Whole-Genome, Single-Cell Measurement of Structural Variation

Unbiased, whole-genome tools to assess risks and accelerate gene therapies to market

Presented by:
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Erin Cross, Director of Whole Genome Research

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Direct, Definitive Genomics

Who We Are

Team of 20 scientists and engineers passionate about directly imaging DNA structure at the lowest possible limit of detection on a single cell basis. Our Focus:

Innovation

- Quantifying location, orientation, size of variants by direct imaging
- Pushing lower the limits of detection with increasing signal strength
- AI automation of imaging and scoring to provide at attractive cost and turn-around time

Execution

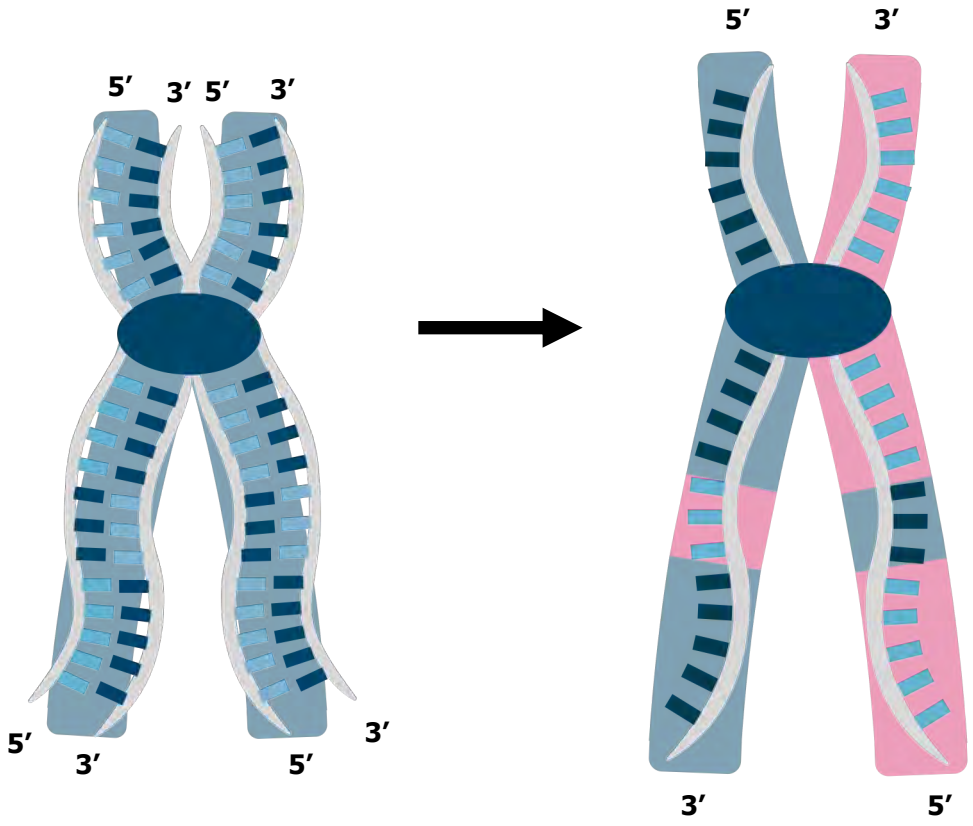
- Creating custom assays & probe designs to answer R&D questions
- Supporting GLP Tox Studies/IND filings via structural analysis
- Assuring cell line genomic stability and clonal selection
- Executing assays for clients in rapid fashion

Cytogenetics Support

- Provide routine cytogenetics & related testing

Comprehensive, High-Definition Genomic Structural Measurements

Direct and Robust Visualization of the Genome



Analyte

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

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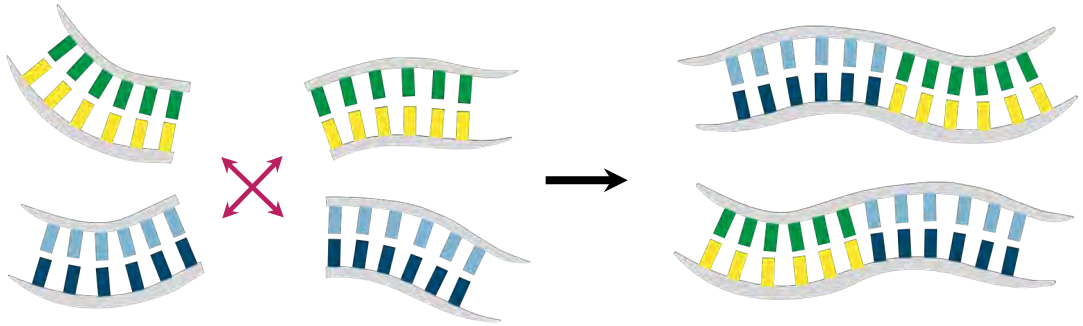
Williams, E., & Bailey, S. (2009). Chromosome Orientation Fluorescence In Situ Hybridization (CO-FISH)

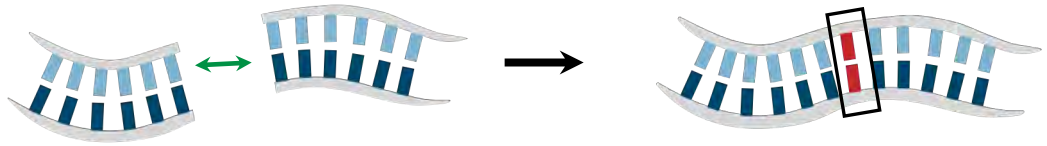
Sequence Variation Requires Structural Context

