# Genome-TALER™ TALEN and TALE-TF Products and Services

## User Manual

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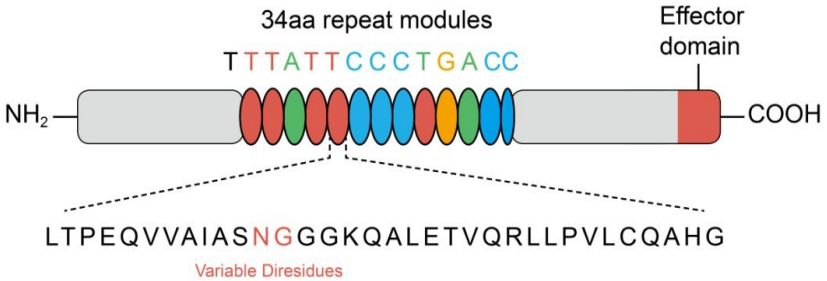
# USER MANUAL

# Genome-TALER™ TALEN and TALE-TF Products and Services



## I. Introduction of TAL effector

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. 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 (Figure 1).




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

**Figure 1. Top:** Schematic of a TAL effector. **Bottom:** Typically-used RVD recognition code.

TAL effectors have been utilized to create site-specific gene-editing tools by fusing target sequence-specific TAL effectors to nucleases (TALENs), transcription factors (TALE-TFs) and other functional domains (Figure 2). 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 allow researchers to be able to specifically target whatever sequence they want.

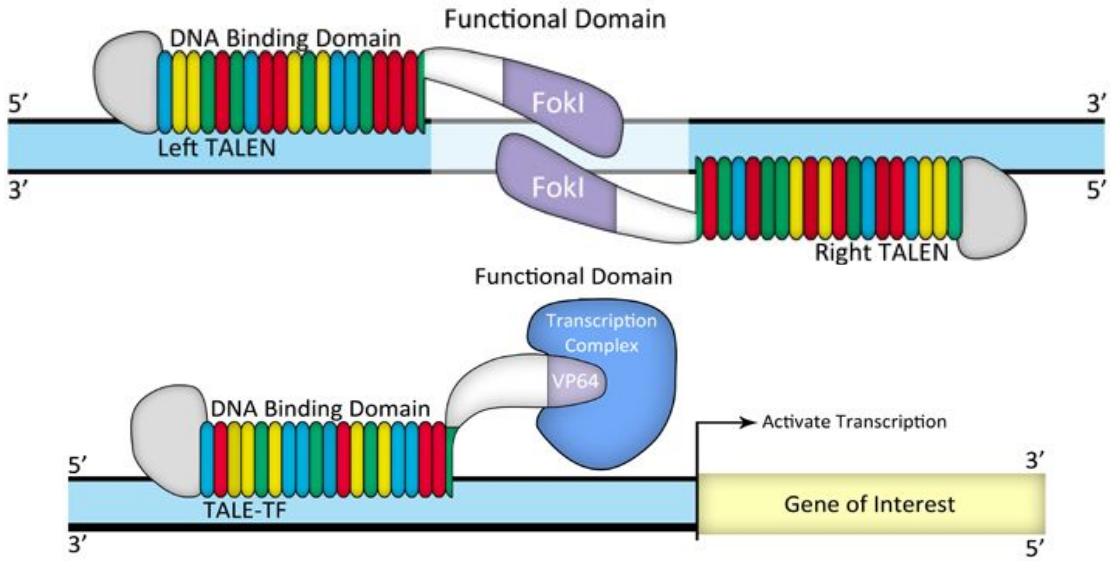



**Figure 2. Top:** Typical TALEN design strategy. **Bottom:** Typical TALE-TF design strategy.

## Advantages

Targeting any gene in any cell

Highly sequence-specific genome editing

For gene knockout, knockin, mutagenesis, activation, repression and more

Flexible TAL effector design of binding and functional domains, such as TALEN and TALE-TF

