Methods 69 (2014) 151–170

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

# Efficient strategies for TALEN-mediated genome editing in mammalian cell lines

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ARTICLE INFO

*Article history:*
Received 19 December 2013
Revised 24 June 2014
Accepted 25 June 2014
Available online 15 July 2014

*Keywords:*
TALEN
Gene editing
Gene tagging
Gene inactivation
Gene insertion
Methylation

ABSTRACT

TALEN is one of the most widely used tools in the field of genome editing. It enables gene integration and gene inactivation in a highly efficient and specific fashion. Although very attractive, the apparent simplicity and high success rate of TALEN could be misleading for novices in the field of gene editing. Depending on the application, specific TALEN designs, activity assessments and screening strategies need to be adopted. Here we report different methods to efficiently perform TALEN-mediated gene integration and inactivation in different mammalian cell systems including induced pluripotent stem cells and delineate experimental examples associated with these approaches.



## 1. Introduction

Transcription activator-like effectors (TALEs), a group of bacterial plant pathogen proteins, have recently emerged as new engineerable scaffolds for production of tailored DNA binding domains with chosen specificities [1]. A TALE DNA binding domain is composed of a variable number of 33–35 aminoacids repeat modules that are nearly identical to one another except for two variable aminoacids named Repeat Variable Di residues (RVD) located at positions 12 and 13. The nature of residues 12 and 13 determines base preferences of individual repeat module and it is now well established that there is a preferential pairing between A, C, G, T nucleotides and the repeat modules harboring respectively NI, HD, NN, and NG RVDs. Based on this specificity cipher, engineered TALE DNA binding domains have been generated and fused to different active domains to generate a vast portfolio of gene editing tools with custom DNA specificity [1,2]. It includes fusions to transcriptional activator [3] and transcriptional repressor domains [4] as well as chromatin remodeling enzyme [5–7] and recombinase [8]. More importantly it also includes fusion to different nuclease domains such as FokI, I-TevI, PvuII and I-AniI [9–12] enabling the generation of the transcription activator like effector nuclease named TALEN[1] or compact TALEN.

Today, TALEN is one of the most widely used TALE-based tools in the field of genome editing. It was used to specifically inactivate, integrate or correct genes of interest for biotechnological and therapeutic applications [13,14]. Each of these different applications was reported to be achieved with high success rate in various organisms including human, mouse, rat, zebrafish and plant as non exhaustive examples [13,14]. Regarding gene inactivation in mammalian cells, Reyon et al. reported that among 96 loci tested in U-2 OS mammalian cells, 88% displayed insertion and deletion (indels) events above 3% [15]. Such high efficiency was further confirmed by Kim et al. that reported that 98% of the 103 loci studied in HEK293 cells, displayed indels $\geqslant$0.5% [16]. Concerning gene integration in mammalian cells, it has been reported to be successfully promoted by TALEN at different loci including AAVSI, OCT4,

*Abbreviations:* TALEN, transcription activator like effector; NHEJ, non homologous end joining; HR, homologous recombination; indels, insertion deletion; 5mC, 5-methylated cytosine; CpG, cytosine-phospho-guanine; GOI, gene of interest; POI, protein of interest.

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[1] TALEN™ is a trademark owned by Cellectis bioresearch.

http://dx.doi.org/10.1016/j.ymeth.2014.06.013
1046-2023/

PITX3 and tyrosine hydroxylase loci found in human embryonic stem cell (hESc), human induced pluripotent stem cells (HiPSC) and in fertilized zebrafish eggs [17–19]. Although to a much lower throughput, mammalian gene correction was also successfully performed on COL7A, HBB, DMD and XPC genes, respectively involved in the Recessive dystrophic epidermolysis bullosa, in the sickle cell disease, in the Duchenne dystrophy and in the xeroderma pigmentosum syndrome [20–23].

Although very attractive, the apparent simplicity and high success rate of TALEN technology could be misleading for novices in the field of gene editing. Indeed, they are a number of different key parameters that need to be taken into account to get successful editing outcomes. Depending on the application, specific TALEN designs, activity assessments and screening strategies need to be adopted. Here we report different methods to efficiently perform TALEN-mediated gene integration and inactivation and delineate experimental examples associated with these approaches.

