Base Editor Genomic Safety Assessment:

keeping pace with emerging FDA expectations

Robert A Policastro, Stephano Spano Mello, Cicera Lazzarotto, Edward Salinas, David Goldfarb, Ashley Lester, Yanjie Qiu, Zachary Lemmon, Huei-Mei Chen

MaxCyte, Inc., Cell and Gene Therapy Services, Waltham, MA

seQure™

Overview: A Landmark Period for CRISPR Therapeutics

- **Nov 2023** · VERVE-101
  First Phase 1/2 *in vivo* base editing results
- **Dec 2023** · CASGEVY
  First approved CRISPR therapy; *ex vivo*
- **Mar 2025** · BEAM-302
  First *in vivo* correction of a disease-causing mutation using a base editor
- **May 2025** · Baby KJ
  First bespoke CRISPR therapy built for one patient
- **Apr 2026** · Ionvo-z
  First Phase 3 results and BLA filing for an *in vivo* gene editing therapy

Figure 1. Recent historic milestones for CRISPR-based therapies, 2023-2026.

CRISPR therapies have recently seen historic clinical milestones (Fig.1), including the approval of CASGEVY, the first in vivo base-editing successes, and late-stage clinical progress for *in vivo* gene editing. Across these advances, genomic safety has remained a critical requirement. Off-target editing can generate genomic variants that alter cell function, cause cell death, or promote oncogenic transformation (Fig. 2A). As a result, genomic safety data is essential throughout the product lifecycle, from candidate selection and preclinical development to regulatory filings, clinical trials, and manufacturing quality control (Fig. 2B)."

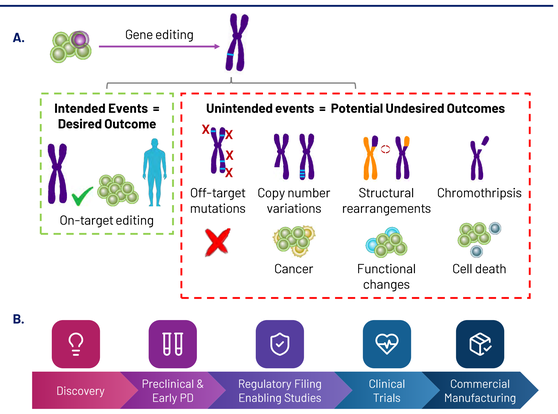
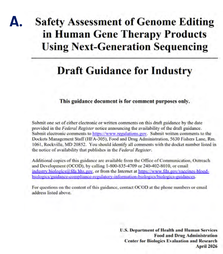


Figure 2. (A) Unintended off-target editing, both small and large variants, can lead to adverse outcomes such as functional changes, cell death, and oncogenic transformation. (B) Genomic safety data can help reduce these risks at every stage of product development.

Regulatory-Aligned Genomic Risk Assessment

In April 2026 FDA's Center for Biologics Evaluation and Research issued draft guidance intended to help standardize how sponsors evaluate off-target genomic risk using NGS in investigational and regulatory submissions (Fig 3A). CRISPR-Cas systems have diversified across Cas proteins with distinct PAM requirements and editing chemistries, and fusion of Cas proteins to deaminases, reverse transcriptases, and methyltransferases has expanded the modality landscape further. Prior to this guidance, methods, thresholds, and reporting formats were left largely up to sponsor discretion and case-by-case FDA feedback. This new draft starts codifying advice related to sequencing strategy (Fig. 3B) and depth, sample selection, off-target nomination and confirmation (Fig. 3B), analysis parameters, and the tabulated formats in which findings should be submitted.


A.

Safety Assessment of Genome Editing in Human Gene Therapy Products Using Next-Generation Sequencing

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/vaccines-blood-biologics-guidance-compliance-regulatory-information/guidances-vaccines-and-biologics-guidances.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
April 2026

B.

