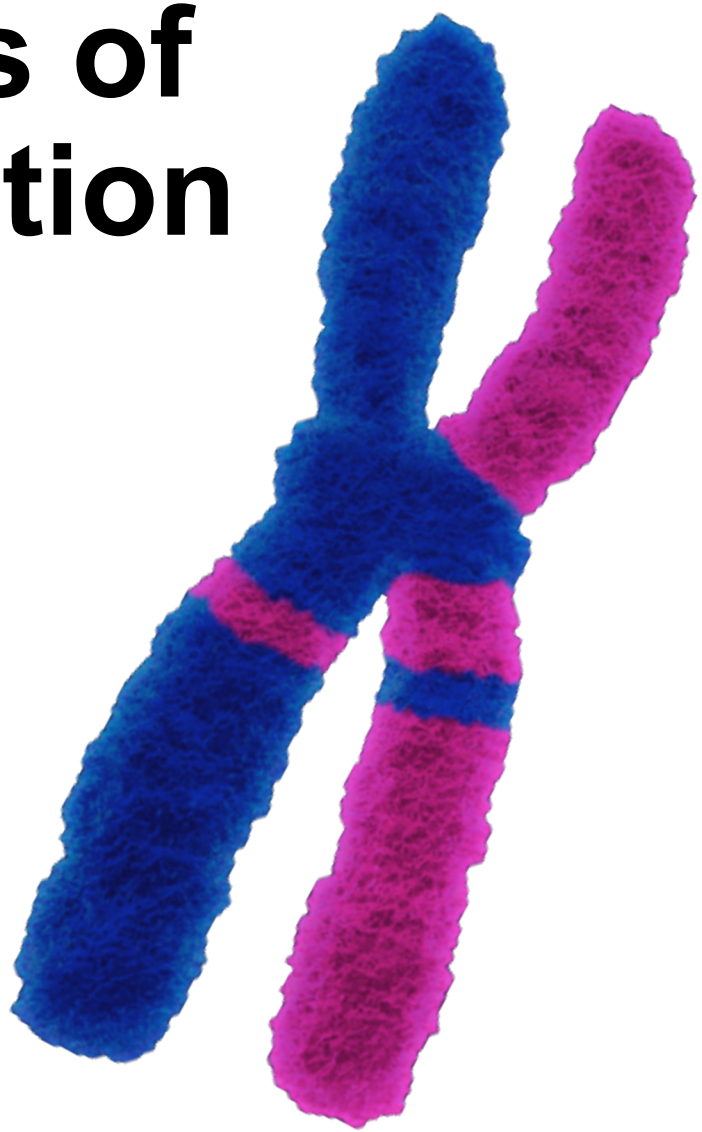
Comprehensive Analysis of Genomic Structural Variation



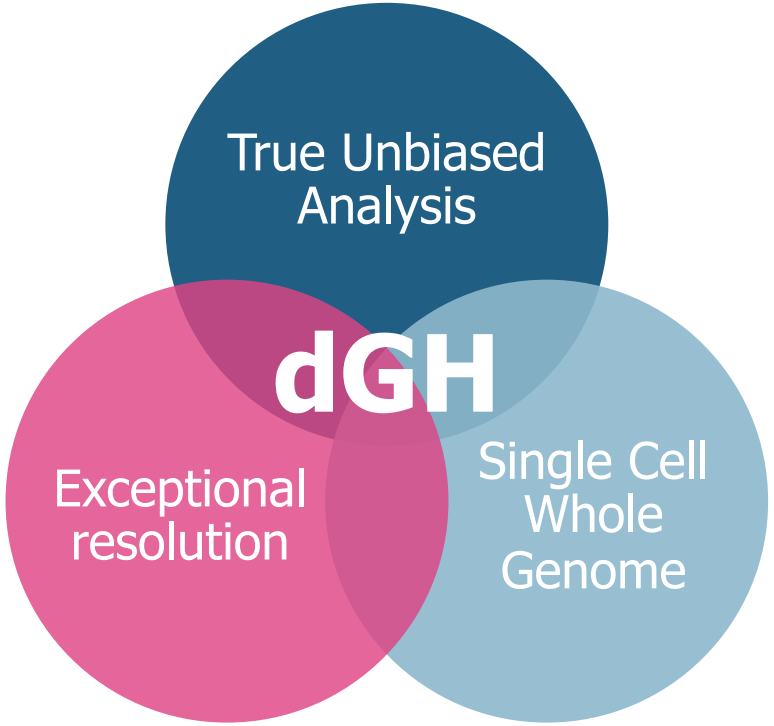
March 30th, 2022

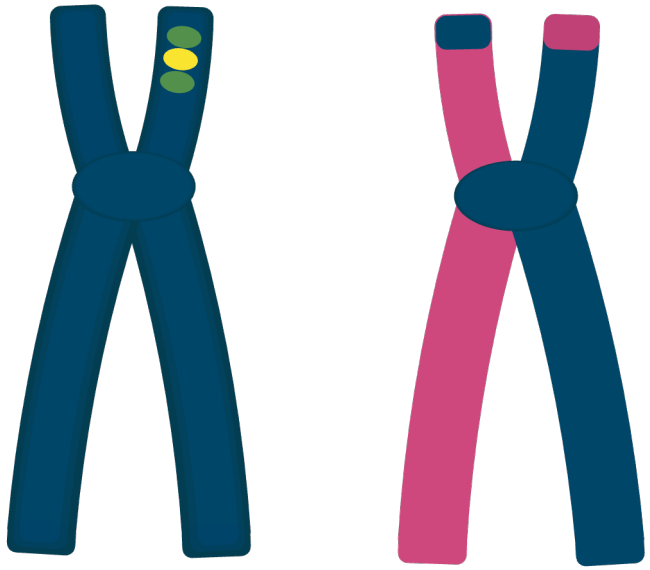
Next Gen Omics Conference
Boston Massachusetts

KromaTiD

Directional Genomic Hybridization

Map genomes, identify structural variation, and profile structural heterogeneity



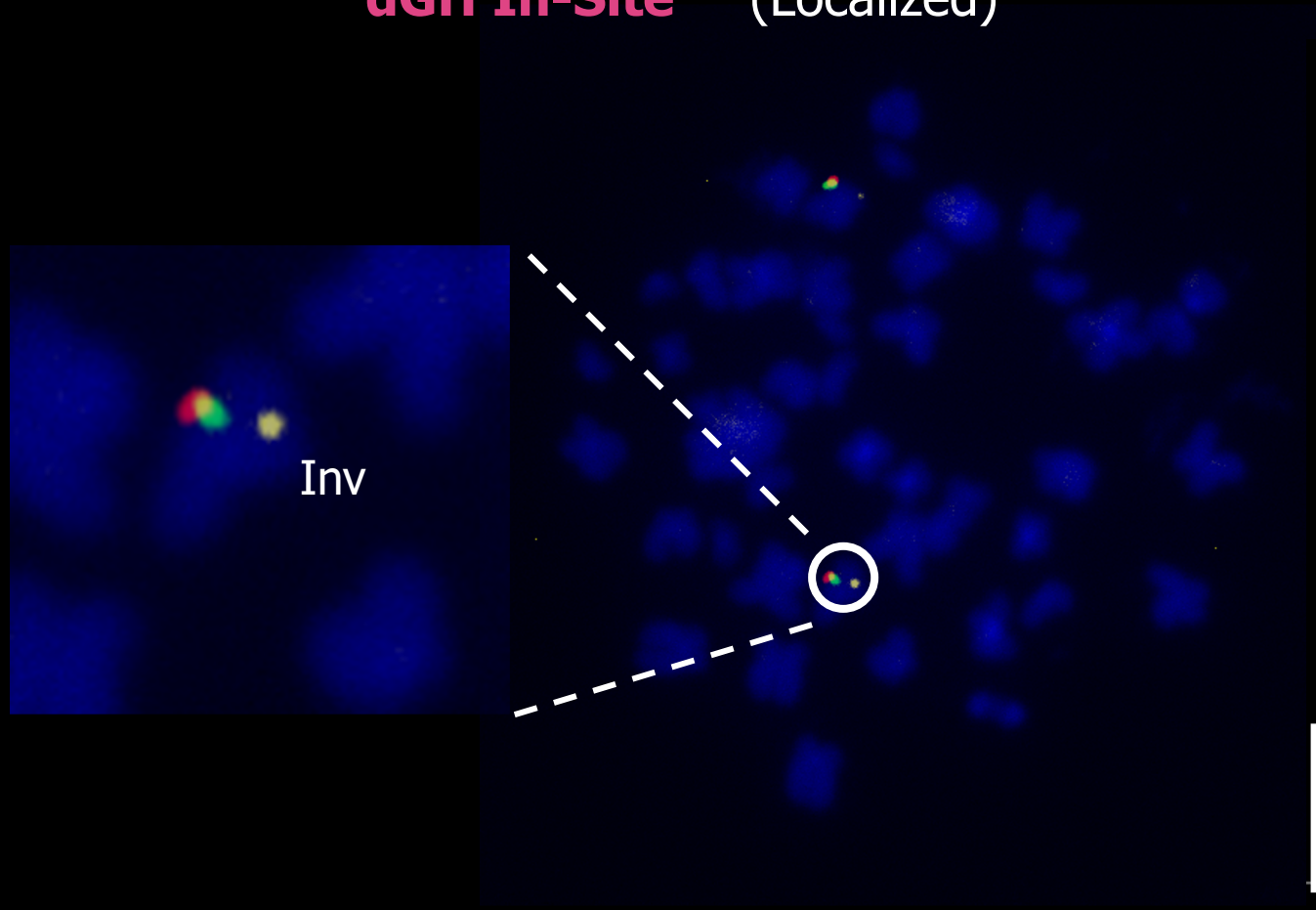


dGH In-Site **dGH SCREEN**

To provide a complete structural genomic toolset, KromaTiD combines dGH with Pinpoint FISH (for non-dividing cells) and G-Banding (for orthogonal confirmation)

dGH™: Single Cell Measurements of Many Cells

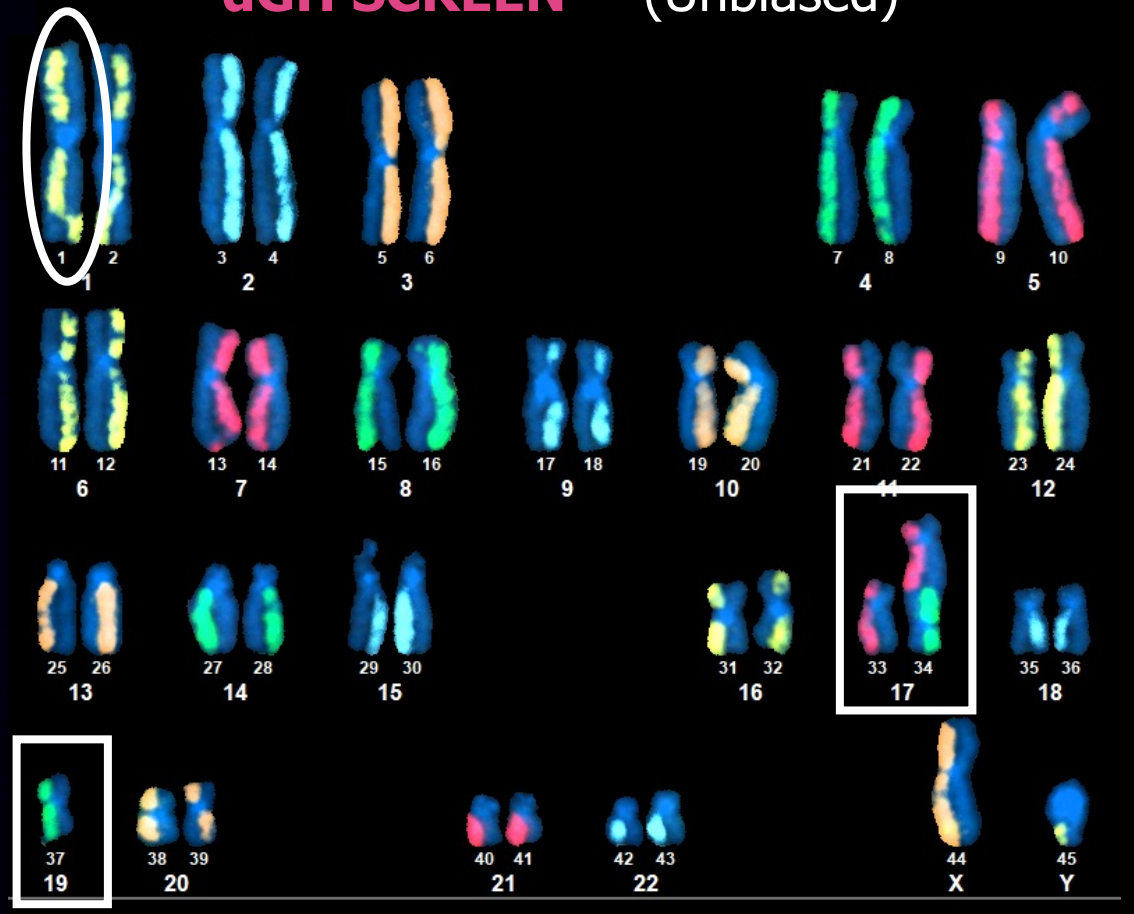
dGH In-Site™ (Localized)



