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TECHNICAL NOTE

IndelCheck™: A Powerful CRISPR/TALEN Validation & Screening Tool

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Introduction

Genome editing by CRISPR or TALEN often requires substantial screening work to identify correctly-modified cell clones or animals, leading to a need for effective validation and screening tools to accompany these reagents. Perhaps the most widely-used validation and screening tool is the "mismatch cleavage assay". GeneCopoeia's IndelCheck™ insertion and deletion detection system streamlines the mismatch cleavage assay to help customers with genome editing. In this Technical Note, we discuss the benefits of performing validation assays, and show how the indelCheck™ system is the best option for validation and screening for your genome editing applications.

Why is CRISPR and TALEN functional validation important?

We recommend that you validate the efficiency of your CRISPR sgRNAs or TALENs before carrying out a complete genome editing project. While CRISPR and TALEN provide highly efficient methods for genome editing, the individual editing tools themselves exhibit large variations in efficiency due to the nature of individual target sites. Therefore, it would be highly beneficial to determine which CRISPR or TALEN clones have the highest potential for successfully editing the genome before doing lots of screening work.

We recommend the mismatch cleavage assay, also known as the Surveyor™ assay (Qiu, et al., 2004) for functional validation. The indelCheck™ mismatch cleavage assay is a cell culture-based assay designed to efficiently detect indels resulting from double strand break (DSB)-mediated nonhomologous end joining (NHEJ). The basic workflow for the indelCheck™ mismatch cleavage assay for CRISPR or TALEN functional validation is illustrated in Figure 1.

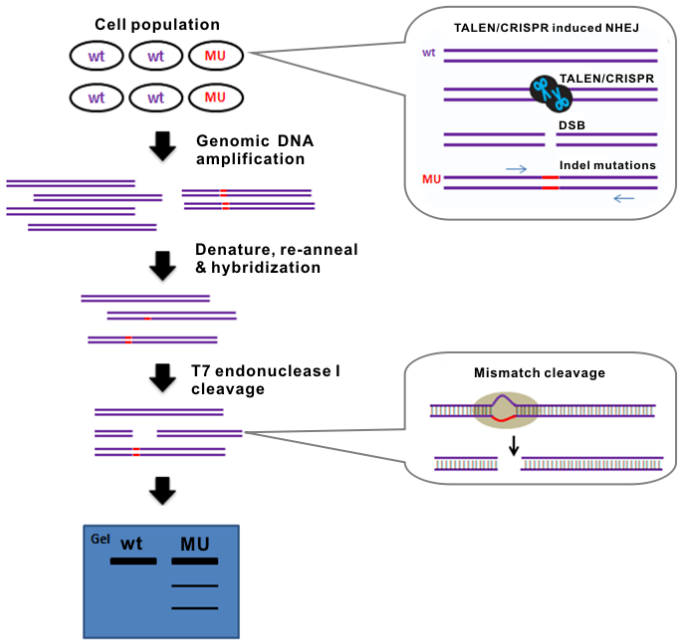

Figure 1. Workflow of the indelCheck™ mismatch cleavage assay. A population of cells that has been exposed to CRISPR or TALEN is subjected to genomic DNA PCR using primers flanking the target site. Denaturation and reannealing of the PCR products creates a mixed population of homoduplex and heteroduplex molecules. The fragments are treated with T7 Endonuclease I, which cuts only the heteroduplex molecules. Cleavage products are detected by standard agarose gel electrophoresis.

Using the IndelCheck™ mismatch cleavage assay for CRISPR or TALEN functional validation

If you are carrying out genome editing in cultured mammalian cells, we recommend using the same cell line you are working with for validation. If you are working *in vivo* in, for example, mice or rats, then perform the validation assay in a model cell line, such as Neuro2A or NIH3T3 for mouse, or PC-12 for rat.

As illustrated in Figure 1, CRISPR Cas9/sgRNA or TALEN clones are used to transiently transfect a cell line. Donor plasmids are not included in these validation transfections, even if your genome editing experiment requires a donor. The indelCheck™ mismatch cleavage assay only detects NHEJ-mediated events, which occur whether or not a donor is present.

