Genome Editing Solutions

Precision genome modification

Genome-TALER™ custom TALEN and TAL effector services
Genome-CRISP™ CRISPR-Cas9 products and services

Validation and more

Functional validation
Donor clone design and construction
Stable cell line development
Transgenic mouse development

Safe harbor genome integration

Human AAVS1 safe harbor gene knock-in kit
Human AAVS1 safe harbor knock-in ORF clones

GeneCopoeia™
Expressway to Discovery

Targeted genome editing at will

One of the most common approaches for analyzing gene function is to alter the sequence of a gene and monitor its effects on the organism. Genome editing is one such type of modification, in which DNA is inserted, replaced or removed from a genome by engineered nucleases. These nucleases induce double-strand breaks (DSBs) at defined sites, leading to modifications resulting from the cellular repair mechanisms of non-homologous end joining (NHEJ) and homologous recombination (HR; Figure 3). Alternatively, this technology can be adapted to target engineered transcription factors to specific sites in order to transiently stimulate or repress gene expression (Figure 2).

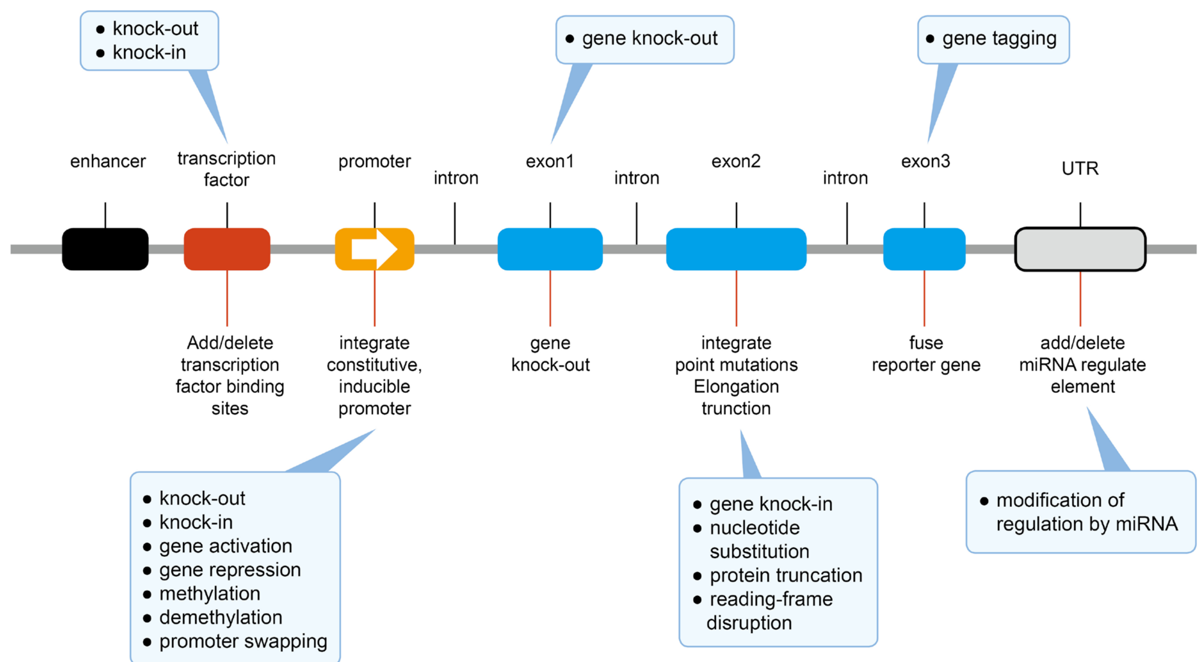


Figure 1. Applications for targeted genome editing

Mechanisms of genome editing

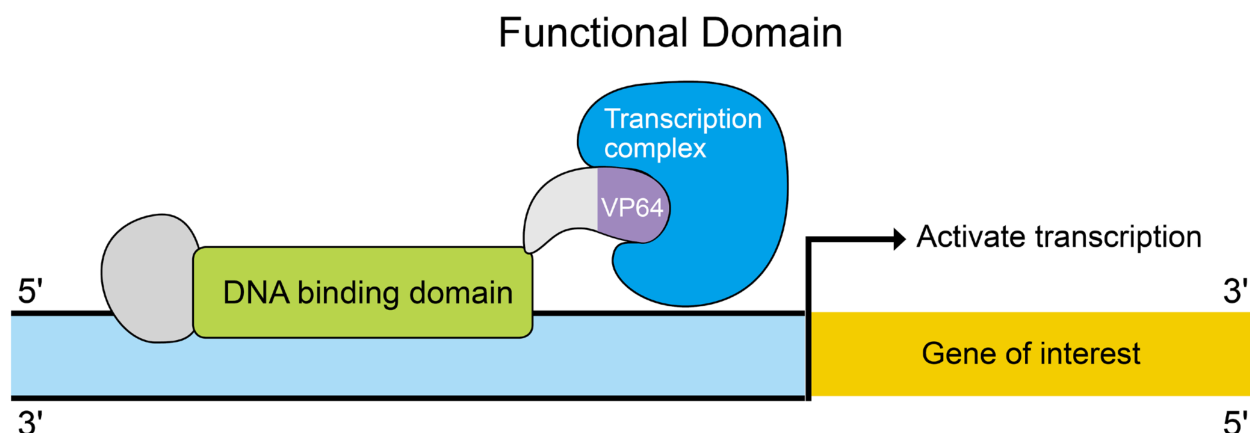


Figure 2. Gene transcription manipulation with engineered transcription factors.

