Measuring CRISPR-Cas9 Genomic Structural Errors with Directional Genomic Hybridization™

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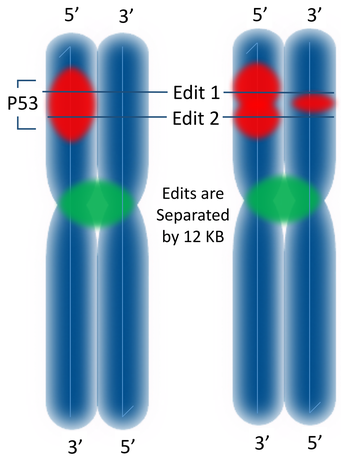
Structural variants, such as translocations and inversions, are unavoidable genome editing errors. Such rearrangements result from faulty repair of the double-strand breaks, which are intentionally (and unintentionally) induced in every gene editing system, including those with the highest therapeutic potential; e.g., CRISPR-Cas9, TALEN, and ZFN.

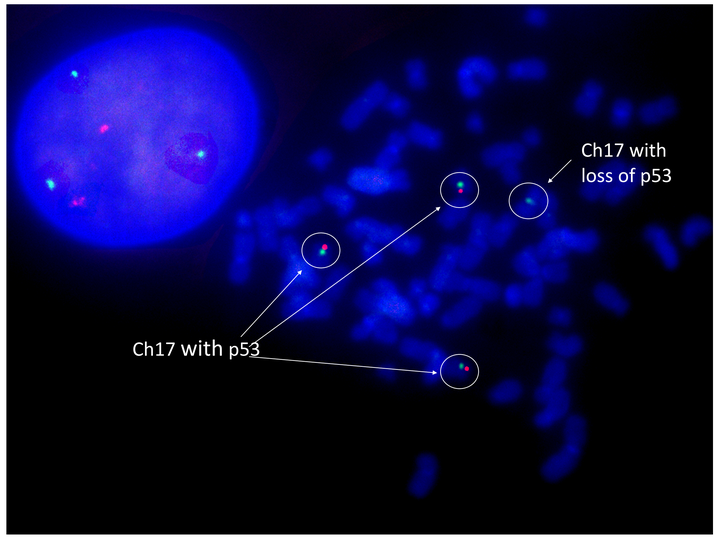
Because mis-repair can occur at any DNA double-strand break, an individual cell may contain more than one error and an edited population may contain a variety of structural variants. In a well-controlled editing process, these individual errors are rare, but in total they can affect a meaningful fraction of the edited cells.



This diagram illustrates relationships between the target p53 gene, the dual CRISPR-Cas9 edit sites, and the hybridization patterns of dGH probes.

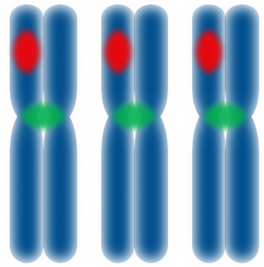
For this assay, the strand-specific red probe intentionally spans the entire p53 gene plus upstream and downstream regions. The green probe is a centromere enumeration probe used to identify chromosome 17.

When two edits are performed to the same chromosome, as here, mis-repair of the edit site breaks can yield an intra-chromosomal inversion. With dGH, these undesired inversions are readily detected as a signal "switch" to the opposite sister chromatid.

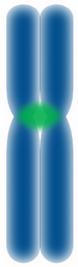


HEK293FT cells were transfected with CRISPR-Cas9 and guide RNAs for two different sites in the p53 gene, then edited cells were enriched via flow cytometry. HEK293FT has four copies of C17—one with a stable p53 deletion which is not edited—leading to a minimum of three double-strand breaks that must be repaired correctly to generate an error free edited cell (above). By combining dual edits with an extra editable chromosome, we have developed a model system enriched in editing errors stemming from mis-repair of induced double-strand breaks.

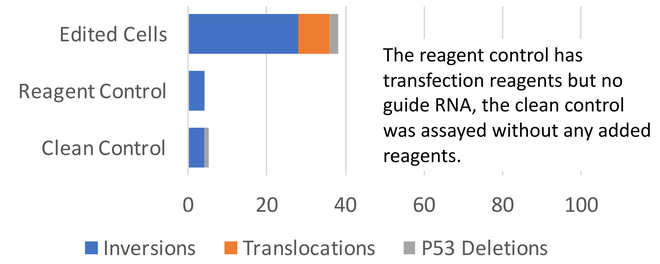
Methods of measuring off-target effects and errors that rely on pooled DNA and bioinformatic reconstruction of structure, such as NGS and PCR, are not suitable for quantitating the heterogeneous populations of low prevalence and complex structural variation characteristic of edited cell populations. Here, we present a cell-by-cell approach based on directional genomic hybridization (dGH) that readily identifies genome editing induced 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).