## II. Related Services

| Services | | Description | Applications |
|---|---|---|---|
| **Validation** | Mismatch cleavage assay | Chromosomal-level functional validation. Detects the presence of indels created by TALEN-mediated NHEJ repair at the specific target site of the chromosome. | TALEN |
| | qPCR assay | Chromosomal-level functional validation. It can measure the change in expression level of the target gene induced by site-specific TALE-TF transcription activator. | TALE-TF |
| **Donor clone services** (see appendix) | Donor clone design and construction | Customized plasmids designed to specifically transfer your gene of interest, selection marker or other genetic elements into targeted sites through homologous recombination (HR) induced by our TALEN. We offer various donor vector choices with different selection markers and genetic elements built in for your experiment purpose. | TALEN |
| **Stable cell line services** (see appendix) | Monoclonal colony | Monoclonal stable cell line with TALEN-mediated genome modifications. | TALEN |
| | Cell bank | Creation of a cell bank of monoclonal stable cell line with TALEN-mediated genome modifications. | TALEN |
| **Transgenic mouse services** | Transgenic mouse | Transgenic mice with TALEN-mediated genome modifications. | TALEN |

## III.Examples

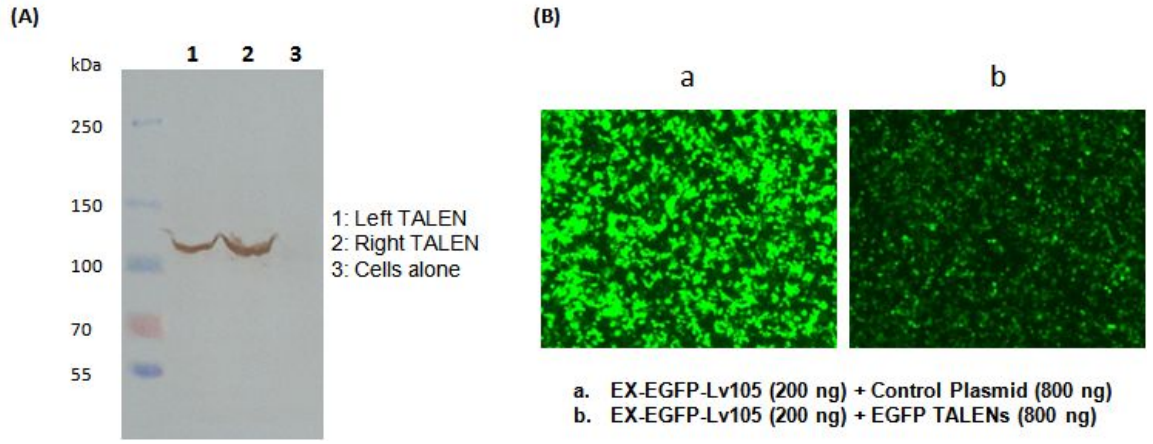



**Figure 3. TALENs knockdown eGFP expression. (A)** eGFP TALENs expression validation: ~80% confluence HEK293T cells were transfected with 0.8 µg plasmid per well in a 6-well plate. The cells were harvested 48 hrs post-transfection. 1/20th of the cell lysate per well was analyzed for western blot using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

