dGH SCREEN™
ASSAY SERVICES

KromaTiD
Direct, Definitive Genomics

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Direct, Definitive Genomics

Who We Are

A team of expert scientists providing unparalleled genomics tools, services and support.

Your partner for

- Biomarker discovery
- Genotoxicity studies
- Assessment of gene editing-associated errors
- Plasmid manufacturing

Our Products

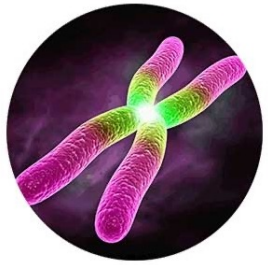
- Patented directional Genomic Hybridization™ (dGH™) technology
- An extensive collection (>700) of chromosome probes and paints
- Improve the sensitivity and specificity of your FISH assays

Our Services

- FISH assays utilizing our patented Pinpoint FISH™ and dGH™ technology
- Plasmid manufacturing (RUO, pre-GMP & cGMP)
- G-banded karyotyping
- Cell culture

What We Provide

Products



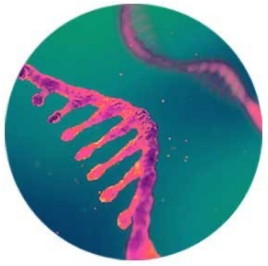
Centromere Probes

Subtelomere Probes

Oncology Probes

Pinpoint FISH™ Kits

Whole Chromosome Paints

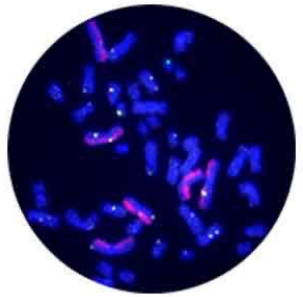


dGH Cell Prep Kit

Services

Cell Culture Services

G-banding Services



dGH in-Site™ Targeted Assays

dGH SCREEN™ Unbiased Assays

Pinpoint FISH™ Assay Services

Plasmid Manufacturing Services

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How are others using KromaTiD?

Chromosome rearrangements and genomic structural measurements

Genome Engineering	Cell Line Selection	Genome Structure
Research Process Development Preclinical/Clinical Development	Genome integrity Cell line QC	Structural biomarker discovery Structural target discovery Biodosimetry

What is dGH SCREEN™ Technology?

dGH SCREEN™ is a 5-color whole-genome karyotyping technology, using single-cell measurement of structural variations.

It is an unbiased assay, utilizing whole-genome tools to assess risks and help accelerate gene therapies to market.

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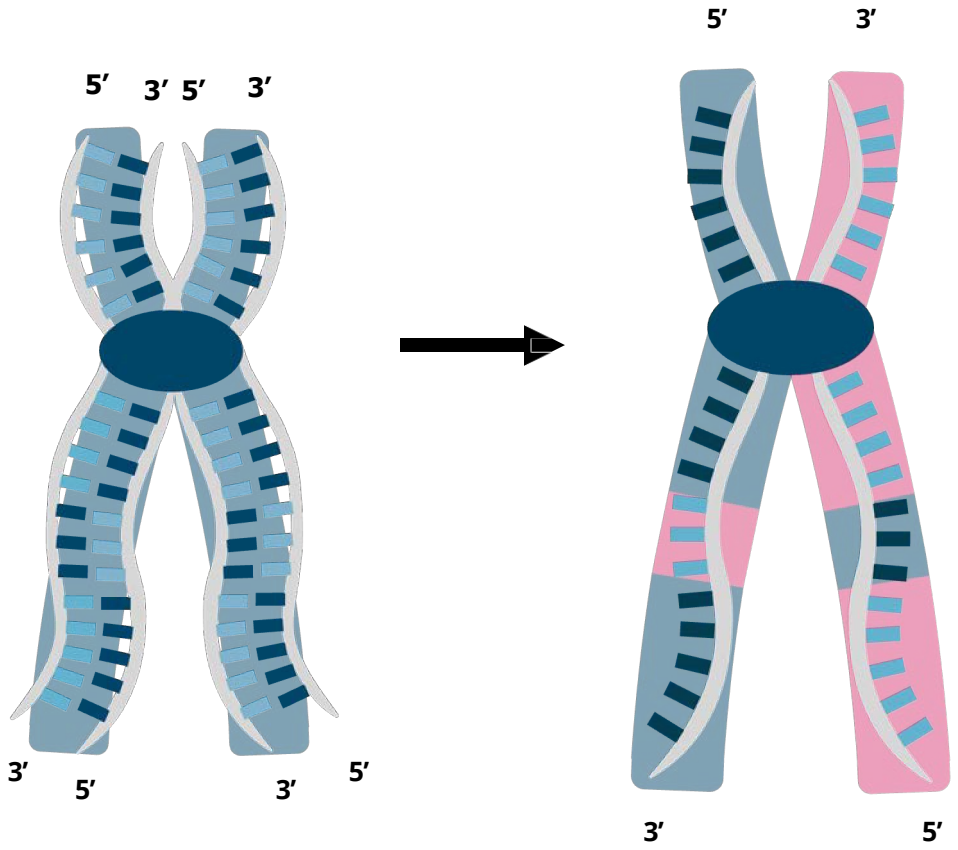
Direct and Robust Visualization of the Genome



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 onlyunique sequences.

Process

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

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

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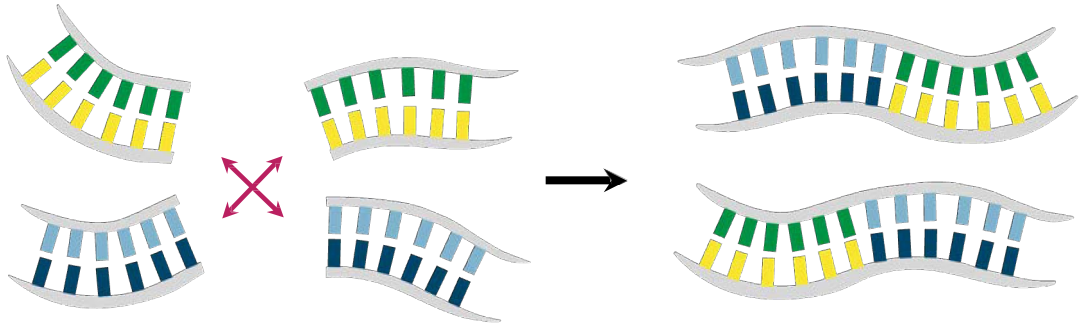
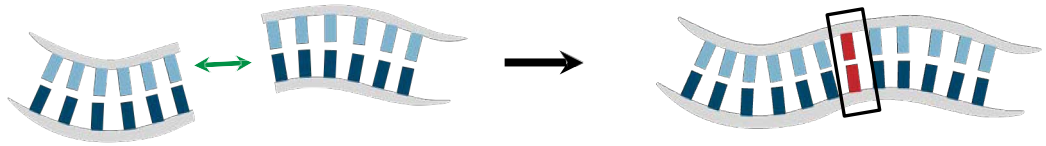
Sequence Variation Requires Structural Context

