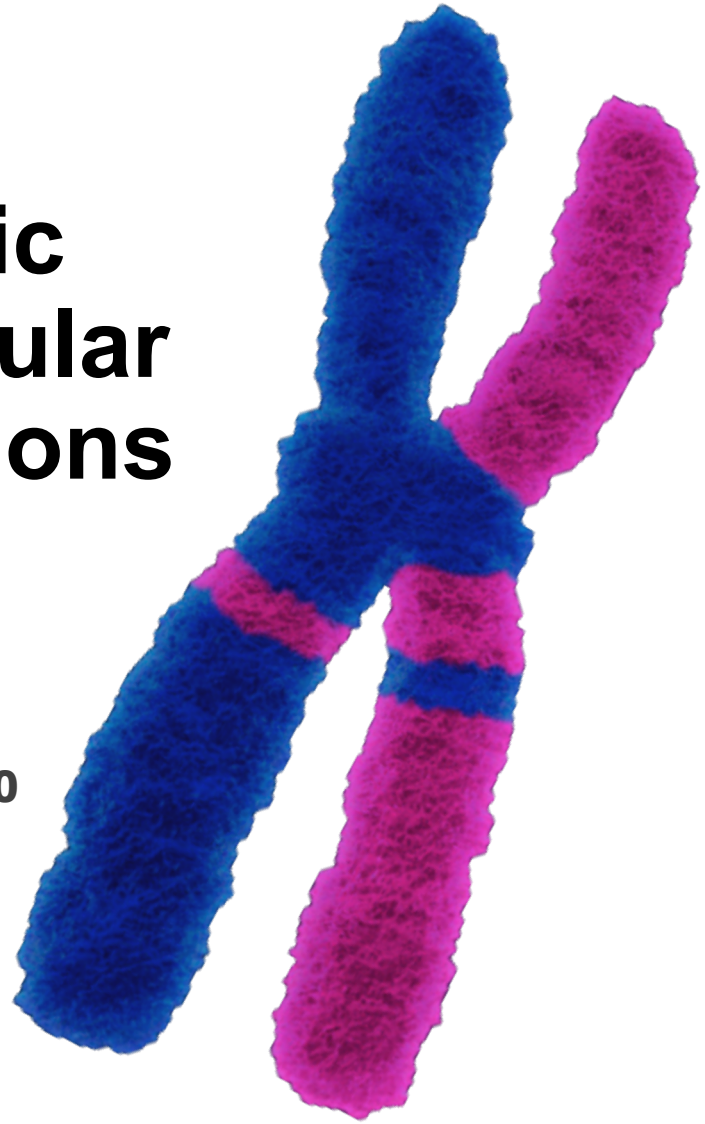
Directional Genomic Hybridization for Cellular Engineering Applications

Presented to:

OXFORD GLOBAL

NextGen Omics 2020

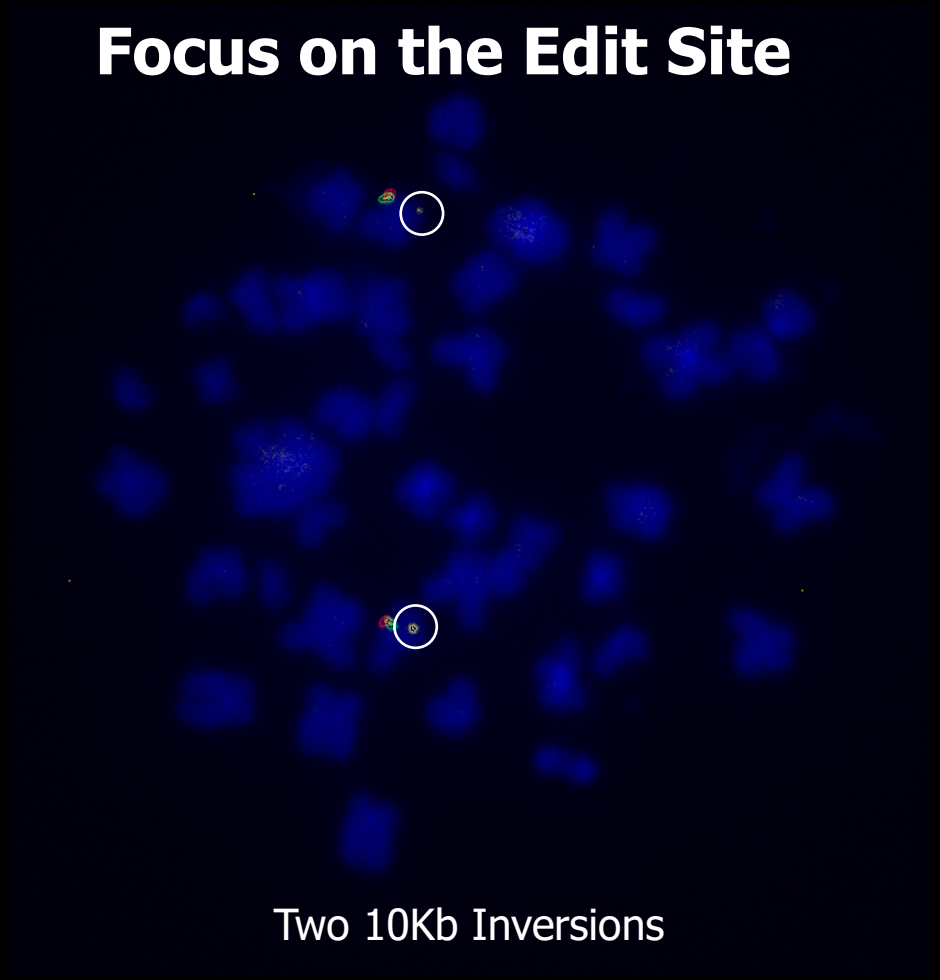


KromaTiD

Direct, Definitive Genomics

Visualizing Genomic Structure with dGH™

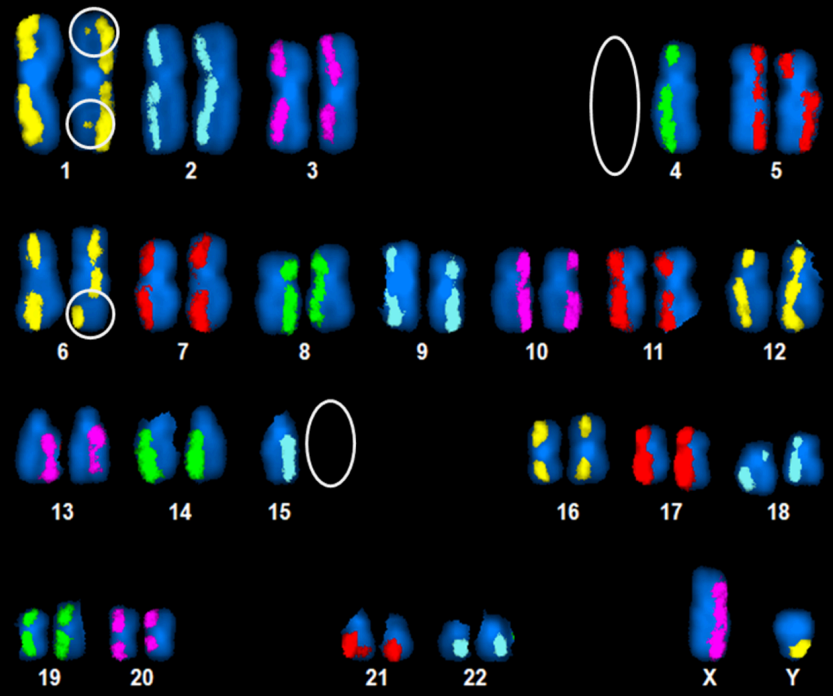
Focus on the Edit Site



Two 10Kb Inversions

OR

The Whole Genome

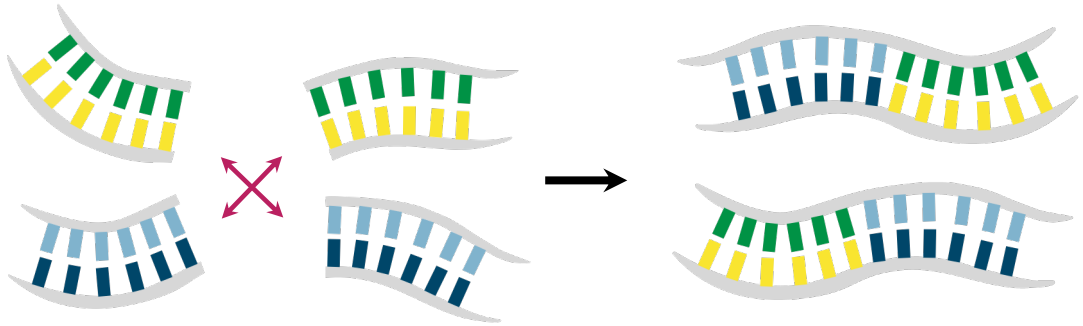


Three Inversions and Two Missing Chromosomes

Direct Structural Measurements -- Definitive Process Outcomes

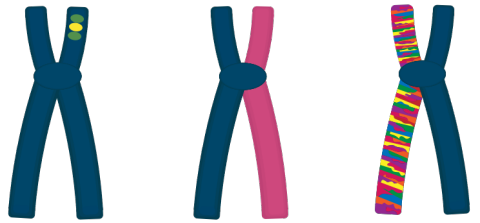
A Comprehensive Genomic Picture Requires dGH