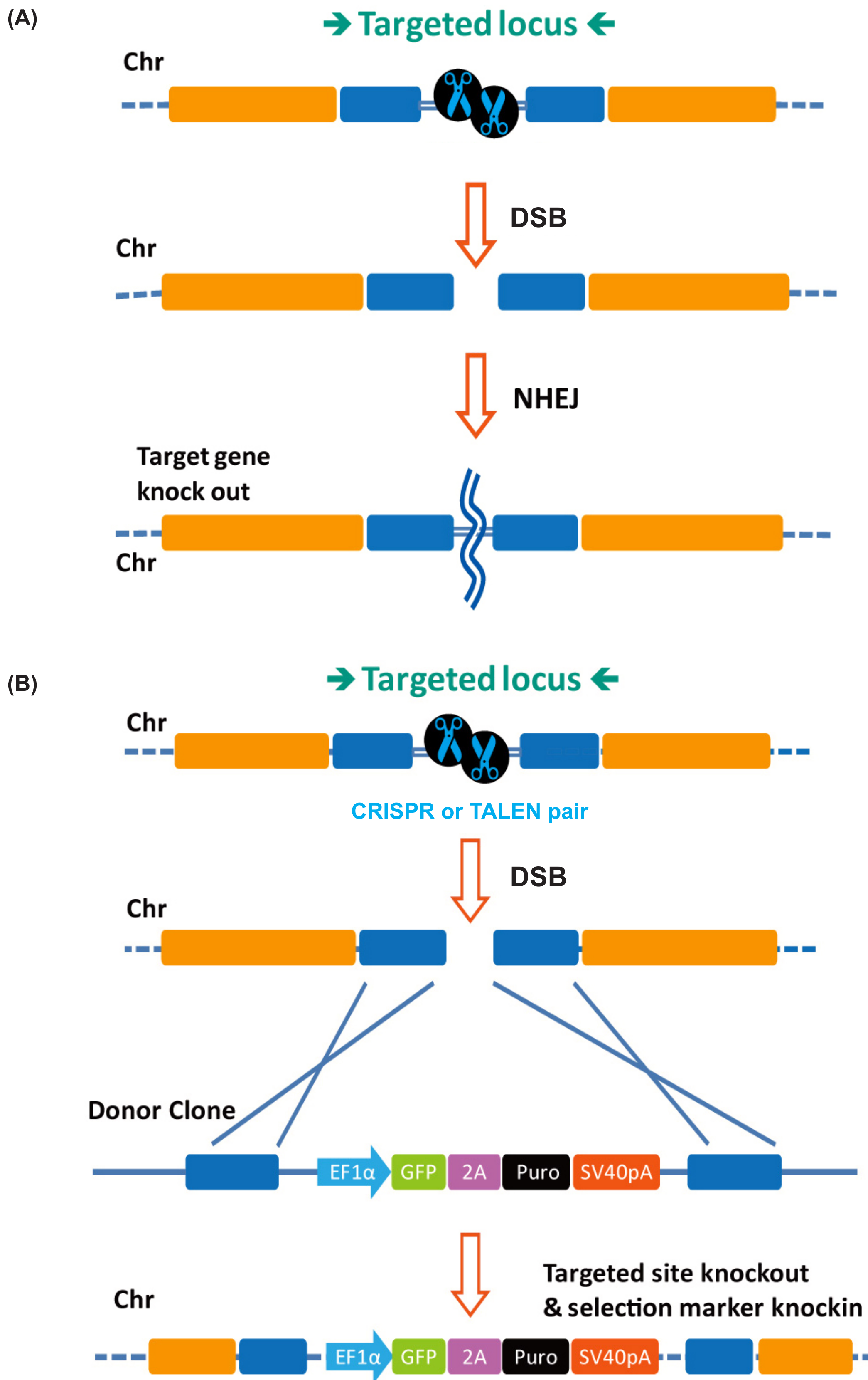


Figure 3. Genome editing with engineered nucleases. (A) DSB created by engineered nucleases are repaired by NHEJ. **(B)** DSB created by engineered nucleases are repaired by the insertion of genes of interest (GOI) & selection markers (or other genetic elements) from a donor plasmid through HR.

Product Portfolio

Genome-TALER™ TAL effectors

Product/Services	Description
TALEN	Sequence-confirmed plasmid pair expressing engineered TALE nuclease specifically targeting your genome site of interest.
TALE-TF	Sequence-confirmed plasmid expressing engineered TALE transcription activator targeting your promoter region of interest.
Validation services	Functional validation of your TAL effector.
Donor clones	Knockin desired sequences to your genomic site of interest via TALEN-mediated homologous recombination. Various vector choices with different reporter genes and selection markers.
Stable cell line services	Monoclonal stable cell lines with TALEN-mediated genome modifications. Cell banking service available.
Transgenic mouse services	Transgenic mice with TALEN-mediated genome modifications.

Genome-CRISP™ CRISPR-Cas9 system

Product /Services	Description
Cas9 nuclease expression clone	Express Cas9 nuclease to create double-strand break at your genomic site of interest in combination with sgRNA(s).
Cas9 nickase expression clone	Express engineered Cas9 nickase to create single-strand nick at your genomic site of interest in combination with sgRNA(s).
sgRNA clones	Transcribe sgRNA(s) to guide Cas9 nuclease to target sites. Various vector choices for transcribing sgRNA alone or with the Cas9 nuclease expression cassette built in.
Validation services	Functional validation of your CRISPR sgRNA(s).
Donor clones	Knockin desired sequences to your genomic site of interest via CRISPR-Cas9-mediated homologous recombination. Various vector choices with different reporter genes and selection markers.
Stable cell line services	Monoclonal stable cell lines with CRISPR-Cas9-mediated genome modifications. Cell banking service available.
Transgenic mouse services	Transgenic mice with CRISPR-Cas9-mediated genome modifications.

Genome-TALER™ human AAVS1 safe harbor

Catalog#	Product	Description
SH-AVS-K100	Human AAVS1 safe harbor gene targeting kit	Includes: AAVS1 TALEN pair (TN-AAVS1) AAVS1 donor cloning vector (DC-DON-SH01) AAVS1 positive control donor (DC-RFP-SH01) knock-in verification primer pairs (HQPAVSHR)
SH-AVS-K000	Human AAVS1 safe harbor gene targeting kit (without donor)	Includes: AAVS1 TALEN pair (TN-AAVS1) AAVS1 positive control (DC-RFP-SH01) knock-in verification primer pairs (HQPAVSHR)
Knock-in ORF Clones	Human AAVS1 knock-in ORF clone	AAVS1 knock-in ORF donor clone containing CMV-driven ORF of customer's gene of interest

Choice of service levels

Services	Engineer	Value	Essential	Premium	Project***
	2 weeks	3-5 weeks	3-5 weeks	7-8 weeks	Various
Genome editing tool design	√	√	√	√	
Clone engineering & sequencing	√	√	√	√	
Plasmid-level functional validation*		√		√	
Chromosomal-level functional validation**			√	√	
Additional or customized services					√

* Nuclease tools: surrogate reporter assay

Transcription activator: surrogate reporter transactivation assay

** Nuclease tools: mismatch detection analysis

Transcription activators: transactivation assay

***Includes donor service, stable cell line service, and transgenic mouse service

Advantages

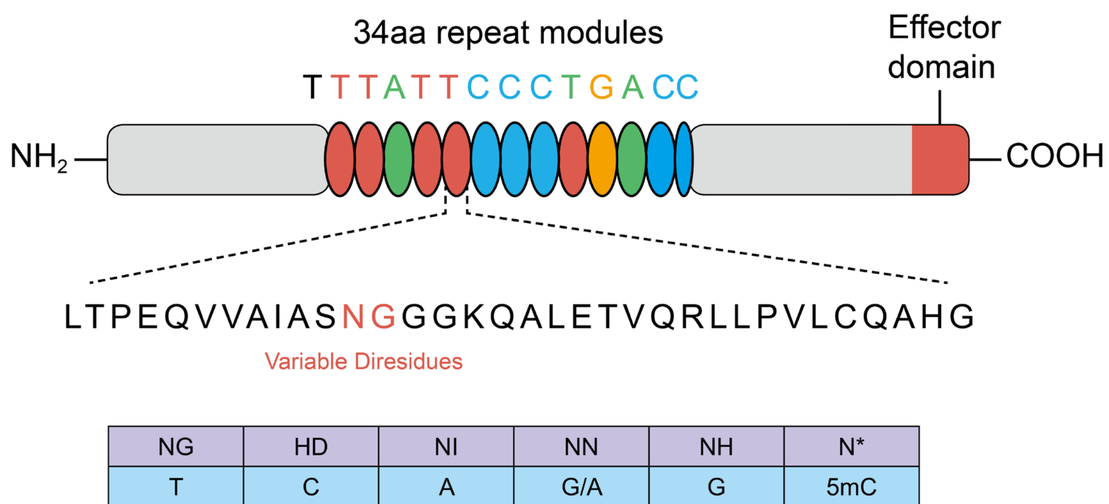
- **Complete solutions.** Genome editing tool design and construction, functional validation services, donor design and construction services, cell or animal model development services for a complete TALE or CRISPR project.
- **Sequence guarantee.** All constructs are sequence verified and guaranteed.
- **Fast delivery.** Fast delivery for both CRISPR and TAL effector constructs. We have pre-built and sequence-verified TAL effector modules for quick assembly.

Comparison between TALEN, CRISPR-Cas9 and ZFN

Property	TALEN	CRISPR-Cas9	ZFN
Type of recognition	Protein-DNA	RNA-DNA	Protein-DNA
Recognition mode	Uses a simple, virtually one-to-one code	Uses Watson-Crick base pairing	Recognizes DNA triplets with context dependence
Methylation sensitivity	Sensitive	Not sensitive	Sensitive
Chromatin structure sensitivity	Sensitive	Sensitive	Sensitive
Off-target activity	Less observed off-target activity than ZFN	More potential off-target activity than TALENs and ZFNs	More potential off-target activity than TALENs
Multiplexing	Rarely used	Capable	Rarely used

TALEN custom services

Transcription activator-like (TAL) effectors are proteins secreted by Xanthomonas bacteria when they infect plants. These proteins can activate the expression of plant genes by recognizing and binding host plant promoter sequences through a central repeat domain consisting of a variable number of ~34 amino acid repeats. The residues at the 12th and 13th positions of each repeat are hyper-variable. There appears to be a simple one-to-one code between these two critical amino acids in each repeat and each DNA base in the target sequence, e.g. NI = A, HD = C, NG = T, and NN = G or A (Figure 4). Recent work has demonstrated that the NH RVD has greater specificity and comparable affinity for G compared with NN. Therefore, the NN RVD has been replaced for G recognition by NH. GeneCopoeia also uses the N* RVD for recognition of 5-methyl cytosine.



