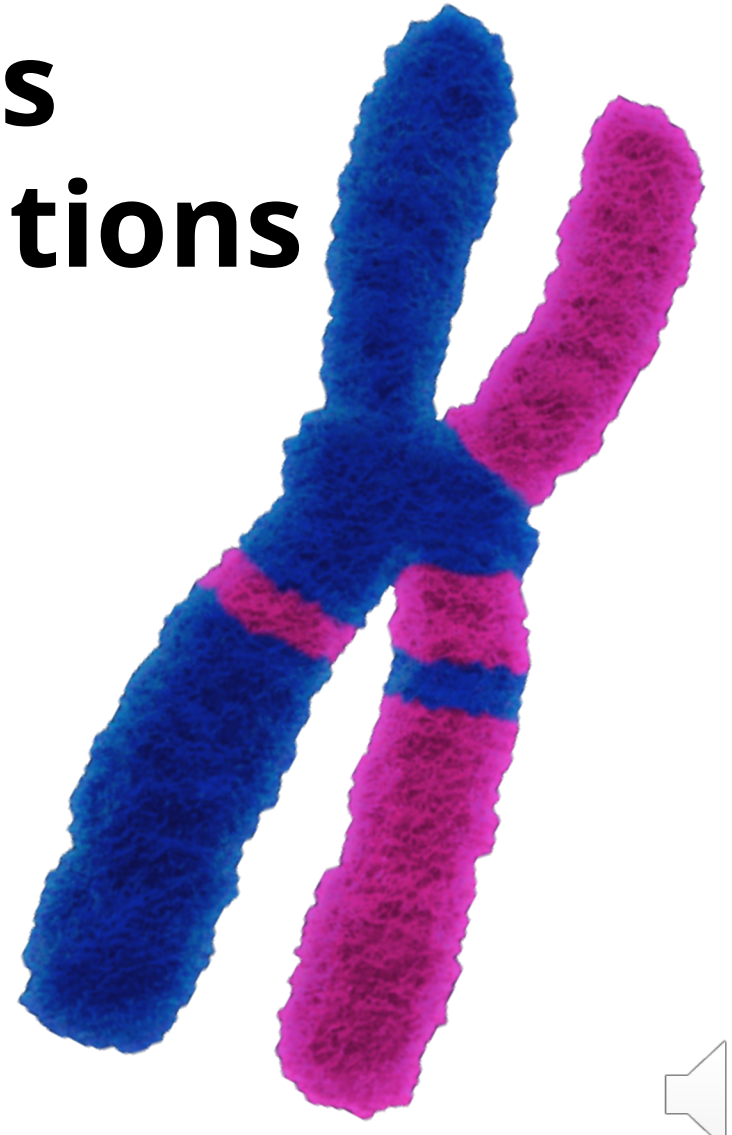
Comprehensive Analysis of Genomic Structural Variations and Off-Target Events

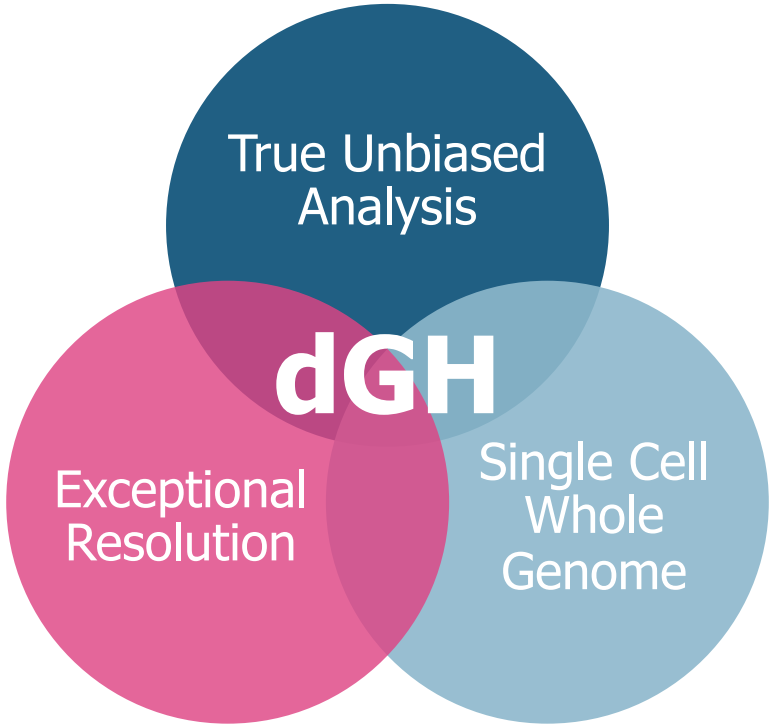


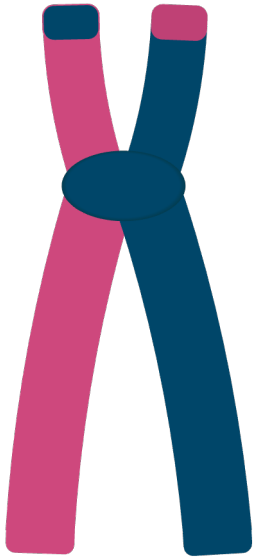
April 27th, 2022

Presentation By:
Chris Tompkins, CTO & Erin Cross, VP of R&D

KromaTiD

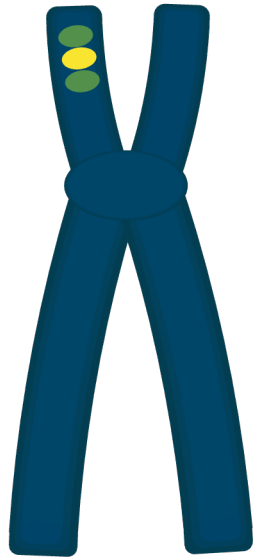
Screen, Discover and Target





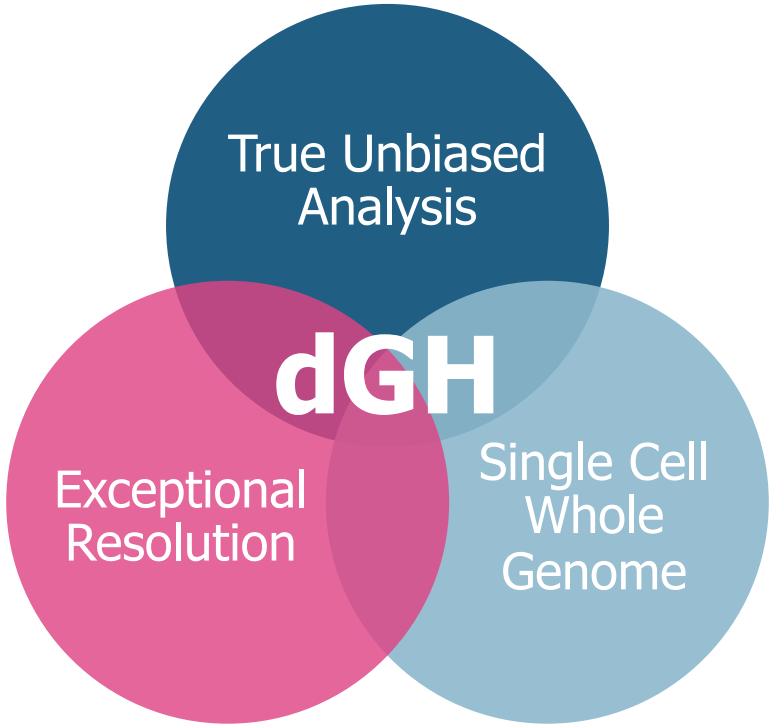
dGH SCREEN

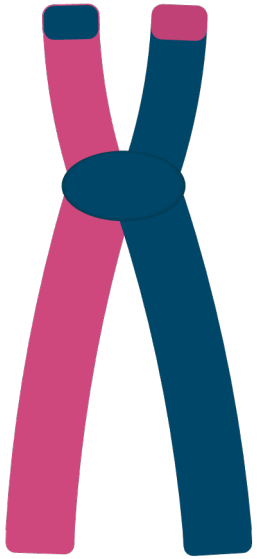
dGH DSCVR



dGH In-Site

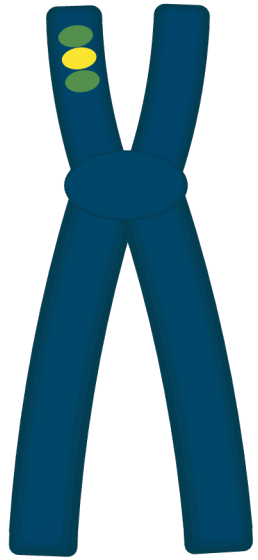
Comprehensive Structural Genomic Analysis



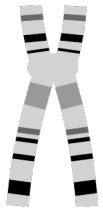


dGH SCREEN

dGH DSCVR



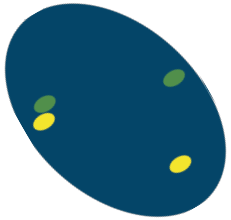
dGH In-Site



G-Band

Orthogonal

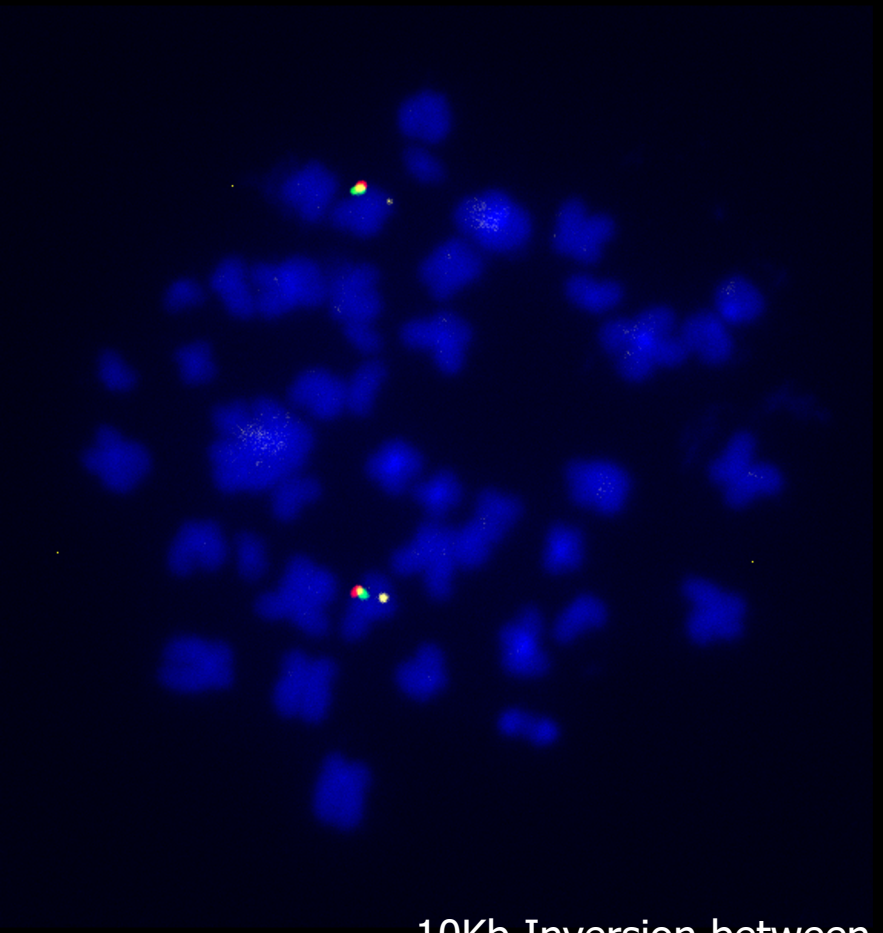
Non-Dividing Cells



Pinpoint FISH

dGH™: Single Cell Measurements of Many Cells

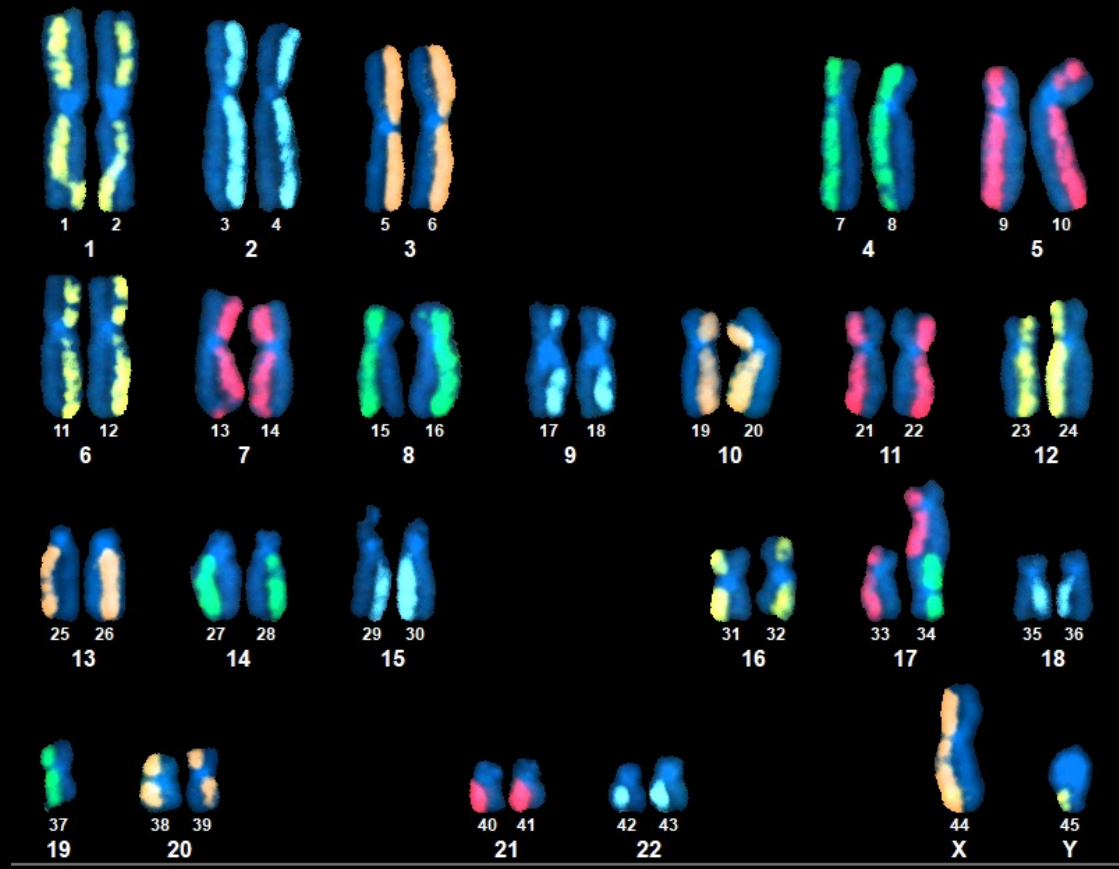
dGH In-Site™ (Localized)



10Kb Inversion between edits

Edits and Integrations

dGH SCREEN™ (Unbiased)

