KromaTiD

Direct, Definitive Genomics

Measuring and Monitoring CRISPR-Cas9 Off-Target Effects with Directional Genomic Hybridization™ (dGH™)

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Widespread adoption of continually improving gene editing technologies such as CRISPR-Cas9, TALEN, and ZFN has led to exciting new developments in both research tools and potential therapeutics. However, editing errors, including off-target effects and random structural variation, result from faulty repair of double-strand breaks in every gene editing system. Some of these effects can be anticipated by analyzing homologous regions in the genome, but the editing process introduces the mechanism for double strand breaks at random locations, and of mis-repair at the target site. Off-target and mis-repair includes single base changes, as well as larger structural variants in a low percentage of edited cells. Though individual errors are rare, their accrual in a population can encompass a significant portion of the edited cells. Therefore, editing systems must be optimized to minimize rates of structural variation. In therapeutic applications, even low prevalence errors and off-target effects pose potential risk to patients and must be quantitated and controlled. Methods of measuring off-target effects and errors that rely on pooled DNA or bioinformatics, such as NGS and PCR, are not suitable for quantitating the low prevalence, random, variable, and complex structural variation characteristic of edited cell populations.

Directional Genomic Hybridization (dGH) is a cytogenetic method for direct visualization of structural rearrangements such as inversions and translocations (Figure 1). dGH uses single cell analysis to quantitate individual low prevalence structural variation across many cells in edited cell populations. Assessment of cell lines or patient samples before and after gene editing identifies effects of the editing process - both the desired edit, and any off-target effects occurring above the baseline of the pre-edit sample. Here we describe the quantitative measurement of off-target effects in a model system. HEK293FT cells were transfected with plasimid-expressing Cas9-RFP and guide RNA in U6-gRNA:PGK-puro-2A-tagBFP plasmid backbone, for two different sites in the p53 gene, and the edited cells were sorted via flow cytometry. These cells were expanded and assessed for structural variation in the p53 region by dGH assays targeting the edit sites and the immediate surrounding sequence (Figure 2). Resulting structural variations are summarized for the system before editing (control), in cells that had been treated with CRISPR reagents but omitting gRNA (reagents-only control), and after editing. Structural rearrangement rates to the p53 region, including translocations, inversions, and deletions of the entire region, were measured in the CRISPR-Cas9 edited cells at elevated rates above both the control and the reagent-only control (Figures 4 and 5).

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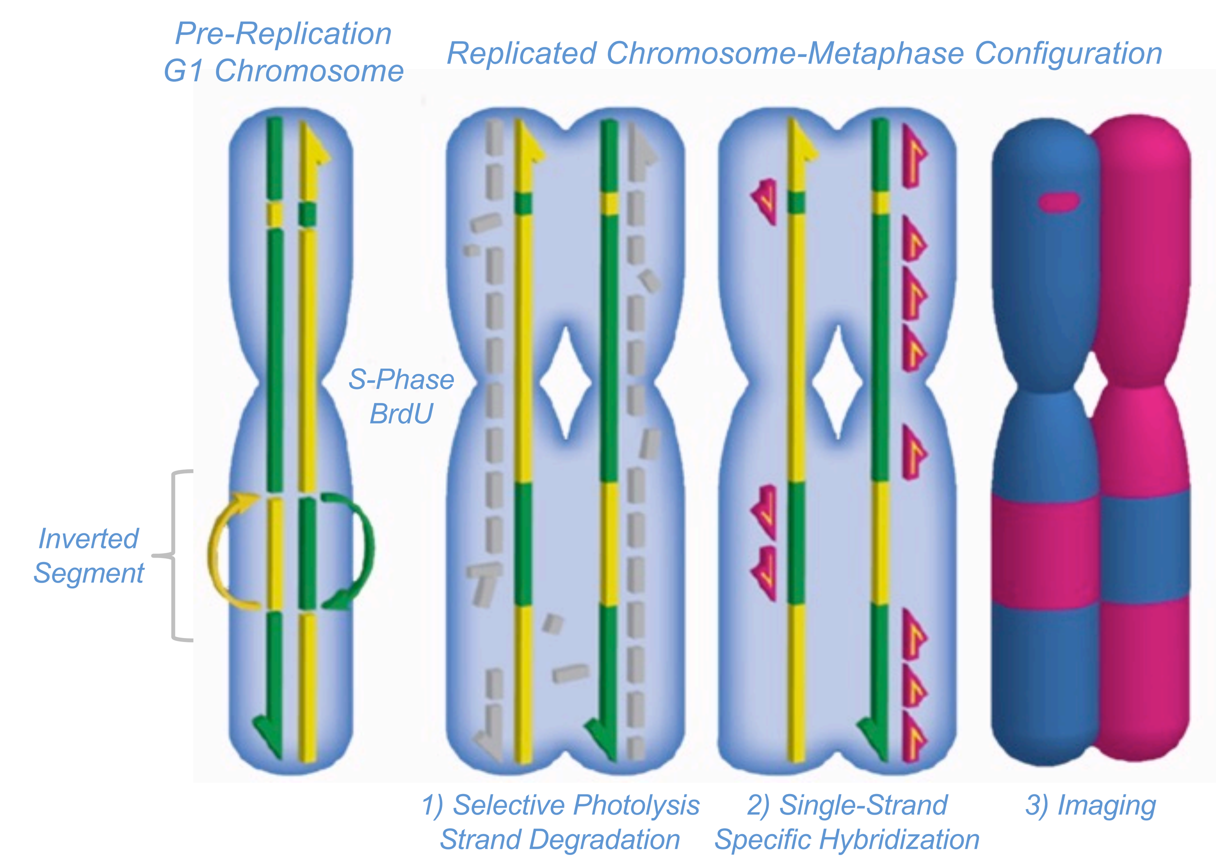