## 2. Material and method

### 2.1. TALEN design

When designing a TALEN, the following key points should be considered:

- Verify that the TALEN target sequence is present in the cell line of interest. Notably, the presence of SNPs should be carefully check.
- Avoid target sites containing (i) at least 5 identical consecutive nucleotides (iii), methylated CpG and (iii) containing AA dinucleotides immediately downstream the first T0.
- For methylated loci, assemble a methyl insensitive TALE DNA binding domain by substituting HD TALE repeat for N*. To our knowledge, up to two substitutions could be performed per TALEN arms although we cannot exclude that a higher number of substitution could lead to an efficient TALEN.
- Identify off-target sites bio-informatically. TALEN showing potential off target sites bearing less than 5 total mismatches compared to the targeted sequence should be left aside.
- Synthesize at least two TALEN per locus to edit. These TALEN should then be validated at the endogenous locus in the biological system of interest. In most of the cases, the fastest and easiest way is to determine the ability of the TALEN to produce mutations via error-prone NHEJ using the different screening methods delineated below. The choice of the final TALEN to use will depend on the balance between the best localization and activity level.

### 2.2. TALEN activity assay in yeast

#### 2.2.1. Generation and transformation of TALEN and TALEN target constructions for single-strand annealing (SSA) assays

- Insert TALEN DNA target into the yeast LacZ reporter vectors (pFL39-ADH-LACURAZ for yeast previously described in [10,24] using the Gateway protocol (Invitrogen). Please note that all targets should contain a control I-SceI target site to monitor baseline SSA activity. In addition, target should contain, between the two LacZ homologous region, a URA gene to enable sorting-out recombined target on a URA-medium (Supplementary Fig. 1).
- Insert left and right TALEN constructions onto Leu2 or G418$^R$ marker-containing plasmids, respectively.
- Transform the TALEN target-containing LacZ reporter vector into *Saccharomyces cerevisiae* strain FYBL2-7B (*Mat alpha, ura3-Δ851, trp1Δ63, leu2Δ1, lys2Δ202*) and select transformants onto solid synthetic medium supplemented by glucose and lacking histidine and uracil.
- Transform the two TALEN constructions into FYC2-6A (*mat alpha, trp1Δ63, leu2Δ1, his3Δ200*) and select transformants onto synthetic solid medium lacking leucine and lysine and supplemented by G418 and glucose.

#### 2.2.2. Mating of TALEN-expressing clones with reporter plasmid-expressing clones and determination of β-galactosidase activity

- Use a colony gridder (QpixII, Genetix) to perform the matting between TALEN-containing yeast strains and those harboring the respective LacZ-TALEN target reporter vector.
- Grid TALEN-containing yeast strains on nylon filters placed on YPGlycerol plates, using high gridding density (about 20 spots/cm$^2$). On the same filter, perform a second gridding by spotting reporter vector-harboring yeast strains. Place membranes on solid agar containing YPGlycerol rich medium and incubate them overnight at 30 °C to allow mating.
- Transfer filters onto synthetic medium, lacking leucine, tryptophan, lysine and histidine, with glucose (2%) as the carbon source and supplemented with G418, and incubated for 3 days at 30 °C to select for diploids carrying the two TALEN and reporter vectors.
- Transfer filters onto YPGalactose rich medium supplemented with G418 for 5 days at either 30 °C (stringent) or 37 °C (standard) to induce the expression of the TALEN.
- Determine β-galactosidase activity resulting from TALEN-induced cleavage of reporter plasmid by placing filters on solid agarose medium with 0.05% X-Gal in 0.5 M sodium phosphate buffer, pH 7.0, 0.1% SDS, 2% agarose, and incubate at 37 °C.
- Scan the resulting filters and quantify each spot using the median values of the pixels constituting the spot. Arbitrary values of 0 and 1 are associated to white and black pixels, respectively. β-galactosidase activity is directly associated with the efficiency of homologous recombination. Relative values are determined with respect to a positive control known to saturate the signal under the conditions tested.

### 2.3. DNA delivery methods

DNA delivery protocol is among the most important parameter to take into account for successful gene editing, thus great care must be taken to set it up. To achieve optimal DNA delivery:

- Explore different transfection reagents or electroporation systems (FuGENE®, lipofectamine®, AMAXA® Nucleofector®, NEON® transfection system and Cytopulse as non exhaustive examples) and select the one that give the best percentage of transfected cells and the higher level of protein expression.
- Optimize quantities of transfected plasmid to find the appropriate balance between TALEN activity and toxicity.
- Consider performing a transient cold shock immediately after transfection. Such treatment has been shown to greatly enhance the outcome of gene processing using Zinc finger nucleases [25].

#### 2.3.1. Transfection of HCT116 and A549 cell lines using lipid-based DNA transfection reagents

*2.3.1.1. Low throughput format.* HCT116 and A549 cell lines, obtained from ATCC (CCL-247™ and CCL-185™ respectively), need to be cultured at 37 °C with 5% $CO_2$ in McCoy's5A (PAA Laboratories, Austria) supplemented with 10% FBS, penicillin (100 IU/ml) and streptomycin (100 μg/ml).

One day prior transfection

- Seed the cells in complete medium at $1 \times 10^6$ cells/10 cm dish or $2 \times 10^5$ cells/6 wells plates.