Simultaneous Mapping of On- and Off-Target Structural Variants, Insertions and Translocations in Engineered CAR-T Cells

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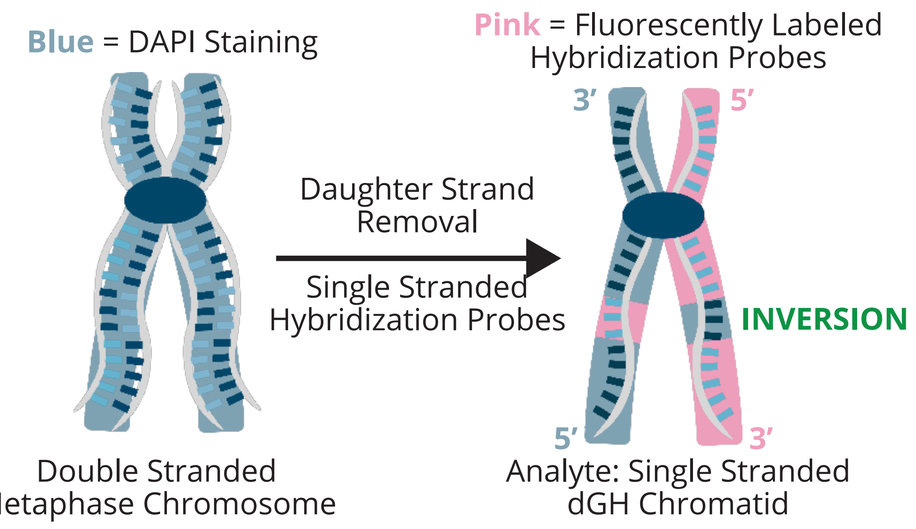
ABSTRACT

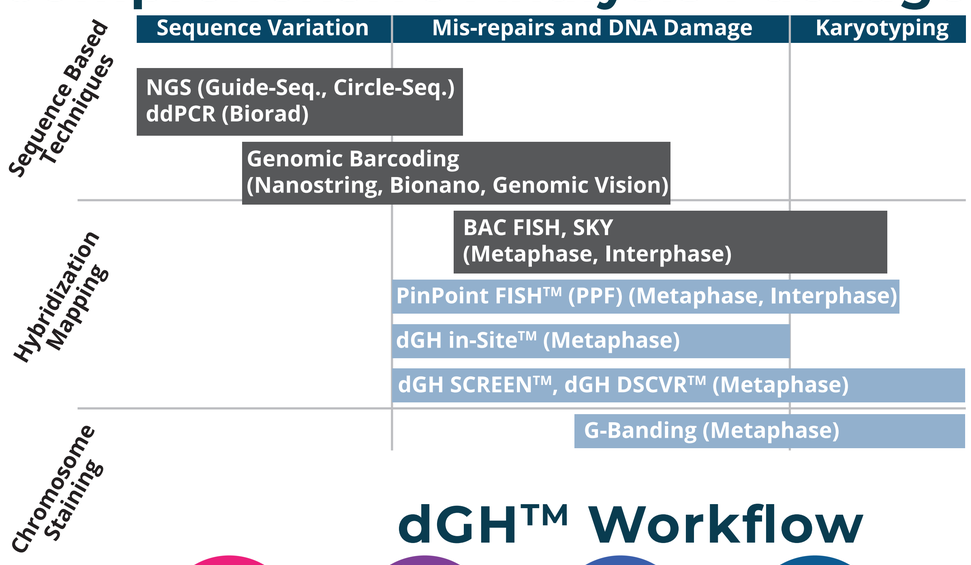
Structural variants, such as inversions and translocations, are common by-products of CAR T-cell engineering processes such as CRISPR-Cas9 editing. These variants are formed by misrepair of DNA breaks, for instance between the TRAC loci and an off-target site. These can result in a heterogenous mixture of low-prevalence variants that involve edit-site, off-target and random breaks. directional Genomic Hybridization™ (dGH™) is a unique cytogenetic technique for mapping the structure and structural variation of many individual genomes in single cells. Based on images of fluorescently labeled DNA probes designed against a reference genome for specific loci and hybridized to metaphase chromosomes, dGH in-Site™ assays provide true *de novo*, unbiased detection of structural variants or CAR insertions as small as 2kb anywhere in the genome. dGH in-Site™ is ideal for measuring these by-products of editing, mapping the on- and off-target locations of transgenes as well as potentially genotoxic outcomes such as sub-clonal outgrowth, insertion at potentially oncogenic sites and chromothripsis.

directional Genomic Hybridization (dGH™)

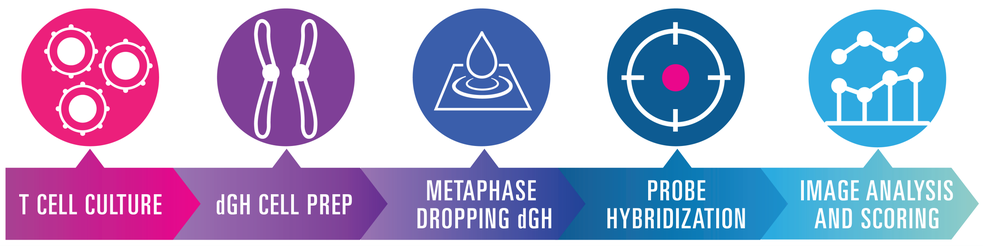


- dGH™ chromosomes contain only the 2 parental DNA strands in opposite orientation.
- Single stranded probes are designed to target only the parental strand (no reverse compliments).
- Signals appear on only one chromatid. Inversions present as signal from the opposite sister chromatid.

