

# Uncovering Structural Variants Missed by Conventional Cytogenetic Techniques



- KromaTiD Overview
- Cytogenetics Techniques and Contrast with dGH
- Introduction to the dGH Assay Suite
- Hypotheticals: Analysis of Cell, of Sample, of Sample Set
- Value of Orthogonal Data and the Role of dGH

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Direct, Definitive Genomics

Presented by Ivan Perez | Technical Applications Scientist, KromaTiD

# Overview of KromaTiD

KromaTiD is staffed by a committed team specializing in delivering top-tier genomic tools and services.

Located in Longmont, Colorado, our primary mission is to offer the tools and assistance required to propel the progress of genomic medicine and research.



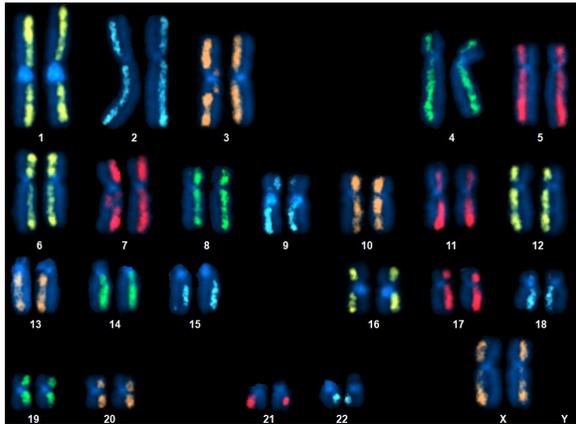
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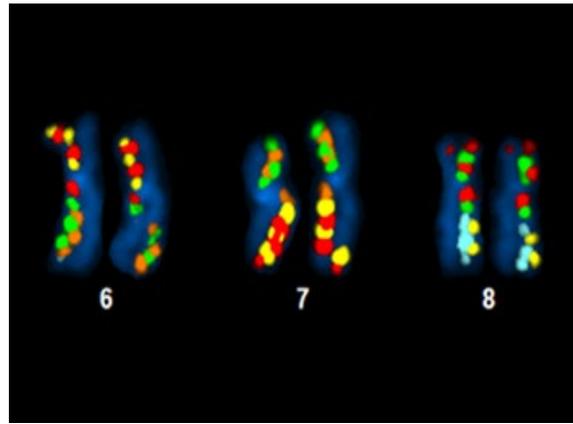


# dGH: Powerful, High-Resolution Cytogenomic Solutions

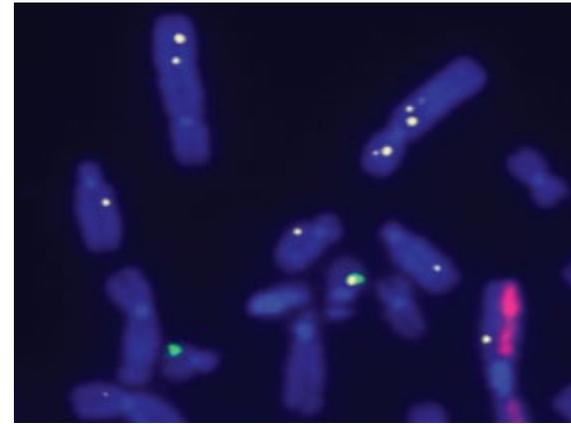
dGH SCREEN



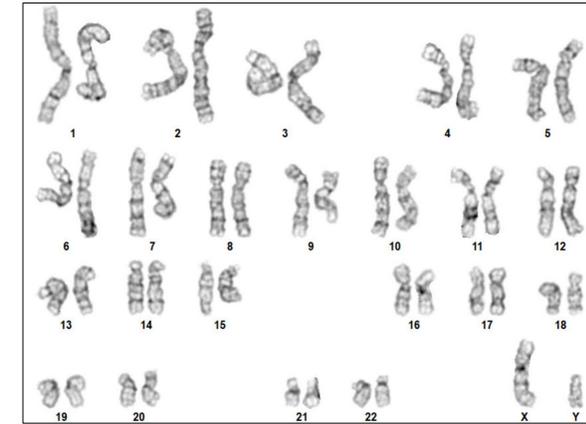
dGH DSCVR



dGH in-Site



G-Banding



Unmatched insights into

- Gene editing outcomes
- Genomic integrity
- Biomarkers of disease

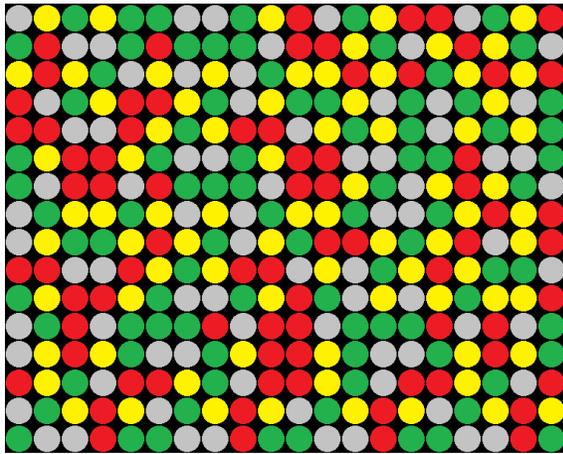
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# Established Cytogenetic Methods

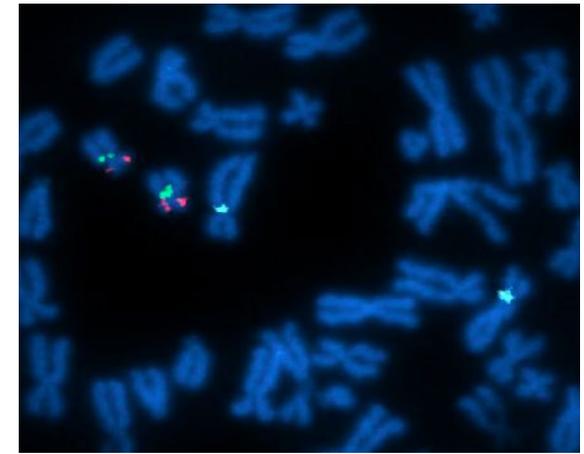
aCGH



G-Banding



FISH

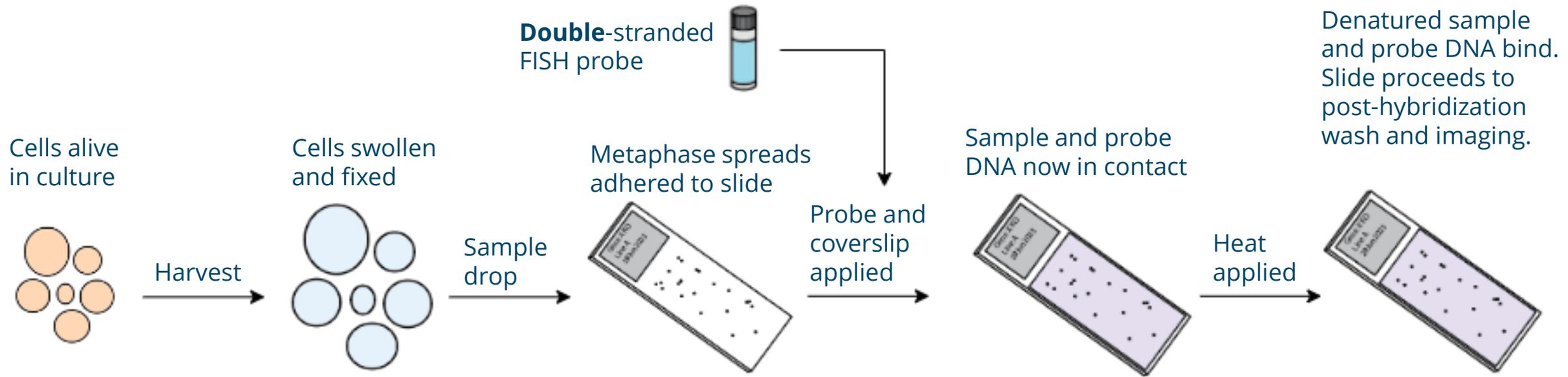


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# Fluorescence In Situ Hybridization (FISH)

