dGH™ Tests for Discovering the Structural Causes of Undiagnosed Genetic Diseases

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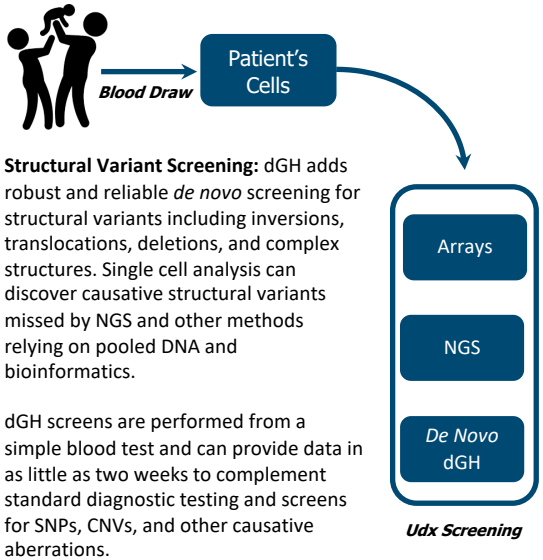
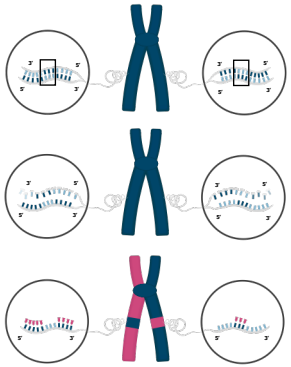
Directional Genomic Hybridization™ (dGH™) is a unique method for discovering causative structural variation in undiagnosed disease patients. dGH allows researchers to read the structure of a genome directly with high specificity and precision and detect structural variations of five kilobases or more. dGH probes, designed to match the reference genome, are hybridized to specially prepared metaphase chromosomes and imaged. Structural variations are then identified from signal patterns. As a single cell method, dGH is uniquely suited to discover causative constitutional structural variants including rare, low-occurrence, and complex rearrangements. Here, we illustrate how dGH can be used to discover structural variation by direct mapping of chromosomes.



Structural Variant Screening: dGH adds robust and reliable *de novo* screening for structural variants including inversions, translocations, deletions, and complex structures. Single cell analysis can discover causative structural variants missed by NGS and other methods relying on pooled DNA and bioinformatics.

dGH screens are performed from a simple blood test and can provide data in as little as two weeks to complement standard diagnostic testing and screens for SNPs, CNVs, and other causative aberrations.

The Principle: dGH involves preparing single-stranded chromosomal DNA to measure sequence location and orientation of causative structural variants. Unique and proprietary dGH probes are hybridized to the prepared metaphase chromosomes and imaged with a simple, accessible method.

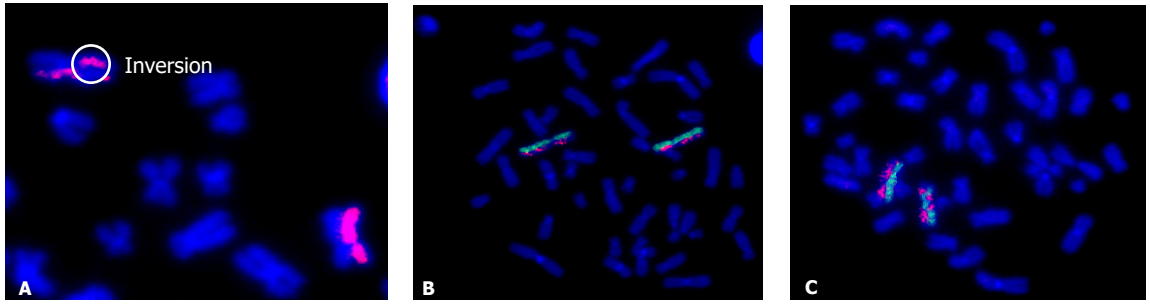


1. Metaphase Chromosome: Double stranded chromosome with inverted segment

2. Sample Prep: Selective daughter strand degradation

3. Screening & Analysis: dGH probe hybridization, imaging and identification of structural variants

Using dGH to Screen for Structural Variants in Undiagnosed Disease Patients: As part of the Undiagnosed Disease Consortium, KromaTiD screened 5 undiagnosed disease patients and their families for causative structural variants. Previously unreported structural variants were found in two patients and one related family member.



A) De novo discovery of an unsuspected inversion on Ch 2 in a patient with an undiagnosed disease. B & C) A antisense probe ladder was used in combination with the Ch 2 de novo dGH screen to identify the breakpoint regions of the structural variant. Figure B shows a normal cell, and C a cell with an inversion.

dGH 2.0 for Undiagnosed Disease Screening: KromaTiD is developing whole genome AI based *de novo* dGH to aide in the identification of currently undetectable causative structural variants. We anticipate beginning screening studies late 2020.

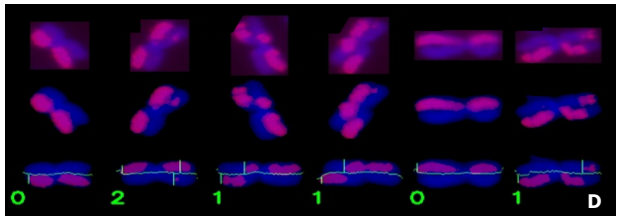
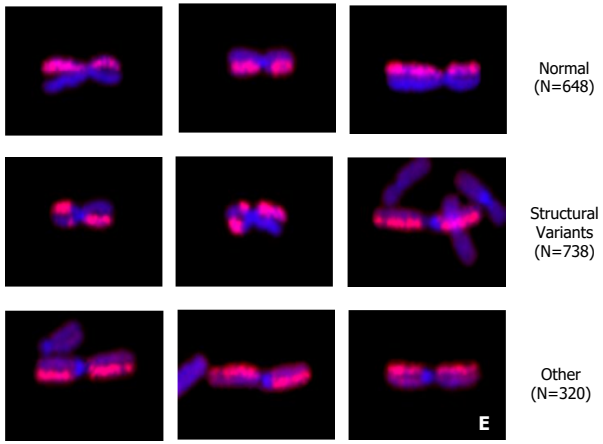


Image D & E: Image D shows AI analysis of breakpoints from a single cell. Image E shows classification of structural variants from a set of 1706 dGH chromosomes



Efficient Screening to Identify Causative Structural Variants: Screening undiagnosed disease patient populations with similar presentations for causative structural variants requires efficient whole genome *de novo* dGH. We are developing AI analysis methods to efficiently discover causative structural variations and identify breakpoints. Image E and Image F show the successful identification of chromosomes with structural variations by a prototype AI from a training set of 400 cells.