Mis-repair: Structural Variation



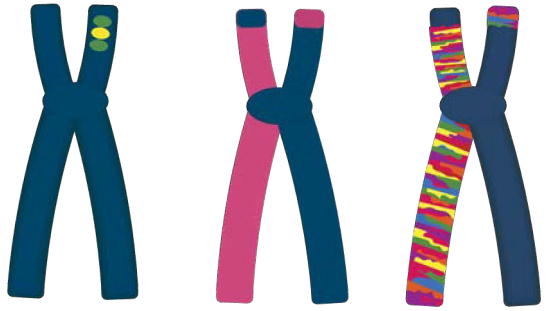
Structural Variant

Mis-Edit: Sequence Variation

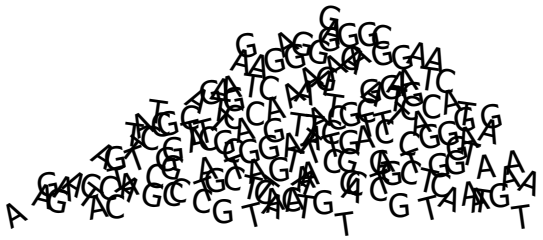


Single Nucleotide Variant

dGH is the Structural Ground Truth



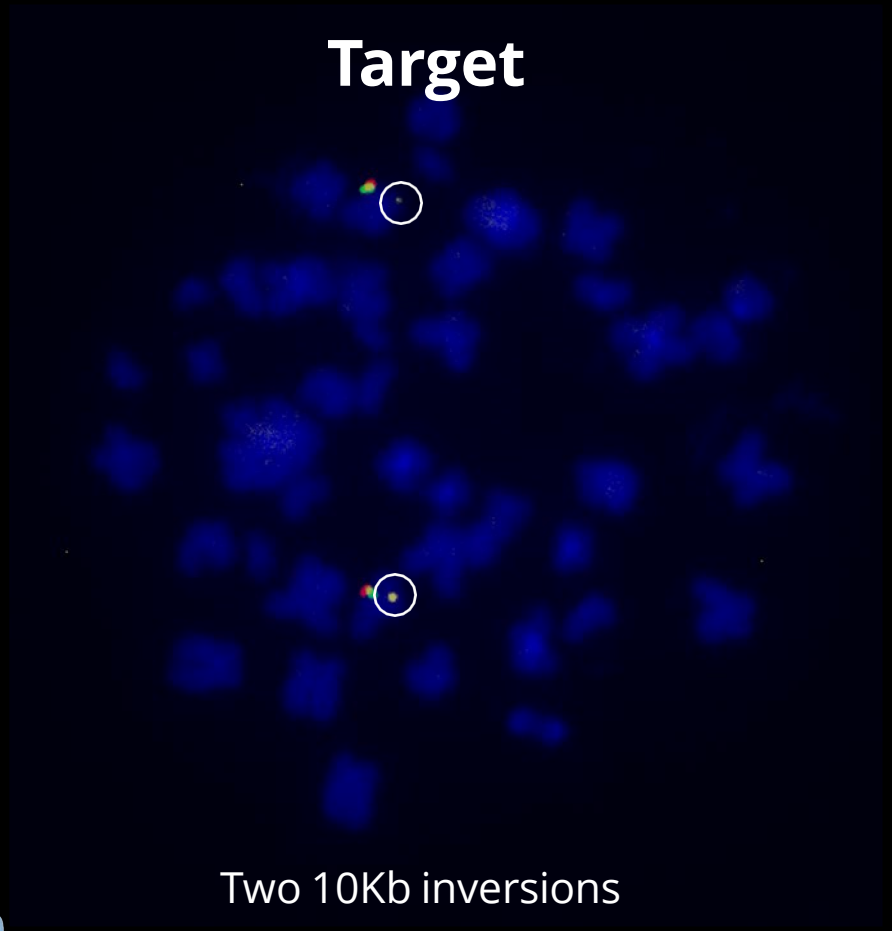
Bioinformatic Structural Hypothesis



Measuring all aberrations with two orthogonal and complementary methods

Visualizing Genomic Structure with dGH™

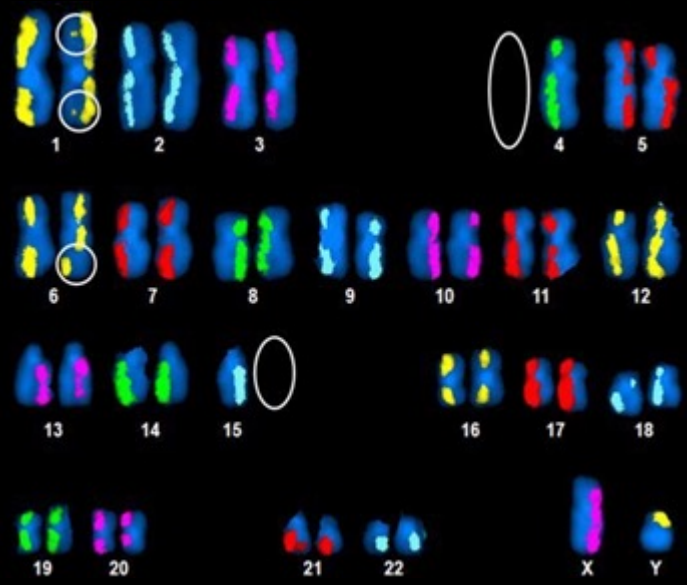
Target



Two 10Kb inversions

dGH in-Site™ (localized)

Discovery



Three inversions and two missing chromosomes

dGH SCREEN™ (unbiased)

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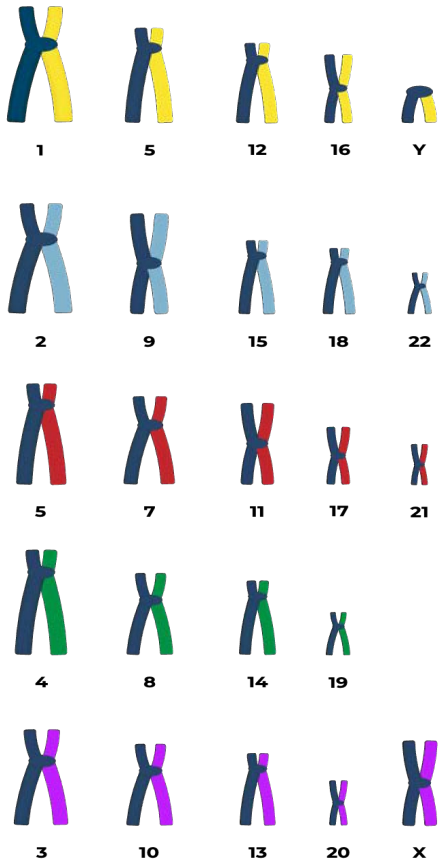
dGH SCREEN™: 5-Color, Whole-Genome Karyotyping



dGH SCREEN:

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

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What you get with dGH SCREEN™

Overall rates of structural events >5 kb in size

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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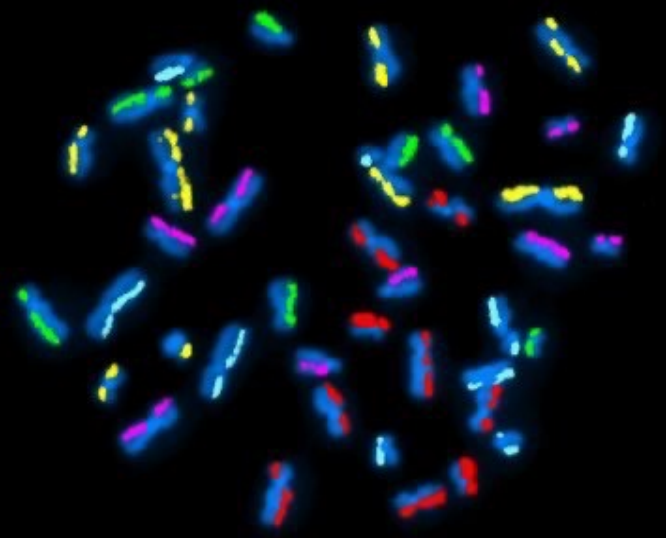
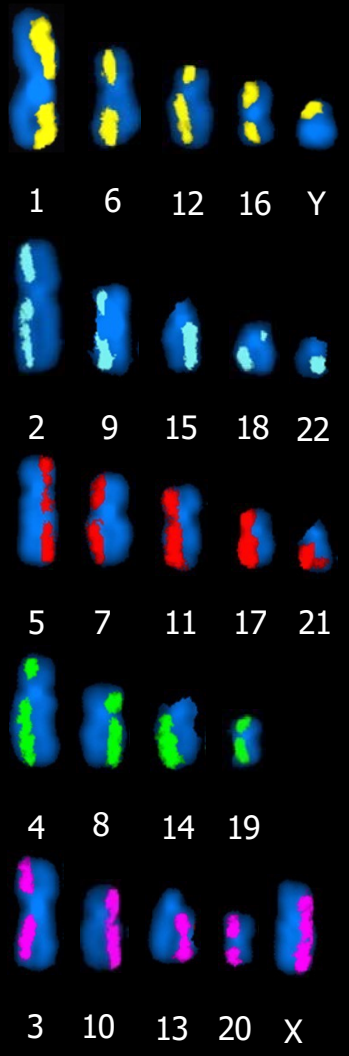
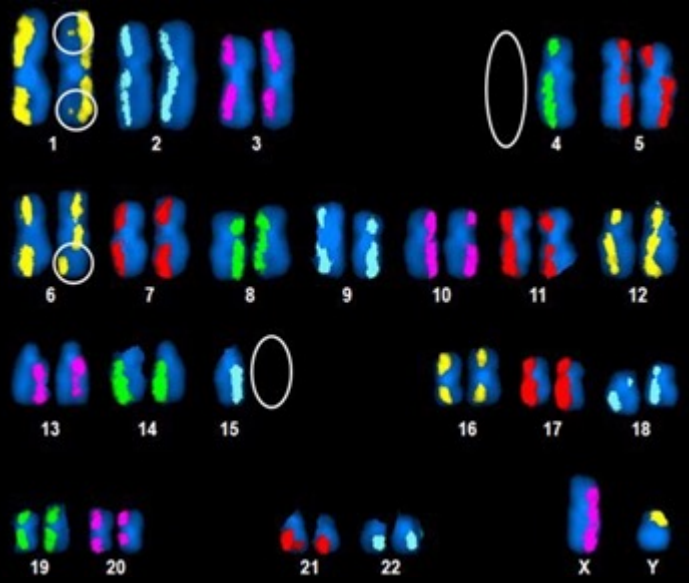
dGH SCREEN™