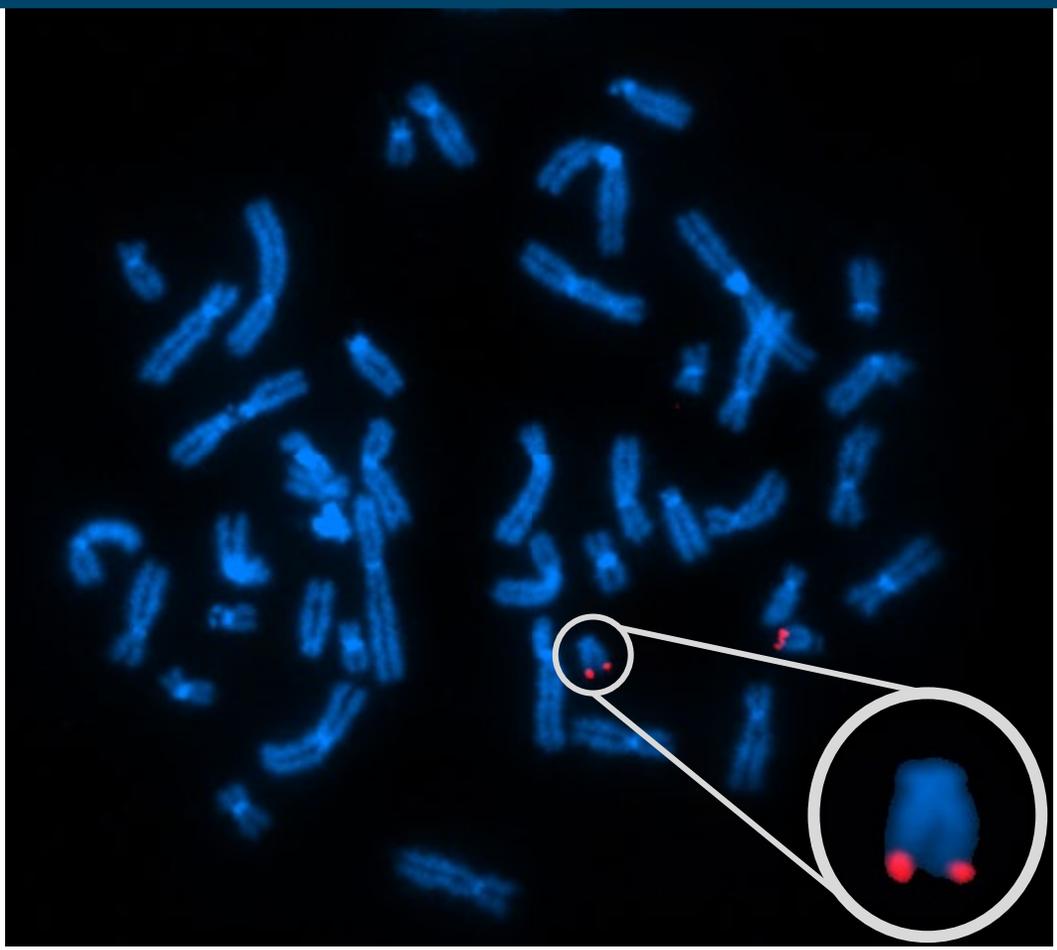


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# Example FISH Image



## Example of FISH hybridization outcome.

Fluorescence is seen in the subtelomeric region of the q-arm of chromosome 21.

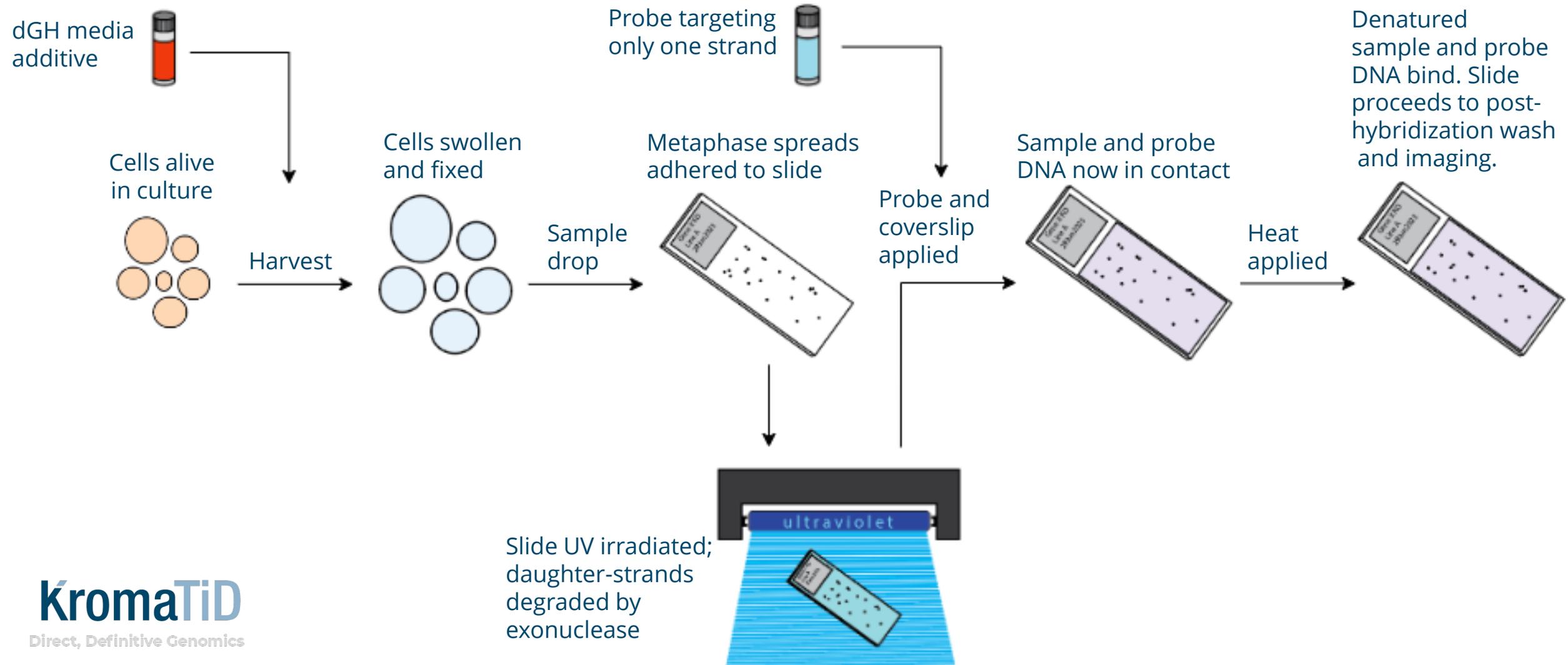
The corresponding target site on each of the two chromatids fluoresces with its own signal.

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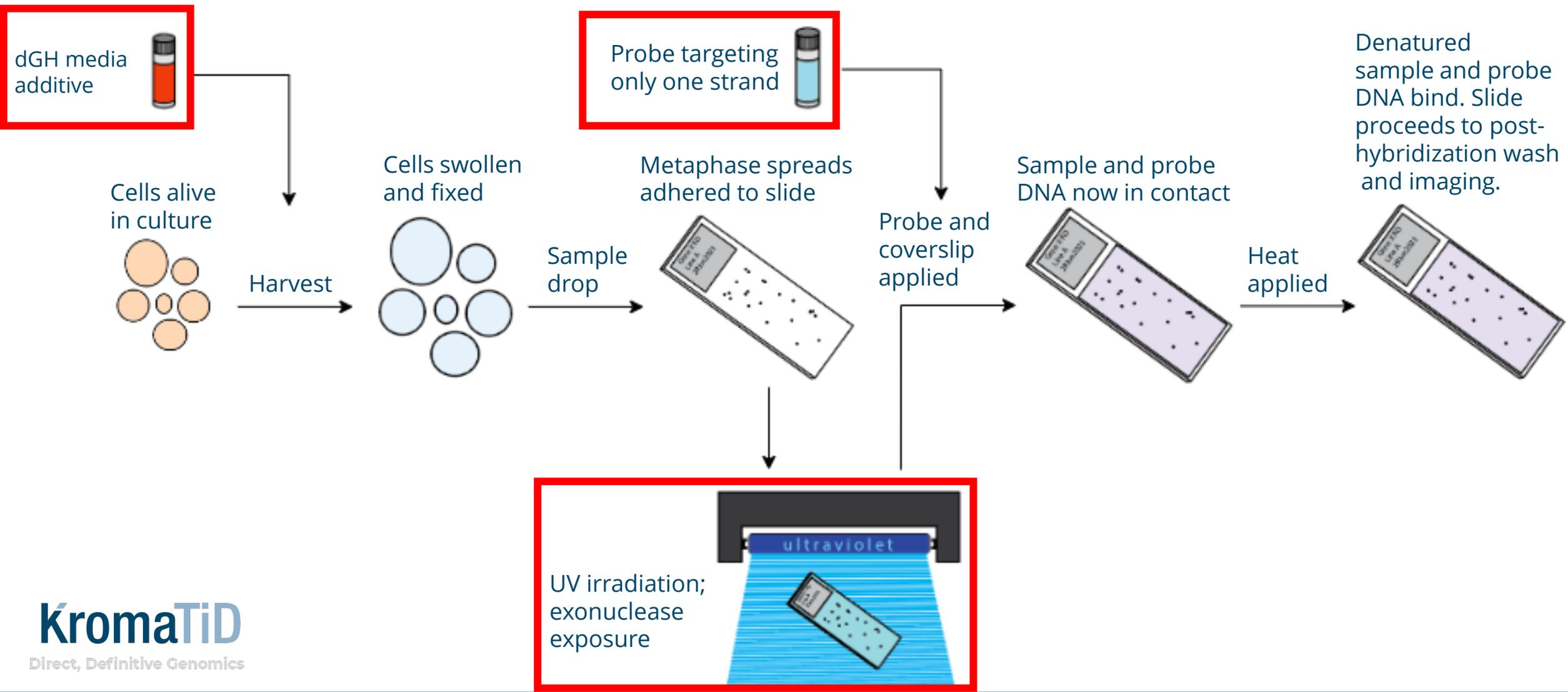


