Directional Genomic Hybridization (dGH™) for Single-cell Detection and Quantitation of Pre-existing and Editing-Associated Structural Variation in Edited Cell Populations. Development of Automated Image Analysis.

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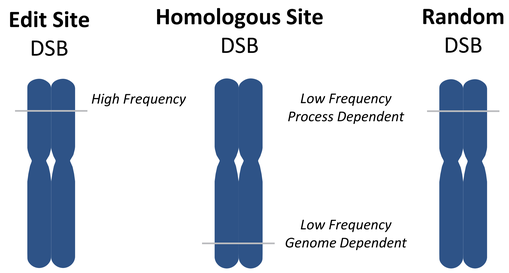
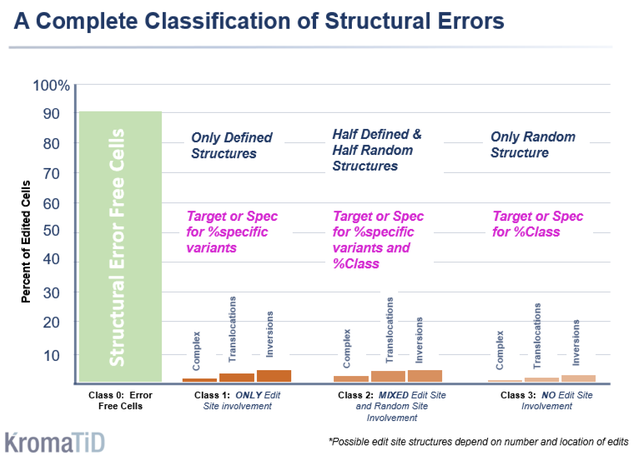
MIS-REPAIRS VS MIS-EDITS: Batches of cells edited by CRISPR/Cas9, zinc finger nucleases, meganucleases, or any technique relying upon double strand breaks (DSBs) will contain unwanted structural variants or errors arising from the mis-alignment of ends during repair by the various endogenous mechanisms. Structural errors arising from mis-repairs are distinct from the more commonly assessed off-target effects associated with cleavage at homologous sites or through faulty edits. Whereas homologous sites and faulty edits typically result in small indels, mis-repair of DSBs leads to large structural variations such as inversions and translocations.

EVERY BATCH OF EDITED CELLS will contain cells with mixtures of high frequency edit site breaks and lower frequency homologous and random site breaks. Because the location and number of breaks varies from cell to cell, editing leads to complex, heterogenous structural errors.

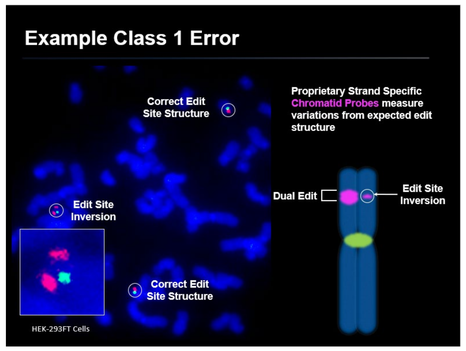


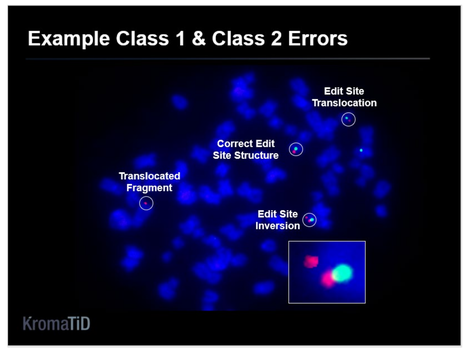
DIRECTIONAL GENOMIC HYBRIDIZATION (dGH™) CAN MEASURE STRUCTURAL VARIATION resulting from mis-repair of double strand breaks (DSB) at the edit site and any other location in the genome. Because the mechanism of break formation leads to different on- and off-target errors, we have developed an error classification system that can inform editing process control, as well provide a framework for understanding patient risks.