Mis-repair: Structural Variation



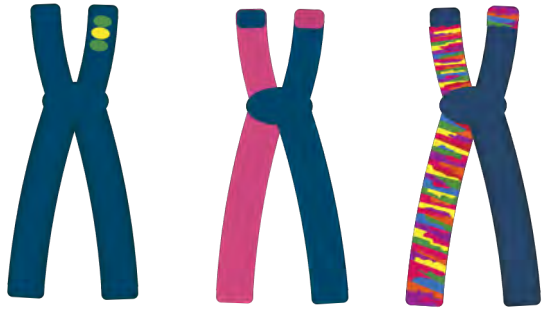
Structural Variant

Mis-Edit: Sequence Variation

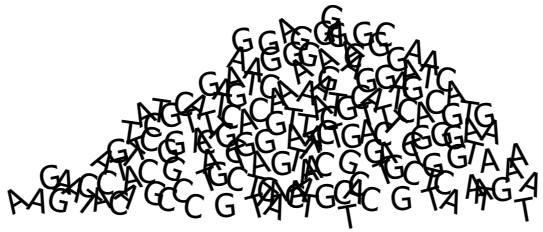


Single Nucleotide Variant

dGH is the Structural Ground Truth



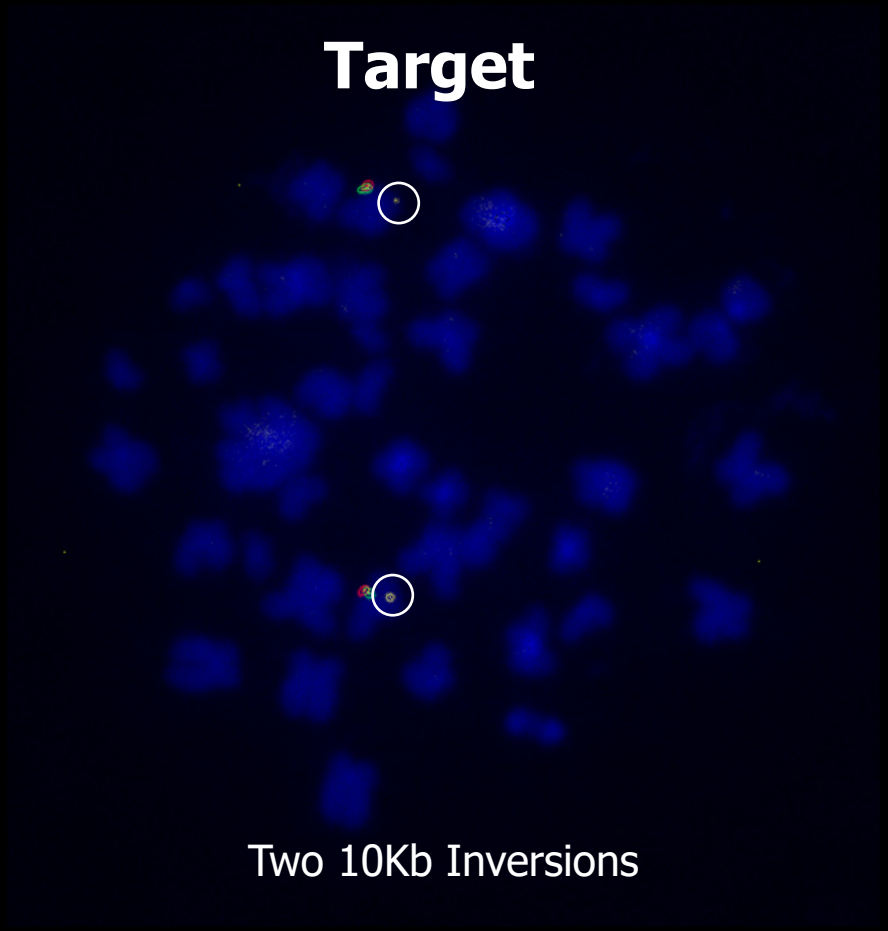
Bioinformatical Structural Hypothesis



Measuring all aberrations with two orthogonal and complementary methods

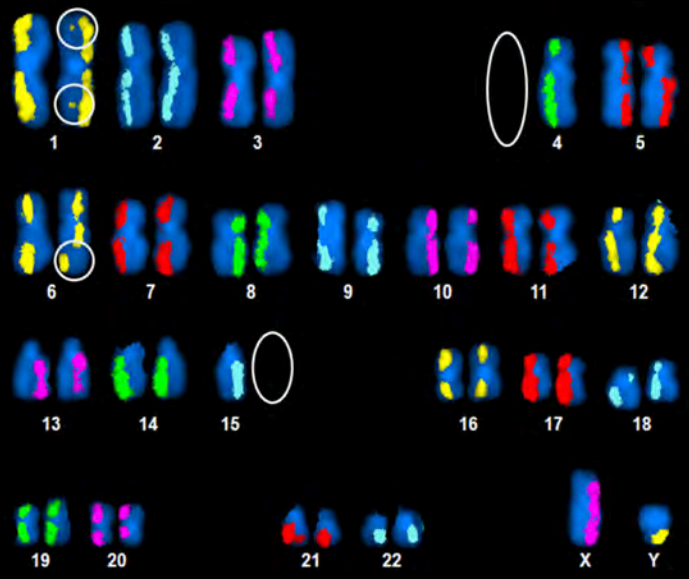
Visualizing Genomic Structure with dGH™

Target



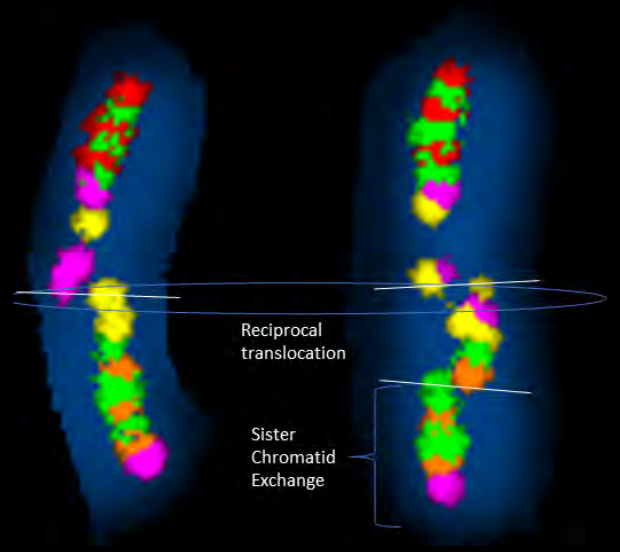
Two 10Kb Inversions

Discovery



Three Inversions and Two Missing Chromosomes

Identification



<1MB Breakpoint Localization

dGH In-Site™ (Localized)

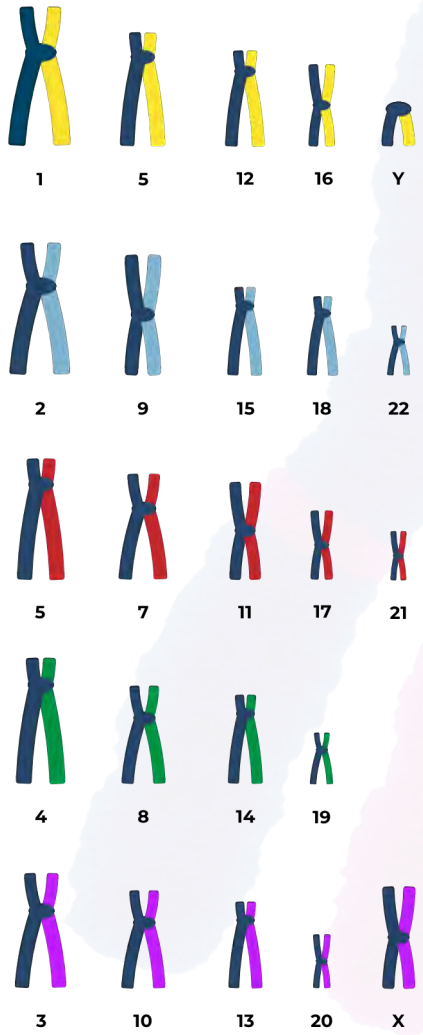
dGH SCREEN™ (Unbiased)

dGH DSCVR™ (Unbiased)

dGH SCREEN: 5 Color, Whole-Genome Karyotyping

dGH SCREEN:

- Whole genome
- Single-cell
- All classes of structural rearrangements
- Chromosomal identification



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What can SCREEN tell you?

- Overall rates of structural events >10-100 kb in size in a sample (combination of random, clonal and/or germline events)
- Distribution of events per chromosome
- Distribution of events or combinations of events across the population

Types of events detected:

- Translocations
- Inversions
- Aneuploidy
- Insertions
- Chromatid-type aberrations (truncation, fusion, chromatid breaks)
- Complex exchanges
- Chromothrypsis and chromosome fragmentation