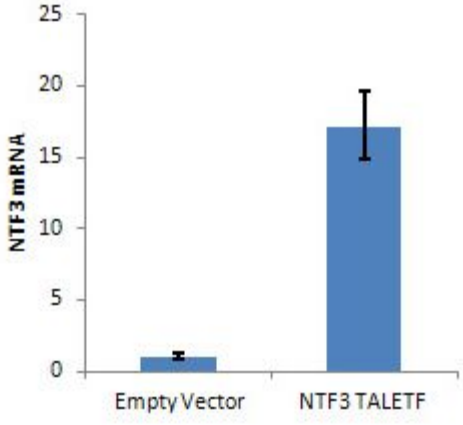
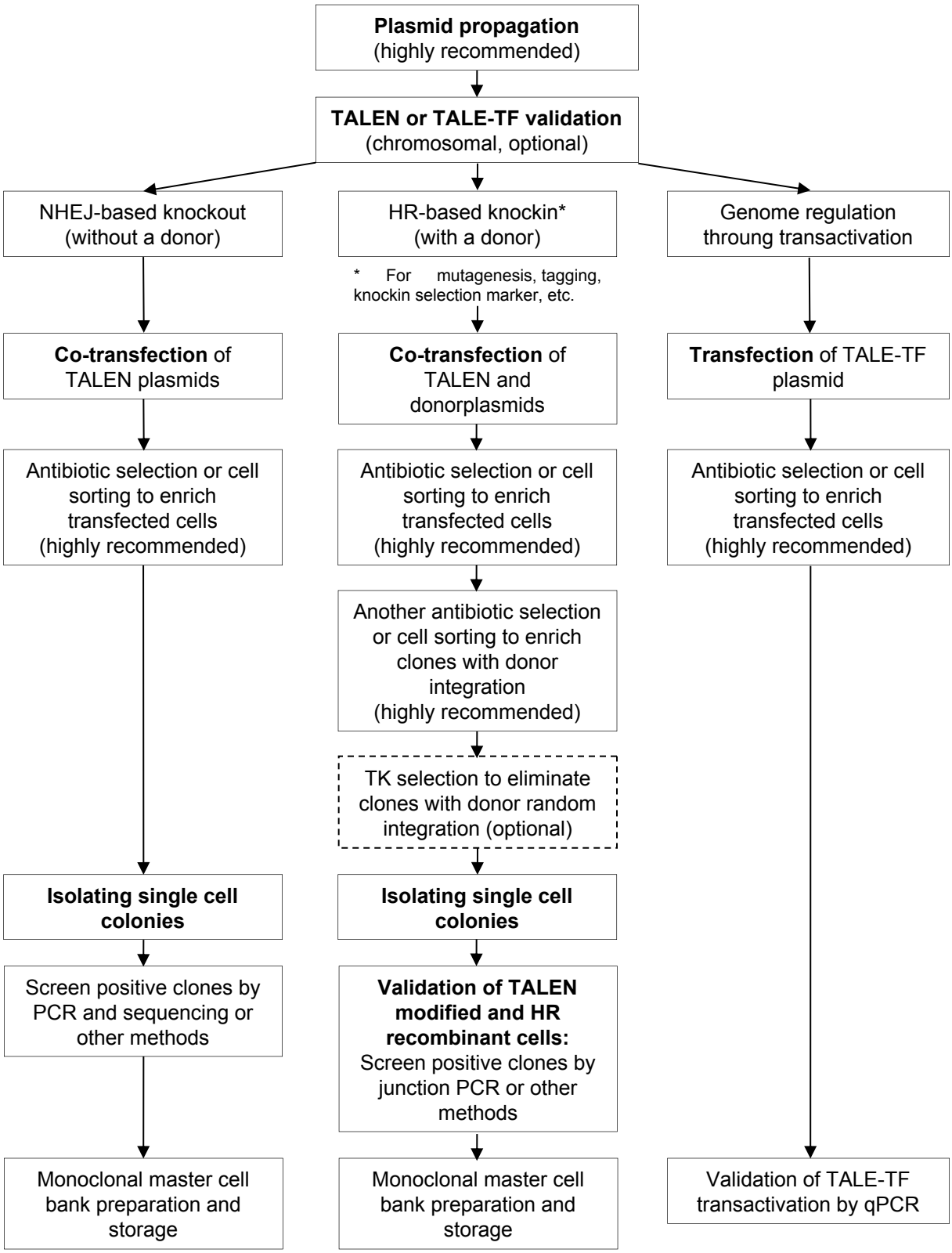



**Figure 4. NTF3 TALE-TF regulates endogenous NTF3 transcription.**

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.

## IV.Overview of Genome Editing Using TALEN and TALE-TF



## V.Critical Steps

### A. Plasmid propagation

We recommend propagating the plasmids provided before your gene targeting experiment. Plasmids can be transformed using standard conditions suitable in any RecA- and EndA- E.coli competent cells.

For transformation of TALE product plasmids, we suggest plating 50-200µl of transformed cells on fresh LB-Ampicillin plates (50µg/ml). Incubate the plates at 37°C overnight. Inoculate colonies from the transformation and grow them at 37°C overnight in ~200ml of LB media containing 50µg/ml Ampicillin. Use an endotoxin-free plasmid DNA maxiprep kit to extract plasmid DNA after overnight growth.

To confirm the integrity of the amplified plasmids, we recommend restriction digestion analysis or direct sequencing.

### B. Characterization of TALE-TF or TALEN modified cells

**(Option A) Measuring TALE-TF transcriptional activation using qRT-PCR**
For TALE-TFs, qRT-PCR quantitatively measures the increase in transcription driven by the TALE-TF. Genecopoeia provides validated qPCR primers for most genes in the human genomes. Full services covering RNA extraction, reverse transcription, Quantitative PCR, data analysis are also available in Genecopoeia.

There are a wide variety of qRT-PCR protocols, we provide brief outlines here.