10Kb Inversion between edits

Edits and Integrations

dGH SCREEN™ (Unbiased)



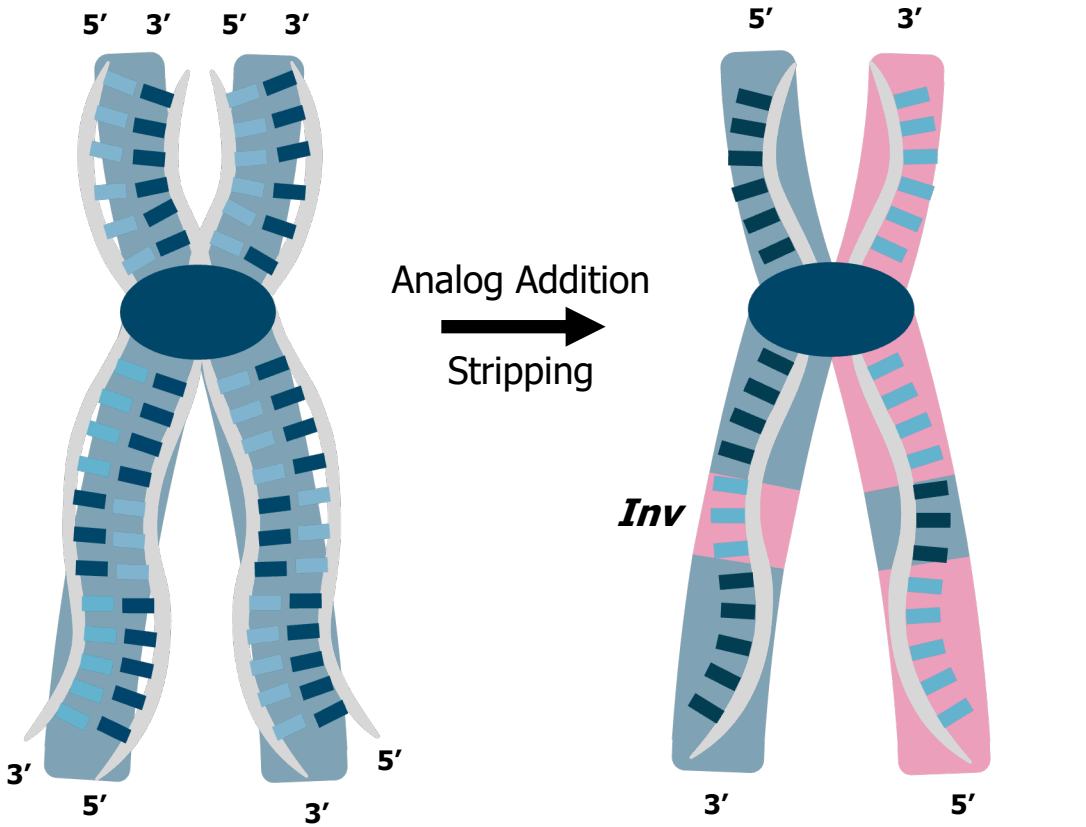
Inversion and Unbalanced Translocation

Whole Genome Map

Adding an Orientation Dimension to Image Data

Blue = DAPI Staining of Chromosome Structure

Pink = Fluorescently Labeled Hybridization Probes



Double Stranded Metaphase Chromatid

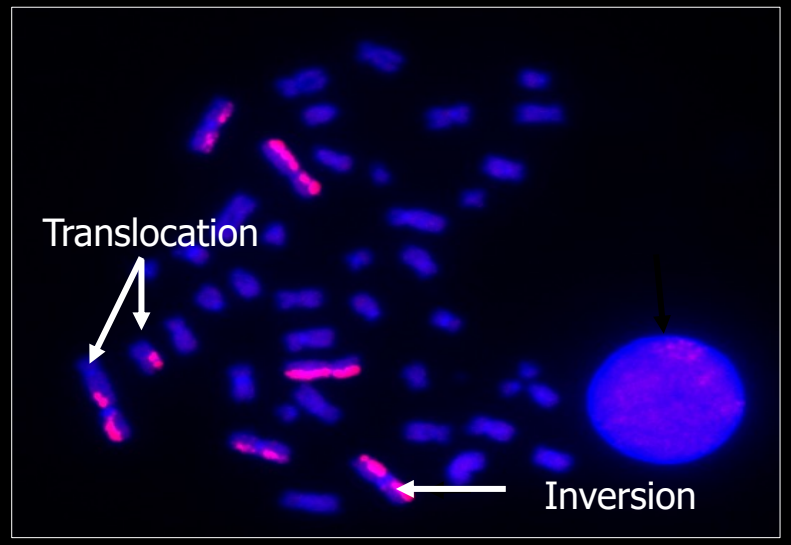
Analyte: Single Stranded dGH Chromatid

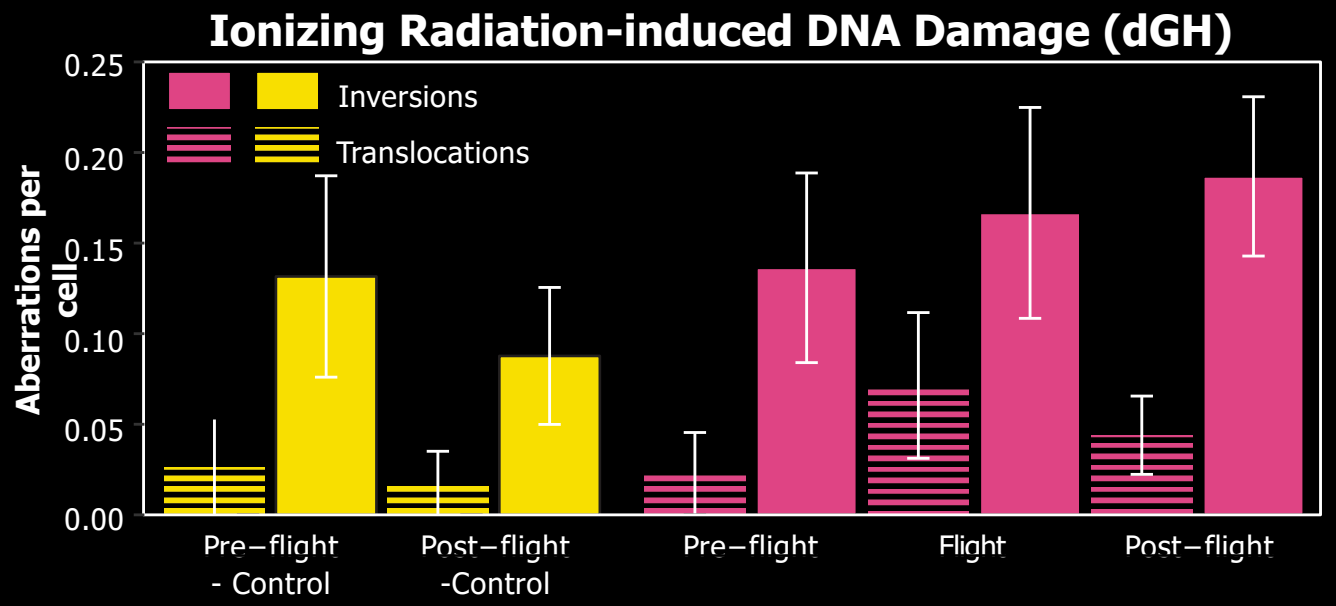
dGH chromosomes contain **2 strands** of oppositely oriented, **Parental DNA only**—NO Daughter Strands

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

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

Case Study: *Undirected DNA Damage*





Increased rearrangements during spaceflight consistent with reported radiation doses

Inversions remain elevated, suggestive of on-going instability damage to stem cells, clonal hematopoiesis.

1. The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight (science.org)
2. Scientists Share Results From NASA's Twins Study : NPR

Case Study: Estimating Baseline Structural Variation

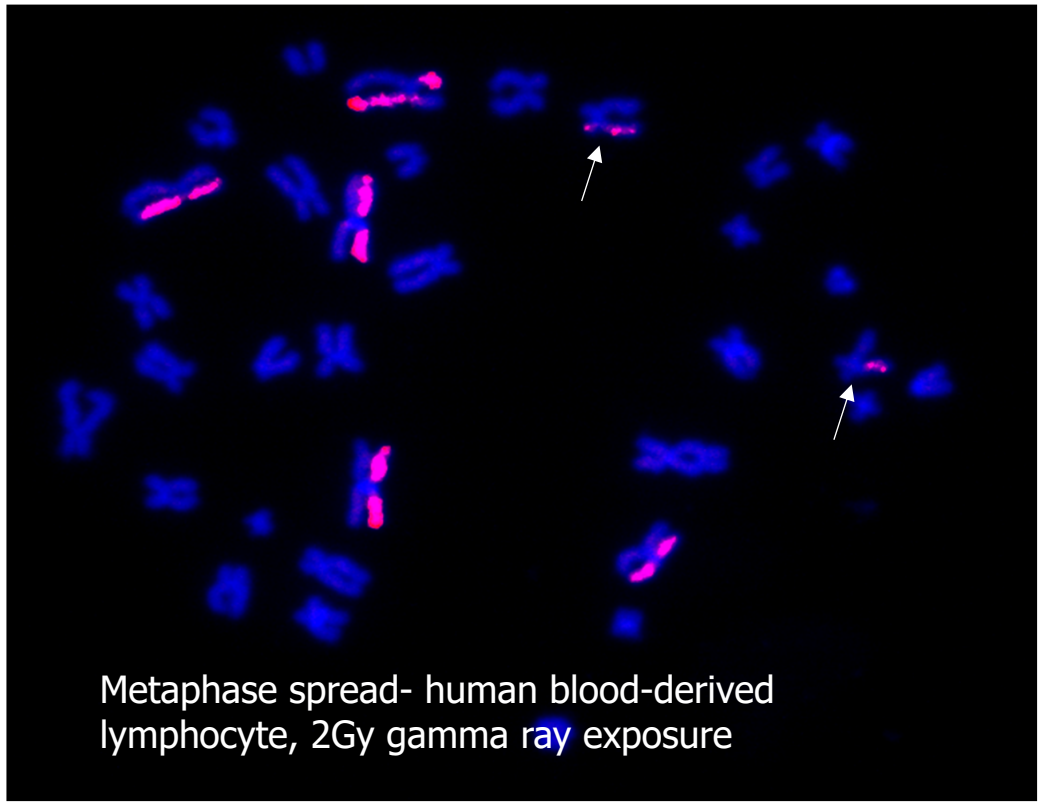


Figure 1: Whole chromosome 1, 2 and 3 paints hybridized to a metaphase spread from a human peripheral blood sample irradiated with 2Gy Cs-137 gamma rays. Structural rearrangements identified by Directional Genomic Hybridization denoted by arrows.