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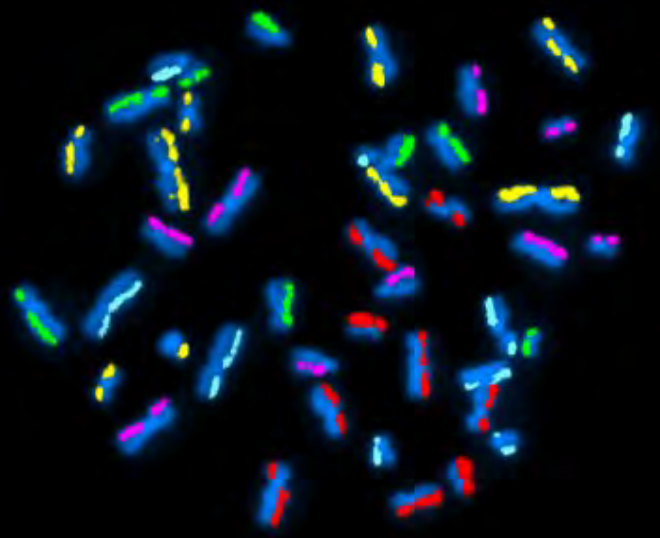
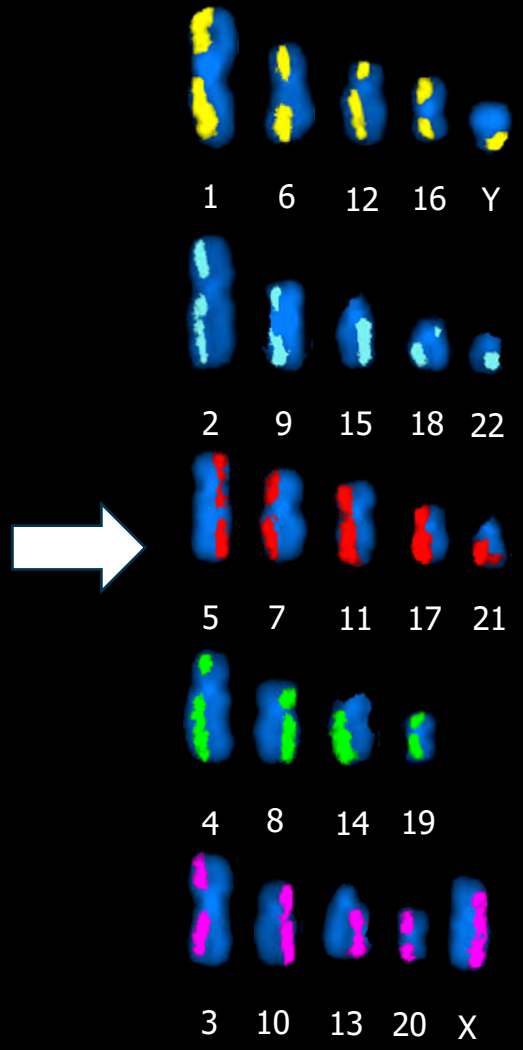
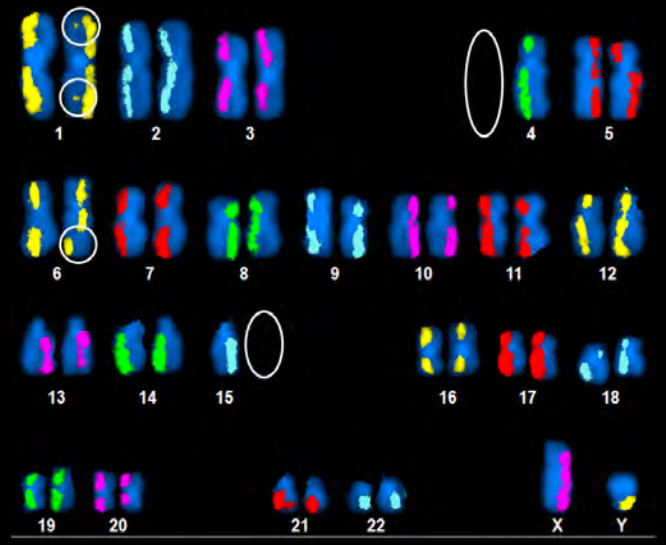
dGH SCREEN™



Spread



Sorting



Karyogram

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3 Use Cases

1. **Three Mile Island Project**
   - Analysis of blood lymphocytes from human subjects

2. **Collaboration with University of Texas Medical Branch**
   - Analysis of clones from an established cell line exposed to several different sources of radiation

3. **NIST Genome Editing Consortium**
   - Analysis of the "Genome in the Bottle" cell line

dGH SCREEN for Biodosimetry

Three Mile Island Project: Use Case for genomic structural event rate assessment as a biodosimeter for radiation exposure.

- Model system for using dGH SCREEN to measure the effects of a genotoxic exposure on overall rates of genomic structural variants present in a sample
- Analogous to measuring off-target effects in a gene editing system.

Traditional Measures of Biodosimetry

Metaphase spread from an irradiated human peripheral blood sample hybridized using dGH

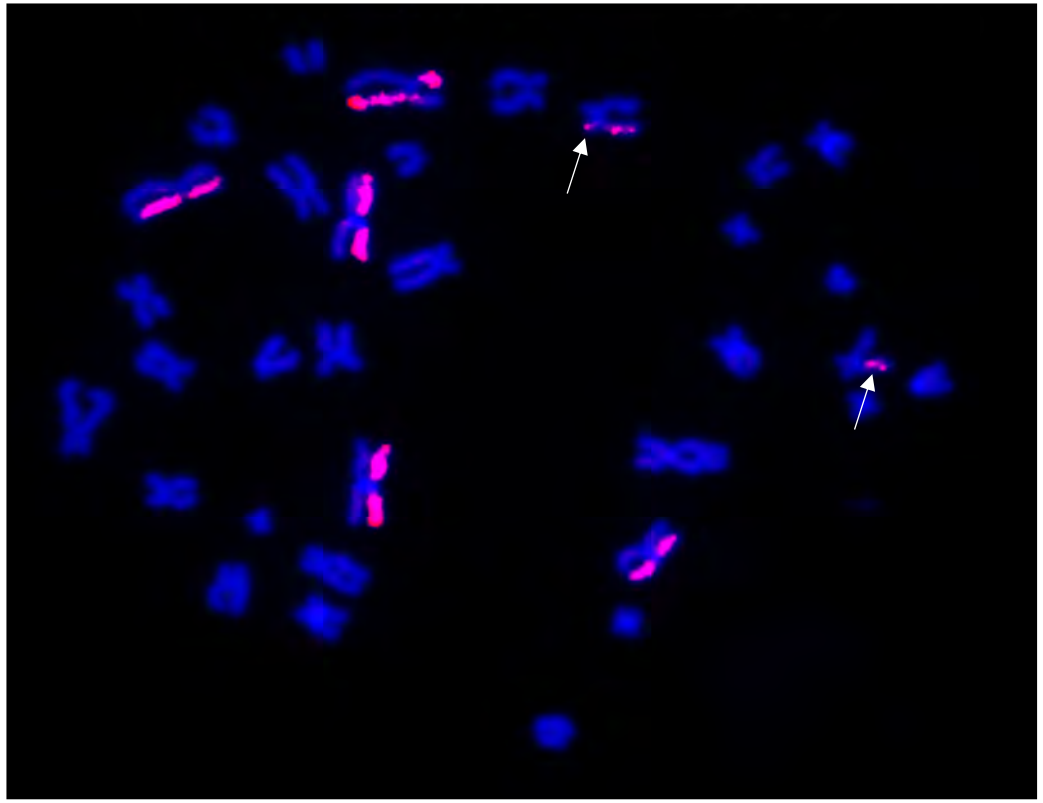


Figure 1: Whole chromosome 1, 2 and 3 paints hybridized to a metaphase spread from a human peripheral blood sample irradiated with 2Gy Cs-137 gamma rays. Structural rearrangements identified by Directional Genomic Hybridization denoted by arrows.

Inversions occur at a higher background frequency and increase at a greater rate per unit dose compared to translocations

