Understanding Genome Integrity and Toxicity Events Associated With Edited Cells:
Next Gen Cytogenetics for Benchmarking Starting Material and Evaluating Edit Effects

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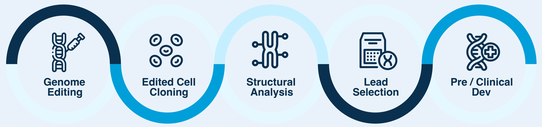
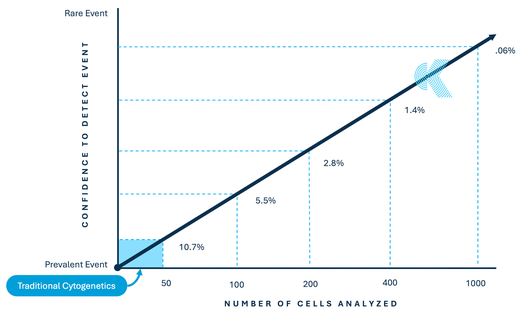
KROMATID

Throughout gene-edited therapy development, it is essential to characterize the genome for changes that could impact the editing process or the patient. Characterizing donors and other starting materials such as pre-engineered cells with advanced cytogenetic analysis, can provide critical insight and benchmarks for genomic integrity.



Holistic Solutions for Genomic Integrity Testing Across the C&GT Workflow



Conventional cytogenetics, a powerful technique for understanding chromosomal mutation falls short for genotoxicity assessment. Classical analysis assesses germline pervasive mutations and does not consider comparison to a reference, nor the cell-by-cell impacts of editing. KROMASURE Next Gen Cytogenetic solutions are fit-for purpose assays and analytical packages for C&GT, with:

1. **MORE CELLS ANALYZED** (significant number of cells to detect rarer structural events)
2. **MORE EVENTS DETECTED** (increased detection power for small events and event classes conventional methods struggle with

KROMASURE provides a baseline comparison for treated cells with an increased capacity that sheds light on the changing structural implications during process development.

Unbiased Cellular-based Assays for the Assessment of Genomic Integrity

KROMASURE K-BAND: Staining based Karyotyping (Giemsa) for characterization of structural mutations (5-10 MB+) with band attribution that shows the batch-to-batch effects of editing or provides a comparison to a baseline rate of chromosomal mutation present in the starting material.

Reference Sample (100 cells analyzed)

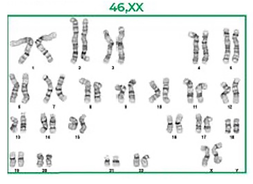
Event Class	# of Cells
No Events	77
Aneuploidy (Loss + Gain)	13
Loss	11
Gain	2
Acentric fragment	0
Additional material of unknown_origin	0
Constitutional anomaly	0
Chromosome break	2
Chromatid break	3
Chromothripsis	0
Deletion	4
Derivative chromosome	0
Dicentric	0
Double minute	0
Duplication	0
Insertion	0
Inversion	1
Marker chromosome	1
Quadriradial	0
Ring chromosome	0
Translocation	1
Triradial	0
Haploid	0
Diploid	100
Triploid	0
Tetraploid	0
Polyploid	0

Edited Sample (100 cells analyzed)

Event Class	# of Cells
No Events	35
Aneuploidy (Loss + Gain)	35
Loss	32
Gain	4
Acentric fragment	0
Additional material of unknown_origin	5
Constitutional anomaly	0
Chromosome break	1
Chromatid break	4
Chromothripsis	0
Deletion	24
Derivative chromosome	6
Dicentric	1
Double minute	0
Duplication	1
Insertion	0
Inversion	0
Marker chromosome	6
Quadriradial	0
Ring chromosome	0
Translocation	14
Triradial	0
Haploid	0
Diploid	97
Triploid	1
Tetraploid	2
Polyploid	0

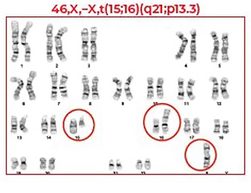
Normal Karyotype:

46,XX



Abnormal Karyotype with Structural Mutations:

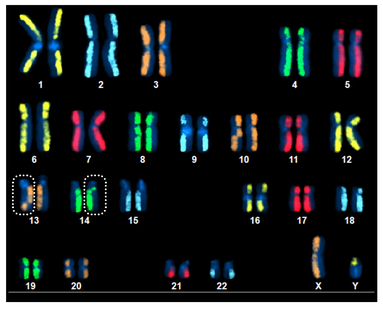
46,X,-X,t(15;16)(q21;p13.3)

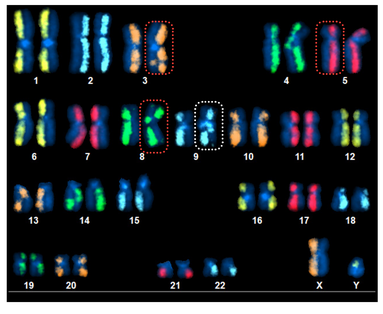


Single Cell Evaluation of Chromosome Structural Variants present across the Genome with Giemsa Staining (G-banding): A Mock control was generated from donor T-cells immediately pre-edit was used to benchmark the structural variants present in the starting material prior to editing. 100 cells from each sample were karyotyped, and the variant calls were categorized by event class. Both sample populations had chromosome structural mutations, with elevated rates of chromosome structural mutations and polyploidy (triploid and tetraploid cells) observed in the edited sample.

Event Classes that are occurring at a statistically significant elevated rate (by Fisher's Exact Test) in the edited sample as compared to the reference sample.

KROMASURE SCREEN: Hybridization-based karyotyping for enhanced characterization of small structural mutations (20kb-5MB+) and complex rearrangements that show the effects of editing batch to batch or provide a comparison to a baseline rate of chromosomal mutation present in the starting material.





Reference Sample (50 cells analyzed)

Event Class	# of Events	# of Cells with Events
Gain	0	0
Loss	6	3
Inversion	34	24
SCE	150	49
Insertion	0	0
Size Diff	95	46
Translocations	2	2
Complex	2	2
No Events	N/A	0

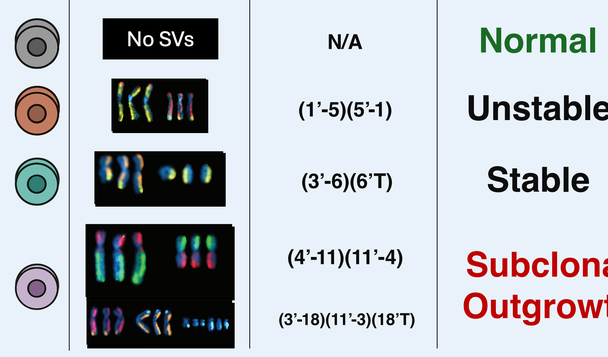
Edited Sample (50 cells analyzed)

Event Class	# of Events	# of Cells with Events
Gain	7	6
Loss	22	8
Inversion	109	48
SCE	164	50
Insertion	11	6
Size Diff	118	46
Translocations	14	12
Complex	15	10
No Events	N/A	0

Event Classes that are occurring at a statistically significant elevated rate (by Fisher's Exact Test) in the edited sample as compared to the reference sample.

Characterizing Distribution of Karyotypic Mutations Pre and Post Edit

Assessing clonal proliferation, selective survival, and dysregulated growth is essential to mitigate the risk of mutagenesis. The image panels below depict translocation events detected in clones isolated from irradiated cells using the KROMASURE SCREEN assay. The stability of karyotypic rearrangements for each clone varied, and the mutation profile changed over several generations of passaging. In this model system, the per-cell attribution of karyotypic events provided by SCREEN enabled population-level analyses of event distribution that was used to study the persistence, stability, and relative impact on growth that the mutations had on the population of treated cells.



Chromosomal mutations, whether random or induced by the editing process, can have varying impacts. They may pose minimal risk, lead to cell senescence or death (dropout), trigger an immunogenic response in the patient, or cause cancer. Therefore, it is crucial to evaluate the genomes of edited cells for changes in their chromosomal mutation profiles. KROMASURE SCREEN karyotyping enables the unbiased profiling of individual structural variants, as well as structural variation phenotypes (clones with a signature structural variant profile) which can be tracked, even at low prevalence, via the analysis of hundreds of individual cells per sample. Comparing the edited cell population to the starting material and monitoring each sample longitudinally for genomic instability and subclonal outgrowth (depicted below) is a powerful tool for understanding mutagenesis risk.