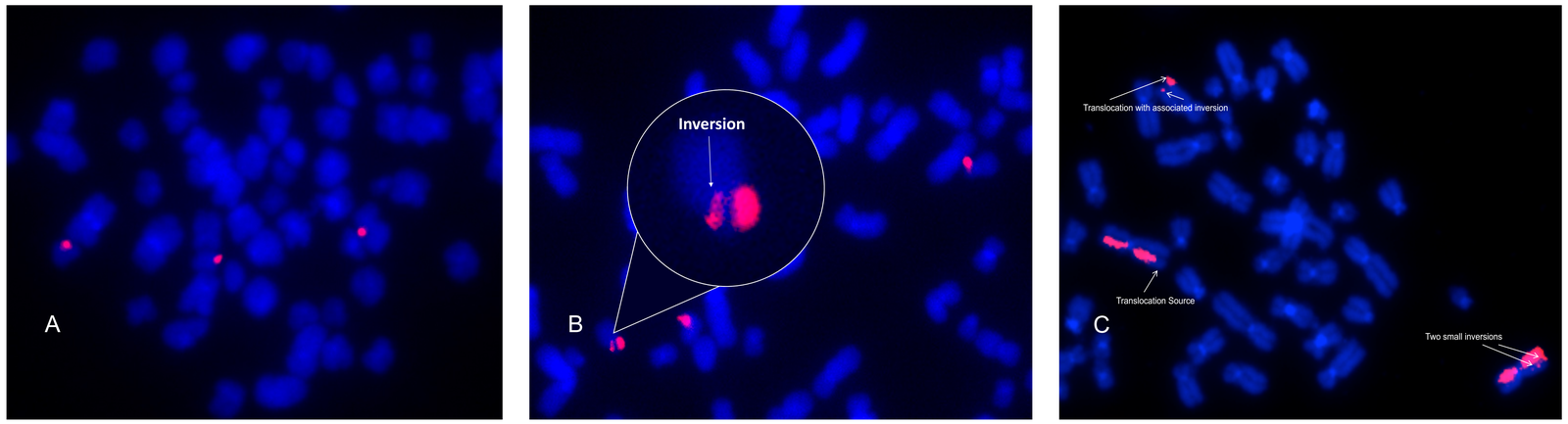
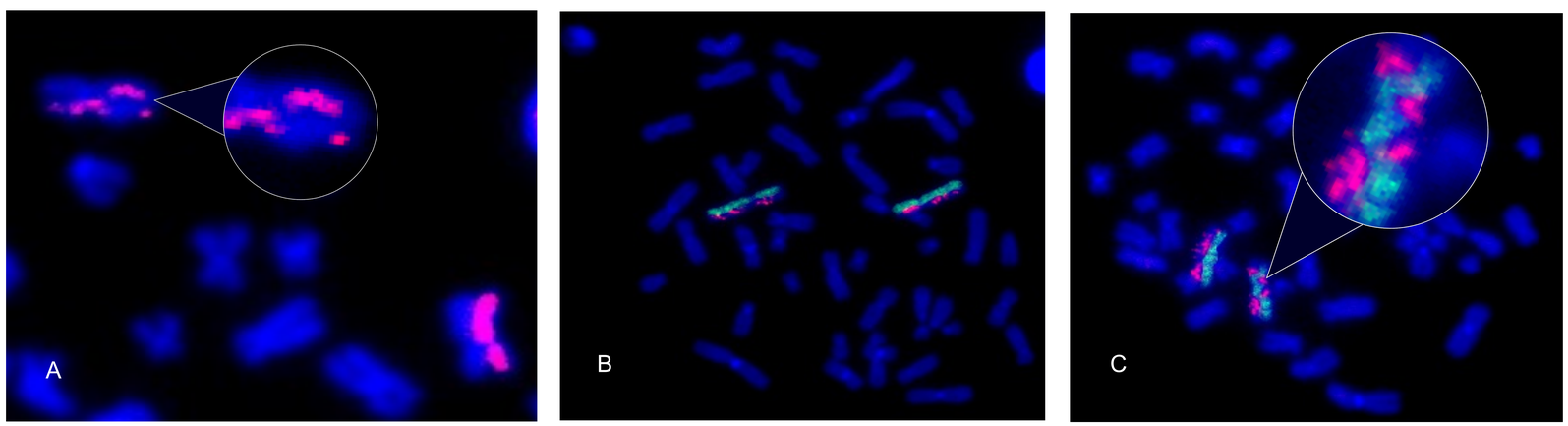
Using Directional Genomic Hybridization™ to Discover and Detect Structural Variation

Erin Robinson[1], Miles McKenna[1,2], Susan Bailey[2], Gretchen Pratt[1], Christopher Tompkins[1], Stephen Hughes[1], Henry Sebesta[1], David P. Sebesta[1], F. Andrew Ray[2], Michael Cornforth[3], Joel Bedford[2], Ed Goodwin[2]

Directional Genomic Hybridization™ (dGH™) is a genomic technique for reading the structure of a genome directly with high specificity and precision, detecting sequence variations of five kilobases or less. Using the reference genome, dGH probes are designed against normal sequence and produced using single-stranded fluorescently labeled DNA. Structural variations from the reference genome are then easily identified from the resulting signal. As a single cell method, paired with automation and automated image analysis, dGH is ideally suited to detecting common constitutional structural variation as well as rare, low occurrence, and complex variation. Here, we illustrate how dGH can be used to complement NGS data by detecting structural variations, using cell-by-cell analysis, as opposed to pooled DNA and bioinformatics.



