



# ToolGen Genome Editing (CRISPR/Cas9) Products & Services

Catalogue 2017

[www.toolgen.com](http://www.toolgen.com)

**We Edit Genome as Nature Does,  
Just more efficiently**

ToolGen is a leading biotech company having focused on development of cutting-edge genome editing technology and transformative products for biomedicine and other industry.

We create and hold intellectual property rights for essential tools and technologies for editing the genetic information in microbial, plant, animal and human cells.

**ToolGen**  
Genome Engineering

 **cosmo** GENETECH

# ToolGen, using CRISPR tools and in partnership with leading research group, is doing the foremost research for

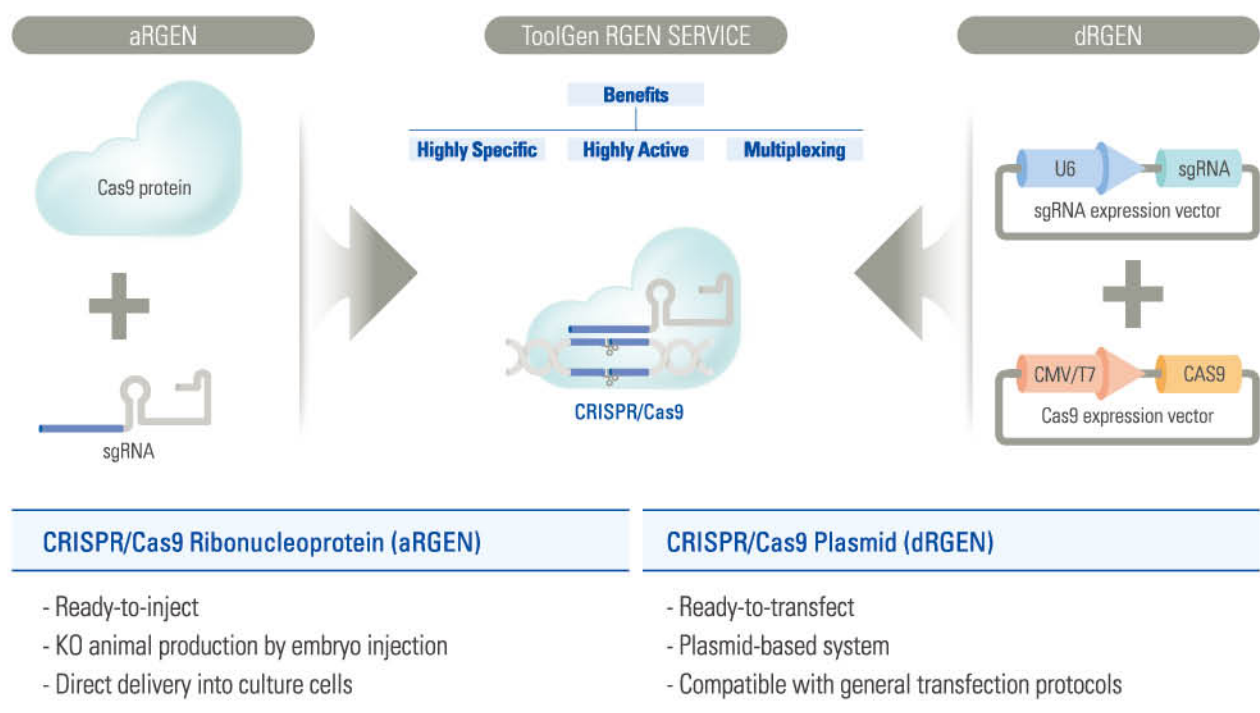
- Gene medicine for hereditary disorder
- Genetic breeding of animals & plants
- Genetic production of new drugs and medical materials

## Background Technology

RNA-Guided ENdonucleases (RGEN), a new generation of engineered nuclease based on a bacterial CRISPR/Cas9 system, is innovative tools for the genome editing.

RGENs recognize a target sequence that is 19~20bps in length and is followed by sequence ending with two guanines (NGG) named PAM. This minimal requirement for target sequence recognition allows RGENs to be designed to edit any region of the genome (every 8bps, in theory). Specific cleavage of your target sequence allows efficient genome editing (knock-out / knock-in / locus deletion) *in vitro* and *in vivo*.

Our recent data is suggesting that the RGENs have a remarkably high efficiency and specificity *in vitro*. Additionally, our RGENs are able to support very robust production of gene-modified cell line, mouse and zebrafish.



**CRISPR/Cas9 is the most sensitive, specific and easy-to-use tool for Genome editing. ToolGen is providing researchers with a comprehensive range of research tools based on CRISPR/Cas9.**

● Gene managing tools for cleavage, insertion, deletion, inversion etc.

● Gene edited cell line, animals

● Other tools accompanying CRISPR/Cas9

## CRISPR/Cas9 Services

Do you want to manage target gene with efficiency and specificity?

## Genome Editing Services

Do you want to get cell or mouse mutated in specific gene you target?

## Reporter Services

Do you want to have cell mutated and separated by surrogation marker?

