SAFER-Detection for Efficient Interrogation of DNA Rearrangements in Gene-edited Human Cells

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Summary

An essential criterion for evaluating the safety of human genome editing products is verification of genomic integrity. This includes an assessment of large insertions or deletions, integration of exogenous DNA, and the potential for oncogenicity or insertional mutagenesis. In this study, we introduce SAFER-Detection (Selective Amplification for Efficient Rearrangement Detection). SAFER-Detection is a tagmentation and next-generation sequencing based method designed to enable quantitative detection of chromosomal rearrangement breakpoints with single base resolution. The method is capable of classifying rearrangements resulting from on-target and off-target editing by programmable nucleases such as CRISPR/Cas and TALENs. SAFER-Detection using Cas9 with a CCR5 guide RNA readily identified intra-chromosomal deletions, insertions, and inversions between the on-target site (CCR5) and off-target or homologous sites in a nearby homolog (CCR2). Inter-chromosomal translocations between the CCR5 target site and off-target sites on chr1 and chr13 were also captured and further validated by PCR. SAFER-Detection exhibits high sensitivity in detecting intra-chromosomal and inter-chromosomal rearrangements mediated by off-target activity or by homologous recombination and is applicable to samples containing low cell numbers. When combined with a sensitive off-target nomination technology such as ONE-seq, SAFER detection provides a valuable method to assess the risk of chromosomal rearrangements in therapeutic genome editing.

