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Measuring and monitoring structural variation associated with gene editing using directional genomic hybridization (dGH) and automated image processing

David Sebesta
KromaTiD Inc, USA

A number of widely used gene editing techniques, including CRISPR/Cas9, ZFNs, TALENs, and meganucleases rely on directed double-strand breaks and endogenous DNA repair mechanisms. Between repair mechanism failure and cellular damage induced by harsh editing system conditions, genomic structural changes are unavoidable. Therefore, it is critical to utilize techniques for the discovery and quantitation of genomic structural rearrangements in populations of cells both before and after editing. While individual errors are rare, even low prevalence errors or off-target effects pose risks to patients and require rigorous quantification and control for therapeutic applications. Directional Genomic Hybridization™ (dGH™) is a highly precise cytogenetic technique, enabling the direct visualization of genomic structural rearrangements of 5kb or less. Using the reference genome, dGH probes are designed against normal sequence and produced using single-stranded fluorescently labeled DNA. dGH probes are hybridized to prepared metaphase chromosomes and imaged, with a simple, accessible method. Structural variations from the reference genome are then easily visualized from the resulting signal. Assessment of cell lines or patient samples before and after gene editing elucidates the effects of the editing process on the desired edit site and identifies any off-target effects occurring above the baseline rate of the pre-edit sample. In this poster, we illustrate how dGH is used to discover and detect structural rearrangements missed by NGS and other methods that use pooled DNA, precisely detecting the prevalence of multiple and variable rearrangements occurring on a cell by cell basis. We describe our progress toward automated image analysis in control and edited cell populations. Process development such as AI-based image analysis and scoring will be presented.

Biography

KromaTiD provides innovative solutions for the discovery, detection and quantification of genomic structural variations. With our dGH™ platform, we are able to observe gene editing associated rearrangements in their chromosomal structural context. The data is complementary to sequencing and our assays provide researchers and innovators in gene editing with an additional dimension of data to support optimization of process variables, quantitation of structural offtarget effects, and tracking of durable genomic changes over time.

David.sebesta@kromatid.com

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