On-target site assessment	**§ V** · Targeted sequencing at the intended site. Long-read methods where indels > 50 bp are possible.
Off-target site nomination approach	**§ VI.B–C** · Method chosen based on editor MOA. Orthogonal approaches encompassing biochemical, cell-based, and/or *in silico*.
Variant-aware nomination	**§ VII** · Consideration of human genetic variants during *in silico* and/or biochemical nomination.
Off-target site confirmation	**§ VI.D** · Targeted or probe-based sequencing of nominated sites in relevant cells.
Chromosomal integrity analysis	**§ VIII** · Sensitive and quantitative NGS approach for translocations between on- and off-target sites.

Figure 3. (A) April 2026 draft FDA guidance for NGS genomic safety assessment of gene editing therapies. (B) Outline of select analysis expectations in the guidance.

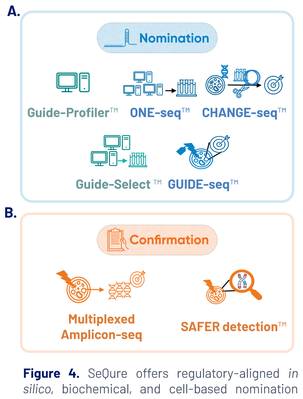
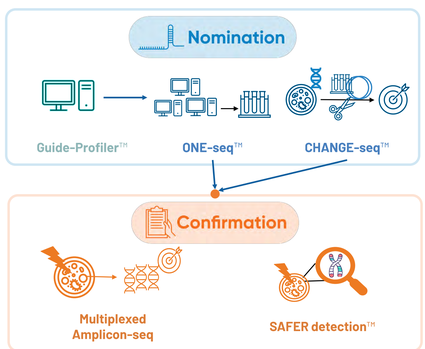


Figure 4. SeQure offers regulatory-aligned *in silico*, biochemical, and cell-based nomination assays for CRISPR base editors and nucleases (A), as well as high-sensitivity confirmation and translocation detection assays (B).

SeQure's portfolio is regulatory-aligned for CRISPR nuclease and base-editing therapies. For nomination, Guide-Profiler™ performs variant-aware *in silico*, homology-based site prediction, and Guide-Select™ couples this with a biochemical screen. ONE-seq™ allows variant-aware biochemical nomination, CHANGE-seq™ allows unbiased biochemical nomination in purified genomic DNA, and GUIDE-seq™ allows nomination with intact cellular context (Fig. 4A). CHANGE-seq™ and ONE-seq™ are available for both nucleases *and* base editors. For confirmation, sensitive multiplexed amplicon sequencing quantifies editing frequency across nominated sites, and SAFER detection™ delivers the high-sensitivity, quantitative translocation analysis recommended for chromosomal integrity (Fig. 4B). The result is a complete genomic safety package for both CRISPR nucleases and base editors.

Integrated Off-Target Assessment for Base Editors



Guide-Profiler *in silico* — Exhaustive search in reference and variant genomes

ONE-seq Biochemical, BE compatible, Variant-aware — Experimental nomination at both reference *and* variant sites

CHANGE-seq Biochemical, BE compatible, Genomic — Genome-wide and unbiased

A hypothetical off-target strategy for a CRISPR-based editor program (Fig 5). Nomination method orthogonality is satisfied through variant-aware in silico nomination (Guide-Profiler™) coupled with biochemical methods (CHANGE-seq™ and ONE-seq™). Both biochemical methods are base editor-compatible to satisfy FDA recommendations, and choosing two assays helps derisk guides by avoiding assay-specific blind spots. The requirement for a sensitive and quantitative method for on- and off-target editing rates, and translocation detection, in relevant cells, is then satisfied at the confirmation stage using Multiplexed Amplicon-seq and SAFER™ , respectively.

Figure 5. A hypothetical regulatory-aligned genetic safety strategy for a CRISPR-based editor therapy that includes variant-aware orthogonal *in silico* and biochemical nomination, as well as sensitive and quantitative editing confirmation and translocation detection.