Graphic abstract of SAFER-Detection

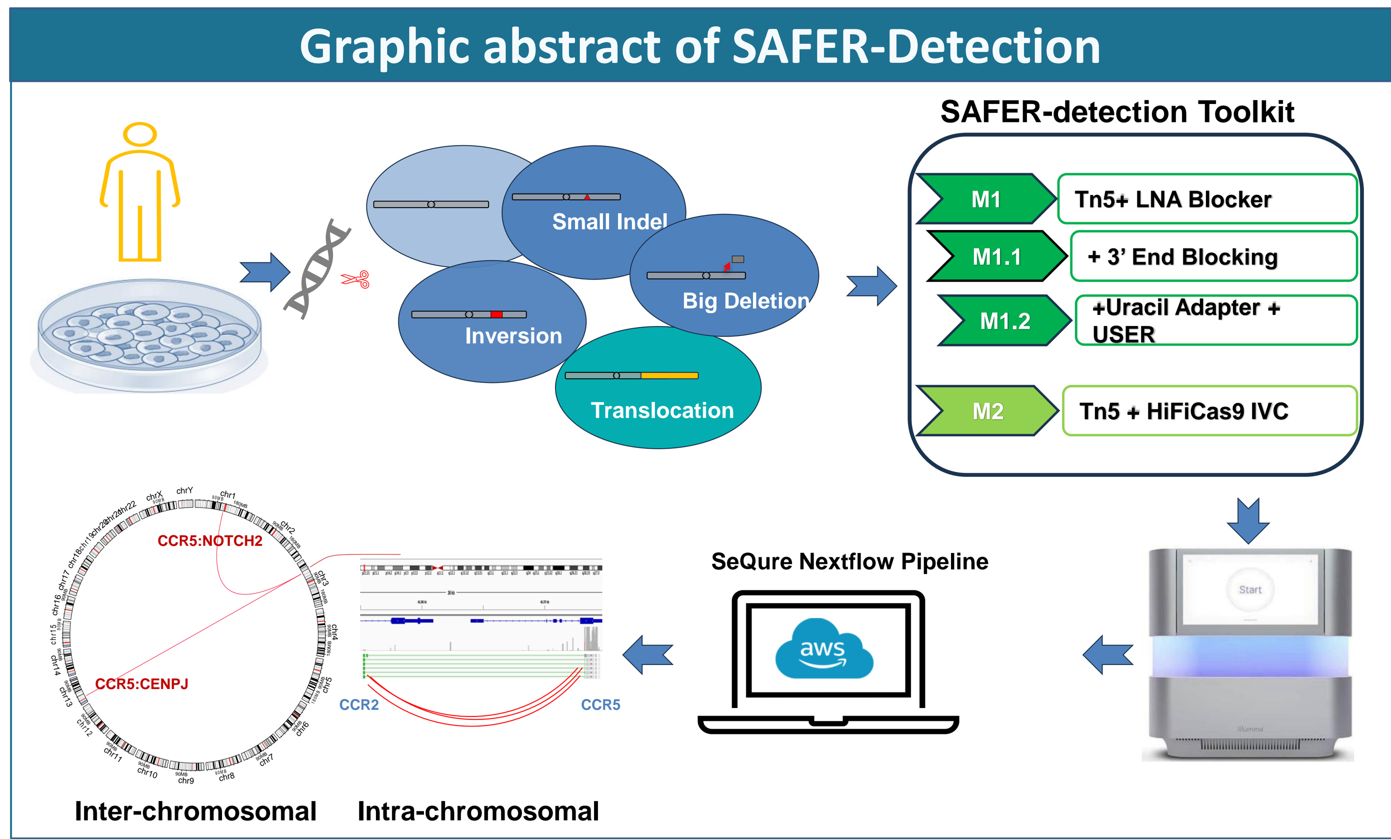


Figure 1. SAFER-Detection Strategy (Method 1)