## In-Del Analysis Services

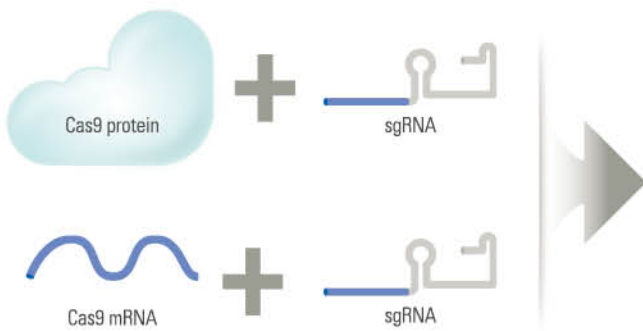
Do you want to have any mutated gene sequenced?

**Why don't you design your research plan with CRISPR tools?  
We are here to support you!**

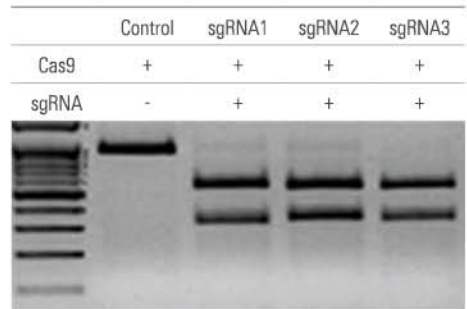


## CRISPR sgRNA Service (Active RGENs, aRGEN)

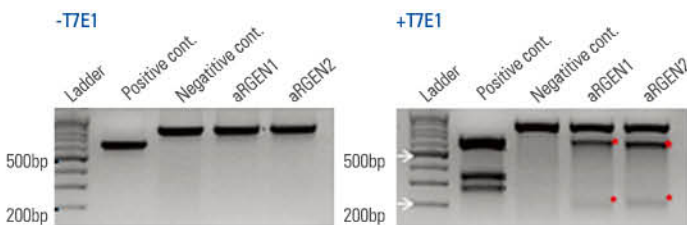
ToolGen's CRISPR sgRNA service provides ready-to-use reagents for genome engineering experiments. When combined with appropriate supporting products (Cas9 mRNA or recombinant Cas9 protein), sgRNAs will form ready-for-action engineered nucleases for your target sequence, both *in vitro* and *in vivo*. The service provides a highly efficient and specific way to apply genome editing in your target cells. The reagents can be injected into one-cell embryos without additional processing



### In vitro cleavage assay

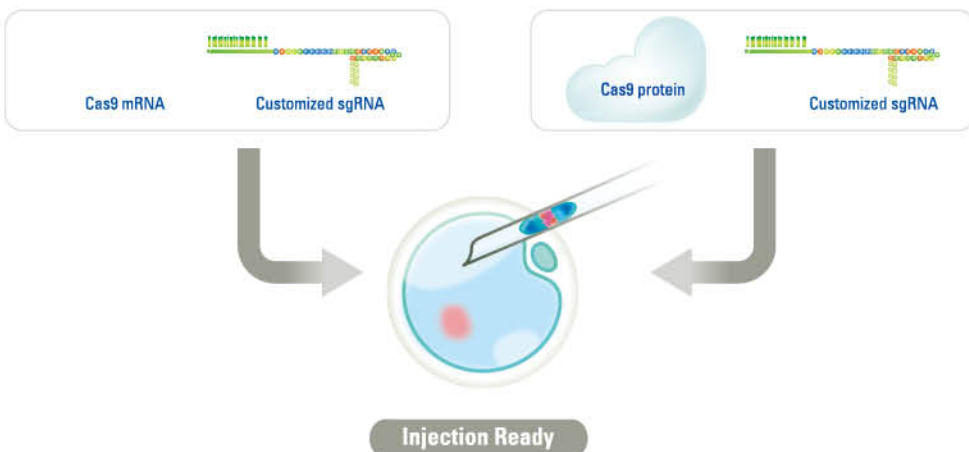


### T7E1 assay in cell(NIH3T3)



### aRGEN \_ In-Del Analysis

	Total Sequences	Mutation efficiency		Indel frequency
		Insertions	Deletions	
sgRNA #1	17014	2609	11338	13947 (82.0%)
sgRNA #2	18512	1347	12550	13897 (75.1%)
sgRNA #3	18512	4678	22459	27137 (91.3%)
sgRNA #4	18512	7864	18068	25932 (81.6%)



## Ordering Information

### aRGEN Synthesis

#### Cat No# TGEN\_aRS1

- Product : aRGEN(sgRNA. 50ug) +pRGEN-Cas9-CMV/T7 (5ug)
- Service Period : 2~3 week
- Warranty period : 4 month

### aRGEN Validation

#### Cat No# TGEN\_aRV1

gDNA(1~2ug) be given for other than Human / Mouse

- Product : aRGEN(sgRNA. 50ug) + pRGEN-Cas9-CMV/T7 (5ug) + Purified Cas9 protein (50ug)
  - In vitro assay
- Service Period : 4~5 week
- Warranty period : 4 month

#### Cat No# TGEN\_aRV2

Cell be given for other than Human / Mouse

- Product : aRGEN(sgRNA. 50ug) + pRGEN-Cas9-CMV/T7 (5ug) + Purified Cas9 protein (50ug)
  - Cell line assay
- Service Period : 4~5 week
- Warranty period : 4 month

### Positive Control (aRGEN Synthesis)

#### Cat No# TGEN\_aRSP1

- Product : Postive control\_EGFP sgRNA (20ug)
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_aRSP2

- Product : Postive control\_HPRT sgRNA (20ug)
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_aRSP3