Spread



Sorting



Karyogram

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3 Use Cases

Three Mile Island Project

Analysis of blood lymphocytes from human subjects

Collaboration with University of Texas Medical Branch

Analysis of clones from an established cell line exposed to several different sources of radiation

NIST Genome Editing Consortium

Analysis of the “Genome in the Bottle” cell line

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dGH SCREEN™ for Biodosimetry

Three Mile Island Project

The Three Mile Island accident was a partial meltdown of the Three Mile Island, Unit 2 reactor in Pennsylvania, United States.

It began at 4 a.m. on March 28, 1979. It is the most significant accident in U.S. commercial nuclear power plant history.

- 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
- Analogous to measuring off-target effects in a gene editing system

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Traditional Measures of Biodosimetry

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

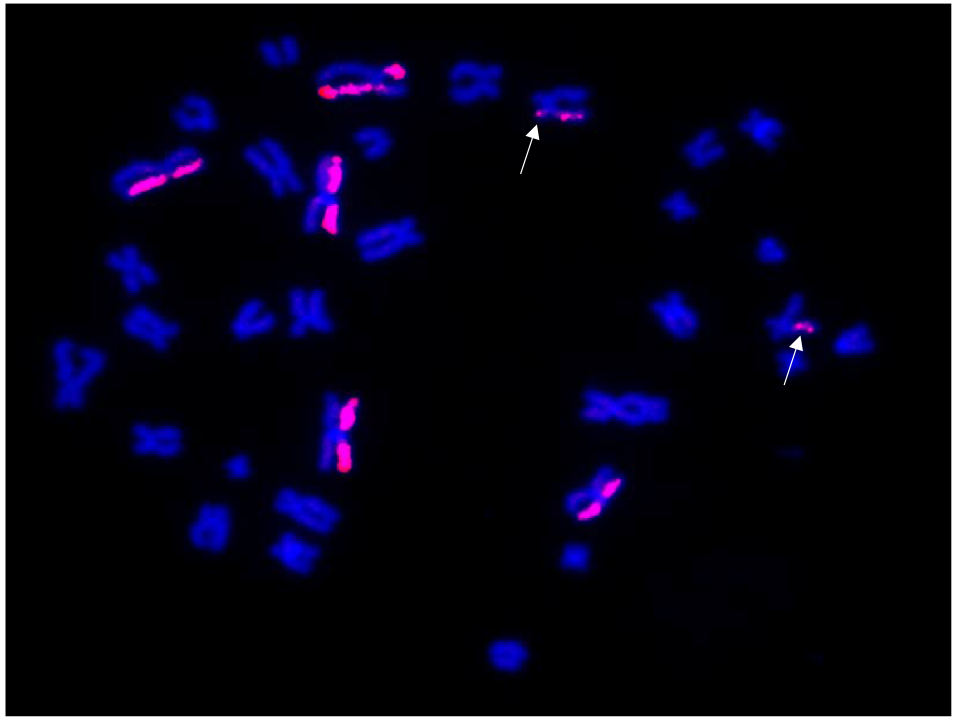

Figure 1: Whole chromosome 1, 2 and 3 paints hybridized to a metaphase spread from a human peripheral blood sample irradiated with 2Gy Cs-137 gamma rays. Structural rearrangements identified by Directional Genomic Hybridization denoted by arrows.

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

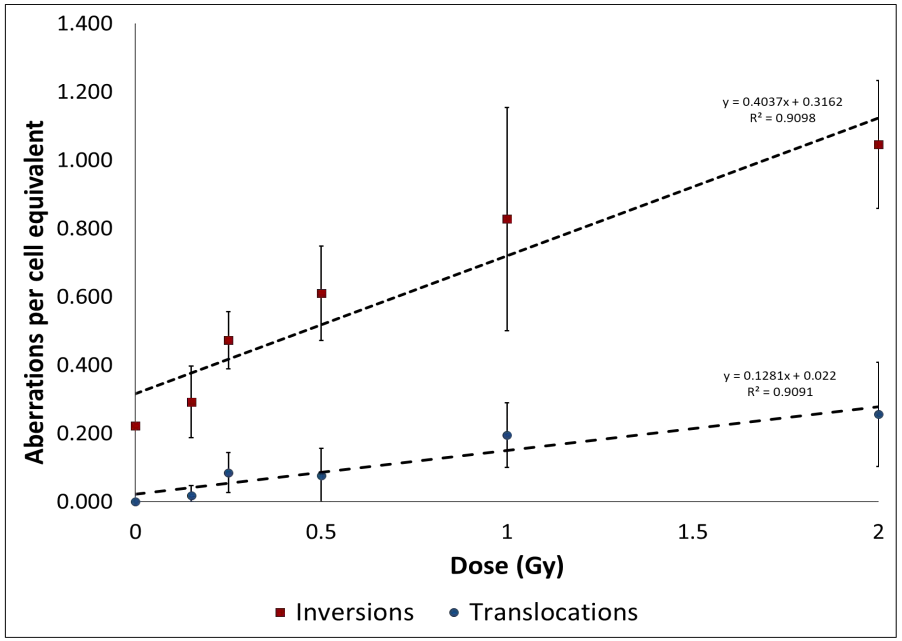

Figure 2: Blood samples from young adult controls were irradiated with Cs-137 gamma rays to establish a dose response (calibration) curve. Males in their mid-20's were selected to account for age at exposure. Inversions (red) had a higher natural background rate compared to translocations (blue); however,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