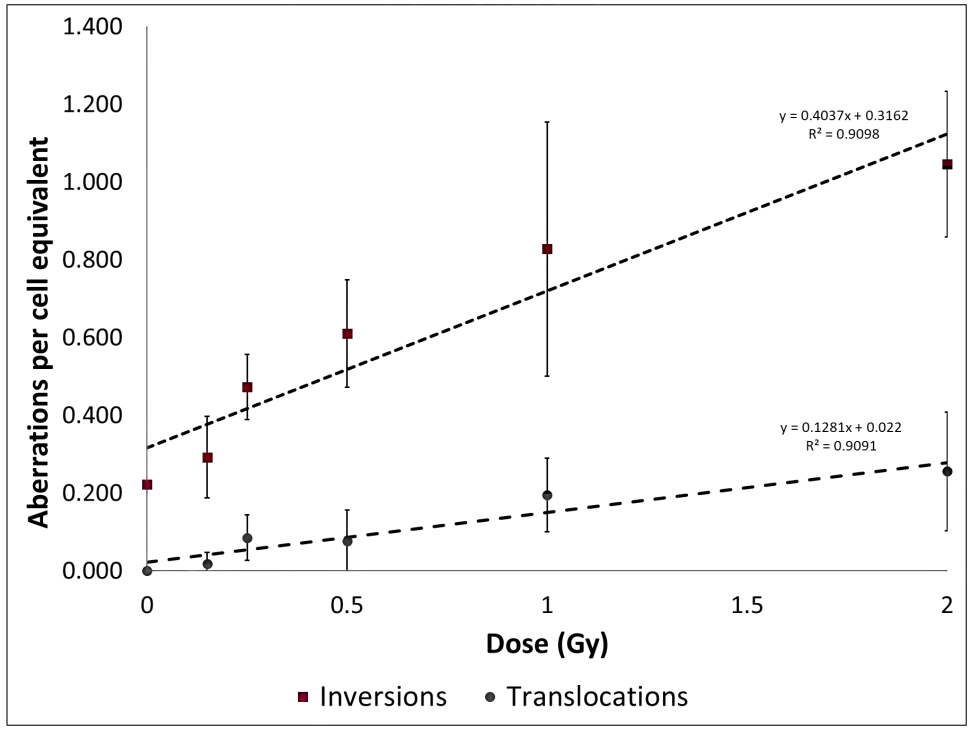
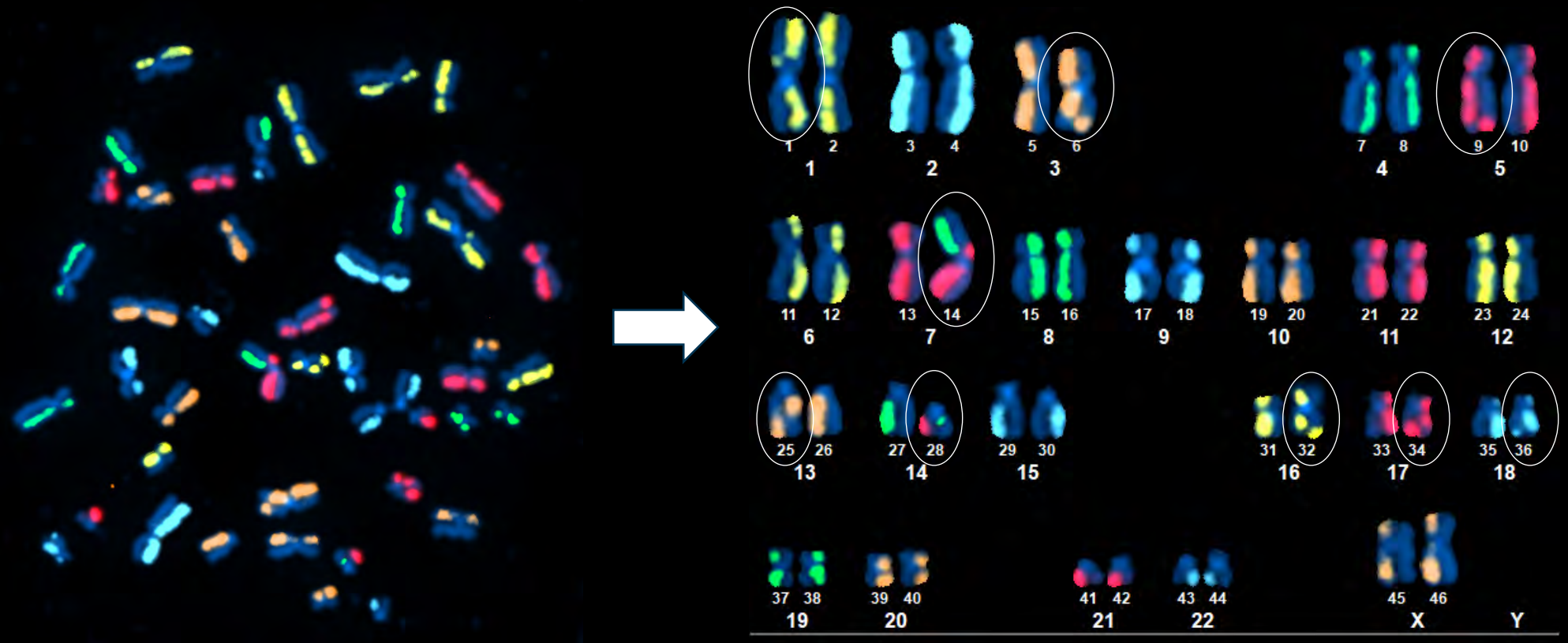


Figure 2: Blood samples from young adult controls were irradiated with Cs-137 gamma rays to establish a dose response (calibration) curve. Males in their mid-20's were selected to account for age at exposure. Inversions (red) had a higher natural background rate compared to translocations (blue); however,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

McKenna MJ, Robinson E, Taylor L, et al. Chromosome Translocations, Inversions and Telomere Length for Retrospective Biodosimetry on Exposed U.S. Atomic Veterans. Radiation Research. 2019 Apr;191(4):311-322. DOI: 10.1667/rr15240.1. PMID: 30714852; PMCID: PMC6492561.

Preliminary SCREEN Data



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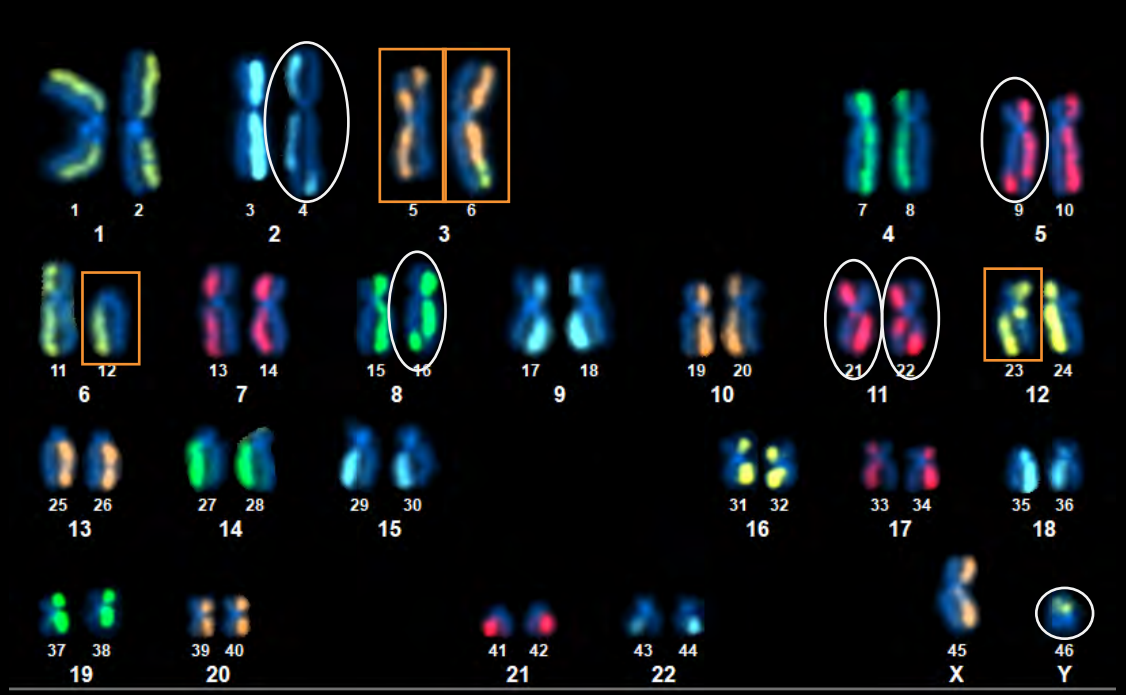
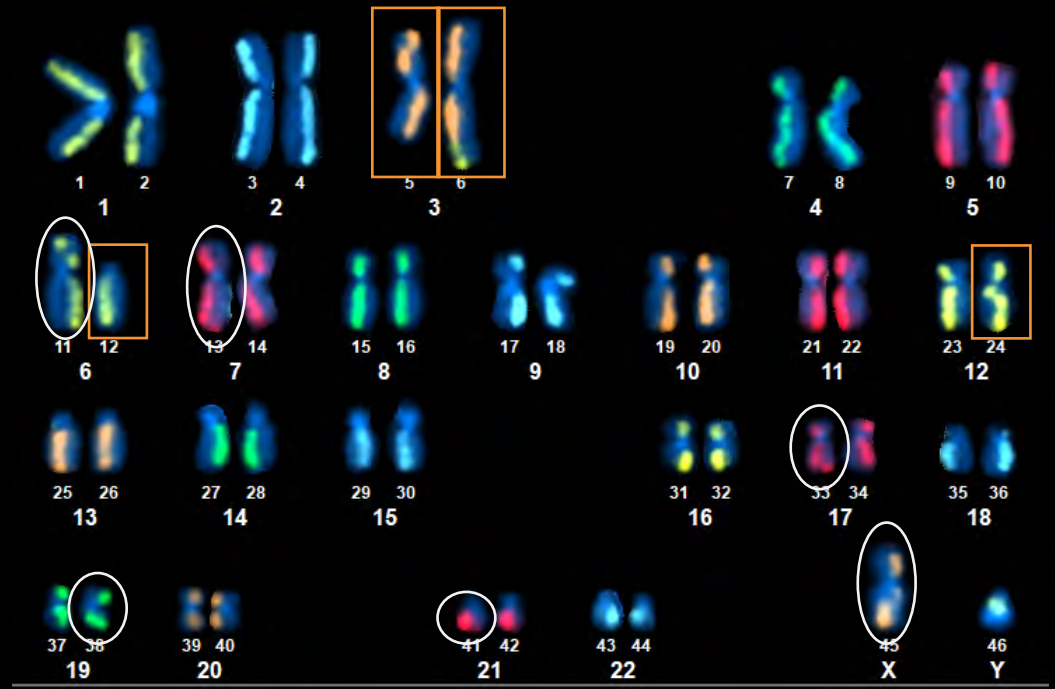
Measuring Recurrent Translocations and Inversions