- Product : Postive control\_CCR5 sgRNA (20ug)
- Service Period : 2 week
- Warranty period : 4 month

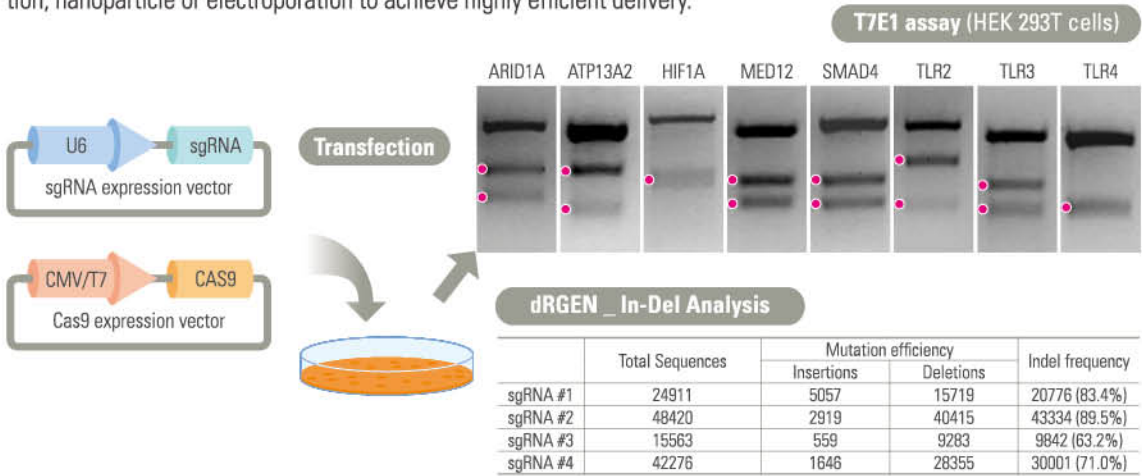
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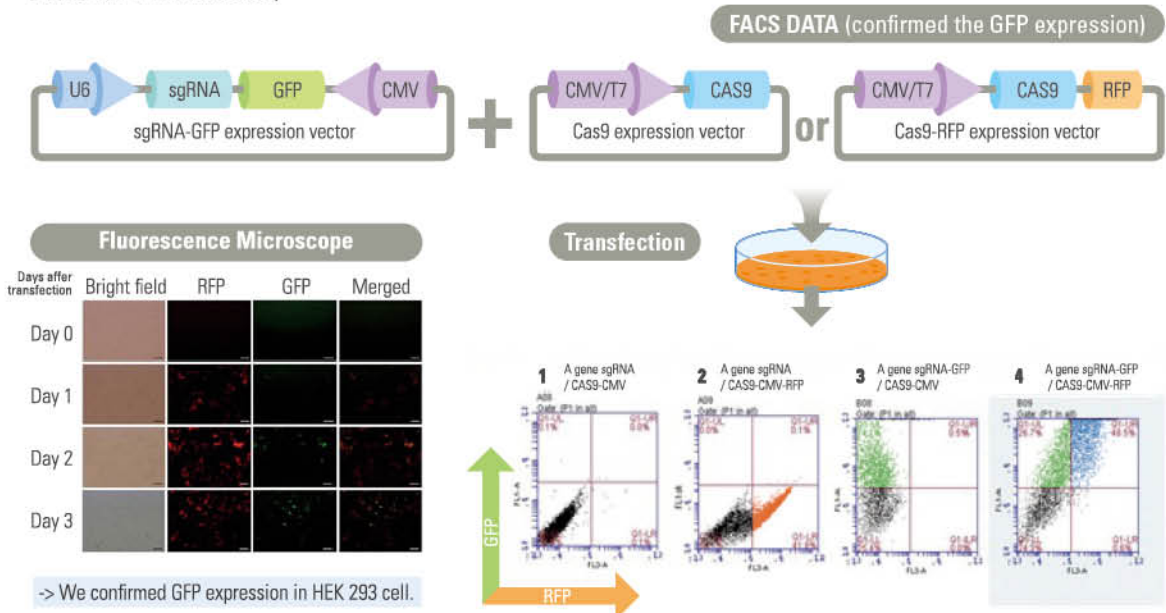
## CRISPR plasmid(DNA-directed RGENs, dRGEN)

ToolGen CRISPR plasmid services are efficient, affordable and convenient tools for genome editing experiments. Custom sgRNA expression plasmids are provided together with Cas9 expression plasmids(human codon optimized, WT/Nickase/Dead form available). They can be delivered to your cell of interests by any standard methods like lipofection, nanoparticle or electroporation to achieve highly efficient delivery.