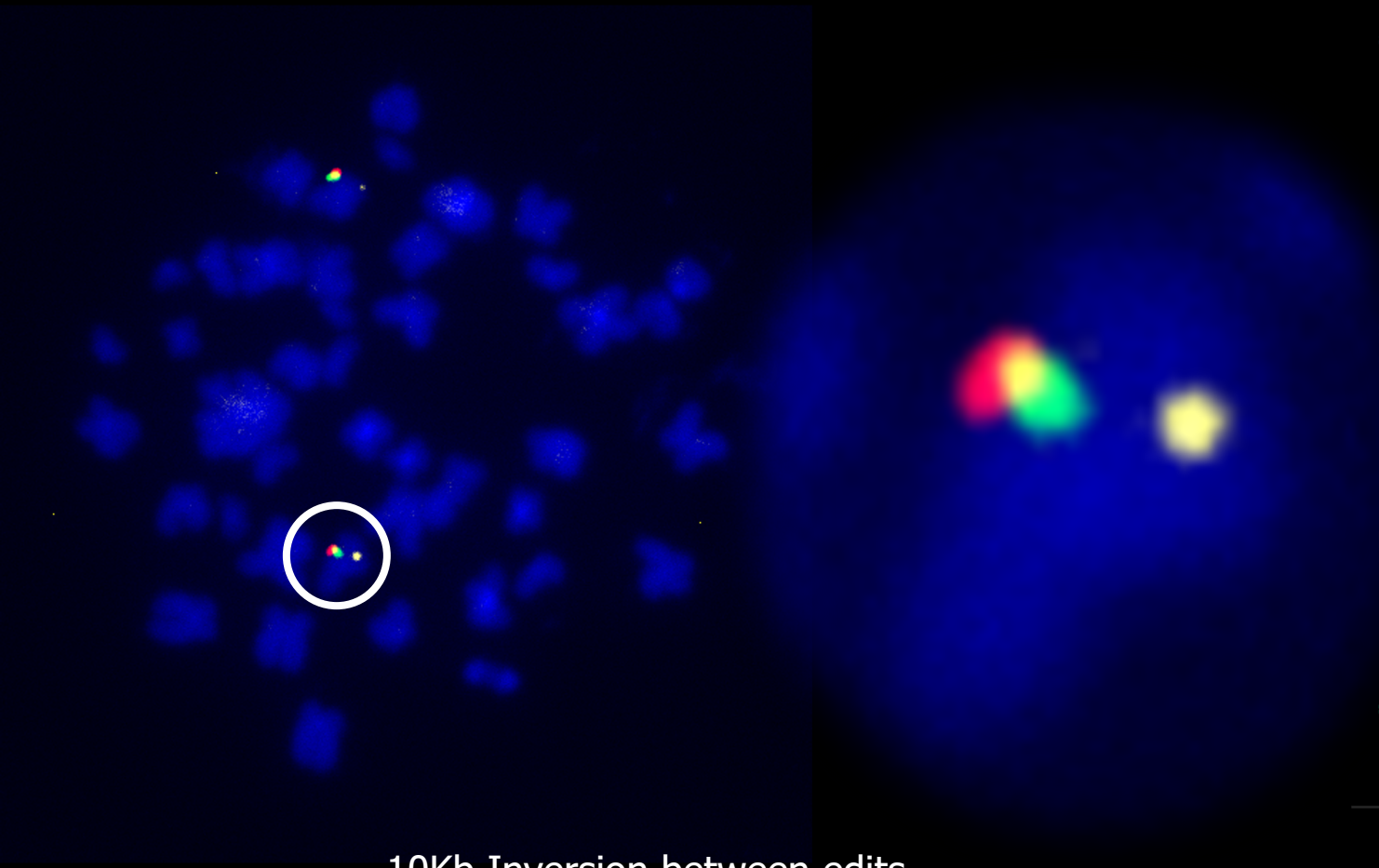


Inversion and Unbalanced Translocation

Whole Chromosome Map

dGH™: Single Cell Measurements of Many Cells

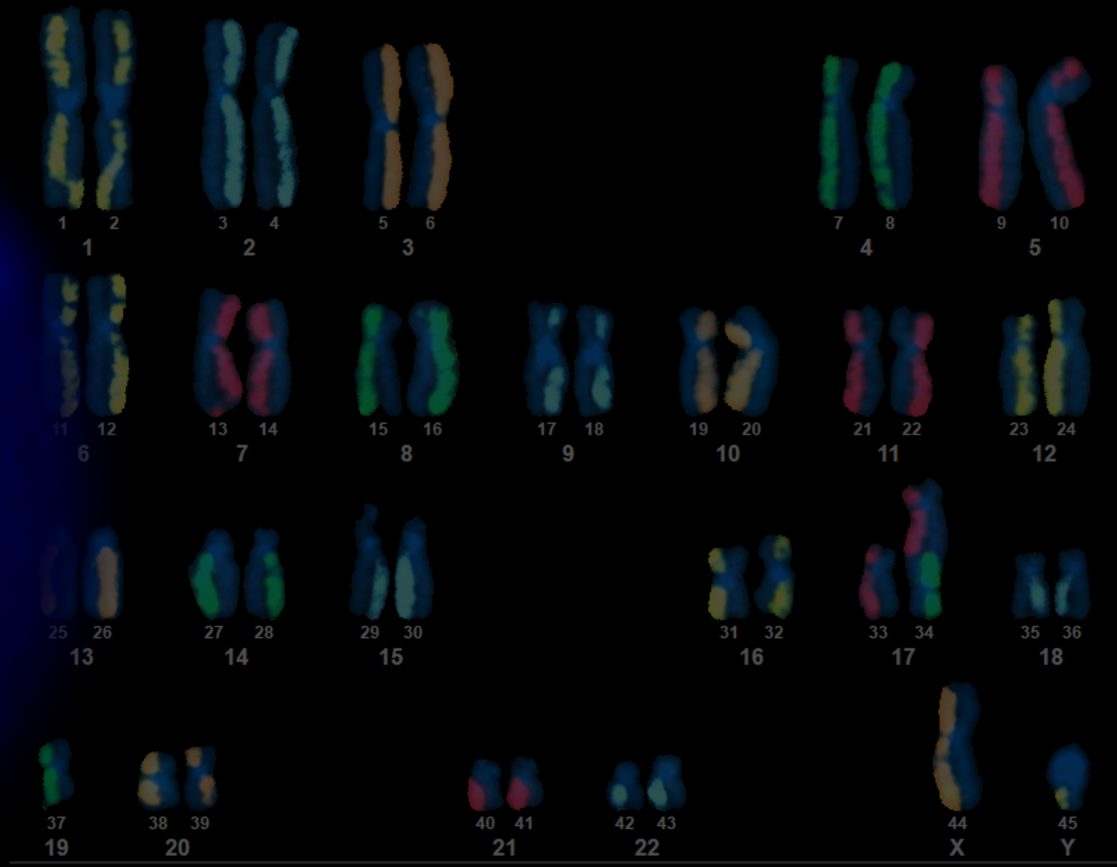
dGH In-Site™ (Localized)



10Kb Inversion between edits

Edits and Integrations

dGH SCREEN™ (Unbiased)



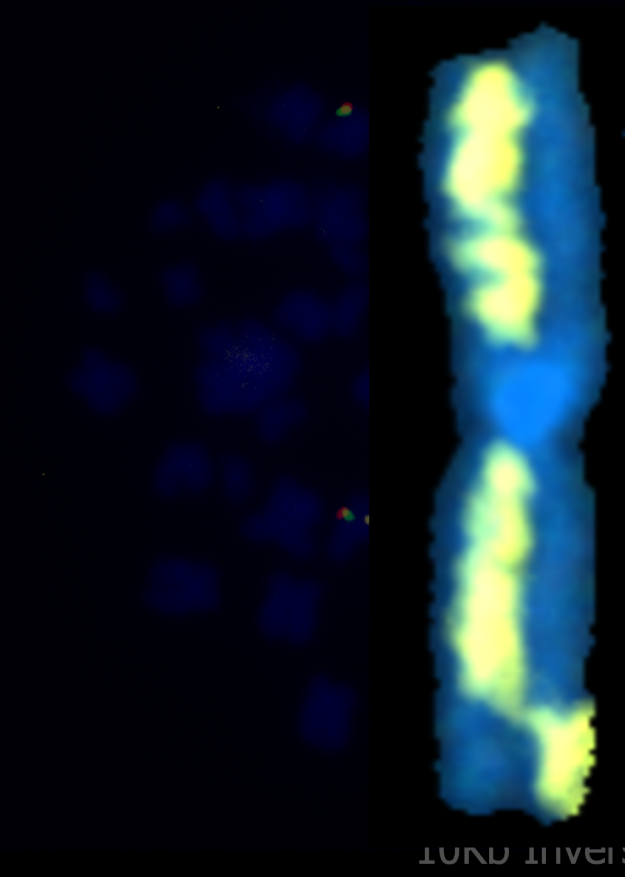
Inversion and Unbalanced Translocation

Whole Chromosome Map

dGH™: Single Cell Measurements of Many Cells

dGH In-Site™ (Localized)

C1 Telomeric Inversion

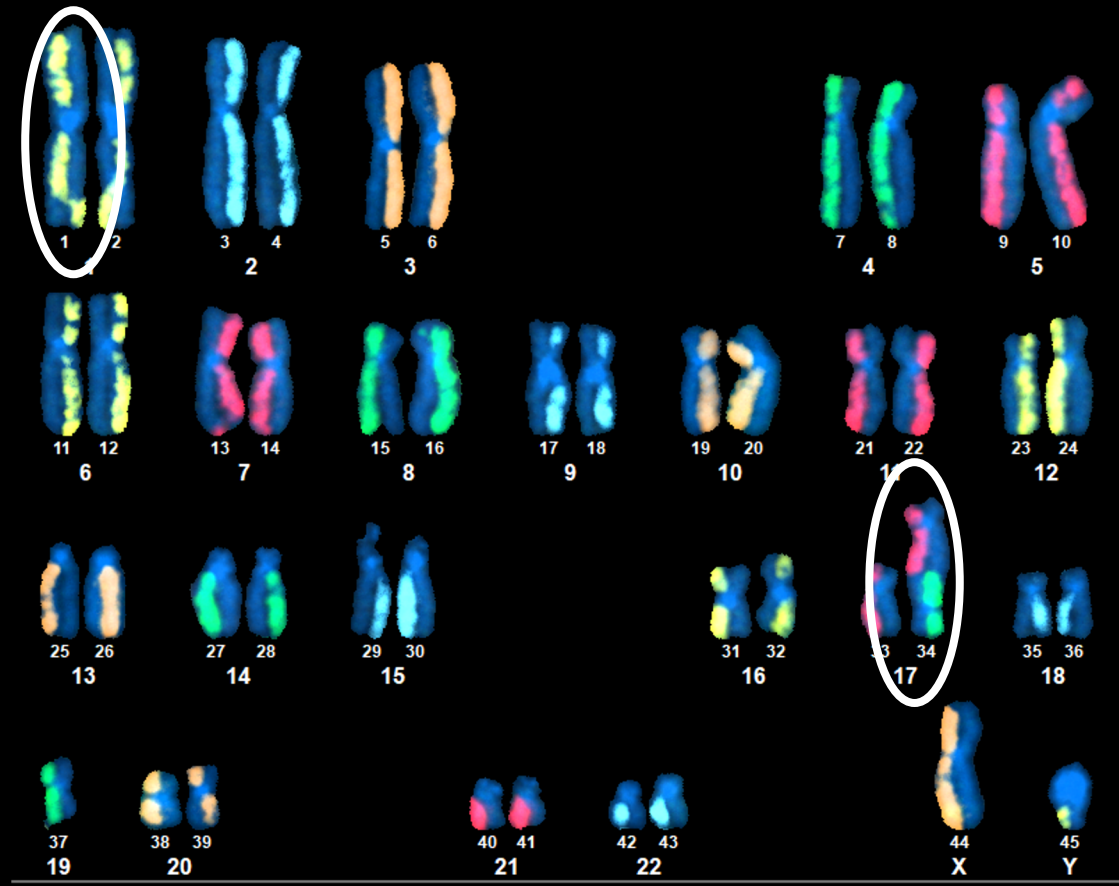


Translocation
C19 to C17

10Kb Inversion between edits

Edits and Integrations

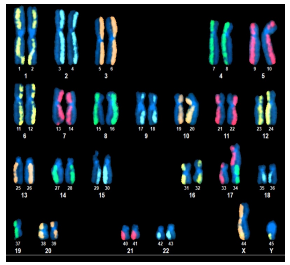
dGH SCREEN™ (Unbiased)



Inversion and Unbalanced Translocation

Whole Chromosome Map

Single Cell Measurement of Many Cells



Hundreds (or more) Cells

Constitutive Inversion from Donor Cells

Recurrent Inversion Between Edit Sites

Edit Site Translocations

Aneuploidy

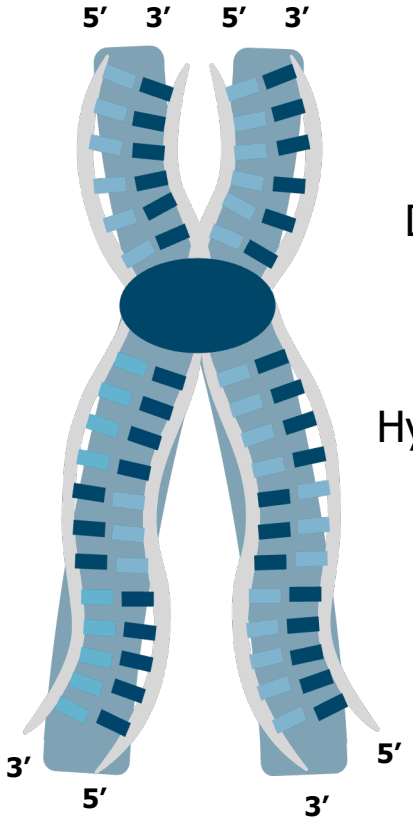
25
20
15
10
5
0

Background DNA Repair Activity

C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 C18 C19 C20 C21 C22

Chromatid Painting with dGH™

Blue = DAPI Staining of Chromosome Structure

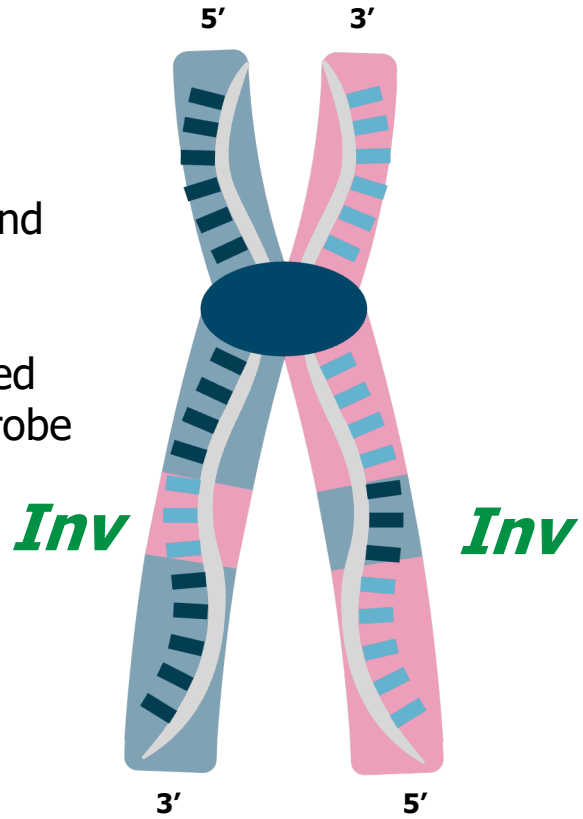


Double Stranded Metaphase Chromatid

Daughter Strand Stripping

Single Stranded Hybridization Probe

Pink = Fluorescently Labeled Hybridization Probes



Analyte: Single Stranded dGH Chromatid

DNA Orientation from Image Data

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

Single-stranded probes designed are to target *only* the Watson strand. **Signal appears on one sister chromatid only**

Inverted targets appear on the opposite sister chromatid

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

A Complete Measure of Outcomes Requires dGH

Workflow

Genome Engineering → **Edited Cell Cloning** → **Structural Analysis** → **Lead Selection** → **Pre/Clinical Dev**

Cellular Engineering
- **Insertion Mapping**
- **Insertional Copy No.**
- Edit Optimization
- Process Optimization
- Qualification

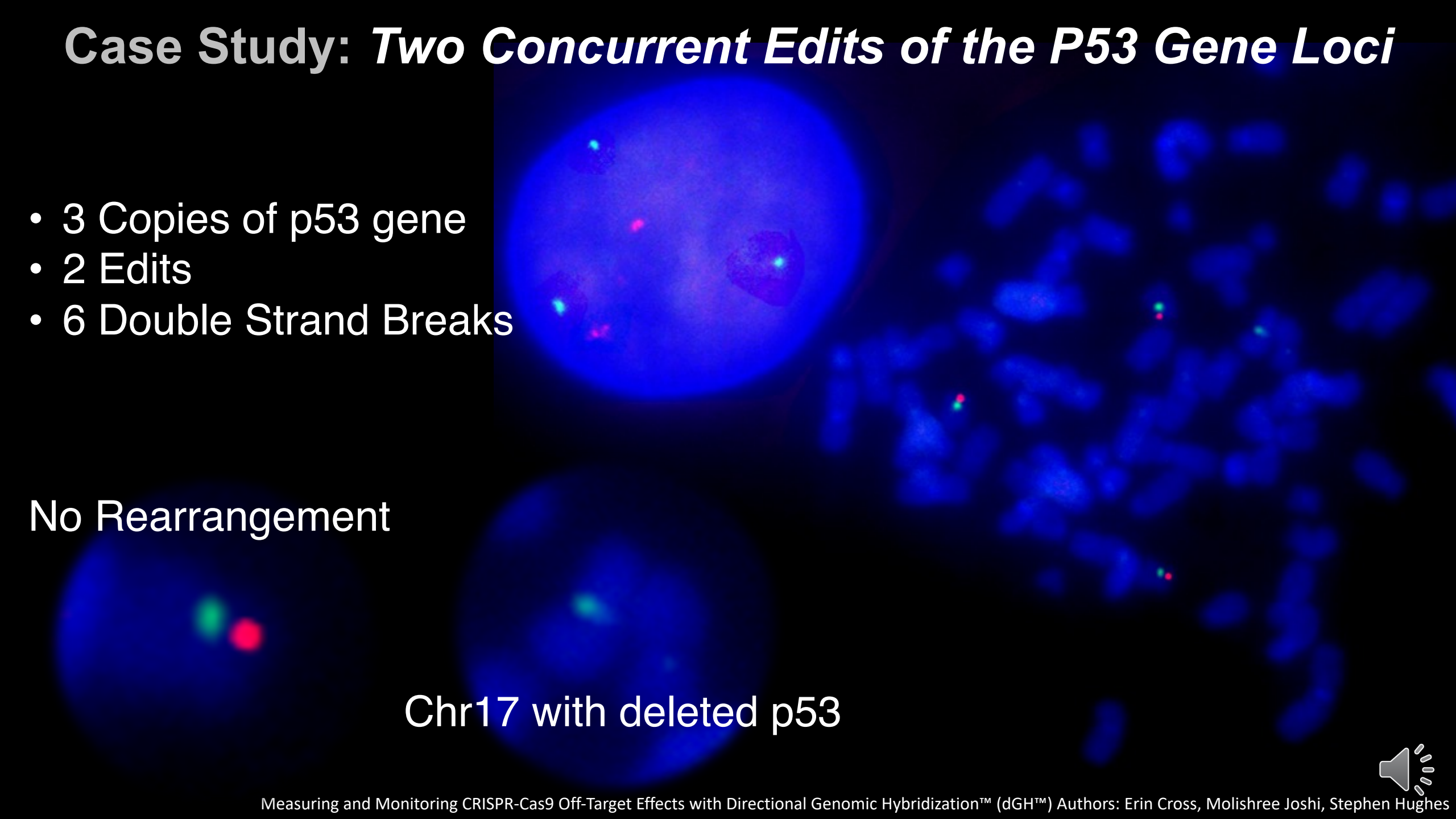
Single Cell Cloning
- Clone Selection
- Clone Qualification
- **Clone Stability**