Mis-repairs: Faulty Repair



Structural Variant

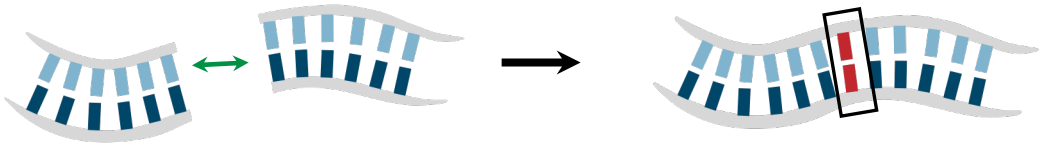
dGH is Direct Visualization



In-Site™ SCREEN™ DSCVR™

Direct measurement of simple, complex and heterogenous structural variation

Mis-edits: Accurate Repair: Faulty Edit

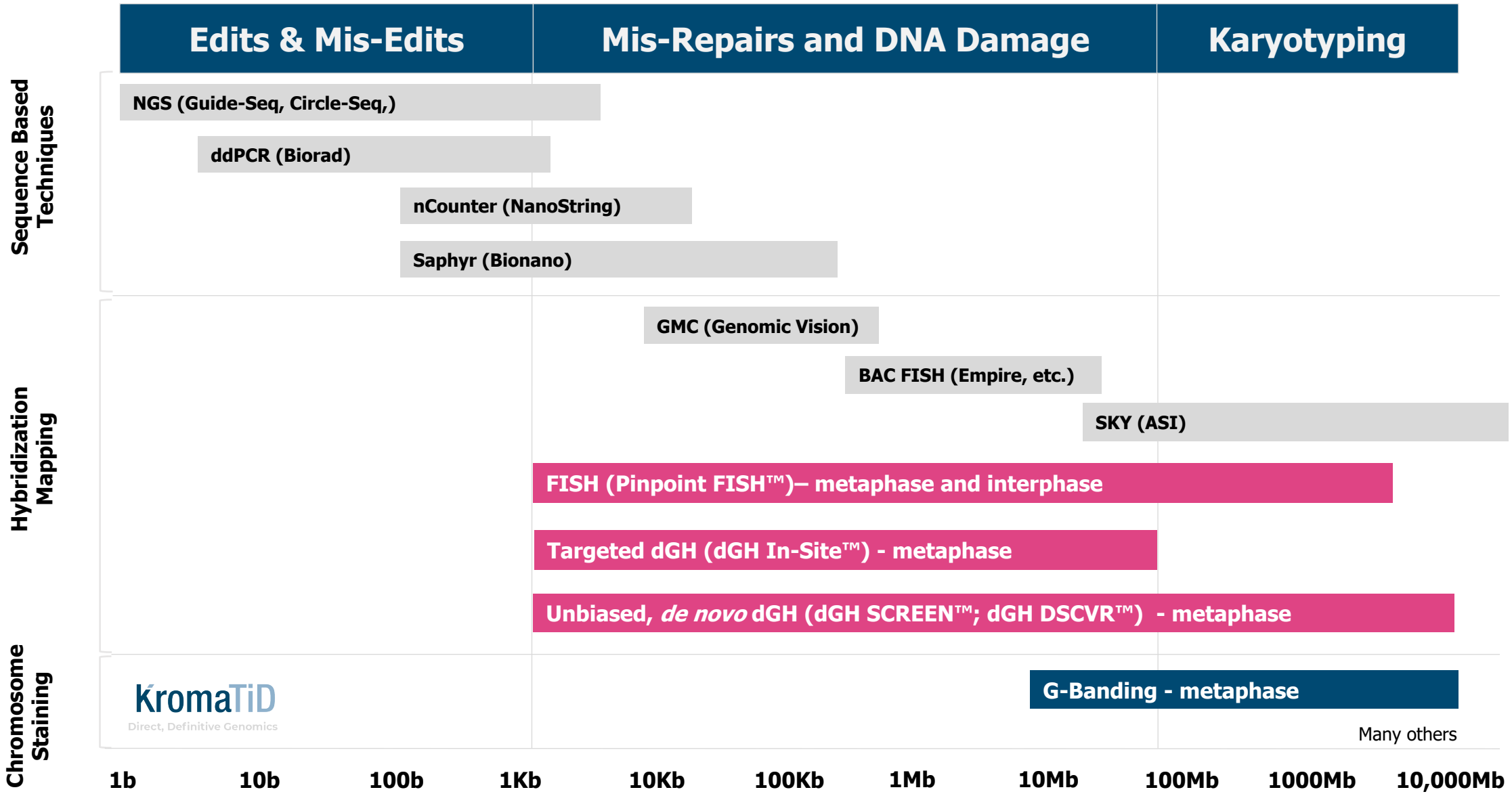


Single Nucleotide Variant

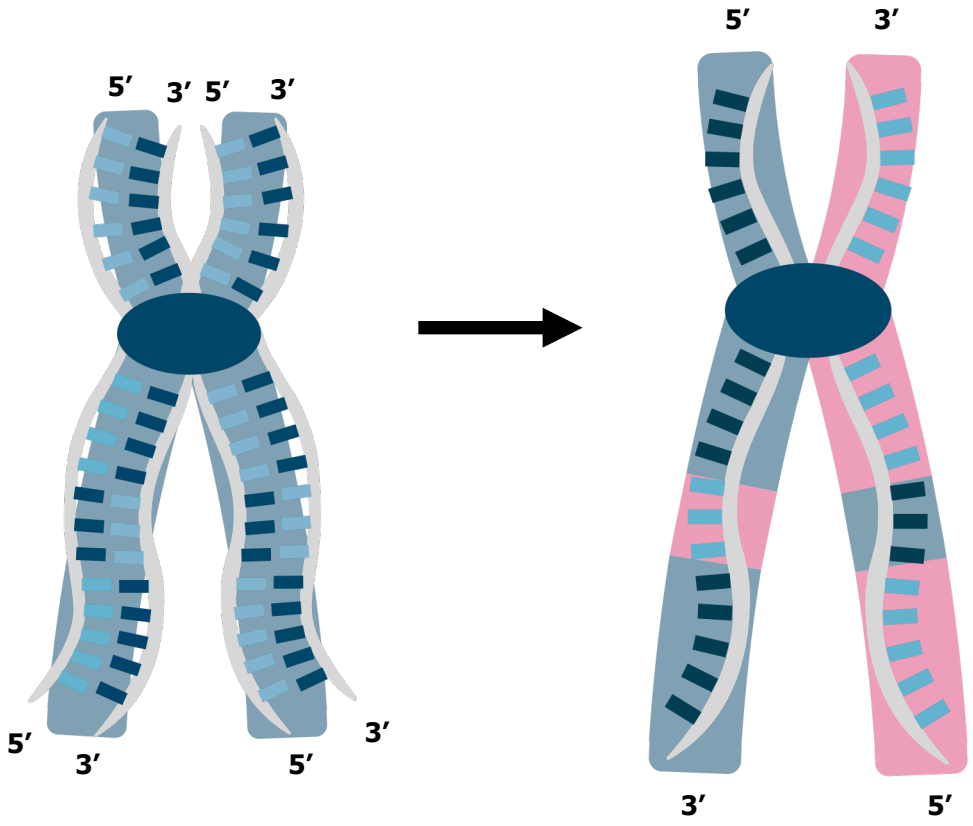
Sequencing uses pooled DNA

Measuring all editing outcomes & risks requires only two orthogonal and complementary methods

Genomic Structure on a Genome Wide Scale



A Robust Method



Analyte

dGH chromosomes contain **2 strands** of oppositely oriented, **Parental DNA only**—NO Daughter Strands

Single-stranded probes are designed to **target *only* one strand** and *only* unique sequences

1. *Grow cells through one cell cycle*
2. *Incorporate analog during replication*
3. *Strip daughter strands*
4. *Hybridize with proprietary single stranded probes*
5. *Image and analyze*

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Williams, E., & Bailey, S. (2009). Chromosome Orientation Fluorescence In Situ Hybridization (CO-FISH)

Using dGH in a Cellular Engineering Workflow

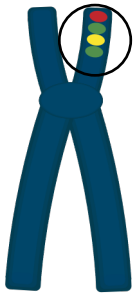
	Genome Modification	Edited Cell Cloning	Structural Analysis	Lead Selection	Pre/Clinical Dev
 In-Site™	Integration outcomes Insert distribution	Edit Clonality	Stability of Edits Distribution of Inserts	GLP Tox Custom Assays Structural Variation Distribution	GLP & CLIA Assays
 SCREEN™	Baseline Structural Variation Edit Process Optimization	Edit & Quality Control Structural Variants	Instability of Genome Subclonal Expansion		Integrity of Edits & Genome

Comprehensive genomic structural measurements throughout therapy development

Mapping Edit Site Structural Variation

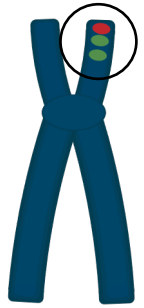
Two concurrent edits to a single chromosome lead to four or more double-strand breaks and a high probability of mis-repair

Desired Edit Site Structure



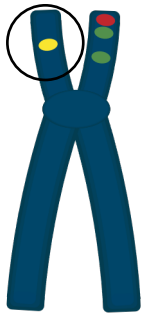
- Sub-telomeric probe
- Bracketing probes
- Inter-edit sequence (10kB)
- Bracketing probes

