KROMATID

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Precision Single Cell Analysis for Assessing Insertional Mutagenesis

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SECTION 1
Intro: What's the Problem?

Insertional mutagenesis analysis remains an indispensable yet imperfect approach for assessing gene therapy safety. Addressing the biases, ambiguities, and interpretive limitations requires a collaborative approach combining technological innovation, computational rigor, and regulatory foresight.

Current analysis methods, such as NGS sequencing and ddPCR, suffer from technical and interpretive limitations that obscure real risk and impede confident decision-making. Many assays fail to quantify the true diversity of integration events in vivo, especially in early preclinical stages. Short-read platforms are not designed to resolve integration into repetitive loci, which are often hotspots for regulatory elements and fragile sites.

In addition, no current pipeline measures copy number and modal distribution of integration events at the chromosome and cell level. Most traditional assays analyze too few cells to capture the true diversity of integration patterns. Critically, this hides rare, and potentially dangerous, clones with unintended insertions. Insertions near cancer promoting oncogenes may be missed without a robust sample size and analysis. As cell and gene therapies continue to advance, so will the risk profile requirements of regulatory bodies. Accurately capturing integration site distributions and clonal representation will be critical to ensure the safe production of life-saving therapies. In answer, Kromatid has developed a molecular cytogenetics platform (KROMASURE Pinpoint FISH) that utilizes proprietary technology to designed custom probes, to enablinge direct visualization of insertion sites at single-cell resolution.

SECTION 2
The Solution

Kromatid's KROMASURE PinPoint solution is designed to deliver high-throughput single-cell analysis of transgene integration. to address critical gaps in existing methodologies. Pinpoint leverages Interphase-based detection, allowing for direct analysis of non-dividing cells, a key limitation of metaphase dependent assays. Optimized for modern genomic research, PinPoint detects integration events as small as 2kb, enabling a deeper understanding of vector behavior and genomic safety.

A defining advantage of the PinPoint assay is its ability to determine the true modal distribution of insertional copy number per cell. While traditional bulk assays, like qPCR and NGS, provide an averaged copy number, PinPoint can detect high-risk clonal populations that may expand over time. (Figure 1). Understanding how many cells exceed regulatory thresholds (e.g., >5 copies/cell) is essential for accurate risk characterization and batch release criteria.

PinPoint uniquely supports high-throughput workflows capable of analyzing hundreds to thousands of individual cells per slide. This enables statistically powered analysis across manufacturing batches or timepoints and is scalable for GMP lot release testing, comparison studies, and preclinical evaluations.

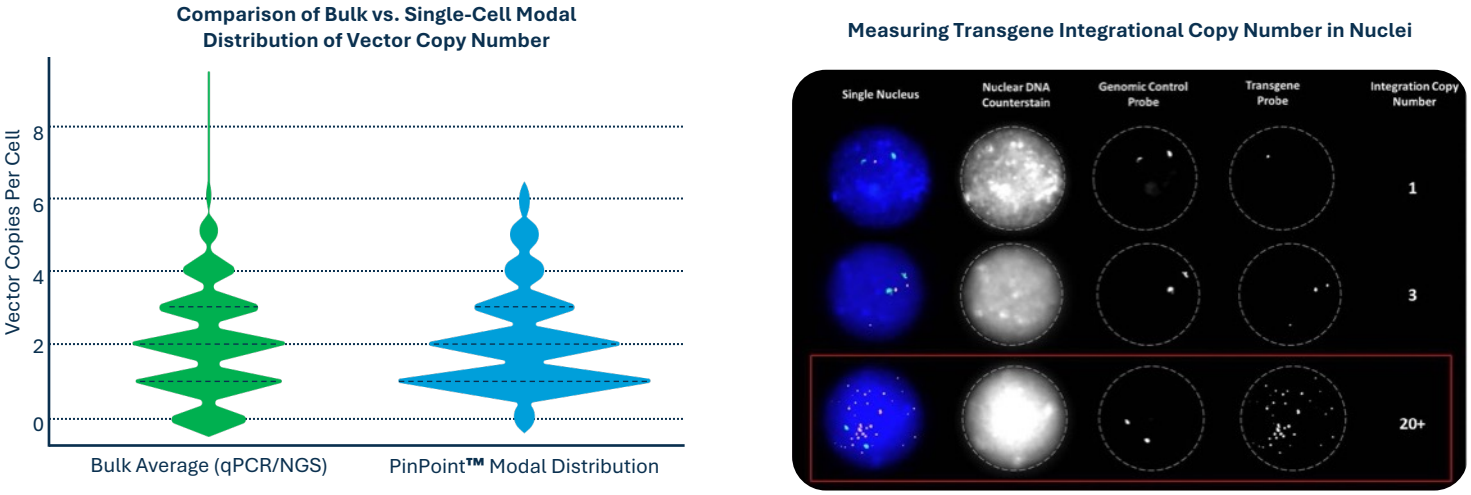

Figure 1. PinPoint modal distribution: Reveals that most cells have 1–2 vector copies, but also clearly identifies cells with 4–6 copies that could pose regulatory or safety concerns.

SECTION 3
Conclusion

As the complexity and scale of engineered cell products grows, insertional analysis must evolve to provide structural resolution and single-cell specificity. High-throughput sequencing is foundational, but new tools are enhancing the field's ability by resolving insertional events at the single-cell and chromosomal level, filling key analytical gaps in regulatory workflows for cell and gene therapy.

Integrating the KROMASURE™ PinPoint solution offers therapy developers a way to obtain more accurate safety assessments, support regulatory filings with single-cell data, and optimize vector design based on high-resolution insertion site maps. Such a solution is not only a scientific imperative, but also a strategic opportunity for companies to de-risk assets, accelerate development, and meet the rising expectations of regulators and investors alike.

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