# Directional Genomic Hybridization (dGH)



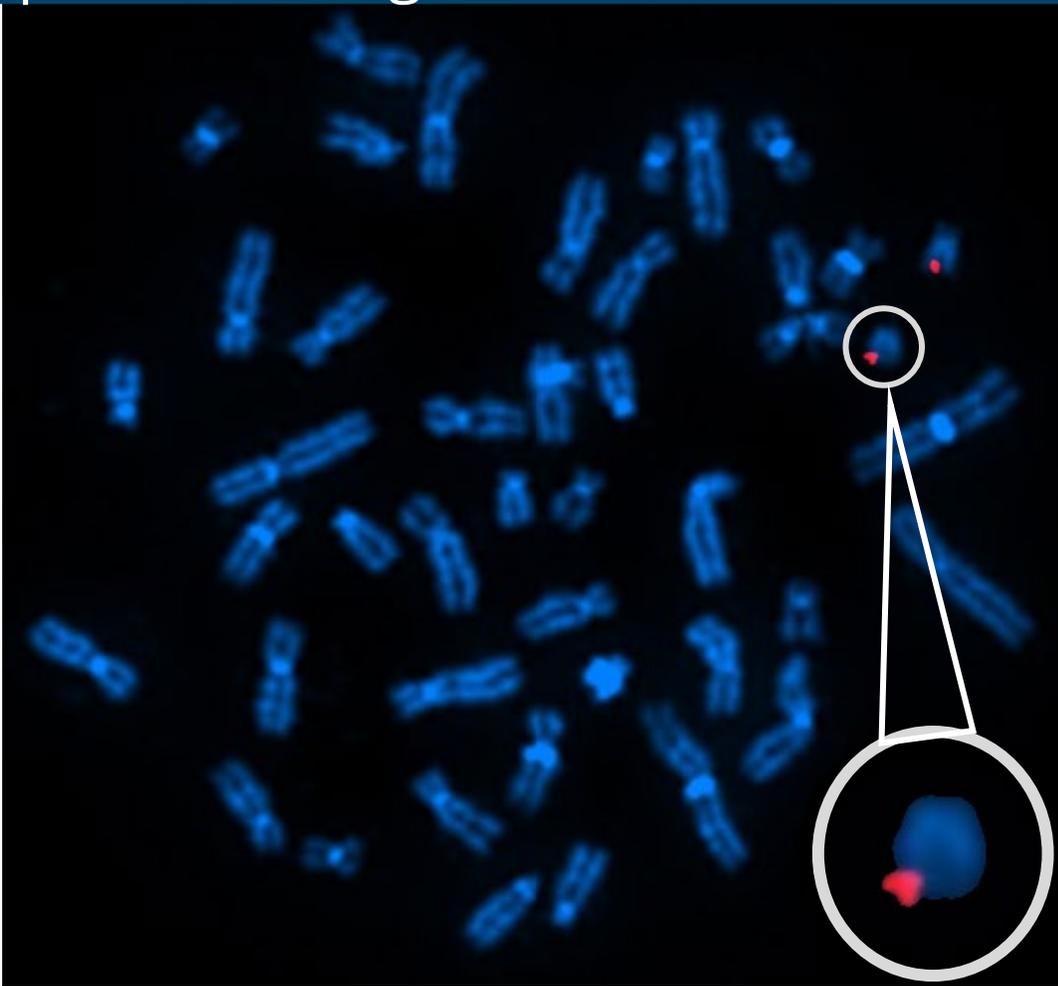


# Directional Genomic Hybridization (dGH)





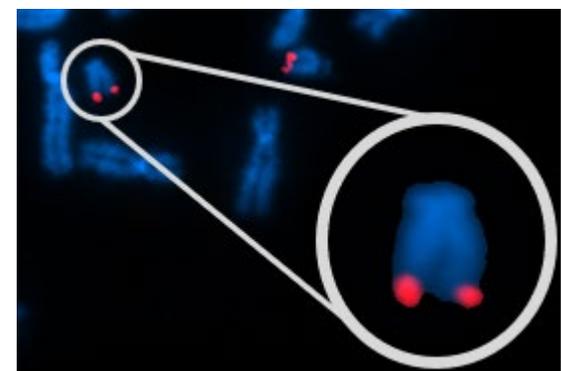
# Example dGH Image



## Example of dGH hybridization outcome.

Fluorescence is seen in the same subtelomeric region of chromosome 21 as with FISH, but now the chromosome has only one target site.

Only one chromatid has DNA complementary to the probe sequences.



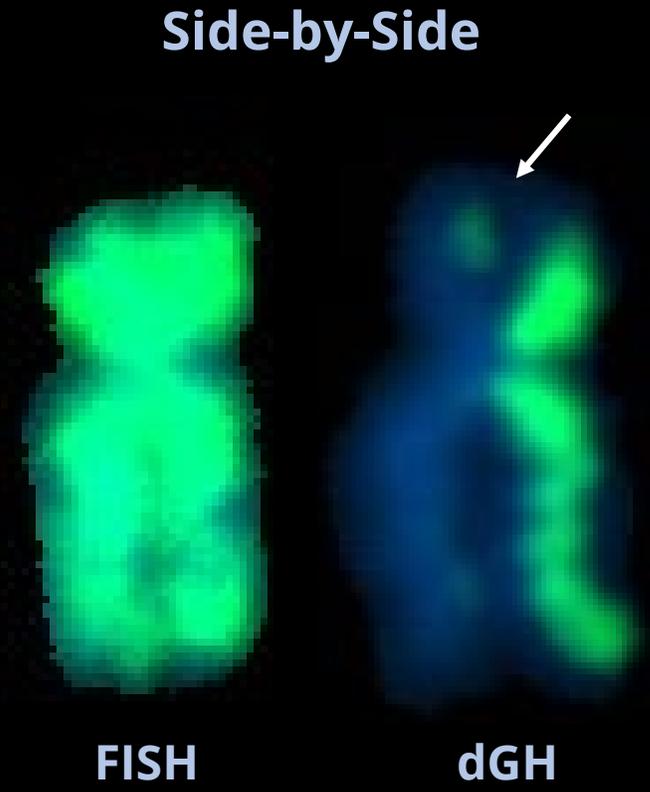
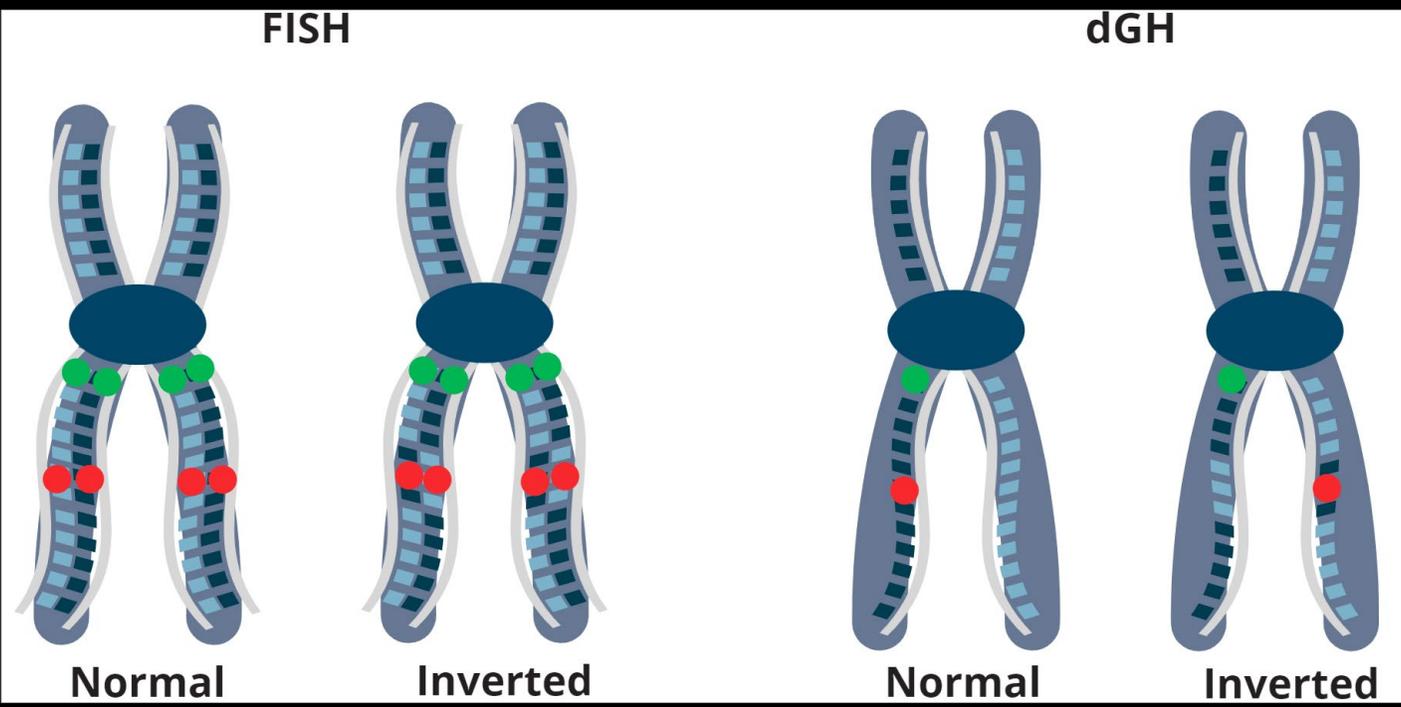
Previous FISH example.

