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NGS applications to assess vector mediated genotoxicity in genetic medicine

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Currently, the development of gene editing (GE)-based cell and gene therapies involves the use a variety of NGS-based methods for detecting off-target editing. However, none of these has achieved gold standard status since all have certain limitations.

INDUCE-seq was specifically developed to overcome these. As a result, this has greatly improved the efficiency and precision of detecting off-target break sites in the genome following GE. At present all methods, including INDUCE-seq, generate hierarchical lists of potential off-target editing sites based on frequency of GE-induced break formation, coupled with breaks located at sites with homology to the guide sequence. These combined potential off-target lists are subsequently analysed to determine the mutation frequency found at each off-target site. Subsequently, these are compared to the mutation frequencies found in unedited (control) cells. This information is used to aid safe and efficacious guide selection to manufacture the cell and gene therapy product.

Here, we describe the use INDUCE-seq to report the detection of off-target editing for a range of different guides employed during CRISPR-Cas9 gene editing. Furthermore, we report the measurement of the corresponding mutation frequencies at these locations using error-corrected NGS by Duplex-seq. We reveal the relationship between the transient formation of editing-induced DNA breaks versus fixed mutational endpoints.

At present off-target break nomination still relies upon relatively simple frequency-based calling of off-targets, with arbitrary thresholds applied to generate hierarchical break lists. We extend our analysis of the genomic break data generated by INDUCE-seq to demonstrate that it is replete with additional genomic information. We show that the adaptation of AI algorithms and the development of additional bioinformatics pipelines make it possible to extract latent information from INDUCE-seq datasets that will advance the safe and efficacious design of future cell and gene therapies.

Keywords:
Gene editing; CRISPR-Cas; Off-targets; INDUCE-seq; AI.