ONE-seq™ Provides Experimental, Variant-Aware Off-Target Nomination

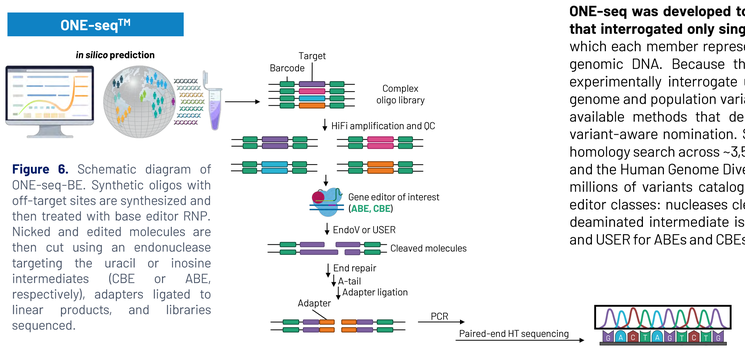
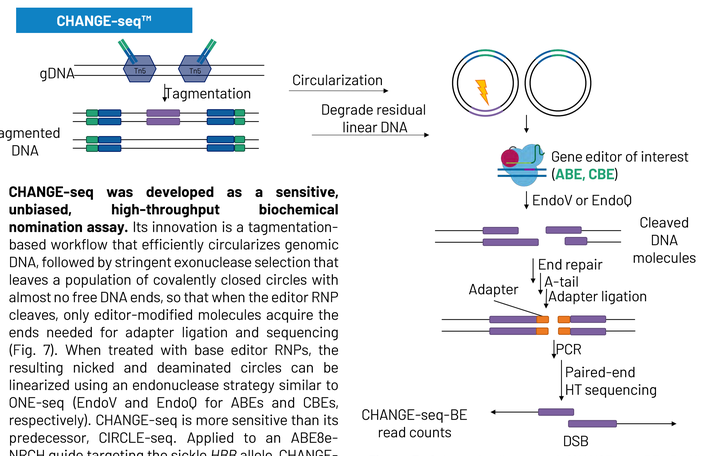


Figure 6. Schematic diagram of ONE-seq-BE. Synthetic oligos with off-target sites are synthesized and then treated with base editor RNP. Nicked and edited molecules are then cut using an endonuclease targeting the uracil or inosine intermediates (CBE or ABE, respectively), adapters ligated to linear products, and libraries sequenced.

ONE-seq was developed to overcome the limitations of previous methods that interrogated only single genomes. A synthetic barcoded oligo library, in which each member represents a candidate off-target site, is used instead of genomic DNA. Because the substrate is synthesized, a single assay can experimentally interrogate up to 250,000 sites spanning both the reference genome and population variants, making ONE-seq one of the few commercially available methods that delivers experimental, rather than purely *in silico*, variant-aware nomination. SeQure designs these libraries from an exhaustive homology search across ~3,500 genomes drawn from the 1000 Genomes Project and the Human Genome Diversity Project (Fig. 6), together with the hundreds of millions of variants cataloged in gnomAD. This method accommodates both editor classes: nucleases cleave the library directly, while for base editors, the deaminated intermediate is converted to a double-strand break using EndoV and USER for ABEs and CBEs, respectively.

CHANGE-seq™ Enables Sensitive, Unbiased Genome-Wide Off-Target Nomination



CHANGE-seq was developed as a sensitive, unbiased, high-throughput biochemical nomination assay. Its innovation is a tagmentation-based workflow that efficiently circularizes genomic DNA, followed by stringent exonuclease selection that leaves a population of covalently closed circles with almost no free DNA ends, so that when the editor RNP cleaves, only editor-modified molecules acquire the ends needed for adapter ligation and sequencing (Fig. 7). When treated with base editor RNPs, the resulting nicked and deaminated circles can be linearized using an endonuclease strategy similar to ONE-seq (EndoV and EndoQ for ABEs and CBEs, respectively). CHANGE-seq is more sensitive than its predecessor, CIRCLE-seq. Applied to an ABE8e-NRCH guide targeting the sickle *HBB* allele, CHANGE-seq-BE recovered 53% more off-targets and a 2.1-fold higher average enrichment ratio at validated sites [Lazzarotto, Katta, Tsai, et al. Nature Biotech, 2026].