1. 24 or 48hr post-transfection, collect cells to extract total RNA.
2. Measure the RNA concentration using a UV spectrophotometer.
3. Reverse transcription to get cDNA.
4. Quantitative PCR
5. Analyze data and calculate the level of gene activation using the $\Delta\Delta$Ct method.

**(Option B) Measuring TALEN cutting efficiency using mismatch cleavage assay**
TALEN-modified DNA will have a few bases of sequence deletion near the TALEN cut site because of NHEJ exonuclease activity. We recommend using the GeneCopoeia's IndelCheck™ CRISPR/TALEN insertion or deletion detection system (ICPE-050, ICPE-200). Alternatives include the Cel1, mung bean and S1 nucleases.

The T7 endonuclease I assay is carried out according to the user manual of the IndelCheck™ detection system. We provide brief details here.

**1.** 24 or 48 hr post-transfection, collect cells for lysis (recommended) or for genomic DNA extraction.

**2.** Use target PCR kit (TPCR-050, TPCR-200) to amplify the region surrounding the sgRNA target site.

**3.** Check the PCR result by running 5 µL of PCR product on a 2% agarose gel. For all templates, it is important to make sure that there is only a single band corresponding to the intended product for the primer pair. The size of this band should be the same as calculated from the distance between the two primer annealing sites in the genome.

*CRITICAL STEP: If multiple amplicons are generated from the PCR, redesign the primers and reoptimize the PCR conditions to avoid off-target amplification. In difficult cases in which a single-band product cannot be achieved, it is acceptable to gel-extract the correct-length band before proceeding with heteroduplex reannealing and Surveyor nuclease digestion.*

**4.** DNA heteroduplex formation. At this point, the amplified PCR product includes a mixture of both TALEN-modified and unmodified genomic DNA. Place 300 ng of the PCR product in a thermocycler tube and perform the cross-hybridization.

**5.** T7 endonuclease I assay. Treat the cross-hybridized homo- and heteroduplexes with T7 endonuclease I assay kit to determine CRISPR cleavage efficiency.

## C. Transfection of TALE-TF or TALEN into target cells

**Option A if you are using TALE-TF for transcriptional modulation, or option B if you are using TALEN for testing nuclease activity.**

**1.** Plate ~100,000 to 300,000 cells/well in a 6-well plate according to established recommended conditions for the cell type(s) being transfected. Scale up and down the culture if needed. On the day before transfection, trypsinize and count the cells. The number of cells plated in each well should be determined so that they are 70-80% confluent at the time of transfection.

**2.** The next day, prepare transfection complexes of TALEN and TALE-TF using a suitable transfection reagent according to the manufacturer's recommended instructions. Leave the transfection complexes on the cells to react for >6 hours.

**Option A)** 2.0µg of TALE-TF

**Option B)** 1.0µg of LEFT TALEN + 1.0µg of RIGHT TALEN

*Notes: including appropriate controls according to your experiment.*

Tech Notes:

1) Since transfection efficiencies vary across different cell lines, we recommend optimizing the input of plasmid to transfection reagent for best results.

2) For optimal results, we recommend complexing of DNA with transfection reagent in serum- and antibiotic-free media and cells growing in complete media.
3) For hard-to-transfect cells (e.g. primary, stem, hematopoietic), it may be advisable to utilize a nonpassive transfection method such as NucleoFection (Lonza) or Neon system (Life Technologies). Please follow recommended transfection guidelines provided by the manufacturer for specific cell type(s) being transfected.

**3.** 24 hours post-transfection, remove transfection media and split the cells 1:10 and 1:20 in complete growth media w/antibiotics. Plate cells into 6-well plates and save a set of plate(s) for characterization. Allow cells to recover for 24 hours.

**4.** Begin antibiotic selection 48 hours post-transfection. We recommend optimizing concentration of antibiotic for best results.

Tech Note:
Establishing a kill-curve on untransfected cells can determine the effective working antibiotic concentration for a target cell line. The concentration of antibiotic that kills >90% of cells after 48hours of selection is the correct dose for the cells being selected.