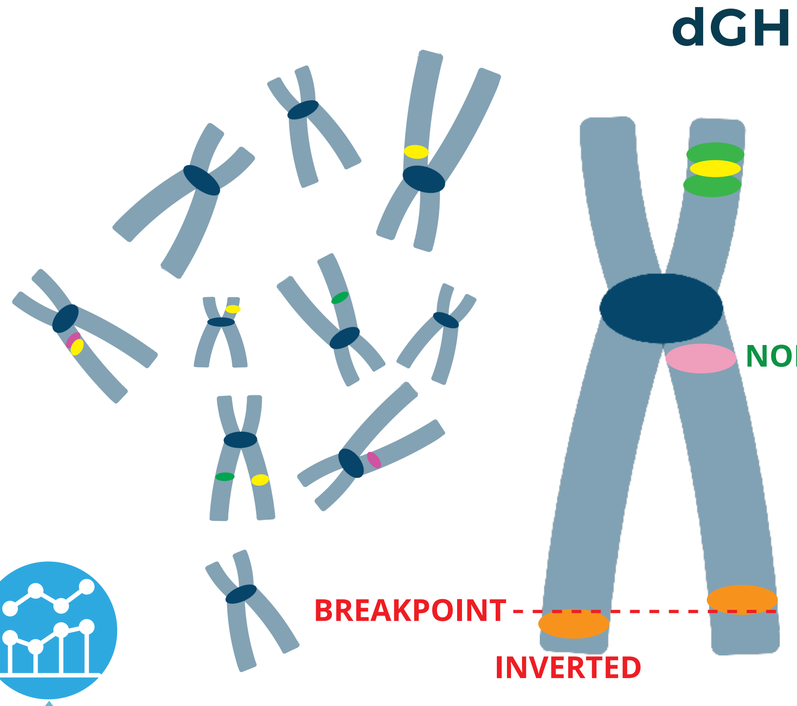
Comprehensive Analysis Package



dGH™ Workflow

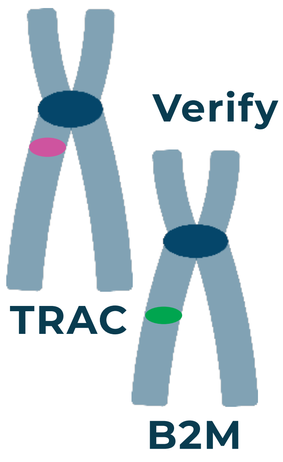


dGH in-Site™ Strategy



- **Sequence:** identify target chromosome location & copy number variation (CNV) by probe color.
  - Loci Marker Probe
  - Non-genomic Insert
  - Centromere Enumeration Probe
  - Telomere Enumeration Probe
- **Orientation:** opposite sister chromatid indicative of inverted target.
  - **TEP** signals indicate **BREAKPOINT** in telomeric region. Probe signal on the opposite sister chromatid indicates **INVERSION**.
- **Location:** map out Loci positions on chromosome arms.
  - **CEP** identifies chromosome, position on Q-arm **NORMAL**.

dGH in-Site™ for CAR-T



Verify the Structure of Target Loci

TRAC: marker probe spans 0.85Mb
B2M: marker probe spans 1.1Mb

- Inversions
- Translocations
- Chromosomal copy number
- Target loci copy number
- On-target insert verification

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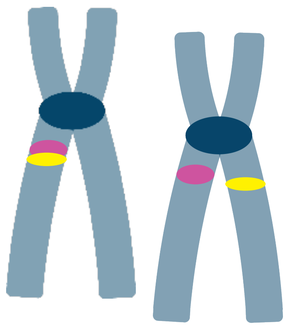
Measure & Locate CAR Insertions



- **CAR Transgene Probes**
  - Insertion signals down to 2kb
  - On-target copy number
  - Off-target copy number
  - Inverted inserts

