Cytogenetics-based Tools for Process Characterization, Optimization and Control of CRISPR/Cas9 (or any other) Gene Editing

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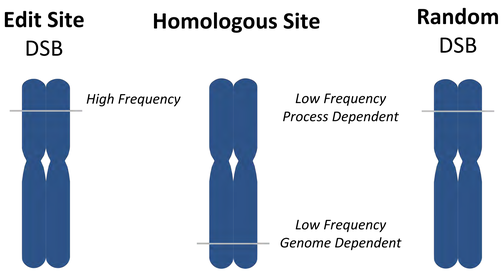
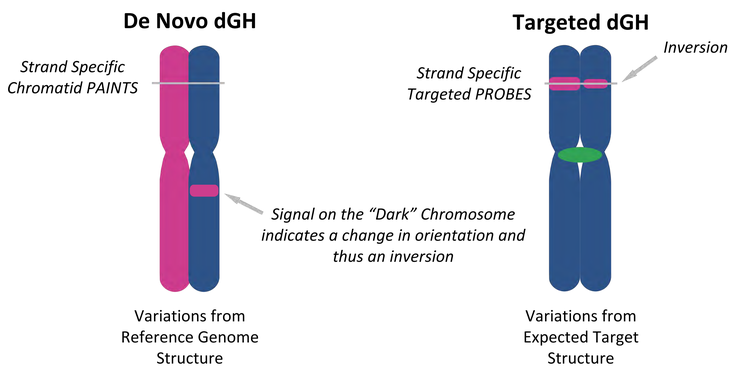
dGH for Gene Editing

Structural variation resulting from DNA mis-repair occurs regardless of the editing method used. Directional Genomic Hybridization or dGH can discover and track pre-existing structural variation, as well as structural genomic changes resulting from faulty repair of double strand breaks (DSB) at the edit site, between the edit site and other locations, and variation at random locations in the genome.

Directional Genomic Hybridization (dGH), developed originally as a sensitive biodosimeter, is uniquely suited to characterize the complex variation associated with editing.

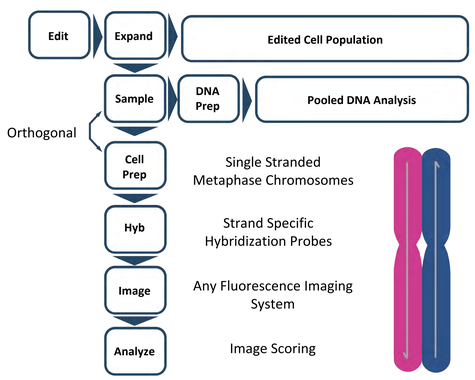


Single Cell Analysis Whole Genome

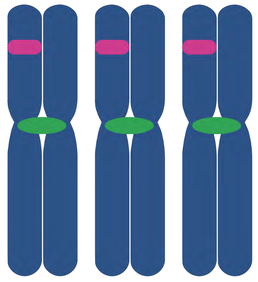


dGH Integrates with Gene Editing Workflows:

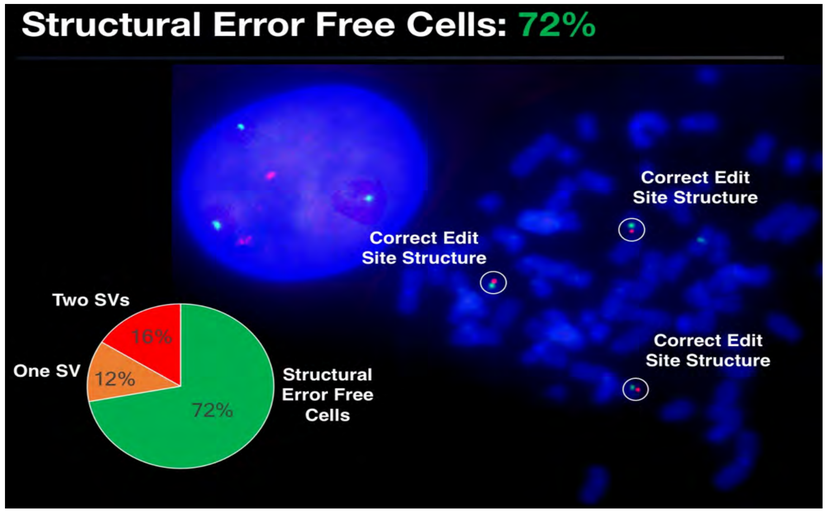
- dGH is Orthogonal to Sequencing and other techniques relying on pooled DNA
- dGH can measure baseline rearrangement in subjects and cells