**Example: For HEK293T cells using EndoFectin reagent, transfect TALEN or TALE-TF**

1) Plate cells
Plate HEK293FT cells onto six-well plates ~24 h before transfection. The number of cells plated in each well should be determined so that they are 70-80% confluent at the time of transfection

2) Prepare the DNA–Opti-MEM mix.
**Option A):** mix 2.0 μg of TALE-TF plasmid DNA with 50 μl of Opti-MEM.
*Notes: include controls (e.g., a reporter plasmid or mock transfection) to monitor transfection efficiency and cell health, respectively.*
**Option B):** mix 1.0 μg of each LEFT TALEN and Right TALEN (2 μg total) with 50 μl of Opti-MEM.
*Notes: control transfections can be done by omitting one or both of the TALENs. Include controls (e.g., a reporter plasmid or mock transfection) to monitor transfection efficiency and cell health, respectively.*

3) Prepare the EndoFectin™–Opti-MEM solution
Dilute 6 μl of EndoFectin™-Plus with 50 μl of Opti-MEM. Mix the solution thoroughly at room temperature.

4) Prepare DNA- EndoFectin™ complex
Add the diluted EndoFectin™ reagent drop-wise to the DNA solution while gently vortexing the DNA-containing tubes. *(Note: Do not reverse the addition sequence.)* Incubate the mixture for 10-25 minutes at room temperature to allow the DNA-EndoFectin™ complex to form.

5) Transfect cells
Add the DNA-EndoFectin™ complex directly to each well and gently swirl the plates/dishes.

## D. Validation of TALEN modified and HDR recombinant cells

**1.** To confirm donor vector integration specifically at target site, junction-PCR can be performed using PCR primer pairs that flank the 5' homology arm and 3' homology arm.

**2.** Protocol for Junction-PCR

1) Primers should be diluted to 10 μ M before use. Validation of either the 5' or 3' homology arms for donor integration is usually sufficient; however, both arms can be done for additional confirmation.

2) Protocol details for junction-PCR assay:

a) Isolate genomic DNA from positive control cells or test sample cells using a suitable genomic DNA miniprep kit. Please follow the protocol recommended by the manufacturer.

b) Perform junction-PCR (PCR reaction below)

| Reagent | TALEN cut+ positive control donor | Positive control donor only |
|---|---|---|
| Genomic DNA(60~100ng/μl) | 1 μ l | 1 μ l |
| 10 μ M 5' (or 3') PCR Primer Mix | 1 μ l | 1 μ l |
| 5×UltraPFTM Buffer (Mg2+ free) | 5 μ l | 5 μ l |
| 10 mM dNTPs | 0.5 μ l | 0.5 μ l |
| 20mM MgSO4 | 2.5 μ l | 2.5 μ l |
| UltraPF(5U/ μ l) | 0.25 μ l | 0.25 μ l |
| PCR-grade distilled water | 14.75 μ l | 14.75 μ l |
| **Total** | **25 μ l** | **25 μ l** |

98°C, 5min

98°C, 20sec
55°C, 30sec
72°C, 1min
} 35 cycles

72°C, 7min

Hold at 4~16°C

Run the PCR reaction out on the 1% Agarose/EtBr gel in 1X TAE buffer to confirm the Junction-PCR result.

Sample results for 5' and 3' Junction-PCR Assay depend on design.

Tech Note:

1) The 3'junction PCR band and 5' junction PCR band may differ in brightness because the amplification efficiency may be different due to the nature of the chromosomal structure, modification and sequence around that region.
2) One positive in junction PCR is sufficient to confirm the integration.
3) Though rare, it is possible that random integration can coexist with site-specific integration. Negative selection can be used to detect coexisting random integration.

## E. Clonal isolation of cell lines

Serial dilution is widely used to isolate single clone with desired modifications, followed by an expansion period to establish a new clonal cell line. Like most clonal isolation methods, there is no guarantee that the colonies arose from single cells. A second round is advised to increase the likelihood of clonal isolation. Also, it is worth noting that cell types can vary substantially in their responses to single-cell isolation, therefore literature specific to the cell type of interest should be consulted.