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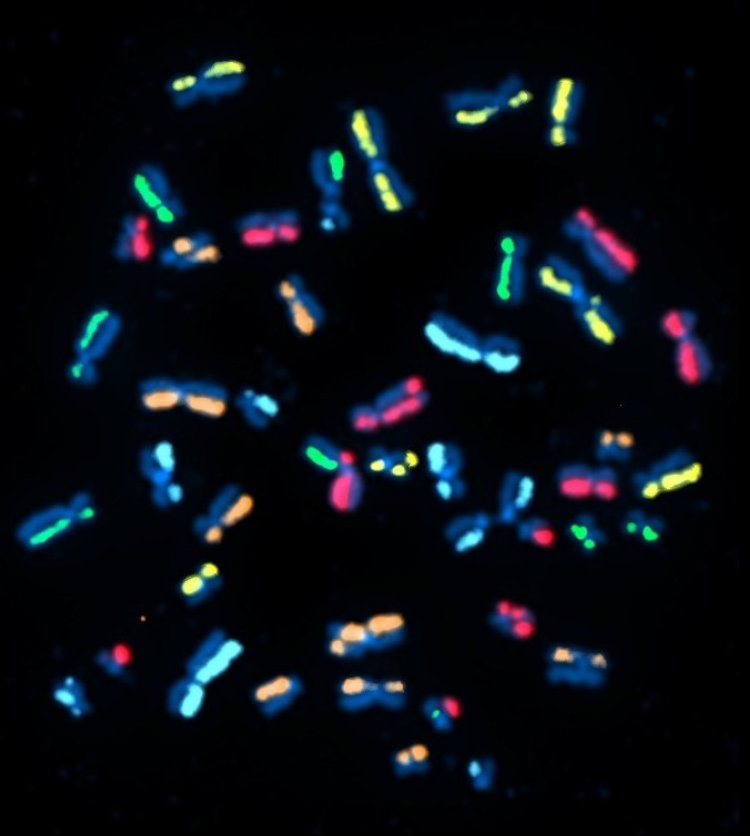
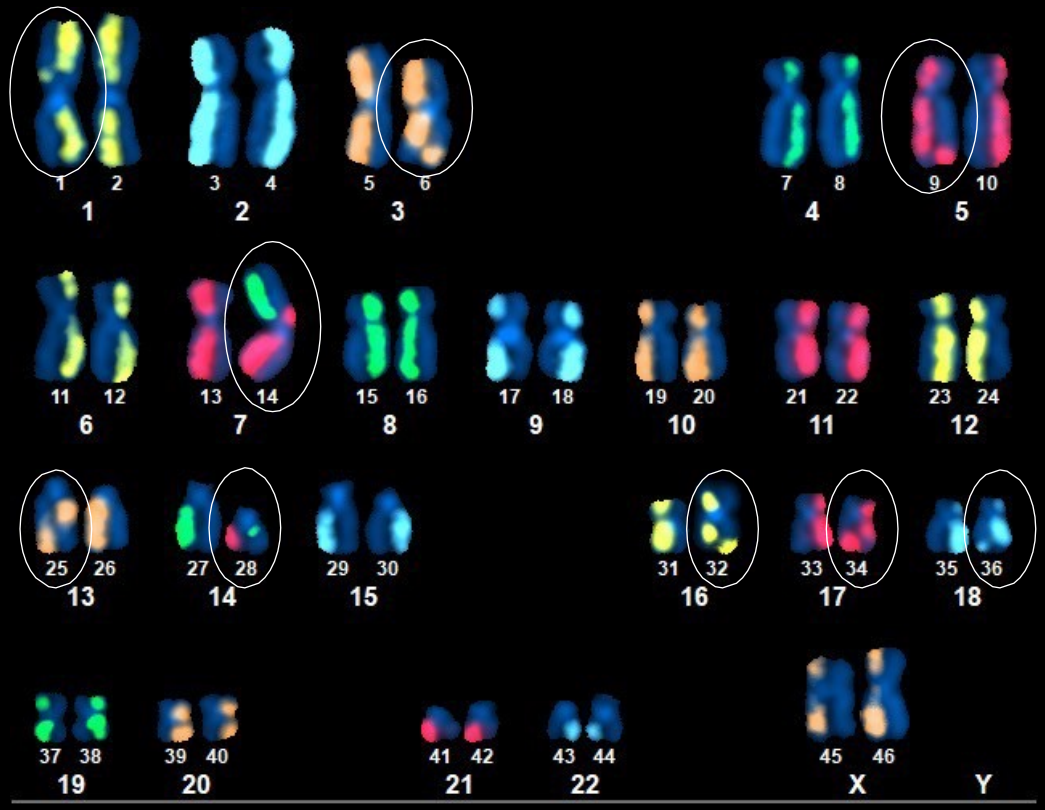
McKenna MJ, Robinson E, Taylor L, et al. Chromosome Translocations, Inversions and Telomere Length for Retrospective Biodosimetry on Exposed U.S. Atomic Veterans. Radiation Research. 2019 Apr;191(4):311-322. DOI: 10.1667/rr15240.1. PMID: 30714852; PMCID: PMC6492561.

Preliminary SCREEN™ Data





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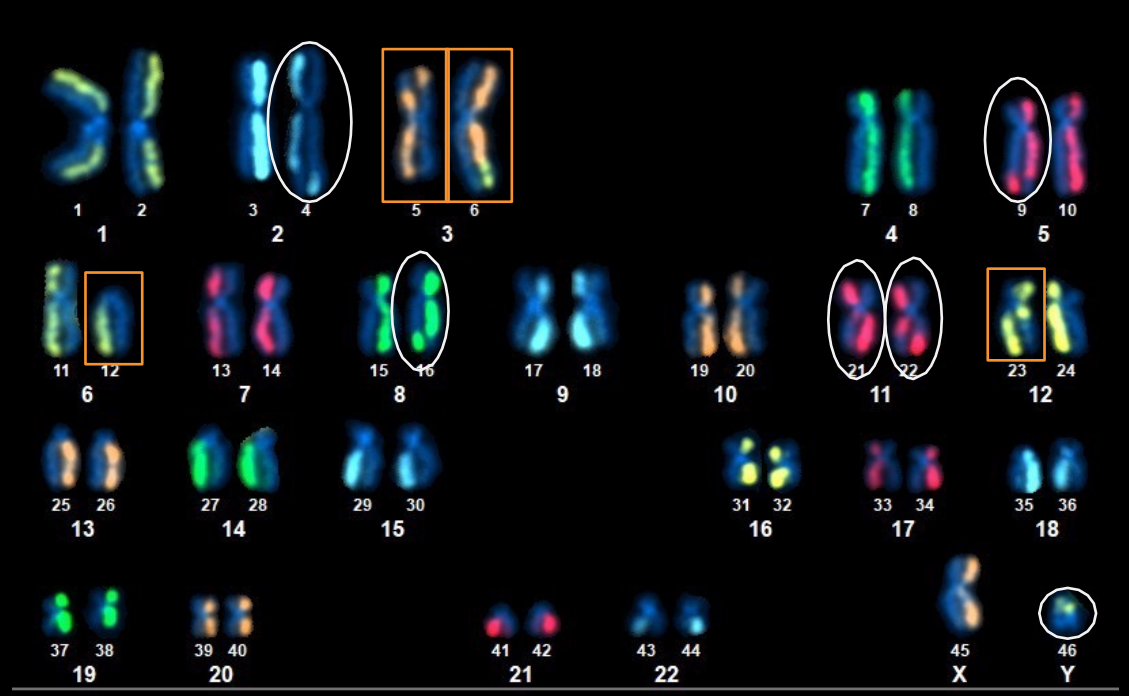
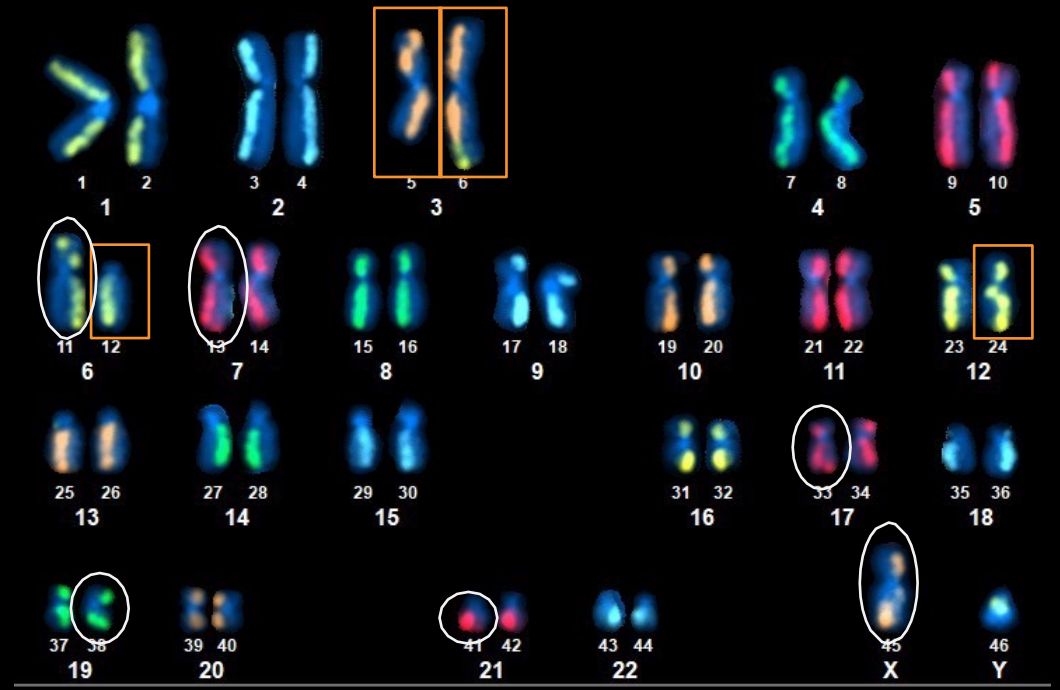
Measuring Recurrent Translocations and Inversions





