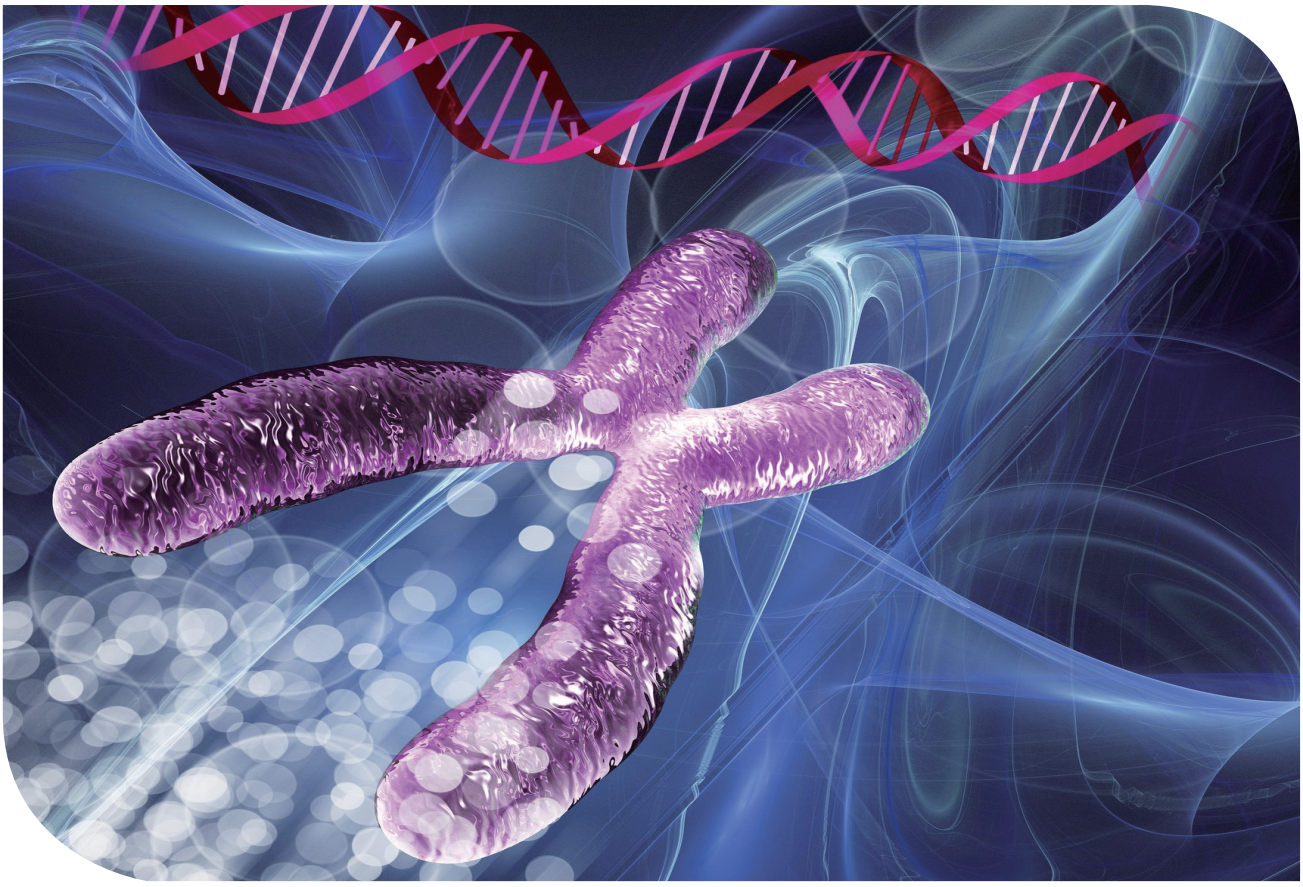
Genome Editing Tools



TAL Effector Custom Services

Design
Engineer
Validate

Safe-Harbor Genome Integration

Safe-harbor TALEN targeting kit
Donor DNA construction services

GeneCopoeia™
Expressway to Discovery

Targeted genome-editing at will

TAL (transcription activator-like) effectors are proteins secreted by Xanthomonas bacteria when they infect plants. These proteins can activate the expression of plant genes that aid bacterial infection 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.