NG	HD	NI	NN	NH	N*
T	C	A	G/A	G	5mC

Figure 4. Top: Schematic of a TAL effector. Bottom: Typically-used RVD recognition code

A TAL effector nuclease (TALEN) contains a TALE DNA binding domain fused to the FokI nuclease. Two TALENs must bind on each side of the targeted site for FokI to dimerize and generate a DSB (Figure 5). The cellular repair mechanism of non-homologous end joining (NHEJ) can then reconnect the DNA and induce insertion or deletion errors at the site of the break. Alternatively, an exogenous double-stranded donor DNA fragment can be used to repair the DSB by homologous recombination (HR). TALENs have been used to generate stably modified human embryonic stem cell and induced pluripotent stem cell (IPSCs) clones, and to generate knockout organisms such as rats, C. elegans, and zebrafish.

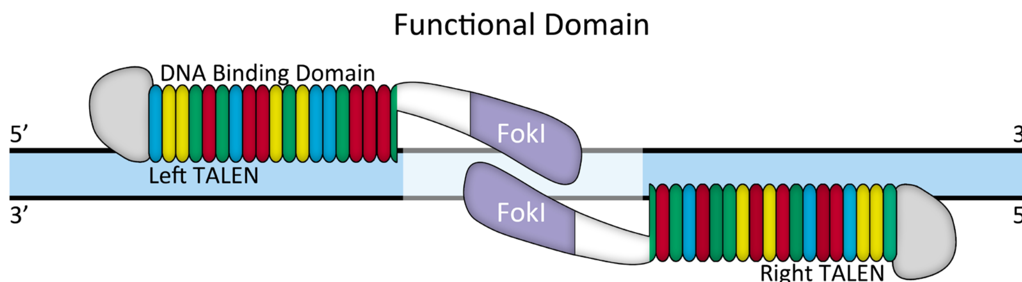


Figure 5. Typical TALEN design strategy

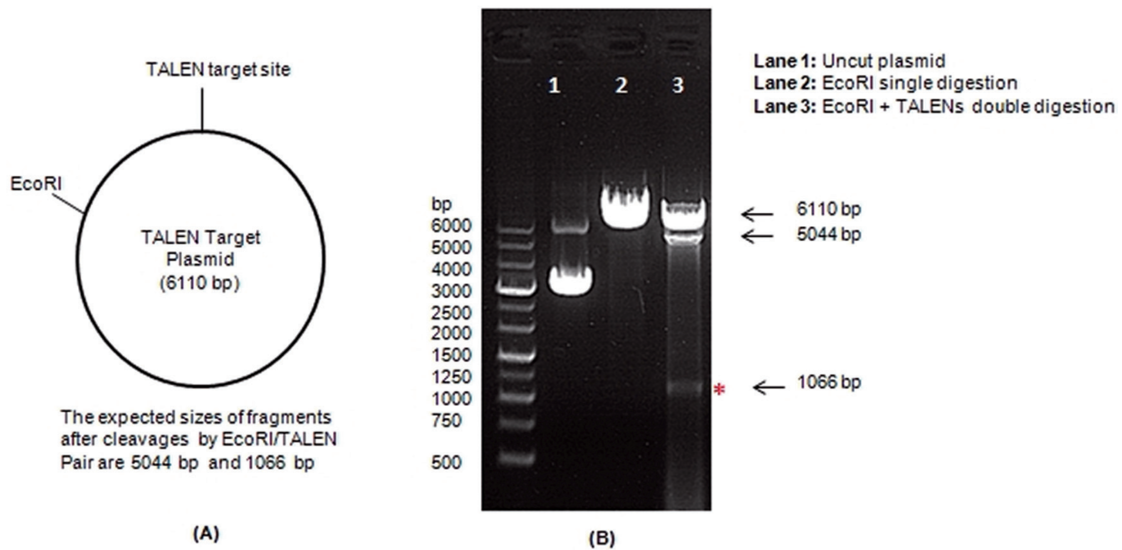


Figure 6. In vitro target DNA cleavage by EGFP-TALENs. (A) The TALEN target plasmid (6110 bp) contains a unique EcoRI site and an eGFP TALEN target site. The two sites are1066 bp apart. (B) 1 μg of the plasmid was incubated with the indicated enzymes for 30 min at 37°C. 0.5 volume of the digestion reaction was analyzed by agarose gel electrophoresis. * The indicated fragment was analyzed by PCR (data not shown)

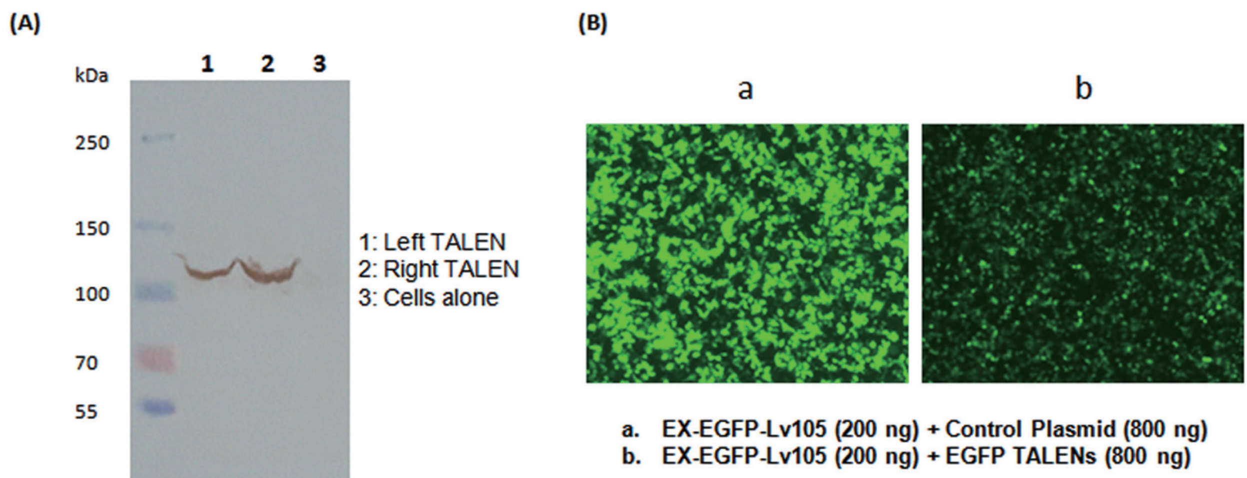


Figure 7. TALEN-mediated knockdown of eGFP expression. (A) eGFP TALENs expression validation: ~80% confluence HEK293T cells were transfected with 0.8 μg plasmid per well in a 6-well plate. The cells were harvested 48 hrs post-transfection. 1/20th of the cell lysate per well was analyzed by western blot using anti-Flag antibody in an SDS-PAGE (8%) gel, with the untransfected cell lysate as the negative control. (B) TALENs knockdown eGFP expression: HEK293T cells in a 6-well plate were co-transfected with EX-EGFP-Lv105 and TALEN plasmids or control plasmid. EGFP expression was checked under microscope (Nikon Eclipse Ti, exposure time: 600ms) 48hrs post-transfection.