## CRISPR –GFP Plasmid (dRGEN-GFP) SERVICE (TGEN\_dRS3~4)

CRISPR-GFP plasmid is prepared by inserting a GFP construct in the existing CRISPR plasmid. This CRISPR-GFP plasmid allows you to confirm the activity level in cells by fluorescent microscope. Custom sgRNA expression plasmids are provided together with Cas9 expression plasmids (human codon optimized, WT/Nickase/Dead forms are also available)



## Ordering Information

### dRGEN Synthesis

#### Cat No# TGEN\_dRS1

- Product : 1 dRGEN ( sgRNA plasmid, 1~2ug) + pRGEN-Cas9-CMV/T7(5ug)
- Service Period : 2~3 week
- Warranty period : 4 month

#### Cat No# TGEN\_dRS3

- Product : CRISPR-GFP plasmid(dRGEN, 50ug)+ pRGEN-Cas9-CMV/T7(5ug)
- Service Period : 2~3 week
- Warranty period : 4 month

#### Cat No# TGEN\_dRS2

- Product : 1 dRGEN ( sgRNA plasmid, 50ug) + pRGEN-Cas9-CMV/T7(5ug)
- Service Period : 2~3 week
- Warranty period : 4 month

#### Cat No# TGEN\_dRS4

- Product : CRISPR-GFP plasmid(dRGEN, 1~2ug)+ pRGEN-Cas9-CMV/T7(5ug)
- Service Period : 2~3 week
- Warranty period : 4 month

### Pre-Designed dRGEN Synthesis

#### Cat No# TGEN\_dRPS1

- Product : 3 dRGEN ( sgRNA plasmid, 1~2ug) + pRGEN-Cas9-CMV/T7(5ug)
- Service Period : 2~3 week
- Warranty period : 2 month

#### Cat No# TGEN\_OP1

- Product : able to buy individual pRGEN-Cas9-selection marker
- pRGEN-Cas9-CMV/T7
- pRGEN-Cas9-CMV/T7 (wild type or Puro-RFP or Hygro-EGFP)
- pRGEN-Cas9-Ef1a (wild type or Puro-RFP or Hygro-EGFP)
- pRGEN-Cas9-CMV/T7 (nickase(D10A) type or Puro-RFP or Hygro-EGFP)
- pRGEN-Cas9-Ef1a (nickase(D10A) type or Puro-RFP or Hygro-EGFP)
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_dRPS2

- Product : 3 dRGEN ( sgRNA plasmid, 1~2ug) + pRGEN-Cas9-CMV/T7(50ug)
- Service Period : 2~3 week
- Warranty period : 2 month

### dRGEN Validation

#### Cat No# TGEN\_dRV1

- Possible to provide only in the Human/Mouse
- Product : Validated 1 dRGEN ( sgRNA plasmid, 1~2ug) + pRGEN-Cas9-CMV/T7(5ug)+ data/setup condition (T7E1 assay)
  - possible to provide additional dRGEN 1ea
  - Service Period : 4~5 week
  - Warranty period : 4 month

#### Cat No# TGEN\_dRV2

- Possible to provide only in the Human/Mouse
- Product : Validated 1 dRGEN ( sgRNA plasmid, 1~2ug) + pRGEN-Cas9-CMV/T7(5ug)+ data/setup condition (FACS assay)
  - possible to provide additional dRGEN & Reporter 1ea
  - Service Period : 4~5 week
  - Warranty period : 4 month

### Kit Service (dRGEN Targeted Integration Kit - AAVS1/Rosa26)

#### Cat No# TGEN\_AAVS1

- AAVS1 RGEN Knock-In Kit
- : pRGEN\_Hs\_AAVS1-U6\_SG (2ug)
- pRGEN\_Cas9(CMV) (5ug)
- AAVS1 T7E1 primer set(100pM, 35ul)
- AAVS1\_donor vector(2ug)

#### Cat No# TGEN\_Rosa26

- Rosa26 RGEN Knock-In Kit
- : pRGEN\_Mm\_Rosa26-U6\_SG(2ug)
- pRGEN\_Cas9(CMV)(5ug)
- Rosa26 T7E1 primer(100pM, 35ul)
- Rosa26\_donor vector(2ug)

### Cj Cas9 Plasmid

#### Cat No# TGEN\_Cj\_OP2

- Product : pRGEN\_CjCas9\_CMV(5ug)
- Service Period : 2~3 weeks
- Warranty Period : 4 months

#### Cat No# TGEN\_Cj\_S1

- Product : CjdRGEN(sgRNA plasmid, 1~2ug)+ pRGEN\_CjCas9\_CMV(5ug)
- Service Period : 2~3 weeks
- Warranty Period : 4 months

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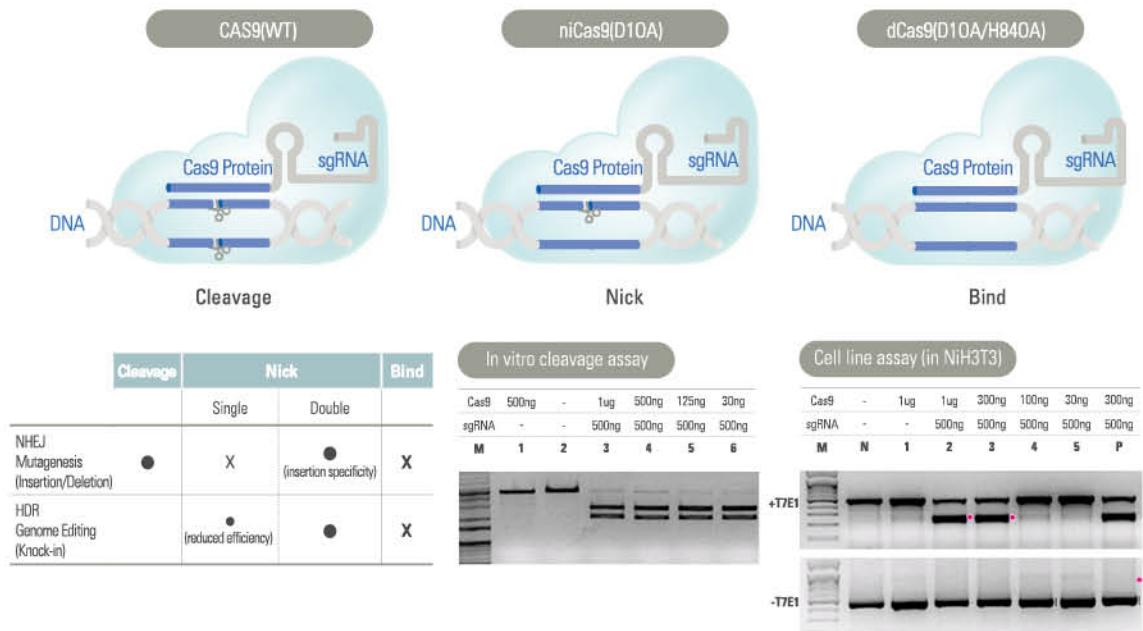
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## Recombinant Cas9 protein (*S.pyogenes*)