Measurement of Class 1 & 2 Structural Errors with Targeted dGH

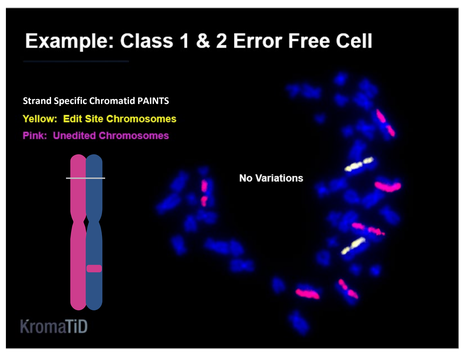
TARGETED DGH is uniquely suited to characterize complex, heterogenous variation involving an edit site or sites. Unidirectional, highly specific fluorescent probes enable detection and quantitation of inversions, translocations or other complex edit site-associated structural changes.

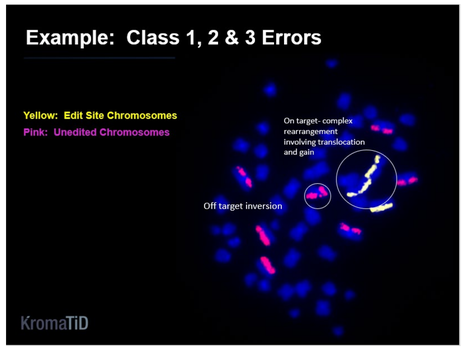




Measurement of Class 1, 2 & 3 Structural Errors with de Novo dGH

DE NOVO DGH: In this example, human CD4+/CD8+ T-cells were edited with CRISPR-Cas9 ribonucleoprotein (RNP) complexes. The edited chromosome is painted yellow (2 homologs) and 3 unedited chromosomes (2 homologs each) are painted pink. In the cell on the lower right, a complex edit site-associated mis-repair involving two edited homologs and a fusion at the edit sites (along with copy number gain) can be observed (Class 1 error), as well as an inversion in one homolog of an unedited chromosome (Class 3 error).





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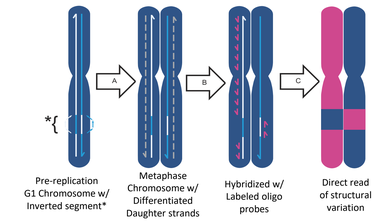
dGH 2.0: De Novo Genome Wide Measurement of Structural Variations

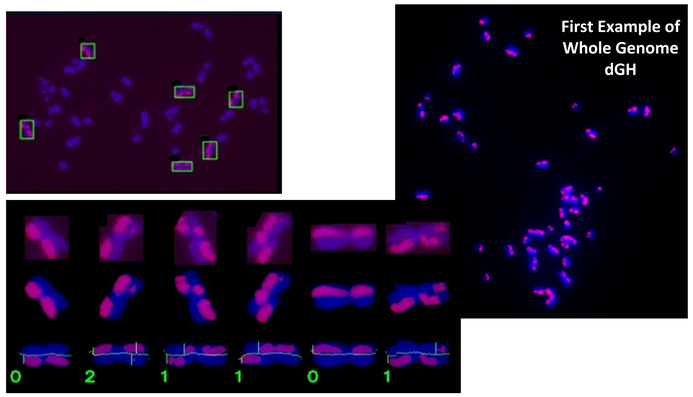
DE NOVO DETECTION of low prevalence structural variations (e.g., <0.1%) requires the analysis of thousands of individual cells per sample**.** We are using machine learning and AI image analysis to efficiently screen samples for structural variants and identify breakpoints



A) Cell Division and dGH sample prep

B) Daughter strand degradation (UV) and chromatid painting

C) Imaging and scoring of structural changes



NEXT STEPS: By implementation of available sample handling laboratory automation, along with automated image analysis and scoring, a whole genome, high-throughput dGH-based platform will be available as an orthogonal, complementary and synergistic analytical technique to sequencing-based options such as Circle-Seq™, Change-Seq™ and UDiTaS™.