TALE-TF custom services

A key application for TALEs is the targeted activation and repression of target genes in cells by fusing transactivation domains to TALE DNA binding domains (Figure 8). The TALE-TF construct is a powerful tool to selectively modulate gene expression in eukaryotic cells with exquisite specificity. The TALE-TF contains a TALE DNA binding domain fused to the VP64 transcription activator.

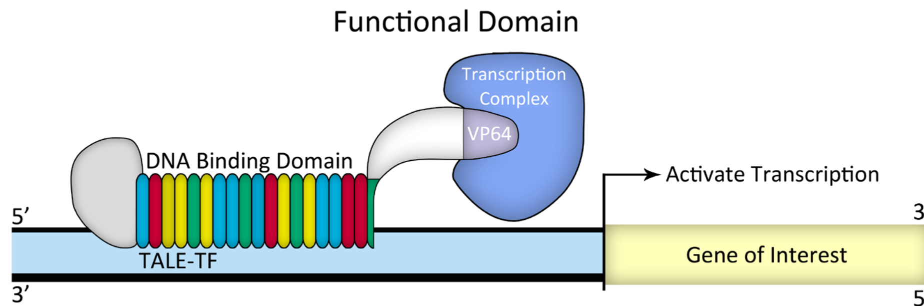


Figure 8. Typical TALE-TF design strategy

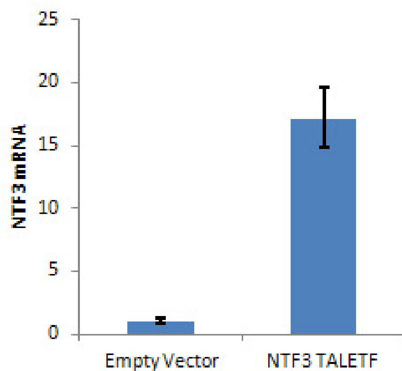


Figure 9. Endogenous NTF3 transcription activation by TALE-TF: HEK 293T cells transfected with the NTF3 TALE-TF (6 well plate, 1 μg plasmid per well) exhibited a 17-fold increase in the amount of NTF3 mRNA compared to cells transfected with an empty vector. Measurements were performed in triplicate.

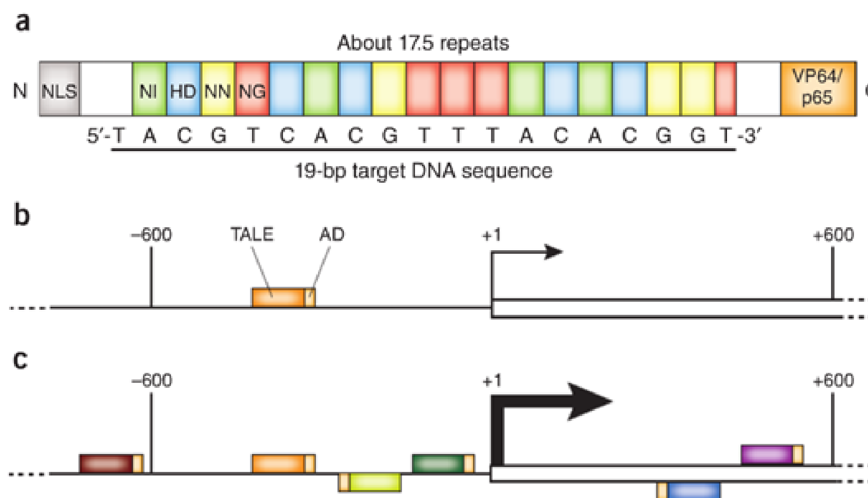


Figure 10. Synthetic TALE activators act synergistically to express human genes. (a) Cartoon of a TALE. The indicated amino acids in each repeat recognize the base below. NLS, nuclear localization signal; VP64/p65, activation domains (ADs). (b) Single TALEs induce target human genes with variable efficiencies. (c) Combinations of TALEs targeting either DNA strand allow for much higher gene induction rates. (Nature Methods. 2013 Vol. 10. No. 3: 207-208)

CRISPR-Cas9: RNA-guided genome editing

The clustered, regularly interspaced, short palindromic repeats (CRISPR)-associated protein (Cas) systems are adaptive mechanisms evolved by bacteria and archaea to repel invading viruses and plasmids. Recently, efficient genome editing by the CRISPR-Cas system has been shown in multiple organisms, including zebrafish, mice, rats, C. elegans, plants, and bacteria. Several groups have demonstrated that compared with zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR-Cas–mediated gene targeting has similar or greater efficiency in cells and zebrafish.

In the CRISPR-Cas9 systems, the complex of a CRISPR RNA (crRNA) annealed to a trans-activating crRNA (tracrRNA) is sufficient to guide the Cas9 endonuclease to a specific genomic sequence to generate DSBs in target DNA. This system can be simplified by fusing crRNA and tracrRNA sequences to produce a synthetic chimeric single-guided RNA (sgRNA). The selected target sequence consists of a 20bp DNA sequence complementary to the crRNA or the chimeric sgRNA, followed by the trinucleotide (5'-NGG-3') protospacer adjacent motif (PAM), which is recognized by Cas9 itself and is essential for cleavage (Figure 10).

This RNA-guided DNA recognition mechanism of CRISPR-Cas9 provides a simple but powerful tool for selected genome engineering. One of the most important advantages of CRISPR-Cas systems is that the Cas9 protein can be guided by individual sgRNAs to modify multiple genomic target loci simultaneously.

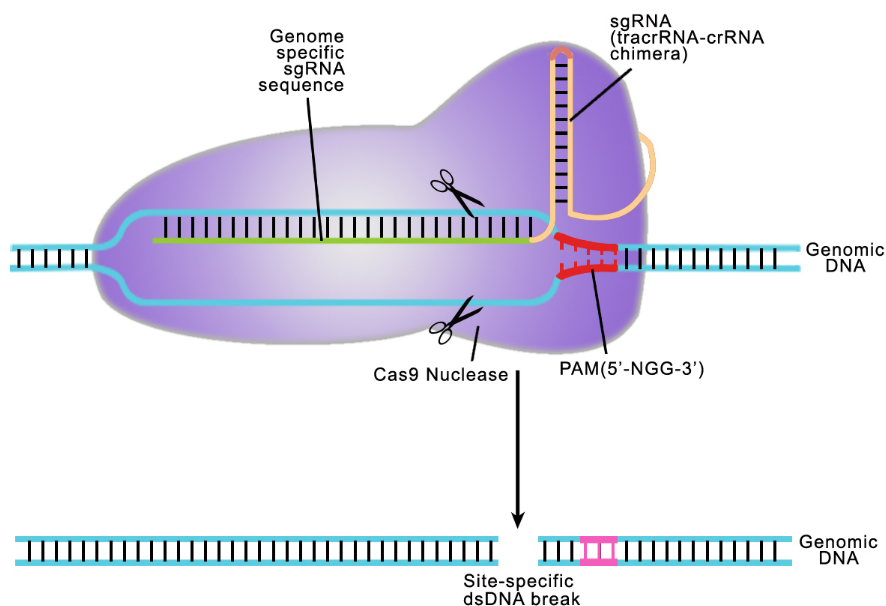


Figure 10. Illustration of CRISPR/Cas9-mediated genome editing

Cas9 expression clones

Genome-CRISP™ Cas9 Nuclease Expression Clone

A Cas9 nuclease expression clone is a premade clone containing the sequence of engineered Cas9 nuclease. In the presence of crRNA and tracrRNA (or chimeric sgRNA), Cas9 nuclease can be guided to induce site-specific DSBs in the host genome, which stimulates the cellular repair mechanism for further modification. (Figure 11)

