Genome-TALER™ Custom TALEN and TALE-TF

General Guide

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I. Introduction

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.

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. 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.

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. 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.

Plate ~100,000 to 300,000 cells/well in a 6-well plate according to established recommended conditions for cell type(s) being transfected. Scale up and down the culture if needed. Include wells for the following:

Option A) 2.0 µg of TALE-TF
Option B) 1.0 µg of LEFT TALEN + 1.0 µg of RIGHT TALEN
Notes: including appropriate controls according to your experiment.

Next day, prepare transfection complexes of TALEN and TALE-TF using a suitable transfection reagent according to the manufacturer's recommended instructions.

Tech Notes:

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

b) For optimal results, we recommend complexing of DNA with transfection reagent in serum- and antibiotic-free media and cells growing in complete media.

c) 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.

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.
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.
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.

III. Characterization of TALE-TF or TALE-TF 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 48 hr 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 ΔΔ*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 Surveyor mutation detection kit for standard gel electrophoresis (Transgenomic, cat. no. 706025) for such assay. Alternatives include the Cel1, T7, mung bean and S1 nucleases.

The Surveyor procedure is carried out according to the manufacturer's protocol and is described in greater detail in the Surveyor manual. We provide brief details here.

1) 24 or 48 hr post-transfection, collect cells to extract genomic DNA.

2) PCR amplification of the region surrounding TALEN 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 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 cross-hybridization.

5) Surveyor Nuclease S digestion. To treat the cross-hybridized homo- and heteroduplexes using Surveyor Nuclease S to determine TALEN cleavage efficiency.

IV. Related Products

- Genome-TALER™ TALEN and TALE-TF custom services
- Genome-TALER™ Human AAVS1 safe harbor gene knock-in kit
- Genome-TALER™ human AAVS1 safe harbor knock-in ORF clones

V. Limited Use License and Warranty

Limited Use License
Following terms and conditions apply to use of the Genome-TALER™ Human AAVS1 Safe Harbor Gene Targeting Kit (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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