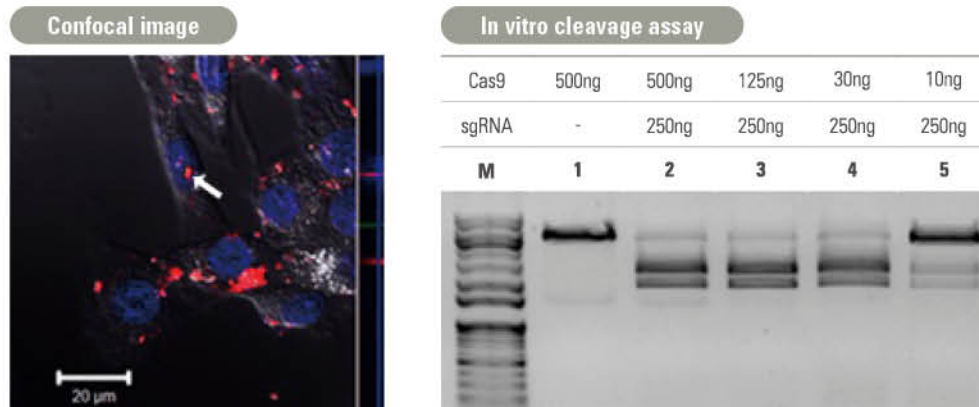
Recombinant Cas9 proteins (~160 KD) are ready-to-use reagents for your genome engineering experiments. Together with custom-designed guide RNA, Cas9 proteins will form ready-to-inject reagents suitable for an accelerated development of gene-modified animals via one-cell embryos injection. Additionally, they can also be used to cut directly the target sequence *in vitro*.

Cas9 variants for various genome manipulation are available as Cas9 (WT) for cleavage, niCas9 (D10A) for nicking and dCas9 (D10A/H840A) for simple binding. Usage of cas9 variants for genome manipulation is summarized as below.



## Cy3 Cas9 protein SERVICE (TGEN\_Cy3P1/2)

In the Cy3 Cas9 protein, cysteines residues are coupled to a Cy3 dye molecule. This modification allows easy identification of the Cas9 protein after delivery to the nucleus.





## Ordering Information

### Cas9 mRNA

#### Cat No# TGEN\_CR1

- Product : Cas9 mRNA, lyophilized, 10ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CNR1

- Product : nickase(D10A) Cas9 mRNA, lyophilized, 10ug
- Service Period : 2 week
- Warranty period : 4 month

### Cas9 Protein

#### Cat No# TGEN\_CP1

- Product : Cas9 WT protein, Injection ready, lyophilized, 50ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CP2

- Product : Cas9 WT protein, Injection ready, lyophilized, 100ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CP3

- Product : Cas9 WT protein, Injection ready, lyophilized, 250ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CP4

- Product : Cas9 WT protein, Injection ready, lyophilized, 500ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_Cy3P1/2

Cy3 Cas9 protein, solution, 25µg/50ug

### Cas9 nickase(D10A) Protein

#### Cat No# TGEN\_CNP1

- Product : nickase(D10A) Cas9 protein, Injection ready, lyophilized, 50ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CNP2

- Product : nickase(D10A) Cas9 protein, Injection ready, lyophilized, 100ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CNP3

- Product : nickase(D10A) Cas9 protein, Injection ready, lyophilized, 250ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CNP4

- Product : nickase(D10A) Cas9 protein, Injection ready, lyophilized, 500ug
- Service Period : 2 week
- Warranty period : 4 month

### Cas9 dead(D10A/840A) Protein

#### Cat No# TGEN\_CDP1

- Product : dead(D10A/840A) Cas9 protein, Injection ready, lyophilized, 50ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CDP2

- Product : dead(D10A/840A) Cas9 protein, Injection ready, lyophilized, 100ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CDP3

- Product : dead(D10A/840A) Cas9 protein, Injection ready, lyophilized, 250ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_CDP4

- Product : dead(D10A/840A) Cas9 protein, Injection ready, lyophilized, 500ug
- Service Period : 2 week
- Warranty period : 4 month