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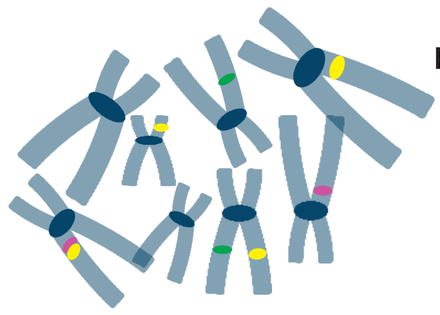
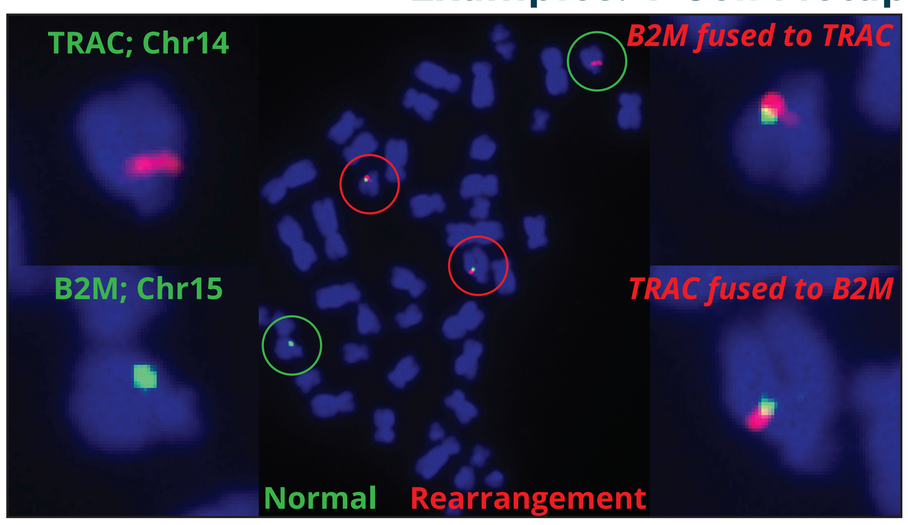
Single-cell Measurements of Many Cells

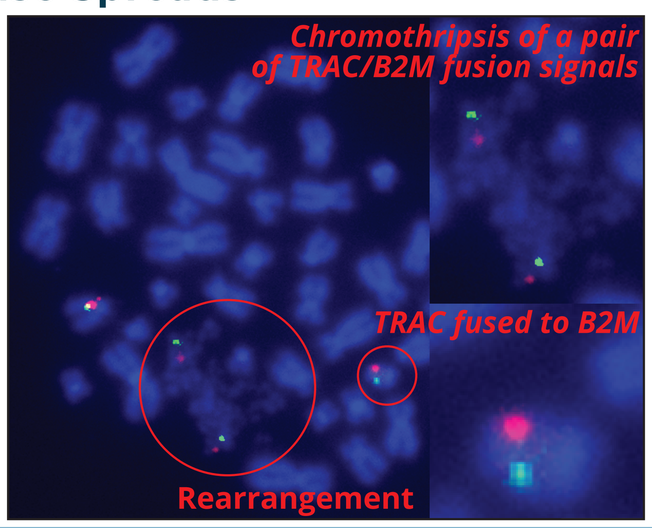


Percent Occurance of:

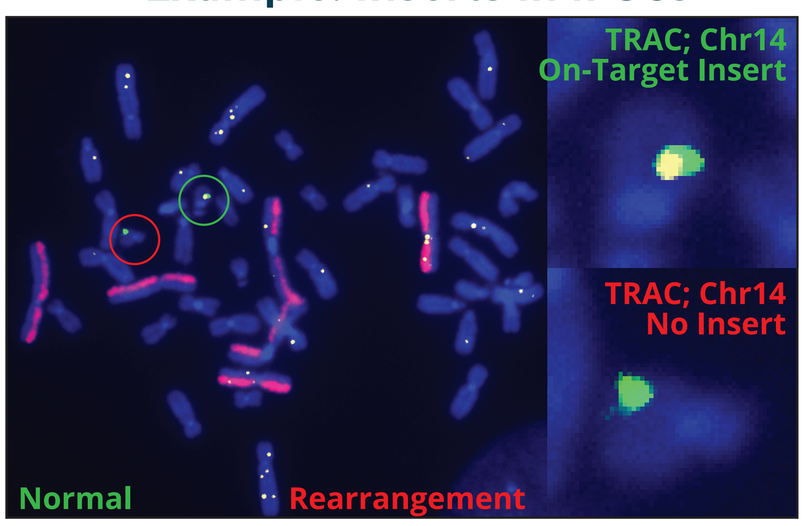
- Normal loci
- Inversions/ SCE's of loci
- Translocations of loci
- On-target insertions

Examples: T Cell Metaphase Spreads





Example: Inserts in iPSCs



Cellular Engineering → Single-cell Cloning → Structural Analysis → Lead Selection → Pre / Clinical Development

Structural Analysis: <u>Genome Mapping</u>
Lead Selection: <u>Genome Toxicity</u>
Pre / Clinical Development: <u>Quality Control</u>

Chromothripsis: Easy to measure with G-Banding, dGH in-Site™ and SCREEN™
Clonal Outgrowth: Measure with dGH in-Site™ timecourse study
Insertional Mutagenesis: Mark high risk loci and track insertions with dGH in-Site™
Structural Variation: Single-cell detection and mapping of variants with dGH in-Site™
Genomic Instability: Early detection of instability with dGH SCREEN™
Aneuploidy: Single-cell detection with dGH SCREEN™ and G-Banding

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