Genome Mapping
- Normalcy
- **Structural Variants**
- **Variation Rates**
- DNA Damage
- Variation Discovery
- Variant Identification

Genotox
- Insertional Mutagenesis
- Clonal Outgrowth
- Chromothyrpsis
- **Genomic Instability**
- Karyotypic Abnormalities
- Model Animal Studies

Quality Control
- Batch Variability
- Cell Line Stability
- Subject Qualification
- Batch Release

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

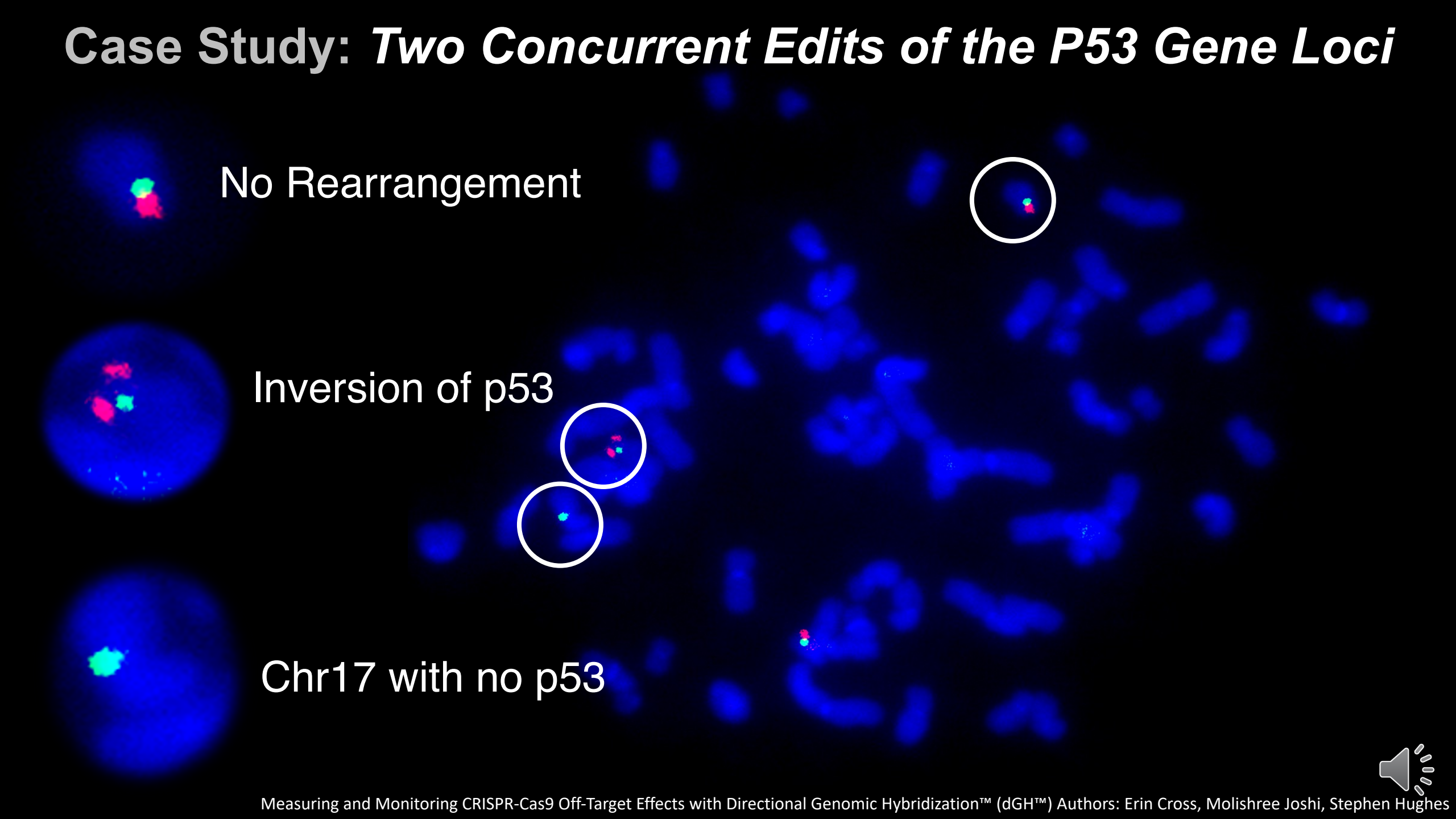


- 3 Copies of p53 gene
- 2 Edits
- 6 Double Strand Breaks

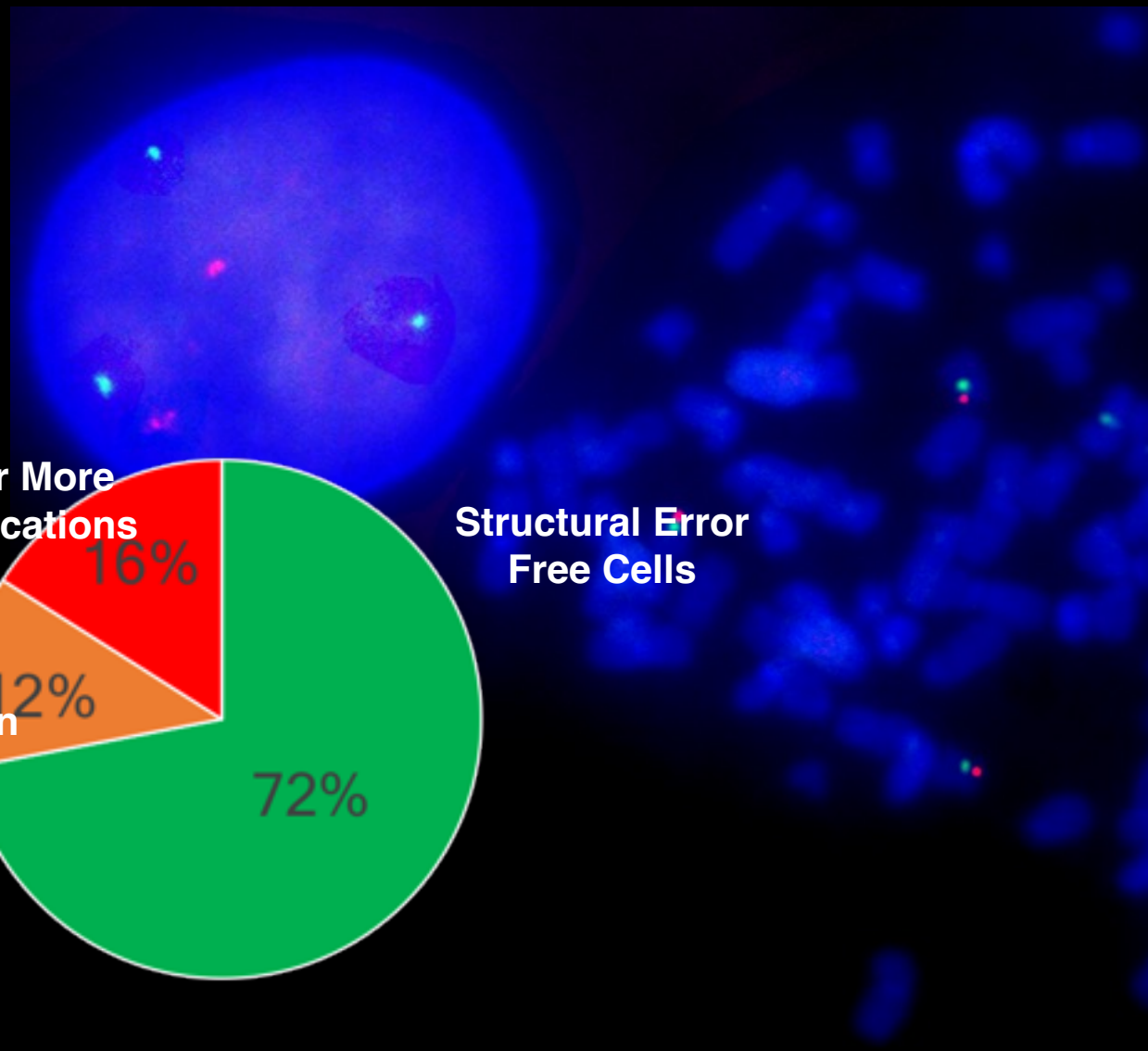
No Rearrangement

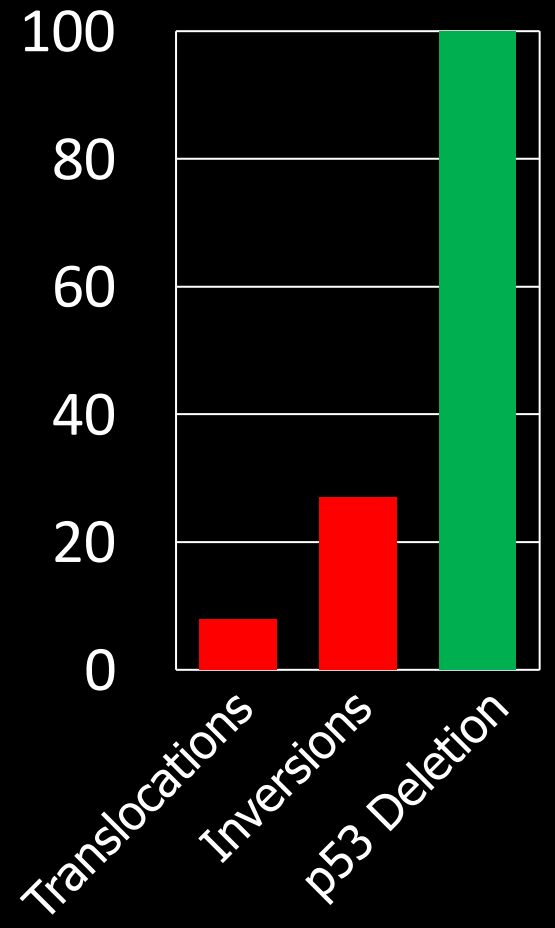
Chr17 with deleted p53

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

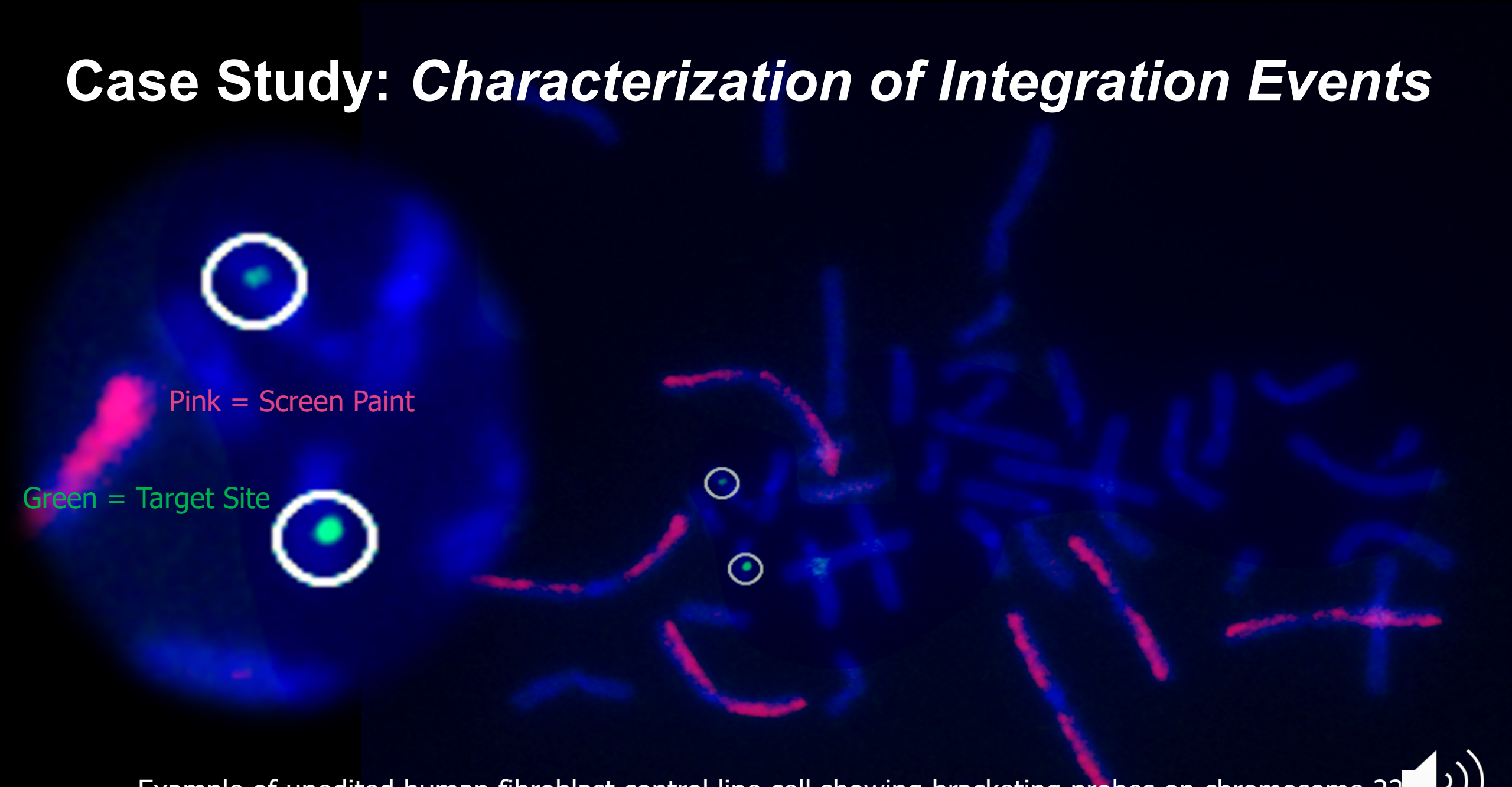


Case Study: *Two Concurrent Edits of the P53 Gene Loci*



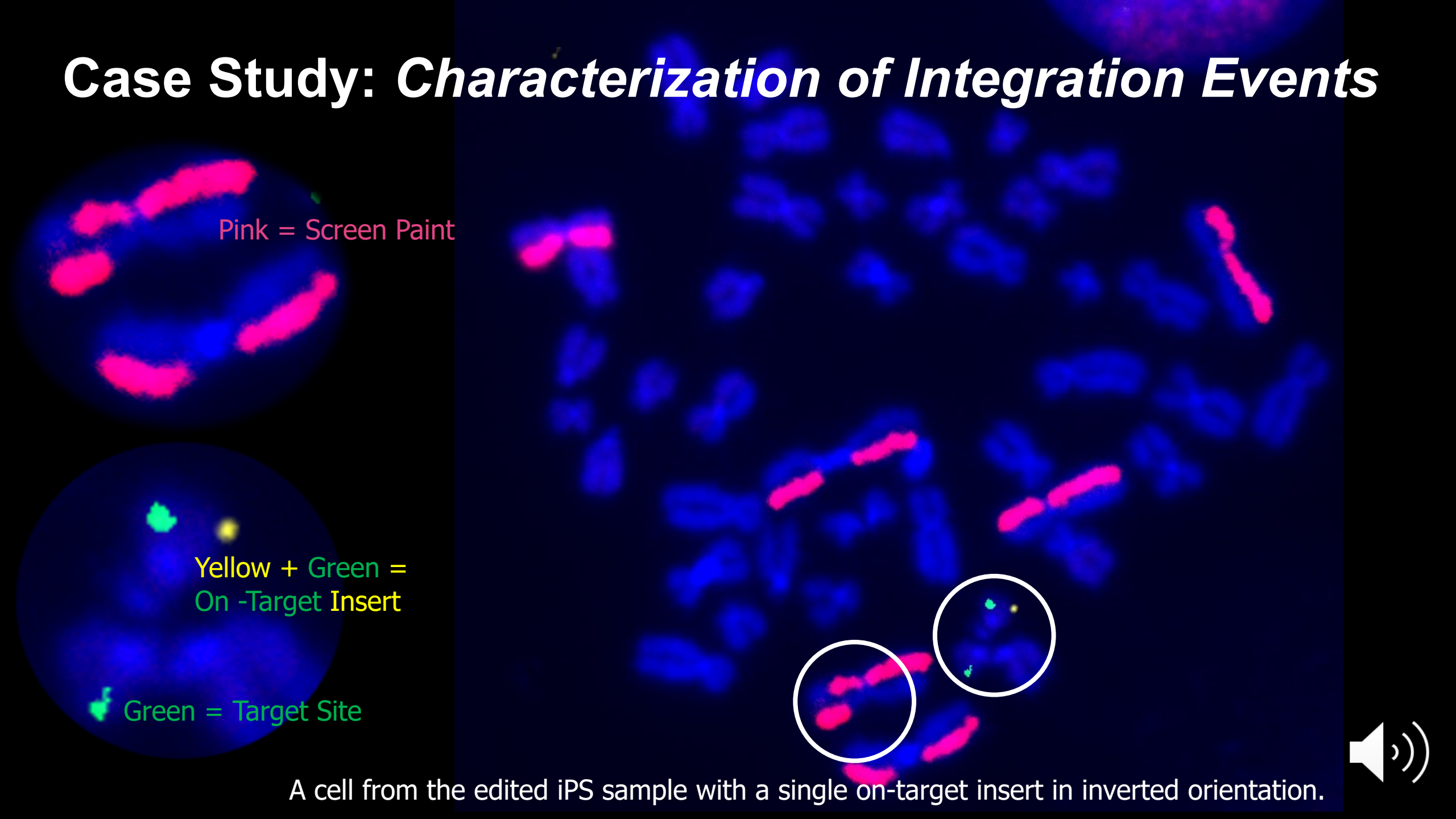


Case Study: *Characterization of Integration Events*



Example of unedited human fibroblast control line cell showing bracketing probes on chromosome 22, dosimetry paints on chromosomes 1, 2 and 3 and no inserts

Case Study: *Characterization of Integration Events*



A cell from the edited iPS sample with a single on-target insert in inverted orientation.