A) HEK control cell triploid for Ch17, with probe on p53 gene.
B) HEK cell line with CRISPR edit, 2 sites within the p53 gene, with an inversion in one homolog.
C) In an irradiated clonal cell line, dGH shows multiple complex rearrangements; one copy of Ch 3 with two small inversions, and a complex translocation from Ch 3, with an associated inversion.

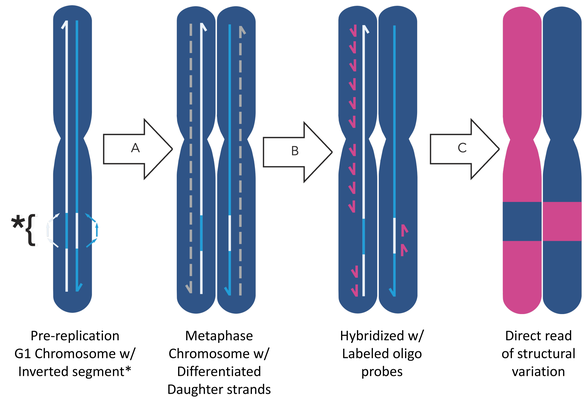


A) De novo discovery of a previously unsuspected large inversion (found in roughly 40% of cells) on Ch 2 in a patient with an undiagnosed disease.
B & C) Follow up assay for sample in Figure A, a probe ladder was designed for the antisense strand, and used in combination with Ch 2 chromatid paint to narrow down the breakpoint region locations. B shows a normal cell, and C shows a cell with an inversion.

KromaTiD

1. KromaTiD, Inc. 2. Colorado State University 3. University of Texas Medical Branch

dGH probes are hybridized to prepared metaphase chromosomes and imaged, with a simple, accessible method. dGH involves stripping newly synthesized daughter strands from metaphase chromosomes, yielding single-stranded target DNA for hybridization of single-stranded directional probes.



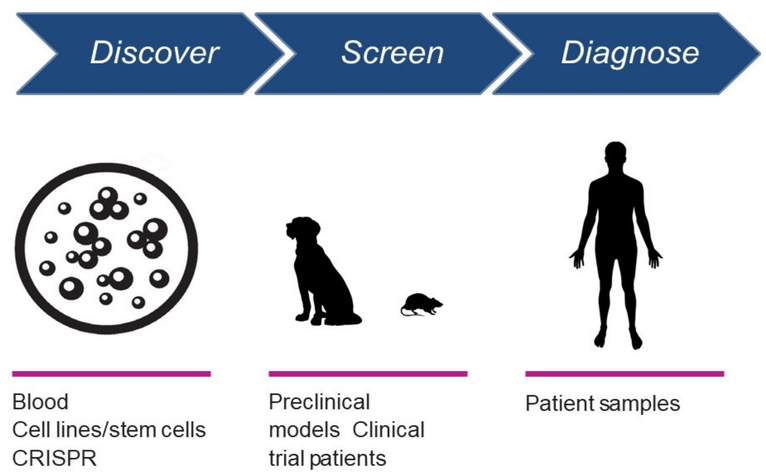
A) Cell Division and dGH sample prep

B) Daughter strand degradation (UV) and chromatid painting

C) Imaging and scoring of structural changes

	dGH	aCGH	Sequencing
Balanced Translocation Detection	High	None	Low
Unbalanced Translocation Detection	High	Medium	Medium
Deletion Detection	Medium	High	Medium
Duplication Detection	Low	High	Medium
Specific Sequence Detection	High	High	High
Inversion Detection	High	None	Low

Single cell analysis can discover and detect rearrangements missed by NGS and other pooled DNA methods, precisely detecting the prevalence of multiple and variable rearrangements occurring in a single sample. As an orthogonal technique to NGS, dGH can be used to confirm simple structural variations determined by NGS bioinformatic analysis in a wide range of research, preclinical and clinical samples.