#### Cat No# TGEN\_Cy3DP2/4

Cy3 Cas9 protein, solution, 50ug/250ug

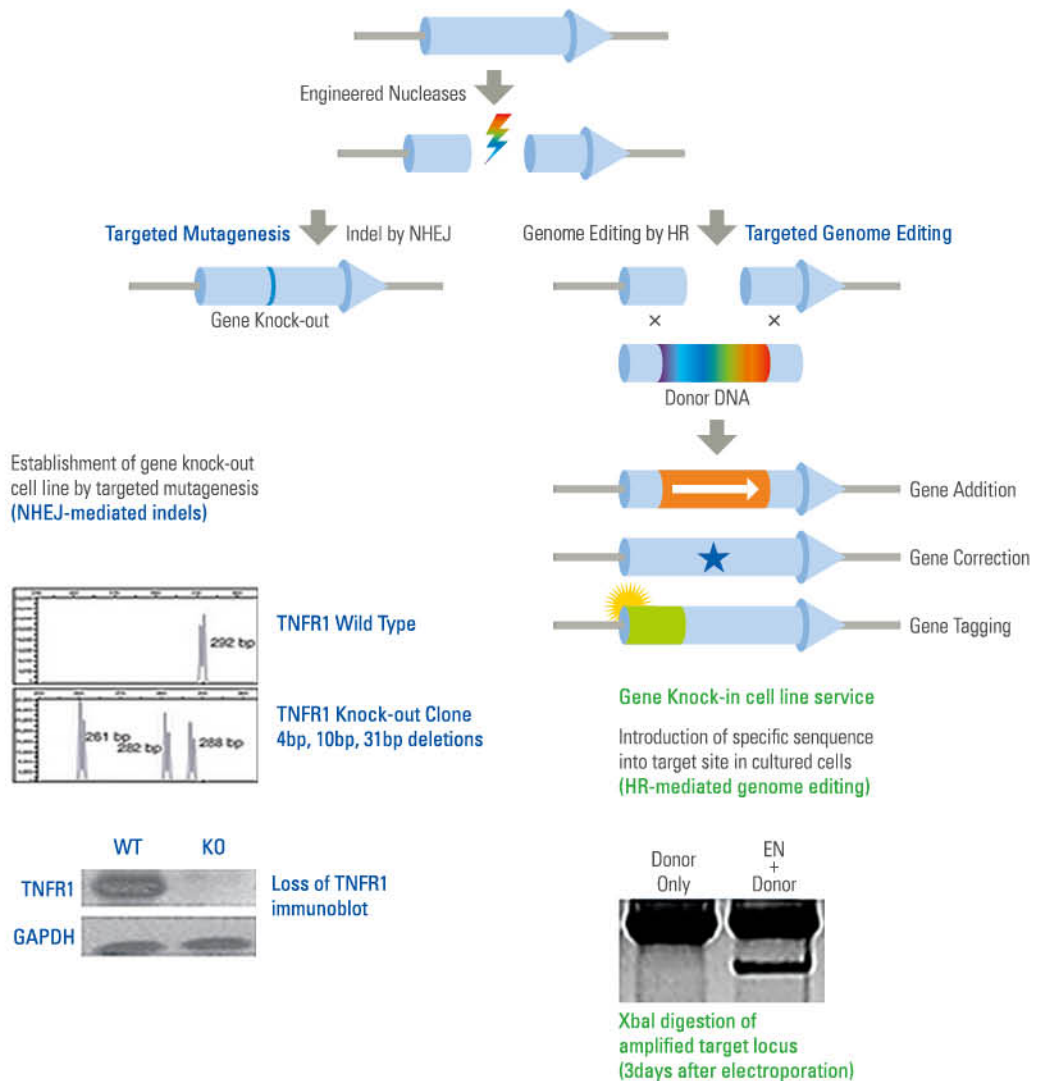
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## Genome-edited Cell Line Service

Engineered nucleases enable efficient and specific modification of a target locus in cells. Utilizing the engineered nuclease development and application technology, ToolGen provides a comprehensive service to establish cell lines with specific genome engineering events at your target gene/locus.



We can provide service on cell line as followings

HCT116, Hep3b, HEK293, HEK293T, HeLa, SNU-423, Huh7, MNT-1, PAN02, 22RV1, RAW(mouse)  
MDA-MB-231, kind of cancer cell line.

## Ordering Information

### Custom KO cell

#### Cat No# TGEN\_CK01

- Product : Hyperploidy available Commonly-used cell line (HeLa, HCT116 recommended, MCF7, HEK293, et al)
- Service Period : 3-6 month
- Warranty period : 1 Year

### Custom KI cell

#### Cat No# TGEN\_CK11

- Product : point mutation, gene insertion  
Specific insertion of reporter gene into target locus (included price of Donor DNA design and synthesis)
- Service Period : 3-6 month
- Warranty period : 1 Year

### Option

#### Cat No# TGEN\_aRSP3

- Product : Toolgen Cell line option service (If allele is more than 3)

#### Cat No# TGEN\_CPP2

- Product : Park2 hetero knockout (+/-) pig fibroblast cell line

### Donor Service

#### Cat No# TGEN\_SSD1

- Single Strand Donor(100uM. 40ul)

#### Cat No# TGEN\_DSD2

- Double Strand Donor(5ug)  
(between 2kb and 3kb, additional charge when more than 3kb)

#### Cat No# TGEN\_DSD1

- Double Strand Donor(5ug) (less than 2kb)