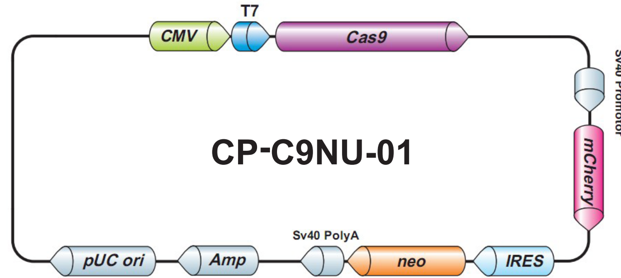


Figure 11. Map of Cas9 Nuclease Expression Clone

Genome-CRISP™ Cas9 Nickase Expression Clone

A Cas9 nickase expression clone is a premade clone containing the sequence of engineered Cas9 nickase (Figure12), which contains an amino acid mutation at position D10A. This mutation inactivates the nuclease catalytic activity to the complementary strand, converting a Cas9 nuclease to a “nickase” enzyme which generates a single-stranded break at the target site on the binding strand. (Figure 13)

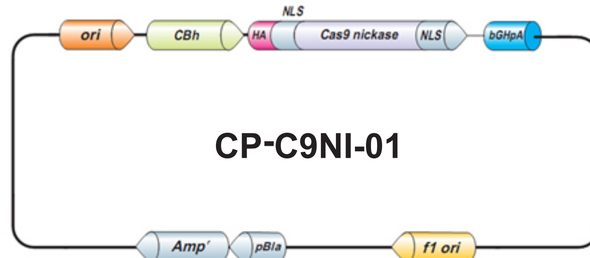


Figure 12: Map of Cas9 Nickase Expression Clone (CP-CPNI-01)

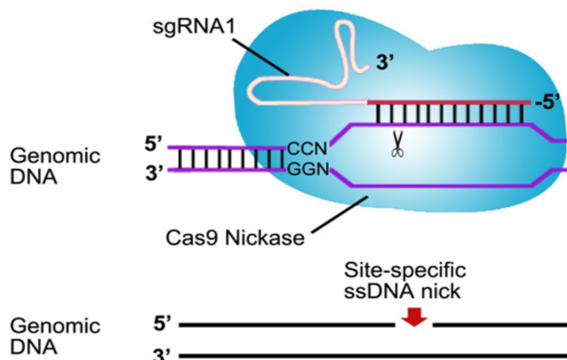


Figure 13: Illustration of Cas9 nickase generating a single-stranded break on its binding strand

Genome-CRISP™ sgRNA clones

GeneCopoeia offers single-guide RNA (sgRNA) design and cloning services for the customer's target gene of interest. sgRNA clones express a single-stranded chimeric sgRNA, consisting of crRNA and tracrRNA. In the presence of the co-transfected Cas9 endonuclease, an sgRNA can guide the Cas9 nuclease to a target site to create a DSB for genome editing applications, including gene knockout, knockin, mutagenesis, and more. Multiple sgRNA clones can be constructed and co-transfected with one Cas9 clone to enable simultaneous editing of several sites within the genome, offering greater efficiency and flexibility for the experiment design.

Vector Types

Vector	Promoter	sgRNA	Cas9 Nuclease	Selection Marker/ Reporter Gene
pCRISPR-SG01	U6	1 or multiple	Sold separately	Hygromycin
pCRISPR-CG01	U6	1 or multiple	CMV-driven Cas9 in the same vector	Neomycin / mCherry
pCRISPR-CG02	U6	1	CBh-driven Cas9 in the same vector	N/A

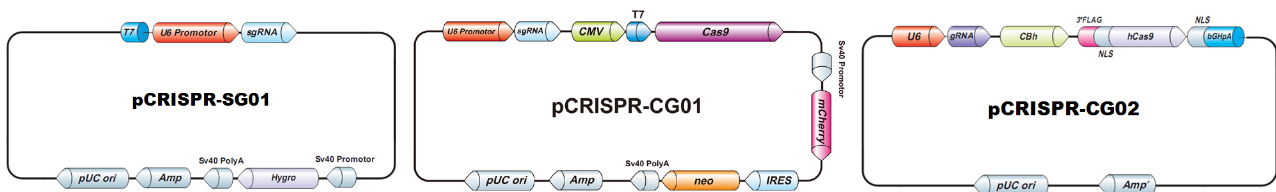


Figure 14. Maps of sgRNA vectors

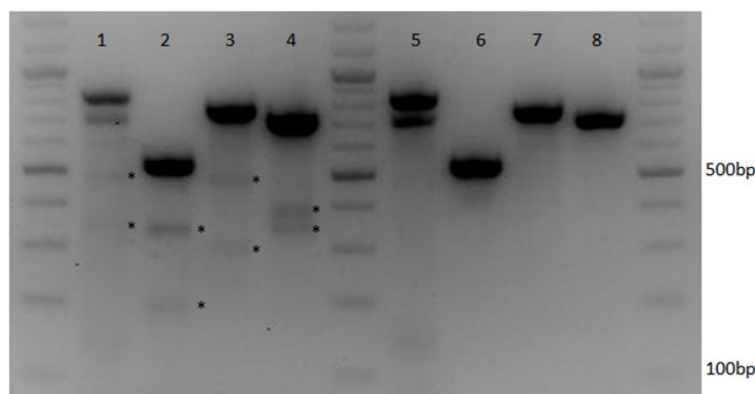


Figure 15. CRISPR-Cas9 multiplexing to target multiple genes. HEK293T GFP-stable cells were co-transfected with plasmids expressing Cas9 plus multiple sgRNAs targeting p53, HUWE1, NCL3 and GFP (Lanes 1-4) or Cas9 plus a scrambled sgRNA (Lanes 5-8). The genomic DNAs were analyzed for co-existence of indels in multiple target sites using T7 endonuclease I (ENI) assays. The * indicates that the Cas9 plus multiple sgRNAs efficiently introduced indels to each target site respectively (Lanes 1-4). PCR product sizes and T7ENI-cleaved product sizes: GFP: 720bp (intact), 340bp + 380bp (cleaved); NCL3: 765bp (intact), 295bp +470bp (cleaved); HUWE: 520bp (intact), 190bp + 330bp (cleaved); P53: 825bp (intact), 475bp + 350bp (cleaved).

Services portfolio

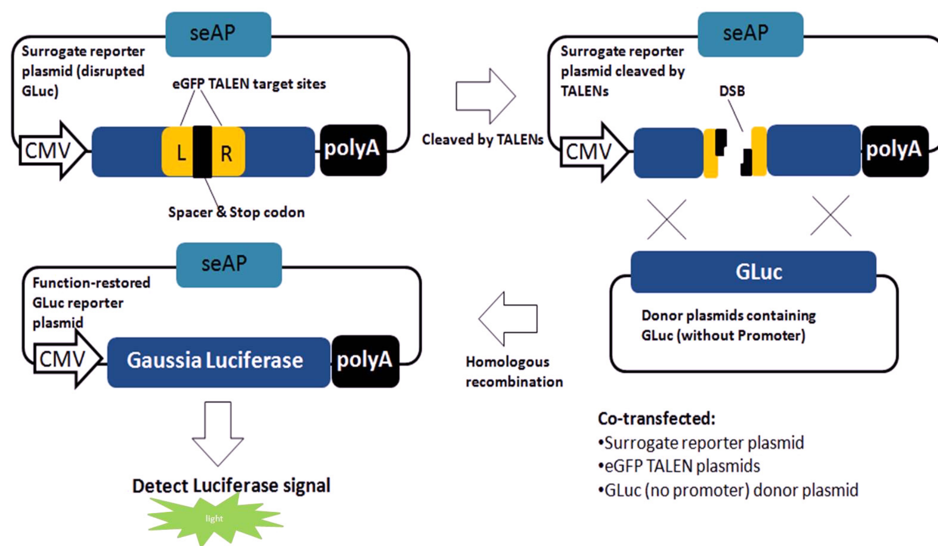
Services		Description	Application
Validation	Surrogate reporter assay	Plasmid-level functional validation. Detects activities of genome editing tools by observing the expression level of a surrogate reporter gene.	TALEN, TALE-TF, CRISPR-Cas9
	T7 endonuclease I assay	Chromosomal-level functional validation. Detects the presence of indels created by TALEN- or CRISPR-mediated NHEJ repair at the specific target site of the chromosome.	TALEN, CRISPR-Cas9
	qPCR assay	Chromosomal-level functional validation. Measures changes in expression level of the target gene induced by site-specific TALE-TF transcription activator.	TALE-TF
Donor clone services	Donor clone design and construction	Customized plasmids designed to specifically transfer your gene of interest, selection marker or other genetic elements into targeted site through homologous recombination (HR) induced by our genome editing tools. We offer various donor vector choices with different selection markers and genetic elements built in for your experiment purpose.	TALEN, CRISPR-Cas9
Stable cell line services	Monoclonal colony	Monoclonal stable cell line with TALEN- or CRISPR-Cas9-mediated genome modifications.	TALEN, CRISPR-Cas9
	Cell bank	Create cell bank of monoclonal stable cell line with TALEN or CRISPR-Cas9-mediated genome modifications.	TALEN, CRISPR-Cas9
Transgenic mouse services	Transgenic mouse	Transgenic mice with TALEN- or CRISPR-Cas9-mediated genome modifications.	TALEN, CRISPR-Cas9