Recurrent

Random

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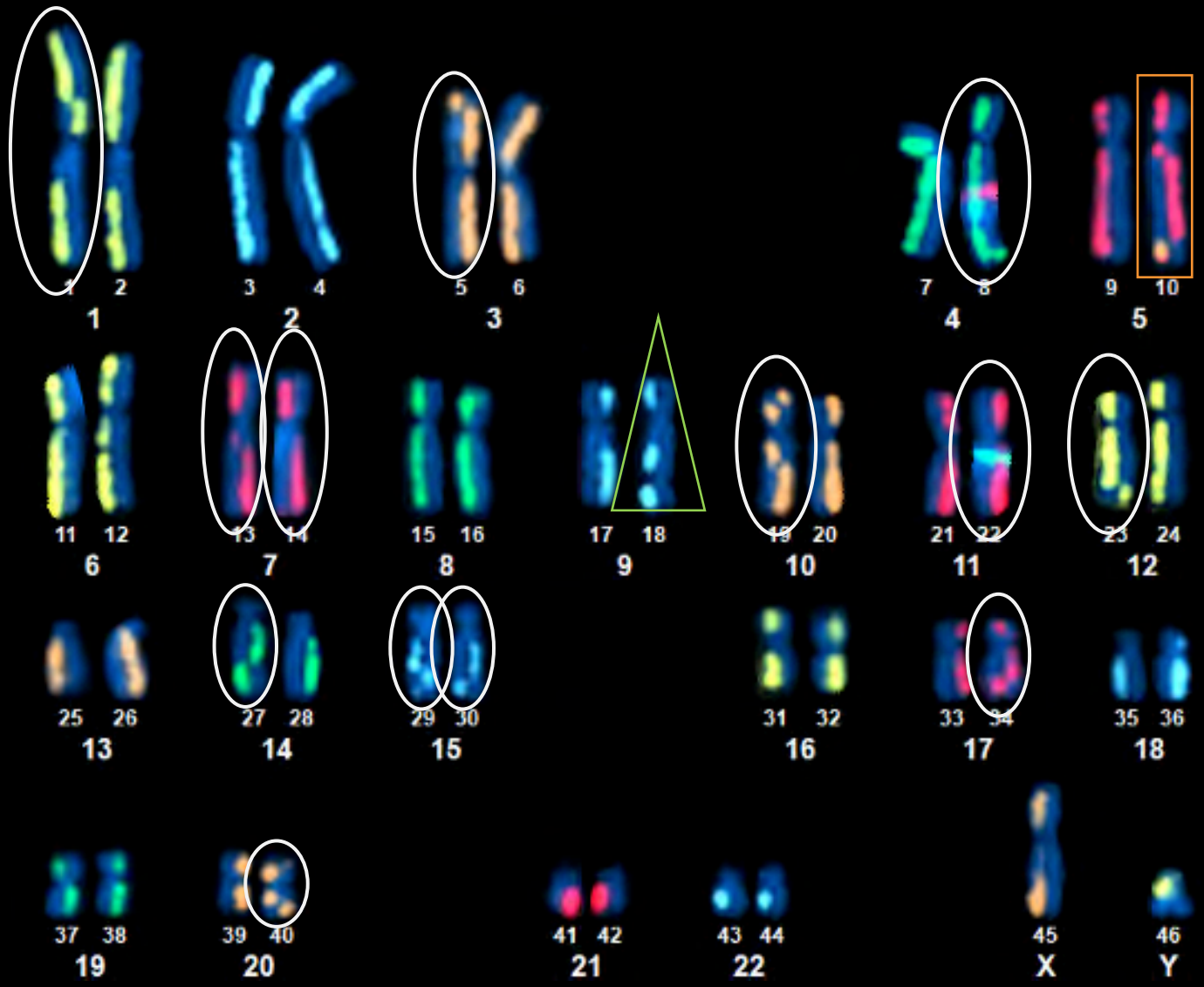
dGH SCREEN Data



Translocation

Inversion or SCE

Chromatid break

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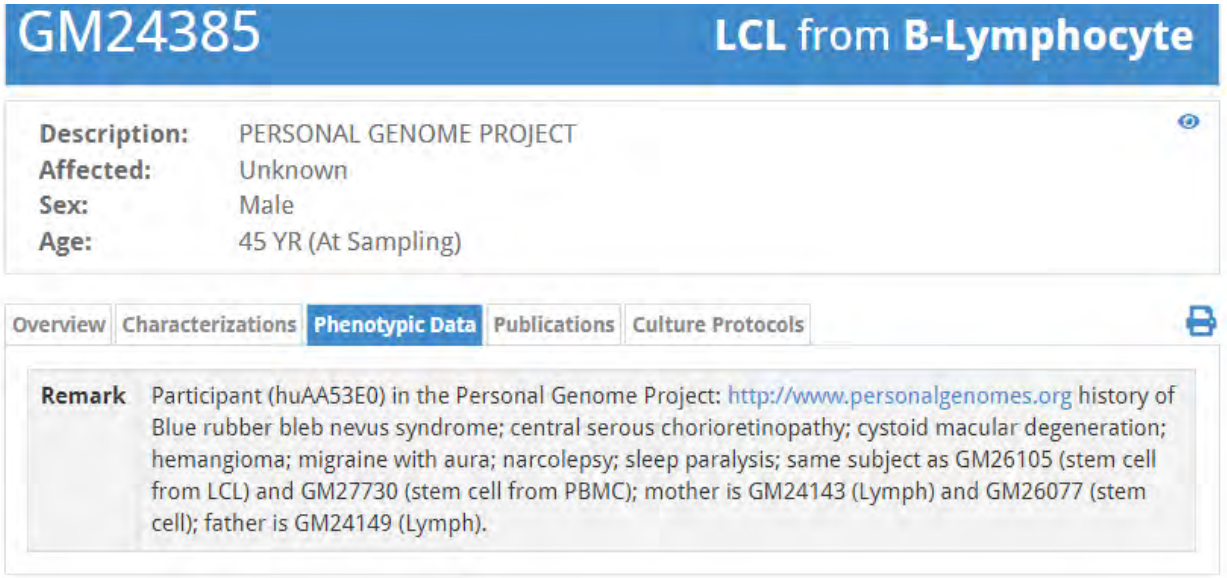
dGH SCREEN for Cell Line QC