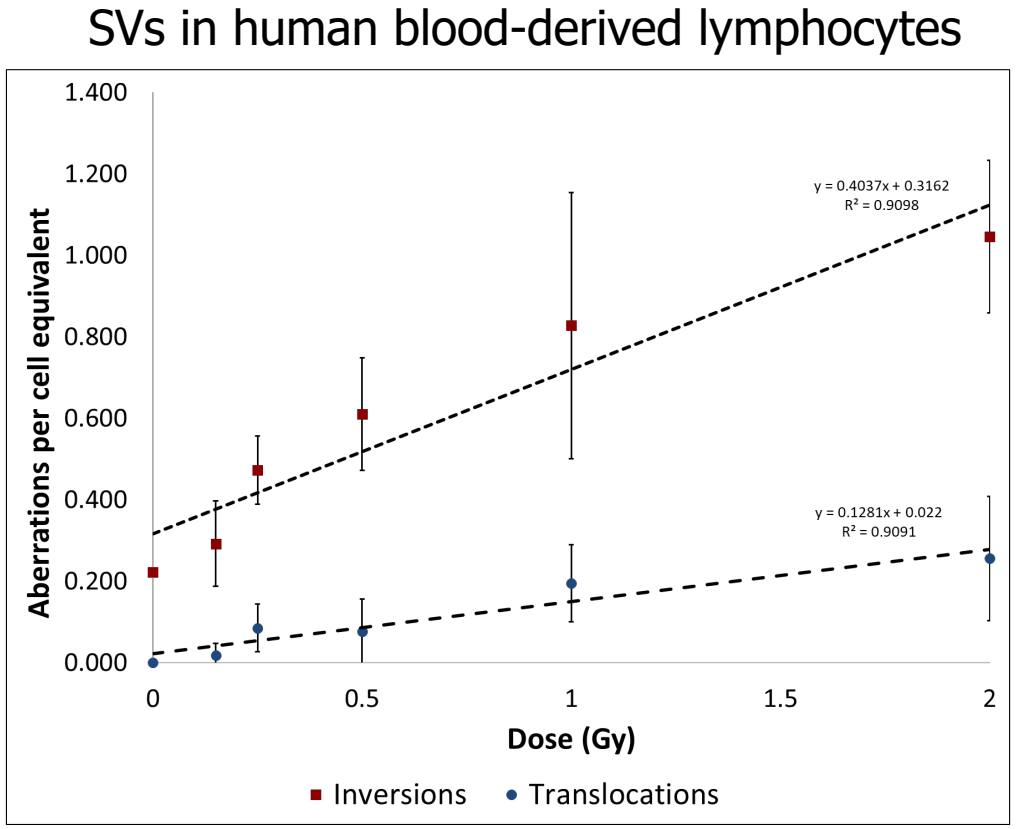
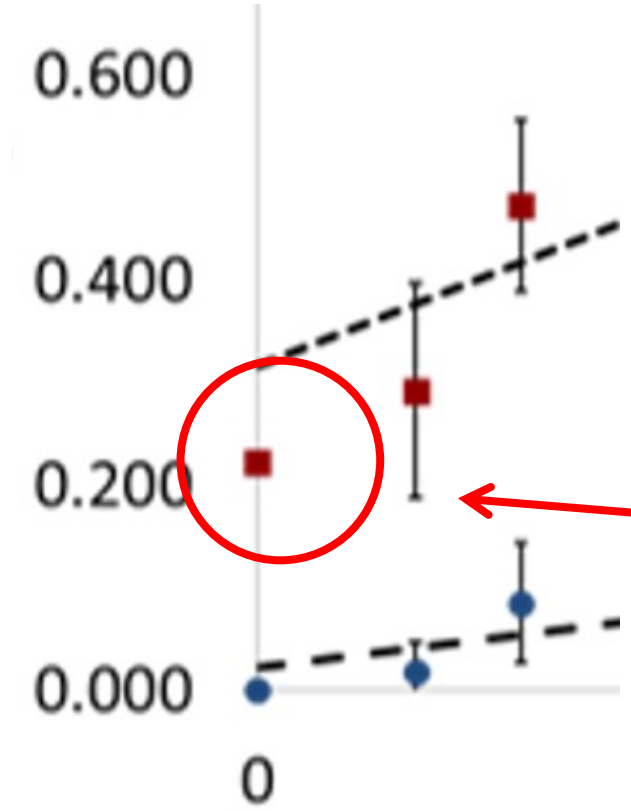


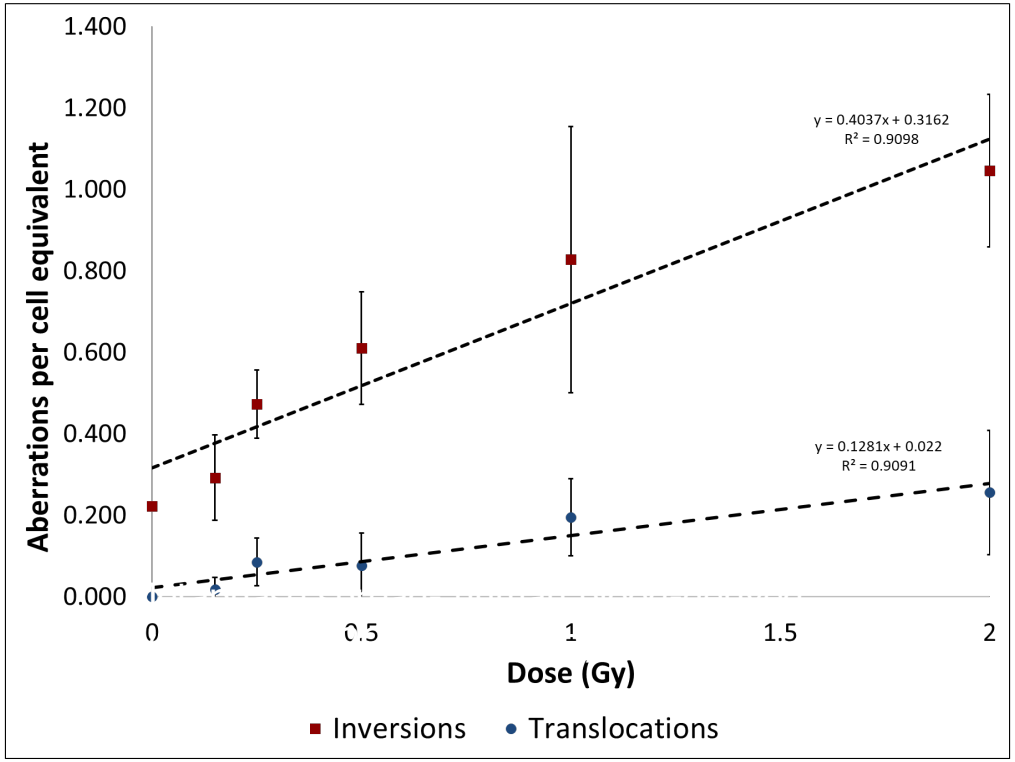
Figure 2: Blood samples from young adult controls were irradiated with Cs-137 gamma rays to establish a dose response (calibration) curve. Males in their mid-20's were selected to account for age at exposure. Inversions (red) had a higher natural background rate compared to translocations (blue); however, inversions formed at a higher rate per unit dose.

Chromosome Translocations, Inversions and Telomere Length for Retrospective Biodosimetry on Exposed U.S. Atomic Veterans - PubMed (nih.gov)

Case Study: Estimating Baseline Structural Variation



SVs in human blood-derived lymphocytes

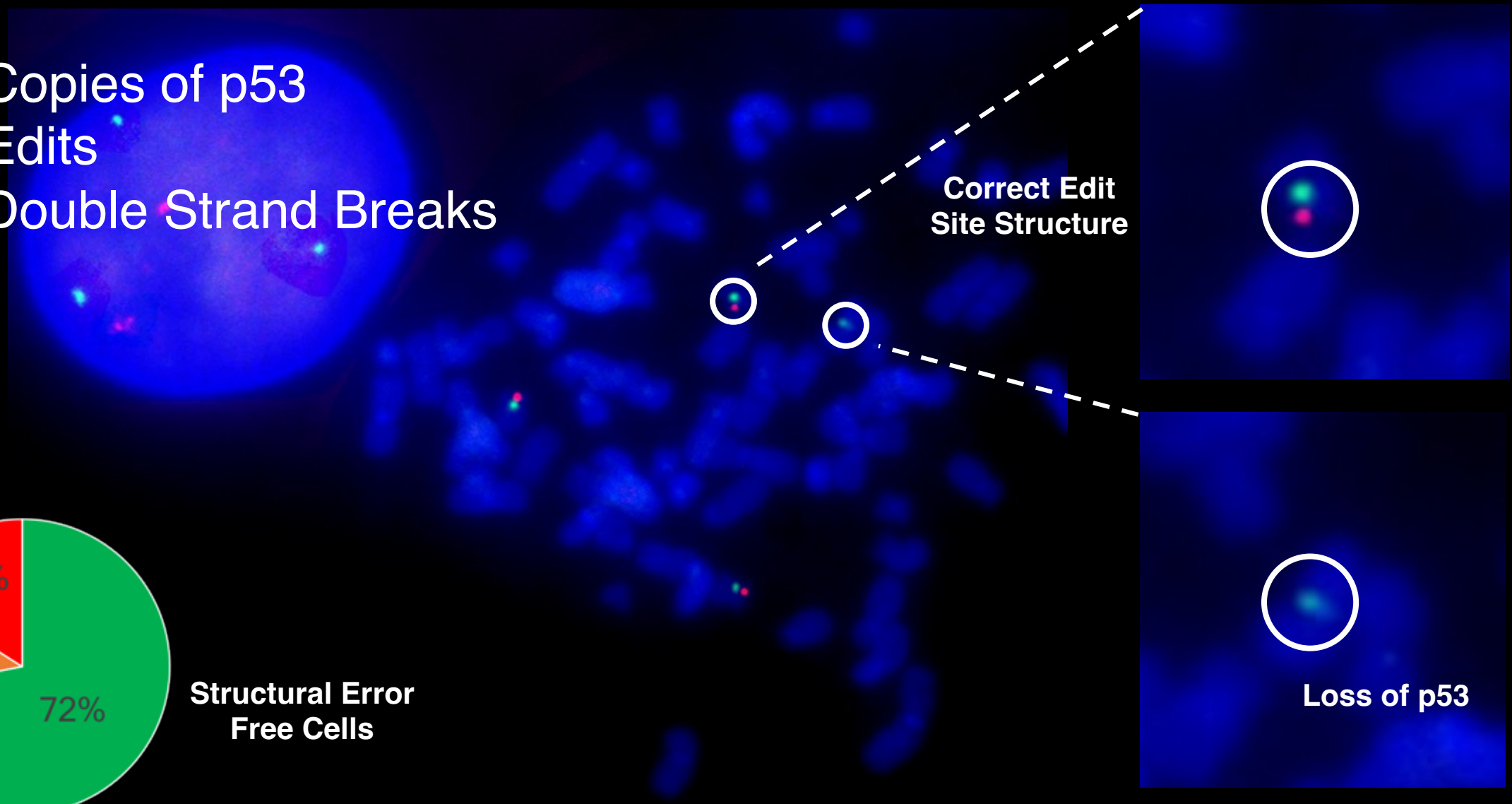


~0.5 aberrations per cell equivalent. Unexposed adult non-smokers. Average age 26yr