**1.** Fill each well of a sterile 96-well plate with 100μl of medium except for well A1, which should remain empty.

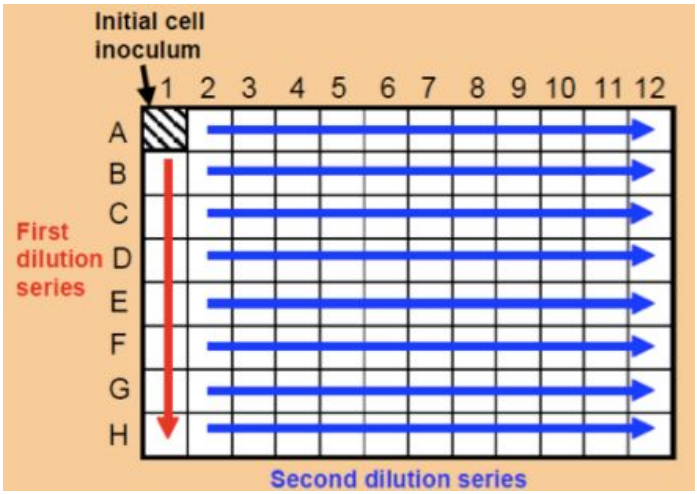



**Figure 5:** Illustration of serial dilution.

**2.** Add 200μl cell suspension to well A1. Mix 100μl from A1 with the medium in well B1. Avoid bubbles. Continue this 1:2 dilution through column 1. Add 100μl of medium back to column 1 so that wells A1 through H1 contain 200μl.

**3.** Mix cells and transfer 100μl of cells from column 1 into column 2. Mix by gently pipetting. Avoid bubbles. Repeat these 1:2 dilutions through the entire plate. Bring the final volume to 200μl by adding 100μl of medium to all but the last column of wells.

**4.** Incubate plates undisturbed at 37℃.

**5.** Cells will be observable via microscopy over 3 days and be ready to score in 5-8 days, depending on the growth rate of cells. Mark each well on the cover of the plate indicating which well contains a single colony. These colonies can later be subcultured from the well into larger vessels.

Tech Note:

1) Adding 4000 cells in well A1 ($2\times10^4$ cells/ml) is a good starting concentration. Increase the concentration for more difficult to grow cell lines.
2) If the reporter gene is fluorescent, determine observe which of these colonies express it. If the reporter gene is not observable you will have to wait until later in the culture process.
3) Label each well with a single colony using a unique identification number and record this number on the plate and in your notebook.

## VI.References

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# VII.Appendix

## 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 | LoxP Site |
|---|---|---|---|---|
| pDonor-D01 | EFa1 | copGFP | Puromycin | N/A |
| pDonor-D02 | CMV | copGFP | Neomycin | N/A |
| pDonor-D03 | CMV | N/A | Neomycin | N/A |
| pDonor-D04 | CMV | N/A | Puromycin | N/A |
| pDonor-D05 | EFa1 | N/A | Neomycin | N/A |
| pDonor-D07 | EFa1 | copGFP | Puromycin/TK | Loxp |
| pDonor-D08 | CMV | copGFP | Neomycin/TK | Loxp |
| pDonor-D09 | EFa1 | N/A | Puromycin/TK | Loxp |
| pDonor-D10 | CMV | N/A | Neomycin/TK | Loxp |
| pDonor-D11 | PGK | copGFP | Puromycin/TK | Loxp |
| pDonor-D12 | EF1a | copGFP | Hygromycin/TK | Loxp |
| pDonor-D13 | PGK | copGFP | Neomycin/TK | Loxp |
| pDonor-D14 | PGK | N/A | Puromycin/TK | Loxp |