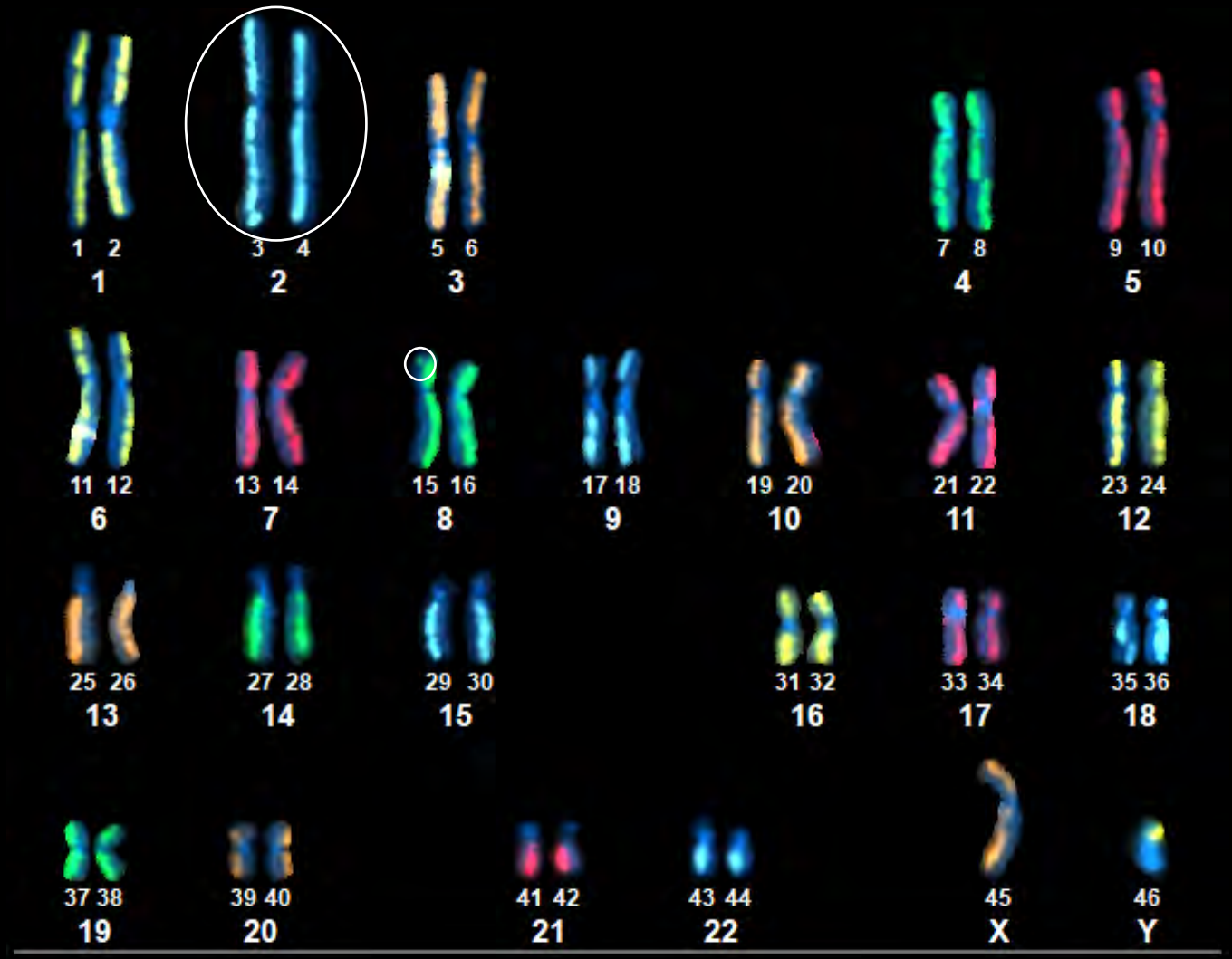
Whole genome dGH analysis and stability screen of the "Genome in a Bottle" progenitor cell line in preparation for engineering of large variant controls by NIST partners



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 (Kromatid)
  - Confirmed inversion on 3q26.3q29
  - Discovered telomeric inversion on 3q
  - Discovered centromeric inversion on 3q

GM24385 p12



S73_c54, Cell 14

Structural Variant Summary*

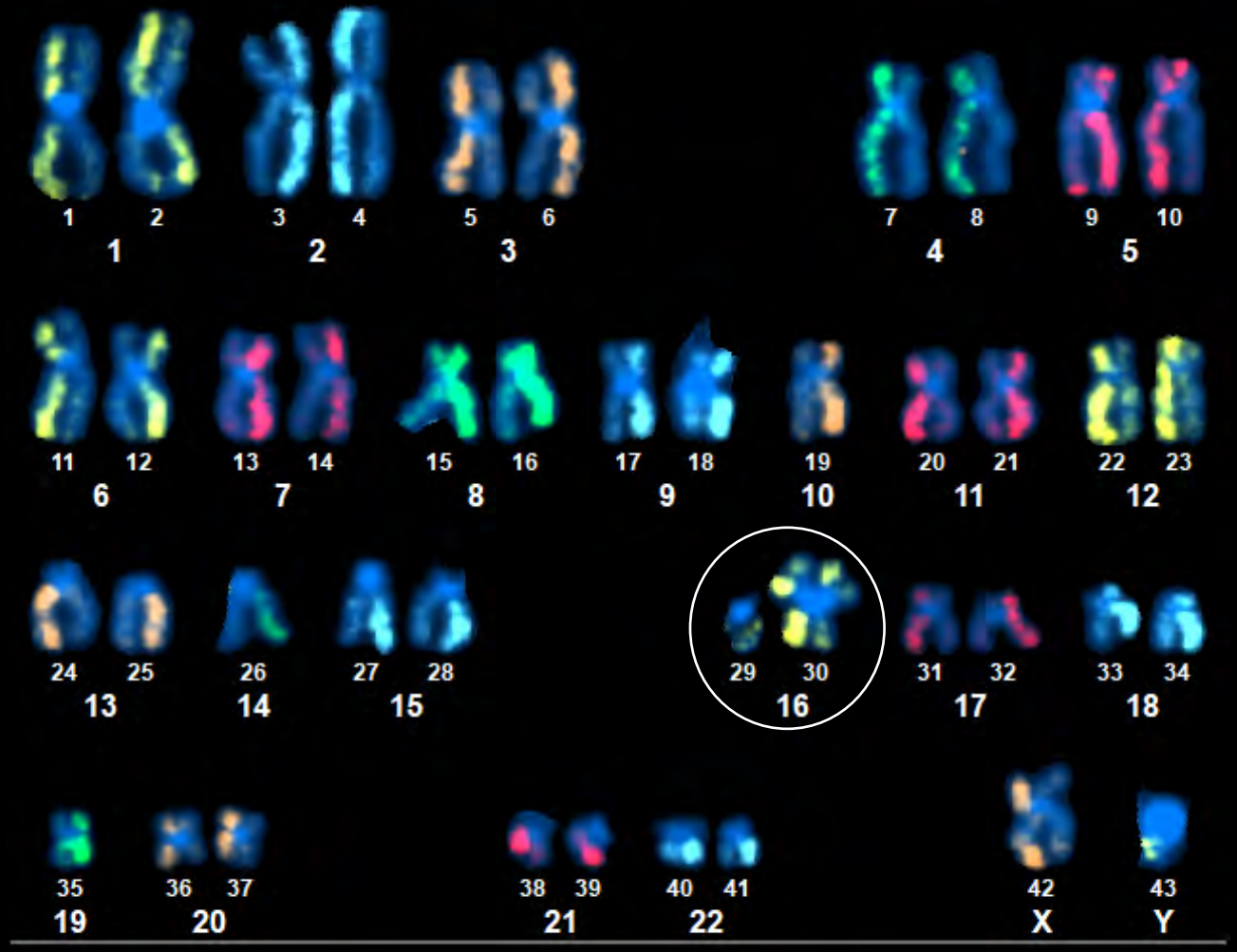
- 4 translocations (heterogenous)-8% of cells
- 34 inversions of > 8% occurrence
- 18% variable monosomy
- 4% variable trisomy
- Low level of complex events, including one cell with chromothripsis of Chr 19, one cell will whole arm deletion of Chr 19, two cells with chromatid-type breaks of Chr 3, and two cells with centromere abnormalities (Chr 9 and Chr 11)

Other observations

- Likely condensation defect (observed in 62% of cells)

*Total 50 cells

GM24385 p18



Structural Variant Summary*

- 4 translocations (random), Chr 16 involved in 2 of the 4 translocations
- 10 inversions (events seen in >8% of cells)
- Likely condensation defect presenting as a size difference between homologs observed in 93% of cells, often involving more than one chromosome.
- 41% variable monosomy
- 5% variable monosomy

Complex Rearrangements:

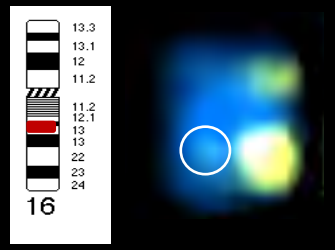
- Elevated rate of complex events in Chromosome 16. Large deletions, radial whole-arm gain, chromothripsis, decondensation and centromere "spindling" observed in 41% of cells
- Centromere abnormalities were also frequently observed in Chr 1 and Chr 9.