Chromosome Translocations, Inversions and Telomere Length for Retrospective Biodosimetry on Exposed U.S. Atomic Veterans - PubMed (nih.gov)

Case Study: *Two Concurrent Edits of the P53 Gene Loci*

- 3 Copies of p53
- 2 Edits
- 6 Double Strand Breaks



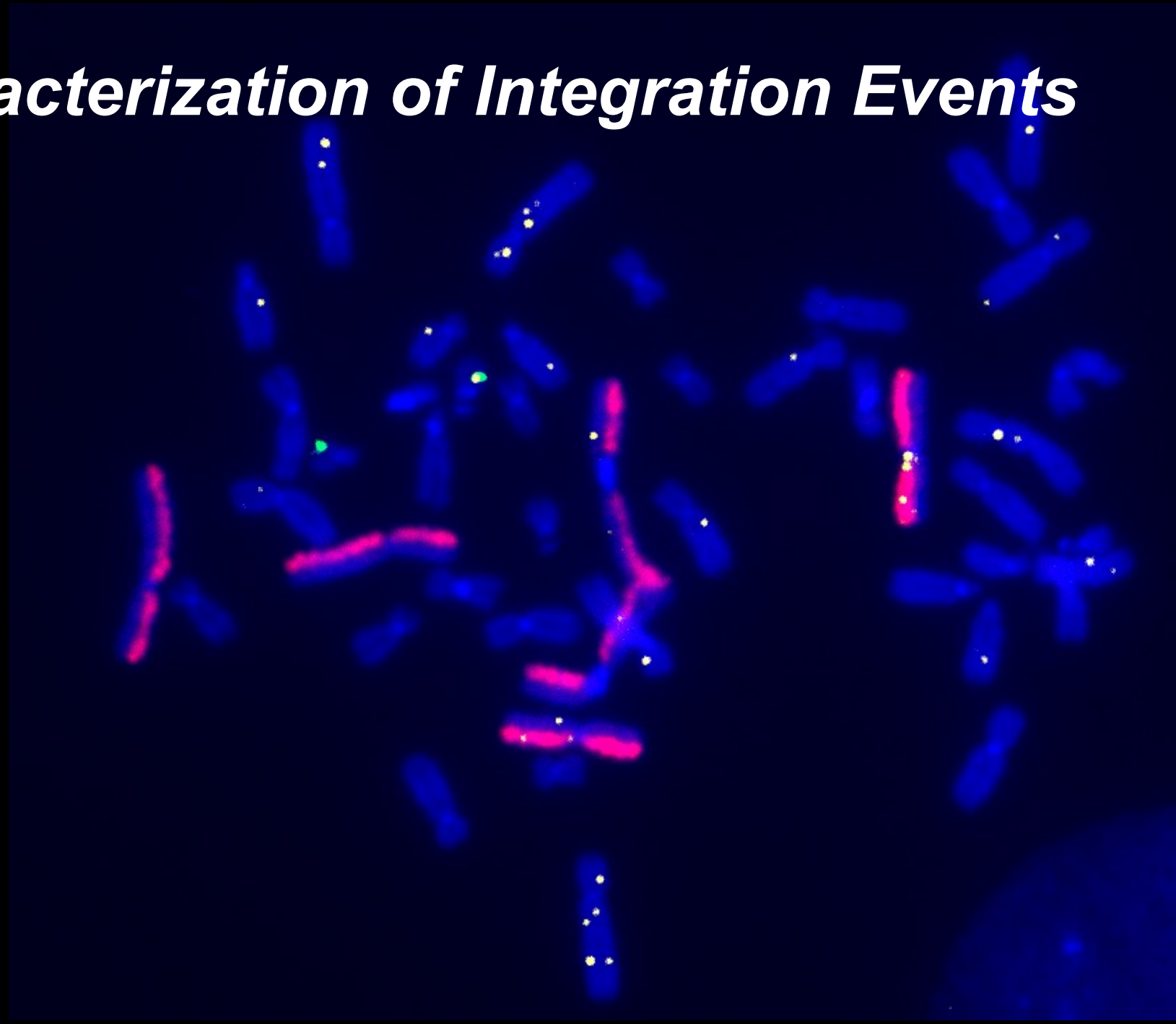
Case Study: *Characterization of Integration Events*

Yellow = Off-Target Insert

Yellow + Green =
On -Target Insert

Green = Target Site

Pink = Screen Paint

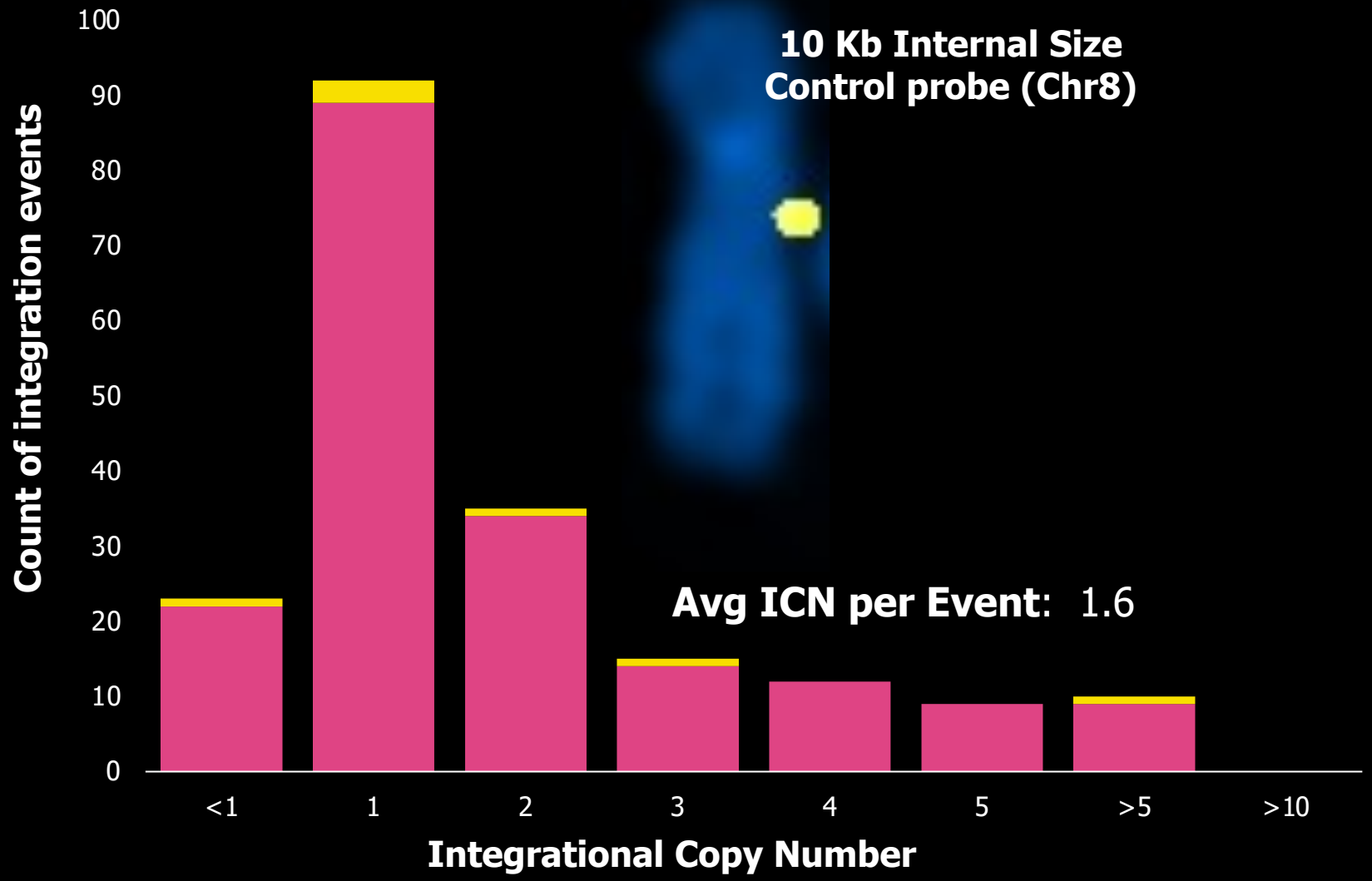


Case Study: *Characterization of Integration Events*

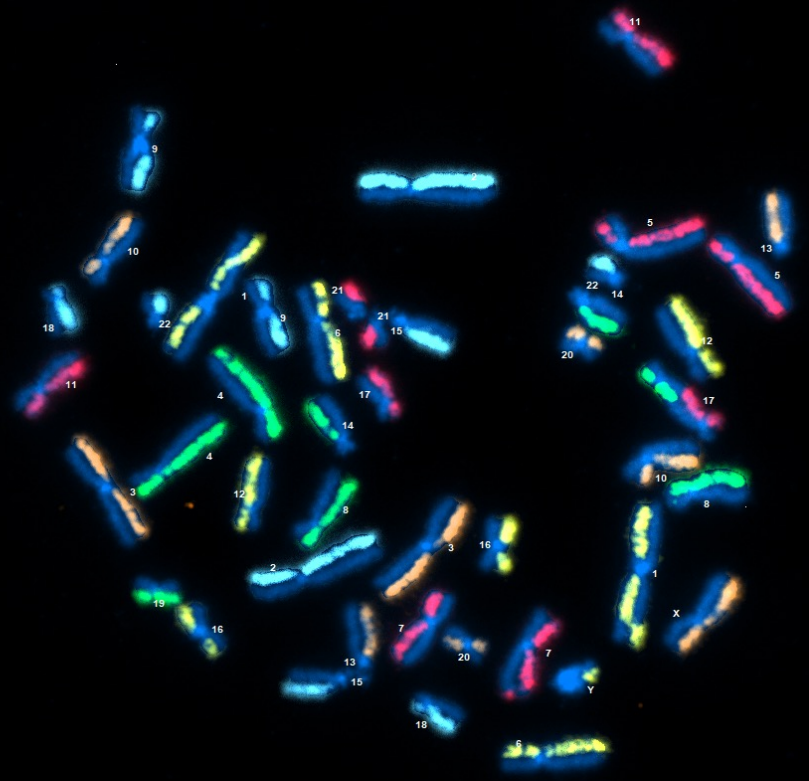
Average Integrations per cell : 7.8

- On-target only: 2%
- On-target plus off-target: 14%
- Off-target only: 77%
- None: 7%

