KROMATID

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A Fit-for-Purpose Genomic Integrity G-Banding Solution for Cell & Gene Therapy (G&GT)

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SECTION 1
Intro: What's the Problem?

G-Banding chromosome analysis is the industry standard for the diagnosis, monitoring, and management of a variety of genetic disorders. Of significance, G-Banding has improved detection of cancers such as leukemia and lymphoma by identifying chromosomal translocations and other rearrangements. In a clinical setting, the results of G-Banding analysis are directly tied to patient management. Identification of specific chromosomal abnormalities guides treatment decisions, helps to assess prognosis, and can be used to monitor for relapse. In a disease like cancer, a single abnormal cell starts to proliferate uncontrollably creating a clonal population of cells with the same genetic abnormalities. Therefore, the G-Banding analysis performed in a clinical setting typically only reports clonal activity or closely related abnormal chromosome complements (two or more cells with the same structural rearrangement). (ISCN ref).

Outside of the clinic, C&GT is a rapidly expanding field of research for the design and application of edited cells to treat disease. These types of therapies offer a possible solution to previously uncurable diseases. However, there are unintended chromosomal alterations that can occur during gene editing or cell manipulation. Editing tools like CRISPR-Cas9, for example, can cause breakage, fusion or other unintended causes of chromosomal instability. As cells are modified and expanded, there may be unwanted rearrangements, or structural changes in chromosomes. The viral vectors used to deliver genes into cells can also cause unintended mutagenesis.

The clinical / classical karyotype reporting may be applicable to some abnormalities in the context of C&GT samples, but such specific criteria can exclude meaningful biological variants in a heterogeneous population from consideration (Figure 1). This raises the question of whether a different method of data presentation would provide a better understanding of the overall genomic stability in an engineered cell population.

Accurate analysis of C&GT samples requires more in-depth assay scope and a presentation of the data that accounts for heterogeneity in the cell sample. Typical clinical G-Banding reports on analysis of 20 cells, which is not intended or sufficient to detect low-prevalence events. The number of cells analyzed directly impacts the potential and reliability of detecting chromosomal abnormalities. A method that analyzes a greater number of cells provides a more comprehensive view of the chromosomal composition, improving the accuracy and reliability of the test. For C&GT programs, this level of analysis captures critical information and ensures that the results are representative of the sample's cell population.

To address these challenges, Kromatid has developed KROMASURE™ KBand, a more rigorous version of the classical method. KBand analysis measures and reports the prevalence of all genomic events, including low prevalence and variable abnormalities often missed by standard G-Banding analysis and reporting. KBand reports provide fit-for-purpose event breakouts that allow the observed frequencies of events from the edited cells to be statistically compared to the baseline rate established in the unedited cells. This approach offers a tool specifically designed to help bring C&GT safely to clinics.

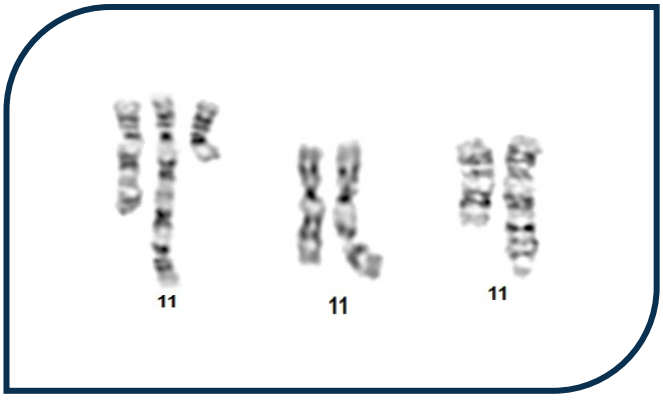


Figure 1. Non-clonal aberrations observed on chromosome 11.

SECTION 2
The Solution

Chromosome analysis in clinical oncology and in cell & gene therapy development share the same staining technique and the same analysis scoring rules (ISCN 2020: An International System for Human Cytogenomic Nomenclature) to examine chromosomal structure and number. However, their focus, purpose, and application differ significantly due to the nature of the questions/concerns being addressed. The critical difference lies in the purpose and application: oncology uses G-Band analysis to understand and manage disease, while C&GT uses it to ensure that the cells used for treatment are thoroughly characterized and genetically sound. In C&GT, the focus is on ensuring that the cells used as a therapy (e.g., stem cells, genetically modified cells) are free from unwanted chromosomal alterations before they are introduced into the patient. KBand was developed specifically to provide C&GT developers with customized data analysis and reporting to highlight any aberrations that may impact the safety of their therapeutic products. Although the chromosome analysis remains the same as conventional clinical G-Banding, an increase in the number of cells combined with specifically designed data presentation and reporting makes this method ideal for C&GT products.
-Reporting