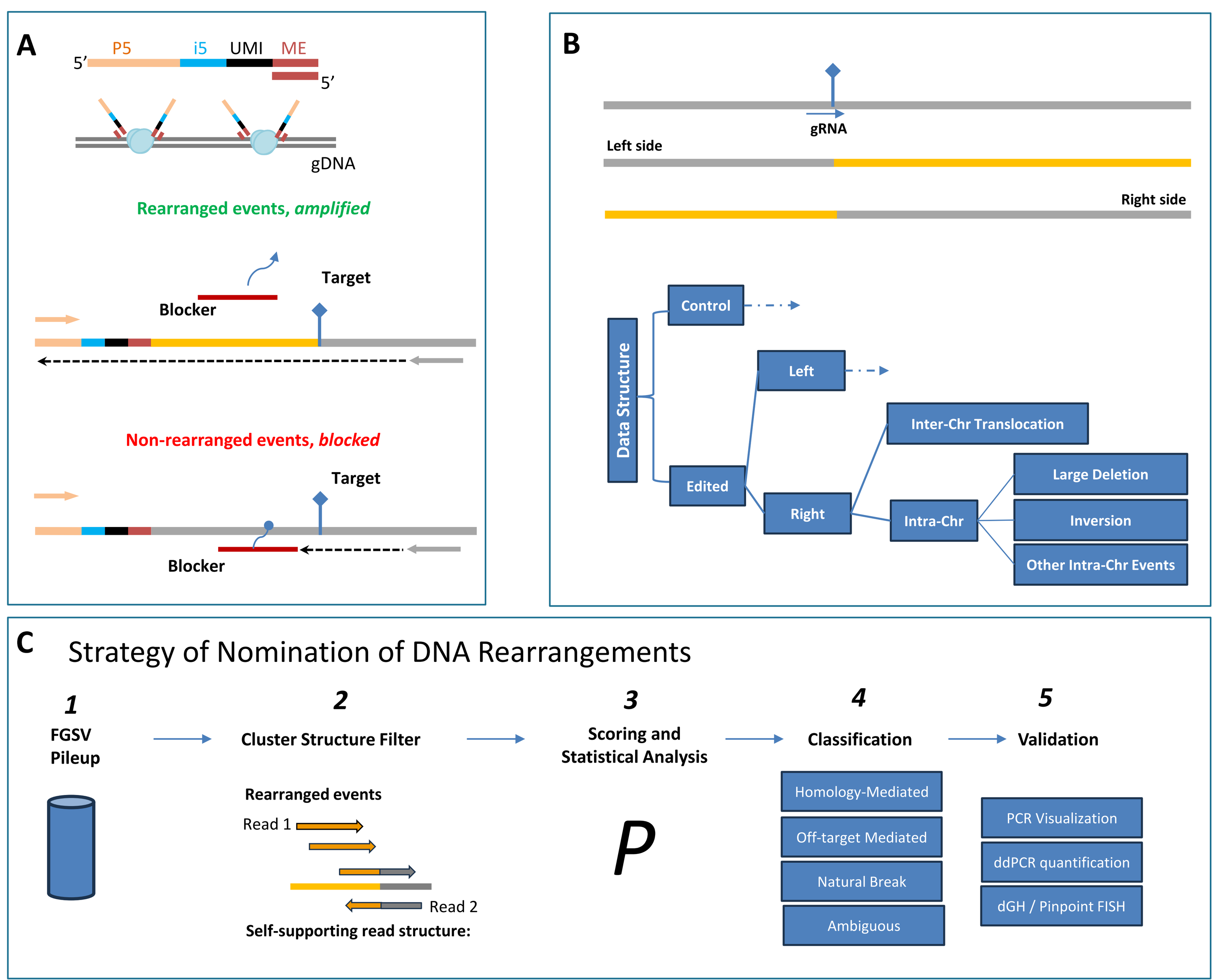
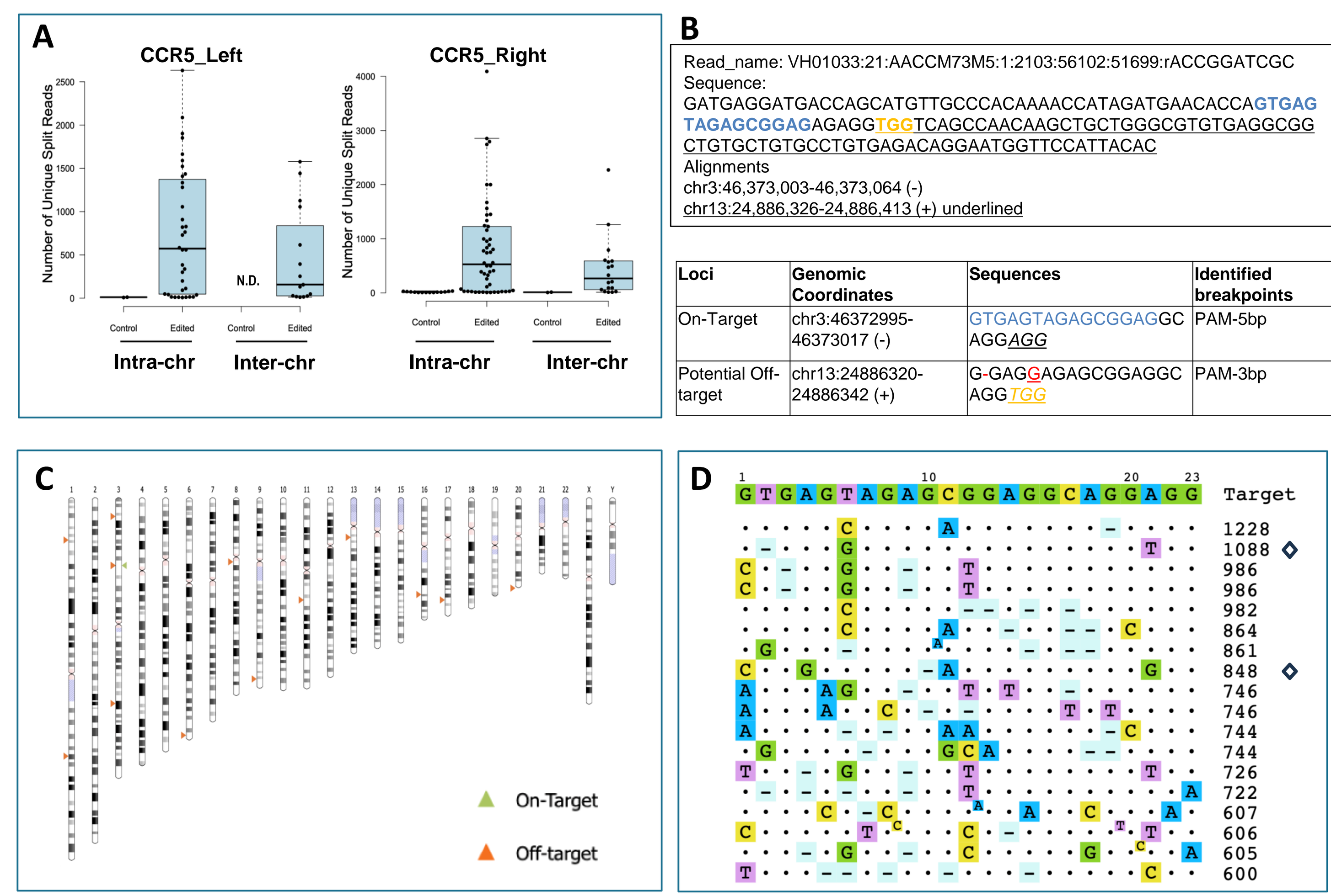