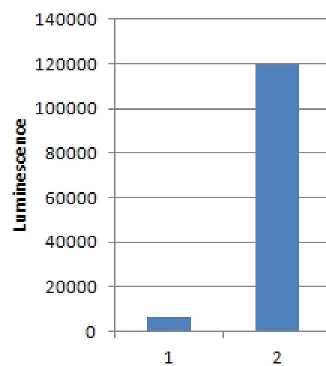
Episomal validation

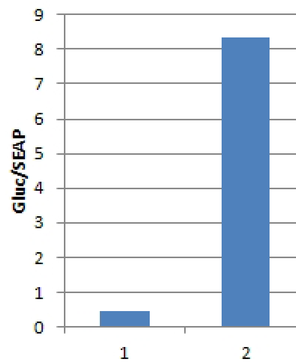
The surrogate reporter assay is a plasmid-level functional validation. The surrogate reporter plasmid consists of a reporter gene expression cassette and the target sequence of the genome editing tool being validated.

To validate a site-specific transactivator (e.g. TALE-TF), the promoter region of a reporter gene expression cassette is replaced with the target sequence. After co-transfection, a functional transactivator will recognize and bind to the target sequence, activating the transcription of reporter gene.

To validate a site-specific nuclease (e.g. TALEN or CRIPSR-Cas9), a surrogate reporter plasmid is constructed by disrupting the reporter gene ORF with an in-frame stop codon followed by the target sequence. A donor plasmid with a promoter-less wild type reporter gene ORF is also co-transfected. A functional site-specific nuclease will generate a double-strand break on the target sequence, stimulating homologous recombination between the surrogate reporter plasmid and donor plasmid. Thus the reporter gene ORF in the expression cassette is repaired, and up-regulation of reporter gene expression will be detected. (Figure 16)







Sample	1	2
Control TALENs	+	
eGFP-TALENs		+
Surrogate reporter*	+	+
Donor plasmid**	+	+

*The surrogate reporter plasmid was constructed by disrupting a CMV-driven Gaussia luciferase (GLuc) with an in-frame stop codon followed by eGFP TALEN target sequences.

**The donor plasmid contains a promoter-less wild type GLuc, which can replace the interrupted GLuc in the surrogate reporter plasmid and restore GLuc expression through homologous recombination, which is enhanced by TALEN cleavage.

Figure 16. TALENs enhance homologous recombination. HEK293T cells in a 6-well plate were co-transfected with the eGFP-TALEN pair (1 μg), the surrogate reporter plasmid (0.5 μg) and the donor plasmid (0.5 μg). 48hours post-transfection, the restored Gluc activity was determined to evaluate the TALEN function. Internal control SEAP activity was used for normalization.

Chromosomal validation

To validate a site-specific transactivator (e.g. TALE-TF) at the chromosomal level, qPCR primers are designed and qPCR performed post-transfection to measure the change in expression level of the target gene induced by the transactivator.

To validate a site-specific nuclease (e.g. TALEN or CRISPR-Cas9), we can use the mismatch cleavage assay to detect the presence of indels caused by NHEJ-mediated DSB repair at the specific target site of the chromosome. Genomic DNA is extracted and PCR amplified using primers specific to the target gene post-transfection. The PCR products are purified, denatured and reannealed, and then digested with a mismatch cleavage enzyme (e.g. T7 endonuclease I). The expected digestion product sizes will be detected if the site-specific nuclease is functional.

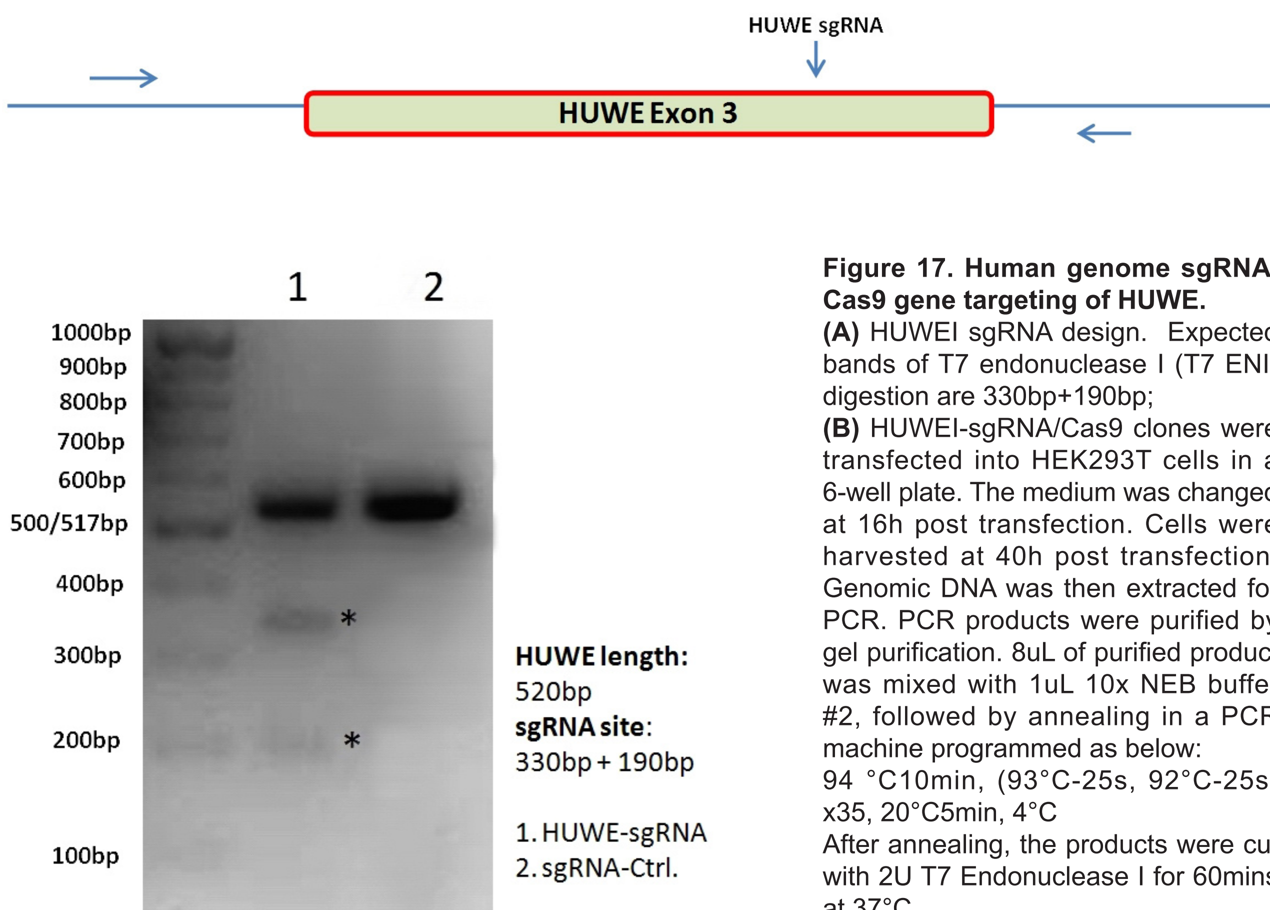


Figure 17. Human genome sgRNA/Cas9 gene targeting of HUWE.
(A) HUWEI sgRNA design. Expected bands of T7 endonuclease I (T7 ENI) digestion are 330bp+190bp;
(B) HUWEI-sgRNA/Cas9 clones were transfected into HEK293T cells in a 6-well plate. The medium was changed at 16h post transfection. Cells were harvested at 40h post transfection. Genomic DNA was then extracted for PCR. PCR products were purified by gel purification. 8uL of purified product was mixed with 1uL 10x NEB buffer #2, followed by annealing in a PCR machine programmed as below:
94 °C10min, (93°C-25s, 92°C-25s) x35, 20°C5min, 4°C
After annealing, the products were cut with 2U T7 Endonuclease I for 60mins at 37°C.

