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Case Study

Superb-seq Uncovers Hidden Off-Target Edits with CRISPR + Single-Cell Precision

Superb-seq uses simultaneous CRISPR-Cas9 edit and single cell transcriptomic profiling to reveal on-target and widespread off-target events and their effects on gene expression.

Background and Objective

A limitation of current CRISPR-Cas9 technology is the inability to measure the total effects of genome edits at the single cell level.

Perturb-seq links guide RNAs to single cell profiles via scRNA-seq but assumes that the detected guide is solely responsible for the observed transcriptional changes. But guides can edit unintended genes.

Additionally, guides vary significantly in their on-target sites efficiency and often exhibit off-target activity, complicating interpretation and mischaracterization of edits—critical in developing cell and gene therapies.

Mickey Lorenzini, Brad Balderson, and colleagues led by Graham McVicker developed Superb-seq, a scalable, high-throughput method that labels Cas9 edit sites in live cells using T7 in situ transcription (IST), enabling detection of both on- and off-target edits at the single-cell level with Evercode™ scRNA-seq. They also introduced Sheriff, a software package that quantifies edit sites and UMI counts per gene.

Superb-seq Workflow

Donor DNA containing a T7 promoter was electroporated with CRISPR-Cas9 complexes into live cells, inserting the promoter at Cas9 edit sites. Being homology-free, the donor DNA enabled labeling of both on- and off-target edits. Cells were then fixed, combinatorial barcoding was performed, and T7 in situ transcription (IST) generated edit-reporting RNAs. This IST preserved cellular context while identifying edits.

Transcripts from 10,000 K562 cells were sequenced using Evercode WT Mini, enabling simultaneous analysis of edit sites and the transcriptome (Figure 2).

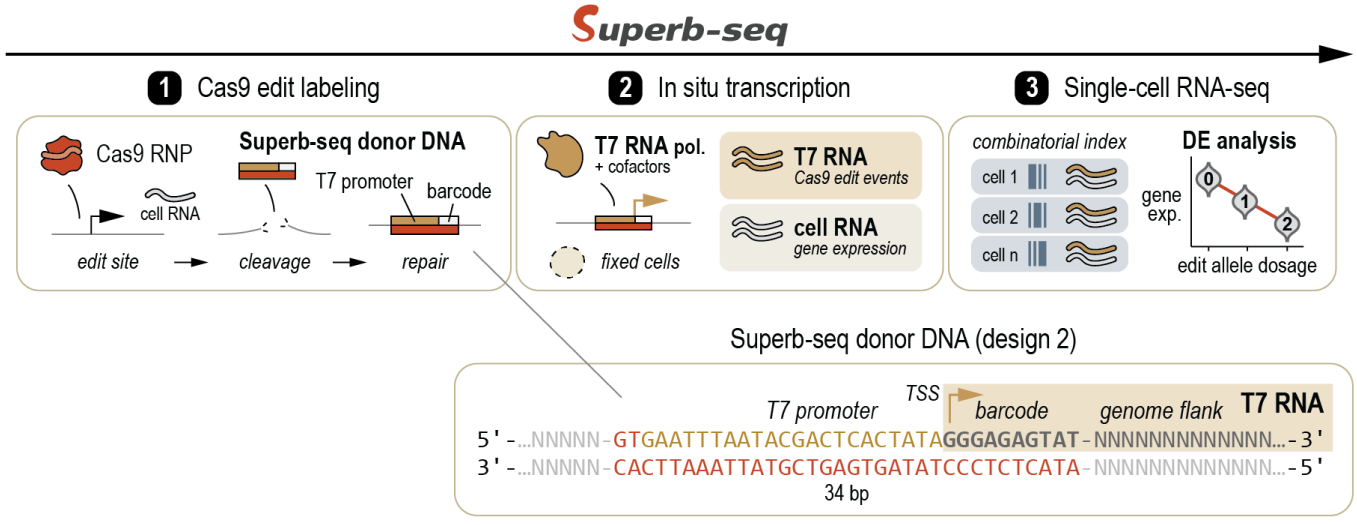

Figure 1. Superb-seq has three steps: Cas9 edit labeling with T7 promoters, IST of edit-marking T7 RNA in fixed cells, and joint combinatorial scRNA-seq of T7 and cellular RNA. The sequence of the 34 bp Superb-seq donor encodes an optimized T7 promoter and T7-transcribed barcode sequence.