Next, one-to-two days post-transfection, the cells are harvested as a pool, and PCR using primers surrounding the DSB site is carried out. A typical PCR design strategy is illustrated in Figure 2.

Figure 2. PCR primer design strategy for the indelCheck™ mismatch cleavage assay. A PCR product is generated using primer sequences highlighted in yellow. GeneCopoeia recommends amplicon sizes in the range of 500-1,000 base pairs. In addition, PCR primer sequences should be chosen such that the DSB sites are offset from the middle by at least 100 bp. The amplicon above is a 989 bp region of the human HDAC6 gene (NCBI GeneID: 9636). This sequence contains 3 CRISPR sgRNA target sites (highlighted in green, cyan, and magenta) being tested. Successful mismatch cleavage products of 341 bp + 648 bp (green), 555 bp + 434 bp (magenta) and 616 bp + 373 bp (cyan) are expected.

The next step is to carry out the PCR on the population of cells. Indel formation by NHEJ is a stochastic process; both the type (insertion or deletion) and length of indels differ from one edited individual to another, as shown in Figure 3. So, if CRISPR- or TALEN-mediated indel formation is successful, the pool of cells will consist of a mixed population, with each cell carrying a different allele at the target site. The mixture can be highly complex, consisting of unmodified alleles and different mutant alleles.

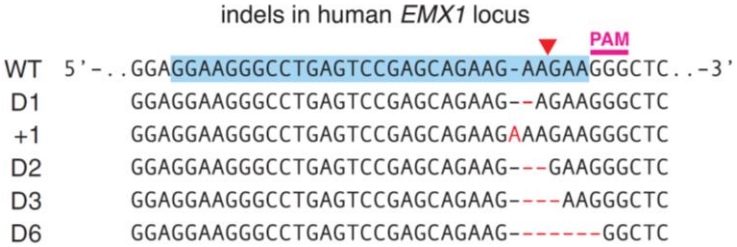

Figure 3. Example of allele distribution resulting from NHEJ-mediated indels. Top: Wild-type (unmodified) CRISPR target sequence in the human *EMX1* gene, followed byfour different deletion alleles (D) and one insertion allele found among edited clones. From Cong, et al. (2013) .

PCR products are denatured by heating to 95^0C, then allowed to re-anneal by slowly cooling to room temperature. The cooling step causes both matched and mismatched strands to anneal to each other, leading to formation of both homoduplex and heteroduplex DNA, respectively (Figure 1).

Finally, the re-annealed DNA is digested with T7 Endonuclease I, an enzyme that cleaves heteroduplex, but not homoduplex, DNA. Cleavage products are electrophoresed on a standard agarose gel. Typically, if NHEJ occurred, three bands will be observed: One band corresponds to undigested (homoduplex) DNA, while the other two bands are the cleavage products of heteroduplex DNA (Figure 4).

