Comparison of the Guide-it Mutation Detection Kit with a CEL nuclease-based assay

Simple method to identify insertions or deletions in mammalian cells:
Amplify genomic regions directly from cells without the need for DNA purification, and detect mutations with the highly efficient Guide-it Resolvase enzyme

Faster and more efficient than CEL nuclease-based assays:
The Guide-it mutation detection protocol is several hours shorter, more sensitive, and less prone to non-specific cleavage

Introduction

Recently-developed genome editing tools such as zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system allow precise manipulation of virtually any gene. All of these editing techniques can be used to introduce double strand breaks at a target DNA sequence that are repaired by the error-prone non-homologous end joining (NHEJ) DNA repair pathway, resulting in introduction of insertion or deletion mutations (INDELs). Detecting these types of induced INDELs at target loci requires a simple and robust method.

Results

A PCR-based method to confirm the presence of mutations

Mutation detection is often based on PCR amplification of the region of interest and detection of mismatches in heteroduplexed DNA. With the Guide-it Mutation Detection Kit, the target sequence is amplified directly from cells, without genomic DNA extraction/purification (Figure 1, step 1). Then, the PCR products are melted and rehybridized, forming mismatched targets that can be cleaved by the Guide-it Resolvase (Figure 1, steps 2 and 3).

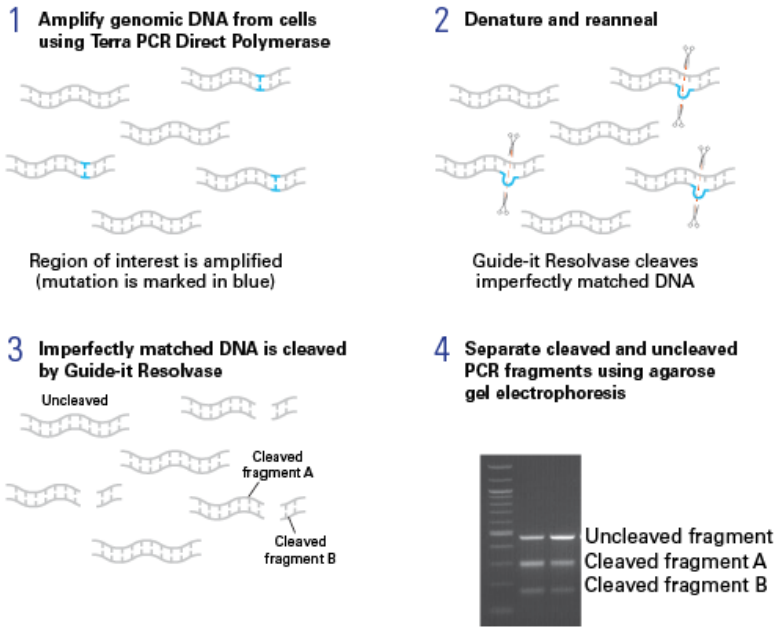

Figure 1. Overview of the Guide-it Mutation Detection Kit method to confirm the presence of mutations in genomic DNA.

Comparison of the Guide-it Mutation Detection Kit with a CEL nuclease-based assay

The key component of the Guide-it Mutation Detection Kit is the Guide-it Resolvase, a mismatch-specific nuclease that recognizes heteroduplexed DNA. This enzyme is more efficient and more robust than other similar nucleases, such as Cel1.To compare the Guide-it system and an assay based on CEL nuclease for detecting CRISPR/Cas9-introduced mutations in mammalian cells, 293T cells were transfected with plasmids encoding Cas9 and an sgRNA specific for the AAVS1 locus. Transfected cells harvested 48 hours post-transfection were mixed with untransfected cells at varying ratios (Figure 2, top). A DNA fragment containing the AAVS1 locus was generated by PCR using Terra Direct Polymerase, and the products were purified and cleaved with either Guide-it Resolvase (Guide-it Mutation Detection Kit) or the Cel1 enzyme (Company T). Mutations were easily discernible when using the Guide-it kit (Figure 2, bottom). In contrast, the CEL assay showed considerable smearing, making it difficult to determine cleavage efficiency and reducing the ability to detect lower levels of mutation (Figure 2, bottom).

	1	2	3	4	5	6
transfected	100	80	60	40	20	0
Non-transfected	0	20	40	60	80	100

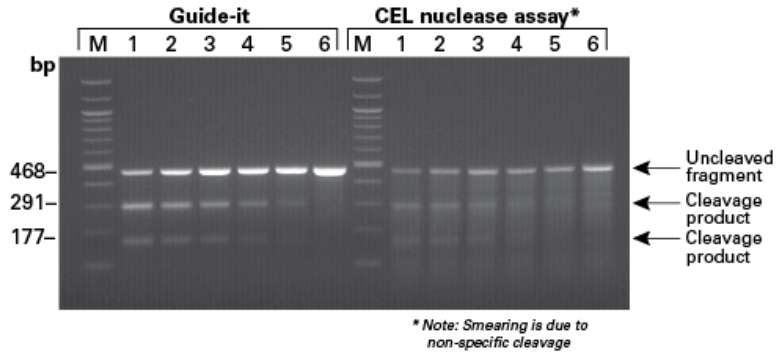

Figure 2. Comparison of the Guide-it Mutation Detection kit and a CEL nuclease assay for detecting CRISPR/Cas9-introduced mutations in mammalian cells. Mutations were easily discernible when using the Guide-it kit (estimation of cleavage, 1: 59%, 2: 46%, 3: 28%, 4: 15%, 5: <10%, 6: 0%). In contrast, the CEL nuclease assay showed considerable smearing, making it difficult to determine cleavage efficiency and reducing the ability to detect low levels of mutation.

Conclusions

The Guide-it protocol, which amplifies genomic regions directly from cells without the need for DNA purification, reduced assay time from the 6 hours required by existing mismatch detection protocols to just 3.5 hours. In addition, this protocol provided increased mutation detection efficiency and demonstrated less sensitivity to buffers used in the PCR reaction than existing protocols.

In summary, compared to a CEL nuclease assay, the Guide-it mutation detection protocol is several hours shorter, more sensitive, and less prone to non-specific cleavage.

Learn more about the Guide-it Mutation Detection Kit »

Related Products

Cat. #	Product	Size	License	Quantity	Details
631443	Guide-it™ Mutation Detection Kit	100 Rxns		*	

The Guide-it Mutation Detection Kit contains all the reagents needed for PCR-based identification of insertions or deletions generated during cellular non-homologous end joining (NHEJ) repair. The first step is the amplification of the putative target sequence directly from cells. This kit uses Terra PCR Direct Polymerase Mix and Buffer, so there is no need to extract genomic DNA from your cell population prior to amplification of your target sequence. The amplicon is then melted and hybridized to form the mismatched targets for cleavage by the Guide-it Resolvase. Sufficient material is provided for 100 amplification and cleavage reactions.

Documents	Components	You May Also Like	Image Data	Resources

631448	Guide-it™ Mutation Detection Kit		25 Rxns		*

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