This characteristic of TAL effectors has been utilized to create targeted gene-editing tools by fusing engineered TAL effectors (target sequence specific) to nucleases(TALENs), transcription factors (TALE-TFs) and other functional domains. These fusion proteins can recognize and bind chromosome target sequences specifically and execute their gene-editing functions, such as gene knockout, knockin (with donor plasmid), modification, activation, repression and more. Unlike zinc fingers' nucleotide triplet recognition, TAL effector domains recognize single nucleotides, which allows researchers to be able to target whatever sequence they want. Also, the context dependence observed with zinc fingers has not been reported with TAL effectors. This allows TAL effectors to work well out of the box instead of requiring time-consuming and costly optimization to overcome context dependence as zinc fingers do.

Why TAL effector

• **Flexibility**	Unlike zinc fingers (ZF) which recognize a nucleotide triplet, TAL effector domains recognize single nucleotides. This means at least in theory, any gene any sequence can be targeted. ZFNs, however, are much less flexible. Even with hundreds of ZF modules, not every possible sequence is represented, which brings a design challenge.
• **Target specificity**	TAL effector domains do not have the well-known context dependence problem that exist in zinc fingers. The interaction between the fingers reduce their binding specificity and considerable optimization is required to achieve specific targeting.
• **Cost**	Compared to the technical challenges of zinc finger design and optimization, the TAL effector design and engineering are much simpler, which translate into significant cost savings.

Applications

Custom-designed TAL effectors can recognize and bind chromosome target sequences specifically and execute their gene-editing functions, such as:

- Gene knockout
- Gene knockin (with donor plasmid)
- Gene modification (mutagenesis, etc.)
- Gene activation
- Gene repression
- And more

For example, 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.

Advantages of Genome-TALER™ services

- Targeting any gene in any cell
- Flexible TALEN, TALE-TF and other TAL effector designs
- Choice of service level available
- Cell free or cell based functional validations

Service options

Table 1. TAL effector service packages and delivery time

TAL effector custom services	Genome-TALER Engineer™	Genome-TALER Value™	Genome-TALER Premium™	Genome-TALER Project™
	2 wks	3-5 wks	7-8 wks	Various
TAL effector design	√	√	√	
TAL effector engineering & sequencing	√	√	√	
Episomal (plasmid) level validation*		√	√	
Chromosomal level validation**			√	
Additional and customized services				√

* TALEN: in vitro cleavage or surrogate reporter plasmid assay
TALE-TF: surrogate reporter transactivation assay

**TALEN: mismatch detection analysis
TALE-TF: transactivation assay

TALEN custom services

A TAL effector nuclease (TALEN) contains a TALE DNA binding domain fused to the FokI nuclease, which acts as a homodimer. For a typical application, two TALENs must bind on each side of the targeted site for FokI to dimerize and cut. TALENs induce a site-specific double-strand break (DSB), which 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 introduced into the genome at 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.