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# Inversions and dGH





# Manufacturing Process for KromaTiD Probes



In Silico Design



Fluorescently Labeled Oligonucleotides

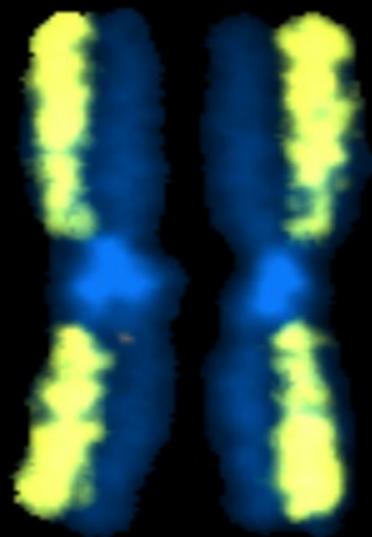
- Selected sequences are synthesized, amplified and fluorescently labeled.
- QC testing ensures clean probe performance.

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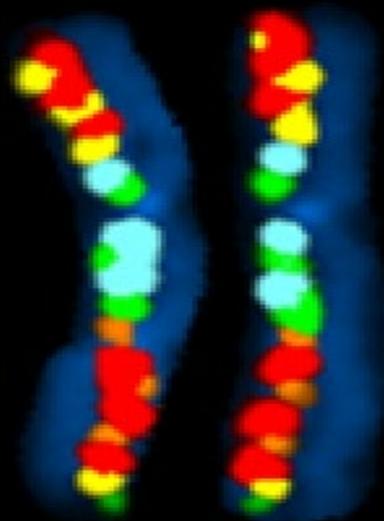
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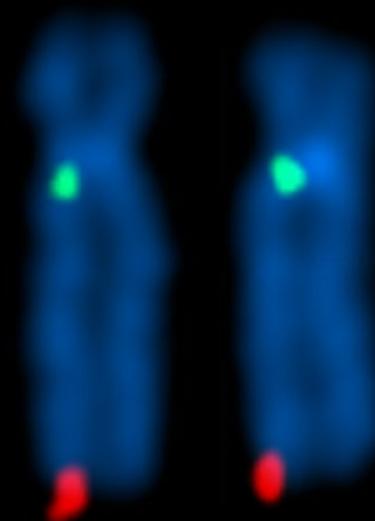
# The dGH Assay Suite: SCREEN, DSCVR, in-Site



dGH SCREEN



dGH DSCVR



dGH in-Site

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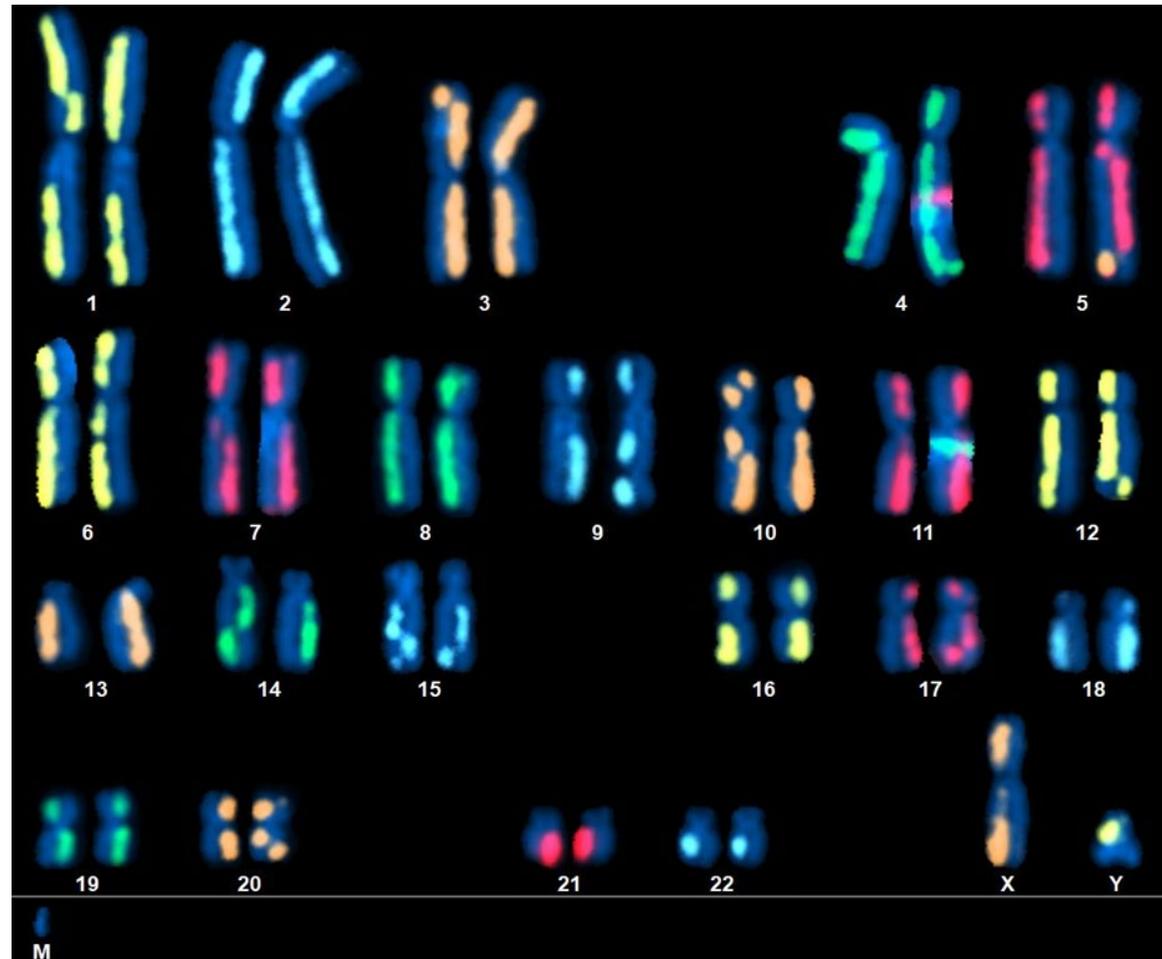
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# dGH SCREEN

## Features of dGH SCREEN:

- Whole-genome, unbiased
- Single-cell
- Direct visualization
- Rearrangements detected include small inversions
- Lower Limit of Detection (LLOD) as low as 10 kb

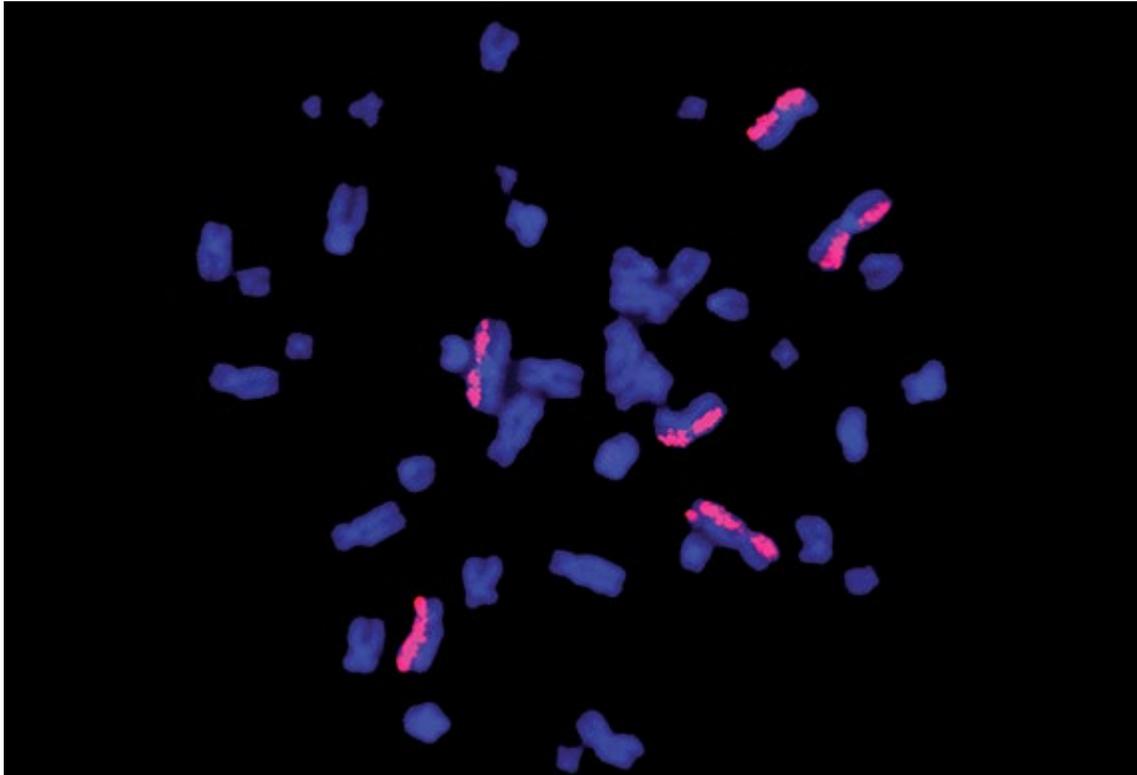


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# Use Case: NASA "Twin's Study" HD Chromosome Paints



KromaTiD work with NASA in the "Twins Study"

- Assay of DNA damage from radiation in Space.
- KromaTiD continues to work with NASA.