Case Study: *Characterization of Integration Events*

Case Study: *Characterization of Integration Events*

Average ICN per cell: 7.8

- On-target only: 2%
- On-target plus off-target: 14%
- Off-target only: 77%
- No Integrations: 7%

Case Study: *Characterization of Integration Events*

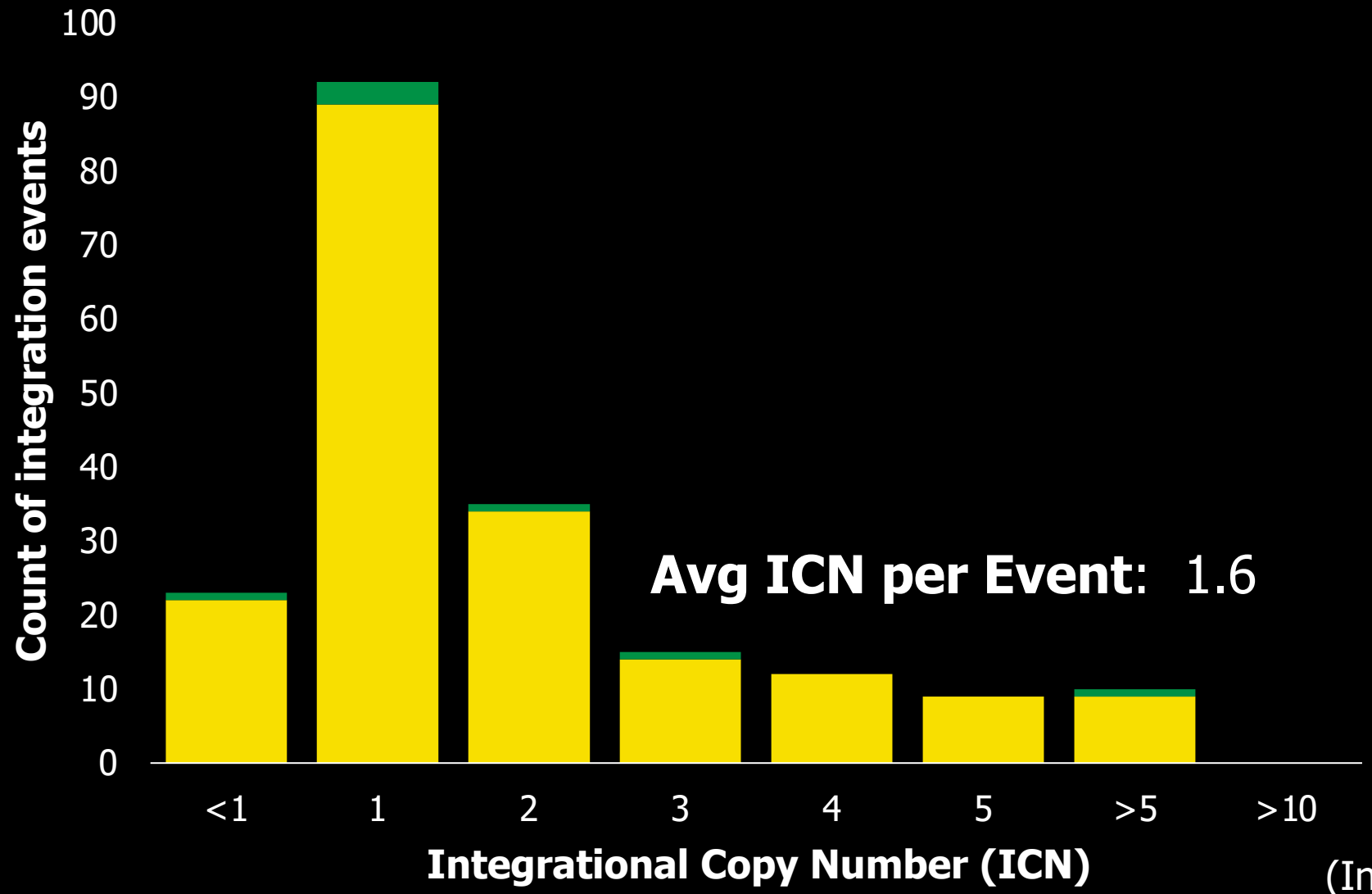
10 Kb Internal Size Control probe (Chr8)

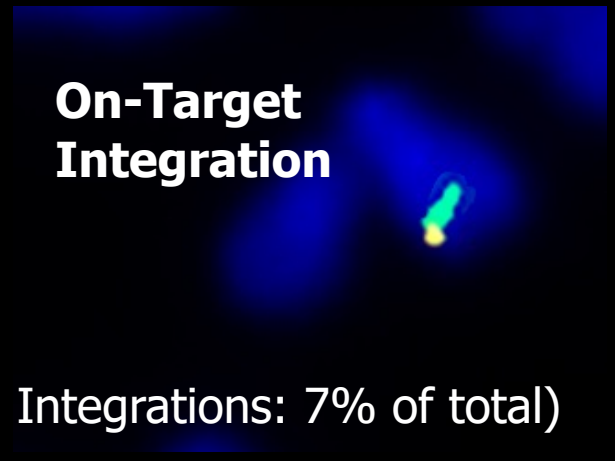
$$ICN_e = S_T / S_c$$

Off-Target Integrations

On-Target Integration

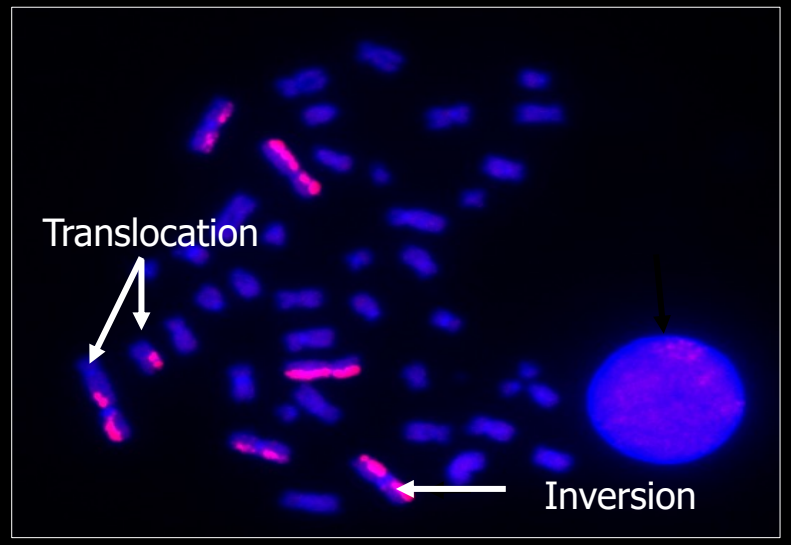
Case Study: *Characterization of Integration Events*

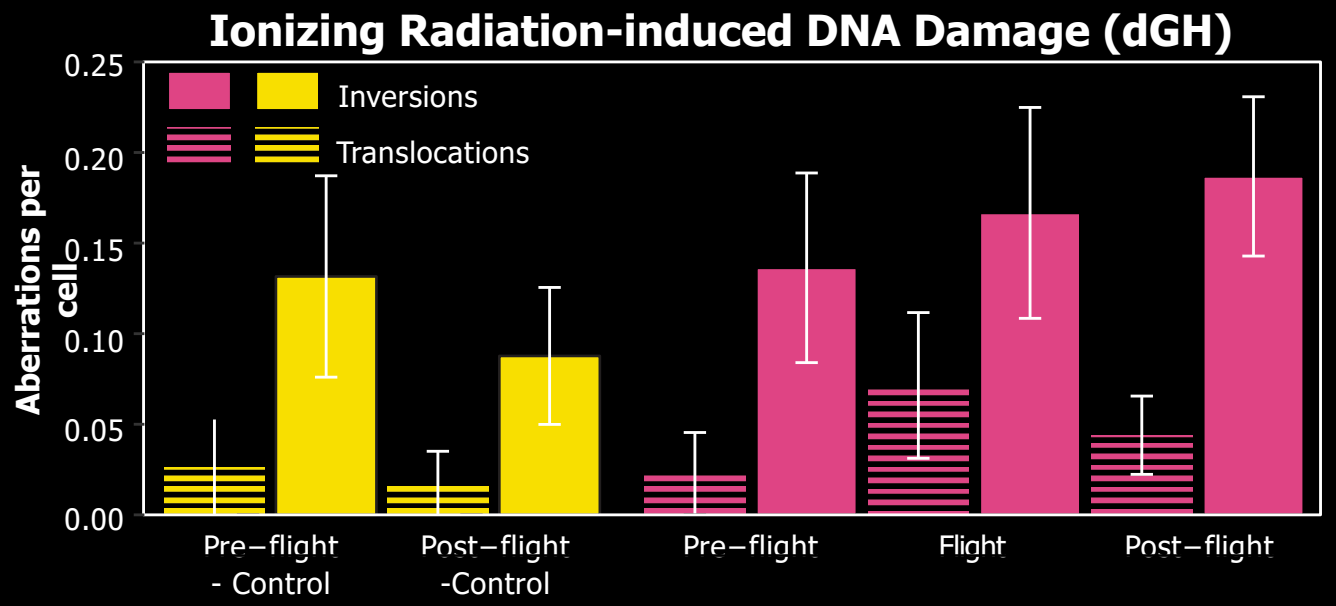




(Inverted Integrations: 7% of total)

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

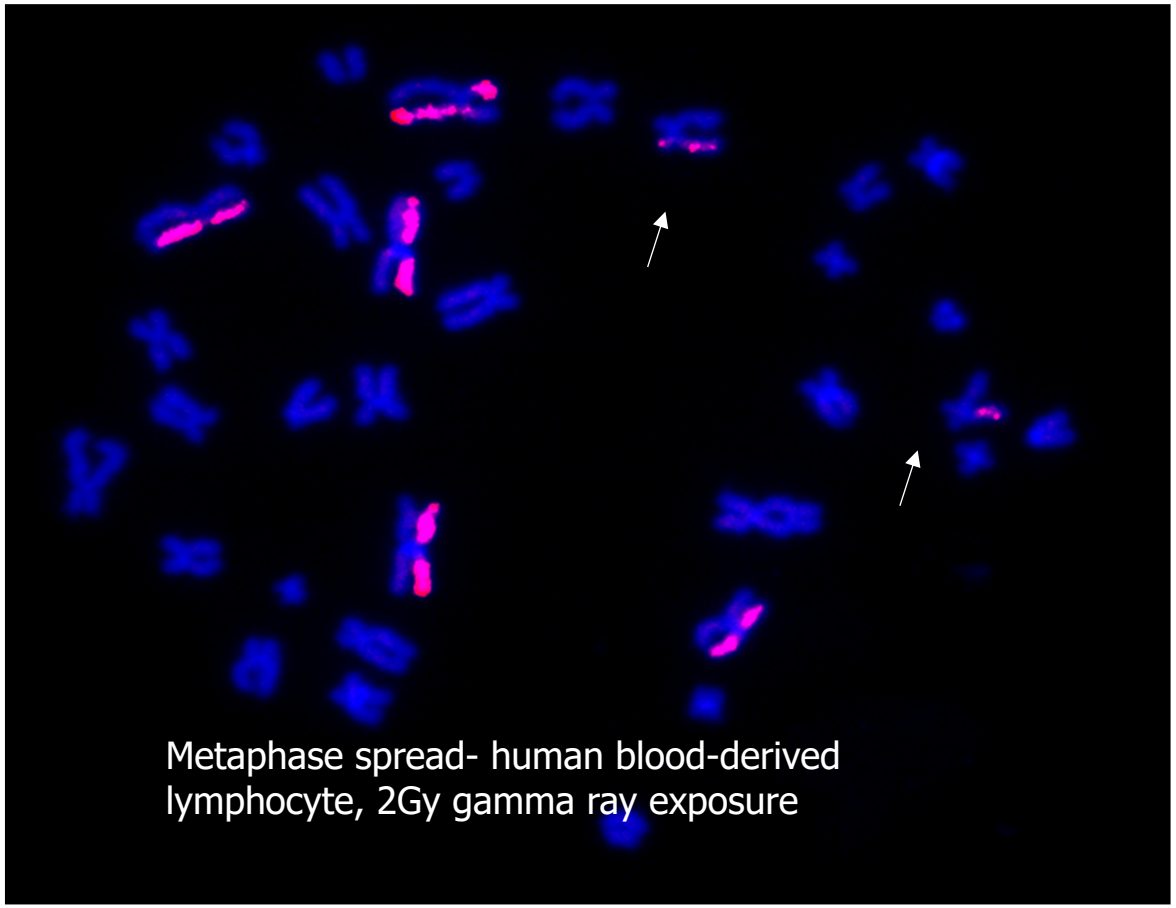


Figure 1: Whole chromosome 1, 2 and 3 paints hybridized to a metaphase spread from a human peripheral blood sample irradiated with 2Gy Cs-137 gamma rays. Structural rearrangements identified by Directional Genomic Hybridization denoted by arrows.