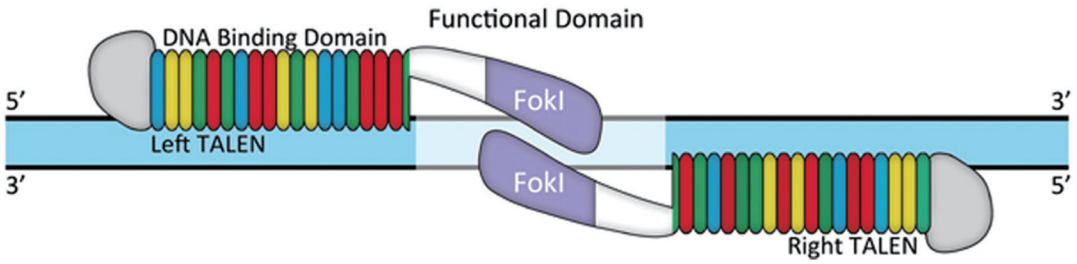


Figure 1. Illustration of TALEN design

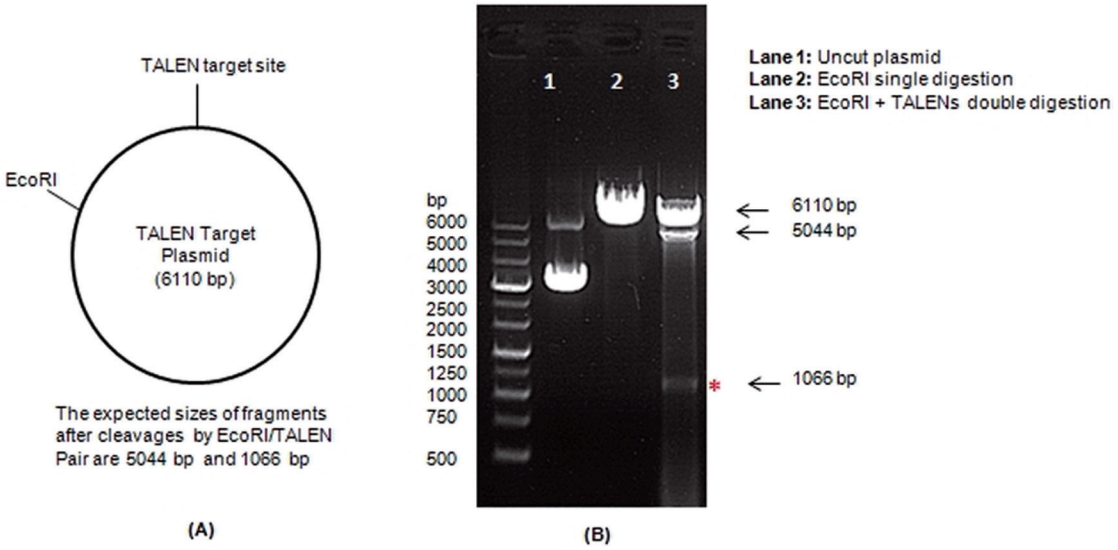


Figure 2. In vitro target DNA cleavage by EGFP-TALENs. (A) The TALEN target plasmid (6110 bp) contains an unique EcoRI site and an eGFP TALEN target site. The two sites are1066 bp apart. **(B)** 1 µg of the plasmid was incubated with the indicated enzymes for 30 min at 37°C. 0.5 volume of the digestion reaction was analyzed by the agarose gel electrophoresis. * The indicated fragment was checked by PCR, data not shown.