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

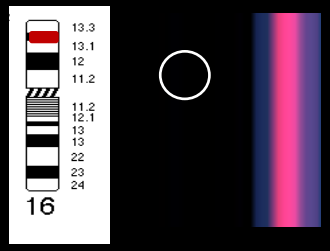
*Total 54 cells

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

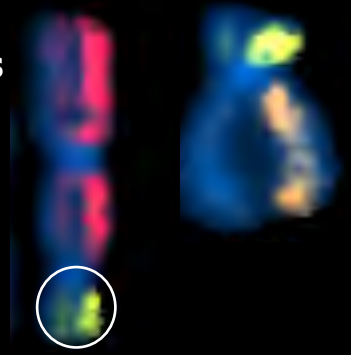
Chr 16q Inversion (37%)
- small, mid-arm
- Observed in 19% of cells



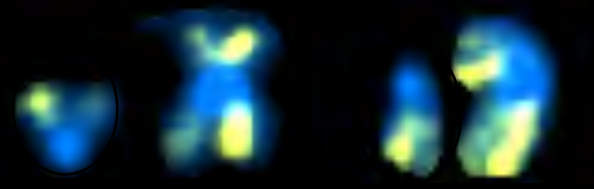
Chr 16p Inversion
- small, mid-arm
- Observed in 19% of cells



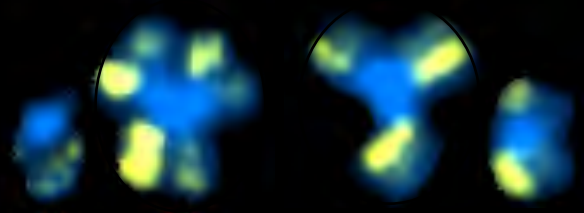
Chromosome 16 translocations (~4%)
- Non-reciprocal, balanced and unbalanced
- Partners Chr7 and Chr10



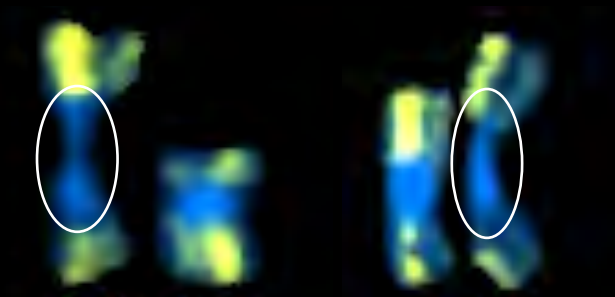
Whole arm deletion
- Observed in 11% of cells



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

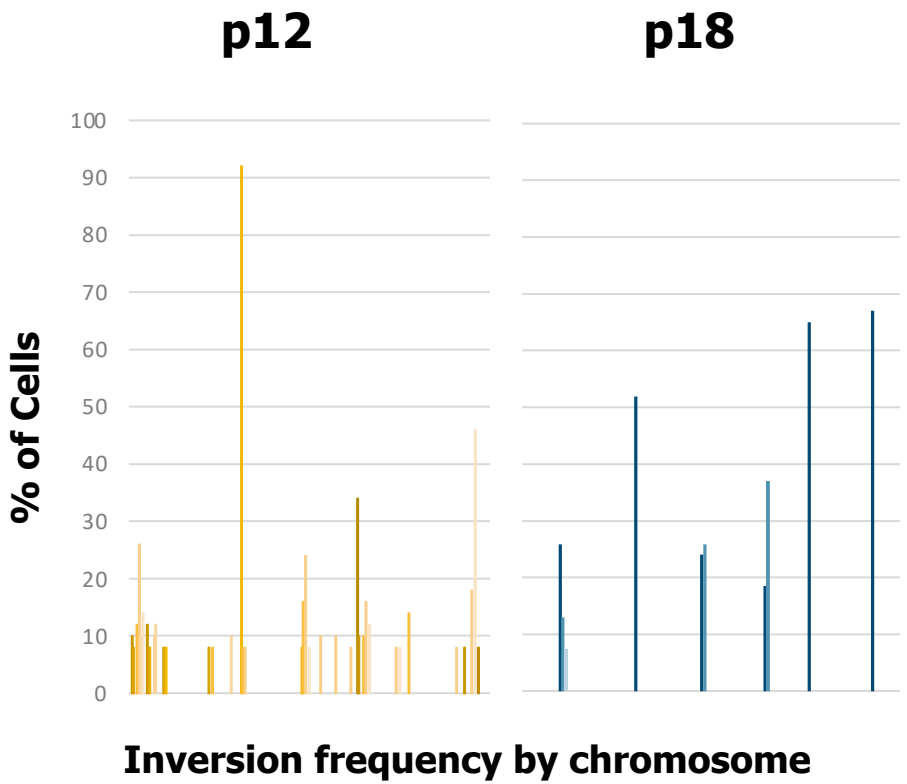


Decondensed/ elongated centromeres and iso-chromosomes
- Observed in 22% of cells



Variable & Complex Structural Variation in Chromosome 16 observed in 41% of cells

Inversions



Inversion frequency by chromosome

Inversions seen in both passages:

Chromosome	Description
8	p-arm, mid size, p22-p21 region
12	q-arm, mid-size, possibly two small inversions in close proximity, q13-q15 region
12	q-arm, small, near telomere, q24 region
16	p-arm, small, mid arm, p13-p12 region
16	q-arm, small, mid-arm, q13-q22 region
19	q-arm, mid-size, near centromere, q12-q13.2 region
X	q-arm, small, near telomere, q27-q28 region

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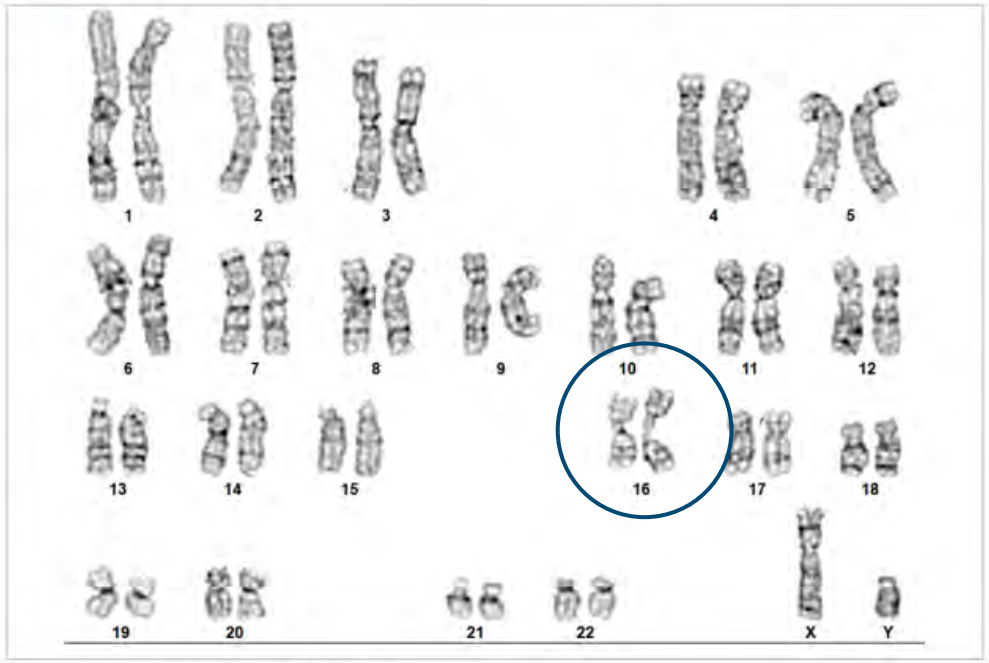
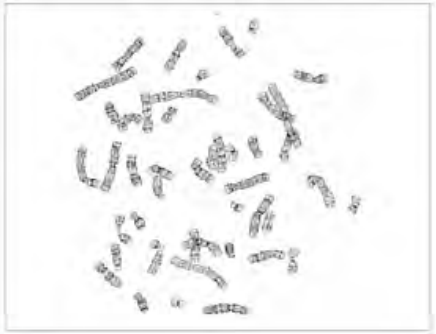
G-banding Confirms Gross Ch16 Result