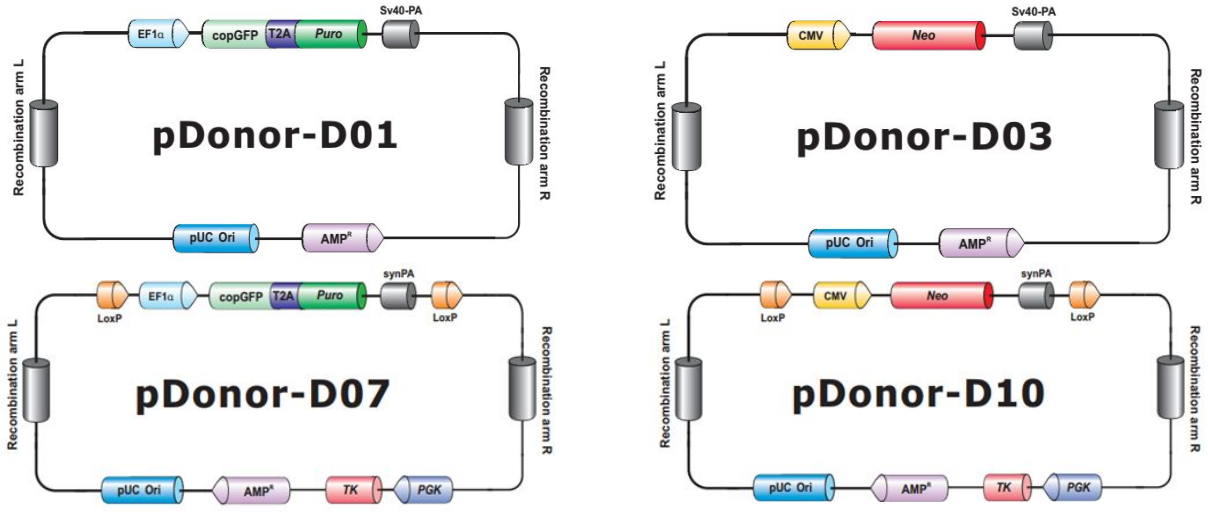
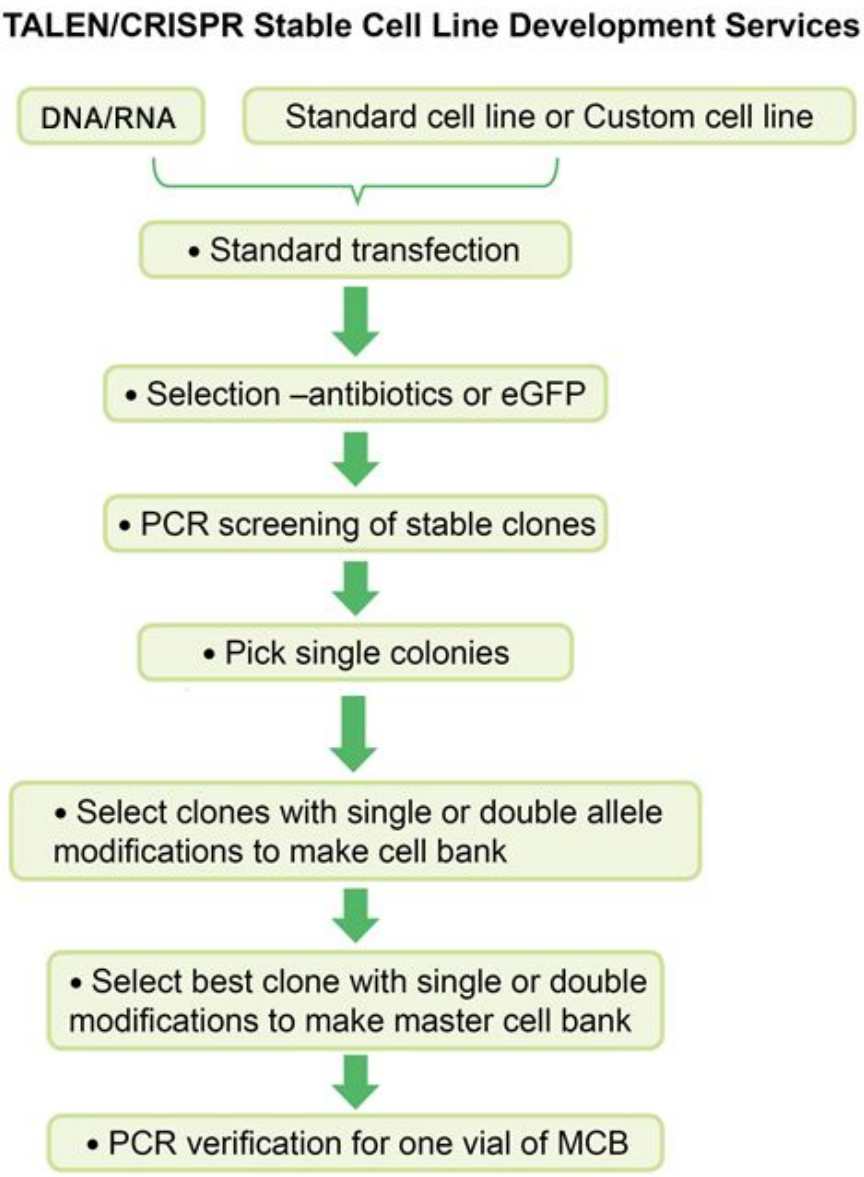