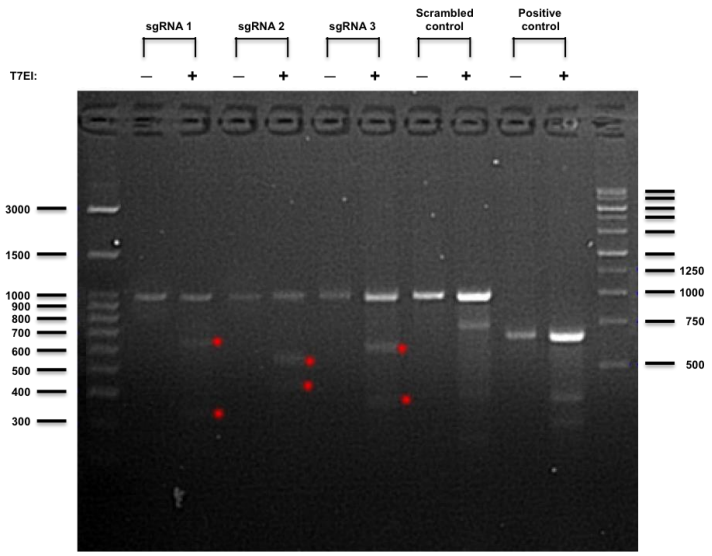

Figure 4. Agarose gel electrophoresis of mismatch cleavage products. indelCheck mismatch cleavage assay was performed following transfection of HEK293 cells with CRISPR clones expressing the sgRNAs in Figure 3. Annealing products before (–) and after (+) digestion with T7 Endonuclease I (T7EI) were separated on a 2% agarose gel. Bands labeled by asterisks occurring in the lanes with T7 Endonuclease I treatment occur at the sizes predicted for successful modification by CRISPR, indicating that each the sgRNAs designed for the experiment are functional. The scrambled control is usually not expected to yield any cleavage bands. However, in this example, cleavage bands are present after T7 EI digestion. This is likely due to the presence of naturally-occurring polymorphisms present between different chromosomes. In this example, the cleavage bands in the scrambled control lane are not the same size as the cleavage bands observed for sgRNAs 1, 2, and 3, and so are not causing false positive results.

Using the indelCheck™ mismatch cleavage assay to screen for edited individuals

The IndelCheck™ system can also be used to screen for NHEJ-mediated knockouts. The procedure for screening is the same as used for validation. However individual cell clones might not contain a mixed population of different alleles. Instead, if a clone is homozygous for the same exact indel mutation, there will be no heteroduplex DNA formed during the IndelCheck™ assay procedure, and so such a clone will not be detected as having been modified. To prevent this type of situation from occurring, we recommend spiking the PCR product DNA with PCR DNA from a negative, unmodified control prior to T7 Endonuclease I treatment. This step will endure the presence of heteroduplex DNA among all modified clones. Once edited clones are identified, the PCR products should be TA subcloned and sequenced to confirm the presence of the modification as well as the structure of the allele.

The indelCheck™ insertion or deletion detection system

The indelCheck™ system consists of components that can be ordered together or separately (Table 1). You can purchase the complete system (catalog numbers ICPE-050 and ICPE-200) consisting of all that's needed to perform both PCR and T7 Endonuclease I cleavage. Target site-specific PCR primers are sold separately. Alternatively, the target site PCR (catalog numbers TPCR-050 and TPCR-200) and the T7 Endonuclease I kits (catalog numbers TENI-050 and TENI-200) can be purchased separately.

Catalog number	Product	Description
ICPE-050	IndelCheck™ CRISPR/TALEN insertion or deletion detection system (50 rxns)	Includes target site PCR kit (TPCR-050) and T7 endonuclease I assay kit (TENI-050)*
ICPE-200	IndelCheck™ CRISPR/TALEN insertion or deletion detection system (200 rxns)	Includes target site PCR kit (TPCR-200) and T7 endonuclease I assay kit (TENI-200)*
TPCR-050	Target site PCR kit, 50 rxns	PCR reagents for amplifying region flanking CRISPR/ TALEN target site, prior to T7 Endonuclease I digestion*
TPCR-200	Target site PCR kit, 200 rxns	PCR reagents for amplifying region flanking CRISPR/ TALEN target site, prior to T7 Endonuclease I digestion*
TENI-050	T7 endonuclease I assay kit, 50 rxns	Cleave mismatched PCR products using T7 endonuclease I to detect indel mutations
TENI-200	T7 endonuclease I assay kit, 200 rxns	Cleave mismatched PCR products using T7 endonuclease I to detect indel mutations

Table 1. Ordering options for the indelCheck™ insertion or deletion detection system. * Target site PCR primers are not included, but are available for purchase. On our web site.

At GeneCopoeia, our Genome Editing team has a wealth of expertise CRISPR and TALEN reagents in mammalian systems. We start at TALEN and CRISPR design and deliver sequence-verified plasmid DNA. We also offer functional validation services for your TALEN and CRISPR constructs, construct stable cell lines or transgenic mice containing your TALEN- or CRISPR-mediated modification of interest, and provide scientific consulting services to help you plan your projects. For more information, visit our website: http://www.genecopoeia.com, call 1-866-360-9531, or email inquiry@genecopoeia.com

References

Cong, *et al*. (2013). Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science* **339**, 819
Qiu, *et al.* (2004). Mutation detection using Surveyor™ nuclease. *Biotechniques* **36**, 702.