Donor services

GeneCopoeia offers customized donor clone design and construction services. Donor clones are customized plasmids designed to specifically transfer your gene of interest, selection marker or other genetic elements into a target site via HR-mediated repair of DSBs induced by site-specific genome editing tools. Donor vectors are available with several options for selection markers and genetic elements to meet your experimental needs.

Donor Vector Types

Vector	Promoter	Reporter Gene	Selection Marker
pDonor-01	EFa1	copGFP	Puromycin/TK
pDonor-02	CMV	copGFP	Neomycin/TK
pDonor-03	EFa1	N/A	Puromycin/TK
pDonor-04	CMV	N/A	Neomycin/TK

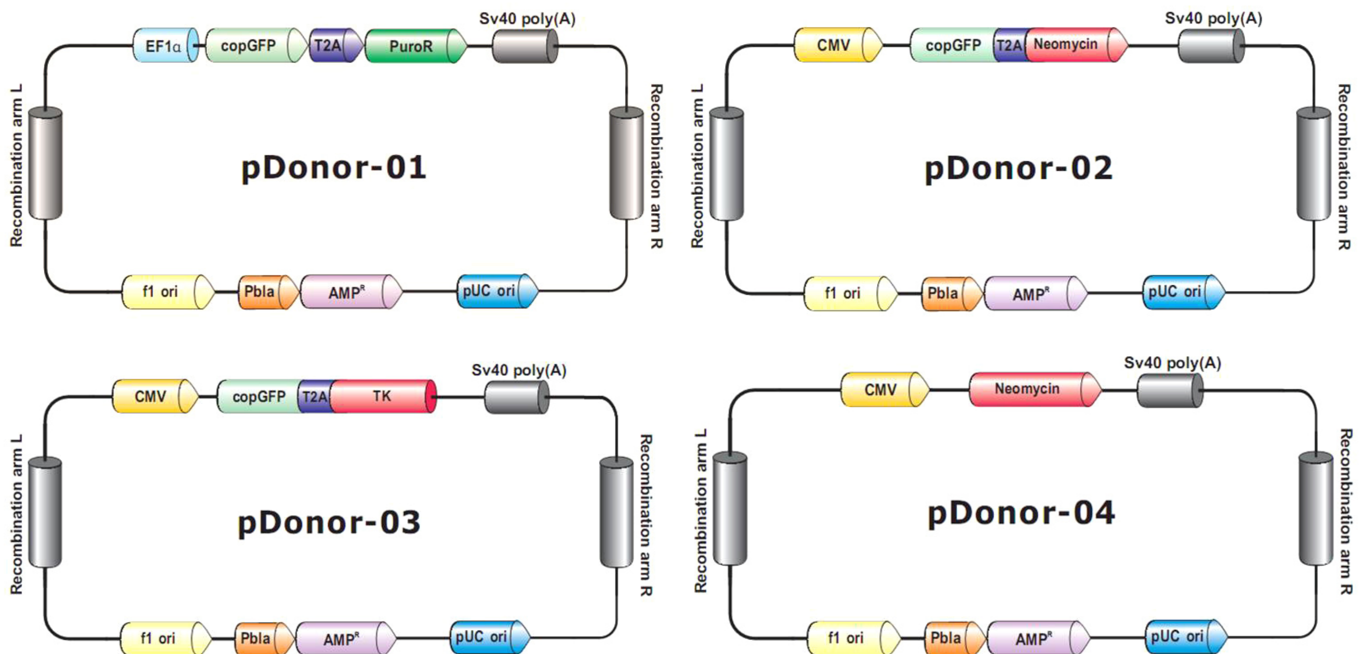
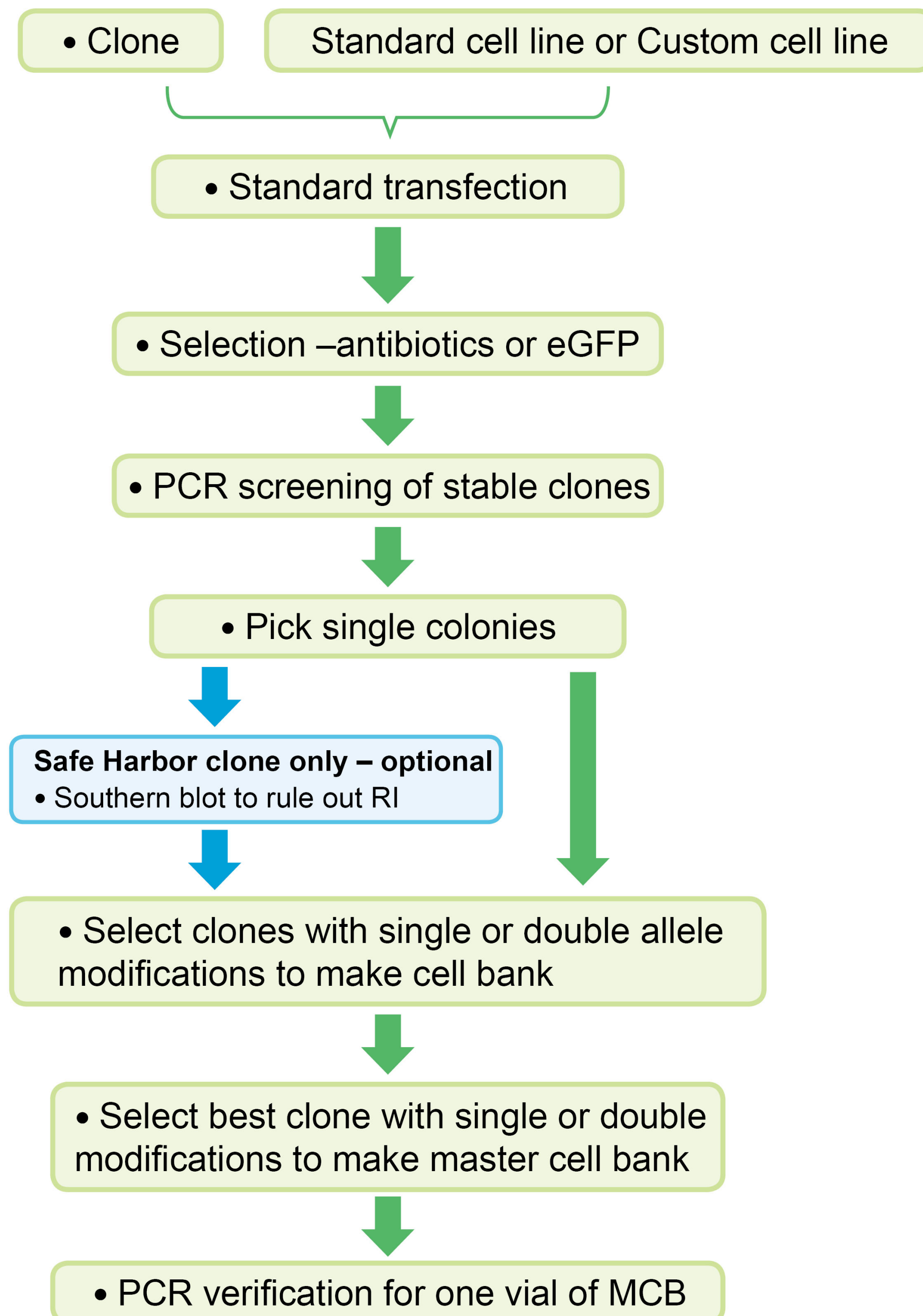


Figure 18. Maps of Donor vectors

Stable cell line services

GeneCopoeia offers monoclonal stable cell line service with customized TALEN- or CRISPR-Cas9-mediated genome modifications. Cell banking service is also available.