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

Cell Notes: Estimated Band Resolution:675



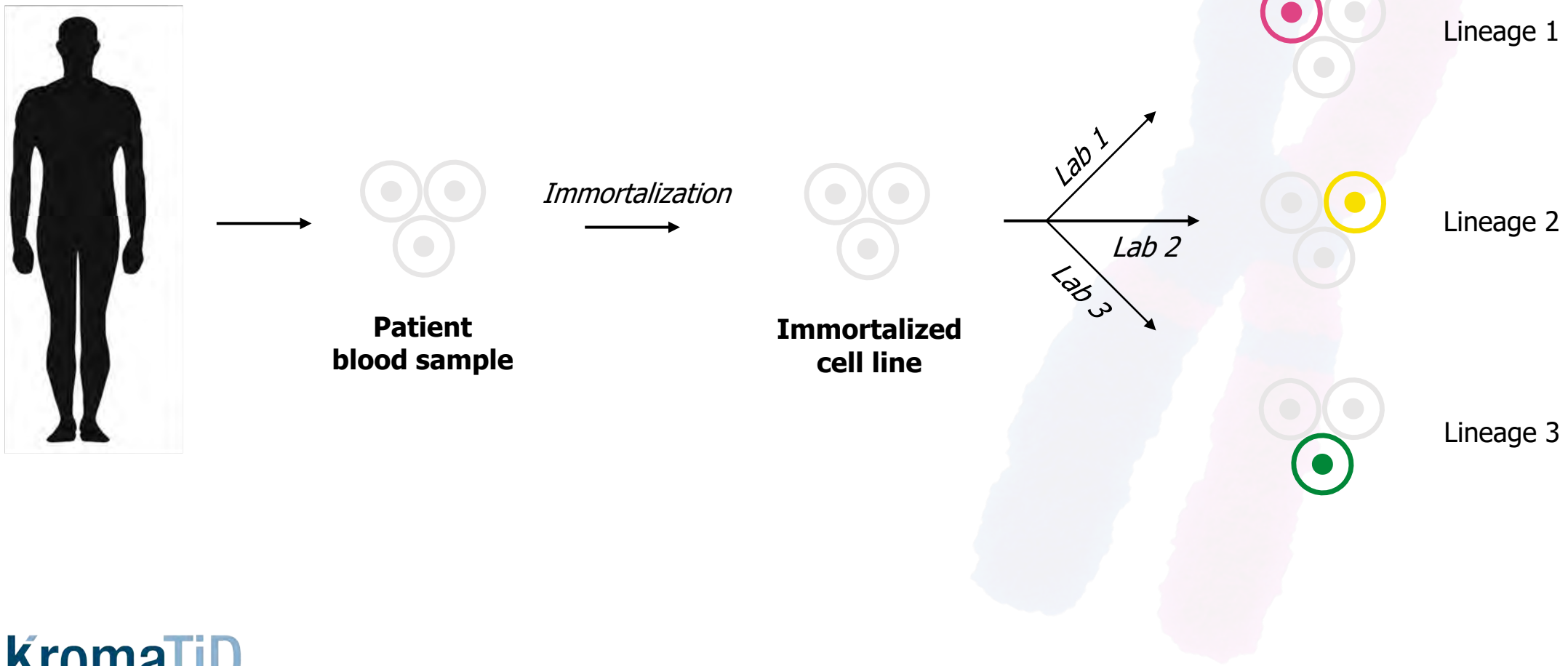
Label - Slide/Cell: S002692 - 8/52

X,Y: 9.9 , 20.2

1. Some rate of potential condensation defects were observed
2. None of the recurrent inversions were detected
3. Instability and gross rearrangement of C16 matched dGH SCREEN observations

46,XY,chbr(1)(q10)[1]/
46,XY,dic(16;17)(p13.2;p13),?add(16)(p)[1]/
46,XY,del(16)(q10),?add(16)(p)[2]/
46,XY,?add(16)(p)?iso(16)(q10)[5]/
46,XY[11]

Parallel studies with inherently unstable cell lines



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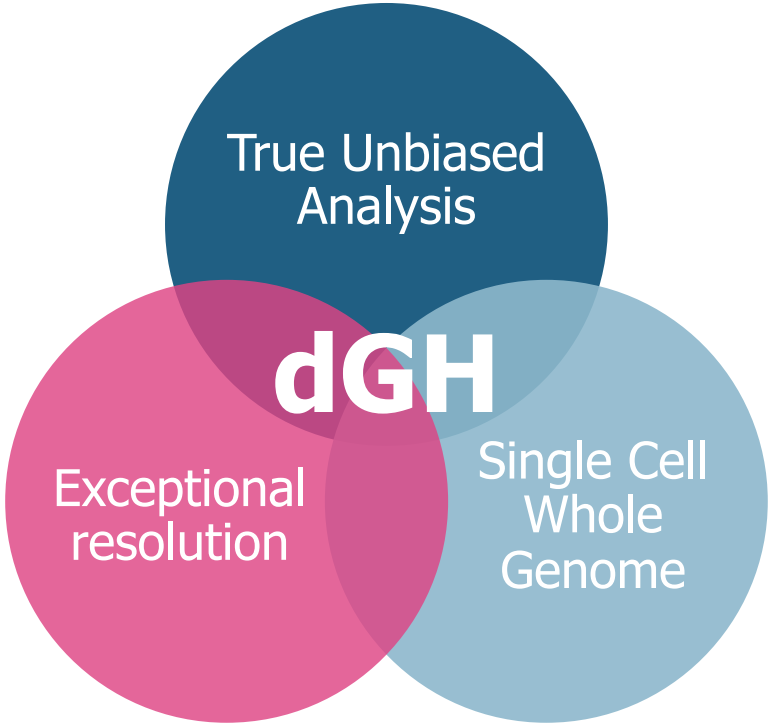
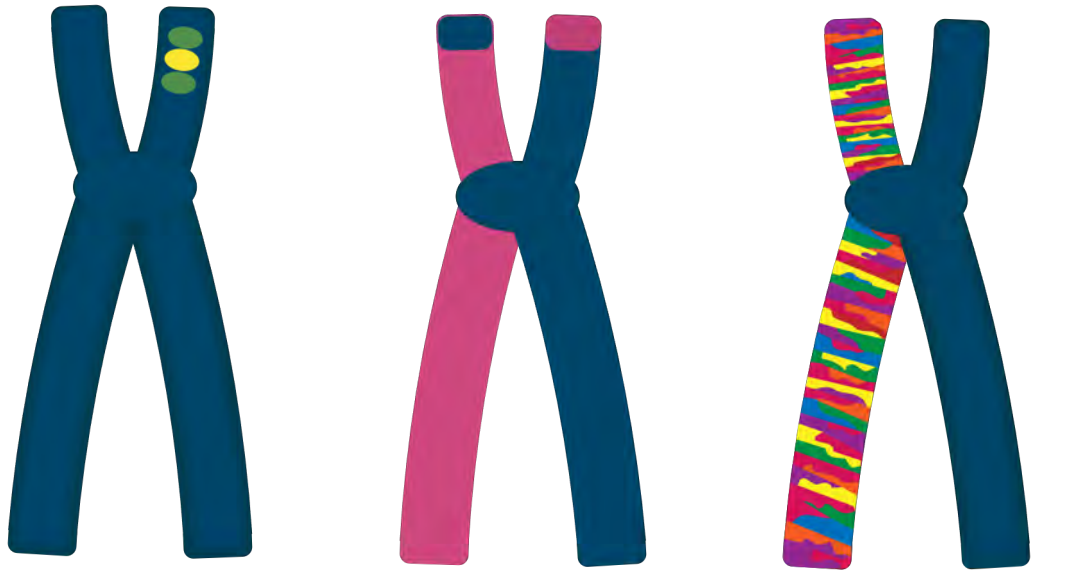
Directional Genomic Hybridization

An unbiased, whole genome, single cell toolset. Map genomes, identify structural variation, and profile structural heterogeneity





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

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We gratefully acknowledge NASA and the NHGRI for providing development funding and support for dGH SCREEN

Thank You..

For more information:

You can visit our website at www.kromatid.com, or check out the latest presentation on our edit site & transgene integration tracking assay here.

Contact us today for all of your cellular engineering or cell line QC needs!

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