Deleted Edit Site Structure



- Sub-telomeric probe
- Bracketing probes
- Bracketing probes

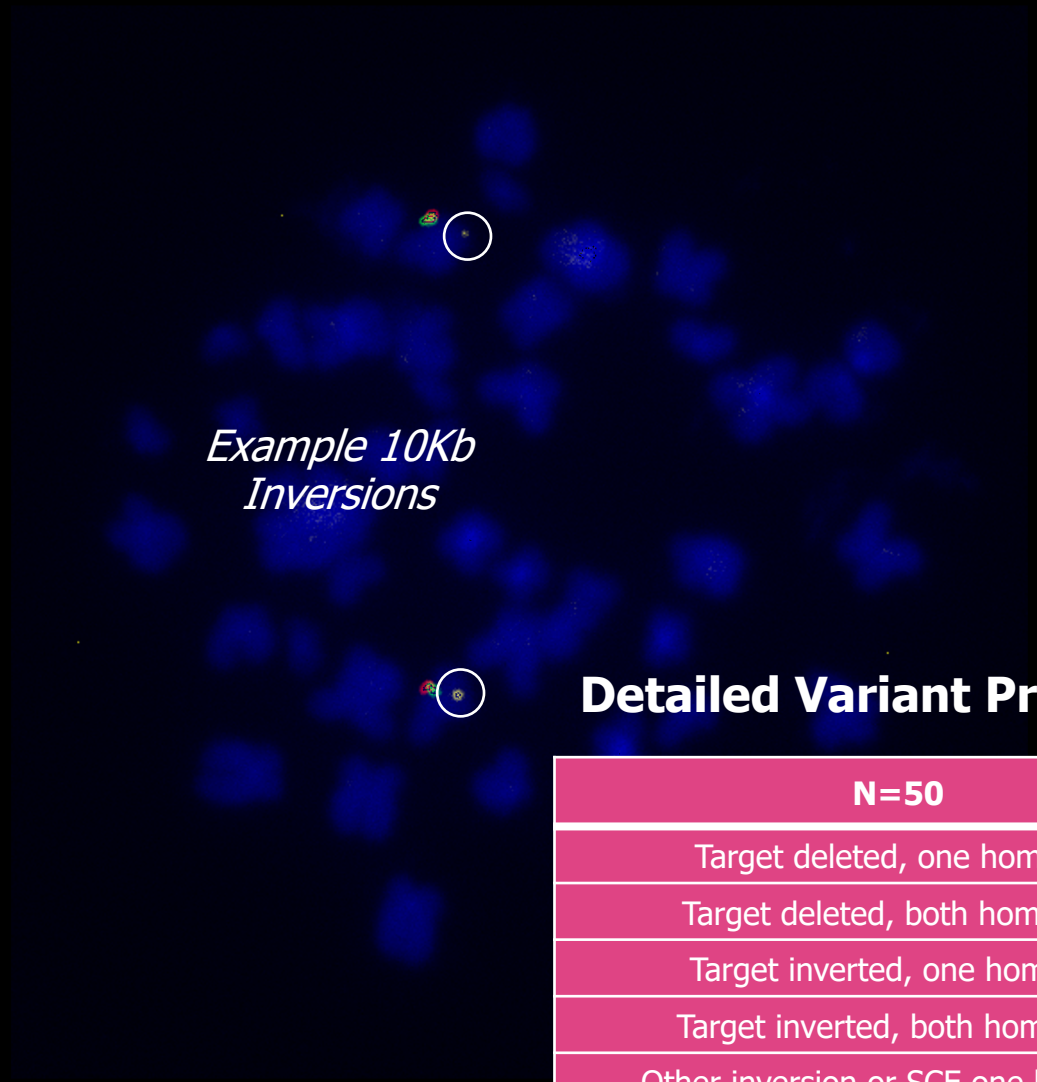
Inverted Edit Site



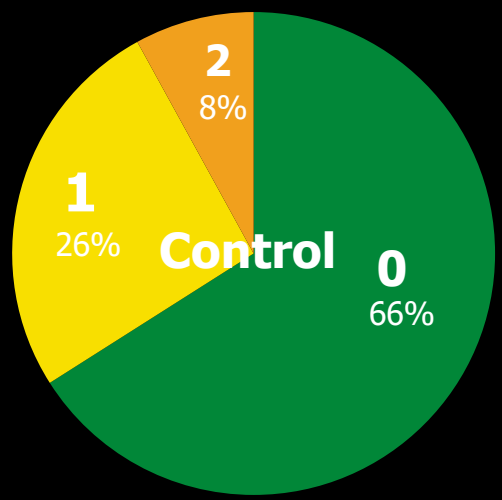
- Sub-telomeric probe
- Bracketing probes
- Inter-Edit sequence (10kB)
- Bracketing probes

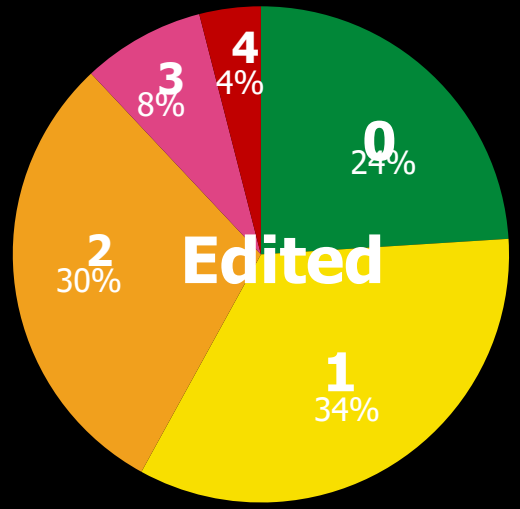
Multiple mis-repairs lead to cells with multiple variants and a complex, heterogeneous batch

Measuring Complex Variants Arising from a Double Edit



Variants per Cell



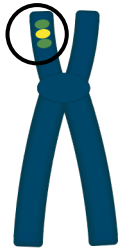


Detailed Variant Profile

N=50	Control	Edited
Target deleted, one homolog	0%	32%
Target deleted, both homologs	0%	6%
Target inverted, one homolog	4%	12%
Target inverted, both homologs	0%	4%
Other inversion or SCE one homolog	12%	22%
Other inversion or SCE both homologs	8%	16%
Target inverted plus other inversion	0%	4%

Single Cell Evaluation of Insertion Quality

On-Target Insert



Ch 22

Chromosome 22
Green Probes define the target site. Yellow signal indicates an on-target insertion

Off-Target Insert

Ch 4-21, X, Y

Other Chromosomes
Off target inserts show as yellow signal

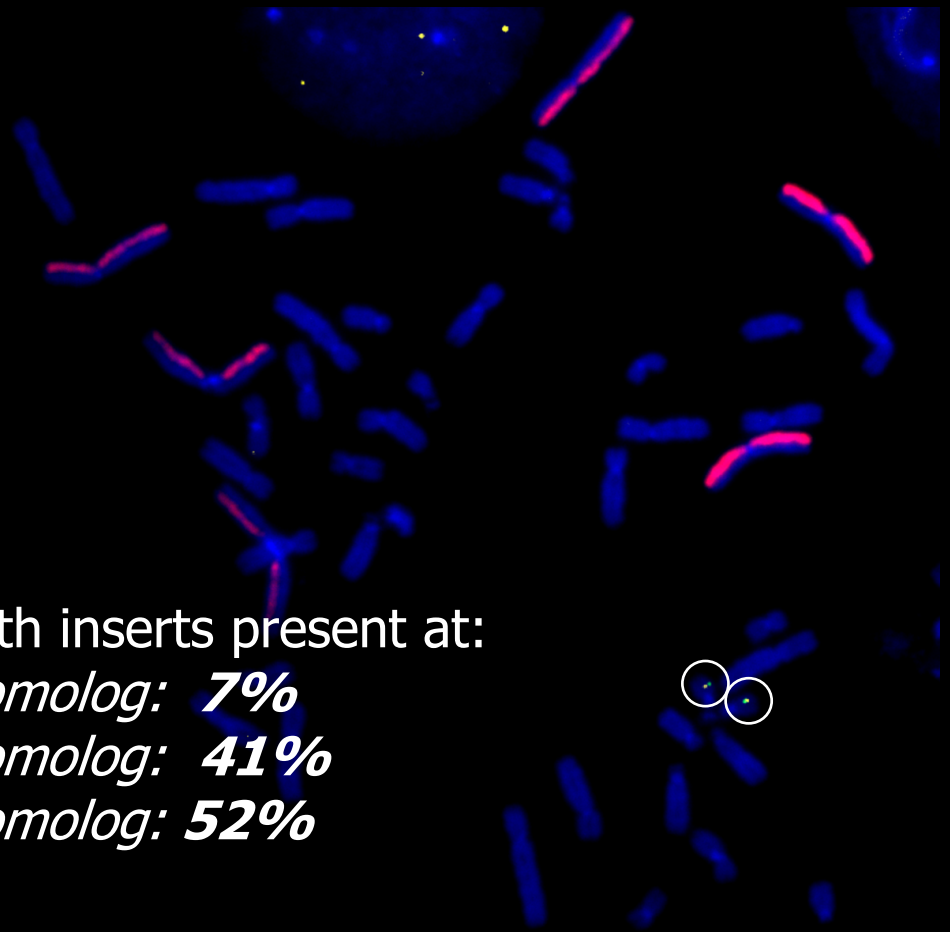
Ch 1, 2, 3

Chromosome 1, 2 and 3
Full chromatid paints to measure rates of random variation and unexpected DNA damage