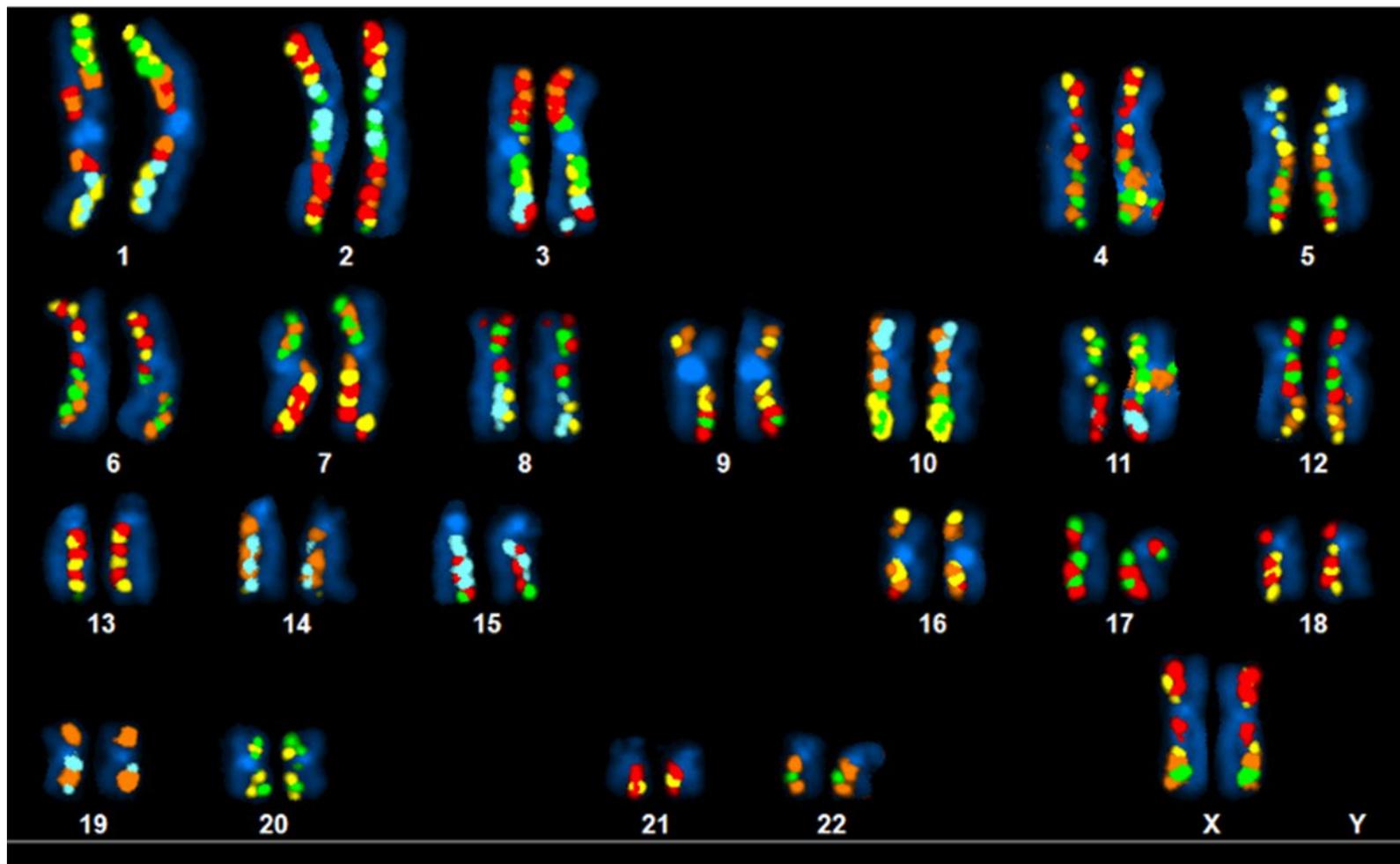
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Fluorescent Paints Spot DNA Damage from Radiation, Gene Editing. (2019). Retrieved from [https://spinoff.nasa.gov/Spinoff2019/hm\\_3.html](https://spinoff.nasa.gov/Spinoff2019/hm_3.html)



## Features of dGH DSCVR:

- Unbiased within locus-of-interest
- Detects all rearrangements affecting that region
- Bands are one to a few Mb
- LLOD same as SCREEN

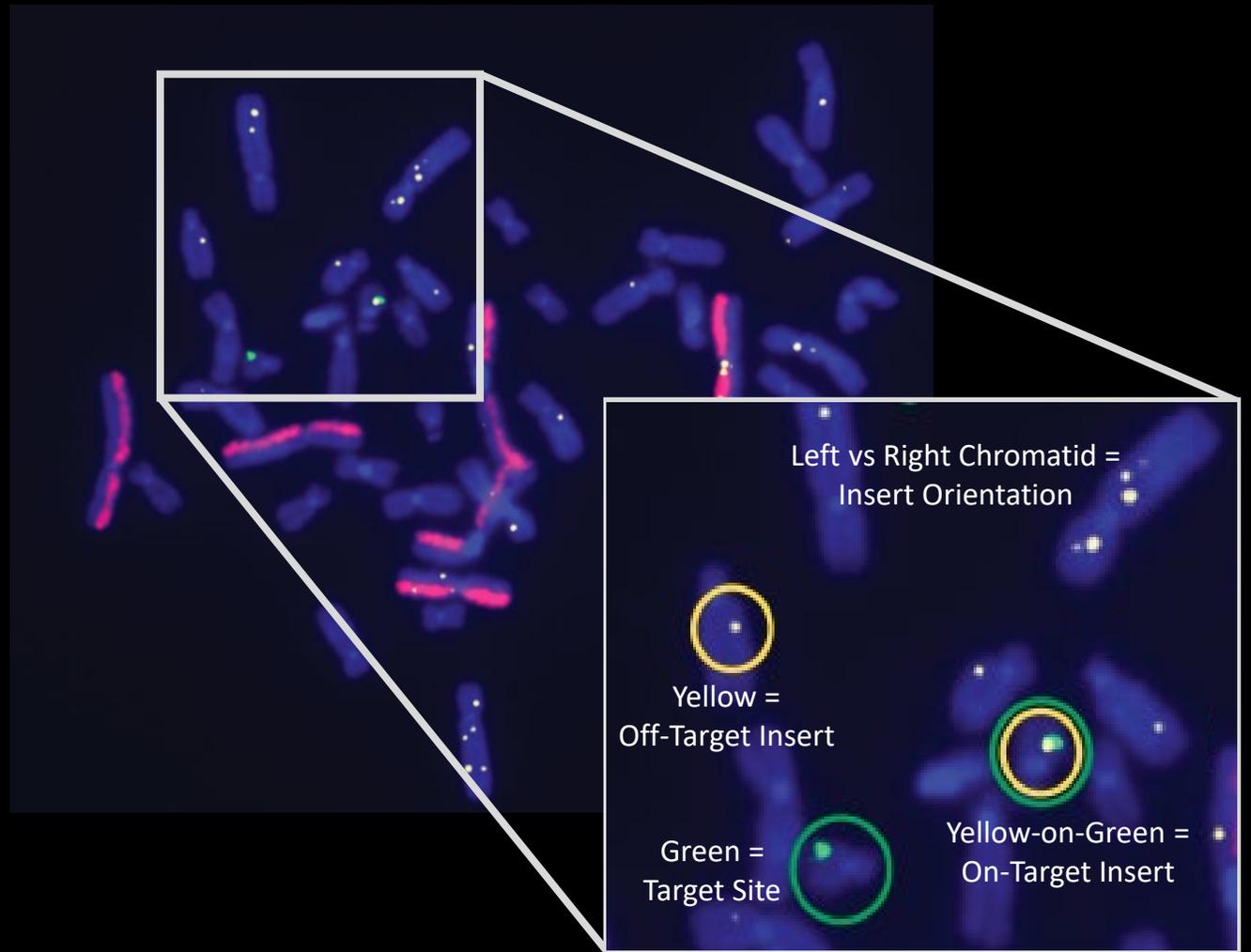
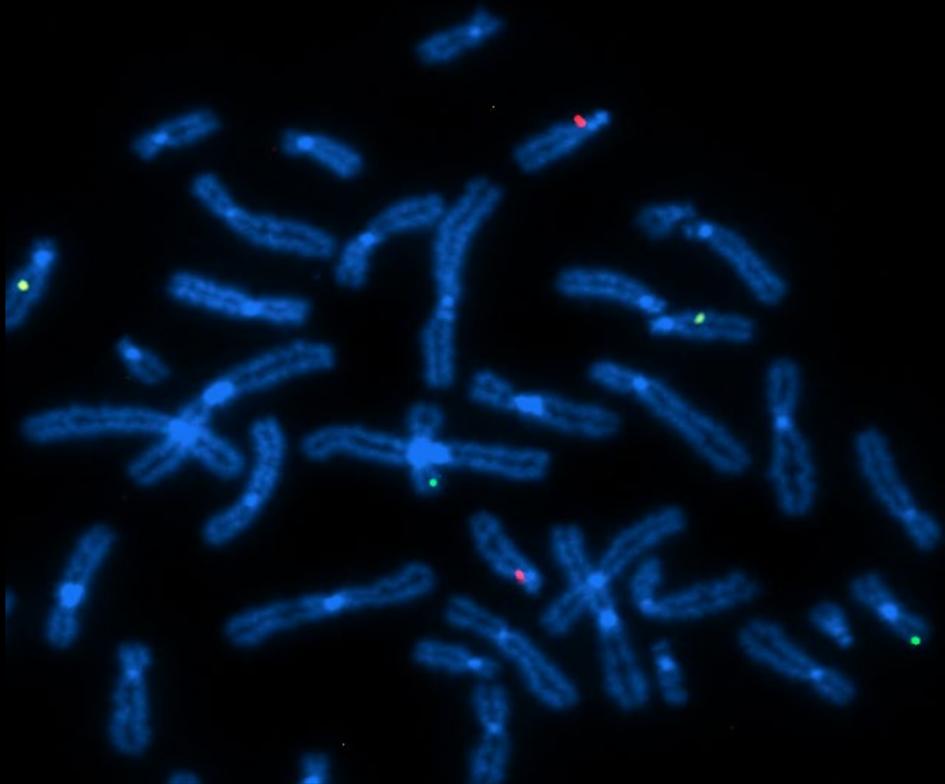