Figure 1. Schematics depicting key components, library prep, data structure, rearrangement grouping, and validation process. A. (upper panel) Tn5 loaded with adapters containing UMI and i5 index. (lower panel) Targeted/rearranged regions are amplified while unedited/small-indel regions are suppressed by an LNA blocker. **B.** Data structure for SAFER-Detection. Potential rearrangements from the left and right sides of the target locus are classified and grouped for analysis. **C.** Strategy for rearrangement event nomination. 1. Reads with potential rearrangements are grouped by FGSV pileup. 2. Hits are filtered by the split-read structure of read 2 and the distance of read 1 to the breakpoint. 3. Hits are scored and evaluated against random sampling from the whole genome. 4. Homology search and off-target site search near the breakpoints are performed to enable classification. 5. Events of interest are then validated using PCR, dPCR, or cytogenetic methods.

Figure 3. Validation of selected intra-chromosomal rearrangement >>>> A. (upper panel) Integrative Genomics Viewer (IGV) plot illustrates the distribution of paired reads mapping to *CCR5* and *CCR2*. (lower panel) Schematic depiction of tested inter-chromosomal translocation events for PCR validation. Green arrows indicate the target region primer; Red arrows indicate the primers tested at the rearranged locus. **B.** PCR validation of rearrangement events with bands amplified at the expected sizes indicated by red arrowheads.

Figure 2. Nomination of DNA Rearrangements



Loci	Genomic Coordinates	Sequences	Identified breakpoints
On-Target	chr3:46372995-46373017 (-)	GTGAGTAGAGCGGAGGC AGGAGG	PAM-5bp
Potential Off-target	chr13:24886320-24886342 (+)	G-GAGGAGAGCGGAGGC AGG TGG	PAM-3bp

Figure 2. Nomination of DNA rearrangements and DNA breakpoints. A. Box plots of DNA rearrangement events captured for the left and right side of a target site in CCR5. Events were filtered by the number of unique split reads (N.D., none detected). **B.** A representative read displaying the split structure, junction, and actual position of DNA breakpoints. **C.** Ideogram of potential breakpoints caused by off-target editing in genome. **D.** Breakpoint site alignment. The reference CCR5 target site is shown at the top. Mismatched nucleotides and indels are highlighted. The alignment score is listed on the right. Diamonds indicate off-target sites detected at validated inter-chromosomal translocation breakpoints.