Case Study: *Characterization of Integration Events*

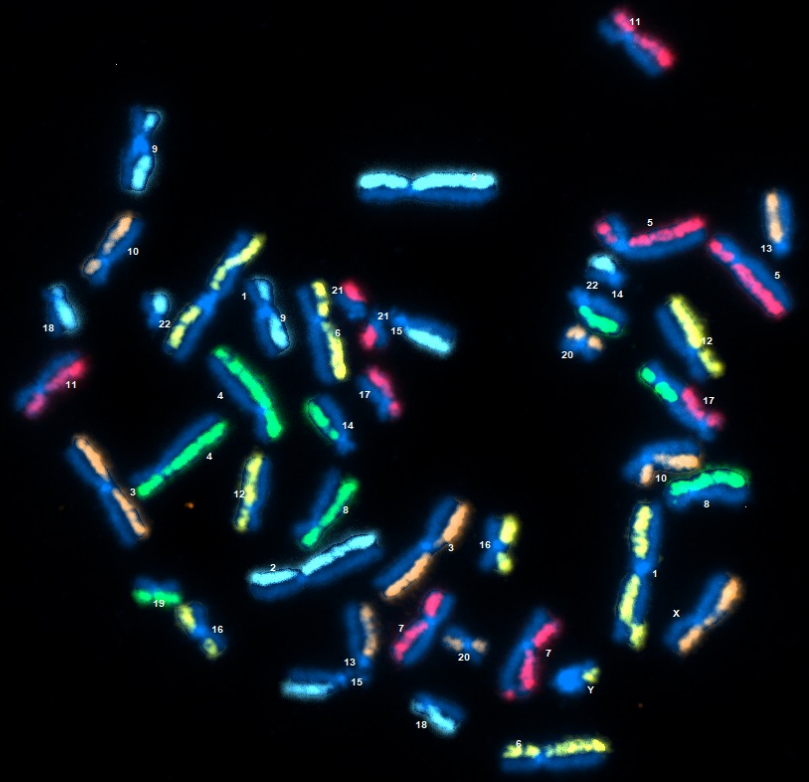


Case Study: *Whole Genome Mapping*

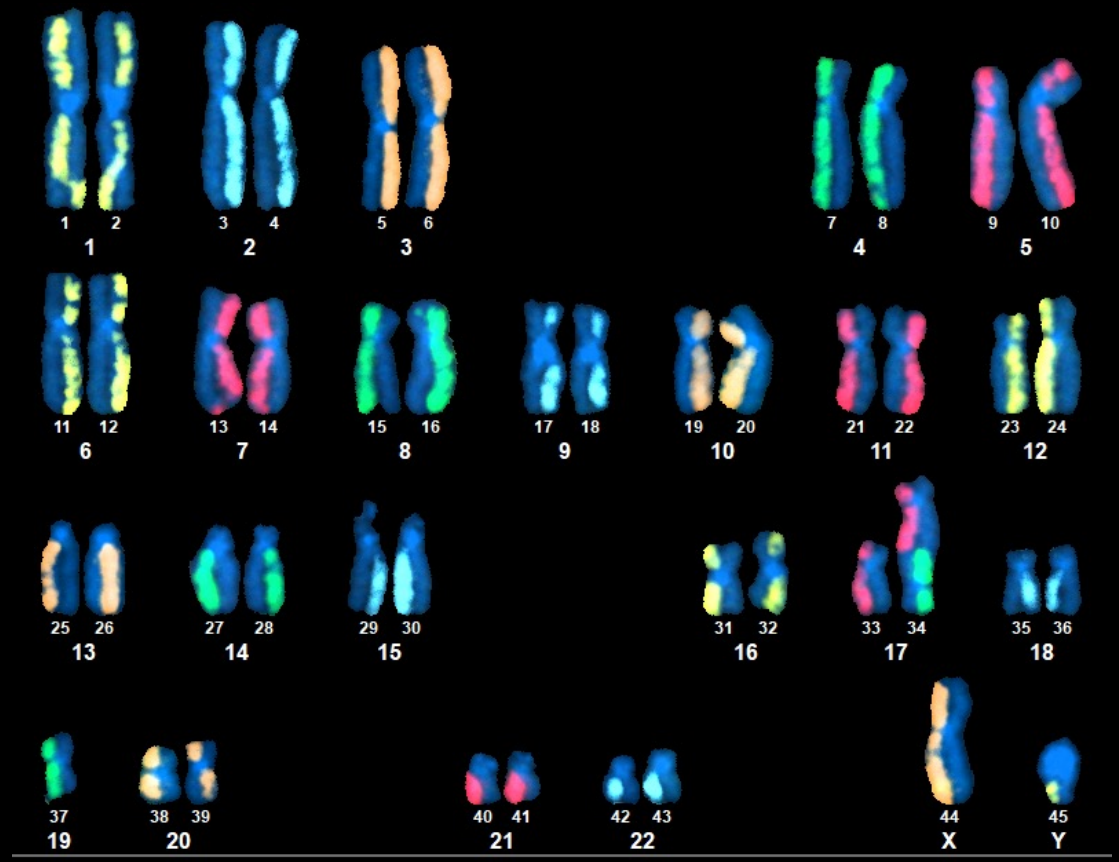


Metaphase Spread

Case Study: *Whole Genome Mapping*

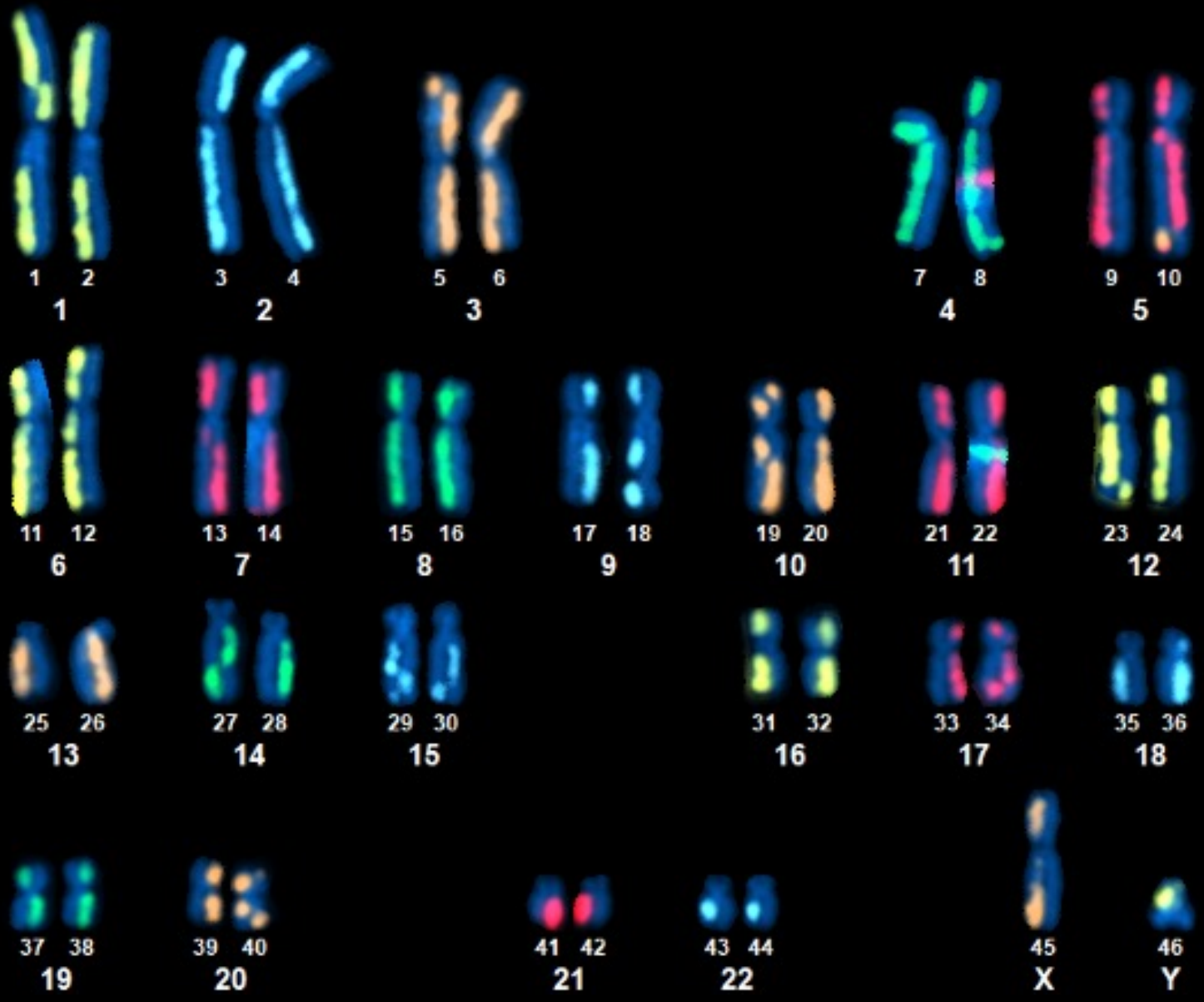


Metaphase Spread

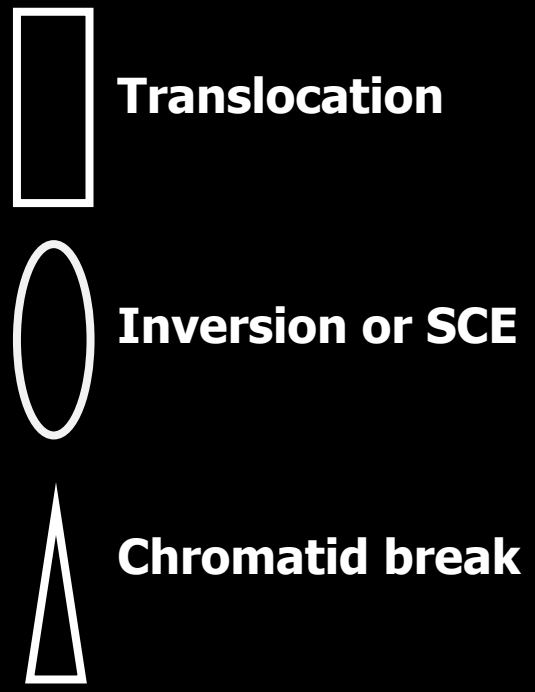


Karyogram

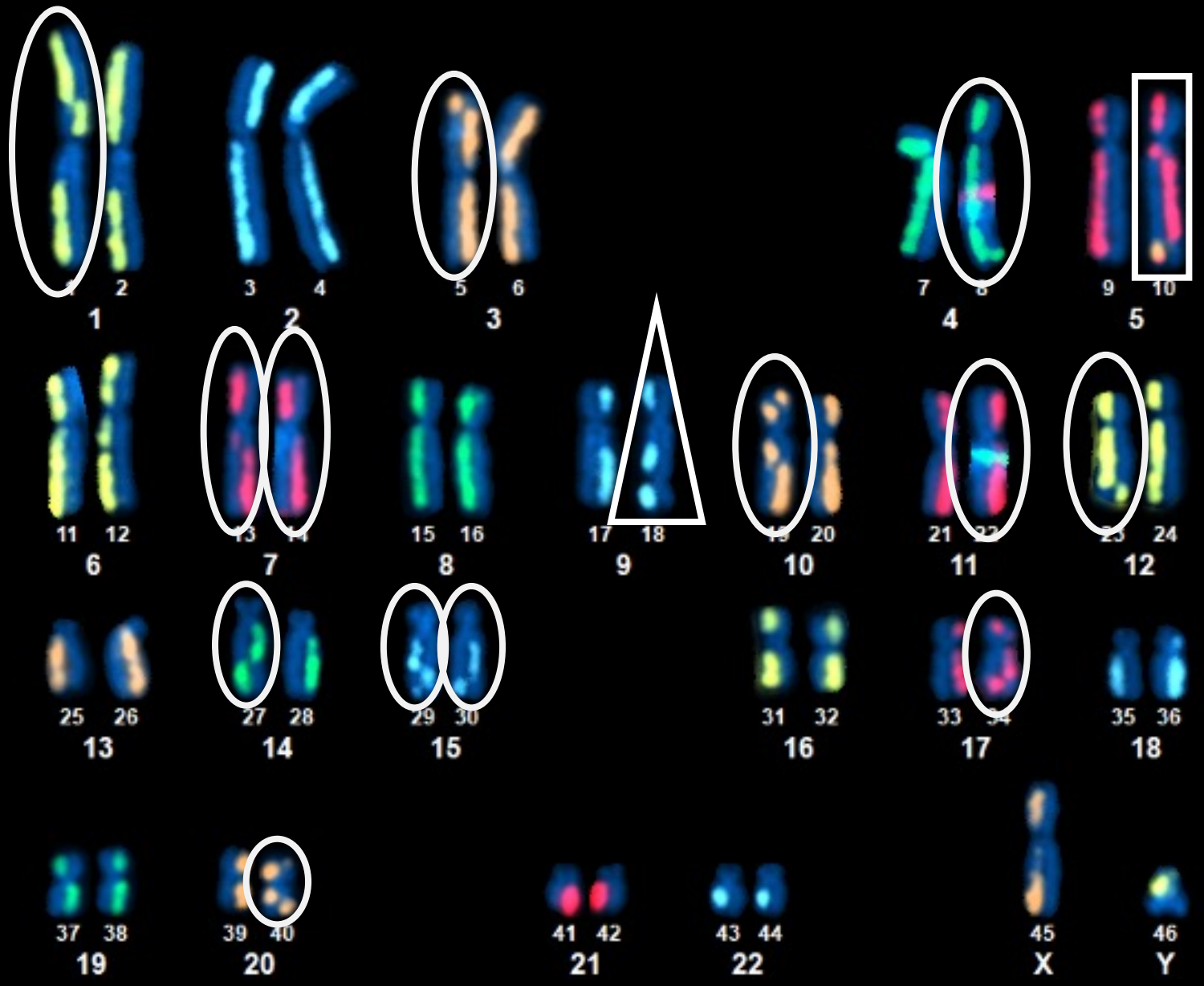
Case Study: Un-Sequenceable Rearrangements