Figure 1: dGH sample preparation and assay method description

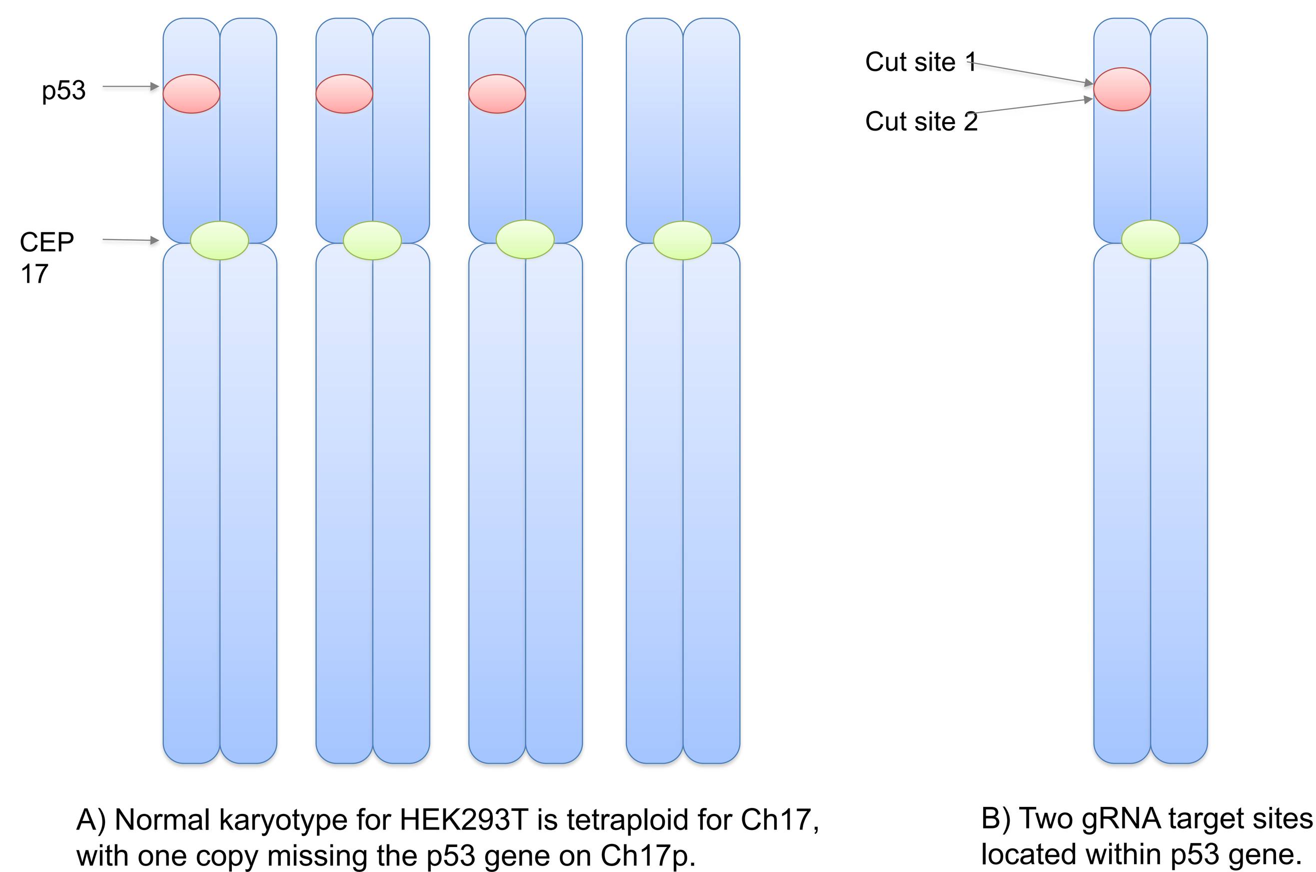

Figure 2: A) Normal karyotype for Ch17 in HEK293T cells B) Location of guide RNA targets for CRISPR-Cas9 edit