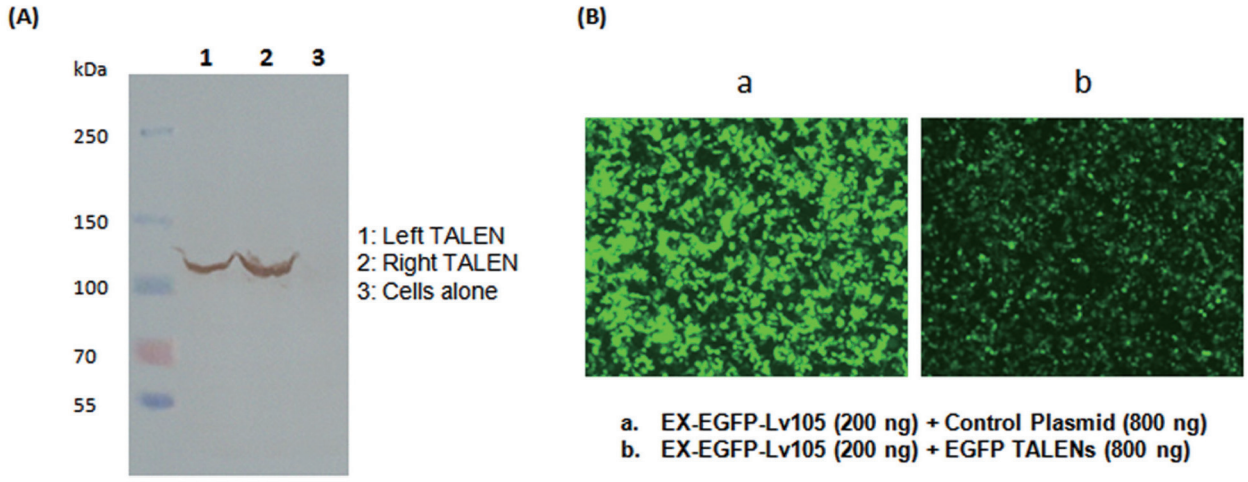
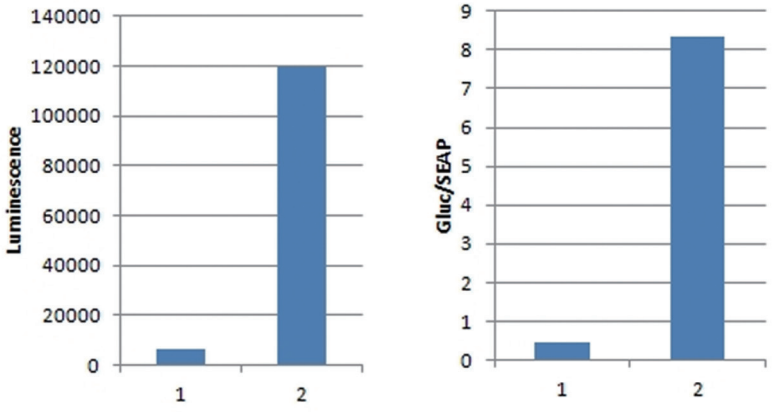


Figure 3. TALENs knockdown eGFP expression. (A) eGFP TALENs expression validation: ~80% confluence HEK293T cells were transfected with 0.8 μg plasmid per well in a 6-well plate. The cells were harvested 48 hrs post-transfection. 1/20th of the cell lysate per well was analyzed by western blot using anti-Flag antibody in a SDS-PAGE (8% resolving 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.



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

*The surrogated 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 the GLuc expression through homologous recombination, which is enhanced by TALEN cleavage.

Figure 4. 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.

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 . 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.

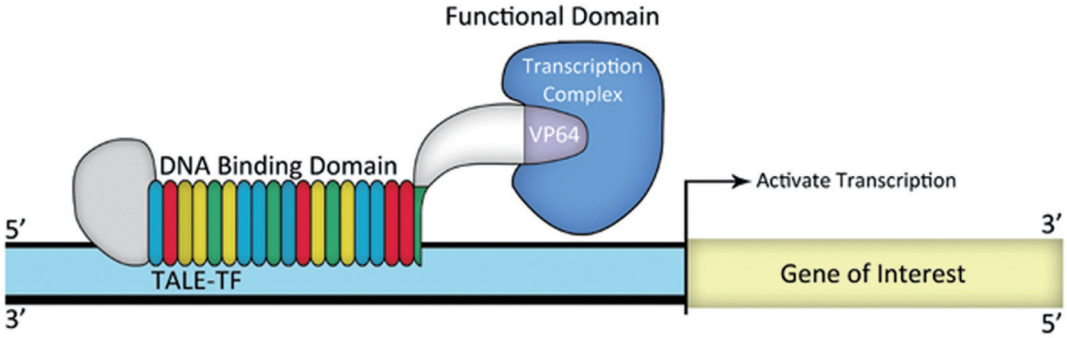


Figure 5. Illustration of TALE-TF design

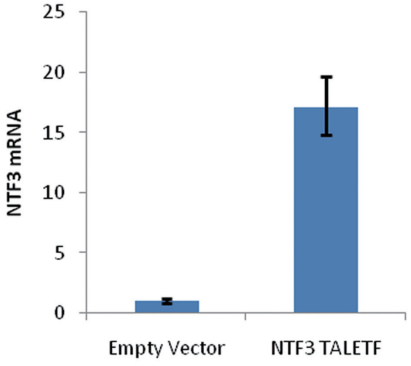


Figure 6. NTF3 TALE-TF regulates endogenous NTF3 transcription. Endogenous NTF3 transcription activation by TALE-TF: HEK 293T cells transfected with the NTF3 TALE-TF (6 well plate, 1 µg plasmid per well) exhibited a 17-fold increase in the amount of NTF3 mRNA compared to cells transfected with an empty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