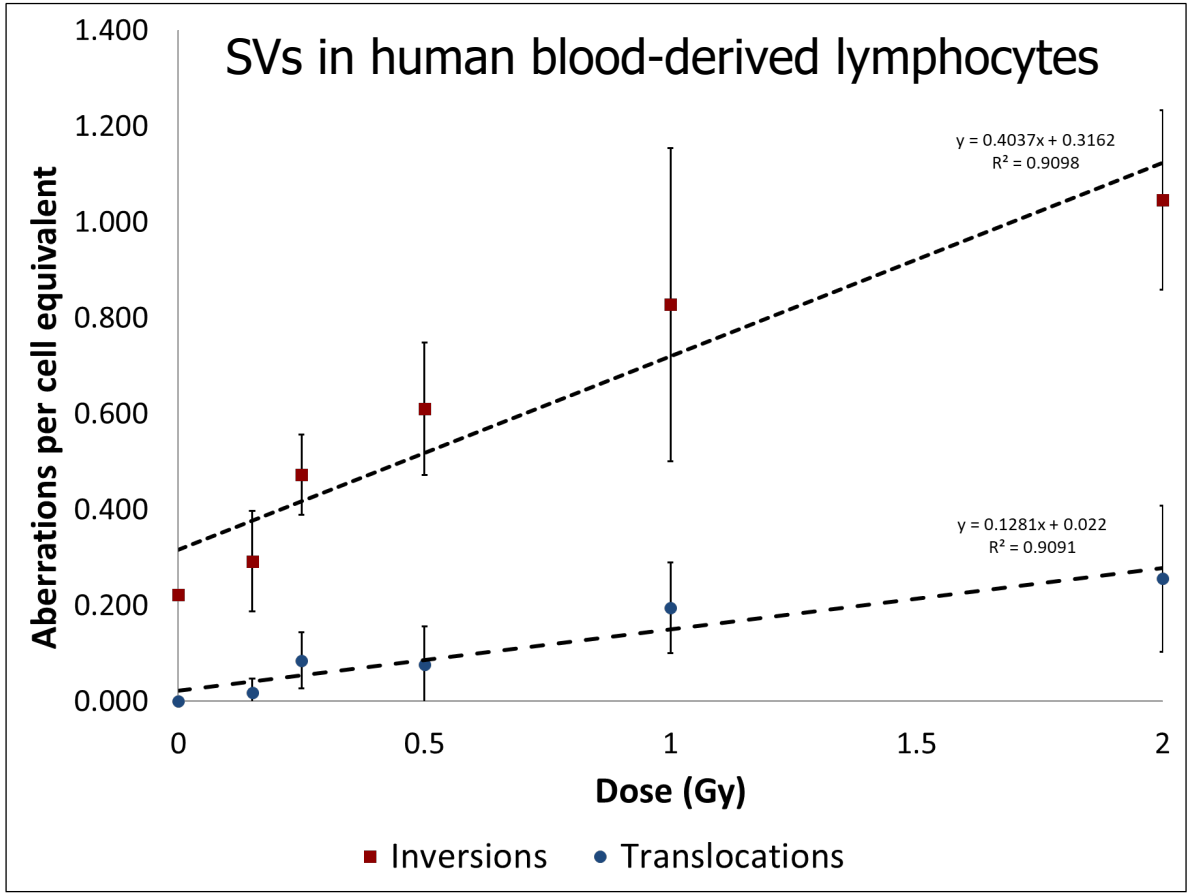
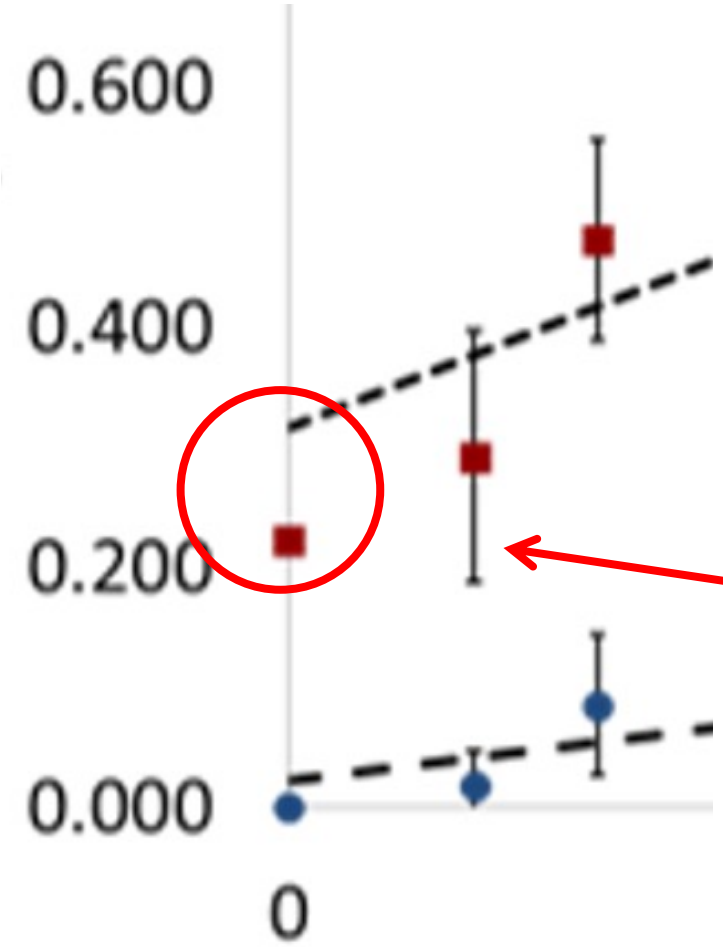
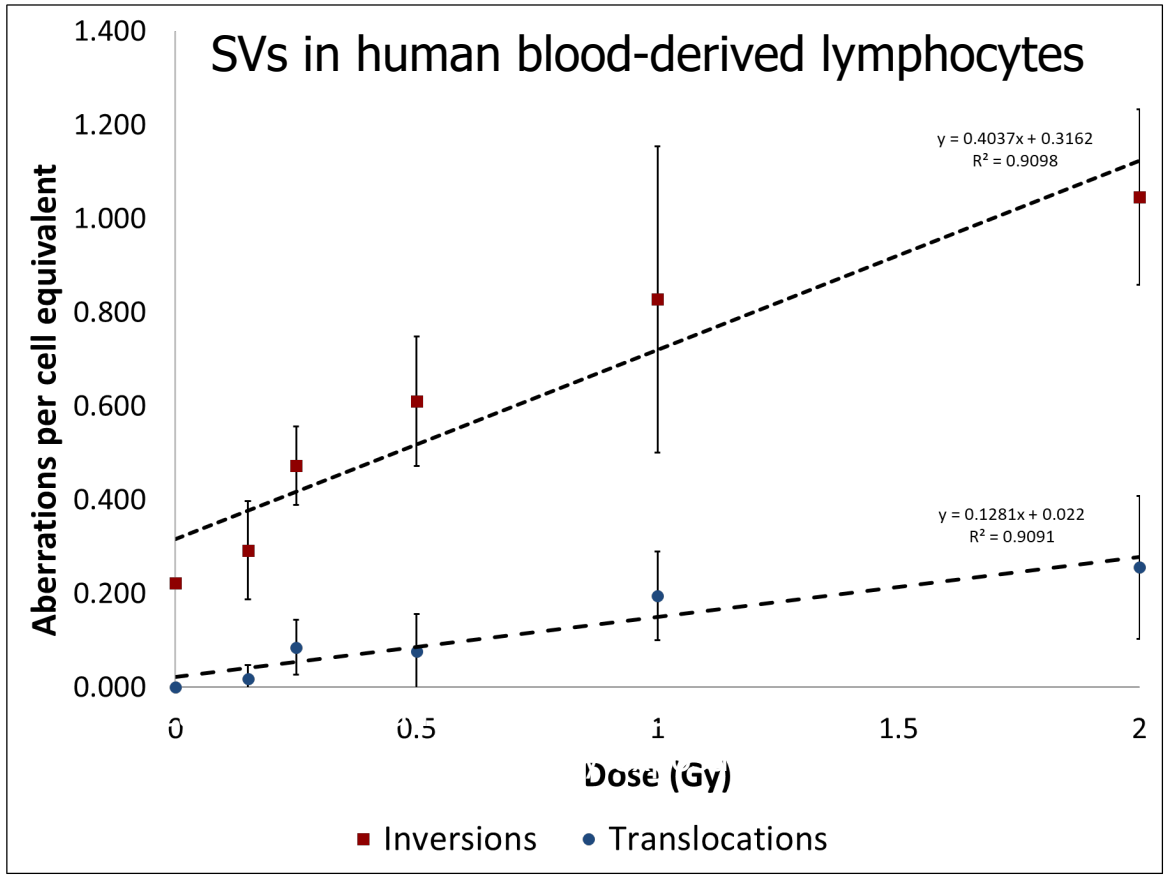


Figure 2: Blood samples from young adult controls were irradiated with Cs-137 gamma rays to establish a dose response (calibration) curve. Males in their mid-20's were selected to account for age at exposure. Inversions (red) had a higher natural background rate compared to translocations (blue); however,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



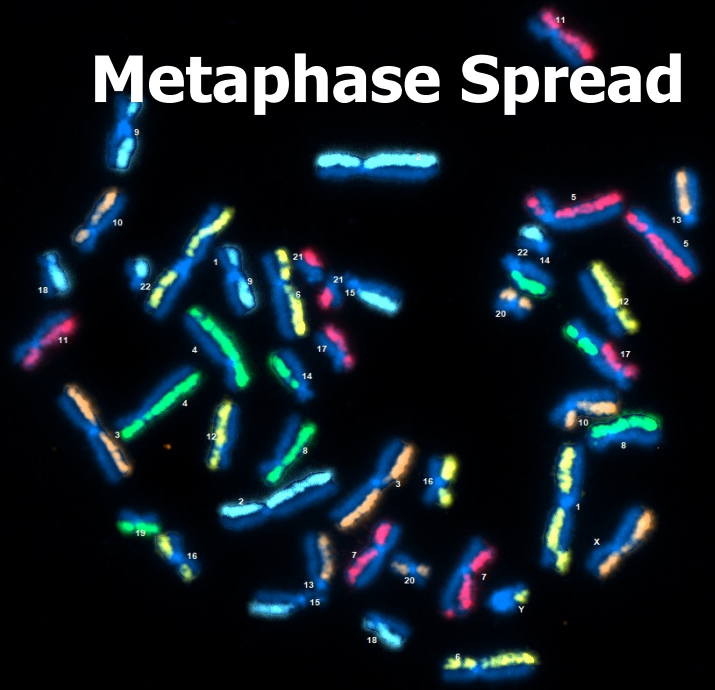


~0.5 aberrations per cell equivalent. Unexposed adult non-smokers. Average age 26yr

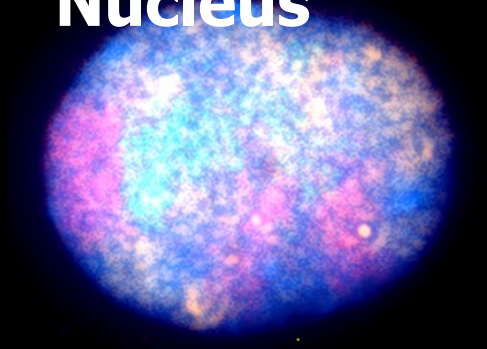
Chromosome Translocations, Inversions and Telomere Length for Retrospective Biodosimetry on Exposed U.S. Atomic Veterans - PubMed (nih.gov)