#### T7E1 enzyme

#### Cat No# TGEN\_T7E1

- T7E1(200rxn)\_1ea
- 10X T7E1 buffer\_1ea
- Control product

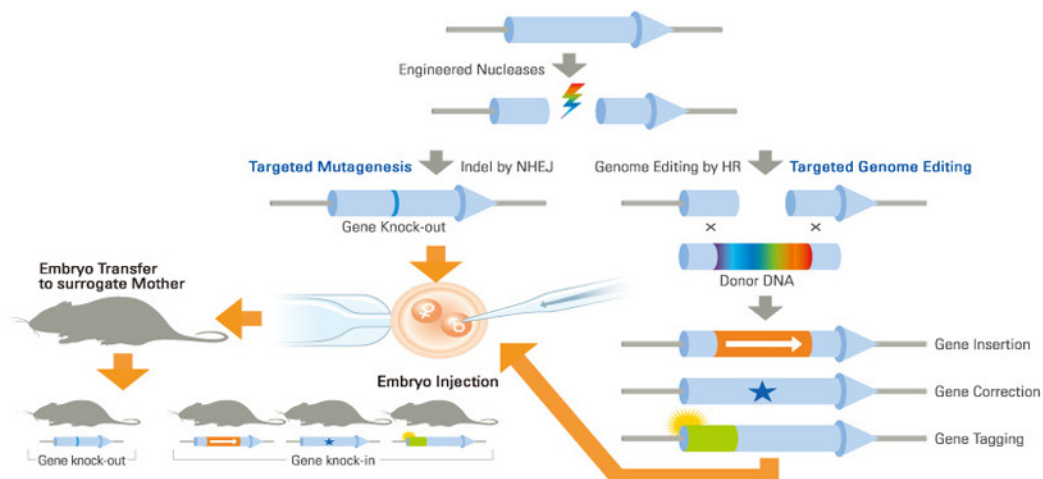
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## Genome-edited Mouse Service

Engineered nucleases enable efficient and specific modification of a target gene/locus in Mouse. Utilizing the engineered nuclease development and application technology, ToolGen provides a comprehensive service to establish Mouse with specific genome engineering events at your target gene/locus.



## Ordering Information

### KO Mouse

Cat No# TGEN\_MK01

- Product : • F0 mutant mouse with >10% mutation rate • C57BL/6N strain is used for KO (Option : Sperm freezing & embryo freezing )
- Service Period : 4 Month - Warranty period : 6 Month

### KI Mouse

Cat No# TGEN\_MK11

- Product : • F0 mutant mouse with >10% mutation rate • C57BL/6N strain is used for KI (Option : Sperm freezing & embryo freezing ) (If Donor cloning is necessary, price will be calculated based on size)
- 1kb is 200,000 KRW - Service Period : 8-12 Month - Warranty period : 6 Month

### Conditional KO Mouse

Cat No# TGEN\_MCK01

- Product : • F0 mutant mouse with >10% mutation rate • C57BL/6N strain is used for KO (Option : Sperm freezing & embryo freezing )
- Service Period : 8-12 Month - Warranty period : 6 Month

### KO Rat

Cat No# TGEN\_RK01

- Product : F0 mutant, (Option : Sperm & Embryo freezing)
- Strain : Sprague dawley(possible with other strain)
- Service Period : 4-6 months
- Warranty Period : 6 month

## Surrogate Reporter Synthesis(Plasmid)

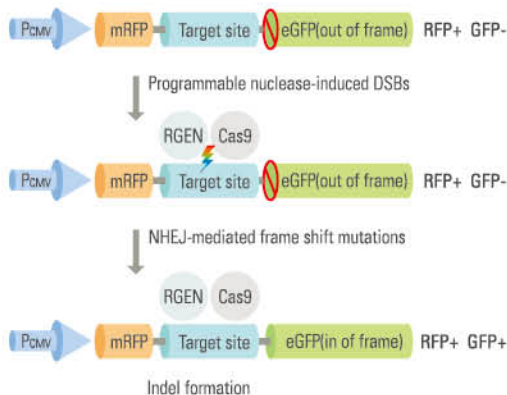
Our engineered nuclease surrogate reporters are very useful tools for observing and tracking the activity of engineered nucleases in cells as well as for enriching cells that express such nucleases.

In all cases, a constitutively expressed RFP gene functions as an indicator of DNA delivery. An EGFP expression, which can be possible by the action of functional engineered nucleases, indicates the presence of engineered nuclease activity. EGFP expression can easily be observed by fluorescence microscopy or flow cytometry. Furthermore, those cells expressing EGFP can be isolated using FACS to enrich cells with high engineered nuclease activity; such cells also contain the desired modification at the target locus with greater frequency.

In one case, the expression of a cell-surface protein allows selection by magnetic-activated cell sorting; in the other case, expression of an antibiotics-resistance gene allows selection by transient exposure to antibiotics. These two selection methods are gentler and more accessible than FACS.