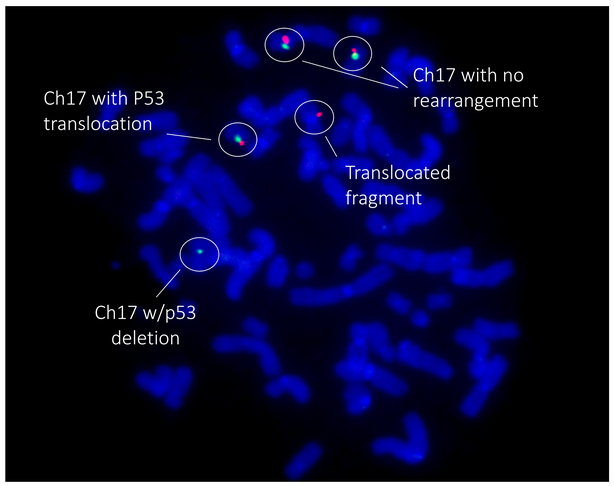
Percentage of Cells with Structural Errors



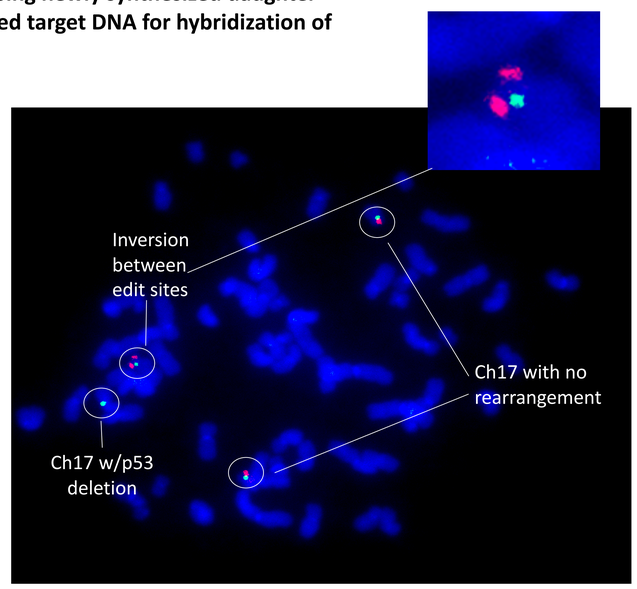
The reagent control has transfection reagents but no guide RNA, the clean control was assayed without any added reagents.

The graph above illustrates the predominance of inversions between the dual p53 edit sites. Translocations result from mis-repair of an edit site with an additional random double-strand break, and are thus less common. With a minimum of three double-strand breaks available for mis-repair, cells with more than one structural error are common in this model.

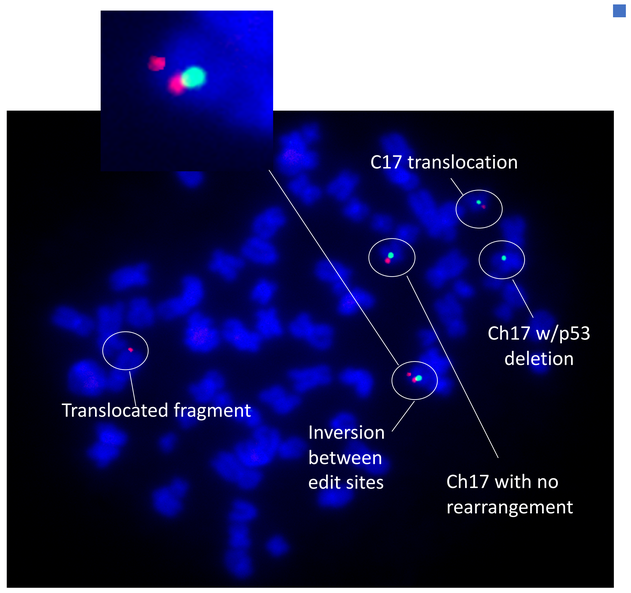
Directional Genomic Hybridization (dGH) is a single cell, cytogenetics-based method for direct visualization of structural rearrangements. dGH involves stripping newly synthesized daughter strands from metaphase chromosomes, yielding single-stranded target DNA for hybridization of single-stranded directional probes.



Translocations are a common structural error as seen here on one copy of C17. *The chromosome receiving the translocated fragment is not identified. The chromosome with the p53 deletion and two copies of C17 appear as expected for a correctly edited chromosome.*



Inversions involving the p53 site are a very common structural error shown here on one homolog of C17. *The chromosome with the p53 deletion and two copies of C17 appear as expected for a correctly edited chromosome.*



As expected, complex rearrangements involving several chromosomes are also common in this model system. This cell contains both a translocation and an inversion to the edit sites. *The chromosome receiving the translocated fragment is not identified.*

Structural Errors per Cell