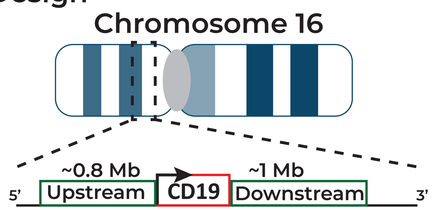
dGH in-Site™: An Assay System for the Simultaneous Single Cell Measurement of Edit Associated Structural Variation and Transgene Localization

Erin Cross, Stephen Gross, and Christopher Tompkins
KromaTiD, Longmont, CO

Cell and Gene therapy products require complex editing systems combining nuclease-based editing with insertion of one or more transgenes into the target genome. This complexity leads to additional opportunities for on- and off-target double-strand breaks which can mis-repair to form structural variants, such as inversions and translocations. Even in systems with highly specific targeted transgene insertions, there is the potential for off-target insertions at random or off-target editing loci within the genome. These number (and sometimes location) of the transgenes in these complex edited products can be measured on average by NGS or dPCR methods. However this averaging divorces structural variant and transgene data from any cellular context, making it impossible to observe co-localization of events or distribution of transgenes in a batch of edit cells; with only dPCR data, it is not possible to differentiate one cell with ten transgenes from two cells with five transgenes. This type of quantitation is best performed by a comprehensive, single cell method such as directional Genomic Hybridization (dGH™). We have designed and qualified a single cell assay system for the simultaneous measurement of edit associated structural variation and transgene localization in the same single cell. Using a model transgene system we demonstrate the potential of this system for understanding the true structural heterogeneity in batches of edited cell products.

in-Site™ Assay Design

Probe	Span	Library Size
Upstream	831 kb	2,333
Downstream	1,060 kb	1,978
Insert	7.3 kb	52

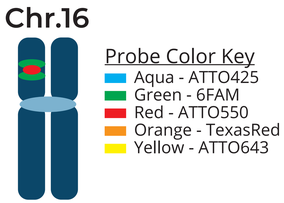


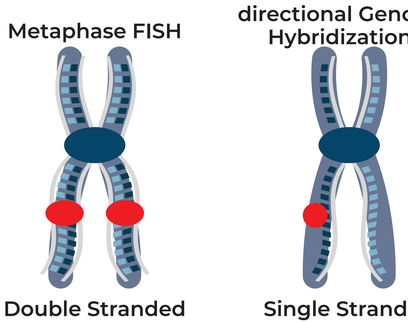
Problem Statement:
- Transgene Insert of 7.2 kb into the cd19 gene locus of chromosome 16 (positive strand).
- Sequence data shows 45% insertions rate, both heterozygous and homozygous knock-ins.

"We would like to assess the rate of integrations, alongside structural rearrangements to the edited locus; including on- and off-target integrations, inverted integrations, copy number expansion as well as any translocations, deletions and inversions present in the flanking sequence."

Bioinformatics Design Strategy:
- Single-stranded bracketing probes targeting the proximal regions upstream and downstream of the editing site.
- Custom probe targeting their transgenic cargo (7.2 kb - 52 fluorescently labeled oligonucleotides).

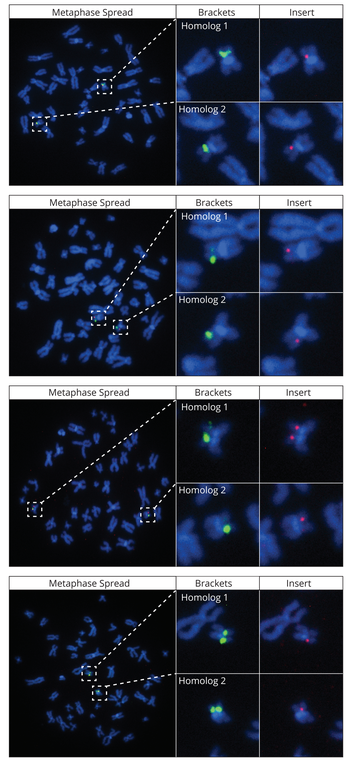
Measurement	Expected Assay Performance
Number of on-target trangene integrations	Yes
Number of off-target trangene integrations	Yes
Number of on-target inverted transgene integrations	Yes
Number of on-target inverted, duplicated trangene integrations	Yes
Translocations, inversions, and deletions inolving flanking regions	Yes
Structural rearrangements involving off-target	No
Significant copy number gain at an integration site	Limited (single v multiple)





directional Genomic Hybridization™ (dGH™)
dGH™ contains only the 2 parental DNA strands in opposite orientation

in-Site™ in Action

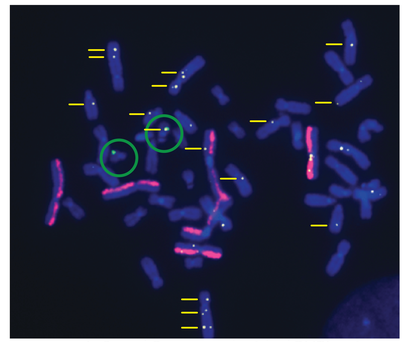


Custom dGH in-Site™ Assays

dGH in-Site™ provides high-resolution, single-cell visual data on transgene integration events throughout the genome.

Track on- and off-target insertions of any transgene!

Multiplex colors to differentiate genomic loci of interest

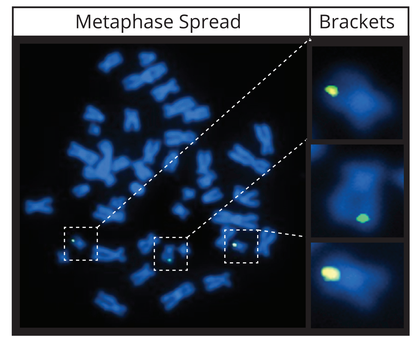


Above: Chromosomes 1, 2 and 3 targeted with dGH™ paint probes labeled in Atto 550 (pink). Custom dGH in-Site™ probe in Atto-643N targets all transgene insertion sites (yellow).

Readily Configurable Assay to Suit All of Your Detection Needs!

Detect Edit Site Translocations

Track multiple transgene insertions in one assay!



Above: Custom dGH in-Site™ probe in Atto-643N targets all transgene insertion sites (yellow). Bracketing Probes labeled in 6-FAM (green). Shown is a reciprocal translocation of the green probe to an off-target chromosome, likely occuring at the cut site.