Case Study: *Whole Genome Mapping*

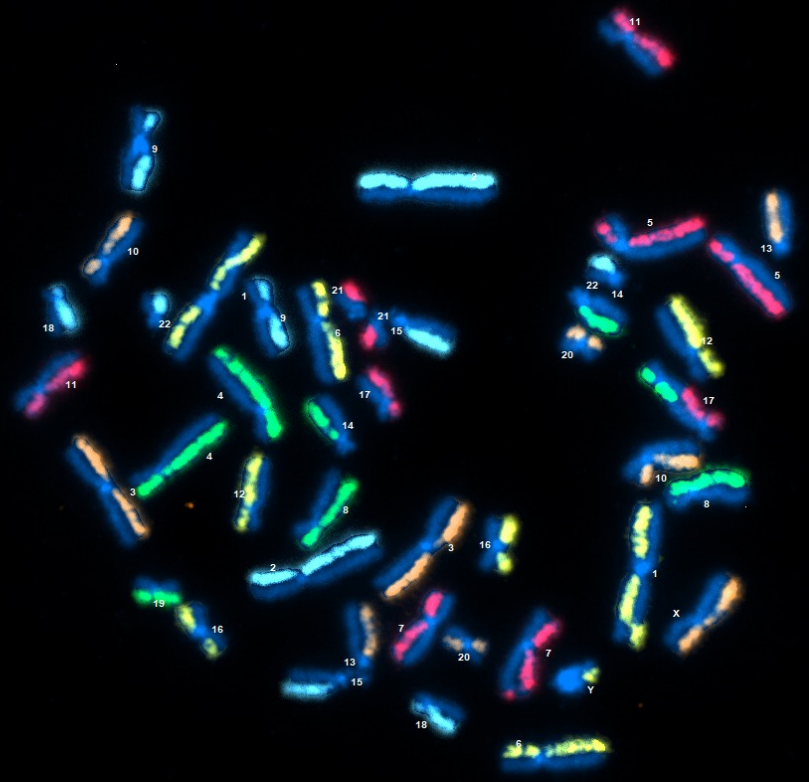
Metaphase Spread



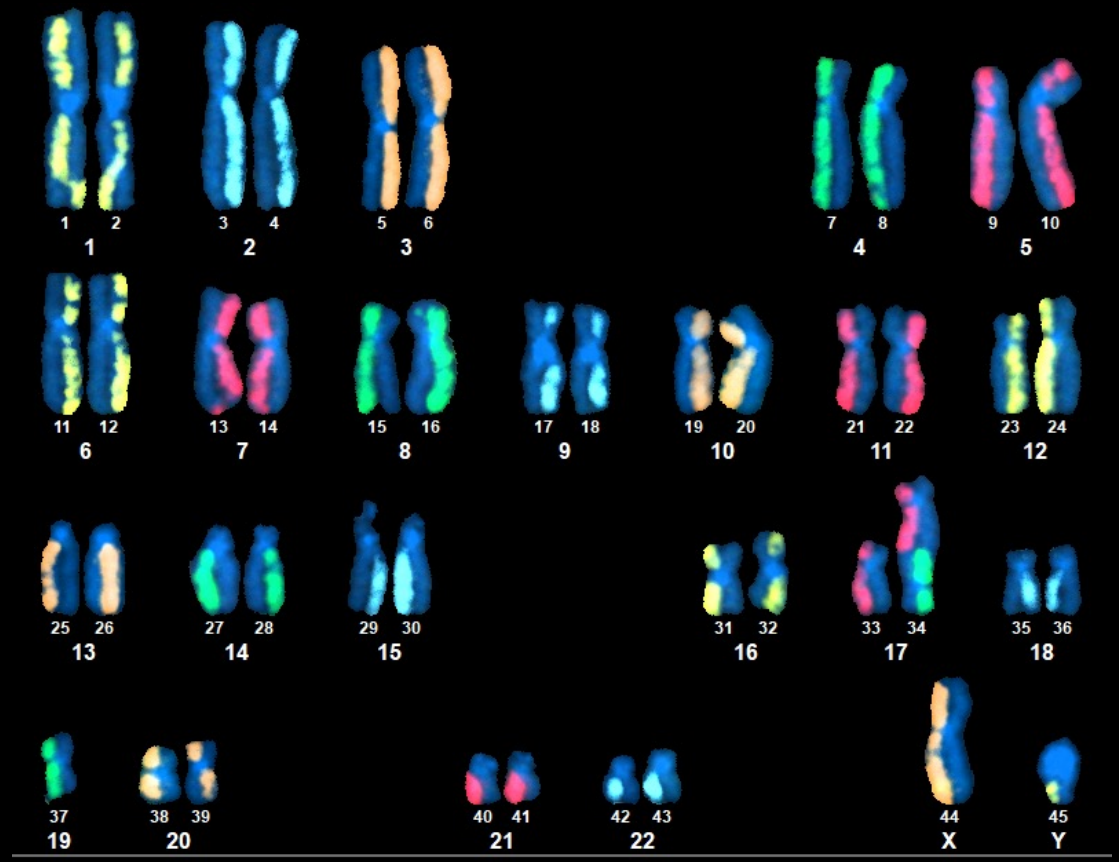
Nucleus



Case Study: *Whole Chromosome Mapping*

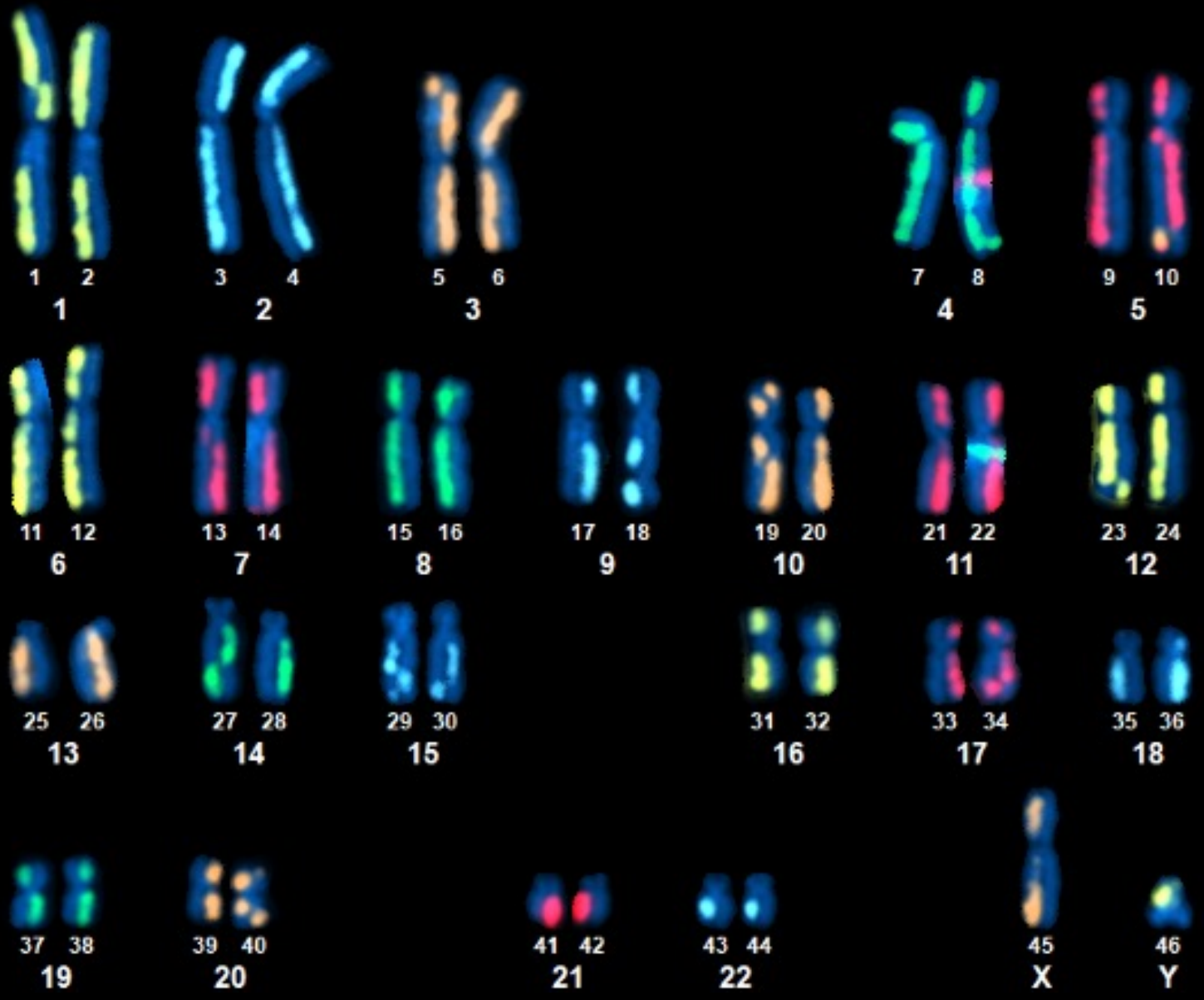


Metaphase Spread

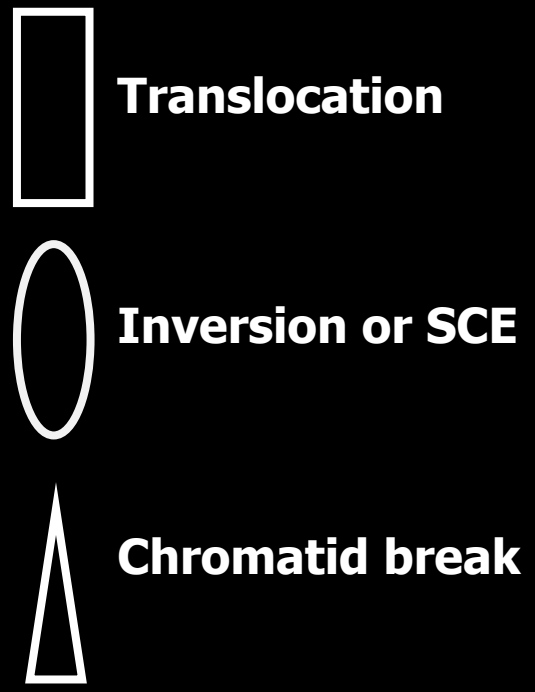


Karyogram

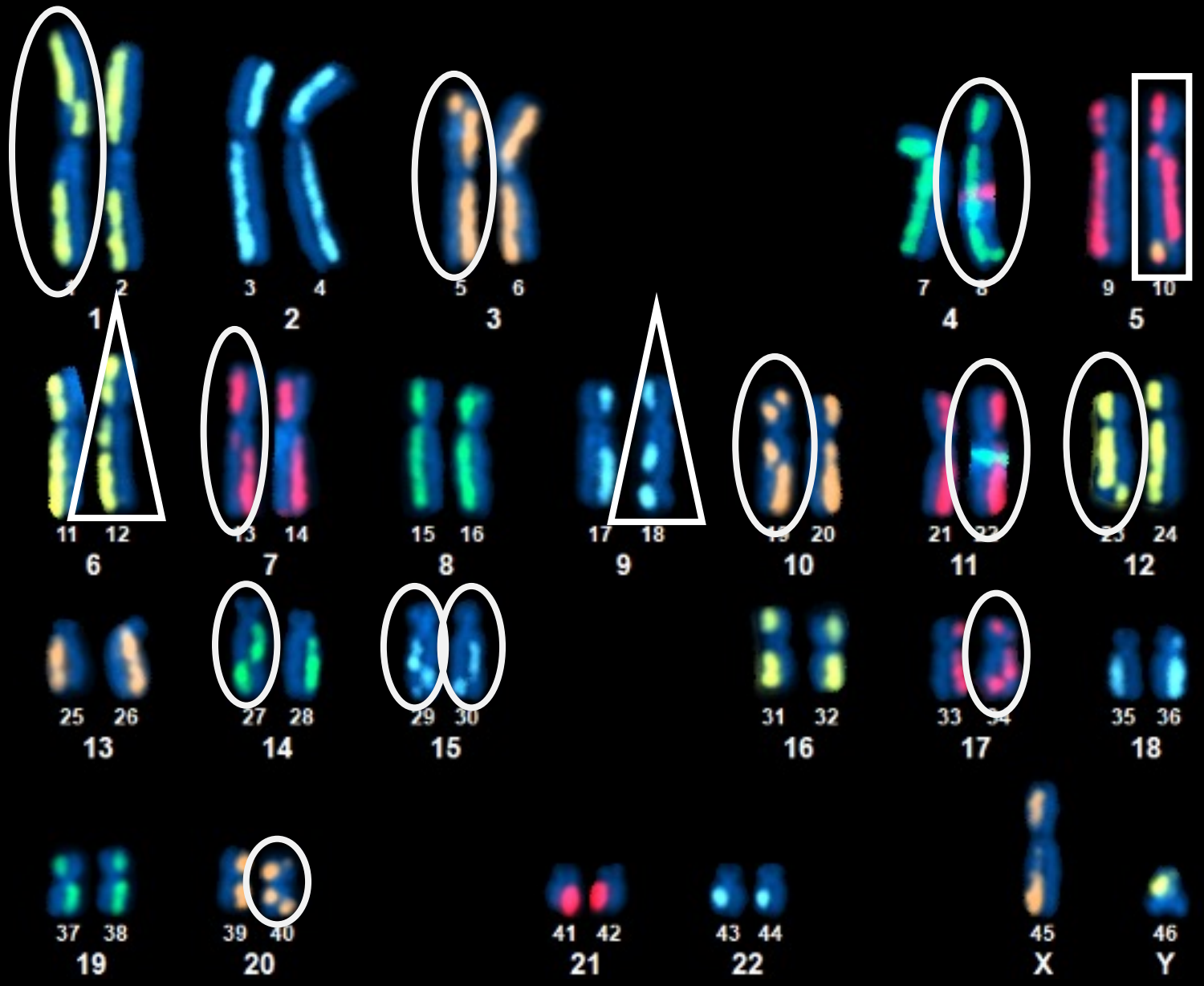
Case Study: Un-Sequenceable Rearrangements



Aberration Types:



Case Study: Un-Sequenceable Rearrangements



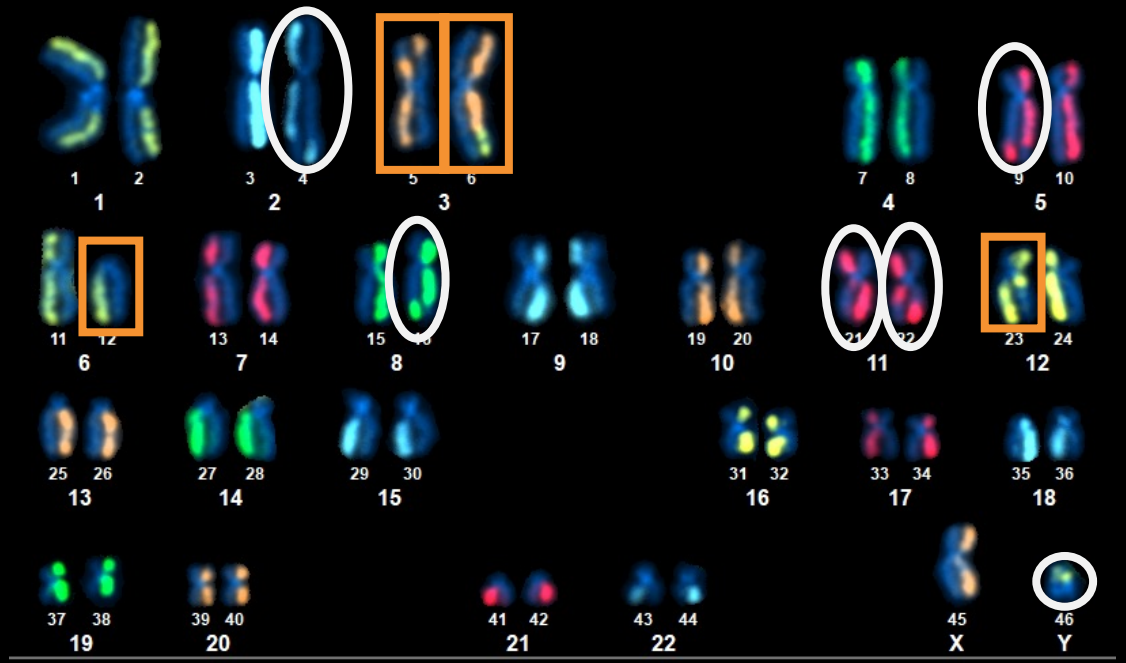
Aberration Types:

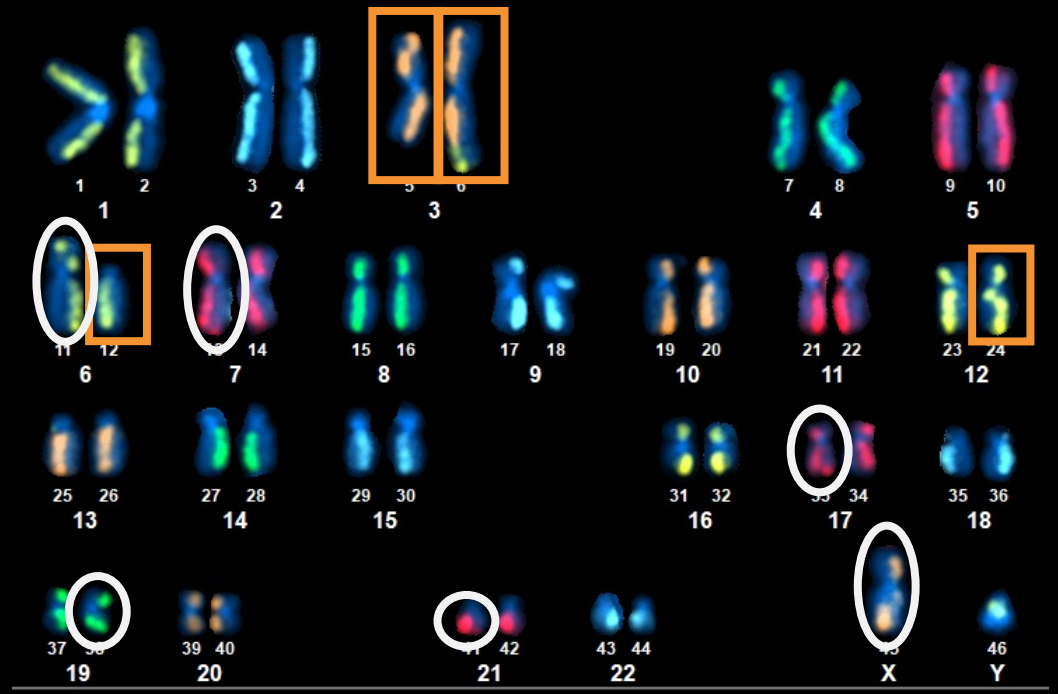
Translocation

Inversion or SCE

Chromatid break

Case Study: Measuring Recurrent Translocations and Inversions

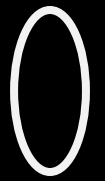




Aberration Types:

Dgh is capable of distinguishing between recurrent and random structural rearrangement at a very high sensitivity

Recurrent



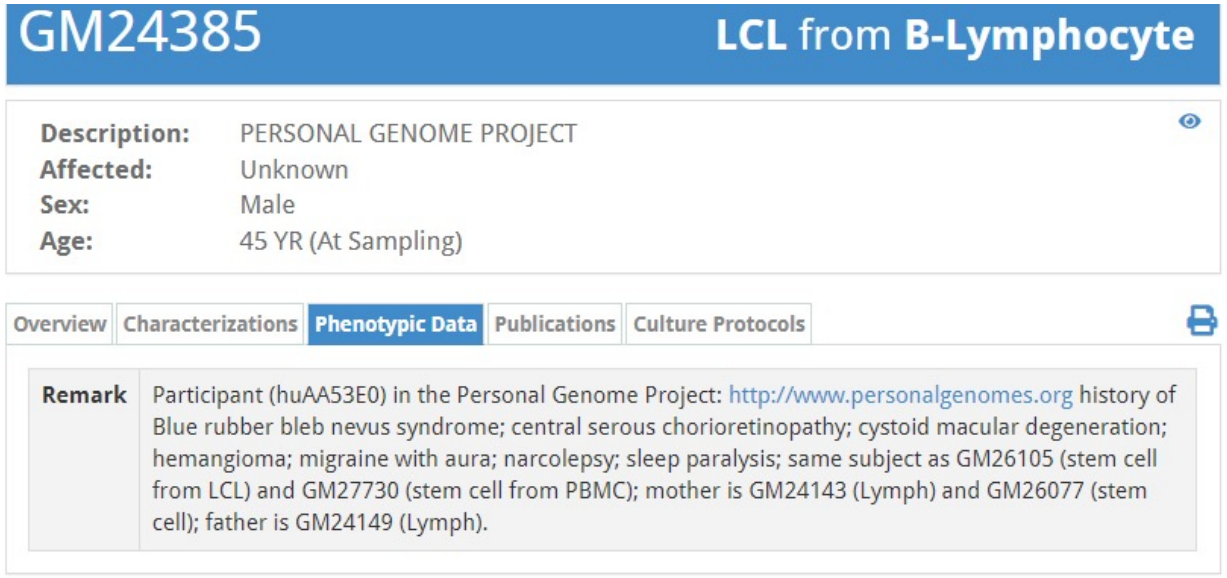
Random

KromaTiD

Direct, Definitive Genomics

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



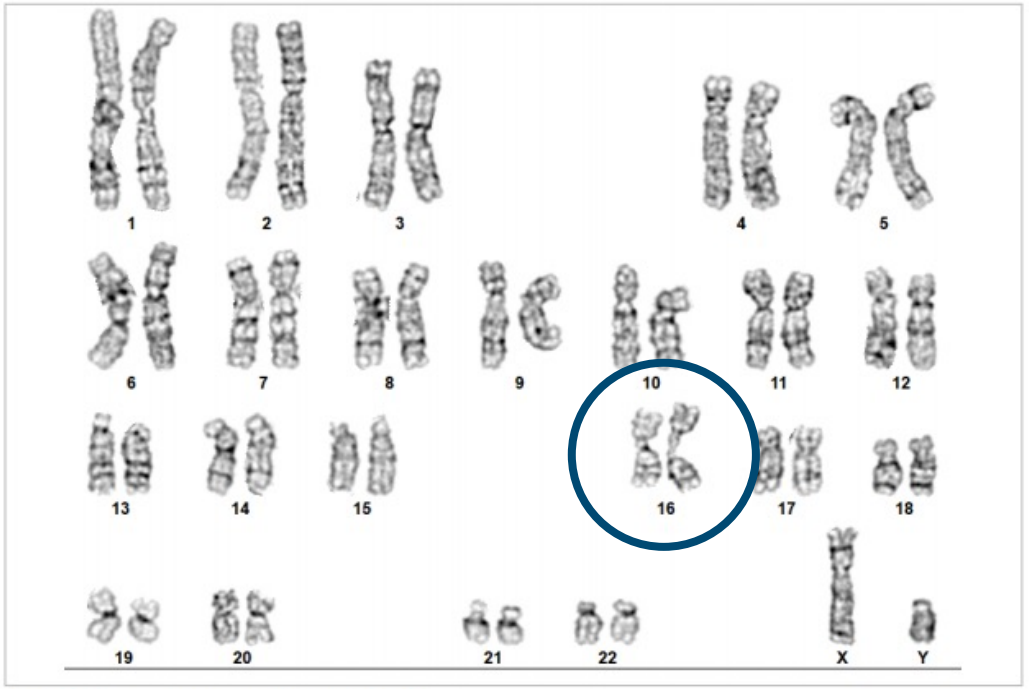
Previous GM24385 Genome Structural Characterization:

- 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 telomeric inversion on 3q
  - Discovered centromeric inversion on 3q

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

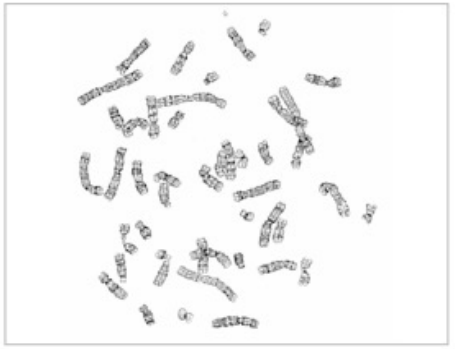
Some rate of potential intra-chromosomal CNV (band expansions) was observed