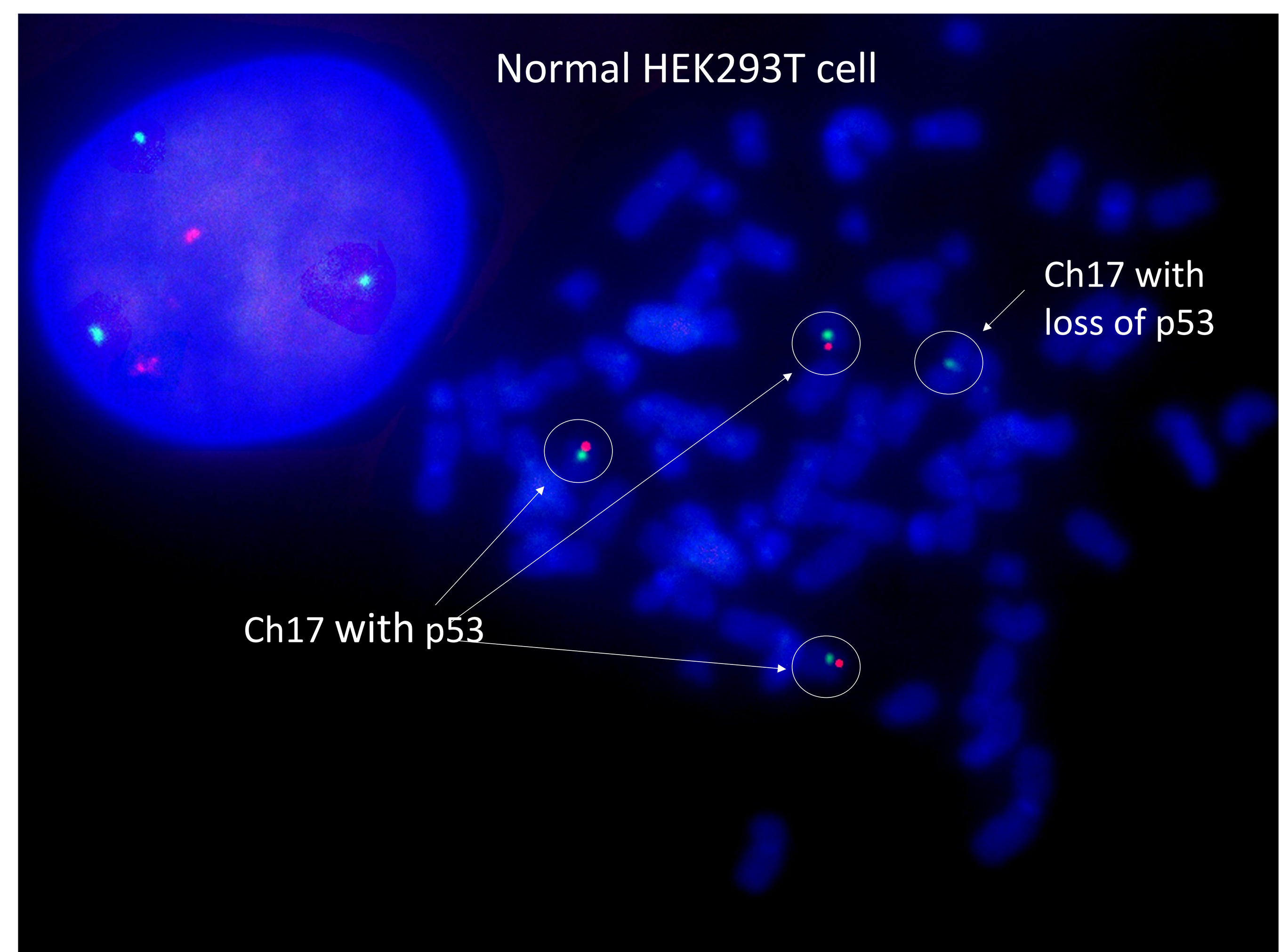

Figure 3: dGH assay for p53 in a normal HEK293T cell