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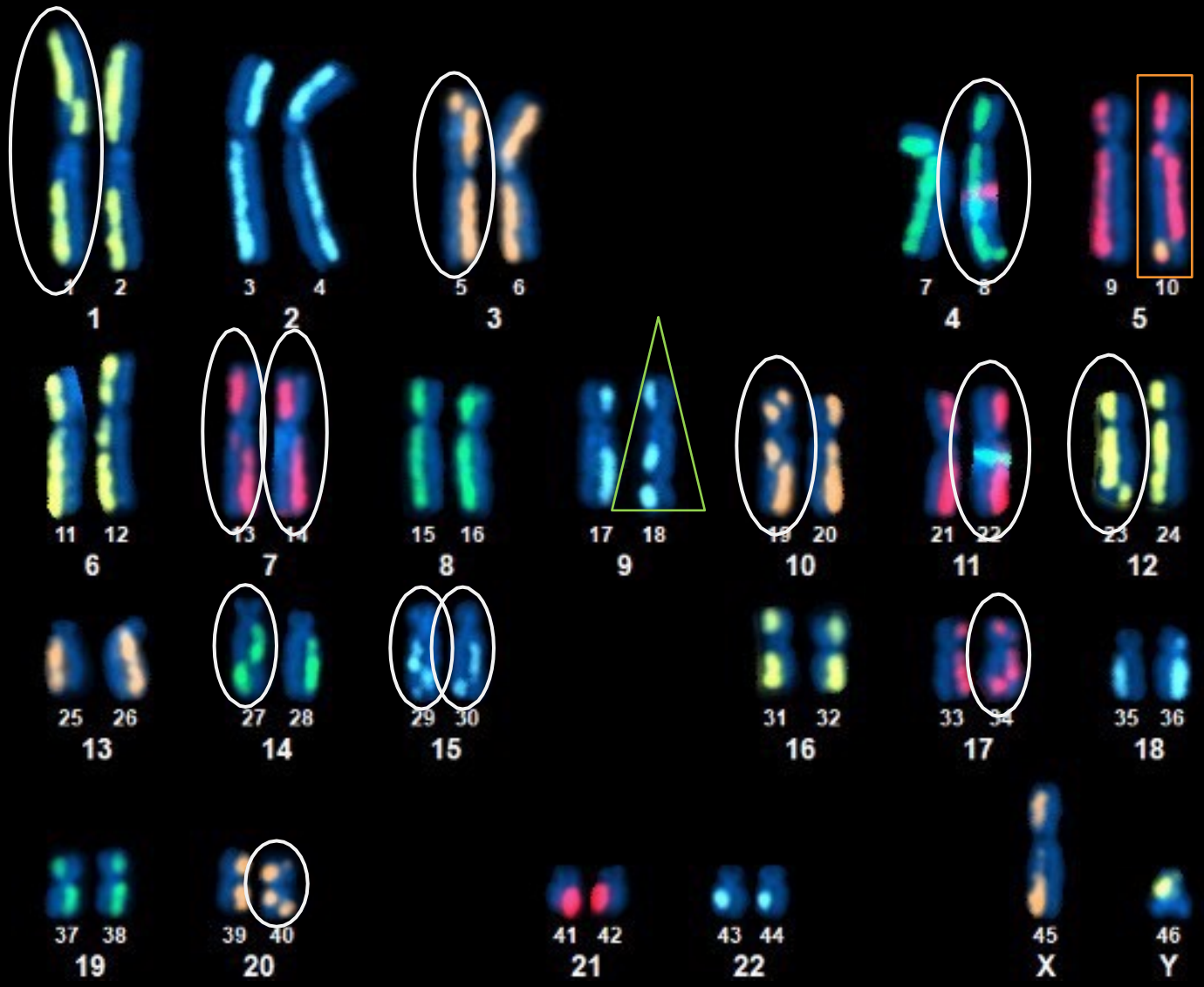
Recurrent

Random

dGH SCREEN™ Data



Translocation

Inversion or SCE

Chromatid break

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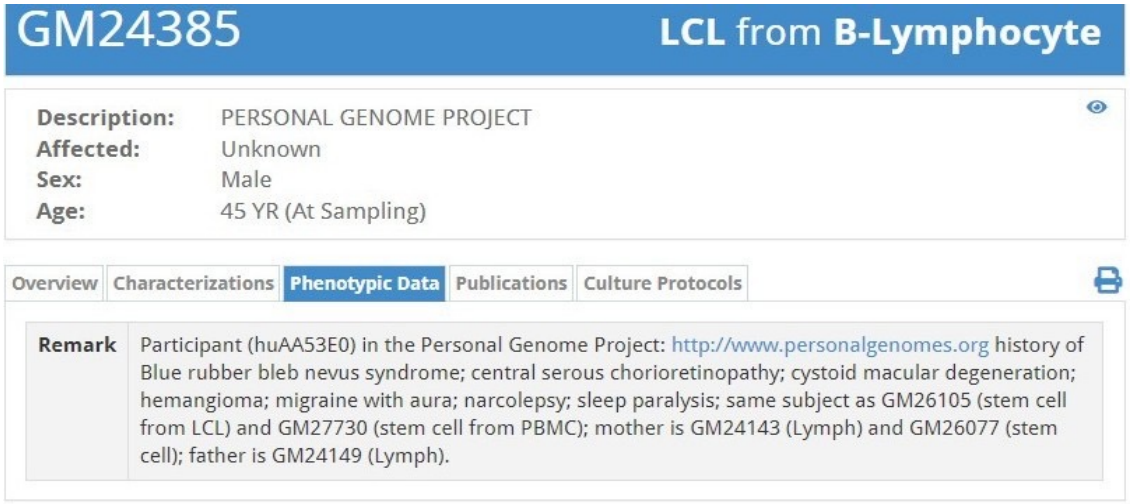
dGH SCREEN™ for Cell Line QC

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



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 (Kromatid)