Figure 7. Schematic diagram of CHANGE-seq-BE. Purified gDNA is tagmented, circularized, and then treated with the base editor RNP. Nicked and edited circular molecules are then linearized using an endonuclease strategy similar to ONE-seq, adapters ligated to cut products, and then libraries sequenced.

CHANGE-seq of *FANCF* CBE nominates hundreds of off-target sites. In total, 838 sites were nominated (Fig 9A), each having a CHANGE-seq score above the minimal assay threshold. CHANGE-seq scores for each off-target site are calculated as the ratio of reads at those sites versus the on-target site, and sites are only nominated if their ratio is not less than 0.1%. Analysis of the target sequences of the top off-target sites by read counts shows the canonical Cas9 NGG PAM preference, and most protospacer variation clustering distal to the PAM, outside of the seed sequence (Fig 8A). Interestingly, the top two off-target sites by read count fall within identical sequences of the paralogous genes RASA4 and RASA4B. Manhattan plots of the read counts for off-target sites across the main chromosomes are provided for illustrative purposes (Fig 8B).

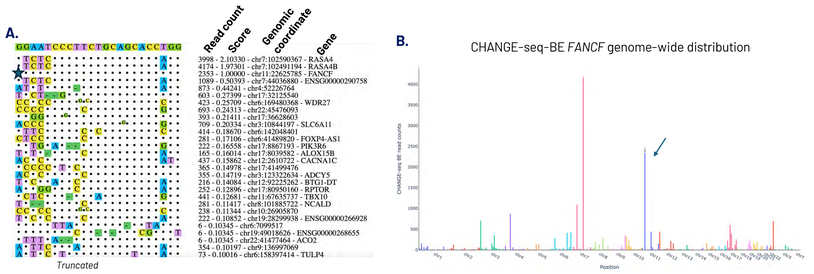


Figure 8. *FANCF* CBE CHANGE-seq on- and off-target sites results. (A) Allele, read count, score (read count fraction relative to on-target), genomic coordinates, and gene annotations. (B) A Manhattan plot of read counts at each site.

Orthogonal Off-Target Nomination for a Comprehensive Risk Profile

Orthogonal approaches capture a more complete picture of off-target sites. Among both methods, 1060 unique off-target sites were captured in total. Of those, 271 were found by both methods, while 567 and 222 were found uniquely in CHANGE-seq and ONE-seq, respectively (Fig 9A). All sites were categorized into tiers based on their proximity to, or effect on, specific genes and functional genomic elements, with tier 1 considered the highest risk. After annotation, 327 of the 1060 sites fell within the highest tiers (1 and 2a/b) (Fig 9B), with these loci distributed across global populations and influenced by population-specific genetic variation (Fig. 9C). A representative subset of these high-risk off-target sites were moved onto confirmation, where Multiplexed Amplicon-seq was performed. Strong on-target C>T editing (~50%) was observed at the on-target site, while off-target editing was above background at 2 of the selected sites (Fig 9D).

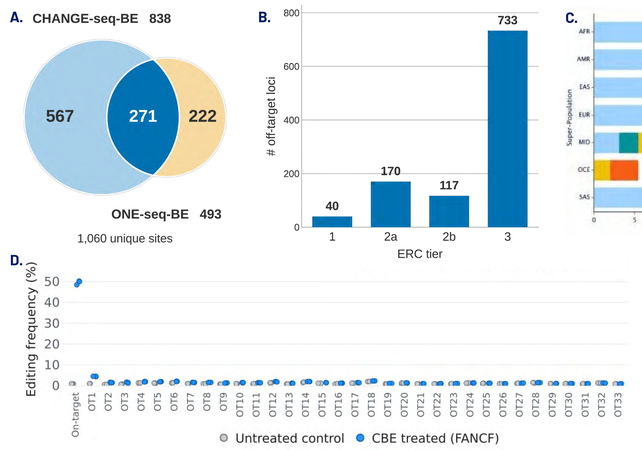


Figure 9. (A) Overlapping and unique *FANCF* CBE off-target sites in CHANGE-seq vs. ONE-seq. (B) The concern score of all nominated off-target sites. Concern score is a composite risk score assigned to off-target sites based on their proximity to, or effect on, certain genes and functional genomic elements. (C) Bar plots comparing Allele Frequencies (Afs) of off-targets by superpopulation. Bars are colored by AF for each superpopulation. (D) Multiplexed amplicon-seq results for select nominated *FANCF* CBE off-target sites. The x-axis is in descending order by the mean difference between control and treated.