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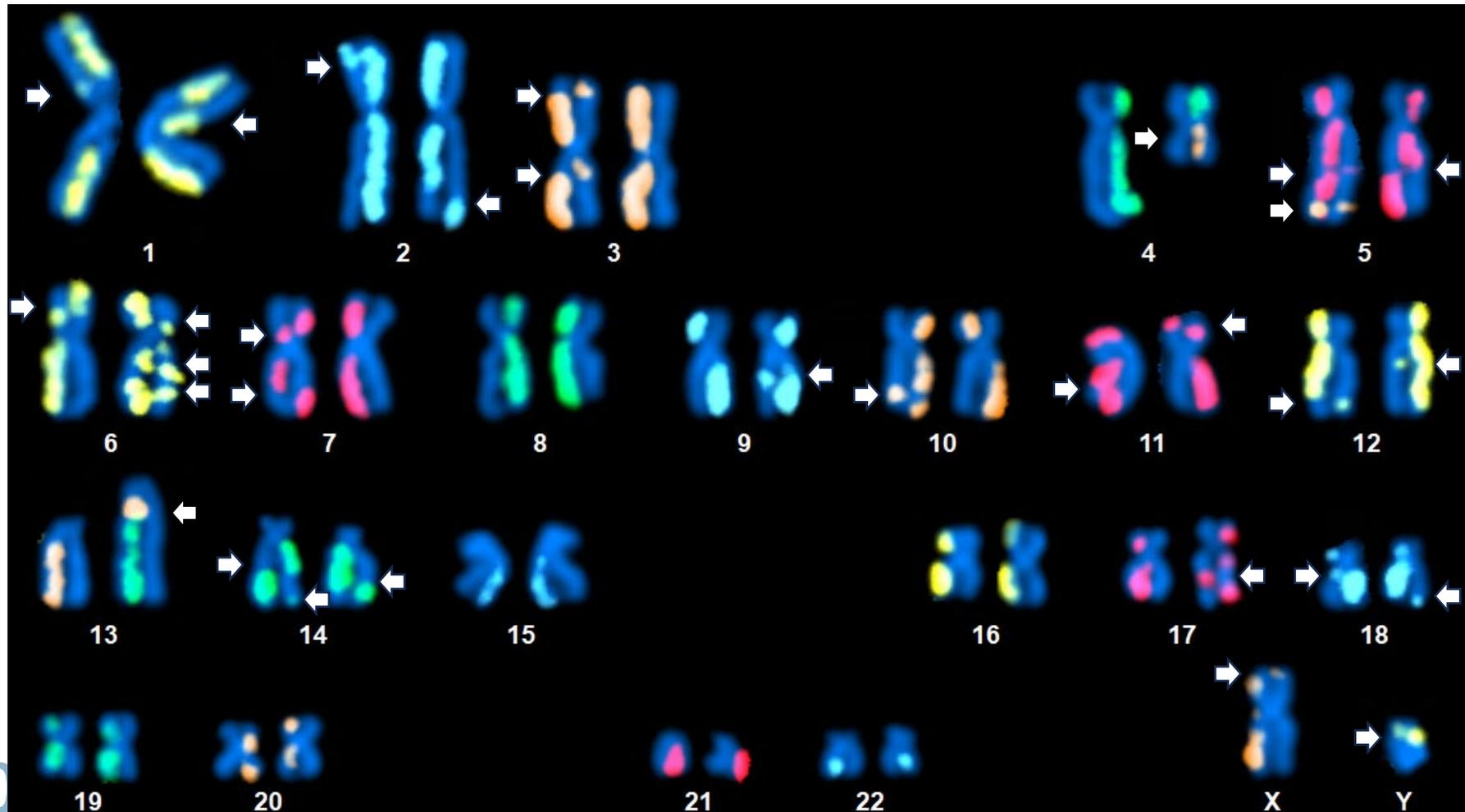


# dGH in-Site





# Hypothetical Scenario: Composite Karyogram



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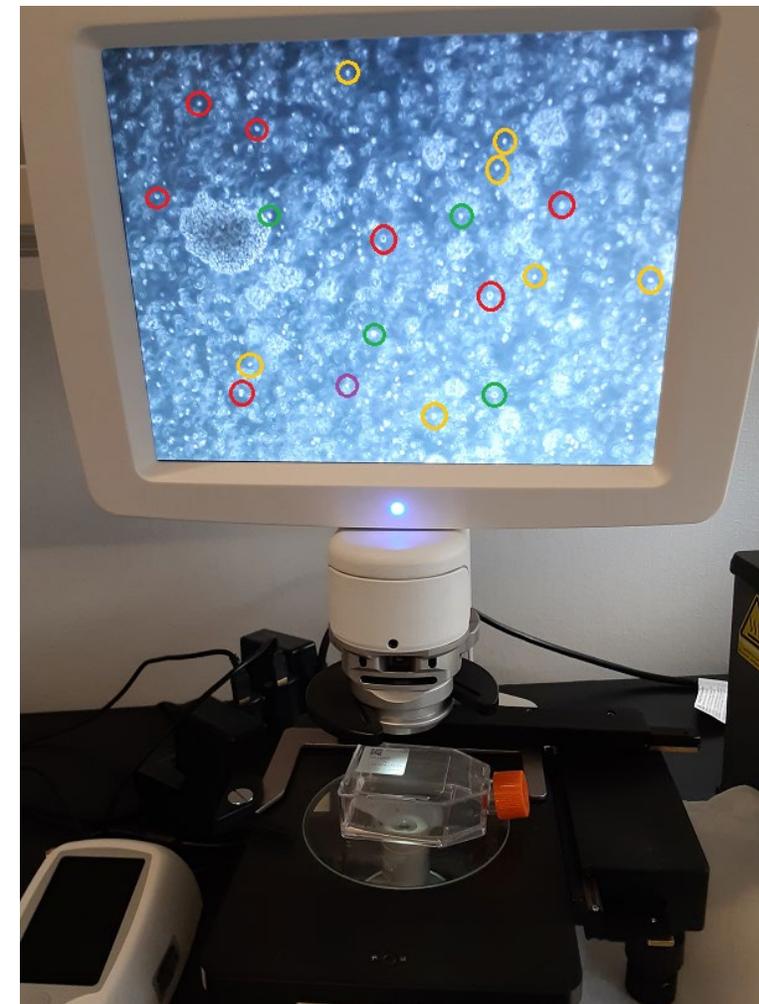


# Hypothetical Scenario: Mosaic Sample



## Multiple Clones within One Sample:

- Clone A; 47% of cells
  - Insertion at all three target sites
  - Translocation triggered between two insertion loci
- Clone B; 41% of cells
  - Insertion at all three target sites
  - Insert sequence duplicated in 2 of 3
- Clone C; 11% of cells
  - Insertion at one of three target sites
  - Insertion is in inverted orientation
  - Delete of tumor-suppressor gene
- **Clone D; 1% of cells**
  - **Subclone of Clone C, has multiple tumor suppressor knock-outs via off-target structural rearrangements**



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# Hypothetical Scenario: Heterogeneous Sample Sets

- Comparing Editing Techniques
- Comparing Donors
- Comparing Culture Conditions or Editing Components
- Comparing Conditions Over Time