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

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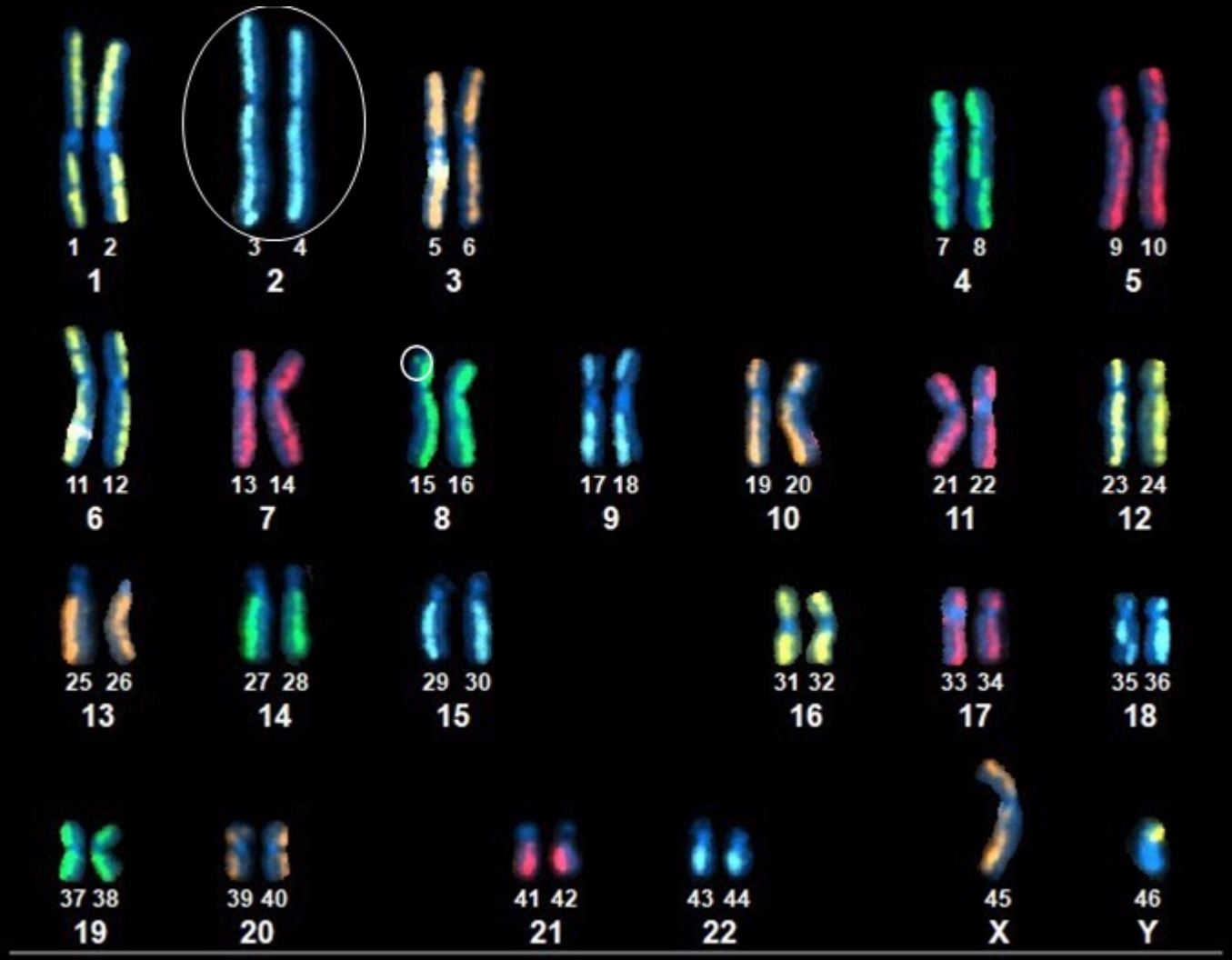
GM24385 p12



S73_c54, Cell 14

Structural Variant Summary*

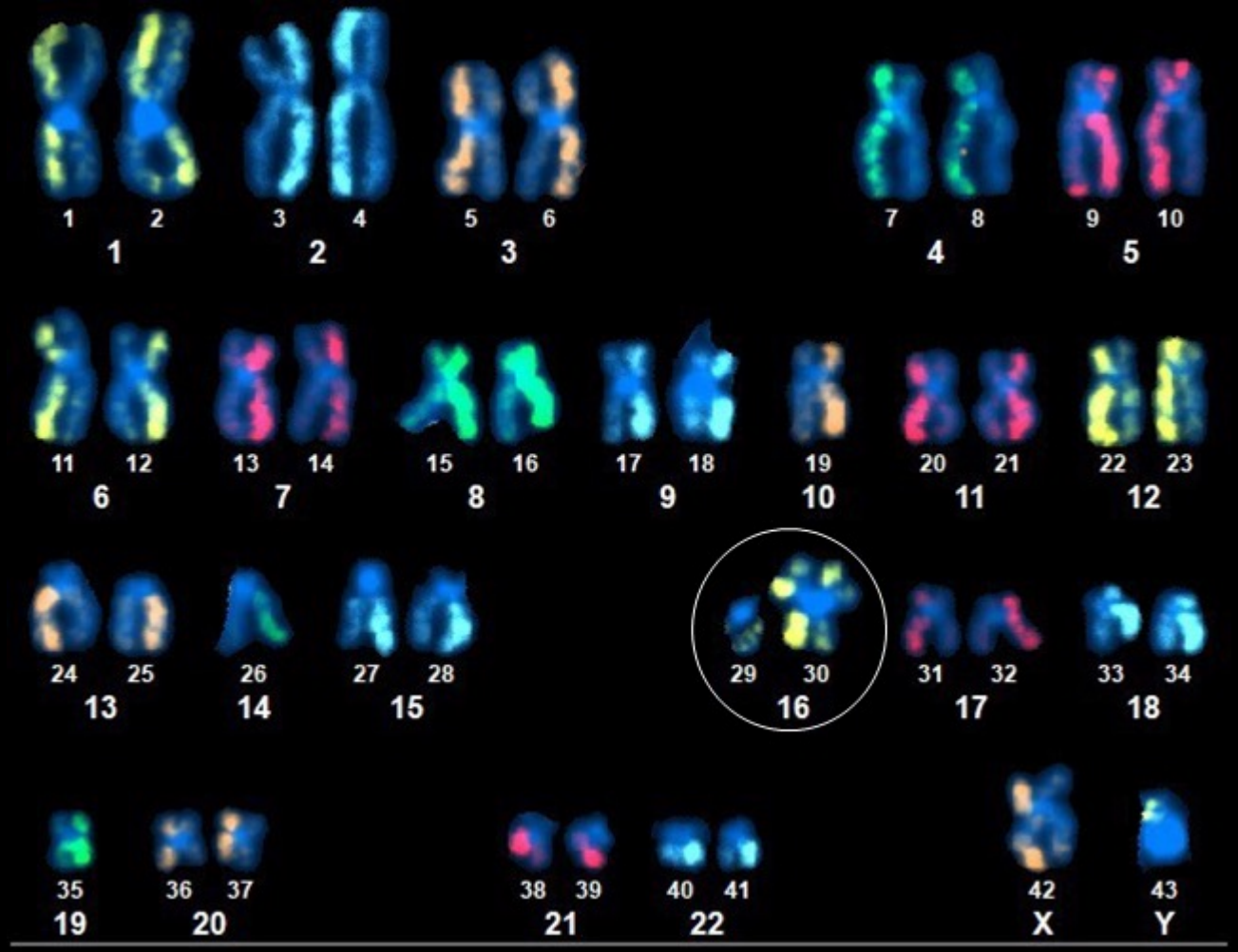
- 4 translocations (heterogenous)-8% of cells
- 34 inversions of > 8% occurrence
- 18% variable monosomy
- 4% variable trisomy
- Low level of complex events, including one cell with chromothripsis of Chr 19, one cell will whole arm deletion of Chr 19, two cells with chromatid-type breaks of Chr 3, and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

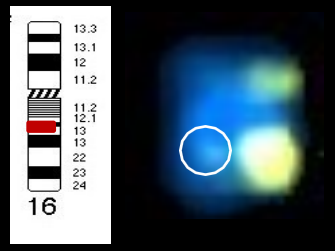
- Elevated rate of complex events in Chromosome 16. Large deletions, radial whole-arm gain, chromothripsis, decondensation and centromere "spindling" observed in 41% of cells
- Centromere abnormalities were also frequently observed in Chr 1 and Chr 9.