dGH In-Site™ *measures quantitative insertion success and quality*

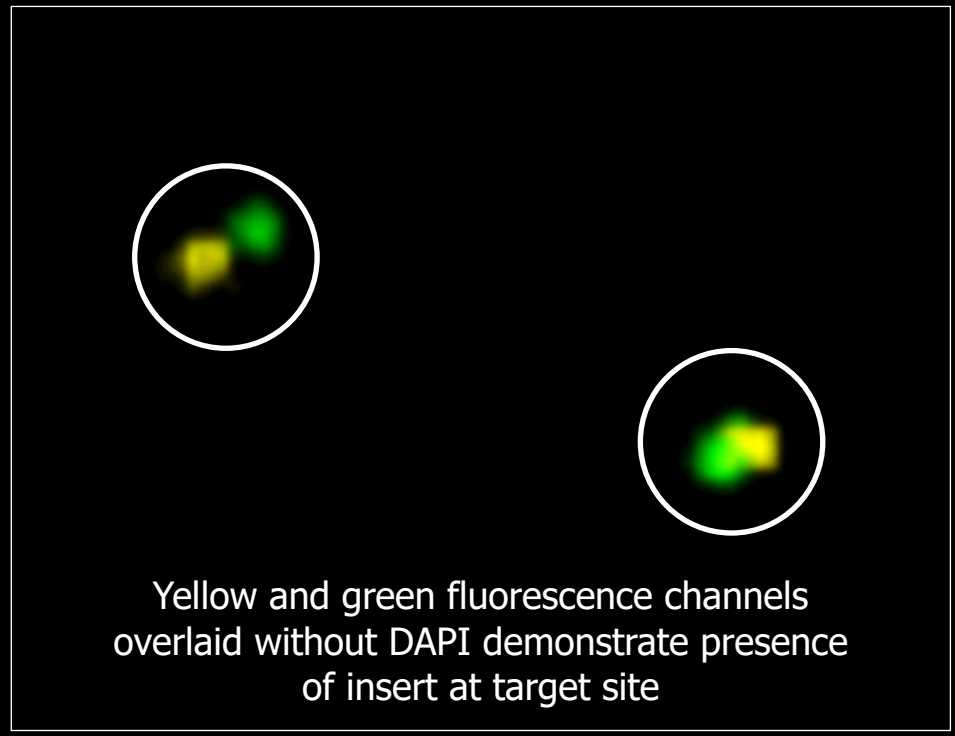
dGH SCREEN™ *measures DNA damage, instability and repair activity* ***concurrently***

48% of cells had at least one on-target insertion



Cells with inserts present at:

- *2 Homolog:* ***7%***
- *1 Homolog:* ***41%***
- *0 Homolog:* ***52%***



Yellow and green fluorescence channels overlaid without DAPI demonstrate presence of insert at target site

8.3% of on-target inserts were inverted and 1 Translocation of the target site was observed

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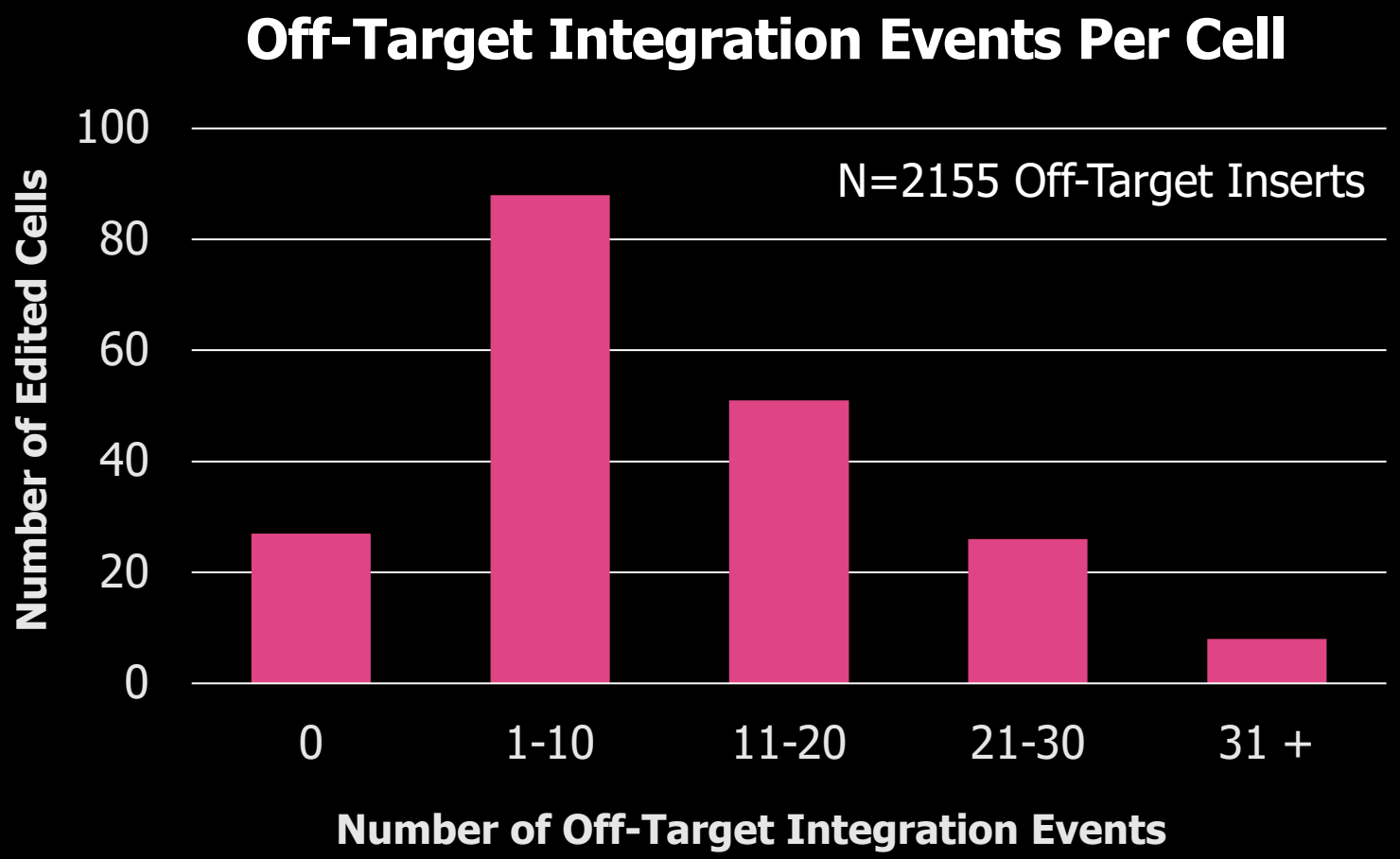
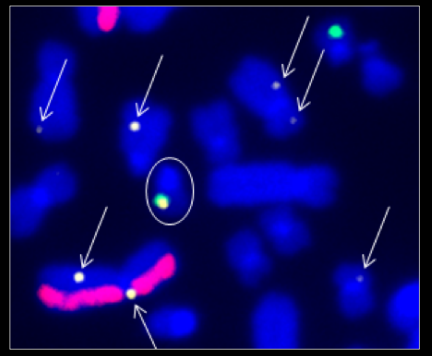
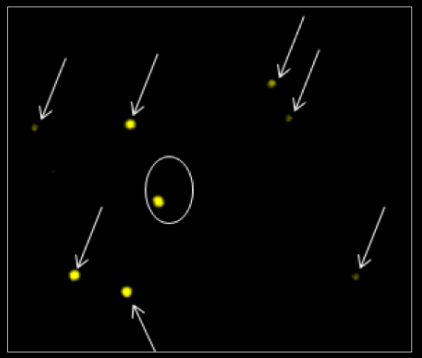
But, Off-Target Insertion was Common