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# Orthogonal Data

G-Band Karyotyping

Unbiased  
Gross Structural Changes

Single Cell, Heterozygosity Data  
Lower Resolution, Approximately 10 Mb

dGH SCREEN

Unbiased, Sequence-Orientation Data  
Rearrangements, Small Inversions Included

Single Cell, Heterozygosity Data  
Higher Resolution, as Low as 10 kb

dGH in-Site

Targeted, Sequence-Orientation Data  
Tracks Inserts, Target Site Rearrangements

Single Cell, Heterozygosity Data  
Can Achieve Resolution Below 2 kb

Targeted NGS

Targeted Sequencing at Loci of Concern  
Accuracy/Bias Are Platform-Dependent

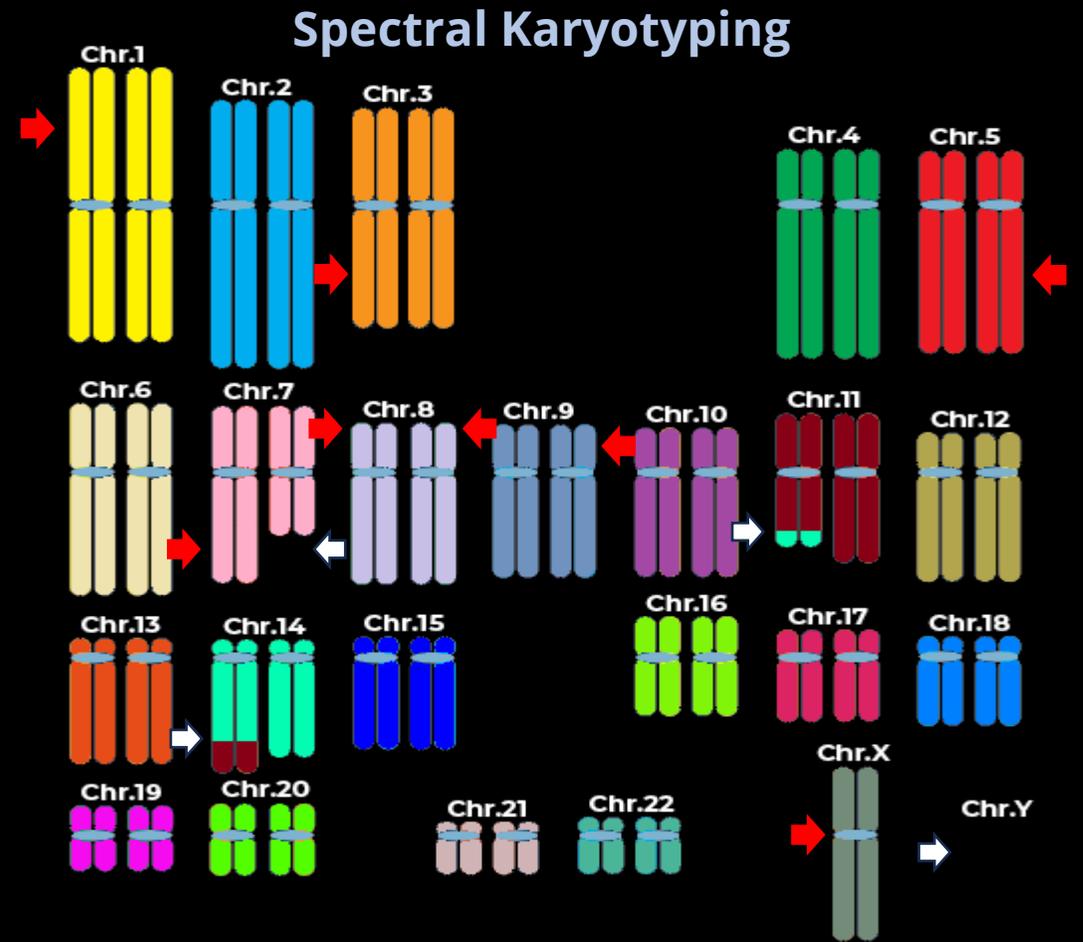
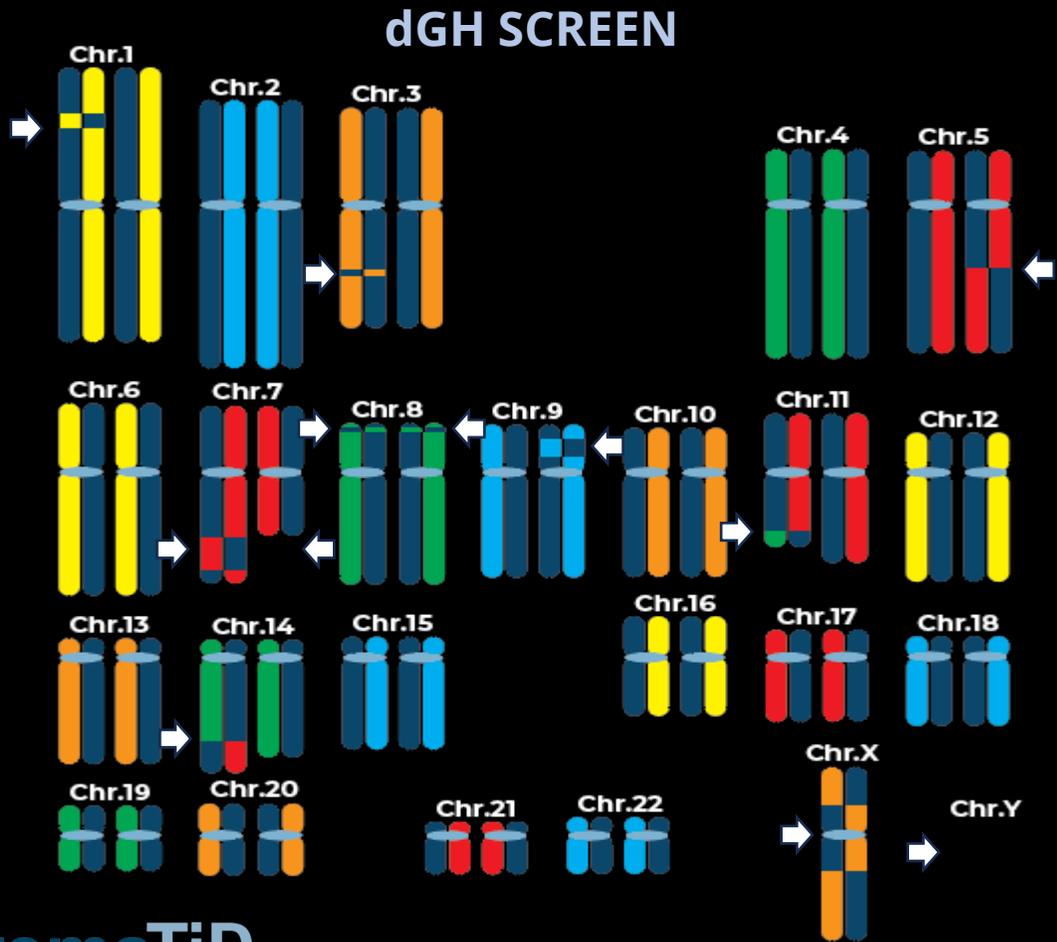
Mingled DNA Strands from Multiple Cells  
Base Pair-Level Resolution

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# Other Multicolored Karyotyping Techniques

