Why do we need to carefully monitor the genomic integrity of iPSCs after CRISPR/Cas9-based gene editing?

The discovery and recent advances in the CRISPR/Cas9 gene-editing technology have profoundly reshaped the field of genome engineering and allowed the generation of a wide range of mutant cell lines at an unprecedented rate.

The delivery of the Cas9 nuclease and a specific guide RNA (gRNA) allow producing DNA double strand breaks at the target site. The subsequent endogenous repair mechanisms will lead to the desired genetic edits, essentially gene knockouts and DNA insertions.
By combining the CRISPR/Cas9 gene-editing technology with human pluripotent stem cells (hPSCs), new opportunities are created to study disease mechanisms and to develop innovative cell products, with huge therapeutic potential[1].

Impact on genomic stability

However, CRISPR/Cas9 gene editing in hPSCs can also pose a threat to the overall genomic integrity.

- Indeed, genetic abnormalities can be introduced directly by impaired repair processes or by a double strand DNA break induced by Cas9 at another locus in the genome (off-target effect).
- Moreover, CRISPR/Cas9 gene editing implies major sequential and potentially stressful events, such as cell transfection, flow cytometry-based cell sorting,

clonal expansion, drug selection, manual colony picking or single-cell dissociation.

- These procedures could favour the generation and selection of genetically abnormal hPSCs, with higher proliferation and survival capacities, or reduced differentiation potential. As they reflect a selection pressure, these defects are frequently observed in hPSC cultures and mainly consist in copy number variations[2-3].

iCS-digital™ PSC solutions

The iCS-digital™ PSC tests (kits and services) offer efficient and rapid methods to control the genetic integrity of CRISPR/Cas9-modified hPSC clones before their use for research purposes or clinical applications.

From one (kits) to three days (services), the "iCS-digital™ PSC-24 probes" digital PCR test allows detecting more than 90% of recurrent genetic abnormalities in hPSCs, including the sub-karyotypic 20q11.21 amplification (which represents >20% of recurrent genomic abnormalities in hPSCs)[3].

The iCS-digital™ PSC range includes other versions of this assay, with different coverage levels for specific needs.

Learn more about how it works

A risk of random transgene integration

One of the major drawbacks of the CRISPR/Cas9 technology is the risk of Cas9 unintended binding to off-target sites where complex genomic events may take place after DNA double-strand breakage.

- This can result in the integration of the transgene at a place different and distant from the selected target. This is particularly true when large transgenes are

delivered using plasmid-based systems.

- To determine whether DNA donor templates have been inserted at off-target sites, specific assays must be used to assess the number of transgenes integrated in the genome.

Ed-digital solution

The Ed-digital test (service only) relies on the digital PCR technology to detect the number of transgene copies present in the genome of gene-edited cells.

The Ed-digital test targets common resistance cassettes (puromycin, neomycin) and the fluorescent reporter genes mCherry and GFP. In less than 3 days, this test provides precise information on: the actual presence or absence of the transgene, the number of integrated transgenes and off-target genomic integration in the case of transgene copy number ≥3.

Learn more about how it works

Let's talk about your research projects and find with our team a tailored testing strategy to meet your needs.

Schedule a meeting

Sincerely,

Alicia Fabre
Marketing Project Manager
Email: alicia.fabre@stemgenomics.com
Phone: +33 985 036 160

1. Mianné J, Bourguignon C, Nguyen Van C, et al. Pipeline for the Generation and Characterization of Transgenic Human Pluripotent Stem Cells Using the CRISPR/Cas9 Technology. Cells. 2020;9(5):1312. Published 2020 May 25.
2. Assou S, Bouckenheimer J, and De Vos J. Concise Review: Assessing the Genome Integrity of Human Induced Pluripotent Stem Cells: What Quality Control Metrics? Stem Cells. 2018; 36: 814-821

3. Assou S, Girault N, Plinet M, et al. Recurrent Genetic Abnormalities in Human Pluripotent Stem Cells: Definition and Routine Detection in Culture Supernatant by Targeted Droplet Digital PCR. Stem Cell Reports. 2020;14(1):1-8

Visit our website

Stem Genomics, 80 Avenue Augustin Fliche, IRMB - Hopital St Eloi, Montpellier Cedex 5, Occitanie 34295, FRANCE, +33 985 036 160