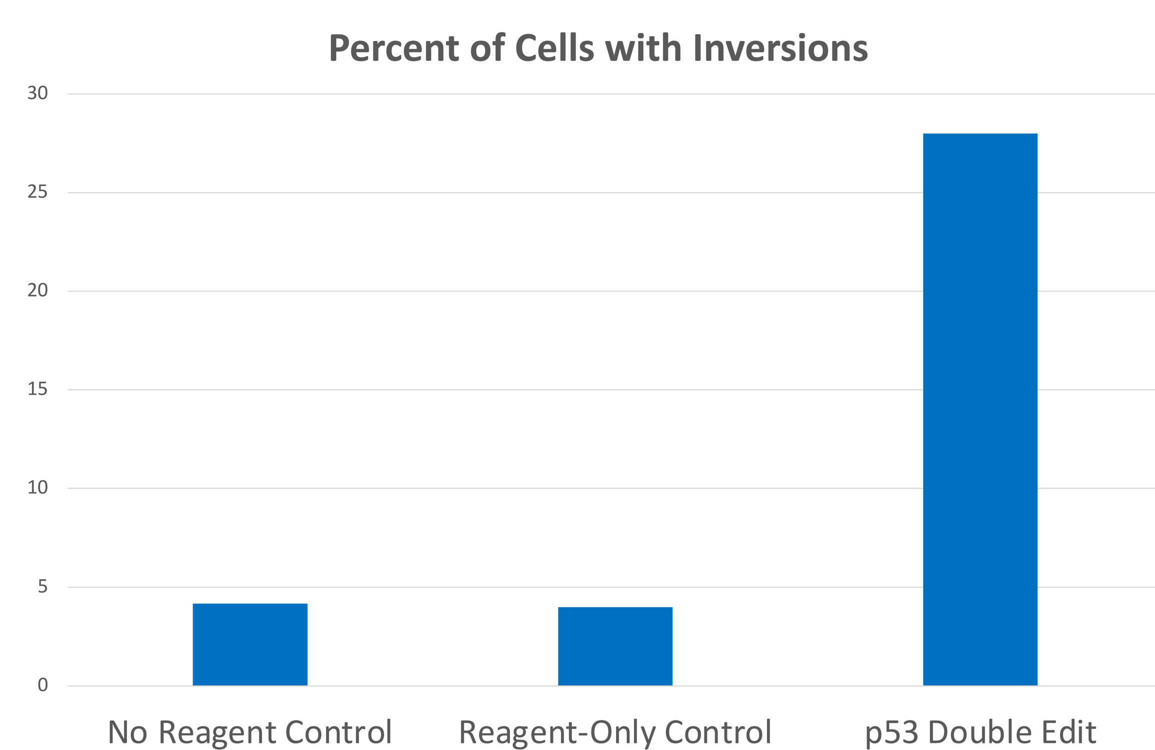

Figure 4: Assay results; percent of cells scored with an inversion in the p53 probe region.