### Principle

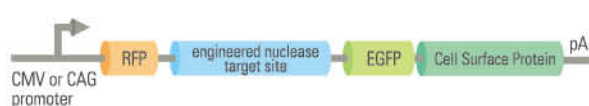
Reporter gene expression (GFP, Cell surface marker) is recovered by frameshift mutation induced by engineered nucleases



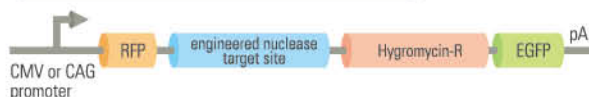
### Fluorescence Surrogate Reporter (pRG2S)



### Cell Surface Surrogate Reporter (pMRS)



### Hygromycin Surrogate Reporter (pHRS)



## Ordering Information

### Custom Reporter Synthesis

#### Cat No# TGEN\_dRRR1

- Product : ToolGen dRGEN RG2S (RFP-GFP-GFP, Kan-R)
- Service Period : 2~3 week      – Warranty period : 4 month

#### Cat No# TGEN\_dRRM1

- Product : ToolGen dRGEN MACS RS (RFP-GFP-H2KK, Kan-R)
- Service Period : 2~3 week      – Warranty period : 4 month

#### Cat No# TGEN\_dRRH1

- Product : ToolGen dRGEN Hygro RS (RFP-GFP, Hygro-R, Kan-R)
- Service Period : 2~3 week      – Warranty period : 4 month

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## In-Del Analysis Services

Targeted DNA resequencing is a high-resolution method of focused DNA sequencing.

It is characterized by amplification of DNA that is bent after being fixed on a plate rather than DNA amplification in solution, as is done for other DNA sequencing techniques. Because targeted resequencing methods specifically focus on the sequencing reads on a small panel of genes, a modestly sized sequencing experiment can achieve high sequencing coverage across many samples.

We can provide FASTQ files obtained through the analysis process.



Total Joined Sequences	With both primers	More than minimum frequency	Insertions	Deletions	Indel ratio
67069	5857	5596	0	4689	83.8%

```

AAATGTTTCAGAGAACAACCTACCAGC-----AGTGGCGTGGGCCTGCTTTCCCGCTAG
AAATGTTTCAGAGAACAACCTACCAGC-----TACTGGTCAGCTCCTTCGGACAAGCCAGTGGCGTGGGCCTGCTTTCCCGCTAG
AAATGTTTCAGAGAACAACCTACCAGC----CATACTGGTCAGCTCCTTCGGACAAGCCAGTGGCGTGGGCCTGCTTTCCCGCTAG
    
```

STEP0.	STEP1.	STEP2.	STEP3.	STEP4.	STEP5.
	Full service			Only running service	
Order	1st PCR	2nd PCR	3rd PCR & Purification	Miseq. Running	Analysis
	Library 제작				

## Ordering Information

### NGS

#### TGEN\_NGS1

Targeted Resequencing (using MiSeq) / 1 sample < X < 90 sample

#### TGEN\_NGS2

Requesting the Only MiSeq running.

## ToolGen's Products & Services

	Product / Service Name	Cat No#
CRISPR sgRNA Service (Active RGENs, aRGEN)	aRGEN Synthesis	• TGEN_ RS1
	aRGEN Validation	• TGEN_aRV1 • TGEN_aRV2
	Positive Control (aRGEN Synthesis)	• TGEN_aRSP1 • TGEN_aRSP2 • TGEN_aRSP3
CRISPR plasmid(DNA-directed RGENs, dRGEN)	dRGEN Synthesis	• TGEN_dRS1 • TGEN_dRS2 • TGEN_dRS3 • TGEN_dRS4
	Pre-Designed dRGEN Synthesis	• TGEN_dRPS1 • TGEN_dRPS2 • TGEN_OP1
	dRGEN Validation	• TGEN_dRV1 • TGEN_dRV2
	Kit Service (dRGEN Targeted Integration Kit - AAVS1)	• TGEN_AAVS1 • TGEN_Rosa26
	Cj Cas9 Plasmid	• TGEN_Cj_OP2 • TGEN_Cj_S1
Recombinant Cas9 protein (S.pyogenes)	Cas9 mRNA	• TGEN_CR1 • TGEN_CNR1
	Cas9 Protein	• TGEN_CP1 • TGEN_CP2 • TGEN_CP3 • TGEN_CP4 • TGEN_Cy3P1/2
	Cas9 nickase(D10A) Protein	• TGEN_CNP1 • TGEN_CNP2 • TGEN_CNP3 • TGEN_CNP4
	Cas9 dead(D10A/840A) Protein	• TGEN_CDP1 • TGEN_CDP2 • TGEN_CDP3 • TGEN_CDP4 • TGEN_Cy3DP2/4
Genome-edited Cell Line Service	Custom KO cell	• TGEN_CK01
	Custom KI cell	• TGEN_CK11
	Option	• TGEN_aRSP3 • TGEN_CPP2
	Donor Service	• TGEN_SSD1 • TGEN_DSD1 • TGEN_DSD2 • TGEN_T7E1
Genome-edited Mouse Service	KO Mouse	• TGEN_MK01
	KI Mouse	• TGEN_MK11
	Conditional KO Mouse	• TGEN_MCK01
	KO Rat	• TGEN_RK01
Surrogate Reporter Synthesis(Plasmid)	Custom Reporter Synthesis	• TGEN_dRRR1 • TGEN_dRRM1 • TGEN_dRRH1
In-Del Analysis Services	NGS	• TGEN_NGS1 • TGEN_NGS2

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Fax: +886-2-26963689  
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Web: http://www.toolsbiotech.com

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