A Fully Integrated and Highly Sensitive Approach to Predicting and Measuring Off-Target Variation in CRISPR Edited Gene Therapy Products

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DISCOVERY

Guide RNA optimization to incorporate population genetic variation at on-target and off-target

Editing System Development

- Guide RNA selection
- Transgene design
- Editing system comparison and optimization

gRNA Risk Profiler — noteSeQ

In-silico preview of off-target search space. Avoid genetic variation at the on-target site. Indicates guide specificity through search-space size proxy. Integrates with standard ONE-seq workflow for additional cost savings. Functional annotation included.

ONE-seq Screen — noteSeQ

Low cost, multiplexed version of ONE-seq designed for screening multiple guide RNAs. Variant aware with tighter search space parameters makes it effective for helping prioritize programs or quickly narrowing large numbers of candidate guides via biochemical assay.

dGH EXPLOR — EXPLOR™

Cost effective, editing system comparisons, guide strand optimization, structural variant detection and breakpoint identification.

Download at sequre-dx.com

Learn more at kromatid.com

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PRECLINICAL

Comprehensive and ultra-high sensitivity nomination of off-target and structural variation

Guide / Nuclease Selection

- On-target confirmation
- On-target transgene mapping
- Off-target **nomination**

ONE-seq — noteSeQ

Gold-standard population-scale variant-aware off-target detection. Candidate off-target enumeration, enrichment, and biochemical IVC combined with deep sequencing results in high sensitivity to low frequency off-target events. Features fully annotated reporting.

DEUX-seq — noteSeQ

Orthogonal, unbiased in-vitro off-target editing assay. PCR-free whole genome IVC combined with deep NGS sequencing.

GUIDE-seq — noteSeQ

Available as either a fully turn-key assay with SeQure Dx performing the editing, LC, sequencing and analysis, or kitted. Orthogonal, unbiased in-vivo off-target editing assay provides an easy start to any project. Ask us about licensing opportunities.

dGH DSCVR — DSCVR™

Determines location and orientation of structural variants, and breakpoint identification.

Selected Guide / Nuclease Program Development

- Off-target **nomination**
- Off-target **confirmation**
- Off target transgene **mapping**

Amplicon-Seq — scopeSeQ

High sensitivity confirmation of off-targets for post-edited cell-lines. Informed by our NoteSeQ assays, we know exactly where we need to look to confirm editing.

SAFER Detection — scopeSeQ

Confirm structural rearrangements off-targets and viral delivery integrations (insertional, copy number). NGS interrogation allows for resolution down to the exact coordinates.

dGH in-Site — in-Site™

For edit-associated structural variant detection, transgene mapping, enumeration and localization.

dGH SCREEN — SCREEN™

For high resolution, unbiased structural variant detection, genomic instability, DNA damage, and chromothripsis.

IND Enabling

- Off-target **confirmation**
- Specification setting
- Genomic Integrity and stability
- **Structural variant** evaluation
- Transgene mapping

CLINICAL 1 | 2 | 3

Clinical Trial Initiation

- Structural variant evaluation
- Genomic integrity testing

Patient Inclusion Criteria

- Individual off-target risk assessment
- Rule in/rule out tests for trial participant enrollment based on population-specific off-target risk(s)

Genomic Integrity — Gi™

Subclonal Outgrowth, high resolution genomic integrity testing, structural variant evaluation.

SEQURE Profile — clinicSeQ

CLIA certified clinical diagnostics, providing individualized off-target risk assessment, aiding participant enrollment.

POST CLINICAL MARKET

Commercial Product Launch

- Structural variant evaluation

Treating Physician Decision Support

- Individual off-target risk assessment

Post Treatment Follow-up

- Individual off-target observation
- Patient testing

SEQURE Monitor — clinicSeQ

CLIA certified clinical diagnostics, monitoring gene therapy patients following their treatment. Monitor for off-target safety signals and on-target clinical efficacy.

In the past 6 months, gene therapy evaluation guidelines have evolved, emphasizing orthogonal methods, genetic variation effects, increased assay sensitivity, testing on target donor cells, genomic rearrangement assessment, and biological consequence evaluation. Recent adverse events reported to the FDA have underscored the importance of these guidelines. To address this, we propose a comprehensive assay and analysis suite to detect all genomic variation induced by CRISPR, including structural variants. Our approach includes off-target nomination, biochemical confirmation, and cytogenetic methods with sensitive DNA probes and NGS sequencing to detect low-frequency variation. This suite of tools also identifies phenomena like chromothrypsis, sub-clonal outgrowth, aneuploidy, or genomic instability, providing comprehensive risk assessment for patients. By combining best in class methods for nomination, confirmation and routine testing of all classes of variants, this integrated suite of tools meets the needs of guide selection, donor selection, guide characterization, drug product QC and patient testing.