TALEN/CRISPR Stable Cell Line Development Services



Safe-harbor genome integration

The modification of the human genome by insertion of genes of interest and other genetic elements in unique site(s) of chromosome(s) is of great value for cell engineering. However, random integration of the transgene can present a threat of unpredicted insertion or mutagenesis. The AAVS1 (also known as PPP1R2C locus) in human chromosome 19 is a well-validated “safe harbor” for hosting DNA fragments with expected function. It has an open chromatin structure and is transcription-competent. Most importantly, there are no known adverse effects on the cell resulting from the inserted DNA fragment of interest.

Safe-harbor gene knock-in kit

The Genome-TALER™ human AAVS1 safe harbor gene knock-in kit is designed to specifically transfer your gene of interest, selection marker or other genetic elements from a donor plasmid into the AAVS1 safe harbor site on human chromosome 19 via TALEN-mediated homologous recombination (HR) for long term, stable expression.

Product name	Description	Included in (Cat#)
AAVS1 TALEN pair clones	Create DSB at the AAVS1 locus on human chromosome 19 to stimulate HR.	SH-AVS-K100, SH-AVS-K000
AAVS1 donor vector	For cloning GOI to be knocked in. Contains two AAVS1 flanking arms for HR as well as GFP and puromycin for detection and selection.	SH-AVS-K100
AAVS1 RFP control	Positive control. Contains two AAVS1 flanking arms for HR as well as RFP/GFP and puromycin for detection and selection.	SH-AVS-K100, SH-AVS-K000
5’ HR primer pair	PCR primer pair for detecting 5’ recombination site at AAVS1 locus	SH-AVS-K100, SH-AVS-K000
3’ HR primer pair	PCR primer pair for detecting 3’ recombination site at AAVS1 locus	SH-AVS-K100, SH-AVS-K000

Safe-harbor ORF knock-in clones

Human AAVS1 safe harbor ORF knock-in clones are a collection of more than 18,000 ORF knock-in donor clones constructed for specially transferring the ORFs of customers' genes of interest from an AAVS1 donor plasmid to the AAVS1 site for safe integration and single copy gene expression. These clones are compatible with the Genome-TALER™ human AAVS1 safe harbor gene knock-in kit, and gene transfer occurs via TALEN-mediated HR.

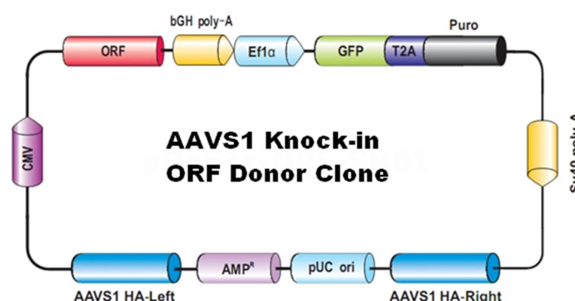


Figure19. Human AAVS1 safe harbor knockin clone

Knockin ORF clones by disease or gene families

Disease Families	ORF cDNAs
Cardiovascular diseases	1596
Congenital anomalies and genetic diseases	3978
Digestive system diseases	864
Diseases of the blood and blood-forming organs	1886
Endocrine, metabolic and nutrition diseases	1784
Immunologic diseases	3644
Infectious diseases	3536
Mental disorders	1805
Musculoskeletal system diseases	946
Neoplasms	8950
Nervous system and sense organs	2404
Respiratory system diseases	565
Urologic and genital diseases	1304
Skin and connective tissue diseases	866
Symptoms and general pathology	2022

Gene Families	ORF cDNAs
Cytokines	315
Cytokine receptors	152
Druggable target genes	6245
G protein-coupled receptors	718
Histone modification enzymes	38
Histone proteins	66
Ion channels	463
Membrane-bound proteins	2138
Nuclear hormone receptors	105
Proteases	625
Protein kinases	933
Protein phosphatases	293
Surface antigens (CD)	263
Transcription factors	1096
Organelle markers	77
Other kinases	201

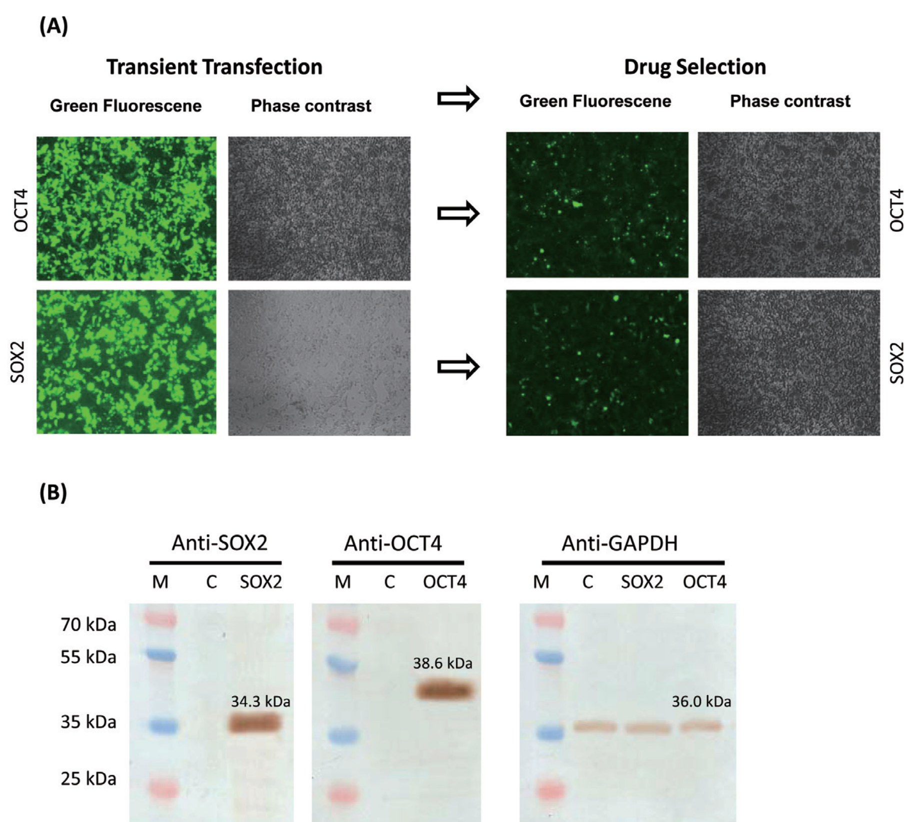


Figure 20: (A) OCT4 or SOX2 ORF knockin clones were co-transfected with the AAVS1 TALEN Pair into HEK293T cells. Cells were subcultrured for 48 hr post-transefection and selected with puromycin (1µg/ml) for 2 weeks. The expression of CopGFP was detected using a microscope (Nikon Eclipse Ti) 48h post-transfection or after 2 weeks of drug selection. (B) Western blot analysis of proteins from HEK293T cells stably integrated with SOX2 or OCT4 at the AAVS1 site, with cells alone as negative control where endogenous Sox2 or OCT4 protein levels were too low to be detected in the same blot.

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