Instability and gross rearrangement of C16 matched dGH SCREEN observations



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

Cell Notes: Estimated Band Resolution:675

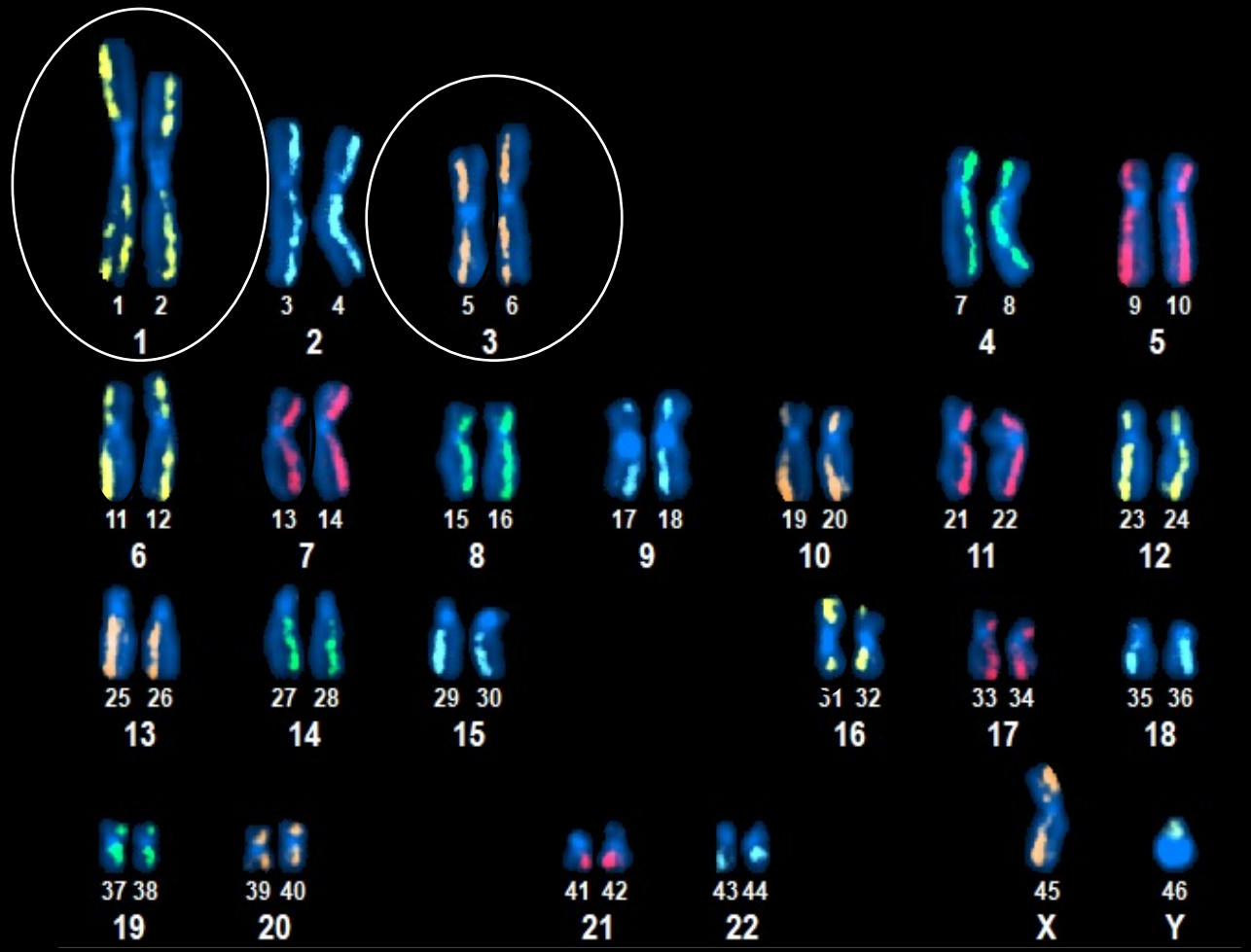


Label - Slide/Cell: S002692 - 8/52

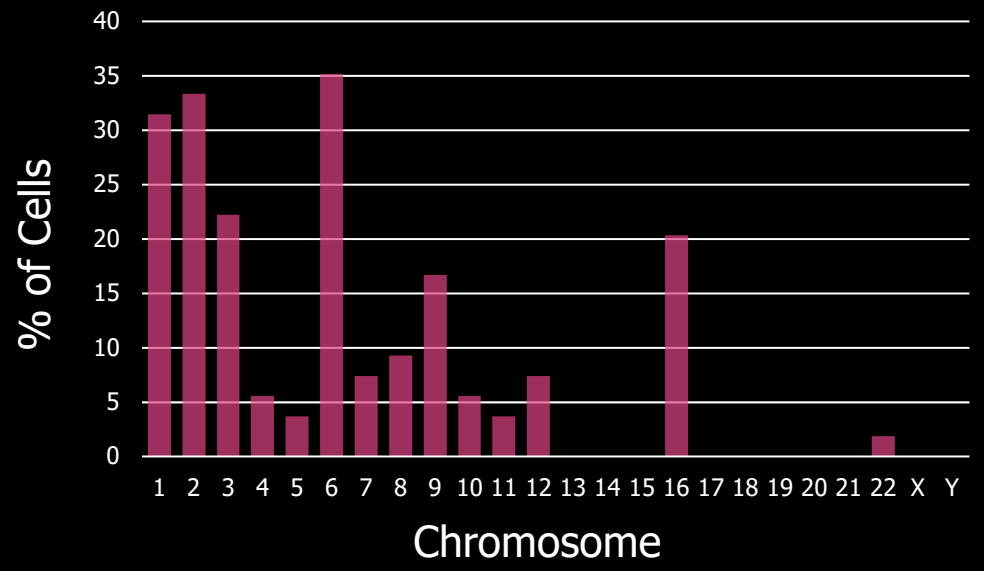
X,Y: 9.9 , 20.2

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

Case Study: *Cell Line Stability*

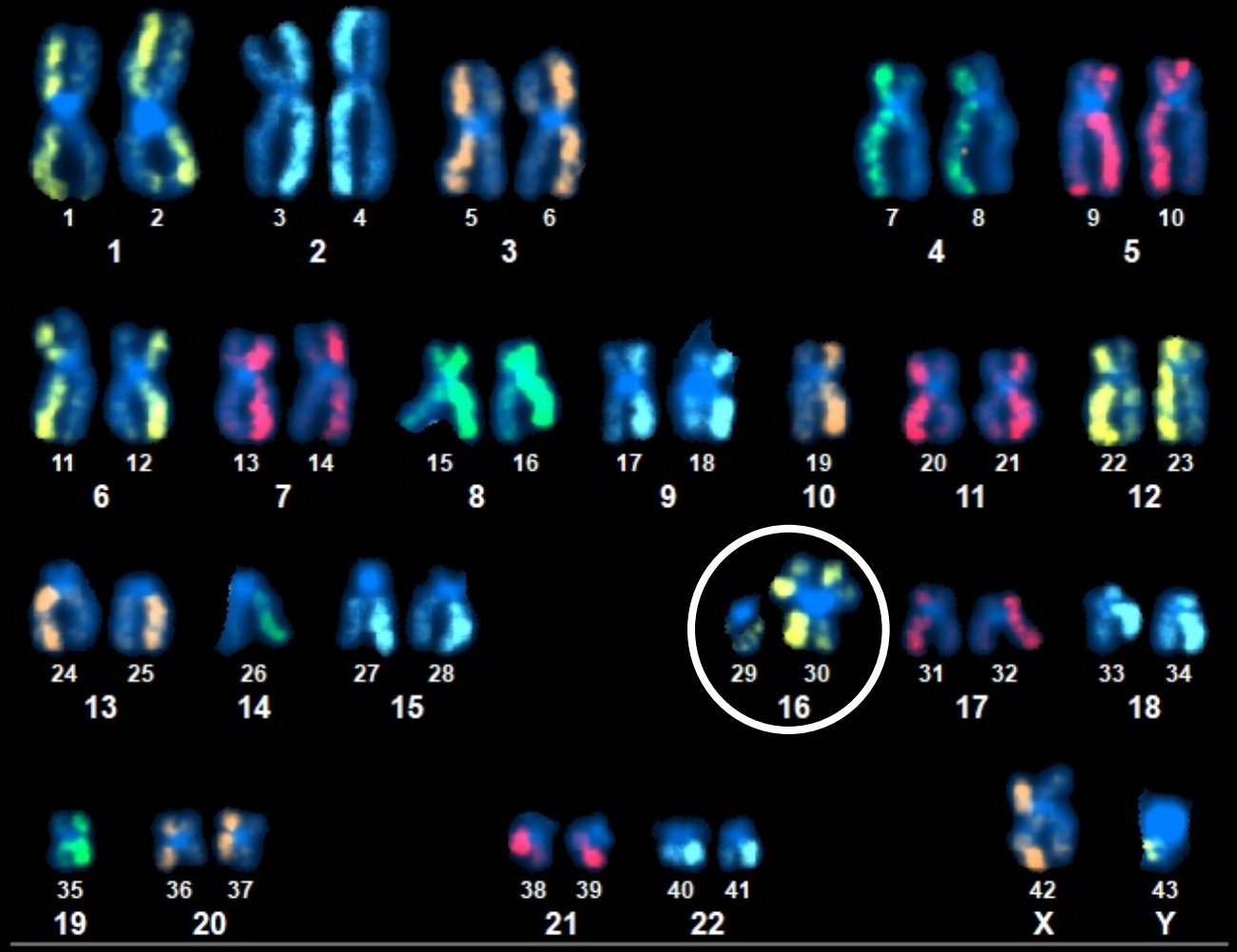


Large intra-chromosomal duplications



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

Case Study: *Cell Line Stability*

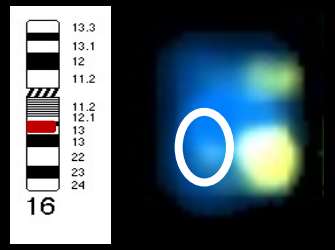


S73_c54, Cell 14 Passage 18

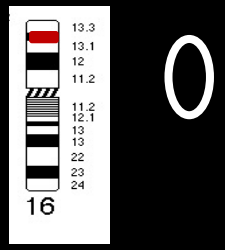
GM24385 (coriell.org), NIST GM24385 Reference Material Certificate
National Institute of Standards & Technology (nist.gov)

Case Study: *Cell Line Stability*

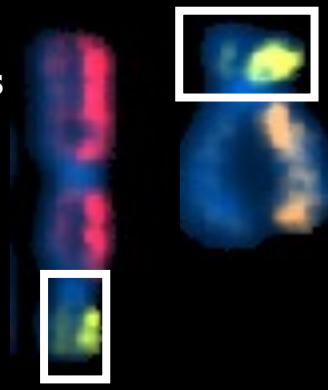
Chr 16q Inversion
- small, mid-arm
- Observed in 37% of cells



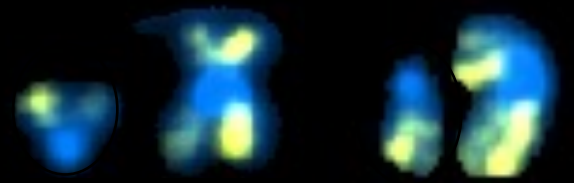
Chr 16p Inversion
- small, mid-arm
- Observed in 19% of cells



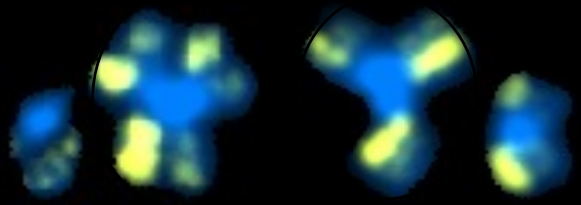
Chromosome 16 translocations (~4%)
- Non-reciprocal, balanced and unbalanced
- Partners Chr7 and Chr10



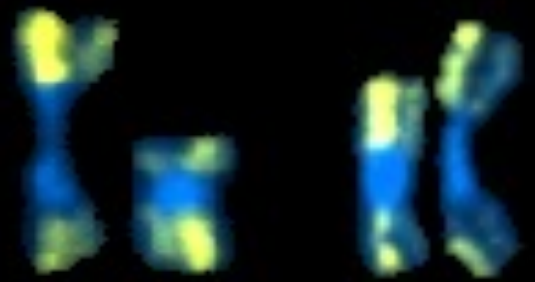
Whole arm deletion
- Observed in 11% of cells



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



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

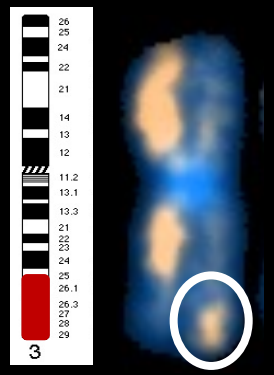


Chromosome 16 Complex Structural Variation in p18 indicates transformation and instability of cell line

Recurrent Inversions: Location, Size, and Prevalence

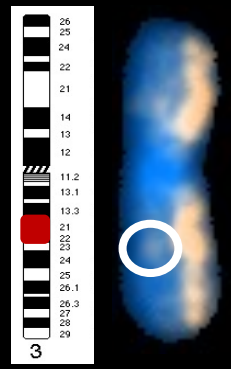
Chr 3q Inversion (7%)
- **large, telomeric**

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



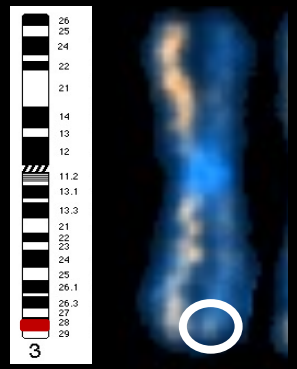
Chr 3q Inversion 2 (26%)
- Small, mid-arm

Confirmation of p3 dGH Results (2014)



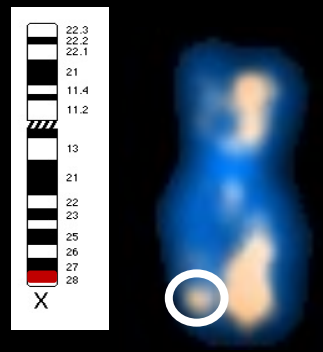
Chr 3q Inversion 3 (13%)
- Small, telomeric

Confirmation of p3 dGH Results (2014)



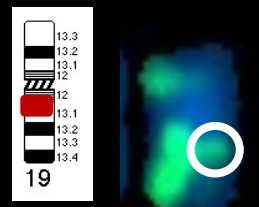
Chr Xq Inversion (67%)
- **Small, telomeric**

Newly Discovered



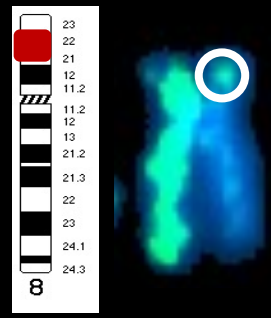
Chr 19q Inversion (65%)
- **Mid-size, centromeric**

Newly Discovered



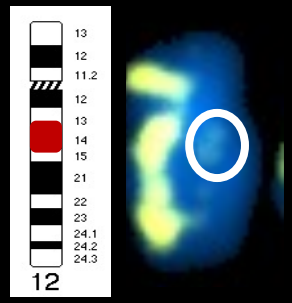
Chr 8p Inversion (52%)
- **Mid-size, mid-arm**

Newly Discovered



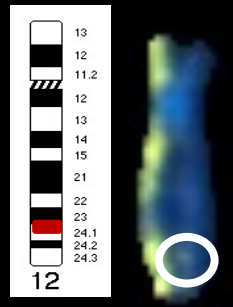
Chr 12p Inversion (26%)
- Mid-sized, centromeric

Newly Discovered



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

Newly Discovered

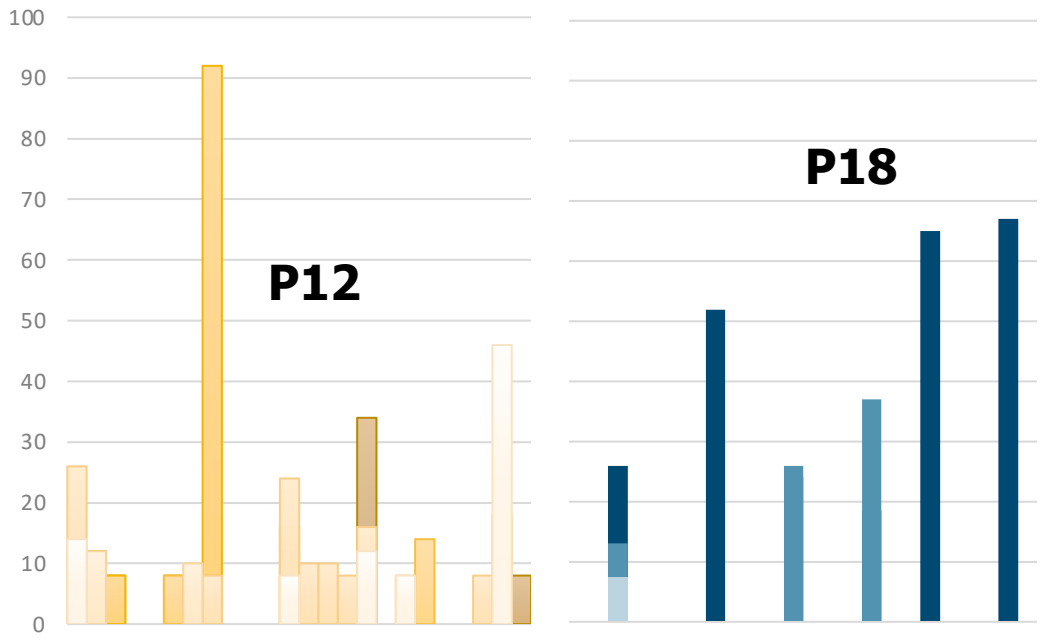


Case Study: *Cell Line Stability*

Differentiation from P12 to P18

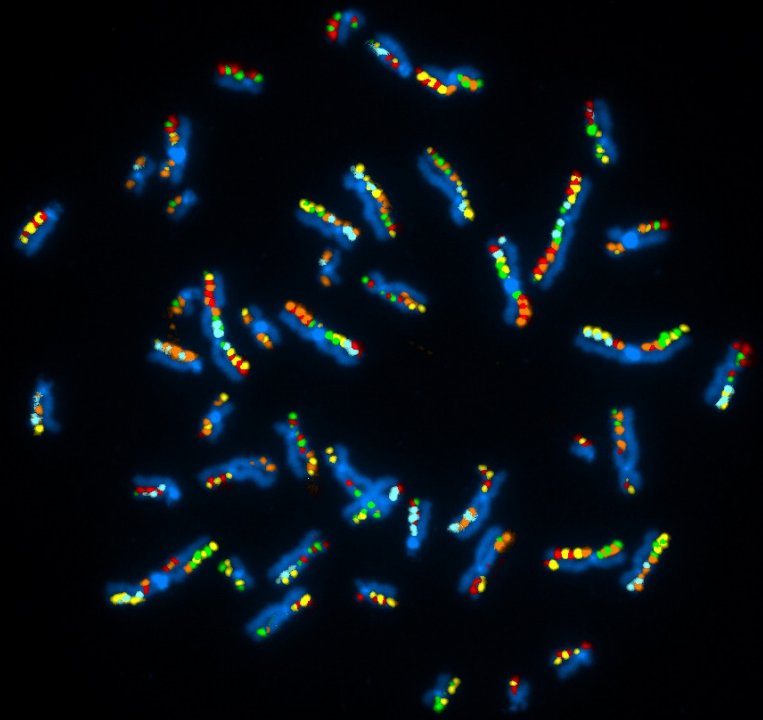
Event	P12	P18
Monosomy	1	4.8
Trisomy	1	1
Recurrent Inversions	1	0.033
Translocations	1	0.8
Large insertions	1	1
Complex events	1	3.9

