to 139 bases	**Quality Control:**	MALDI-TOF MS
Synthesis scale:	10 nmol • 40 nmol • 200 nmol • 1000 nmol • 2.5 µmol • 5 µmol • 10 µmol*	**Format:**	Dried
Backbone:	DNA, RNA, LNA®, 2'O-Me RNA, PNA and all linkages	**Packaging:**	2 mL tube, 96-well or 384-well plates
Modifications:	More than 300 modifications!	**Documentation:**	Technical data sheet
Purifications:	SePOP desalting, RP-Cartridge-Gold™, HPLC, PAGE, Dual HPLC, UltraPureGold™	**Shipping:**	At room temperature

GenScript – One Stop Solution for CRISPR/Cas9

To accelerate your research, GenScript offers validated CRISPR products, services and resources to help you harness the power of CRISPR genome editing. As a leader in gene synthesis and genome editing, and through partnership with Feng Zhang's laboratory at the Broad Institute of MIT and Harvard, GenScript offers validated CRISPR products, services and resources to help you harness the power of CRISPR genome editing for your research.

CRISPR gRNA Libraries

- In-stock GeCKO libraries for genome-wide knock-out
- SAM libraries for genome-scale transcription activation

CRISPR-edited Mammalian Cell Lines

- Fully-validated KO cell lines and cell pools using CRISPR technology

Purified Cas9 Proteins

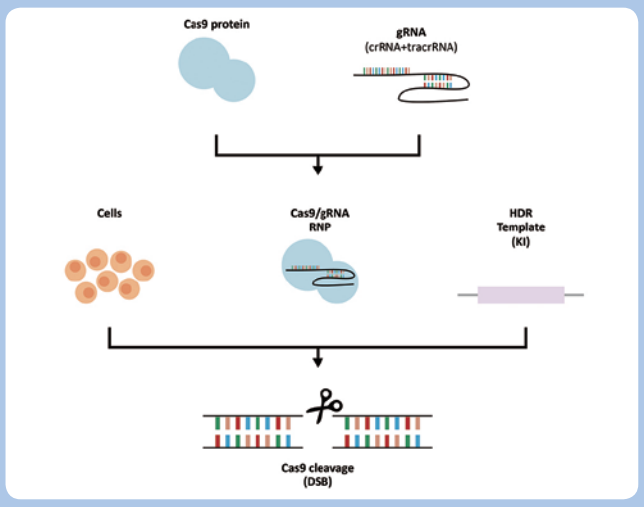
- Get high-efficiency gene editing with purified Cas9 proteins and other products

CRISPR gRNA Constructs

- All-in-one or dual SpCas9, Nickase & SaCas9 constructs for single guide RNA expression
- SAM constructs for transcription activation

New Synthetic sgRNA Service

- DNA-free, rapid gene editing in any cell type
- Pre-synthesized, validated crRNA:tracrRNA sequences



Advantages of Cas9 RNP Service:

- **sgRNA design included:** choose from Broad-validated gRNA sequences or design your own
- **Delivered as duplexed crRNA tracrRNA oligos:** no need for in vitro transcription
- **Ready for transfection:** just prepare the complex and deliver into your cells!
- **Flexible:** quantity options

GenCRISPR™ Mammalian Cell Line Services

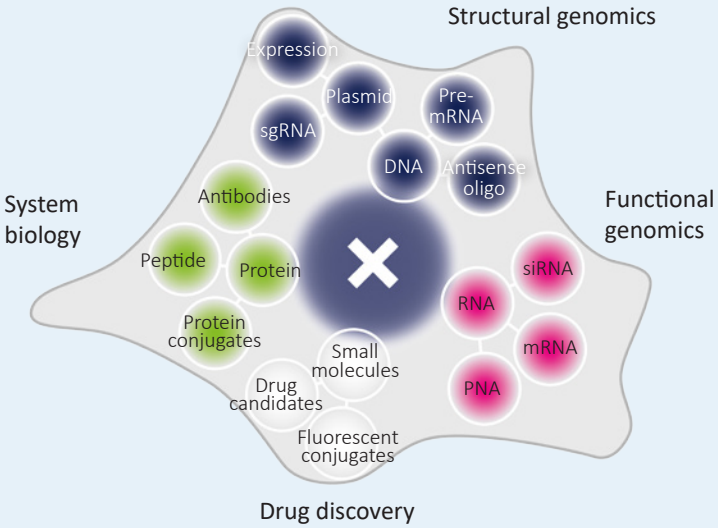
GenScript is pleased to introduce GenCRISPR™, a full CRISPR-based gene editing service, to produce a genetically modified cell using any mammalian cell line and targeting any gene. Our scientists are experts at performing gene editing with CRISPR, from designing gRNA constructs for CRISPR to transfection and single clone generation of a wide range of cells, including difficult-to-transfect and tumor cell lines.

Host cell characterization	gRNA design and synthesis	Transfection	Single cell cloning	Genotyping
• Clonality test • Transfection efficiency optimization with up to 10 method tests • Promoter validation • **3-5 weeks**	• Design up to 5 gRNAs • **3-4 weeks**	• Transfection or viral-based delivery • Cell pool examination by sequencing trace analysis or surveyor assay • Enrichment of transfected cells by FACS sorting or antibiotic selection • **2-6 weeks**	• Plate up to 10 to 96-well plates • **5-8 weeks**	• High-throughput screening of up to 150 single cell clones by sequencing • Validation at mRNA or protein level upon request • **3-5 weeks**

Lonza

Successful Transfection of CRISPR using Nucleofector™ Technology

All genome editing tools require co-transfer of several substrates into the cell type of interest for successful modification of genomic DNA. Lonza's non-viral Nucleofector™ Technology has been shown to work as a reliable and efficient method for transferring the required DNA- or RNA-based components into various cell lines and primary or stem cells, e.g., primary T cells, human embryonic stem cells (hESC) or induced pluripotent stem cells (iPSCs).



Nucleofector™ Features

- High transfection efficiencies for a broad range of cell types, including iPSCs
- Efficient co-transfection of various substrates
- Same conditions for transfecting plasmids, DNA, mRNA or PCR cassettes, ssODN
- Proven for ZFN, TALEN and CRISPR by more than 30 publications, including high ranking journals including Nature

The Nucleofector™ Technology Relies on a Unique Combination of:

- **A Nucleofector™ Device:** Delivering optimized electroporation parameters pre-programmed for each optimized cell type
- **Nucleofector™ Kits for Primary Cells or Cell Lines:** Containing a specific Nucleofector™ Solution that provides a protective environment that allows for high transfection efficiency and cell viability, while helping to maintain physiologically relevant cellular functions
- **Cell-specific Optimized Protocols:** Providing optimal Nucleofection™ Conditions and comprehensive guidance, including tips for cell sourcing, passage, growth conditions and media, and post-transfection culture

BioNordika
YOUR SCIENTIFIC SUPPLIER