SAFER™ Enables Sensitive Detection of Structural Rearrangements

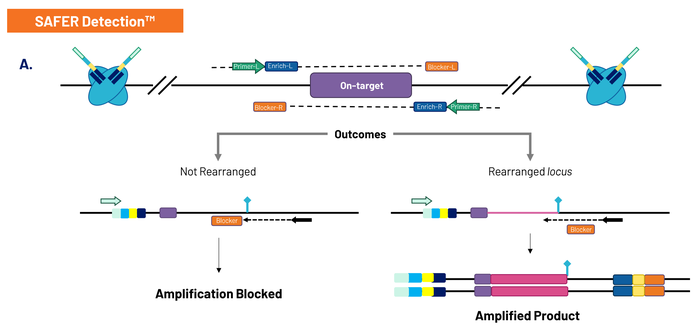
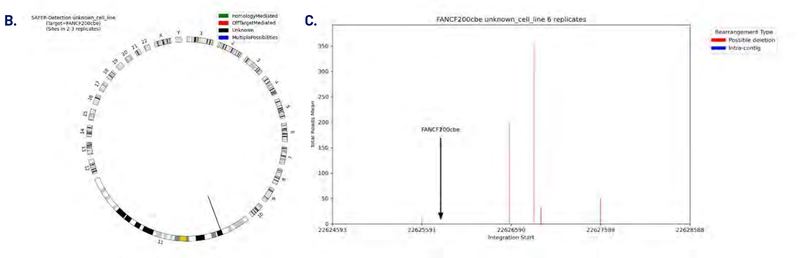


Figure 10 (A) Schematic diagram of SAFER. DNA is tagmented, and nested PCR is performed using a primer anchored to the site of interest. DNA fragments with translocations and other large structural variants are enriched by blocking the amplification of unaltered molecules. (B) A circos plot showing genome-wide rearrangement sites, and (C) a bar plot showing large variants proximal to the on-target site.

SAFER is a sensitive, quantitative method for detecting structural rearrangements at sites of interest. Tagmented genomic DNA is amplified by nested PCR from a primer anchored at the site of interest, and a blocking oligonucleotide positioned downstream suppresses amplification of intact molecules. Only fragments whose sequence has been displaced by a rearrangement escape the block and are amplified (Fig. 10A). Recovered junctions are then classified by likely origin: whether the partner site corresponds to a nominated off-target, falls within a region of homology to the target, or reflects other mechanisms. Figure 10B and 10C show output from the *FANCF* CBE experiment: a circos plot showing few large structural rearrangements, and a barplot resolving a few variants proximal to the on-target site.



Summary

Modalities
- *ex vivo* · autologous
- *ex vivo* · allogeneic
- *in vivo*

Editors
- Nucleases
- Base editors
- Novel enzymes

Programs
- Rare disease
- N-of-1
- Immuno-oncology
- iPSCs/stem cells

- ✓ Comprehensive off-target nomination and/or confirmation
- ✓ Regulatory-aligned results report
- ✓ Off-target risk characterization based on:
  - ✓ Genomic annotation context
  - ✓ Functional risk
- ✓ Phase-appropriate GLP
  - ✓ Methodology transparency
  - ✓ Reproducibility and traceability

SeQure has helped dozens of clients spanning *in vivo* and *ex vivo* therapies, various nuclease and base editing modalities, and a diverse array of programs, including rare disease, N-of-1, and iPSC/stem cells. All nomination and confirmation reports delivered by SeQure are comprehensive, regulatory-aligned, and phase-appropriate GLP.

MaxCyte®

Corresponding Author: Huei-Mei Chen: mchen@maxcyte.com MaxCyte, Inc., Tel: (240) 552-9480 info@maxcyte.com • www.maxcyte.com