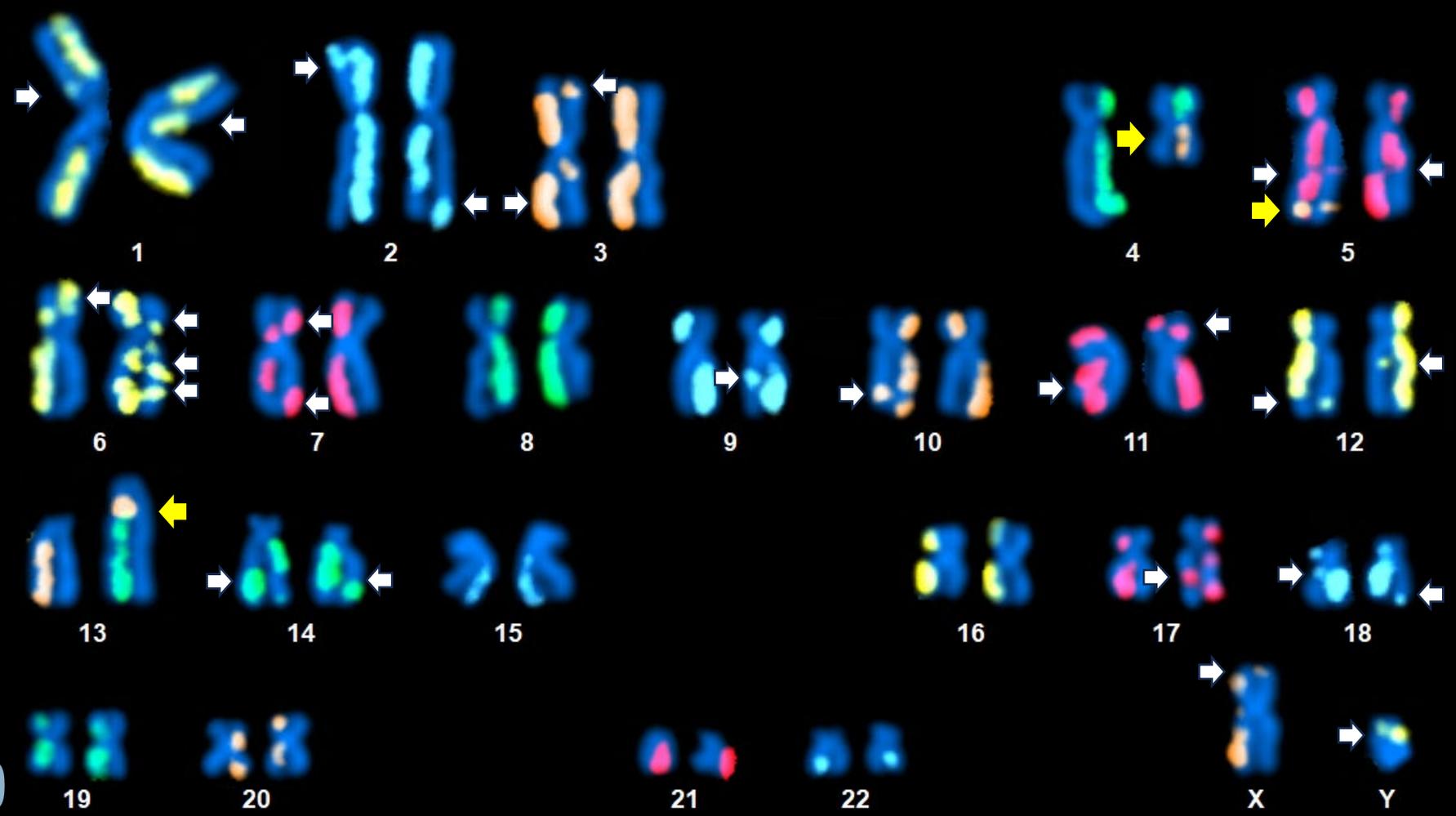


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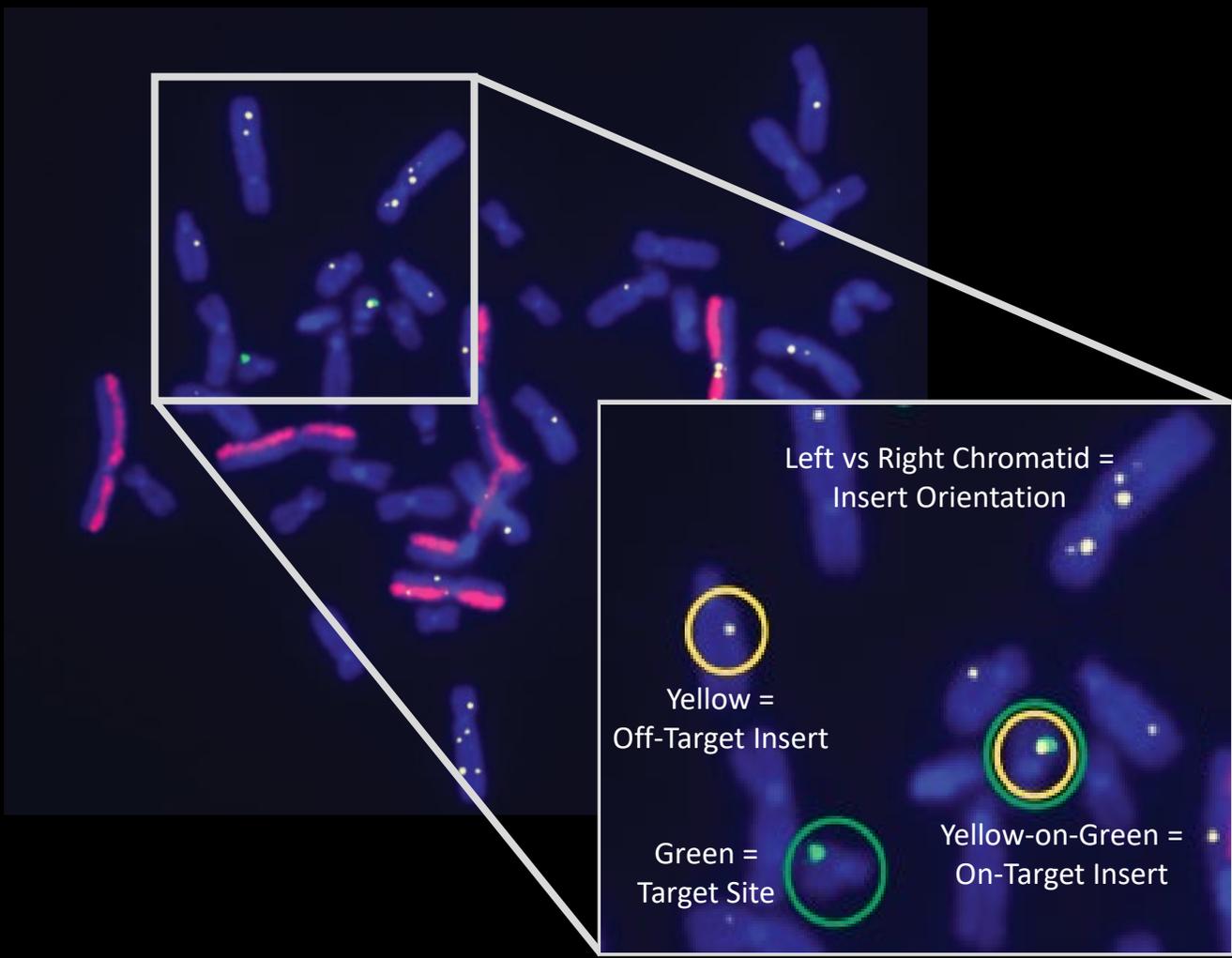
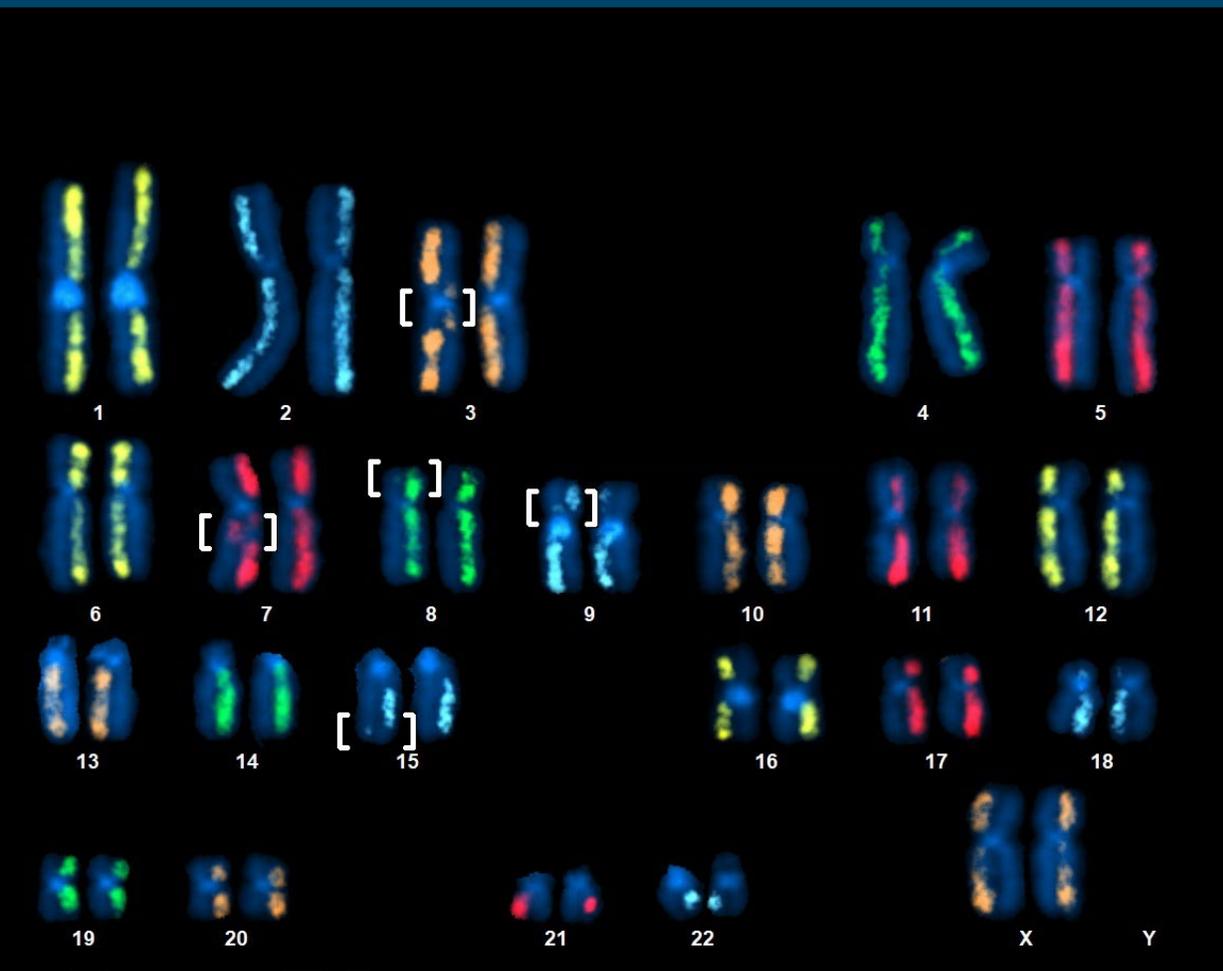
# The Role of dGH



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# The Role of dGH



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# Q & A

[Questions? Contact Us Today!](#)

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