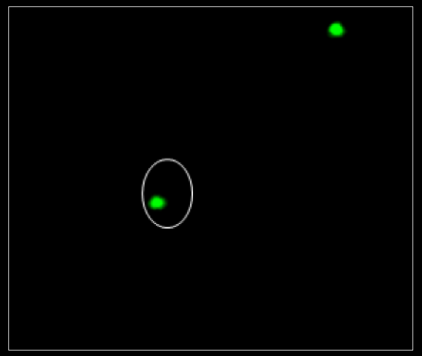




Off-target chromosomes in pink channel



Inserts visible in yellow channel



Target sites visible in green channel

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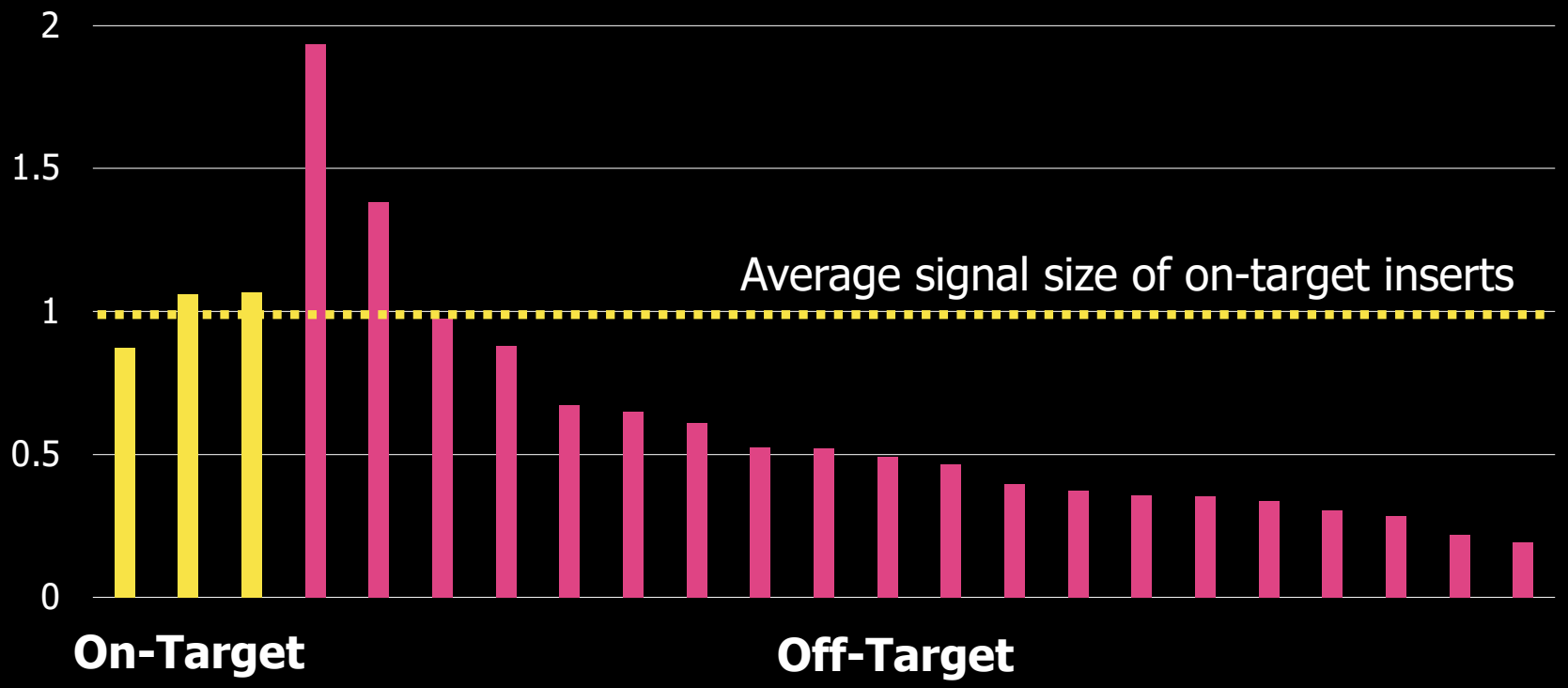
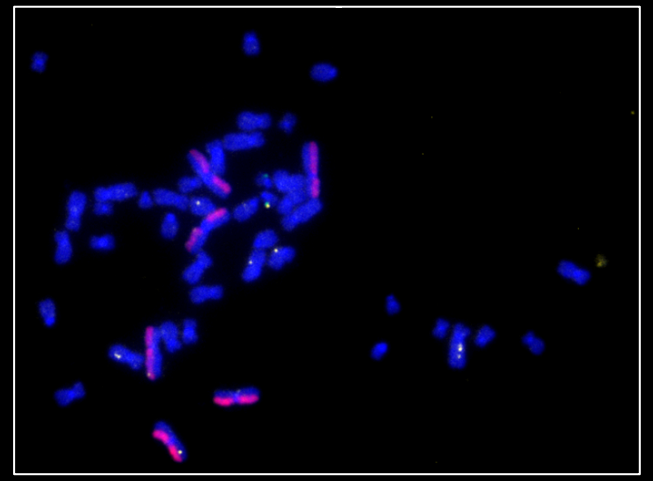
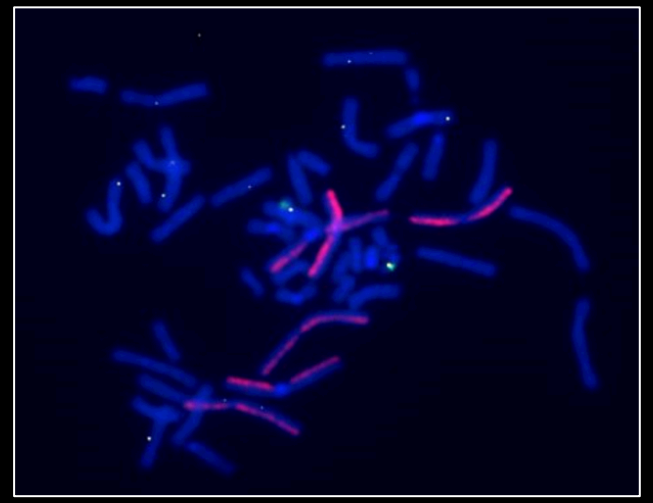
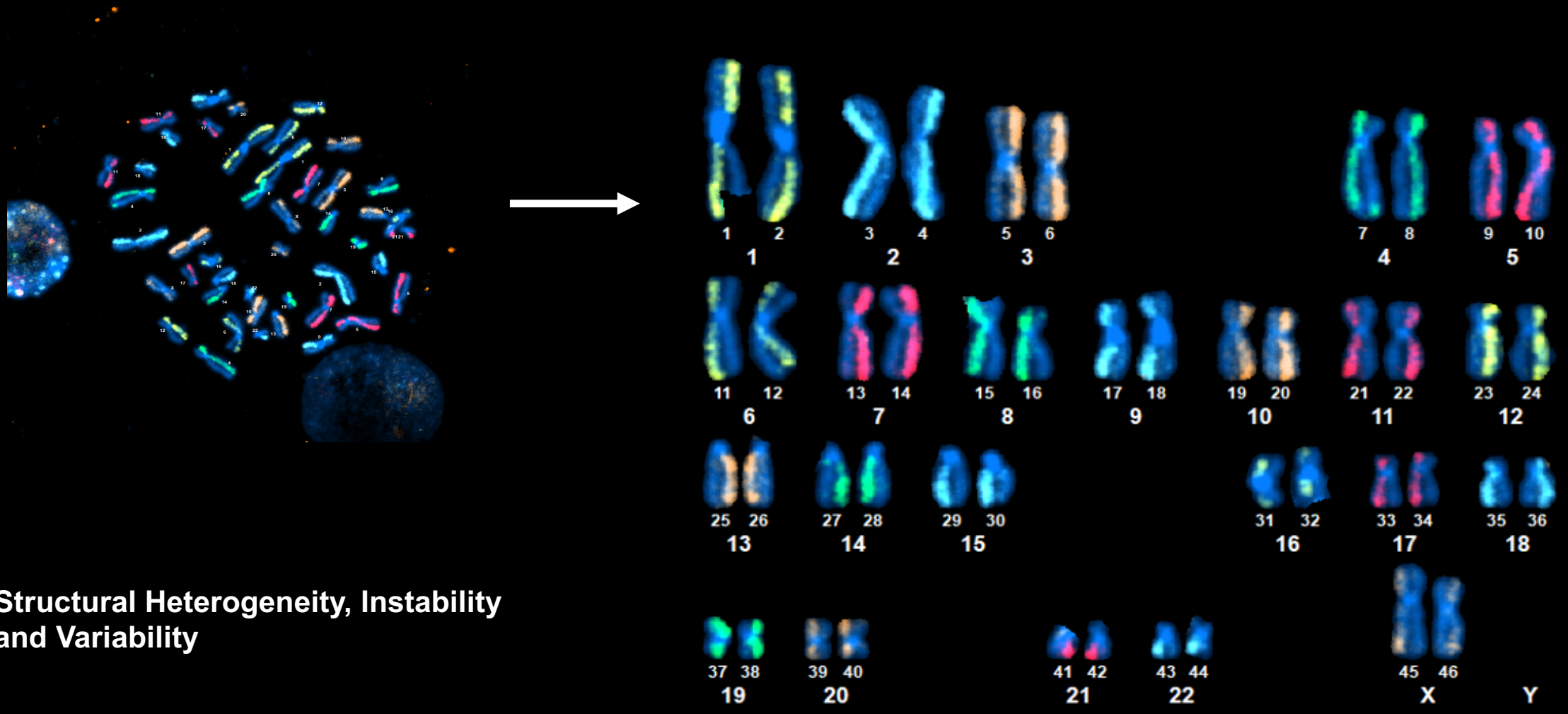
Correlating signal size with insert copy number