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

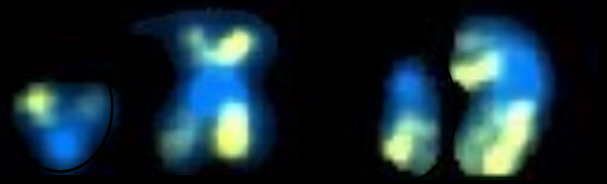
*Total 54 cells

Chr 16 Complex Structural Variation in p18 indicates cell line transformation, instability

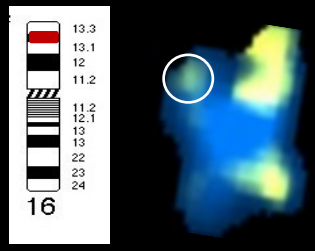
Chr 16q Inversion (37%)
- Small, mid-arm
- Observed in 19% of cells



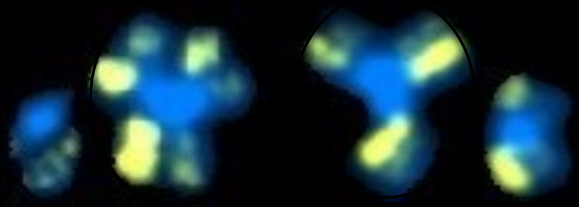
Whole arm deletion
- Observed in 11% of cells



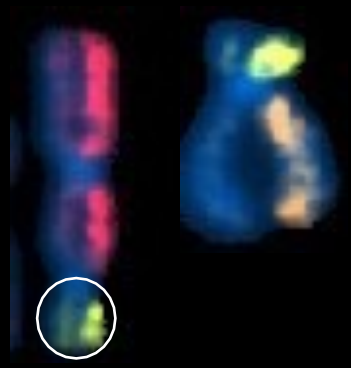
Chr 16p Inversion
- Small, mid-arm
- Observed in 19% of cells



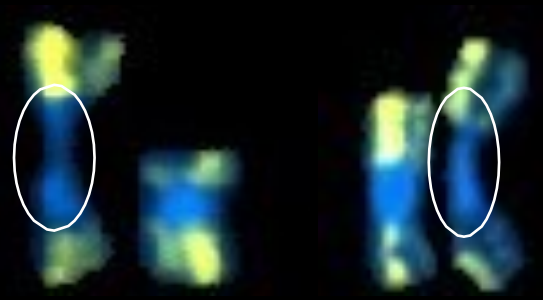
Chromosome 16 multi-radial association
- Observed in 4% of cells



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

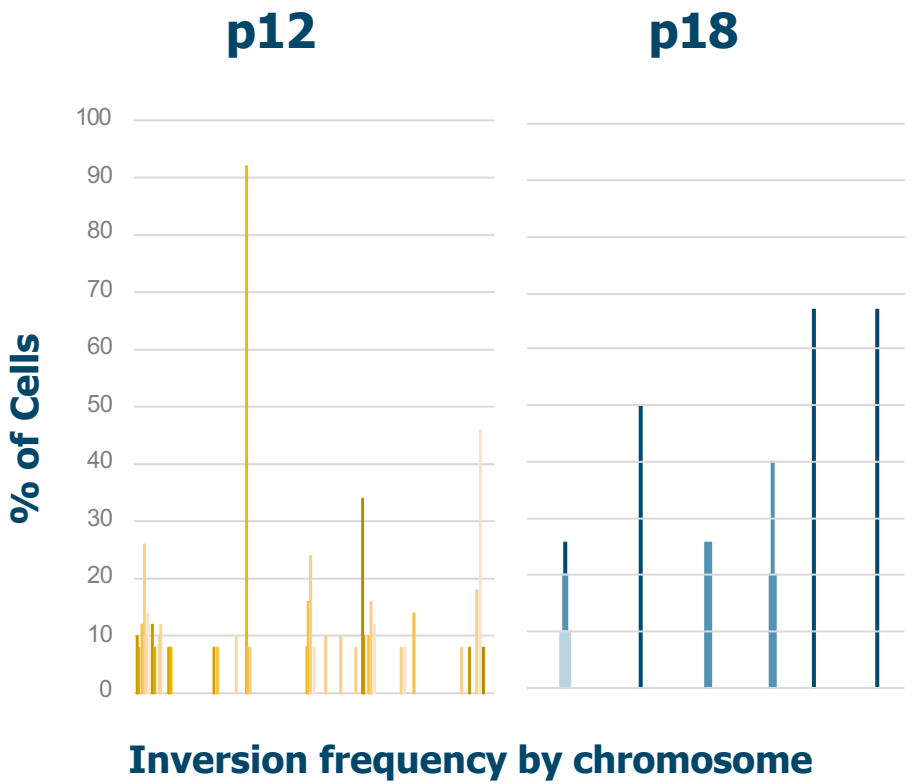


Decondensed/ elongated centromeres and iso-chromosomes
- Observed in 22% of cells



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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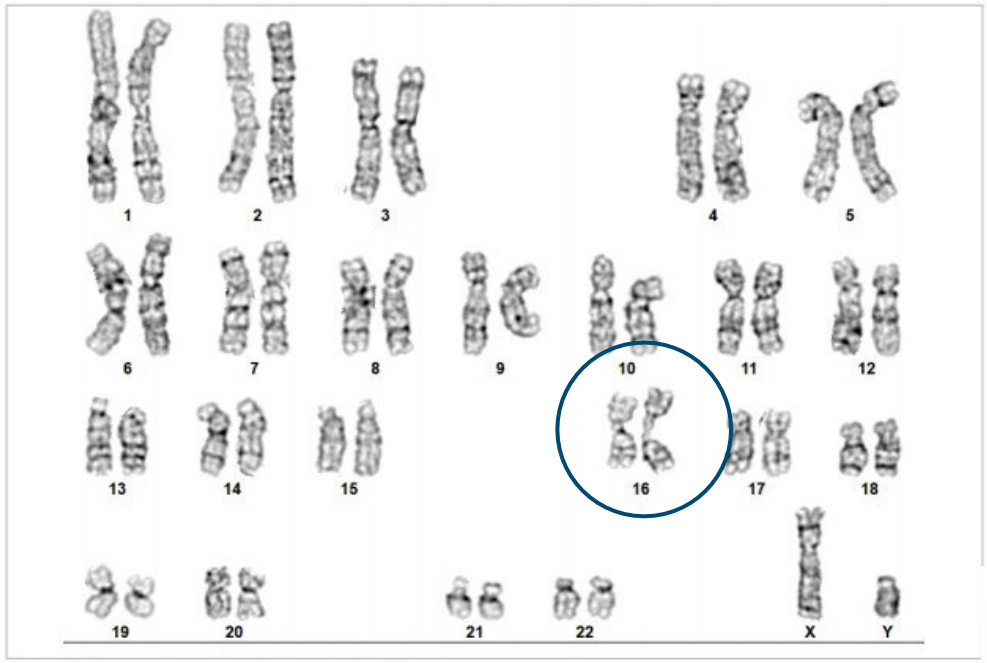
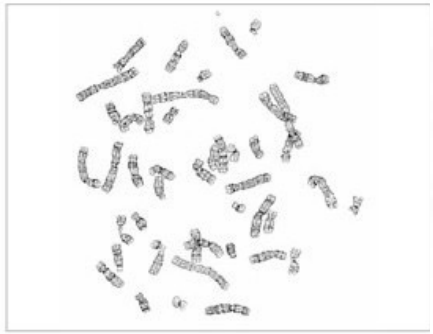
G-banding Confirms Gross Chr16 Result



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]