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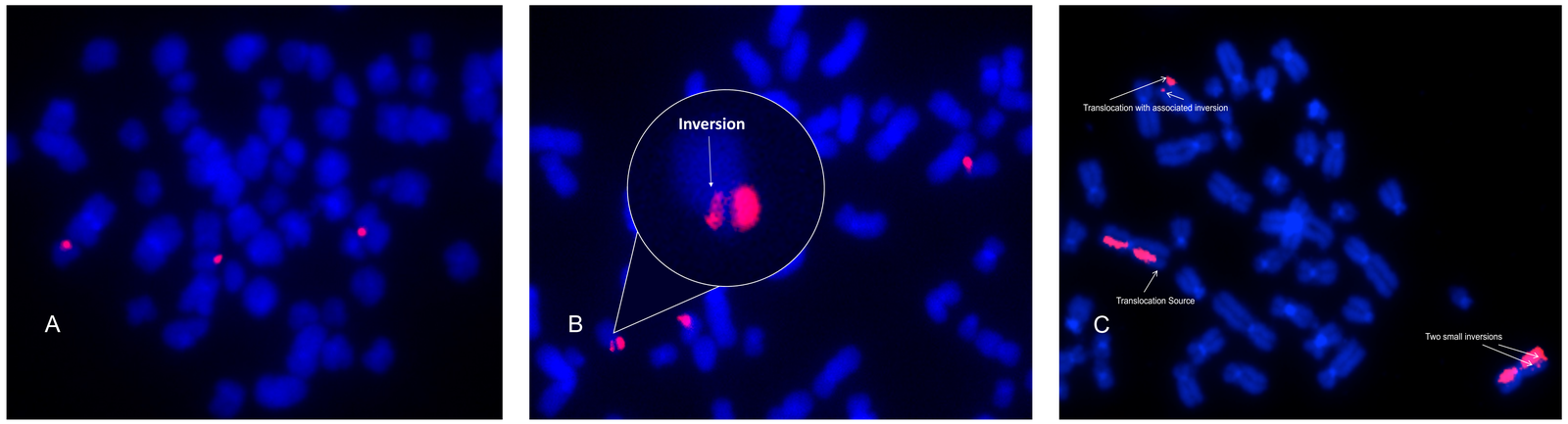

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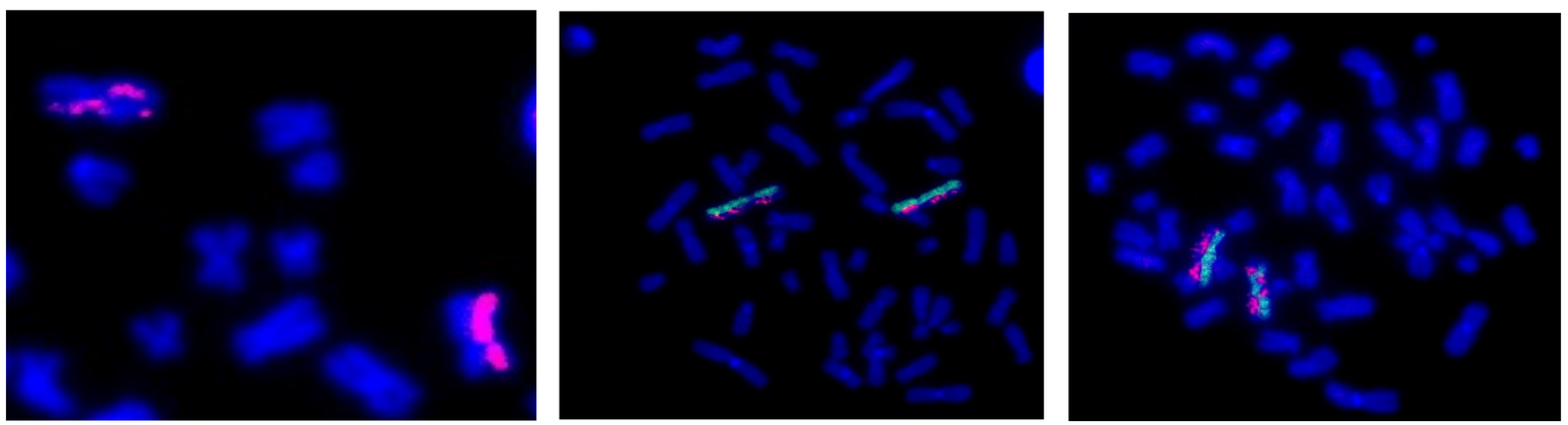

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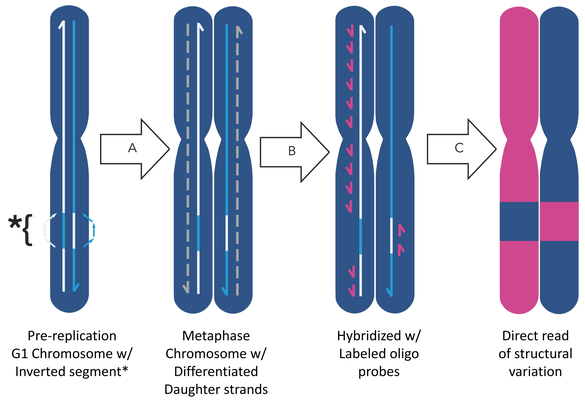

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