Figure 3. Validation of Selected Intra-chr Rearrangements

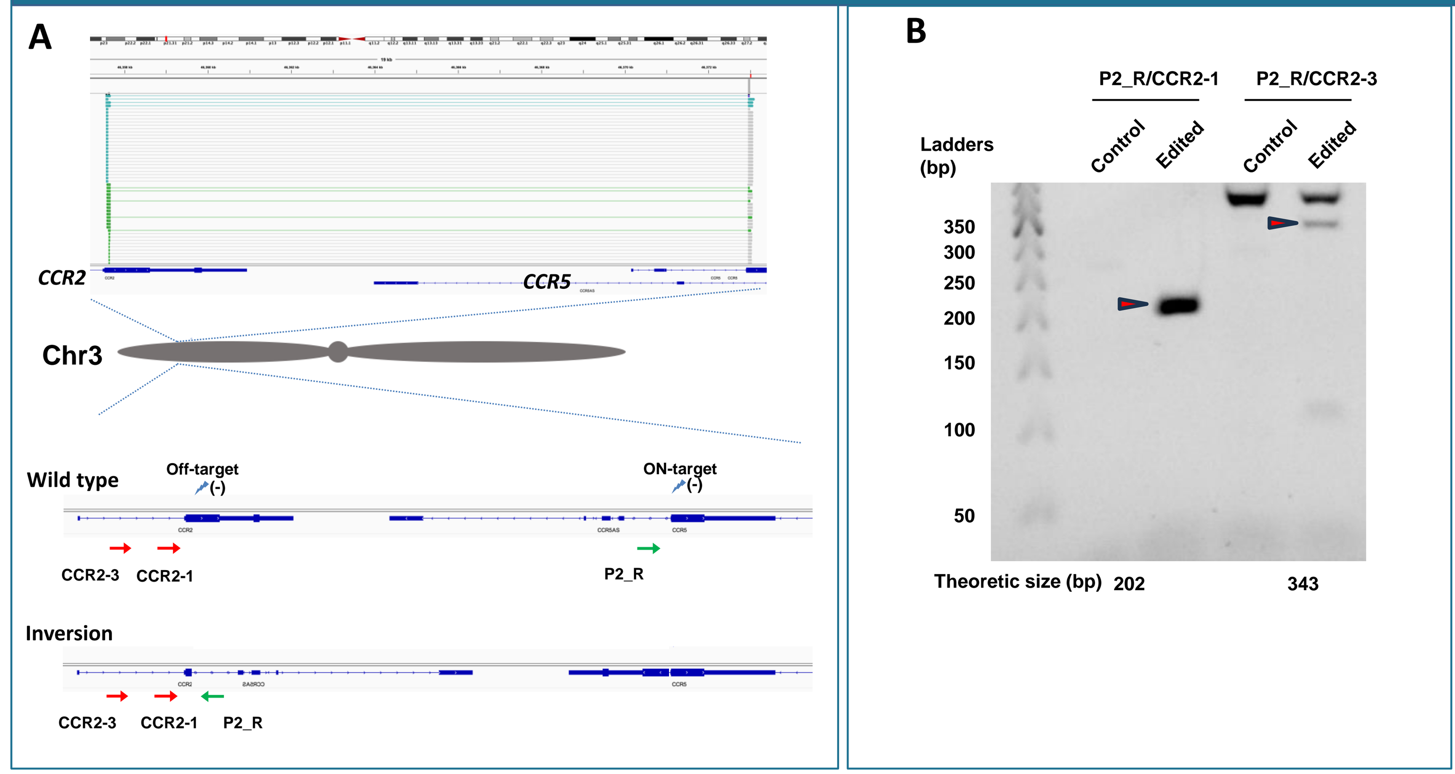


Figure 4. Validation of Selected Inter-chr Rearrangements

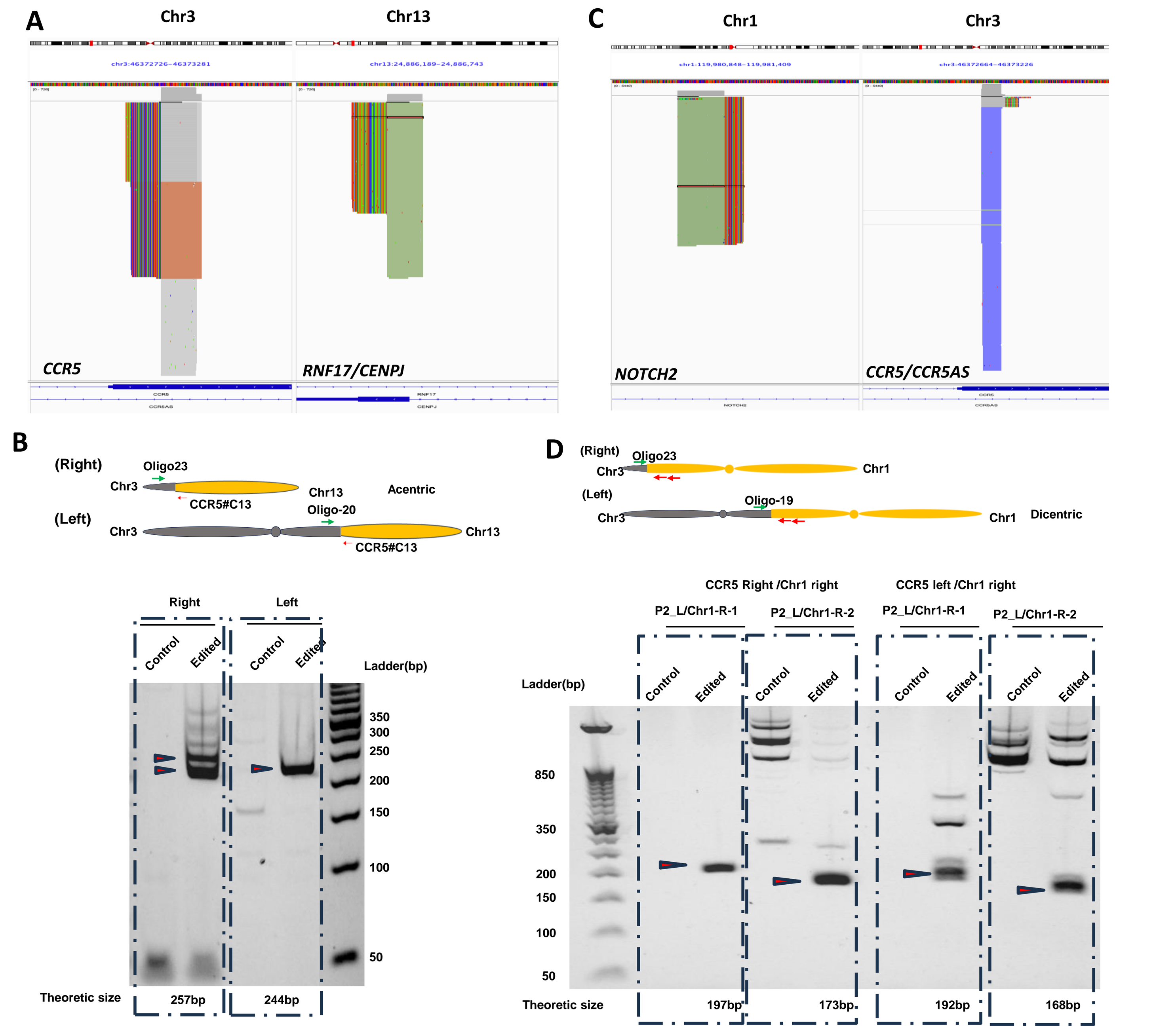


Figure 4. Validation of selected inter-chromosomal translocations. A. IGV plots illustrate paired read distribution at *CCR5*/Chr3 and *RNF17*/Chr13. **B.** (upper) Schematic depiction of inter-chromosomal translocation event; (lower) PCR validation of rearrangement events with bands amplified at expected sizes indicated by arrowheads. **C.** IGV plots illustrate paired read distribution at *CCR5*/Chr3 and *NOTCH2*/Chr1. **D.** (upper panel) Schematic depiction of inter-chromosomal translocation event; (lower) PCR validation of rearrangement events with bands amplified at theoretic sizes indicated by arrowheads. Green arrows in schematics indicate the on-target region primers; red arrows indicate the primers tested at the rearranged locus.

Conclusions

SAFER-Detection identifies rearrangement events with high sensitivity in Cas9-edited samples with low cell numbers.

SAFER-Detection identifies intra-chromosomal and inter-chromosomal rearrangements mediated by off-target activity and by homologous recombination.

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