TAL effector references

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3. Christian, M. et al. Targeting DNA Double-Strand Breaks with TAL Effector Nucleases. DOI: 10.1534/genetics.110.120717
4. Morbitzera, R. et al. Regulation of selected genome loci using de novo-engineered transcription activator-like effector (TALE)-type transcription factors. www.pnas.org/cgi/doi/10.1073/pnas.1013133107
5. Cermak, T. et al. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Research, 2011, Vol. 39, No. 12 e82 doi:10.1093/nar/gkr218
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Safe-harbor genome integration

The long-term stable expression of a transgene is an important application for functional genomics study. However, the random integration of viral-mediated gene transfer sometimes presents a threat of unpredicted insertion or mutagenesis, which can cause gene silencing and tumor development. Designated AAVS1 site (also known as PPP1R2C locus) in human chromosome 19 is a well-validated "safe-harbor" in the human genome. AAVS1 has an open chromatin structure and is transcription-competent. Most importantly, there is no known adverse effect on the cell resulting from its disruption.

Safe-harbor TALEN targeting kit

Genome-TALER™ safe-harbor TALEN targeting kit is designed to knockin the researcher's gene of interest (GOI) to the AAVS1 site in chromosome 19 of human cell lines for long-term stable expression. The kit contains:

A pair of AAVS1 TALENs for specific cleavage of the AAVS1 site
An AAVS1 donor vector for cloning the GOI to be knocked-in. It contains two AAVS1 flanking arms for homologous recombination as well as GFP and puromycin for detection and selection
A positive control AAVS1 donor vector, containing RFP/GFP and puromycin for detection and selection

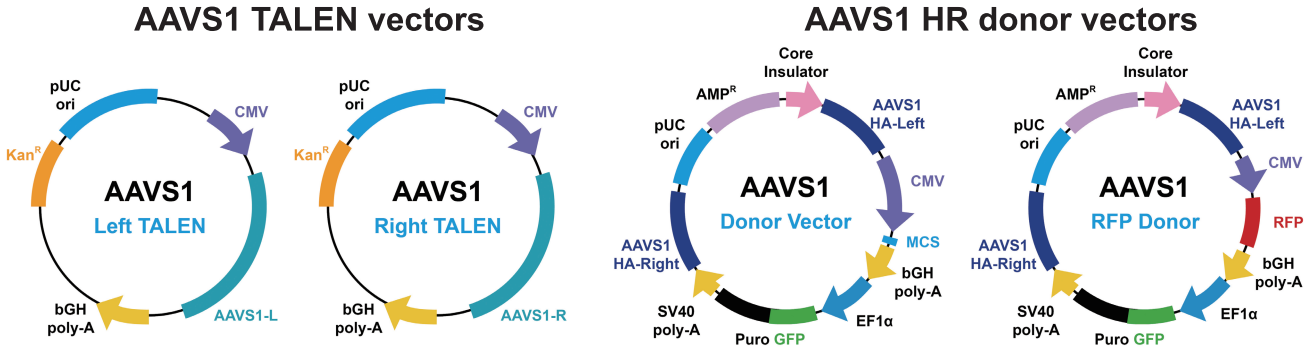

Figure 7. Safe-harbor TALEN target kit plasmids

Safe-harbor genome integration services

GeneCopoeia offers custom services for the AAVS1 donor DNA construction and/or stable cell line generation. Researchers can choose from GeneCopoeia's large collection of ORF cDNA, miRNA, and shRNA as knockin candidates.

1. Zou, J. et al. 2009. Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. Cell Stem Cell. 2009 Jul 2;5(1):97-110
2. Sadelain, M. et al. 2011. Safe harbours for the integration of new DNA in the human genome. Nat Rev Cancer. 2011 Dec 1;12(1):51-8.

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