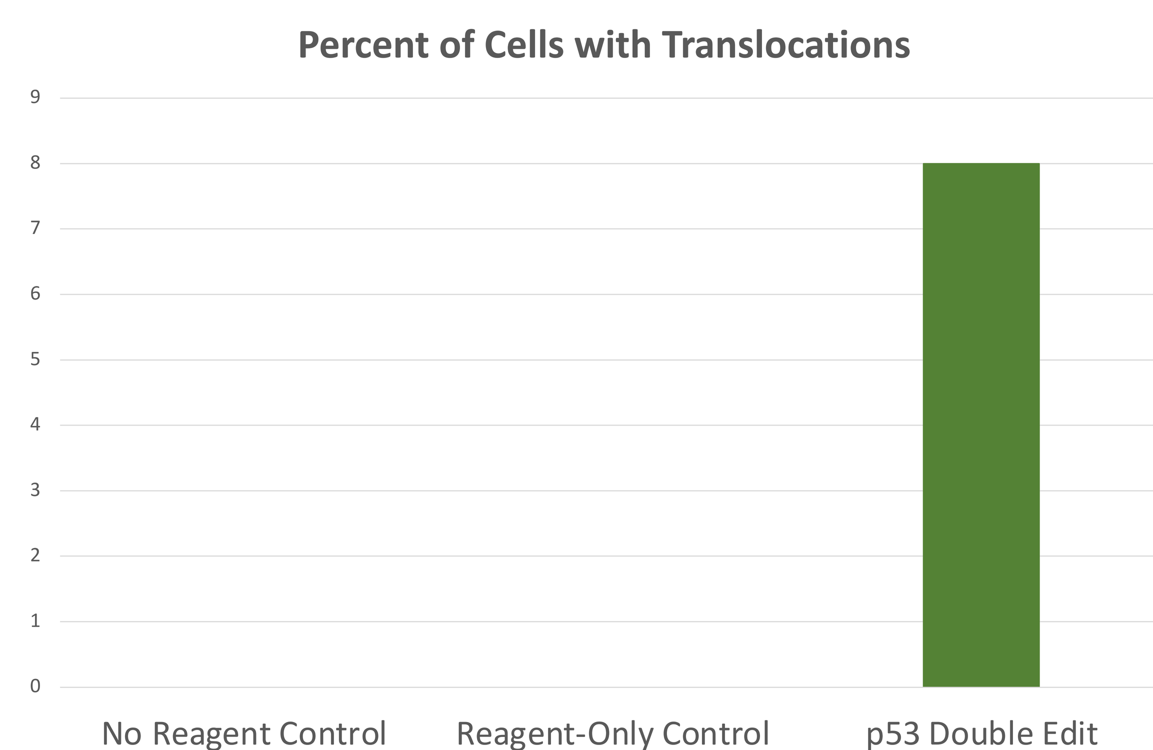

Figure 5: Assay results; percent of cells scored with a translocation in the p53 probe region.

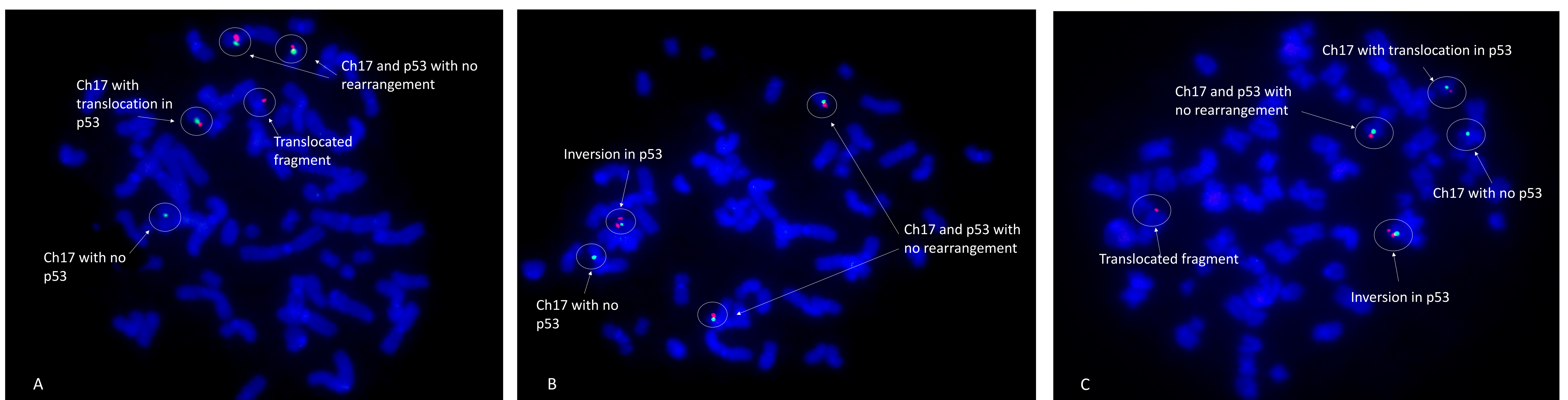

Figure 5: Example images of rearrangements involving the p53 probe in edited HEK293T cells. A) A translocation with a breakpoint in the p53 region on one copy of Ch17. B) An inversion in the p53 probe region on one copy of Ch17. C) An inversion in the p53 probe on one copy of Ch17, and a translocation with a breakpoint within the probe region on another copy of Ch17.

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