As a C&GT developer performing batch to batch analysis, how would you incorporate the following sample karyotypes into your data package/batch evaluation:

Standard Karyotype Summary Report		
Reference	46,XX[100]	Normal Female
Treated	46,XX,del(16)(p13.2)[6]/46,XX,t(14;15)(q11.2;q21)[6]/45,X,-X[5]/45,XX,-14[3]/46,XX,t(14;16)(q11.2;p13.2)[3]/46,XX,add(16)(p13.3)[2]/46,XX[75]	Abnormal Female

The Kromatid Genomic Integrity Report (Table 1, Table 2) uses statistical methods to quantify chromosomal aberrations and assess the overall impact of gene therapy on chromosomal stability. This involves tracking the frequency of specific anomalies and assessing the proportion of cells with abnormal karyotypes. This service is designed to meet the unique needs of investigators by providing detailed reporting on low frequency variants that is not captured by conventional G-Banding services.

Table 1: Number of Cells in Each Sample with Specific Events

Event type	Reference Number of Cells	Treated Number of the Cells	Fisher's Exact* *p*-Value	*P*-Value Significant < .05
del(16)(p13.2)	0	6	0.029	Yes
t(14;15)(q11.2;q21)	0	6	0.029	Yes
-X	1	5	0.212	No
t(14;16)(q11.2;p13.2)	0	3	0.246	No
add(16)(p13.3)	0	2	0.497	No
*dic(14;17)(q11.2;p13)	0	1	1.000	No
*t(7;14)(q35;q11.2)	0	1	1.000	No
*inv(14)(q11.2q32)	0	1	1.000	No
*del(14)(q11.2)	0	1	1.000	No
-14	1	3	0.621	No
-15	0	2	0.497	No
-16	0	2	0.497	No
-21	2	2	1.000	No
chtb(14)(q)	0	2	0.497	No
No Events	90	55	< .001	Yes

** These events are not typically captured in conventional cytogenetics karyotype reports.*

Table 2: Event Rates for Edit Target Chromosomes

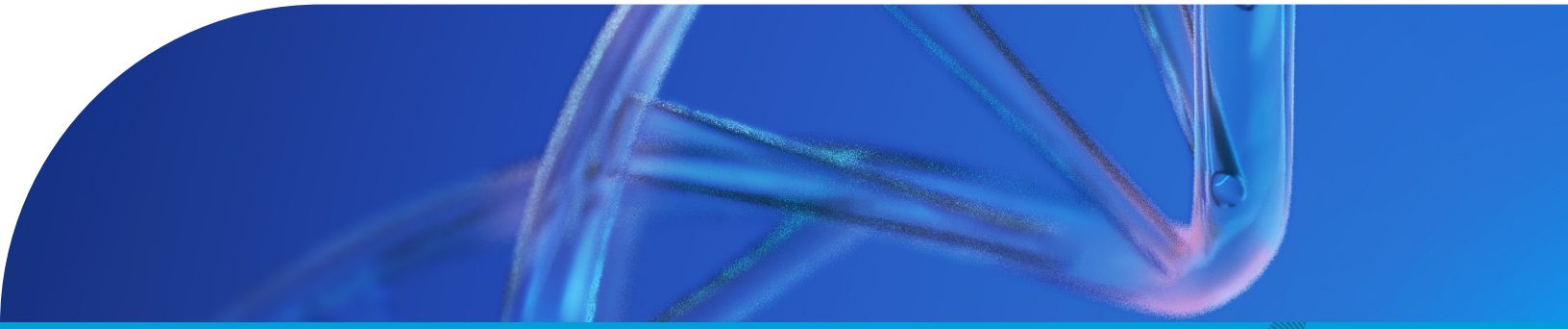
Sample Name		Reference		Treated		
Sample ID		S0XXXXX		S0XXXXX		
Category	# Events	# of Cells	% of Cells	# Events	# of Cells	% of Cells
Chromosome 14	1	1	1.0	18	18	18.0
Chromosome 16	0	0	0.0	13	13	13.0

SECTION 3
Conclusion: Why This Solution Matters

The advancement of cell and gene therapies brings new hope to the treatment of complex and lethal diseases, but these treatments will only reach patients with thorough testing that assures safety. KBand chromosome analysis is a valuable tool for ensuring that the cells used in cell and gene therapy are genetically stable, free of harmful mutations, and suitable for therapeutic applications. When cells are modified by gene editing techniques, KBand monitors chromosomal rearrangements and structural changes that occur during the editing process.

By enhancing a well-established clinical method with data analysis that is fit for its new use case, KROMASURE KBand provides a rigorous assessment of the stability of each batch of engineered cell product. KBand data illustrates the potential risks in each program, allowing investigators to optimize methods, fine-tune process parameters, and file INDs with confidence.

Learn More



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