Recurrent Inversions

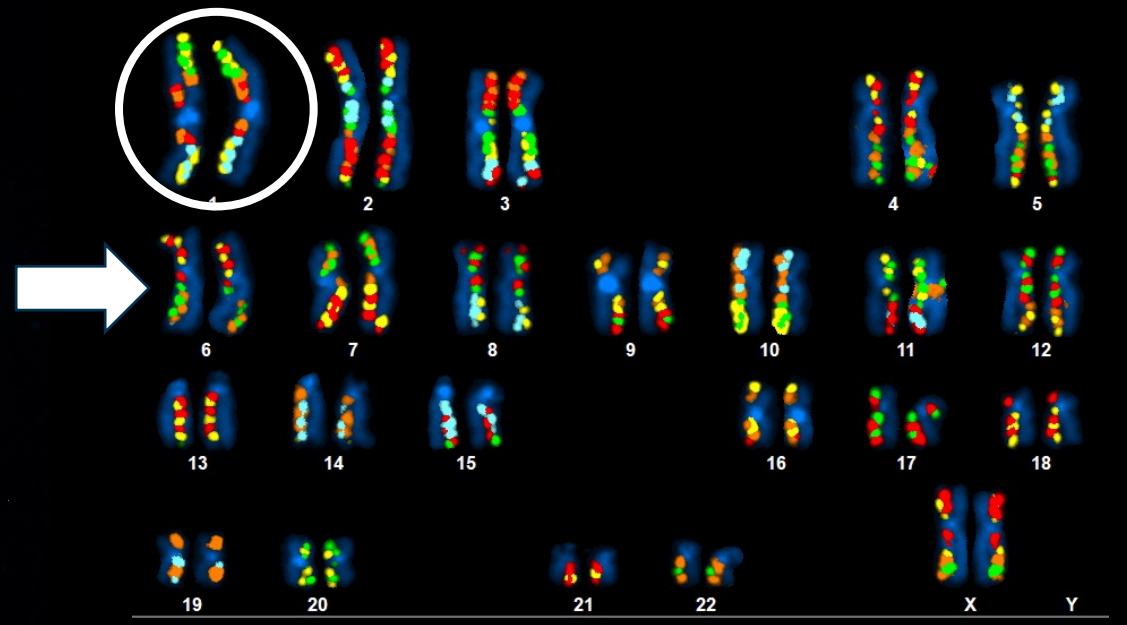


From passage 12 to 18 the culture is unstable but there is signs that clonal outgrowth of a more stable variant is beginning.

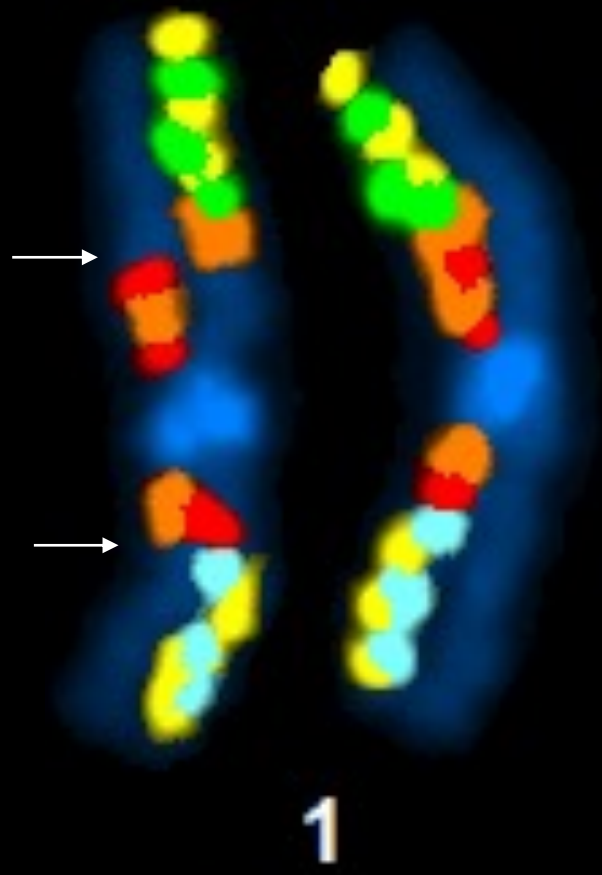
Whole Genome E-Band



Metaphase Spread



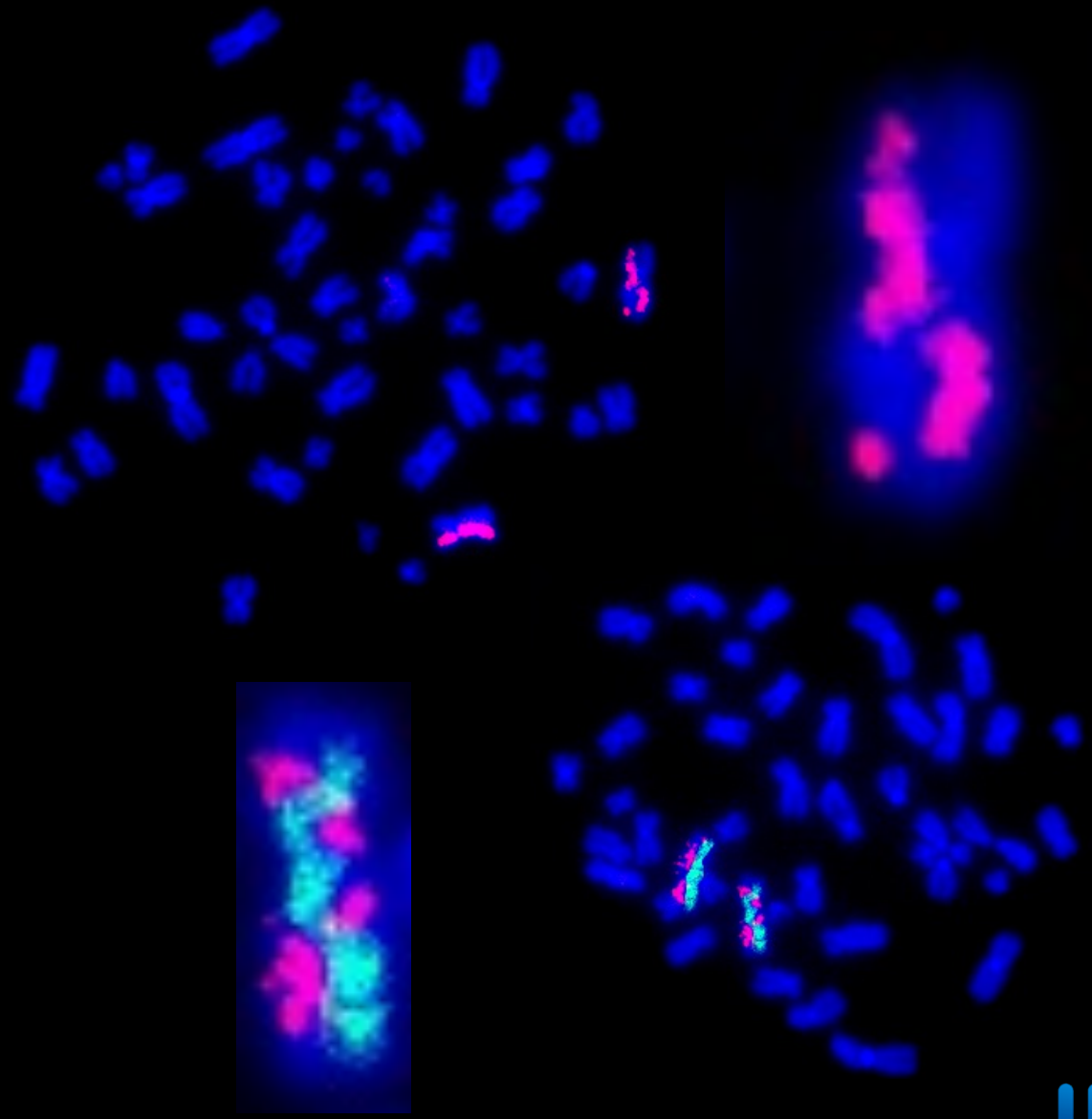
Karyogram



Case Study: *Discovery & Identification of SV in Udx Patients*

Involved Genes:

- DCTN1 (2p13.1)
- Previously identified by WGS in Udx patient and offspring to have point mutations.
- DCTN1 gene mutations are associated with distal motor neuronopathy type VIIB.

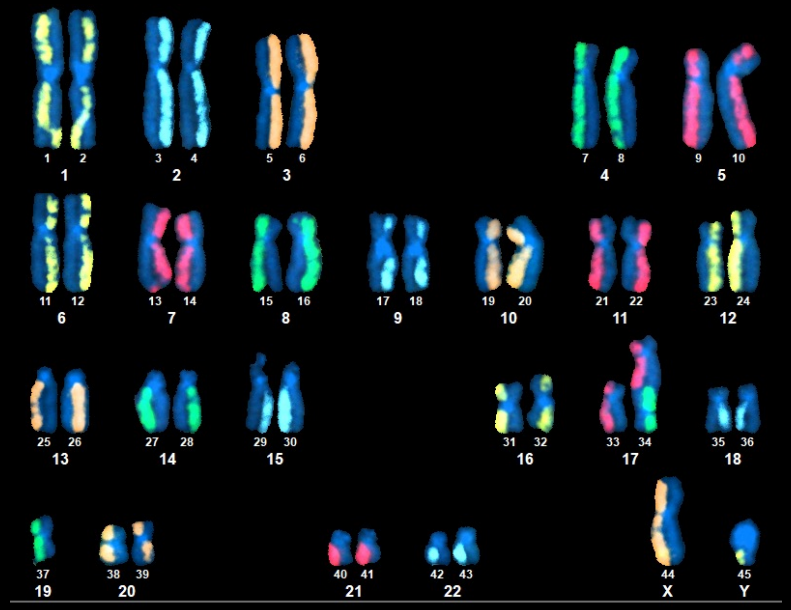


KromaTiD
Direct, Definitive Genomics

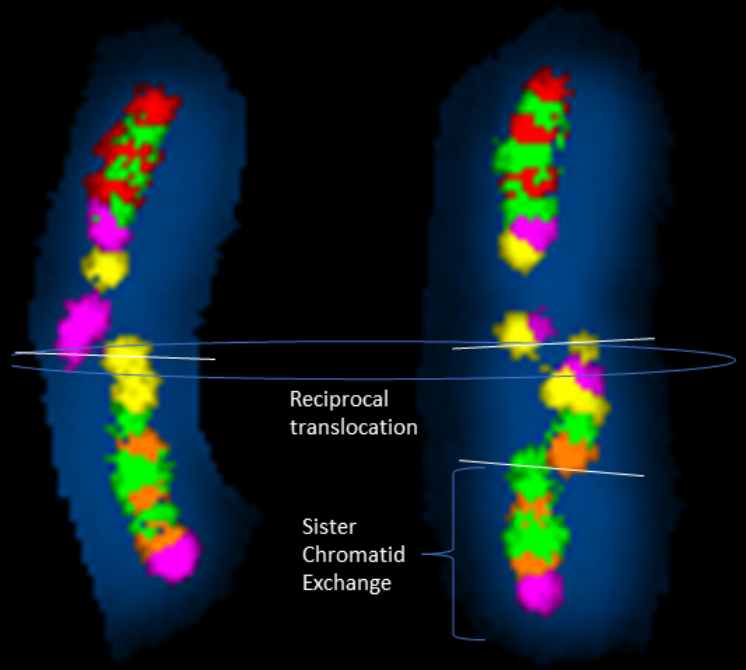
UnD CONSORTIUM
New Technologies + Collaboration = Hope

From Sample to Target

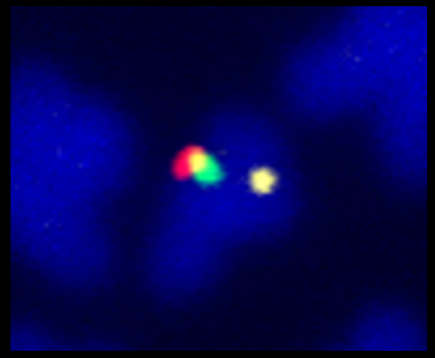
dGH SCREEN™ (Unbiased)



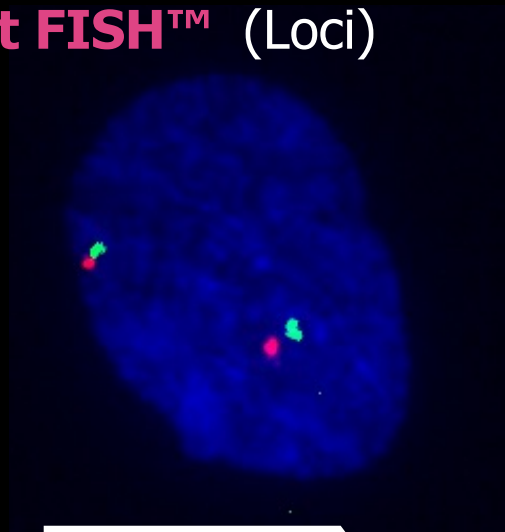
dGH DSCVR™ (Unbiased)



dGH In-Site™ (Loci)

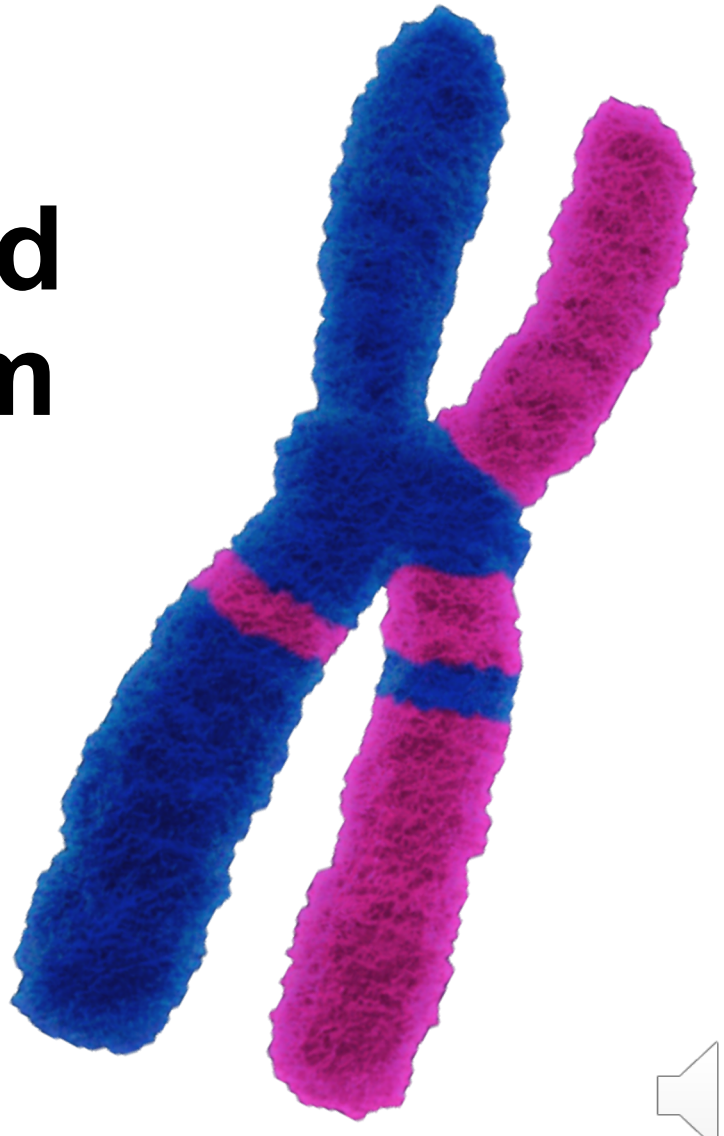


Pinpoint FISH™ (Loci)



Discover → **Identify** → **Target**

PinPoint FISH Probes and Chromosome Paints from KromaTiD



KromaTiD