Aberration Types:



Case Study: Un-Sequenceable Rearrangements



Aberration Types:

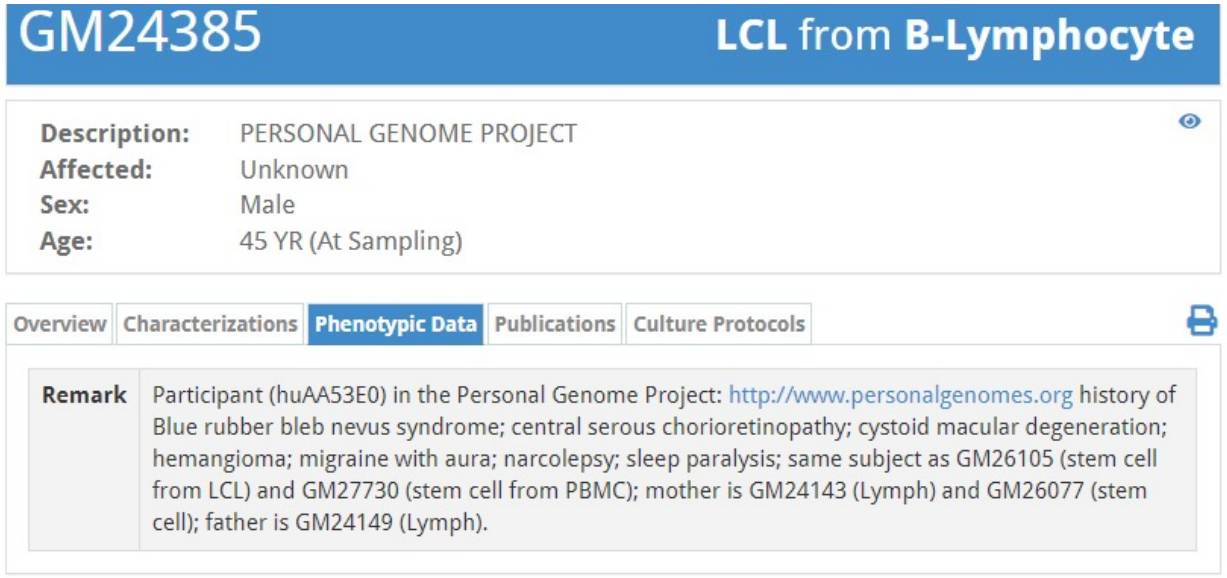
Translocation

Inversion or SCE

Chromatid break

WG Analysis for the NIST Genome Editing Consortium

Whole genome dGH analysis of the "Genome in a Bottle" progenitor cell line in preparation for engineering of large variant controls by NIST partners



GM24385 — LCL from B-Lymphocyte

Description:	PERSONAL GENOME PROJECT
Affected:	Unknown
Sex:	Male
Age:	45 YR (At Sampling)

Overview | Characterizations | **Phenotypic Data** | Publications | Culture Protocols

Remark Participant (huAA53E0) in the Personal Genome Project: http://www.personalgenomes.org history of Blue rubber bleb nevus syndrome; central serous chorioretinopathy; cystoid macular degeneration; hemangioma; migraine with aura; narcolepsy; sleep paralysis; same subject as GM26105 (stem cell from LCL) and GM27730 (stem cell from PBMC); mother is GM24143 (Lymph) and GM26077 (stem cell); father is GM24149 (Lymph).

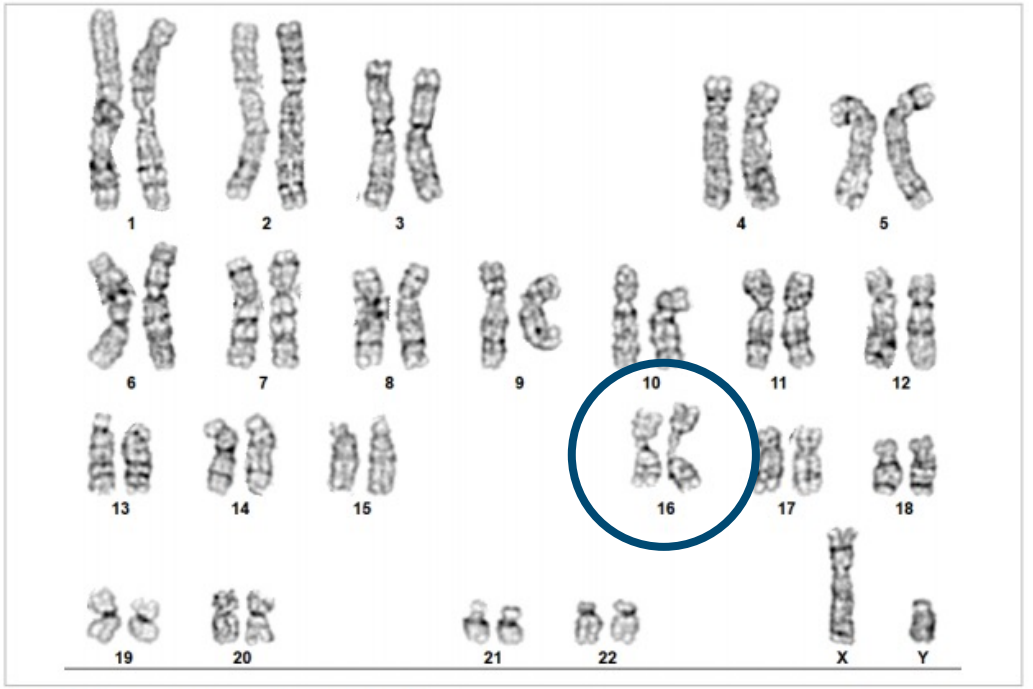
Previous GM24385 Genome Structural Characterization:

- Karyotyping (Coriell):
  - primarily diploid
  - Potential inversion on 3q26.3q29
- Sequencing (GiaB Consortium):
  - Numerous large CNVs
  - No inversion or translocation variant calls
- Whole chromosome dGH on C3
  - Confirmed inversion on 3q26.3q29
  - Discovered telemeric inversion on 3q
  - Discovered centromeric inversion on 3q

Case Study: ***Cell Line Stability***

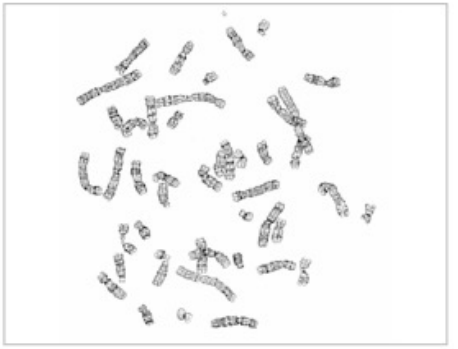
Some rate of potential intra-chromosomal CNV (band expansions) was observed

Instability and gross rearrangement of C16 matched dGH SCREEN observations



Cell Results: Karyotyped: 46,XY,?add(16)

Cell Notes: Estimated Band Resolution:675



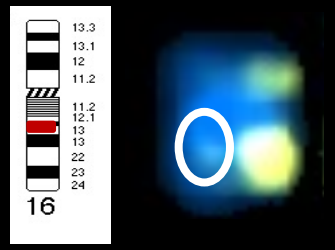
Label - Slide/Cell: S002692 - 8/52

X,Y: 9.9 , 20.2

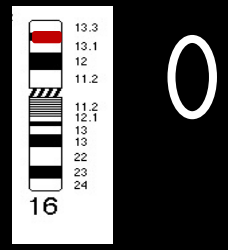
GM24385 (coriell.org) National Institute of Standards & Technology (nist.gov)

Case Study: *Cell Line Stability*

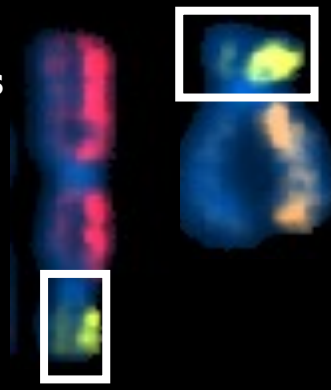
Chr 16q Inversion (37%)
- small, mid-arm
- Observed in 19% of cells



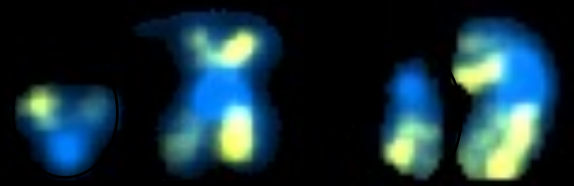
Chr 16p Inversion
- small, mid-arm
- Observed in 19% of cells



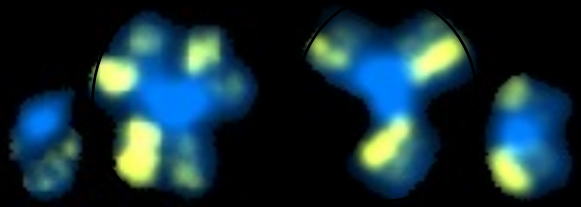
Chromosome 16 translocations (~4%)
- Non-reciprocal, balanced and unbalanced
- Partners Chr7 and Chr10



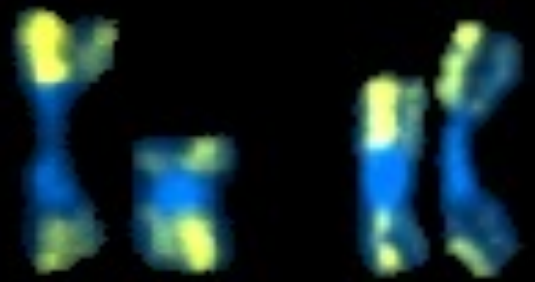
Whole arm deletion
- Observed in 11% of cells



Chromosome 16 multi-radial association
- Observed in 4% of cells



Decondensed/ elongated centromeres and iso-chromosomes
- Observed in 22% of cells

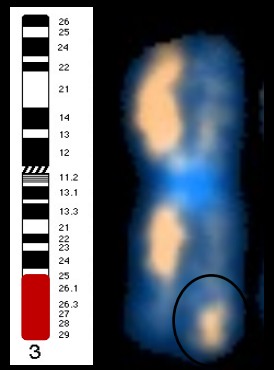


Chromosome 16 Complex Structural Variation in p18 indicates transformation and instability of cell line

Recurrent Inversions: Location, size, and prevalence

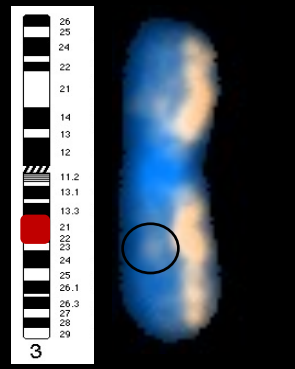
Chr 3q Inversion (7%)
- **large, telomeric**