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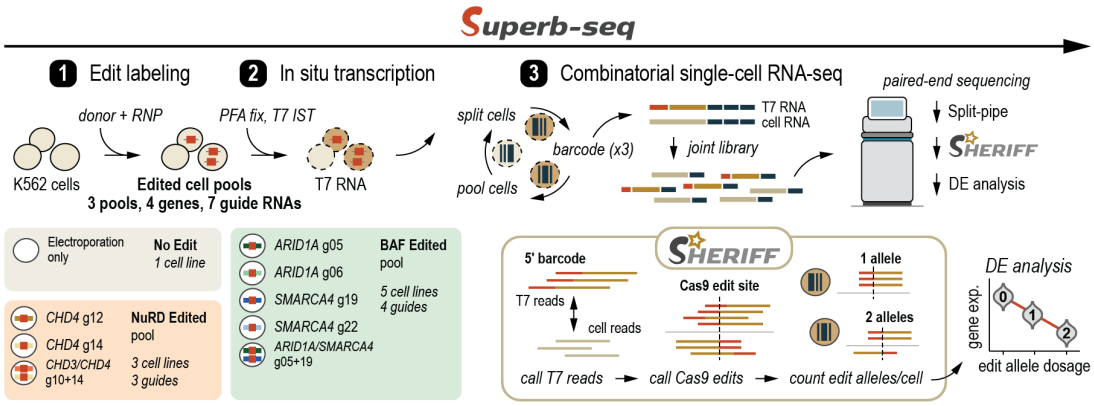

Figure 2. Diagram of a Superb-seq experiment on 10,000 K562 cells targeting four chromatin remodeler genes with seven guide RNAs. In situ transcription and combinatorial barcoding was performed on three pools of unedited, ARID1A/SMARCA4-edited, and CHD3/CHD4-edited cell lines. Paired-end sequencing data were analyzed by Parse Biosciences analysis pipeline and custom Sheriff software.

Identification of Cas9 Edits

To quantify Cas9 edits and the transcriptomic effects at a single cell resolution, the authors developed Sheriff, a custom software suite that processes - Parse Biosciences analysis pipeline — alignments to measure both edit sites and UMI counts per gene. Sheriff detected 7 on-target and 36 off-target sites across seven guide RNAs (Figure 3).

While most off-target edits were less frequent than on-target ones, the SMARCA4 guide produced several off-target edits at higher rates. Many of these closely matched the guide sequence outside the seed region, indicating Cas9 may tolerate more seed mismatches than previously thought

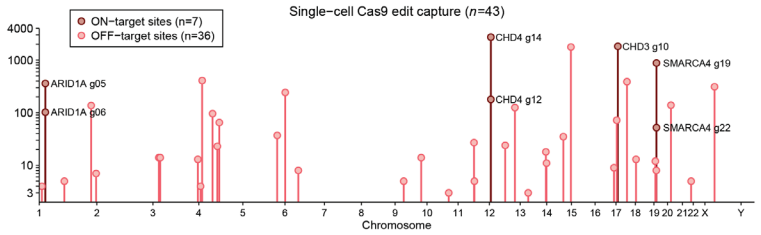

Figure 3. Sheriff quantified single-cell Cas9 edit capture at all seven on-target sites and 36 off-target sites in 6,230 edited cells.

Cas9 Edits Associated with Differential Gene Expression

Cas9 editing significantly impacted gene expression for both on-target and off-target genes. Notably, the guide targeting CHD3 caused an off-target edit in the USP9X gene, reducing its expression by disrupting an SP1 transcription factor binding site in a regulatory region (Figure 4). This highlights how off-target edits can affect gene expression through non-coding elements and interfere with pathways regulated by USP9X, which influences protein abundance and other cellular functions. These findings underscore the broad downstream effects of off-target editing on gene expression and cellular pathways.

Conclusions

Superb-seq is an innovative method that integrates CRISPR-Cas9 editing, T7 in situ transcription, and Evercode combinatorial barcoding to enable high-throughput single cell profiling of both on- and off-target edits and their impact on gene expression. It efficiently captures Cas9 edits and associated transcriptomic changes, identifying and quantifying both on- and off-target effects. The accompanying Sheriff software distinguishes T7 and transcriptome reads to accurately measure edit sites and gene expression.

Superb-seq provides a powerful tool to assess genome editing specificity and its consequences at single cell resolution, essential for functional genomics and therapeutic development.

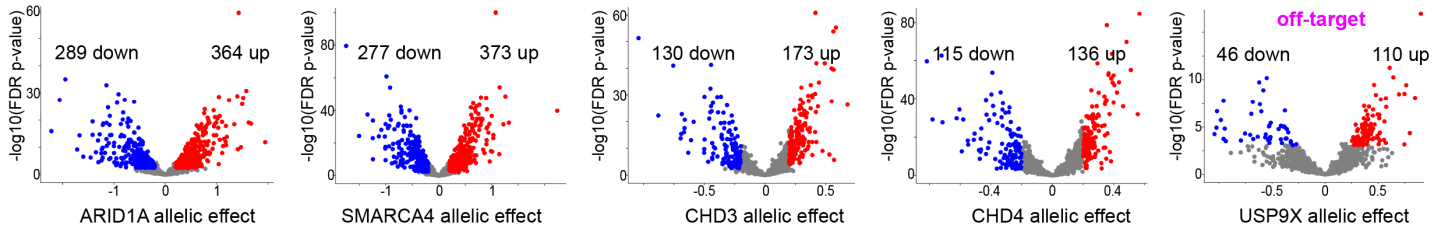

Figure 4. Volcano plots of DEGs associated with on- and off-target edit events. Edit allele effect sizes are on the x-axes and p-values on the y-axes.

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