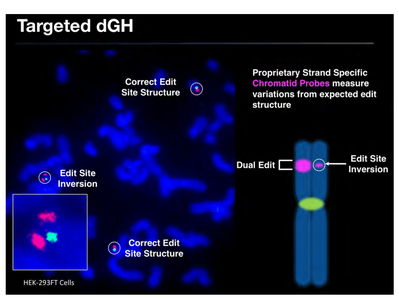
Structural Variants

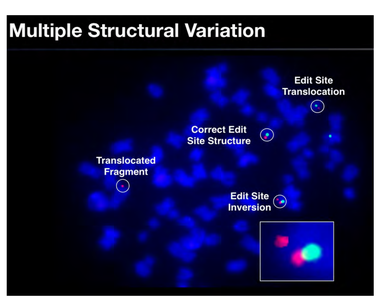


A correctly edited cell will contain 3 copies of C17 with a solid red signal on one chromatid and a green CEP signal (left), plus one copy of C17, exhibiting the stable p53 deletion (no red signal) with only the green signal (right).



HEK293FT cells were transfected with CRISPR-Cas9 and guide RNAs for two different sites in the p53 gene, then edited cells were enriched via flow cytometry. HEK293FT has four copies of C17—one with a stable p53 deletion which is not edited—leading to a minimum of six double-strand breaks that must be repaired correctly to generate an error free edited cell (above).

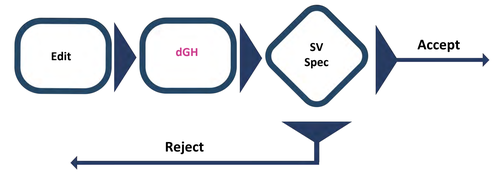




Optimization and Control

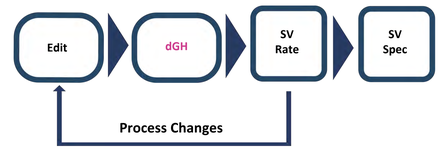
dGH analysis provides useful aggregate parameters to qualitatively and quantitatively characterize edited cell populations.

Quality Control

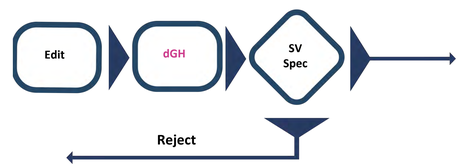


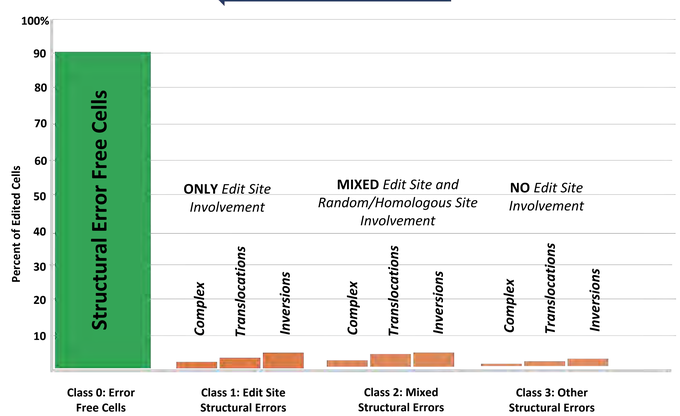
dGH can be used as a functional test for raw material quality, as a method to optimize gene editing process variables and is a necessary final quality control measurement for *ex vivo* edited cells

Optimization



Material Qualification





**Possible edit structures depend on number and location of edits*