Confirmation of p0 G-Banding Result and p3 dGH Results (2014)



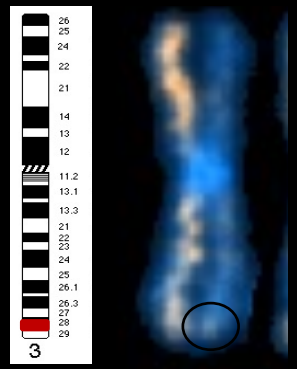
Chr 3q Inversion 2 (26%)
- Small, mid-arm

Confirmation of p3 dGH Results (2014)



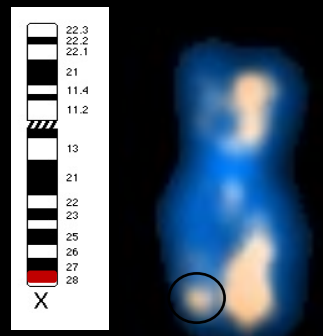
Chr 3q Inversion 3 (13%)
- Small, telomeric

Confirmation of p3 dGH Results (2014)



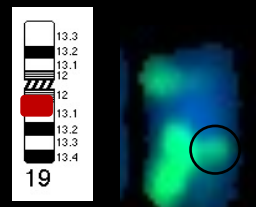
Chr Xq Inversion (67%)
- **Small, telomeric**

Newly Discovered



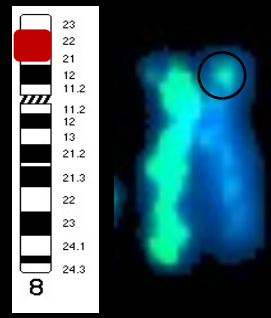
Chr 19q Inversion (65%)
- **Mid-size, centromeric**

Newly Discovered



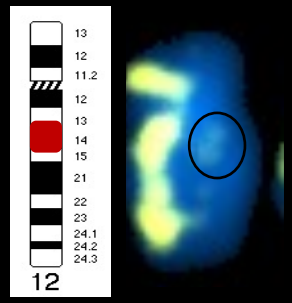
Chr 8p Inversion (52%)
- **Mid-size, mid-arm**

Newly Discovered



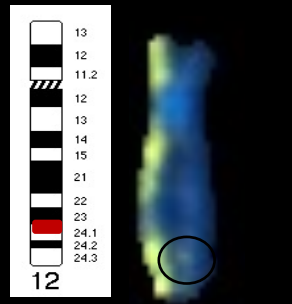
Chr 12p Inversion (26%)
- Mid-sized, centromeric

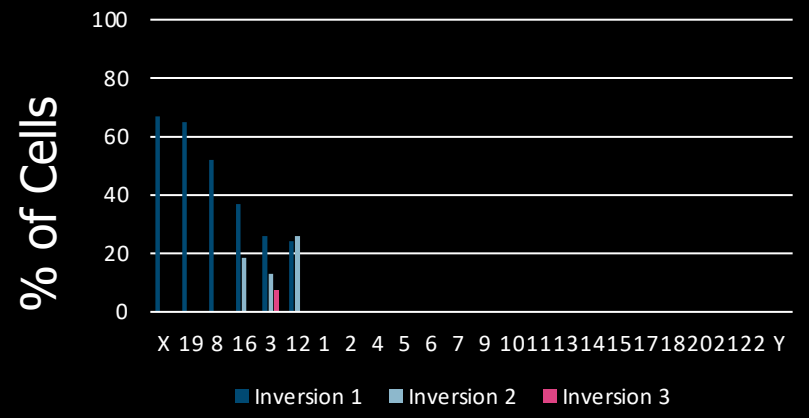
Newly Discovered



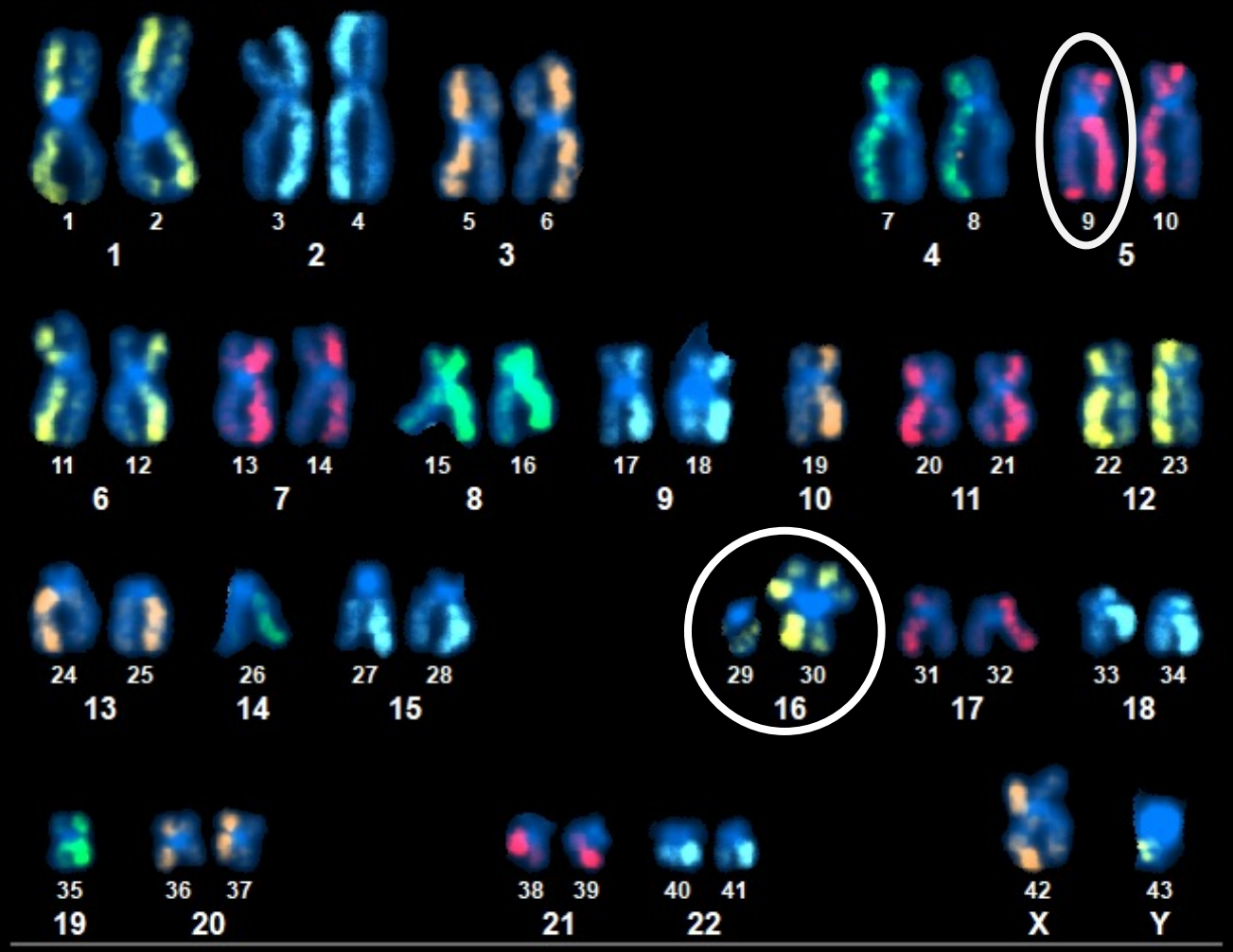
Chr 12p Inversion 2 (30%)
- small, telomeric

Newly Discovered





Case Study: *Cell Line Stability*



S73_c54, Cell 14 Passage 18

Conclusions

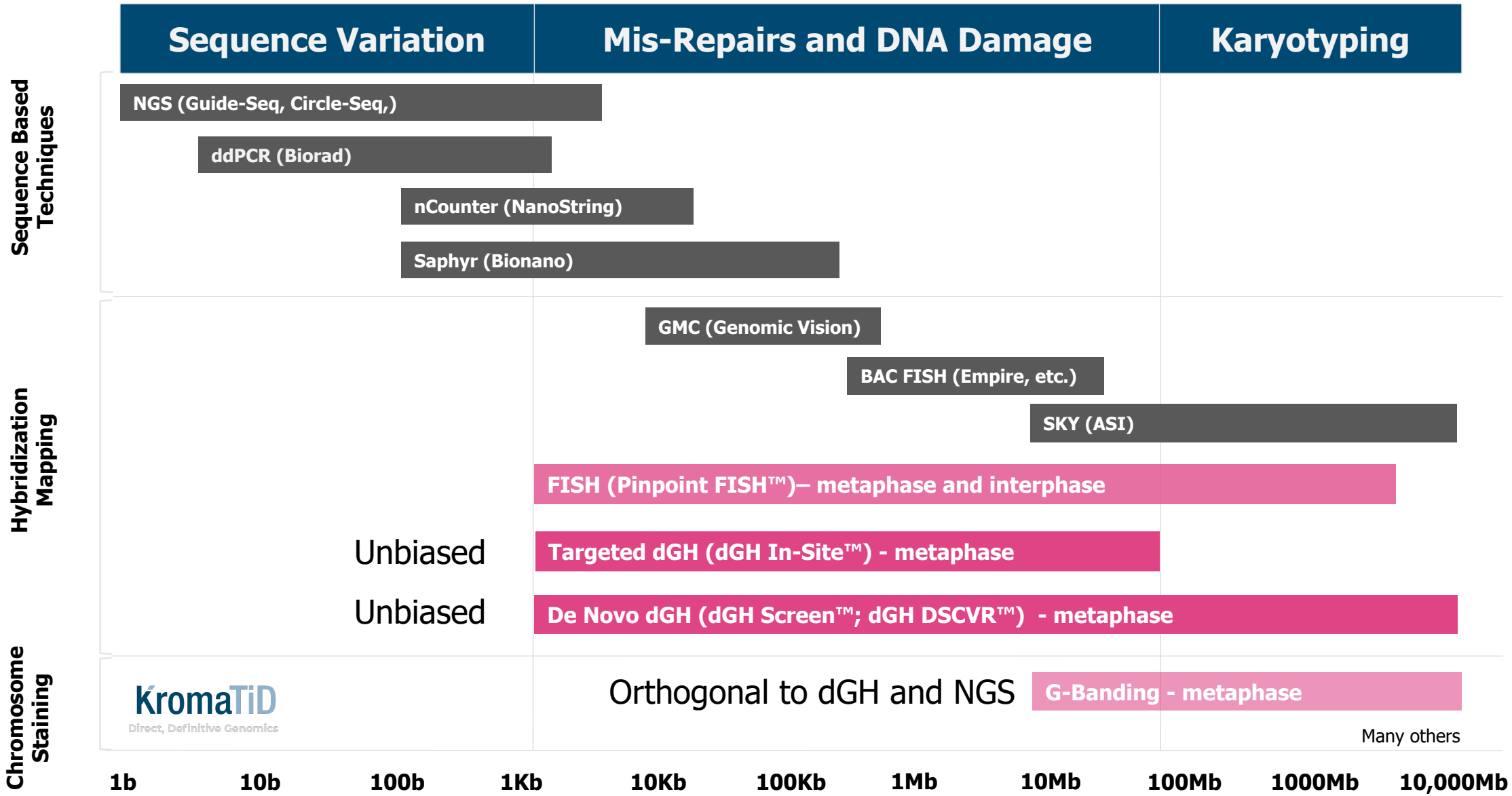
- Expanding number of differing variations from Passage 12 to 18 indicating instability and differentiation
- No consistency of sub-populations

Structural Variant Summary

- 4 Heterogenous translocations
- 34 Heterogenous inversions
- 18% variable monosomy
- 4% variable trisomy
- Low level of Chromothrypsis of C19complex events