**Figure 6.** Maps of some of the donor vectors

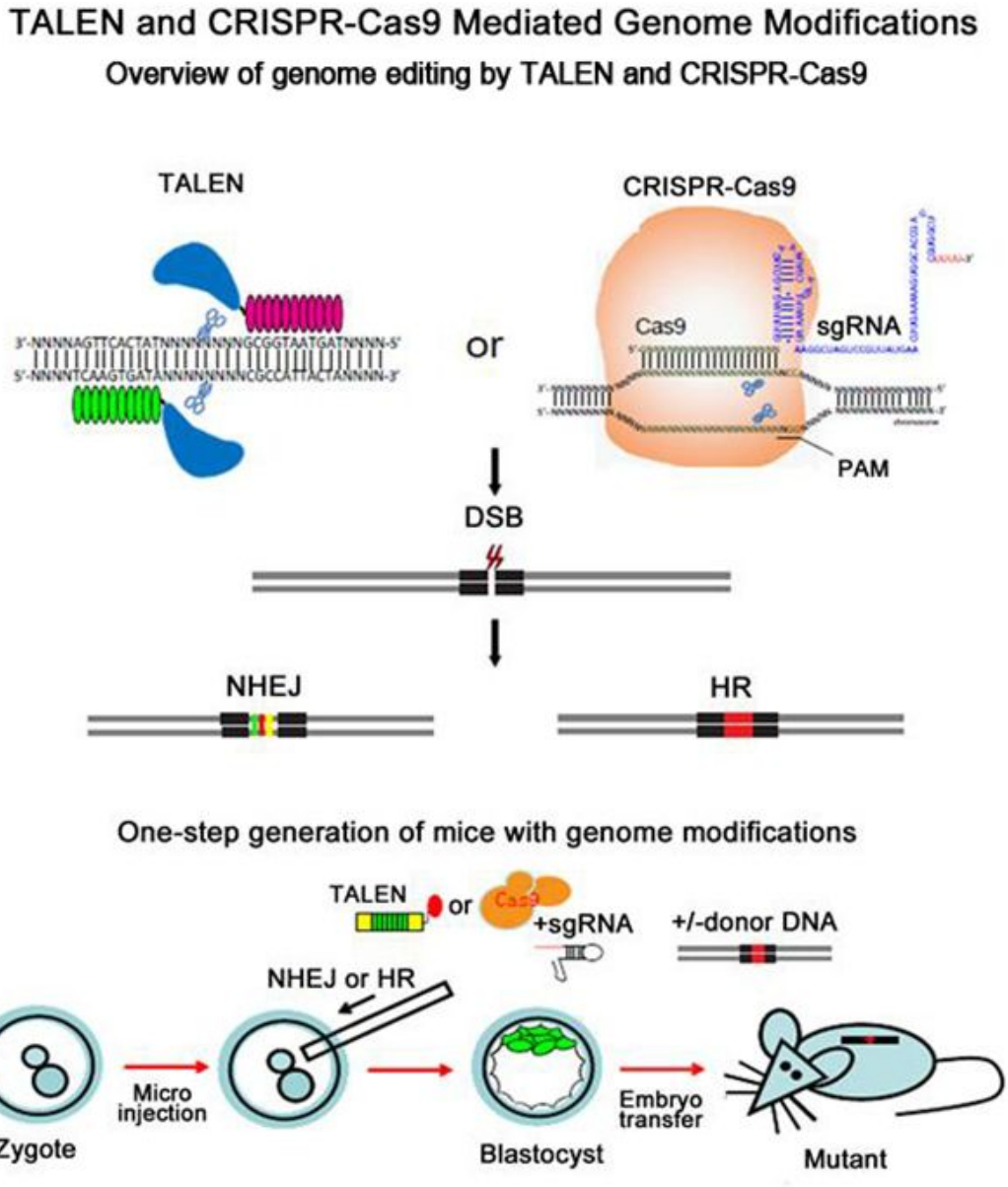
**Stable cell line services**

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



**Transgenic mouse services**

Transgenic mouse models are now considered ideal tools to delineate the molecular mechanisms of the gene products and their interactions with one another that influence all cellular processes that form the basis of physiological systems. . GeneCopoeia offers transgenic mice with customized TALEN- or CRISPR-Cas9-mediated genome modifications.



## VIII. Limited Use License and Warranty

**Limited Use License**
Following terms and conditions apply to use of the Genome-TALER™ TALEN and TALE-TF products and services (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products or deliver information obtained in service without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

**Limited Warranty**
GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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