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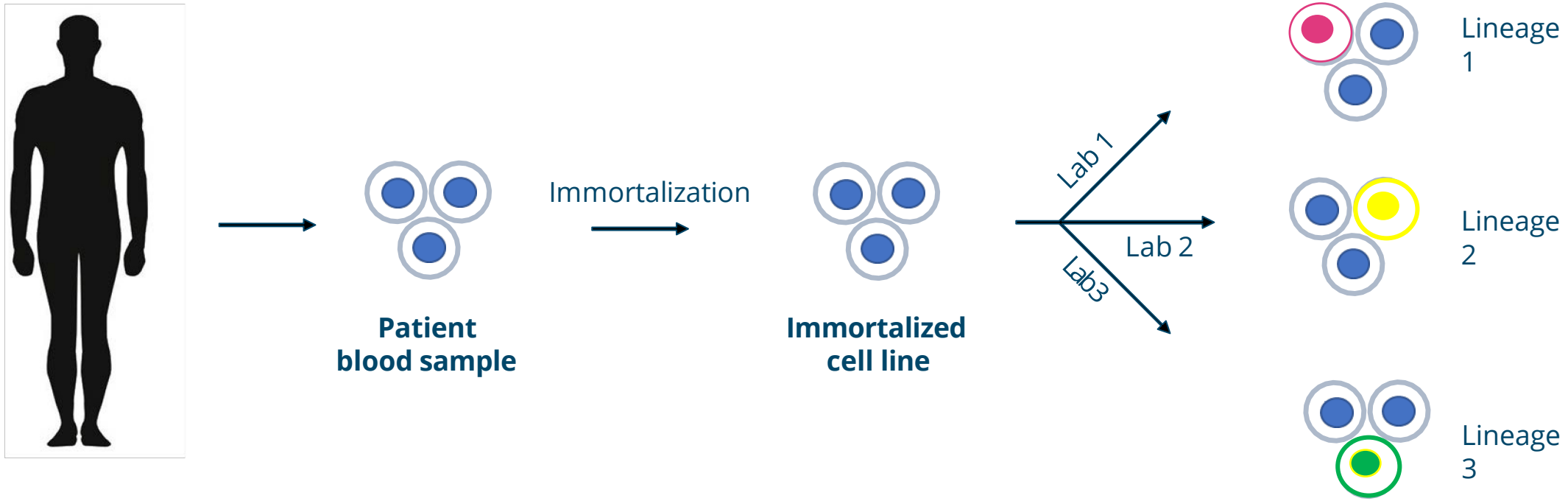
Parallel studies with inherently unstable cell lines



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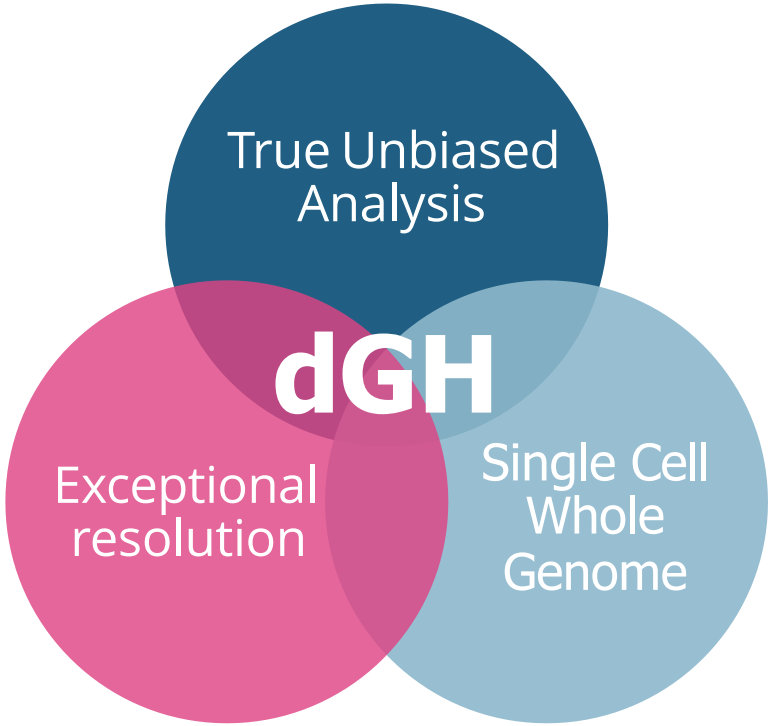
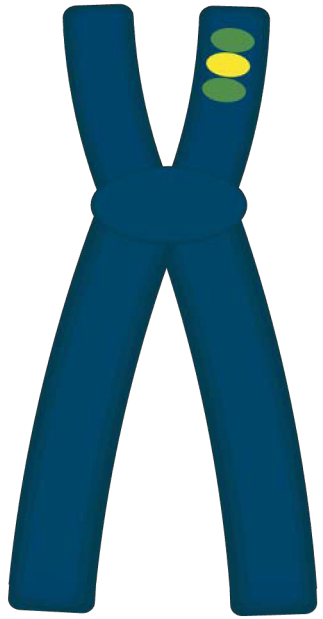
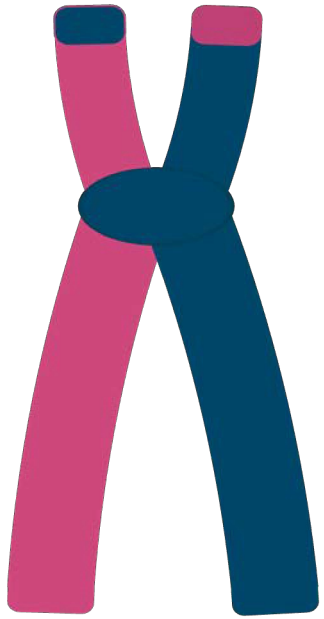
directional Genomic Hybridization™

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





dGH in-Site™



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

dGH SCREEN™ Services Pricing

SKU	Product Description	List Price
SCR-001	SCREEN Culture Development: Thaw, recovery, and harvest optimization	$1,500.00
SCR-002	SCREEN Assay Execution and Analysis: Imaging and scoring for 50 spreads/sample	$5,775.00
SCR-003	SCREEN Assay Execution and Analysis: Imaging and scoring for 20 spreads/sample	$3,465.00
SCR-004	SCREEN Assay Execution and Analysis: Imaging and scoring for 100 spreads/sample	$10,395.00
SCR-005	T-Cell: Thaw, recovery, and harvest optimization	$1,625.00
SCR-006	IPSC: Thaw, recovery, and harvest optimization	$1,750.00
SCR-007	Whole Blood: Thaw, recovery, and harvest optimization	$1,250.00
SCR-008	SCREEN Metaphase Adherent/Suspension Prep and Harvest	$1,500.00
SCR-009	SCREEN T Cells Metaphase Prep and Harvest	$1,625.00
SCR-010	SCREEN IPSC Metaphase Prep and Harvest	$1,750.00
SCR-011	SCREEN Whole Blood Metaphase Prep and Harvest	$1,250.00
SCR-012	dGH SCREEN™ Chr1p E-Banding mini-Analysis on 100 existing cells	$1,050.00
SCR-013	SCREEN™ NK Cells Metaphase Prep and Harvest	$1,625.00
SCR-014	SCREEN™ NK Cell Culture Development: Thaw, recovery, and harvest optimization	$1,625.00

KromaTiD
Direct, Definitive Genomics

Working with KromaTiD is Simple

1. **Identify target and scope**
2. **Probe design and assay proposal**
3. **Design approval and sample shipment**
4. **Assay execution and data analysis**
5. **Data review and discussion**

Example workflow with KromaTiD running in-Site™ or alternative assays on engineered lines in-house.

- Customer
- KromaTiD
- KromaTiD & Customer

KromaTiD is committed to **collaborative excellence** through dedicated project management and **expert technical analysis**.

KromaTiD
Direct, Definitive Genomics

Why You Win With Us

Collaboration: The trusted structural genomics partner for leading gene therapy innovators

Performance: Gold standard products for the measurement of genomic structure and structural variation

Scalability: End to end process automation, high-throughput analysis, AI meta-analysis

Excellence: Experienced team of 20 operating today in a world class, 11,000 square foot genomics facility

Proprietary: Issued patents, broadened applications, trade secret methods, proprietary bioinformatics

Thank you!

KromaTiD
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For Research Use Only. Not for use in diagnostic procedures.

to inquire and for orders
visit: kromatid.com
or contact: sales@kromatid.com