dGH SCREEN: WG, Single Cell Structural Variant Measurement



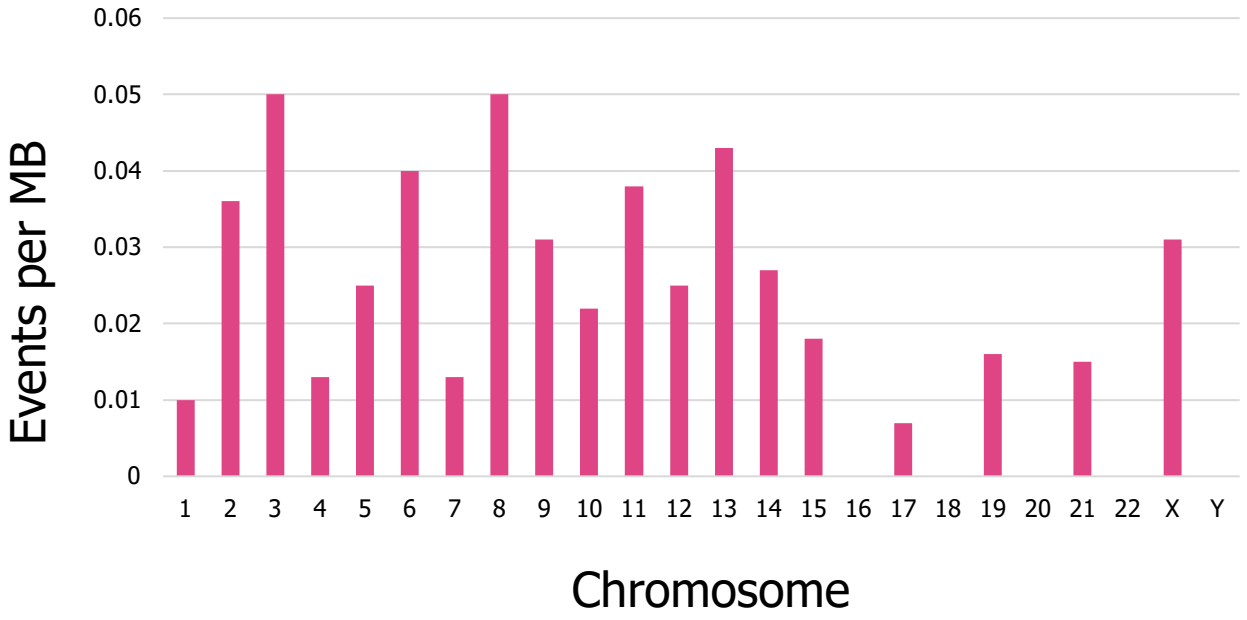
Structural Heterogeneity, Instability and Variability

Ref: Immortalized Leukemia B-Cells https://www.coriell.org/1/NIGMS/Collections/NIST-Reference-Materials

Unbiased Measurements - 3 Kb LLOD – Highest Res Karyotyping

Whole Genome, Single Cell Structural Variation: GM Data

DNA Repair Activity (SCEs)



Hi-Res Structural Mapping

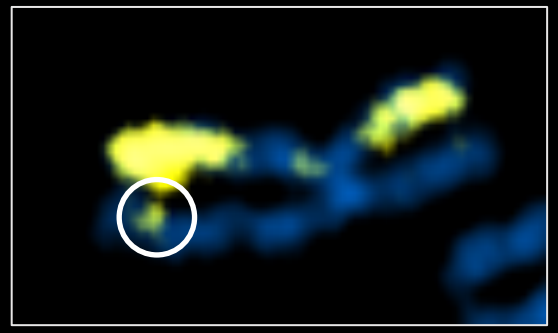
n = 25	Rate
Aneuploidy	40% of cells were aneuploid; monosomy of Chr14 observed in 25% of cells
Random Variants (<3kb)	Not observed
Variants Identified (<3Kb)	Not observed
Degree of Mosaicism	25% for c14 Monosomy
SCE Range: X to Y STD	2.6 ± 1.5

Ref: Immortalized Leukemia B-Cells https://www.coriell.org/1/NIGMS/Collections/NIST-Reference-Materials