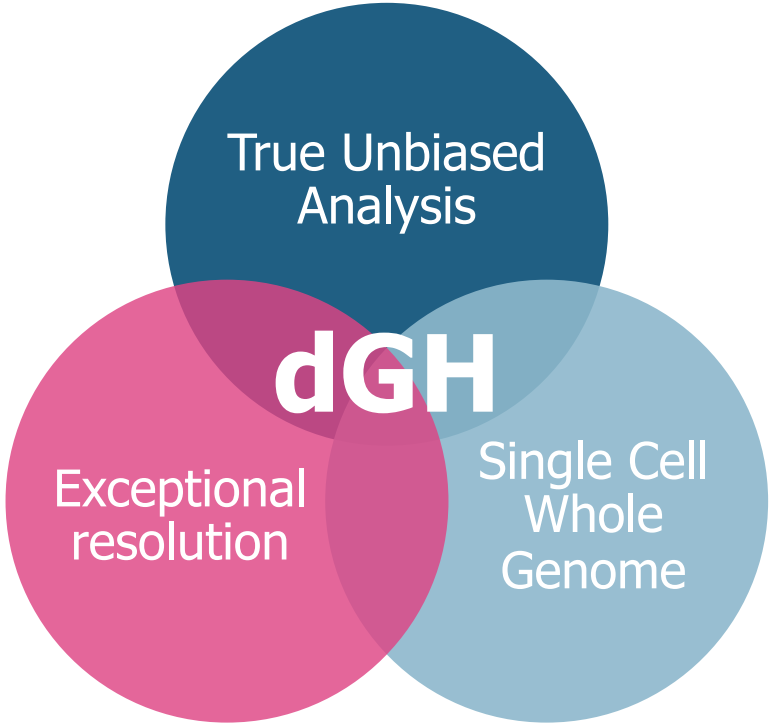
GM24385 (coriell.org), NIST GM24385 Reference Material Certificate National Institute of Standards & Technology (nist.gov)

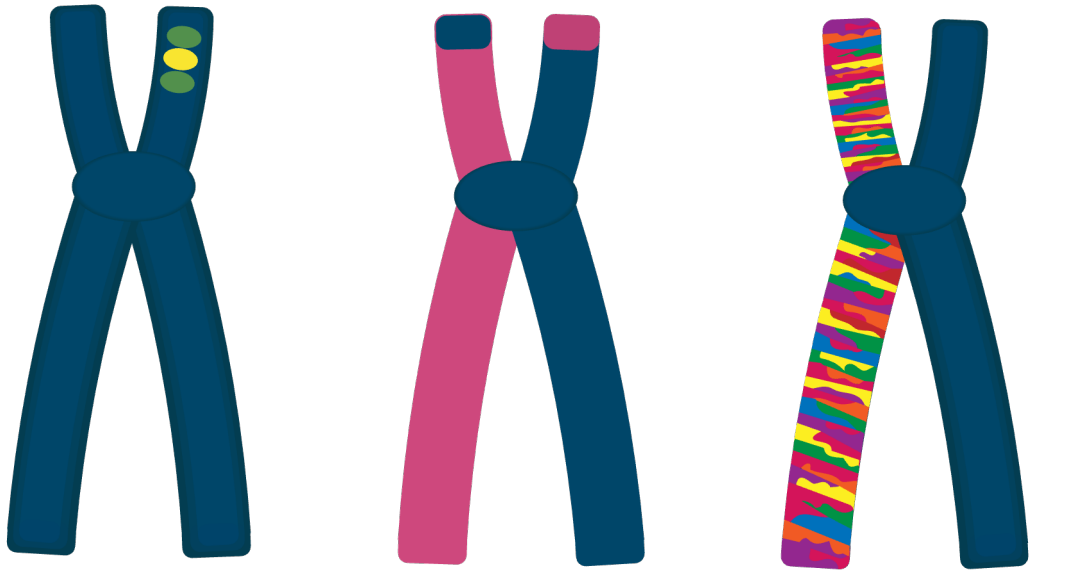
Goal: Measure Any Variation in a Genome



Directional Genomic Hybridization

Map genomes, identify structural variation, and profile structural heterogeneity

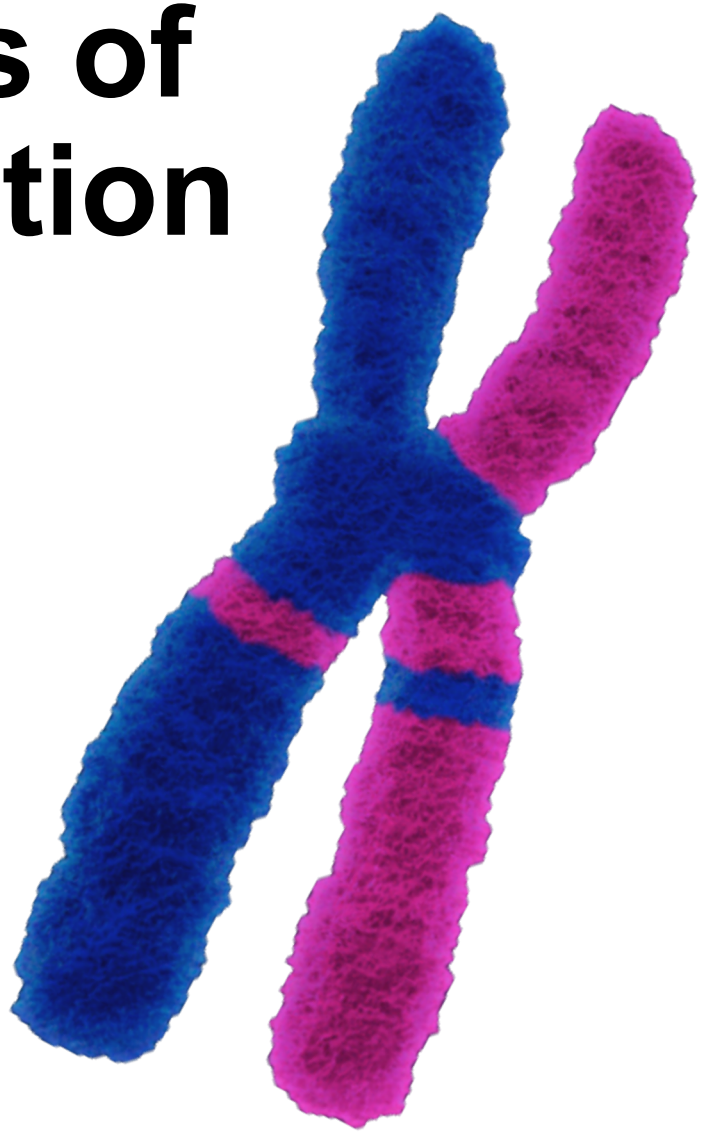




dGH In-Site **dGH SCREEN** **dGH DSCVR**

To provide a complete structural genomic toolset, KromaTiD combines dGH with Pinpoint FISH (for non-dividing cells) and G-Banding (for orthogonal confirmation)

Comprehensive Analysis of Genomic Structural Variation



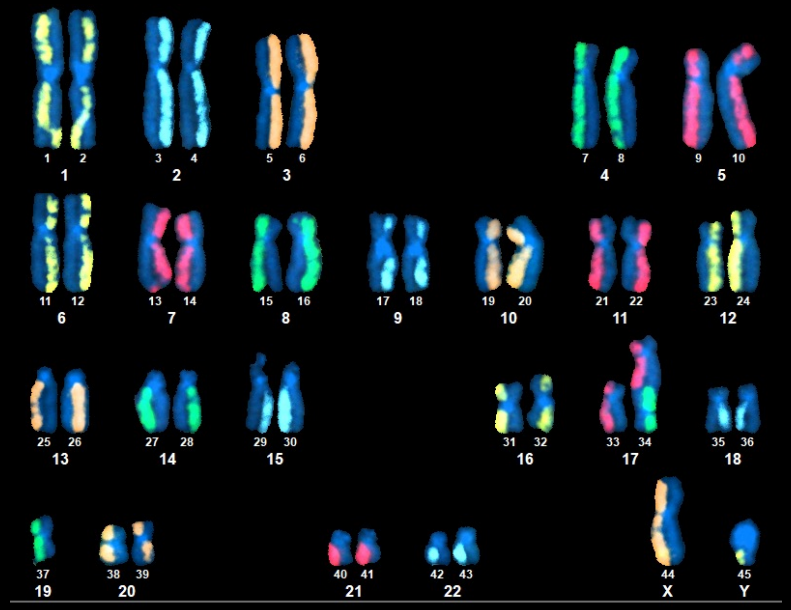
For further information, please contact

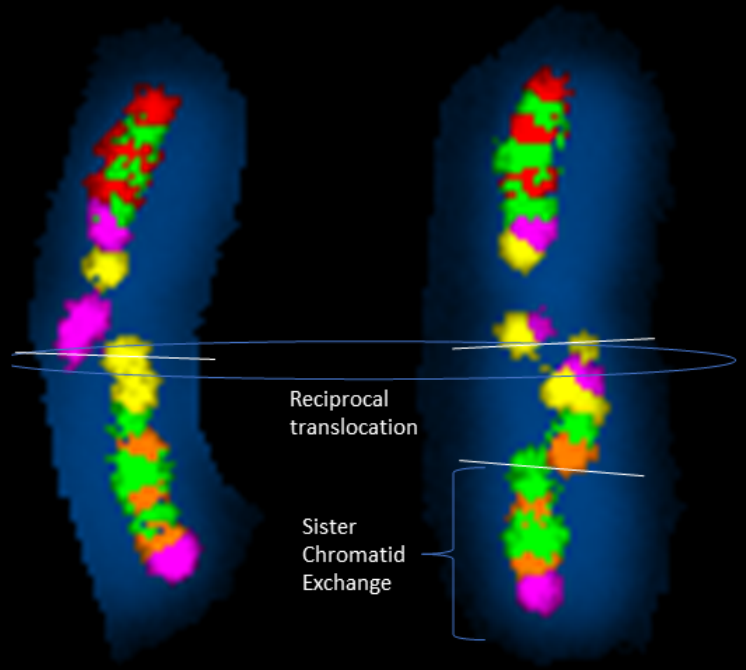
Christopher Tompkins
Chief Technical Officer
ctompkins@kromatid.com

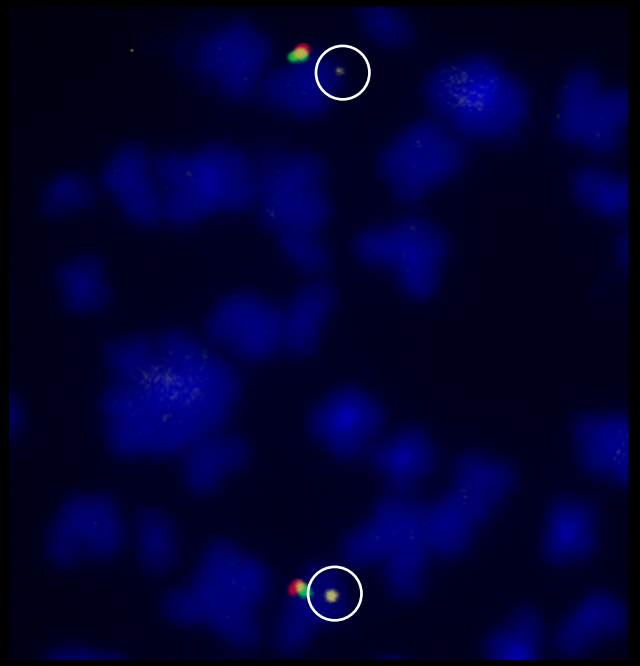
KromaTiD

From Sample to Target

dGH SCREEN™ (Unbiased) **dGH DSCVR™** (Unbiased) **dGH In-Site™** (Localized)







Discover → **Identify** → **Target**