ddPCR for Safety Monitoring in Gene Therapy (MEGA)

MEGA enables a quick and unbiased overview of gene editing outcomes after designer nuclease treatment in therapeutically-relevant cells.

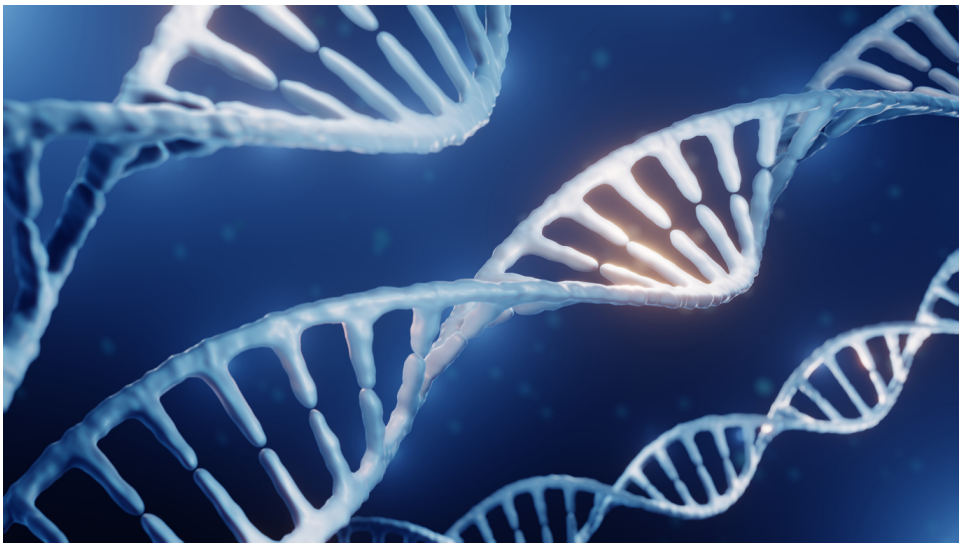


Image credit: I Stock (Artur Plawgo)

Background

Designer nuclease technology is becoming a standard procedure in many laboratories and it has revolutionized not only the basic biology research but also the diagnostic and the gene therapy field, leading to its application in several clinical trials. As gene therapies become more commonplace, it is likely that regulatory authorities will require more stringent and holistic genotoxicity evaluations to be undertaken as a pre-requisite for approval.

At present, designer nuclease activity evaluation is routinely performed with PCR approaches that amplify a specific region surrounding the cleavage site within the window range of 300 to 700 bp. These methods only the evaluate presence of small insertions and deletions (indels) but fail to detect large deletions or mutations that disrupt one primer binding site.

Other than the targeted sequence (On-target) editing evaluation, it is also necessary to assess the safety of the designer nucleases to verify the quality and quantity of chromosomal aberrations induced at ON- and OFF- target sites by double strand breaks (DSBs), as well as-

• Genomic integrity assays

• Chromosomal aberration assays

• Donor DNA homologous recombination and episomal assays

• Loss of heterozygosis assays

Technology overview

The inventors have developed a new method, “MEGA” (Multipurpose Editing Genotoxicity Assessment), which enables a quick and unbiased overview of gene editing outcomes after designer nuclease treatment in therapeutically-relevant cells. This methodology is based on droplet digital PCR technology (ddPCR) and enables the quantification of DSBs in the ON/OFF targeted sites, while discerning the large deletions and the chromosomal aberrations (such as translocations, inversions, unrepaired DSBs). It is also able to quantify the copy number variation (CNV) of the entire targeted chromosome or the possible loss of 5’ or 3’ chromosome arm portions with respect to the cleavage site. In case of a gene addition approach where a DNA donor template (either viral or oligonucleotide based) is utilized to knock-in genetic sequences at the ON-target site, this method can also detect and quantify the amount of integrated and episomal DNA fragment. In addition, MEGA can be used to provide new insights in the DNA repair kinetics in human primary cells by measuring the designer nuclease’s activity at different time points after treatment.

See figure 1.

Applications

The MEGA method can be implemented during the routine controls for designer nucleases activity in the following stages:

1. Screening of designer nuclease activity and safety in proof-of-concept studies;

2. Nuclease activity and safety assessment in pre-clinical studies, including in in vivo settings, in therapeutically-relevant primary cells;

3. Quality release criteria check in treated patient-derived cells pre-transplantation in ex-vivo gene editing approaches;

4. Post-transplant follow-up genotoxicity studies;

5. Determining copy number variation of specific loci, genomic portions or of entire chromosome portions overcoming existing standard techniques such as chromosome genomic hybridization (CGH); and

6. Routine genomic integrity and quantity check after genomic DNA extraction

Patents

• Multipurposing editing genotoxicity assessment PCT/GB2022/052772

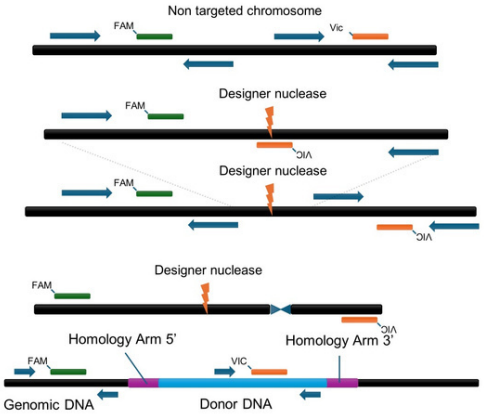
Seeking

Development partner, Commercial partner, Licensing

IP Status

Patent application submitted

ddPCR Based Genotoxicity Overview- MEGA



1st assay Controls
-Reference
-gDNA integrity

2nd assay ON
-Large deletions
-Indel

3rd assay Flanking
-Large deletions
-Chr. Aberration
-Open ends

4th assay LOH
-Chromosome LOH

5th assay HR
-Episomal donor
-KI %