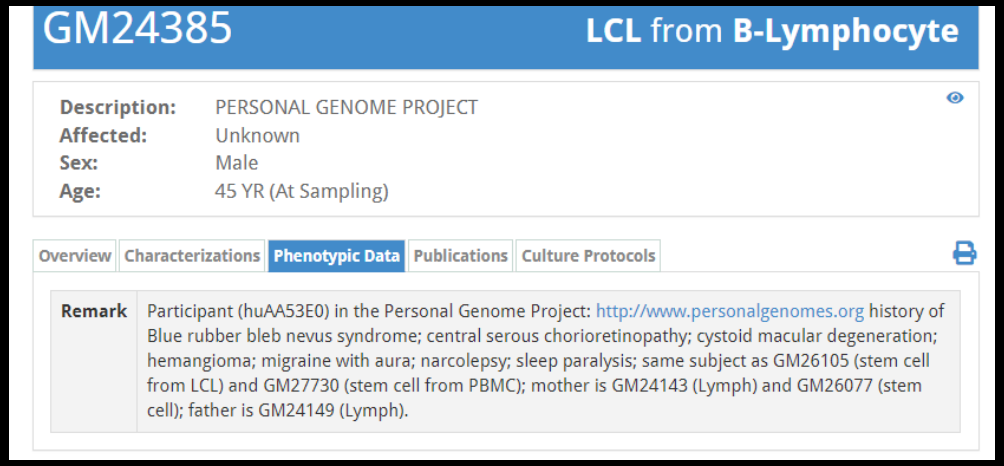
C14 mosaicism; Normal B-Cell repair activity, random aneuploidy; no structural variants

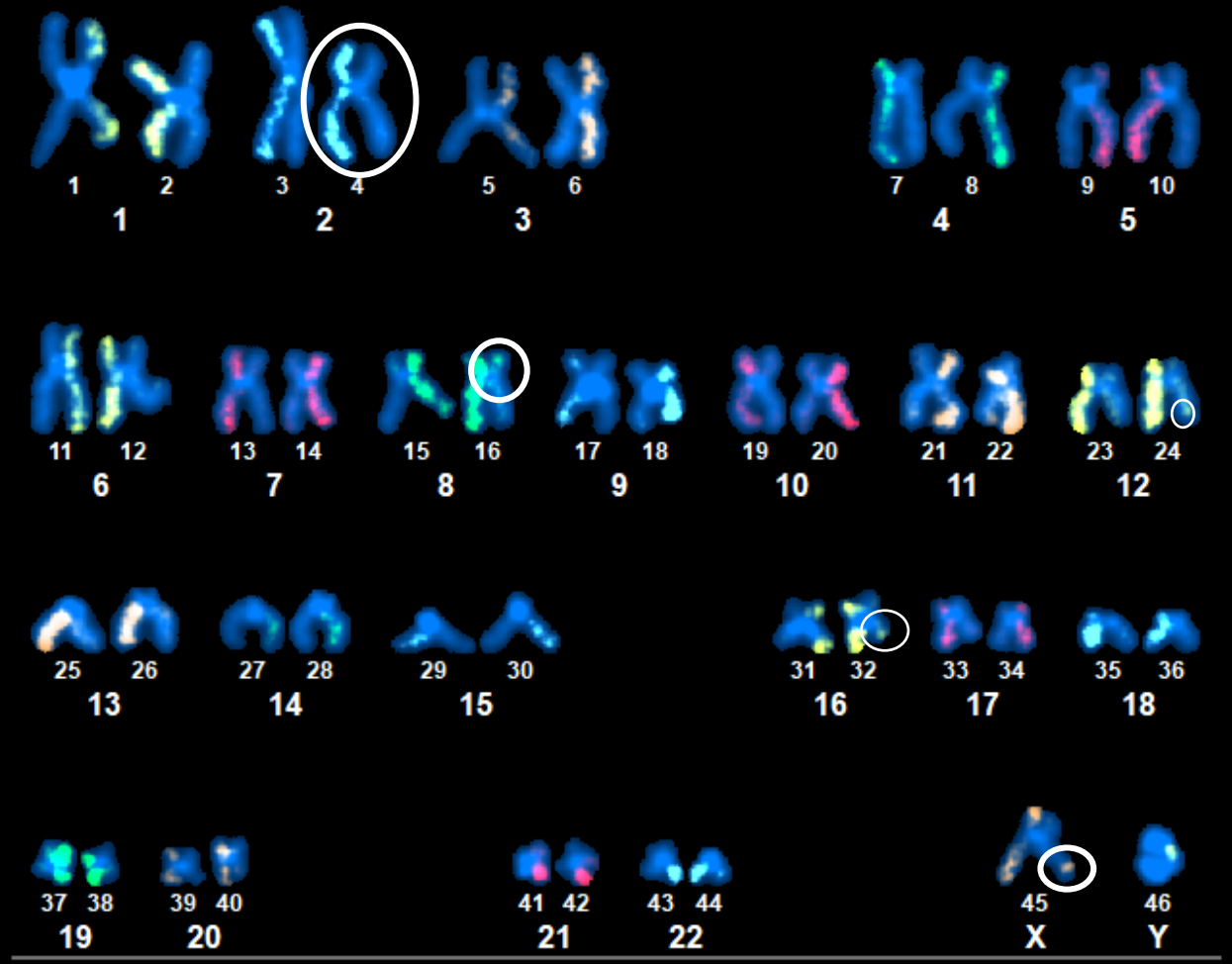
Discovering Variants in Rare Disease Patients

Using dGH SCREEN KromaTiD discovers 7 previously unidentified inversions and confirms 1 expected inversion



Inversion on CX





Indications of other rearrangements and genomic instability were also observed

KromaTiD Genomic Structural Products & Services

Product	Samples	Analyte	Applications	Status
Pinpoint FISH™	Blood, Cell, Tissues	Interphase cells or FISH prepped metaphase chromosomes	**Insert Tracking and Edit Site Profiling** for non-dividing cells and tissues	Launched 2015
dGH In-Site™	Blood or Cells	dGH prepped metaphase chromosomes	**Insert Tracking** and **Edit Site Profiling**	Launched 2020
dGH SCREEN™	Blood or Cells	dGH or FISH prepped metaphase chromosomes	**Unbiased, De Novo Genomic Structural Mapping**. Single chromosome through whole genome; Hi-Res Karyotyping	Launching 2020
dGH DSCVR™	Blood or Cells	dGH prepped metaphase chromosomes	De Novo Structural Variant Identification. Hi-Res Karyotyping	Launching 2021
dGH Research Solutions™	Blood, Cell, Tissues	Interphase cells, dGH or FISH prepped metaphase chromosomes	**Structural Editing Outcomes**. DNA damage, instability and variability	Launched 2015
dGH Custom Solutions™	Blood, Cell, Tissues	Interphase cells, dGH or FISH prepped metaphase chromosomes	**Structural Editing Outcomes**. DNA damage, instability and variability	Launched 2016
dGH GLP Solutions™	Blood, Cell, Tissues	Interphase cells, dGH or FISH prepped metaphase chromosomes	**GLP dGH and PPF analysis** for research and preclinical studies	Launching 2020
dGH Discovery Solutions™	Blood, Cell, Tissues	Interphase cells, dGH or FISH prepped metaphase chromosomes	**Screening, Discovery and Identification** of disease driving structural variants	Launching 2021

Thank You..

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